Title	**The Genetics of Atrial Fibrillation: A Systematic Investigation of Associated Candidate Genes**
Principal Investigator	Emelia Benjamin, MD (email: emelia@bu.edu)
Abstract	To test for an association between atrial fibrillation (AF), and candidate genes for left ventricular mass in the following pathways: 1) cardiac channel pathways; 2) neurohumoral factor pathways; 3) Sarcomere protein pathways; 4) alpha and beta adrenergic receptor pathways; 5) metalloproteinase pathways; 6) signaling pathways; 7) Growth factor pathways; 8) Kinase pathways; 9) Apoptosis pathways; 10) Inflammation pathways; 11) Oxidative stress pathways; and 12) Dilated cardiomyopathy pathways. Our hypothesis is that an association exists between polymorphisms in these pathways and AF. We will rely upon previously genotyped data and will study the AF phenotype. SAS will be used to look for association in unrelated subjects. SAS PROC LOGISTIC will be used to assess associations of each polymorphism with AF.
Title	**Associations of SNP variants in A20 and related genes in the TNFalpha stimulated NFkappaB activation pathway with clinical atherosclerotic cardiovascular disease and subclinical atherosclerosis measures in the Framingham Heart Study**
Principal Investigators	Jan Breslow, MD (email: breslow@rockefeller.edu) Christopher O'Donnell, MD, MPH, FACC (email: chris@fram.nhlbi.nih.gov)
Abstract	We propose to test for associations of genetic variants (haplotype tagging single nucleotide polymorphisms) in A20 and 12 other genes in the TNFalpha stimulated NFkappaB activation pathway with clinical atherosclerotic cardiovascular disease and subclinical atherosclerosis measures in the Framingham Heart Study. The rationale for this study is the discovery of an atherosclerosis susceptibility locus on mouse chromosome 10 (11 cM, logarithm of odds 7.8), noted after we conducted an intercross between atherosclerosis susceptible (C57BL/6J ApoE0) and resistant (FVB/N ApoE0) mice. A candidate gene in this mouse region, A20, is involved in the feedback suppression of NFkappaB activation induced by tumor necrosis factor alpha (TNFalpha), and an amino acid change in A20 results in less effective termination of TNFalpha-stimulated NFkappaB activation by C57BL/6J. These experiments aim to directly translate novel and potentially clinically important findings from mouse atherosclerosis models into human population studies.
Title	**Summer Institute in Biostatistics**
Principal Investigator	Anita DeStefano, PhD (email: adestef@bu.edu)
Abstract	Given the educational nature of this request no abstract is provided.
Title	**Genetic Determinants of Vitamin D Status**
Principal Investigator	David Karasik, PhD (email: karasik@mail.hrca.harvard.edu)
Abstract	Vitamin D is essential for calcium absorption and skeletal health. Most circulating vitamin D is produced in the body when the skin is exposed to sunlight, but it can also be obtained from diet. The blood level of 25-hydroxyvitamin D (25OHD), a liver metabolite of vitamin D, is the best clinical indicator of vitamin D status, and large subsets of the US population are thought to have suboptimal 25OHD levels. Several previous studies suggest that 25OHD

	levels may be genetically influenced. Complex segregation analyses performed to date showed that about 22% of the 25OHD variation was accounted for by a putative major gene effect, but specific genetic mechanisms for this effect have not been examined. The proposed study will investigate the heritability of 25OHD in a well-characterized sample from general population, examine the possibility of a major gene effect on 25OHD levels, and link specific chromosomal loci to 25OHD and measures of bone strength and quality. Extensive genetic, biochemical and physical data related to vitamin D and bone health have been collected from both FHS cohorts. In addition, genome-wide genotyping with a set of 401 microsatellite markers has been performed in a large subset of the population, allowing for analyses that link specific genes to 25OHD and bone measurements. Statistical methods and software designed specifically for genetic analyses will be used to evaluate heritability, conduct segregation and linkage analyses, and control for confounding variables. This study will provide preliminary data needed to examine the genes that influence 25OHD variation in men and women across a wide age range that includes the very elderly. Identification of chromosomal region(s) governing 25OHD levels will allow us to perform further studies of genetic association of candidate genes in these regions with 25OHD and other osteoporosis-related phenotypes. Ultimately, this work may lead to molecular targets for future therapeutic interventions designed to prevent osteoporosis.
Title	**Genetics of Bone Density and Structural Geometry in Framingham Cohorts II**
Principal Investigators	Douglas Kiel, MD, MPH (email: kiel@mail.hrca.harvard.edu) David Karasik, PhD (email: karasik@mail.hrca.harvard.edu)
Abstract	Osteoporosis is a skeletal disorder characterized by compromised bone strength leading to increased risk of fracture. Several genes have demonstrated functional importance in bone metabolism and have been proposed as being associated with bone strength, including: Apolipoprotein E, Bone morphogenetic protein 4, Bone morphogenetic protein receptor, type II, Growth hormone receptor , Insulin-like growth factor 1 receptor and Insulin-like growth factor binding proteins 3 and 4, Matrix Gla protein, /Matrix metalloproteinase 3, and Transforming growth factor, beta 3. Study of both weight-bearing and non weight-bearing bones, namely the femur, calcaneus, spine, and metacarpals, may help to identify both site-specific and general skeletal effects of genes. The specific aim of this study is to examine the association between polymorphisms in several candidate genes, bone mineral density, and biomechanical and structural indices of the proximal femur and of the metacarpals, in participants of the Original and Offspring Cohorts of the Framingham Heart Study. Polymorphisms of potential gene candidates will be associated with indices of bone density as well as geometry at two skeletal sites. The phenotypes to be studied include highly heritable bone mineral density, as well as biomechanical and structural indices of proximal femur and metacarpals, derived from cross-sectional cortical measurements. Single marker and haplotype association analyses will be completed.
Title	**A candidate gene approach to identifying genetic determinants of electrocardiographic QT, RR, and PR interval variation in the Framingham Heart Study**
Principal Investigator	Christopher O'Donnell, MD, MPH, FACC (email: chris@fram.nhlbi.nih.gov)
Abstract	Common genetic variation in genes encoding ion channels, ankyrin B, sarcomere proteins and beta-adrenergic receptors contribute to variation in cardiac repolarization phenotypes. Additional genes which confer susceptibility to left ventricular hypertrophy, associated with increased risk of sudden cardiac death by ventricular arrhythmias, may be associated with

	abnormal myocardial repolarization, as manifest on the electrocardiogram. We seek to define common variation in these candidate genes and to associate such variation to QT, QT-peak, JT, RR and PR intervals measured in the Framingham Heart Study Offspring. We will examine associations with these phenotypes of single nucleotide polymorphisms (SNPs), including haplotype-tagging SNPs and missense SNPs, in candidate genes for cardiovascular disease being genotyped through the NHLBI's Programs for Genomics Applications. Regression analyses will be used to test for associations of individual SNPs with each phenotype and *haplo.score* will be used to test for haplotype association to phenotypes.
Title	**Genetics of Vascular Stiffness**
Principal Investigators	Tom Quertermous, MD (email: Tomq1 @stanford.edu) Daniel Levy, MD (email: dan@fram.nhlbi.nih.gov)
Abstract	An understanding of the genetic basis of vascular phenotypes such as hypertension and accelerated vascular stiffness will provide insight into successful vascular aging and into the pathologic changes in vascular structure and function that contribute to premature CVD. The vascular phenotypes proposed in this Reynolds Project will facilitate the discovery of vascular disease promoting genes by a candidate gene approach. Linkage results from a completed genome-wide scan will be used to further guide positional candidate gene selection. There are convincing data that association (i.e. candidate gene) and linkage (i.e. genome scan) approaches are complementary. The Aims of this project are threefold: 1) To genotype 20-40 candidate genes for vascular stiffness phenotypes and conduct association analyses with vascular stiffness phenotypes derived from arterial tonometry; 2) To extend association analyses of Reynolds candidate genes to include blood pressure phenotypes; and 3) To extend association analyses of Reynolds phenotypes to include candidate genes from other Framingham investigations.
Title	**Heritability, Linkage of Plasma Renin Levels and Urine Sodium Excretion, and Relations to Genetic Variation in the Renin-Angiotensin-Aldosterone Pathway**
Principal Investigator	Vasan Ramachandran, MD (email: vasan@bu.edu)
Abstract	There is now increasing evidence that the RAAS and renal tubular handling of sodium excretion play a fundamental role in susceptibility to hypertension and diseases associated with sodium retention. These observations have led to the consideration of an etiologic role for plasma renin in a number of disease states including heart failure, atherosclerosis, hypertension, and nephrosclerosis. Inter-individual variability in circulating renin levels and urinary sodium excretion may contribute to the differential susceptibility to the disease states noted above. Thus, it is important to the understand sources of this variability. The exact contribution of clinical, environmental, and genetic factors to the variability of plasma renin and to urine sodium excretion have been inadequately investigated. Data on the heritability of plasma renin and urinary sodium excretion, and the relations of plasma renin levels and urinary sodium excretion to common genetic variation in select genes involved in the RAAS are very limited. The objectives of this study are to use extant databases (urine sodium excretion, select SNPs in aldosterone metabolic pathways and the RAAS that have been previously genotyped) and plasma renin (to be measured following funding of pending research grant) to: (1) estimate the genetic contribution to the variance in plasma renin and urinary sodium via a

	heritability analysis; (2) perform linkage analyses to identify candidate genes that influence plasma renin and urine sodium levels, if there is evidence of heritability; and (3) investigate the relations of polymorphisms in the RAAS and mineralocorticoid pathway genes to plasma renin and urinary sodium excretion. Heritability for both plasma renin and urine sodium excretion will be estimated and the existing genome scan data at Framingham will be used for linkage analysis.
Title	**Examining the utility of a new Bayesian method for constructing genetic maps**
Principal Investigator	Andrew George, PhD (email: andrew-george@uiowa.edu)
Abstract	Genetic maps are an invaluable resource when mapping disease genes, calculating risks in genetic counseling and ordering DNA sequence. Unfortunately, genetic maps are not known with certainty. Genotyping errors can significantly bias map distances and most published maps are generated from data containing relative few informative meioses. Genetic maps are most accurately constructed from the joint linkage analysis of multilocus data. Computational limitations, however, greatly restrict the number of loci that can be mapped jointly. Consequently, multilocus data are rarely fully utilized in the construction of genetic maps. To address this problem, we have developed a Bayesian method for mapping many markers jointly using genetic data observed on multiplex families. We avoid the computational difficulties of dealing with many candidate loci orders and calculating multilocus likelihoods by implementing a new Markov chain Monte Carlo procedure. Our Markov chain Monte Carlo procedure approximates the posterior probability of a loci order along with the posterior probabilities of the recombination rates and marker allele frequencies allowing a variety of statistics to be constructed. Our proposal is to demonstrate the performance characteristics of the Bayesian multilocus mapping methodology via the analysis of real and simulated data sets originating from the Framingham Heart Study.
Title	**The Genetics of Age at Natural Menopause**
Principal Investigator	Joanne Murabito, MD, ScM (email: joanne@fram.nhlbi.nih.gov)
Abstract	The goal of this project is to identify chromosomal regions linked to age at natural menopause and to identify associated candidate gene variants in the estrogen receptor alpha gene. Reported age at natural menopause will be the phenotype. The existing Framingham genome scan data will be used. The estrogen receptor 1 *(ESR1) Pvu* II site and five other *ESR1* polymorphisms have already been typed. Heritability for age at menopause will be calculated using a variance component strategy, such as that implemented in GENEHUNTER. The established genome-wide linkage analysis at Framingham will be used for linkage analysis. Multipoint quantitative trait linkage (QTL) analyses will be performed and the multipoint LOD scores will be computed for all 22 chromosomes. SAS PROC MIXED will be used to perform multivariate regression analyses examining the association between the genotype of interest and age at natural menopause.
Title	**Assessment of linkage and association of genotypes returned to the Framingham Heart Study with a range of phenotypes measured in the Framingham Heart Study**
Principal Investigator	L. Adrienne Cupples, PhD (email: adrienne@bu.edu)
Abstract	Genotyping data in the Framingham Heart Study repository will be used for hypothesis

	generating analyses. Association and linkage (when appropriate) analyses will be conducted using the standard unrelated and family plate sets. The traits that will be evaluated are blood pressure, lipids, glycemic measures, anthropomorphic measures and pulmonary function measures. Analytical methods will use variance component analyses for linkage, analysis of covariance for association in unrelated subjects and family-based association tests in families. Conservative alpha levels will be applied to guard against making a Type I Error. Analyses will be reviewed by groups of individuals with expertise in these traits. These groups will recommend whether follow up studies are warranted.
Title	**Genotyping the Framingham third generation by the Mammalian Genotyping Service (Marshfield)**
Principal Investigator	Larry Atwood, PhD (email: lda@bu.edu)
Abstract	The aim of the genetics component of the Framingham Heart Study (FHS) is to detect and locate genes that affect a broad range of phenotypes gathered in FHS. In pursuit of this goal, this project will submit DNA to the Mammalian Genotyping Service (Marshfield) to genotype 400 polymorphic markers in approximately 5370 participants in the study. These participants fall into two categories. The first is approximately 3680 participants in the third generation that are currently being recruited. The second is approximately 1690 parents and grandparents of the third generation that are participants in FHS but have not been previously genotyped by MGS. Common diseases like arteriosclerosis, hypertension, diabetes, and obesity are acknowledged to have a strong genetic component contributing to disease etiology. The genotyped markers will be primarily used to perform genome wide linkage analysis on all of these traits. The proposed genotypes will be added to the general Framingham genome map and thus *will be available not only to Framingham investigators but also to ancillary studies and external investigators* who have permission to analyze Framingham data. Thus, the genotyping will benefit the study of a large number and variety of traits. FHS is truly a national resource.
Title	**Does an Association Exist between *ESR1* polymorphisms and Obesity Phenotypes?**
Principal Investigator	Caroline Fox, MD, MPH (email: caroline@fram.nhlbi.nih.gov)
Abstract	The goal of this project is to test for an association between ESR1, a candidate gene located on chromosome 6q25, and waist circumference, waist hip ratio (WHR), and body mass index (BMI). Our hypothesis is that an association exists between polymorphisms in ESR1 and obesity phenotypes. Waist circumference, waist hip ratio, and BMI are the phenotypes in this project. Six polymorphisms in the ESR1 gene have already been typed by David Housman's group at MIT. Several others are in progress. SAS will be used to look for association in related and unrelated subjects. SAS PROC GLM will be used to assess associations of each polymorphism with waist circumference, WHR, and BMI among unrelated individuals. SAS PROC MIXED and FBAT will be used to examine associations among related individuals.
Title	**Linkage and Heritability to Weight Gain**
Principal Investigator	Caroline Fox, MD, MPH (email: caroline@fram.nhlbi.nih.gov)
Abstract	While loci for body mass index (BMI) and waist circumference have been identified, it is not known whether the same sets of genes underlie the process of weight gain. Thus, we seek to

	assess heritability and linkage to weight gain. We will use the existing Framingham Heart Study genome scan; thus no additional genotyping will be necessary for this project. Variance components linkage-analysis will be performed on 330 families from the Framingham Heart Study original and offspring cohorts using the existing 10 cM genome-wide linkage analysis.
Title	**Somatic Mosaicism and Premature Coronary Artery Disease**
Principal Investigators	Calum Macrae, MD (email: macrae@cvrc.mgh.harvard.edu) Christopher O'Donnell, MD, MPH, FACC (email: codonnell@partners.org)
Abstract	Somatic mutations have been implicated in cancer and in some inherited forms of pulmonary hypertension, but their role in atherosclerotic disease is unknown. Feto-maternal microchimerism has been previously demonstrated in some forms of inflammatory disease. Recently a rare and extreme form of premature atherosclerosis was found to be caused by hemizygosity for mutations in the lamin A/C gene. We are proposing to use quantitative real-time PCR to assess feto-maternal michrocimerism in subjects with premature coronary artery disease (CAD), to specifically test for somatic mutation of the lamin A/C gene in circulating leukocytes, and to assess the role of common haplotypes in the lamin A/C gene in premature CAD and other CVD phenotypes (specifically an integral CVD measure and carotid IMT).
Title	**Genetics of Vasorelaxation and Cardiovascular Responses**
Principal Investigator	David Housman, PhD (email: dhousman@mit.edu)
Abstract	Common genetic contributors to high blood pressure, which afflicts more than 50 million Americans, are unknown. Recent studies have demonstrated that genes controlling blood vessel constriction critically regulate blood pressure, and can affect blood vessel thickness. We hypothesize that variants in genes that control blood vessel contraction are associated with hypertension and blood vessel thickness in humans. To test this hypothesis we will measure the association of genetic variants for candidate genes important for vascular contraction and relaxation with the thickness of the common carotid artery. Polymorphisms that are associated with increased or decreased carotid artery thickness will then be emphasized when we screen for association of these same variants with blood pressure. In addition we will study the association of these markers with the requirement for blood pressure treatment and the timing of hypertension onset. For continuous variables, such as blood pressure, we will use analysis of variance while for a dichotomous variable logistic regression models will be used; potential confounders will be added to each analysis to account for their effect. In summary, we will determine if variants of genes important for blood vessel contraction and relaxation are associated with heart and blood vessel disorders, including blood pressure.
Title	**Genetics of Bone Structural Geometry in Framingham Cohorts II**
Principal Investigators	David Karasik, PhD (email: karasik@mail.hrca.harvard.edu) Douglas Kiel, MD, MPH (email: kiel@mail.hrca.harvard.edu)
Abstract	Osteoporosis is a skeletal disorder characterized by compromised bone strength leading to increased risk of fracture. Several genes have demonstrated functional importance in bone metabolism and have been proposed as being associated with bone strength, including: ACE, ADRB2, AR, COL1A1, CYP19, ESR1 and ESR2, IGF1, IL-6, LRP5, MTHFR, NOS3, OPG

	(TNFRSF11B), PPARG, PTH, TGFB1, TNF, and TNFRSF1B. Study of both weight-bearing and non weight-bearing bones, namely the femur and metacarpals, may help to identify both site-specific and general skeletal effects of genes. The specific aim of this study is to examine the association between polymorphisms in several candidate genes, bone mineral density, and biomechanical and structural indices of the proximal femur and of the metacarpals, in participants of the Original and Offspring Cohorts of the Framingham Heart Study. Polymorphisms of potential gene candidates will be associated with indices of bone density as well as geometry at two skeletal sites. The phenotypes to be studied include highly heritable bone mineral density, as well as biomechanical and structural indices of proximal femur and metacarpals, derived from cross-sectional cortical measurements. Population-based and family-based association methods and genetic linkage analysis will be conducted.
Title	**Heritability, Linkage of Serum Aldosterone Levels, and Relations to Genetic Variation in Aldosterone Synthase and the Mineralocorticoid Receptor**
Principal Investigator	Vasan Ramachandran, MD (email: vasan@bu.edu)
Abstract	There is now increasing evidence that aldosterone has direct effects on the heart and vasculature independent of its salt-retaining effects on the kidney. These observations have led to the consideration of an etiologic role for aldosterone in a number of disease states including heart failure, atherosclerosis, hypertension, and nephrosclerosis. Inter-individual variability in circulating aldosterone may contribute to the differential susceptibility to the disease states considered above. Thus, it is important to the understand sources of this variability. The exact contribution of clinical, environmental, and genetic factors to the variability of plasma aldosterone is currently unknown. Data on the heritability of serum aldosterone and the relations of serum levels to common genetic variation in select genes involved in the aldosterone synthesis pathway and aldosterone receptors are very limited. The objectives of this study are to use extant databases (serum aldosterone, select SNPs in aldosterone metabolic pathways that have been previously genotyped) to: (1) estimate the genetic contribution to the variance in hormone levels via a heritability analysis; (2) perform linkage analyses to identify candidate genes that influence aldosterone levels; and (3) investigate the relations of polymorphisms in the aldosterone synthase and mineralocorticoid receptor genes to serum aldosterone levels. Heritability for serum aldosterone will be estimated and the existing genome scan data at Framingham will be used for linkage analysis.
Title	**Heritability and Linkage of Exercise Treadmill Test Performance Measures**
Principal Investigator	Vasan Ramachandran, MD (email: vasan@bu.edu)
Abstract	Exercise capacity and systolic blood pressure (BP) and heart rate responses to exercise are associated with long-term cardiovascular outcome and mortality. Limited data emphasizes considerable familial resemblance in exercise performance measures, and linkage analyses have identified chromosomal regions that may be associated with these hemodynamic responses. Our objectives are: to examine the heritabilities of exercise BP and heart responses, and exercise capacity during a submaximal treadmill exercise test performed on Framingham Offspring study participants; to identify chromosomal regions linked to exercise hemodynamic responses. Heritability for measures of exercise response will be estimated and the existing genome scan data at Framingham will be used for linkage analyses.

Title	**Epistatic Interactions and the Framingham Heart Study**
Principal Investigator	Denise Daley, PhD (email: denise@darwin.cwru.edu)
Abstract	The purpose of our investigation is to explore the complex trait of hypertension using epistatic models, to further explore the relationship between candidate genes and their relationships to blood pressure, gender and body mass index (BMI) in the Framingham Heart Study population. By incorporating covariates into our analysis of the Framingham Heart Study data, we were able to show that some regions of the genome males with high BMI are providing evidence for linkage, while in other areas women who gain weight over time are providing evidence for linkage. These findings have lead to our investigative hypothesis, that age at onset, and disease prevalence, differ between men and women because the underlying biological mechanisms that lead to hypertension are different between the sexes. Loci that are involved in the development of high blood pressure in males may very well be different than those in females. Our proposal has two two specific aims, 1) Follow-up and more detailed analysis of regions demonstrating evidence for linkage in men or women and 2) Investigation of potential epistatic interactions between loci. These are both undeniably important questions, which can provide significant insight and further understanding to the mechanism of hypertension in men and women.
Title	**Meta-Analysis of genomewide scans to identify regions linked to hypertension**
Principal Investigator	Olle Melander, MD, PhD (email: olle.melander@endo.mas.lu.se)
Abstract	Over the past years a large number of genome wide scans have been performed with the aim to map genetic loci linked to hypertension and blood pressure variation. However, a variability of linkage findings have been reported although the replication of linkage findings would be essential in successful detecting of chromosomal regions containing hypertension susceptibility genes. To combine efforts in this field, we believe a meta-analysis using the novel method of GSMA (genome-search analysis method) could be a powerful tool in assessing the evidence for linkage across hypertension studies. The analysis will be performed using the LOD-/NPL-/ etc. statistics (not including fine mapping data) for each chromosome resulting from as many published genomewide scans on hypertension/blood pressure as possible. Using this method our goal is to produce new information from the results of already published scans on hypertension/blood pressure variation and so reveal new regions linked to hypertension or reassure the significance of regions already discovered.
Title	**Mapping Susceptibility Loci for Nicotine Dependence in Framingham Heart Study samples**
Principal Investigator	Ming Li, PhD (email: lim2@uthscsa.edu)
Abstract	According to the 1996 National Household Survey on Drug Abuse, an estimated 68.8 million Americans reported current use of tobacco products. Therefore, tobacco represents one of the most widely used/abused substances. Many years of twin and family studies have provided strong evidence for a genetic contribution to nicotine dependence (ND). The heritability for ND, as estimated from twin studies, is in the range of 0.28 to 0.84, with a mean of 0.56. We hypothesize that a group of susceptibility genes increases vulnerability to ND. Recently, my research group performed a genome-wide linkage analysis of data from the 313 extended Framingham Heart Study (FHS) families and found evidence for significant linkage of smoking quantity to chromosome 11 and suggestive linkage to chromosomes 4, 7, 9, 14 and

	17. The genomic region on chromosome 9 in the FHS samples was also linked to alcohol consumption in another study reported by this group. To confirm the significance of the initial findings and to narrow the regions of interest in these FHS family samples, we propose to saturate these regions at approximately 1-2 cM with additional markers. Furthermore, we detected more than 40 candidate genes in these regions and plan to genotype 3 to 5 single nucleotide polymorphisms (SNPs) per candidate gene (depending on the availability of SNPs within each gene) in both the FSH family and random samples using the allelic discrimination assay. We expect that the completion of the proposed studies will greatly advance our understanding of genetic determinants of ND and may eventually allow targeting of novel preventive strategies to individuals at risk.
Title	**Relation between the 31bp VNTR in the Cystathionine Beta-Synthase Gene and Vascular Disease Risk**
Principal Investigator	Jacob Selhub, PhD (email: jacob.selhub@tufts.edu)
Abstract	Our purpose is to examine the effect of the 31 bp variable number tandem repeat (VNTR) in the cystathionine β-synthase (CBS) gene on markers of vascular disease risk. Homocysteine is a putative risk factor for vascular disease. The number of VNTR repeated elements in the CBS gene is inversely associated with enzyme activity and positively associated with fasting and PML hyperhomocysteinemia in small case-control samples. It has been proposed that this gene mutation may predispose individuals to a higher risk of vascular disease. We propose to use the Offspring cohort to examine the relation between the number of VNTR elements and three markers of vascular disease risk: 1) fasting and post-methionine homocysteine concentrations, 2) carotid artery intima media thickness measures, and 3) brachial artery reactivity measures. We will determine categories based on the number of VNTR's and compare the values of our vascular disease risk markers across these categories. With a total sample size of approximately 3500, we will have >90% statistical power for our three aims to detect differences of 10% or less between the three most frequent genotypes (17/18, 18/18, and 18/19), which comprise approximately 90% of subjects.
Title	**Alcohol Dehydrogenase-3 and Lewis Genes as Modifiers of the Association between Alcohol Consumption and HDL and Incident CHD: the Framingham Offspring Study**
Principal Investigator	Luc Djousse, MD, DSc (email: ldjousse@bu.edu)
Abstract	While moderate alcohol consumption has been associated with lower risk of coronary heart disease (CHD), little data are available on the gene-environmental interactions influencing the risk of CHD. Using data collected on approximately 1880 unrelated subjects of the Framingham Offspring, the current project will examine whether alcohol dehydrogenase gene (ADH1C) and Lewis gene influence the association between alcohol consumption and CHD. We are hypothesizing that subjects who are slow metabolizer for alcohol (homozygote for gamma allele of ADH1C) will show a greater reduction in CHD risk and higher concentration of HDL and that association between Lewis (a-b-) of the FUT3 gene and CHD will be modified by alcohol consumption. Furthermore, we will assess the influence of ADH1C gene on the relation between alcohol consumption and HDL cholesterol as well as its effects on frequency and quantity of alcohol consumed. MALDI-TOF mass spectrometry will be used to assay single nucleotide polymorphisms for ADH1C and FUT3 genes and multivariate regression models will be used to evaluate the association between alcohol intake and CHD, HDL-cholesterol, frequency, and amounts of alcohol consumed.

Title	**Insulin resistance, obesity, and endothelial dysfunction in the pathogenesis of type 2 diabetes-the Framingham Offspring Study**
Principal Investigators	James Meigs (jmeigs@partners.org)
Abstract	Type 2 diabetes is an increasingly common disease worldwide, and is closely associated with atherosclerotic cardiovascular disease (CVD). Insulin resistance, obesity, and heredity are known risk factors for diabetes, but mechanisms by which they confer increased risk remain uncertain. Recent hypotheses linking abnormal adipocyte signaling and vascular endothelial dysfunction with insulin resistance suggest novel pathways in the genesis of type 2 diabetes and CVD. Objectives of this study are to test hypotheses that insulin resistance syndrome traits (obesity, dyslipidemia, hypertension, and glucose intolerance), abnormal adipocyte signaling (plasma levels of tumor necrosis factor (TNF)-alpha receptors, interleukin-6, c-reactive protein, and adiponectin), endothelial dysfunction (plasma levels of PAI-1, von Willbrand Factor, intercellular adhesion molecule-1; brachial artery flow-mediated dilatation; and microalbuminuria), and associated gene polymorphisms (in the PPAR-gamma, TNF-alpha, endothelial nitric oxide synthase, adiponectin, resistin, and insulin genes) are determinants of type 2 diabetes and CVD. Cross-sectional and prospective analyses will use extensive data available from ~3600 participants of the population-based Framingham Offspring Study. Elucidation of the role of adipocyte signaling, endothelial dysfunction, and insulin resistance in the pathogenesis of type 2 diabetes may suggest new strategies urgently needed to stem the rising tide of this common disease and its and devastating CVD complications.
Title	**CardioGenomics Program in Genomics Applications: The Genetics of Ventriculo-Vascular Remodeling of Hypertension**
Principal Investigator	Emelia Benjamin, MD, ScM (email: emelia@bu.edu)
Abstract	Hypertension and vascular/cardiac remodeling are complex interrelated phenotypes that evolve over a lifetime and are influenced by multiple environmental and genetic factors. We postulate that common variation in key candidate genes, defined by haplotype tagging SNPs, contributes to variation in cardiac structure and function, blood pressure [BP] and carotid intimal medial thickness [IMT], in the community. As part of the NHLBI CardioGenomics Program in Genomic Applications primary aims are: 1) To characterize genetic variation in candidate genes relevant to cardiovascular structure and function; 2) To select for each candidate gene a comprehensive set of SNPs for association studies, and type these SNPs for 180 genes in the FHS cohort; 3)To examine the genetic determinants of alterations in cardiac structure and function assessed by echocardiography and cardiac magnetic resonance (Also, with the Seidman laboratory, we will examine participants with extreme LV wall thickness for rare variants of sarcomere protein and glycogen storage diseases); 4) To investigate the genetic determinants of contemporary, long-term and longitudinal progression of BP and HTN; 5) To study the genetic determinants of alterations in vascular remodeling. The secondary aim of Project 5 is to study the gene-environment interactions.
Title	**Cloning a blood pressure gene on human chromosome 2q32.3**
Principal Investigator	Nanette Steinle, MD (email: nsteinle@medicine.umaryland.edu)
Abstract	The Old Order Amish (OOA) represent a unique closed founder population who are relatively

	genetically homogeneous, and have very large family sizes and well documented genealogies. We identified a region of strong linkage to both diastolic (LOD = 4.23) and systolic (LOD1.61) blood pressures in the OOA. Peak evidence for linkage occurred at map positions 217 and 221 cM from p-ter for diastolic and systolic blood pressure, respectively. Association analysis of 46 SNPS already genotyped further narrows the region to approximately an area of 700kb at 2q32.3. We hypothesize that an important blood pressure susceptibility gene resides on chromosome 2q31-34 in the Amish, and that this same gene will be an important contributor to blood pressure variation in more outbred Caucasian (and possibly other) populations. Although there is no established road map to positional cloning of complex disease genes, we propose to use a parallel approach that has been successful for the positional cloning of other complex disease genes. Using DNA from the Framingham study in addition to our Amish samples, we propose to perform a combination of linkage disequilibrium mapping and exhaustive positional candidate gene analysis within and across Amish and the more outbred Caucasian Framingham population, with the rationale that the latter is likely to have less linkage disequilibrium and thus greater gene-localization power. This proposal includes a highly productive interdisciplinary research team at the University of Maryland. Discovery of genes influencing blood pressure will provide (i) critical insights into molecular mechanisms and new molecular targets for therapeutics; (ii) blood tests for the early detection of susceptible individuals so that targeted preventative interventions can be instituted. These advances will impact substantially on the quality of life of millions of older Americans with hypertension and cardiovascular disease.
Title	**HDL Cholesterol in females in the Framingham Heart Study**
Principal Investigator	Kari North, PhD (email: kari_north@unc.edu)
Abstract	In GAW13, we submitted the contribution, "*HDL cholesterol in females in the Framingham Heart Study is linked to a region of chromosome 2q*" by North et al. We propose to supplement the original findings by introducing additional covariates, such as menopausal status, hormone use, and use of cholesterol lowering medications that are believed to be important correlates of HDL-C in this population. As in our GAW13 contribution, we will examine the evidence for sex-specific linkage of HDL-C in a longitudinal sample of participants from the Framingham Heart Study. We will determine the evidence for linkage of HDL-C at three time points, spaced approximately 8 years apart, corresponding to visits 11, 15, and 20 for the cohort and visits 1, 2, and 4 for the offspring and their spouses. Using variance component methods, for each time point, we will estimate the heritability and genetic correlation of HDL-C, and test for genotype-by-sex interaction at a QTL in male and female samples, both separately and combined.
Title	**Genetic Variants Influencing Blood Pressure and Hypertension**
Principal Investigator	Haralambos Gavras, MD (email: hgavras@bu.edu)
Abstract	The prevalence of hypertension is about 20% in Caucasian-Americans and because of the associated morbidity and mortality, hypertension presents a major public health problem. Quantitative measures of blood pressure and clinical phenotypes, such as hypertension, demonstrate a genetic component. Searches for genetic factors influencing blood pressure or susceptibility to hypertension have often led to conflicting results (e.g. association studies of the angiotensin converting enzyme variants) or results are not reproducible (e.g. evidence of linkage to a specific chromosomal region). A cohort of unrelated Caucasian subjects and a

	family based cohort have been collected through the hypertension clinics at Boston Medical Center and collaborating clinical sites. The unrelated subjects include hypertensive individuals (SBP>140 and/or DBP>90 or on anti-hypertensive medication) and normotensive controls (age greater than 60 and SBP<130 or DBP<80 and not on anti-hypertensive medication). We have investigated polymorphisms in multiple candidate genes and found association between specific variants and hypertension status. We propose to confirm these findings by performing case-control and quantitative trait association in unrelated subjects as well as conducting family based association tests in the Framingham cohort.
Title	**Role of CYPs 2C8 and 4A11 in Human Hypertension**
Principal Investigator	Jorge Capdevila, PhD (email: jorge.capdevila@vanderbilt.edu)
Abstract	Studies with animal models of genetically controlled hypertension, including Cyp 4a knockout mice, suggest important pro- and antihypertensive actions for products of the CYP P450 arachidonic acid (AA) monooxygenases. We have identified a functional variant in human CYP4A11 (F434S) that results in impaired A metabolism, and established and increased frequency of the F434S variants in hypertensive compared to normotensive subjects. Based on these and other studies, we proposed that genetically determined alterations in the activity and/or expression of the Cyp 4A11 AAώ-hydroxylase or 2C8 epoxygenase are associated with the development of hypertension in humans. To test this hypothesis, we will apply high-throughput DNA sequencing methods to determine whether CYP4A11 and 2C8 variants are associated with hypertension and related cardiovascular parameters in the clinically well-characterized Framingham Cohort. The significance of CYP variants in contributing to continuous outcome measures such as level of blood pressure will be determined using analysis of variance. Logistic regression with multivariate analysis will be used to assess the significance of dichotomous variables, such as hypertensive state.
Title	**Joint Linkage Disequilibrium and Linkage Fine-Mapping of Quantitative Trait Loci**
Principal Investigator	Ina Hoeschele, PhD (email: inah@vt.edu)
Abstract	Multi-factorial, polygenic and quantitative traits are ubiquitous and include traits and risk factors related to complex diseases in humans (e.g., cardiovascular disease, diabetes, and hypertension). Gene mapping is one of few promising avenues for advancing our knowledge of the genetic architecture and molecular basis of quantitative traits. Two major steps are required to improve the accuracy and power of gene mapping: 1) The development of statistical methods for joint linkage disequilibrium and linkage fine-mapping of Quantitative Trait Loci (QTL); and 2) The implementation of such methods with efficient computing algorithms, in particular with efficient multi-locus genotype samplers, which enable the analysis of large, multi-generational, complex pedigrees. The goal of this project is to develop, implement, evaluate and compare statistical methods for joint linkage disequilibrium and linkage mapping of genes affecting quantitative traits related to heart disease in simulated populations representing pedigree, clinical trait distribution, and genetic marker structures of actual Framingham Heart Study pedigrees. The best method(s) will be made available in a set of computer programs, which are expected to extend considerably the range of pedigrees, which can be analyzed. An approximate variance components or expectation method for joint linkage disequilibrium and linkage mapping of QTL will be further developed and implemented. A fully parametric or distribution Bayesian analysis for joint linkage disequilibrium and linkage mapping of QTL will be developed. These methods will be then

	compared with the simulated data.
Title	**Identification of single nucleotide polymorphisms with the SIR genes in the oldest Framingham Heart study Cohort members**
Principal Investigator	Leonard Guarente, PhD (email: leng@mit.edu)
Abstract	The seven SIRT genes are human homologs of SIR2, a gene that regulates life span in simple model organisms, such as yeast and C. elegans. To investigate whether any of the human SIRT genes regulate any aspect of aging, we will identify SNPs by sequencing DNA of samples of the Framingham Heart Study. Any SNPs, particularly those that cause amino acid changes, will be correlated with longitudinal patient information on cardiovascular and other diseases of aging. The Framingham population affords a unique opportunity to link a key regulator of aging in laboratory organisms with human aging.
Title	**Inflammation: Correlates and Prognosis in Framingham - RO1 HL64753 Proposal to Submit a Grant Renewal Extending the Previous Work to Include Genetic Analyses**
Principal Investigator	Emelia Benjamin, MD, ScM (email: emelia@bu.edu)
Abstract	Recent experimental and clinical studies support the concept that vascular inflammation is central to the development of atherosclerosis, and that systemic inflammatory markers predict a wide array of CVD events. We have previously measured systemic vascular inflammatory markers, in a population-based sample of 3800 middle-aged and elderly men and women of the Framingham Heart Study offspring cohort and have observed that the inflammatory markers are related to standard CVD risk factors, and prevalent clinical and subclinical CVD. However, a large proportion of the variability of vascular inflammation remains unexplained and the contribution of genes to the variability is largely unknown. The central hypothesis of this proposal is that systemic vascular inflammation represents a complex phenotype that evolves over a lifetime and is influenced by both environmental and genetic factors. We further postulate that variations in the inflammatory phenotype (marker levels) and genotype predispose to the development of CVD. The specific genetic aims are: 1) To investigate the genetic determinants of systemic inflammation via heritability, linkage and genotype-phenotype association studies; 2) To identify the inflammatory phenotypic and genetic determinants of subclinical CVD; and 3) To determine the contribution of inflammatory phenotype versus genotype to prevalent and incident CVD.
Title	**Genes on 10q23-q25 related to type2 Diabetes Mellitus**
Principal Investigator	Alan Herbert, PhD (email: aherbert@bu.edu)
Abstract	Over 14% of FHS Offspring participants meet clinical criteria for the diagnosis of type 2 Diabetes Mellitus (DM2) by age 70 (defined as defined as hypoglycemic medication treatment, fasting plasma glucose level ≥7.8 mmol/l or the 2-h post challenge glucose level ≥11.1 mmol/l). Insulin resistance, glucose intolerance and hyperinsulinemia are also components of the metabolic syndrome. In an effort to identify genes that confer risk for DM2, a number of studies have identified the 10q23-q25 region as one that contains loci predisposing an individual to DM2. Our aim is to discover genetic risk factors for DM2 and pave the way for their use in both the prevention and treatment of this disease. We have

	mapped a locus that affects fasting plasma glucose levels (FPG), the 20-year mean FPG level (mFPG), glycated hemoglobin level (HbA_{1c}) and risk of DM2 to 10q23-25. We hypothesize that allelic variants in one or more genes in this region confer susceptibility. Our aim is to discover the identity of these gene(s) and characterize the polymorphism that are protective and those that confer risk. One strong candidate gene is the insulin-degrading enzyme (IDE, EC 3.4.24.56). IDE is a cytoplasmic metallopeptidase that is thought to play a role in terminating insulin signaling within cells and is a plausible candidate for DM2. Previously polymorphisms in the IDE gene have been associated with DM2 in the Goto-Kakizaki rat model. In this proposal, we plan to examine whether IDE variants account for the associations that we have observed or whether other genes. We also hypothesize that other genes in the 10q23-q25 region also affect susceptibility to DM2. We will use a screening strategy based on bioinformatics microsatellites to fine map gene variants affecting FPG, mFPG, HbA_{1c} and DM2. Additional fine mapping will be performed using SNPs. Variants that increase risk of DM2 will be assessed for their role in the metabolic syndrome.
Title	**A genome-wide scan for quantitative trait loci of hematocrit, and a study on the relationships between hematocrit and other vascular risk factors**
Principal Investigator	JingPing Lin, MD, PhD (email: linj@nhlbi.nih.gov)
Abstract	Many studies including two Framingham studies have shown that hematocrit (HCT) levels are associated with cerebrovascular disease, cardiovascular disease, peripheral vascular disease, and all-cause mortality. HCT is the percent of whole blood that is comprised of red blood cells and is a compound measure of red blood cell number and size. From the rheological viewpoint, the blood viscosity depends largely on HCT value. There is an inverse relationship between viscosity and vascular blood flow. Twin studies in humans have shown that HCT variation is largely determined by genetic factors with heritability estimated as 40%-65%. A series of animal studies also demonstrated that genetic components play an important role in determining the variation of HCT with heritabilities ranged between 30%-80%. A genome scan to map genes controlling HCT in the spontaneously hypertensive rat identified a chromosome region with significant linkage to HCT. However, the locus of erythropoietin, known to be involved in erythropoiesis, was excluded. So far, no linkage analysis on HCT in humans has been reported. Our study is intended to identify the chromosome regions that contain QTLs involved in controlling HCT using variance component linkage method.
Title	**Simulation of Power and Error in the Framingham families**
Principal Investigator	Larry Atwood, PhD (email: lda@bu.edu)
Abstract	The power and error rate of linkage studies in Framingham is largely unknown. However, as more groups use the Framingham families the need for a standard set of power and error rate tables becomes crucial. We will use our newly acquired computing resources to compute the power and error rate for the existing Framingham families and for the third generation. The results of this simulation will be made publicly available for use by investigators interested in analyzing Framingham data. It will also be used to justify additional genotyping from the Mammalian Genotyping Service and for comparison of analytical methods.
Title	**Associations between Surfactant Protein Polymorphisms and the Diagnosis of Chronic Obstructive Pulmonary Disease**

Principal Investigator	Robert Walter, MD, MPH (email: bwalter@lung.bumc.bu.edu)
Abstract	Chronic obstructive pulmonary disease (COPD) is a significant public health issue; however, our understanding of its pathogenesis remains limited. Genetic factors appear to influence the effect of tobacco exposure. We hypothesize that surfactant protein polymorphisms are associated with the phenotype of COPD. To test this hypothesis, we propose using the Framingham Heart Study Standard Plate Set to compare the allele frequencies of putative COPD-associated surfactant gene polymorphisms in subjects with smoking-related airflow obstruction to their frequencies in control subjects without airflow obstruction, adjusting for such covariates as smoking history, sex, age, and anthropomorphic measures, where appropriate. Genotyping will be done using using PCR with electrophoresis for polymorphic tandem repeats and mass spectrometry for SNPs.
Title	**Heritability and Linkage to Measures of Renal Function**
Principal Investigator	Caroline Fox, MD, MPH (email: caroline@fram.nhlbi.nih.gov)
Abstract	The goal of this study is to identify chromosomal regions linked to renal function. The list of phenotypes includes measures of kidney filtration (serum creatinine, change in creatinine, glomerular filtration rate, creatinine clearance, 1/creatinine) microalbuminuria, and uric acid. The existing Framingham genome scan data will be used for analysis. Heritability for measures of kidney filtration, microalbuminuria, and uric acid will be calculated using GENEHUNTER. The established genome-wide linkage analysis at Framingham will be used for linkage analysis.
Title	**Statistical Methods for Adjusting Covariate Effects In Linkage Analysis**
Principal Investigator	Colin Wu, PhD (email: wuc@nhlbi.nih.gov)
Abstract	We are requesting clinical and genetic analysis data from the Framingham Study families for use in developing new statistical methods for family studies. We propose to develop a series of statistical methods for quantifying the effects of covariates in linkage analysis with longitudinal data. Our approaches include establishing regression models for adjusting the covariate effects, developing methods of estimation and inferences with data from longitudinal studies, and evaluating the adequacy of our proposed methods through simulation studies and applications to the Framingham Heart Study. To broaden the scope of our methods, we will develop methods that include both qualitative traits and quantitative traits, investigate longitudinal methods with parametric and nonparametric regression models and study both the theoretical and practical aspects of our proposed methods. Results obtained from this project will be compared with the ones in the literature. The main objective of the project is to bridge the methodological gaps between statistical analysis and linkage analysis, and improve the accuracy for linkage detection. This will be a collaborative research project involving biomedical, epidemiological, genetic and statistical researchers at the National Heart Lung and Blood Institute and other research institutions as well as the Framingham investigators.
Title	**Positional Cloning of an Obesity Susceptibility Gene on Chromosome 11q23-24: Replication of SNPs Demonstrating an Association with BMI**

Principal Investigator	Leslie Baier, PhD (email: lbaier@phx.niddk.nih.gov)
Abstract	A prior genomic scan in Pima Indians living in Arizona indicated an obesity susceptibility locus centered at marker D11S4464 on chromosome 11 (LOD=3.6). Linkage to body mass index (BMI) at this precise genomic region has been replicated in Caucasians from the Framingham Heart Study. Our current goal is to positionally clone the gene(s) responsible for the linkage. Linkage disequilibrium (LD) mapping is being used to narrow the susceptibility region. For initial LD mapping, single nucleotide polymorphisms (SNPs) are being systematically identified and genotyped at 50 kB intervals across the region of linkage. To date, approximately 440 SNPs that span this region of linkage have been individually genotyped in Pima subjects (N=1229) using either Taqman or SNaPshot technology. Of the 440 SNPs preliminarily genotyped, 28 are associated with BMI in the Pima samples. To better evaluate the significance of these and future associations, we plan to replicate the most significant associations in an independent population. Since linkage to BMI at the same chromosomal region has been established in families from the Framingham Heart Study, we propose to replicate the genotyping of 40 SNPs in the Framingham Family sample.
Title	**Heritability of Gene Expression and the Organization of Biological Networks**
Principal Investigator	Alan Herbert, PhD (email: aherbert@bu.edu)
Abstract	In this study, this question will be addressed using a collection of pre-existing cell lines derived from related individuals. In total, 252 offspring cell lines from 78 families will be analyzed. Gene products that are translationally competent, as indicated by their association with the polyribosomal, protein translation apparatus, will be studied. This gene product pool is more closely related to the protein content of the cell than the total gene product pool. Levels of gene product expression will be quantified using microarray analysis. The investigation is intended to be comprehensive and plans to use both the U133A and U133B set of Affymetrix Chips to survey the entire expressed human genome. This information will be used to analyze underlying genetic networks.

Title	**Variance Component Models for Linkage Analysis of Longitudinal Data**
Principal Investigator	Heping Zhang, PhD (email: heping@peace.med.yale.edu)
Abstract	The purpose of this project is to develop statistical models, namely variance component models, to conduct linkage analysis for longitudinally collected trait. We have developed the basic models and conducted simulation studies that provide empirical evidence to support our models. In the next step, we will apply our models to the data from the Framingham Heart Study.
Title	**Study of association and linkage for loci in the region of chromosome 3 containing evidence for linkage to electrocardiographic QT interval**
Principal Investigator	Alan Herbert, PhD (email: aherbert@bu.edu) Christopher O'Donnell, MD, MPH (email: chris@fram.nhlbi.nih.gov)
Abstract	Repolarization abnormalities manifested by QT interval prolongation have been associated with sudden cardiac death. In families with the Long QT Syndrome, heritable ion-channel defects have been identified. However, the role of specific genetic variants in the regulation

	of QT duration in the general population is poorly characterized. Methods: We sought evidence that the QT interval is heritable, and we then examined for evidence of linkage to chromosomal regions in a large population-based cohort. 12 lead ECG tracings were obtained in family members as part of the routine examination cycle for the original cohort and the Offspring cohort of the Framingham Heart Study. ECG intervals in leads II, V2 and V5 were measured using digital calipers. We conducted a 10 cM genome-wide scan in 329 extended families (1688 genotyped subjects, 2259 phenotyped subjects, 2029 phenotyped sibling pairs). Variance-component methods were used to estimate heritability and to perform linkage analysis using sex-specific normalized residuals, adjusted for age and RR interval. Results: The adjusted QT interval was heritable [H^2 0.35 (95% confidence interval 0.29-0.41). The highest multipoint LOD score for the adjusted QT interval was found on chromosome 3 (LOD score 2.52 with a broad peak from 25-46 cM). The next highest LOD score was found on chromosome 9 (LOD score 1.69 at 104 cM). Conclusion: These data suggest there are influential genetic regions contributing to variability in QT intervals in the general population. Defining the genetic determinants of QT duration may provide insights into the pathophysiology of repolarization abnormalities and their role in sudden cardiac death.
Title	**Associations of alpha-2 adrenergic receptor polymorphisms with platelet aggregability and cardiovascular outcomes**
Principal Investigators	Vahid Afshar-Kharghan, MD (email: vahid@bcm.tmc.edu) Christopher O'Donnell, MD (email: chris@fram.nhlbi.nih.gov)
Abstract	The α_{2A}-adrenergic receptor mediates important physiologic responses to sympathetic stimulation, including changes in the blood pressure and aggregation of platelets. We hypothesize that the polymorphisms in the α_{2A}-adrenergic receptor gene are important in causing the interindividual variations in the response to adrenergic stimuli. To test this hypothesis, we will study the relationship between the α_{2A}-adrenergic receptor gene polymorphisms and the prevalence of hypertension, the threshold for epinephrine-induced platelet aggregation, and finally the incidence of cardiovascular disease in Framingham Offspring subjects. In this study, we will investigate the frequency of the polymorphic alleles for polymorphisms in the α_{2A}-adrenergic receptor gene, including polymorphisms in the coding region and in the 3' or 5' non-coding regions of the gene. Polymerase chain reaction and restriction fragment length polymorphism analysis of genomic DNA will be used to detect the frequency of the polymorphic alleles. Linear and logistic regression analyses will be used to examine associations of genotypes and haplotypes with blood pressure, platelet aggregation and cardiovascular with blood pressure, platelet aggregation and cardiovascular disease outcomes.
Title	**Associations of SNP variants in coding regions of genes for thrombospondins and hemostatic factors with clinical coronary heart disease and stroke, subclinical atherosclerosis, and levels of hemostatic factors and platelet function**
Principal Investigators	Stacey Bolk-Gabriel, PhD (email: stacey@genome.wi.mit.edu) Christopher O'Donnell, MD (email: chris@fram.nhlbi.nih.gov)
Abstract	Acute thrombosis in an atherosclerotic plaque—atherothrombosis—is the proximate cause of acute coronary syndromes as well thrombotic stroke. Altered levels of various hemostatic factors (fibrinogen, PAI-1, tPA, factor VII and vWF) and altered platelet aggregability have been measured in the Framingham Offspring cohort and implicated in the onset of coronary disease and stroke. There is a substantial heritable component to these hemostatic factor

	levels and platelet aggregability; however, there is incomplete understanding regarding the role of specific genetic variants in regulation of hemostatic function and progression to subclinical and clinically apparent disease. We propose to genotype single nucleotide polymorphisms densely spaced across genes encoding key hemostatic factor and platelet receptor proteins in unrelated individuals and families in the Framingham Heart Study. We will examine for significant associations of these SNPs and SNP haplotypes with levels of hemostatic factors and platelet aggregability; subclinical atherosclerosis (determined by carotid intimal medial thickness); and clinically apparent cardiovascular disease and coronary heart disease.
Title	**Study of association and linkage for loci in the region of chromosome 12 containing evidence for linkage to internal carotid artery intimal medial thickness**
Principal Investigator	Christopher O'Donnell, MD (email: chris@fram.nhlbi.nih.gov)
Abstract	We have reported that carotid intimal medial thickness (IMT) is a heritable subclinical atherosclerosis trait measured by carotid ultrasonography. We recently reported evidence for genome-wide linkage of internal carotid IMT to a region 161 cM from the tip of the short arm of chromosome 12. In this proposal, we seek to identify candidate SNP(s) underlying the linkage of chromosome 12 for carotid IMT. Within the chromosome 12 region reside a number of candidate genes for atherosclerosis, including the macrophage scavenger receptor, type I (SCARB1), nitric oxide synthase 1 (NOS1), epimorphin (EPIM), paxillin (PXN), and maturity onset of diabetes of the young type 3 (transcription factor 1, TCF1). We propose to densely genotype single nucleotide polymorphisms (SNPs) that span these and other candidate genes within this chromosomal region. We will then test for associations of these SNPs and SNP haplotypes with carotid IMT phenotypes.
Title	**Studies of Variation in Positional Candidate Genes Influencing Lipid Levels and Blood Pressure**
Principal Investigator	Carole Ober, PhD (email: c-ober@genetics.uchicago.edu)
Abstract	Our genetic studies of complex traits in the Hutterites have identified a susceptibility locus on chromosome 2q14 for triglycerides levels in the Hutterites (LOD = 3.6 at 131.2 cM). The gene encoding the diazepam binding inhibitor (DBI), also known as acyl-CoA binding protein, maps to this region and is an excellent candidate, as it is likely involved in triglycerides biosynthesis. Four SNPs in the gene show strong association with triglycerides levels in the Hutterites. To replicate these findings, we propose to genotype these four SNPs in 1888 unrelated individuals from the Framingham samples, using either RFLP or SBE-FP analysis. We will test for association between each SNP, and haplotypes composed of SNPs, and triglyceride level in collaboration with Framingham investigators.
Title	**Association study of adducin genes polymorphisms to cardiovascular and renal phenotypes in Framingham population**
Principal Investigator	Guiseppe Bianchi, MD (email: bianchi.giuseppe@hsr.it)
Abstract	Adducin is a heterodimer composed by related but not identical subunits (alpha, beta and gamma) coded by genes mapping on different chromosomes. Therefore putative epistatic interactions among adducin loci can furnish an additional genetic tool to assess their role in

	blood pressure regulation. Previous studies both in humans and rats suggest the existence of these interactions. However, larger population studies are needed to substantiate these observations. Adducin may favor the development of hypertension and its complications through an increase of the constitutive capacity of the kidney to retain sodium and through the modification of cell adhesion molecules. Since these effects may be age-related, it is important to evaluate possible genotype-phenotype associations in an age dependent manner. The objectives of this study are to 1) to study single and epistatic interactions of adducin loci with blood pressure (including age-dependent changes) and other available cardiovascular and renal phenotypes (proteinuria, creatinine, cardiac hypertrophy), and 2) to study epistatic interactions between adducins and ACE. Allelic discrimination of adducin SNPs will be carried out with and ABI PRISM 7700 Sequence Detection System, using fluorognic probes for the wild and mutated alleles of each SNP. The list of phenotypes includes age-related blood pressure changes, creatinine, proteinuria, cardiac mass and function.
Title	**Sarcomere protein gene mutations and LV wall thickness changes in the Framingham Study Population**
Principal Investigator	Jonathan Seidman, PhD (email: seidman@rascal.med.harvard.edu)
Abstract	Familial hypertrophic cardiomyopathy (HCM) is inherited as an autosomal dominant trait and is often caused by dominant-negative-acting sarcomere protein gene mutations. Mutations in beta-cardiac myosin heavy chain, cardiac actin, cardiac troponin T, alpha-tropomyosin, cardiac troponin I, cardiac myosin binding protein C, and the myosin light chains have all been shown to cause FHC. We propose to measure the prevalence of sarcomere protein gene mutations among individuals with increased left ventricular wall thickness. To this end we will determine the nucleotide sequences of the listed genes in the DNA of about 80 individuals from the Framingham Heart Study who have left ventricular wall thickness greater than 1.3 cm.
Title	**Association of Apolipoprotein E polymorphisms with obstructive sleep apnea among participants in the Sleep Heart Health Study**
Principal Investigator	Daniel J. Gottlieb, MD, MPH (email: dgottlieb@lung.bumc.bu.edu)
Abstract	Obstructive sleep apnea/hypopnea (OSAH) is a heritable trait, with familial factors explaining approximately 40% of the variance in apnea-hypopnea index (AHI) independent of body habitus. Data from the Wisconsin Sleep Cohort Study showed a significant association between ApoE e4 positivity and the presence of OSAH, defined as AHI>15, among men and women aged 30 to 60 years. In contrast, no association was found between ApoE e4 and OSAH in the Honolulu-Asia Aging Study cohort of men aged 80 years and older. We will examine the relation of ApoE genotype to OSAH in a larger and more age-diverse cohort than either previous study, using data from Framingham Heart Study and Cardiovascular Health Study participants enrolled in the Sleep Heart Health Study. The association of the ApoE e4 genotype with OSAH will be analyzed using a dichotomous measure of OSAH defined as AHI>15. The genotype will be considered as ApoE e4 positive if the subject has either one or two e4 alleles present (if the number of homozygous subjects is sufficient, a dose effect will also be examined). Generalized Estimating Equations using the logit link function will be used to assess differences in AHI among genotypes, adjusting for age, sex, and BMI (which are strong predictors of AHI) while accounting for correlated observations from

	individuals within the same sibship. Analyses will be performed for the entire sample, as well as stratified on median age.
Title	**Genetics of sleep apnea and daytime sleepiness: Pilot study**
Principal Investigator	Daniel J. Gottlieb, MD, MPH (email: dgottlieb@lung.bumc.bu.edu)
Abstract	There is evidence that obstructive sleep apnea/hypopnea (OSAH) is a heritable trait, with familial factors explaining approximately 40% of the variance in apnea-hypopnea index independent of the effects of body habitus. Only a single gene polymorphism (APOE ϵ4) has been identified as a potential cause of OSAH, and suggestive evidence for linkage has been reported near the gene encoding pro-opiomelanocortin. There is evidence from twin studies for heritability of sleepiness. Data on snoring and sleepiness are available in approximately 2500 Framingham Heart Study Offspring subjects and objective measures of OSAH in 700. The proposed pilot study will assess heritability of these phenotypes among FHS Offspring subjects. This will allow a determination regarding the feasibility of linkage analysis to identify genetic loci linked to these phenotypes. Sleepiness (Epworth Sleepiness Scale score, range 0-24) and OSAH (operationally defined by questionnaire response in all subjects completing Sleep Habits Questionnaires and by apnea/hypopnea index in the 700 subjects with polysomnograms) will be treated as dichotomous variables and, in the case of sleepiness and apnea/hypopnea index, as continuous variables. For each dichotomous variable, lambda, the risk to relatives, will be calculated for first-degree relatives. For continuous variables, heritability will be assessed by fitting a polygenic model in SOLAR.
Title	**The Molecular Basis of High Density Lipoprotein Deficiency**
Principal Investigator	Margaret Brousseau, PhD (email: mbrousseau@hnrc.tufts.edu)
Abstract	HDL deficiency is the most common lipid abnormality observed among patients with premature CHD. HDL plays a central role in reverse cholesterol transport (RCT), the process by which HDL mediates the transport of excess cholesterol from peripheral cells back to the liver for excretion into the bile. We're proposing to identify novel SNPs in genes involved in RCT (ABCA1, ApoAV, CETP, HL, SR-BI), and to assess the prevalence of well-defined SNPs that are associated with plasma lipid levels and/or CHD risk, in participants from the Veterans Affairs HDL-C Intervention Trial (VA-HIT), a study comprised of men having low levels of HDL-C (<40 mg/dL), normal levels of LDL-C, and CHD. Among our goals is to determine the frequencies of novel and known polymorphisms in VA-HIT and to compare these frequencies with those of CHD-free men in FOS. We hypothesize that the frequencies of polymorphisms that are associated with reduced HDL-C levels and/or increased CHD risk will be significantly increased in VA-HIT. In turn, the frequencies of polymorphisms that are associated with increased concentrations of HDL-C and/or decreased CHD will be significantly decreased in VA-HIT. This work will provide us with important information regarding the role of genetic variation in the RCT pathway in the determination of low HDL-C concentrations and CHD risk.
Title	**Genetics of Bone Structural Geometry in Framingham Cohorts**
Principal Investigator	David Karasik, PhD (email: karasik@mail.hrca.harvard.edu)

Abstract	Bone geometry is a valid measure of bone fragility and risk of osteoporotic fractures. Understanding of the factors contributing to the differences in fracture rates needs detailed knowledge of the genetic and environmental basis of bone geometry. Given the high heritability of bone geometry traits, we propose to examine its genetics using genetic linkage analysis with consequent testing of the candidate genes for association with geometry of both non- and weight-bearing bones. The aims of this study are: 1) To measure biomechanical and structural indices of the proximal femur and metacarpal bones using DXA scans and hand radiographs, respectively, in a pedigree sample from the Original and Offspring Cohorts of the Framingham Heart Study; 2) To determine the heritability of these geometric indices, adjusted for confounders, such as age, sex, weight, height, smoking, alcohol intake, physical activity, serum vitamin D, and for women, estrogen history; 3) To perform a genome scan to identify potential linkage of femoral and metacarpal indices with a set of 401 genome-wide microsatellite markers; and 4) To examine the association between the indices of bone geometry and polymorphisms in several candidate genes, that were previously studied for a role in osteoporosis and/or identified by our linkage analysis.
Title	**Candidate Genes for Bone Mineral Density and Quantitative Ultrasound of Bone**
Principal Investigator	Douglas Kiel, MD, MPH (email: kiel@mail.hrca.harvard.edu)
Abstract	Previous studies of the vitamin D receptor (VDR) polymorphisms and bone mineral density (BMD) have suggested that there may be differences in calcium absorption among groups of women with different VDR genotypes, and that the association may be stronger in younger women. To investigate the association between the VDR polymorphisms and BMD, this study was undertaken in the Framingham Study Cohort and a group of younger volunteers. Subjects from the Framingham Study (ages 69-90) included those who underwent BMD testing and who had genotyping for the VDR alleles (n=328) using polymerase chain reaction methods and restriction fragment length polymorphisms with BsmI (B absence, b presence of cut site). A group of younger volunteer subjects (ages 18-68) also underwent BMD testing and VDR genotyping (n=94). In Framingham Cohort subjects with the bb genotype, but not the Bb or BB genotypes, there were significant associations between calcium intake and BMD at five of six skeletal sites, such that BMD was 7-12% higher in those with dietary calcium intakes greater than 800 mg/day compared with those with intakes <500 mg/day. The data also suggested that BMD was higher in persons with the bb genotype only in the group with calcium intakes about 800 mg/day. No significant differences were found in the Framingham Cohort for age-, sex-, and weight-adjusted BMD at any skeletal site between those with the BB genotype and those with the bb genotype regardless of 25-hydroxyvitamin D levels or country or origin. In the younger volunteers, BMD of the femoral neck was 5.4% higher ($p < 0.05$) in the bb genotype group compared with the BB group and 11% higher ($p < 0.05$) in males with the bb genotype compared with the BB group. There were no significant differences at the lumbar spine. In this study, the association between calcium intake and BMD appeared to be dependent upon VDR genotype. The finding of an association between dietary calcium intake and BMD only in the bb genotype group suggests that the VDR genotype may play a role in the absorption of dietary calcium. Studies that do not consider calcium intake may not detect associations between VDR genotype and BMD. In addition, the association between VDR alleles and BMD may become less evident in older subjects.
Title	**Genetic Analysis of Loci Identified by Genome Scan for Hemostatic Factors and Stroke Phenotype**
Principal	Philip Wolf, MD (email: pawolf@bu.edu)

Investigator	
Abstract	Hemostatic factors and specifically fibrinogen levels and PAI-1 have been widely implicated as risk factors for stroke. In analysis of the original cohort of the Framingham Study population each standard deviation in fibrinogen was associated with an increment of coronary events of 30% in men and 40% in women. Fibrinogen also predicted stroke in Framingham. Given that these hemostatic measures are recognized risk factors for cardiovascular disease, the identification of genes that regulate these hemostatic factors will have significance not only for stroke but for CVD generally. Linkage analysis were conducted in the Framingham study for the traits plasma fibrinogen level, plasminogen activator inhibitor type 1 (PAI-1), tissue plasminogen activator (tPA), factor V and VII and von Willebrand factor. LOD scores > 1.4 were observed on chromosomes 6, 8, 9, 10 and 15. We propose to type microsatellite markers to fine map these regions and to type single nucleotide polymorphism to assess candidate genes in these regions.
Title	**PPAR Gamma Variants, lipoproteins and Gene-diet Interactions**
Principal Investigator	Jose Ordovas, PhD (email: ordovas@hnrc.tufts.edu)
Abstract	The current proposal is to investigate PPAR gamma variants and their relation to lipid phenotypes in the Framingham Offspring see at exam 6. Moreover, gene-diet interactions will be also examined. There is considerable interest in the lipid and glycemic field in this realm, as many of the lipid and glycemic agents have PPAR activity. For instance, fibrates as a class partly exert their effects through PPAR alpha mechanisms and thiazolidinediones act on the PPAR gamma axis. We will focus our research on lipid traits and gene-diet interactions but our research team also has great interest in PPAR gamma and glycemic traits and there will be study the relation between polymorphisms at this locus and impaired fasting glucose, the metabolic syndrome and diabetes mellitus. We will carry out the genotyping using the ABI 3100 and SNAPShot methodology. Our goals are 1) to identify haplotypes that can increase the amount of information provided by single SNPs, 2) to uncover significant gene-diet interactions, and 3) to demonstrate that these interactions between genetic variants at this locus and diet play a very relevant role in the regulation of lipid-related proteins.
Title	**Marker Saturation of Chromosomes 3 and 6 Regions Containing Promising LOD Scores**
Principal Investigator	Jose Ordovas, PhD (email: ordovas@hnrc.tufts.edu)
Abstract	The importance of HDL-C concentrations as a predictor of CHD is well established. While age, weigh, gender, smoking and alcohol consumption are associated with HDL-C levels, a number of investigators have reported on the genetic determinants of HDL-C. Previously we carried out a genome scan of 330 pedigrees in the study. We examined two measures 1) a single measurement recorded in the mid-1970s among parents and offspring of the study, all of whom were adults averaging 46 years of age, and 2) a mean HDL-C level from measurements recorded between the 1970s and late 1990s. Analysis of these data used a pedigree variance component linkage analysis model with SOLAR. These analyses identified several regions of interest (Lod scores close to 2 or above). Specifically, we consider that the regions between 0-30 cM in chromosome 3 and between 120-150 cM in chromosome 6 are worthy of further exploration. Examination of the loci present in these regions did not reveal any gene potentially involved in lipid metabolism. Therefore, we will saturate those regions with additional markers to confirm the significance of the initial findings and to narrow the

	region of interest. Our research could contribute to a better understanding of HDL metabolism.
Title	**Linkage analysis, mathematical modeling, and association study of serum bilirubin, CHD and Uridine diphosphate glycosyltransferase 1 gene--a Framingham Study**
Principal Investigator	JingPing Lin, MD, PhD (email: linj@nhlbi.nih.gov)
Abstract	Our previous genome-wide scan indicated that UGT1A1 may be the major gene controlling serum bilirubin levels in the general population. This study is intended to 1) confirm the linkage between this gene and serum bilirubin, 2) to test whether the common TA insertion/deletion polymorphism of this gene is associated with serum bilirubin, 3) to determine the model of inheritance of this gene on serum bilirubin, 4) and to perform association studies between the UGT1A1 genotypes and CHD. The TA insertion/deletion polymorphism and two microsatellites flanking the gene will be genotyped through SSCP and sequencing. Variance component linkage analysis will be used to confirm the linkage. The TA insertion/deletion genotypings on 330 Framingham families will be used to carry out mathematical modeling to determine the model of inheritance. The measured genotype approach implemented in SOLAR will also be used for association studies between serum bilirubin and the genotypes of the TA insertion/deletion among family members. The unrelated family plates will be used to test the association between CHD and the genotypes. Logistic regression analysis will enable adjustment for covariates of interest.
Title	**Cardiovascular Disease and Estrogen Action II**
Principal Investigator	David Housman, PhD (email: dhousman@mit.edu)
Abstract	The goal of our SCOR project in Ischemic Heart Disease is to extend our previous study of the genetic contribution to atherosclerotic cardiovascular disease (CVD) status of polymorphisms of the estrogen receptors and important genes regulated by these ligand-activated transcription factors. We will continue our ongoing association studies by exploring genetic variation in specific genes and their potential relationship to gender, hormonal status, and estrogen-related variables. The genes to be studied include vasodilator enzymes endothelial nitric oxide synthase (eNOS) and inducible NOS (iNOS), the vasoconstrictor, endothelin-1, the vascular endothelial growth factor (VEGF), monocyte chemotactic protein (MCP-1), the matrix proteins collagen and matrix metalloproteinase 2 (MMP-2), MMP-9, the calcium-activated potassium channel (BKCa), and the programmed cell death genes caspase-3 and fas ligand. We will study the frequency of each variant in relation to specific parameters of cardiovascular function (ECHO, blood pressure, lipid, hormone, and vascular reactivity measures and CVD outcomes) in 1,821 unrelated men and women from the Framingham Offspring Study. Genotyping will be carried out using fluorescence polarization, restriction fragment analysis, allele specific oligo hybridization, or microsatellite analysis. For each marker allele and genotype, frequencies will be calculated and evaluated for Hardy-Weinberg equilibrium. The association between a marker and outcome measures will be evaluated using analysis of covariance regression models (continuous outcome measures) and logistic regression models (dichotomous outcome measures). Haplotype analysis will be performed for closely spaced markers within a gene. All analyses will be carried out separately for men and women with adjustment for potential confounders. These studies will permit an evaluation of the relationship between genotype for key genes involved in estrogen response and cardiovascular function. Furthermore, because this population has been

	examined longitudinally, cardiovascular parameters measured on the same individual both pre- and postmenopausally can be compared, supporting the study of associations between genotype and postmenopausal changes in cardiovascular parameters. Where we obtain statistically significant results we will attempt to confirm these using an independent set of 1,400 individuals, composed of families from the Framingham Heart Study.
Title	**Genome Scan for Bone Age Phenotype in the Framingham Study Cohorts**
Principal Investigator	David Karasik, PhD (email: karasik@mail.hrca.harvard.edu)
Abstract	Bone aging may serve as a model of general aging of an organism. An osteographic score (OSS) has been developed to measure bone age as progression of age-related radiographic changes on hand bones. The aim of the proposed study is to determine genetic contribution to bone aging, and to determine the chromosomal location of these genes, in members of 330 pedigrees from the Original and Offspring Cohorts of the Framingham Study. Different health and activity related factors will be evaluated for covariation with bone aging. For each hand roentgenogram OSS score will be estimated using four groups of features: 1) bony spurs (osteophytes); 2) porosity; 3) osteosclerosis; and 4) non-traumatic joint deformities. The standardized difference between the chronological and the predicted by OSS age, adjusted for covariates, will be used as a measure of bone age. Variance component analysis will be done to evaluate heritability, and a genome scan will be performed to identify potential linkage of bone age with 400 microsatellite markers. Understanding of the genetic mechanisms leading to different rates of bone aging may significantly contribute to the knowledge of mechanisms of degenerative bone disease and genetic sources of aging in general, which will help to improve strategies for increasing longevity and health monitoring.
Title	**Evidence for a gene influencing serum bilirubin on chromosome 2q telomere: a genome-wide scan in the Framingham Study**
Principal Investigator	JingPing Lin, MD, PhD (email: linj@nhlbi.nih.gov)
Abstract	There is an inverse relationship between serum bilirubin concentrations and risk of coronary artery disease (CAD). The strength of the association between serum bilirubin and CAD is similar to that of smoking, systolic blood pressure, and HDL-cholesterol. We carried out a 10 cM genome-wide scan in a community-based Caucasian cohort, the Framingham Heart Study. Our study sample consisted of 330 families with 1,264 individuals being both genotyped and phenotyped, including 1,394 sibling pairs, 681 cousin pairs, and 89 avuncular pairs. Using variance-component methods implemented in SOLAR, our genome-wide linkage analysis demonstrated significant evidence of linkage of serum bilirubin to chromosome 2q telomere with a LOD score of 3.8 (p=0.00001) at location 243 cM. The peak multipoint LOD score is located at about 1 cM away from the location of the Uridine diphosphate glycosyltransferase 1 (UGT1A1) gene. UGT1A1 catalyzes the conjugation of bilirubin with glucuronic acid and thus enhances bilirubin elimination and therefore is an important candidate gene for regulation of serum bilirubin. Gilbert syndrome is a hyperbilirubinemic syndrome, which has a population frequency of 2-19%, and is mainly due to a TA insertion at the promoter region of UGT1A1. Only one other region in the genome produced a multipoint LOD score greater than 1 (LODs = 1.3). Our findings are consistent with the contention that UGT1A1 may be a major gene controlling serum bilirubin levels in the population.
Title	**Analysis of the role of the sterol transporters ABCG5 and ABCG8 in the regulation of**

	cholesterol levels
Principal Investigator	Michael Dean, PhD (email: dean@ncifcrf.gov)
Abstract	The ABCG5 and ABCG8 genes are the body's major gatekeepers for cholesterol absorption from the diet and excretion in bile. These genes encode two halves of a transport protein that can transport cholesterol out of the intestine and out of the liver. These genes are both mutated in patients with sitosterolemia, a disease of cholesterol transport. This disorder is characterized by elevated levels of plant and fish sterols in the blood of affected individuals. In addition many of these subjects have elevated cholesterol levels and increased risk of coronary artery disease. The aim of the study is to examine the role of variants in the sterol transporter genes ABCG5 and ABCG8 in the regulation of cholesterol levels and in the incidence and outcome of coronary disease. By studying the role of variants in these genes in the Framingham cohort we hope to learn about the role of these genes in regulating cholesterol levels, and the influence on disease.

Title	**Analysis of α1 Na, K-ATPase gene as a candidate gene for essential hypertension**
Principal Investigator	Nelson Ruiz-Opazo, PhD (email: nruizo@bu.edu)
Abstract	The overall hypothesis is that "1NK (ATP1A1) and NKC (SLC12A1) genes represent an interacting bilocus genetic paradigm for certain subtype(s) of human essential hypertension. We note that since essential hypertension is a product of many genes as well as environmental factors, the a1NK-NKC bi-locus can be expected to account for only part of the hypertension variance/pathogenesis. The following aims are proposed to investigate the bi-locus model for a1NK-NKC as a basis of hypertension in human cohorts. Aim 1: We hypothesize that the a1NK#4 and NKC#4 alleles are markers in linkage disequilibrium with genetic variants in either coding regions or regulatory elements of these genes that underlie altered sodium transporter functions contributing to essential (polygenic) hypertension. We propose to identify nucleotide sequence variants (single nucleotide polymorphisms, SNPs) in the Sardinian "1NK and NKC alleles associated with hypertension, "1NK#4 and NKC#4. Aim 2: We hypothesize that functional gene variants in a1NK and NKC genes identified in Aim 1 will account for a significant portion of variation in blood pressure and for hypertension in the Sardinian cohort in a bilocus genetic paradigm. We propose to test this hypothesis by genotyping in a case-control paradigm those SNPs identified in Aim 1, and to assess their association to blood pressure and hypertension in a single locus and bi-locus model. These studies are aimed at identifying the hypertension susceptibility SNPs - functional SNPs that increase genotypic relative risk for hypertension in a subtype of essential hypertension represented in the Sardinian cohort. Aim 3: We hypothesize that the hypertension susceptibility gene variants identified in Aims 1 and 2 for the Sardinian cohort will also have a significant role in blood pressure regulation and risk for hypertension in other hypertension cohorts. We propose to test for functionality by testing the association of the hypertension susceptibility SNP (SNP found to increase genotypic relative risk for hypertension in the Sardinian cohort) to blood pressure and hypertension in a community based population, the Framingham cohort. These studies are aimed at determining the broader relevance of hypertension susceptibility gene variants or SNPs detected in the northern Sardinian cohort with respect to essential hypertension in another well-characterized cohort. Concordant results in both Sardinia and Framingham hypertension cohorts will affirm functionality of hypertension susceptibility SNPs, which will then be further tested in future in vitro functional transport and/or regulatory assays to elucidate the putative physiological mechanisms involved.

Title	**Chemokine System Polymorphisms and Risk of Atherosclerosis**
Principal Investigator	David McDermott, MD (email: dmcdermott@nih.gov)
Abstract	Atherosclerosis, the disease that causes strokes and heart attacks, is increasingly being understood as a disease of chronic inflammation of the arteries. Additionally, it is clear that there is a genetic predisposition to this disease in certain individuals. We are studying genetic variants in the immune system that affect inflammation with a focus on the chemokine system. Chemokines are small proteins secreted in response to injury that are capable of recruiting activated white blood cells (leukocytes) from the blood to the site of inflammation. Both the chemokines and their cellular receptors have common genetic variants that influence their activity and therefore can affect how the immune system responds to injury. Because the Framingham Heart Disease Study has such careful and long-term follow-up of its participants, we feel that we will be able to make meaningful advances in the knowledge of how the immune system affects atherosclerosis risk. Identifying individuals at increased risk could allow earlier conventional treatment and prevention measures. In addition, genotypes associated with disease can identify new drug targets for selective control of the immune system so that heart disease and strokes can be prevented with fewer side effects.
Title	**Genetic Factors in Presbycusis**
Principal Investigator	Clinton Baldwin, PhD (email: baldwin@mbcrrc.bu.edu)
Abstract	Presbycusis is the most common cause of hearing loss in adults; nearly 25 million Americans are affected and this number is increasing as the population ages. Presbycusis contributes to decreasing quality of life and depression. With the current population trends, remediation of presbycusis will consume an ever greater proportion of healthcare expenditures. At present there is no prevention or cure for presbycusis. Presbycusis is manifest as a progressive bilateral decrease in hearing sensitivity with difficulty understanding speech. The etiology is multifactorial and includes the accumulated effects of all agents that affect hearing with age, of which noise damage and aging are the chief. The marked variability in hearing among subjects of similar ages has made research into the pathophysiology of presbycusis difficult. We may now postulate that presbycusic hearing loss results from one or all of three distinct processes 1) outer hair cell (OHC) loss, 2) age-related atrophy of the stria vascularis, and 3) central nervous system dysfunction due to aging. All of these processes have both environmental and genetic components. The differential expression of these elements in any given case undoubtedly contributes to the variability within and between groups. Finding reliable clinical and genetic markers to identify these subtypes in the living person motivates this research. Such markers would be critical for localizing which cells and molecules are affected, a necessary step for selecting cases for the types of molecular otologic therapy being pursued in research centers around the world.
Title	**Linkage Analysis of Height in the Framingham Genome Scan. To explore linkage methods for quantitative traits in a large random sample study.**
Principal Investigator	Daniel Weeks, PhD (email: dweeks@watson.hgen.pitt.edu)
Abstract	Height is an ideal example of a polygenic quantitative trait shared by all humans. Previous linkage analysis studies have focused on growth-related factors and individuals with abnormally short stature. Since findings from plants and animals suggest that often a large

	proportion of the variance for a polygenic trait is controlled by a small number of genes with significant effects, we analyzed data from 1,720 individuals from 333 families from the Framingham Heart Study in an attempt to map loci influencing normal adult height. These individuals were ascertained in two cohorts, the first ascertained in 1948 via a random sample of households in Framingham, MA, and the second Offspring cohort in 1971. Individuals in the original cohort have undergone biennial examinations since inception of the study, while individuals in the Offspring cohort have been examined every 4 years (except between examinations 1 and 2 with an 8 year gap). At each exam, height was measured with subjects standing erect with their heads in the Frankfort plane. The first height measurement for individuals between the ages 20-55 years were analyzed using variance component linkage analysis on extended pedigree structures and extended Haseman-Elston linkage analysis on the component nuclear families. Sex and cohort were included as covariates. Marker data were generated by the Marshfield Mammalian Genotyping Service for 391 markers on chromosomes 1-22, 24 markers on X, and 2 markers in the pseudo-autosomal region of the X chromosome. Nine chromosomes with 11 peaks with log P-values greater than 2.0 were detected. Three chromosomes demonstrated peaks with log P-value greater than 3.0. None of the regions with significant log P-values corresponded to the location of known genes in the neuro-endocrine growth hormone axis. We are currently evaluating (in silico) the regions under these peaks for candidate genes contributing to final adult height.
Title	**Genomics of Cardiovascular Development, Adaptation and Remodeling**
Principal Investigator	Seigo Izumo, MD (email: sizumo@caregroup.harvard.edu)
Abstract	We propose to examine common polymorphisms in approximately 200 candidate genes identified by literature review and by mouse and human studies in other CardioGenomics Projects (http//www.cardiogenomics.org; Program in Genomics Applications). The Whitehead Institute Center for Genome Research will perform genotyping by MALDI-TOF methods to determine which of the candidate gene SNPs are associated with echocardiographic cardiac structure and function among members of the Framingham Heart Study (FHS). The objective is to test the association of these polymorphisms with left ventricular mass, left ventricular and left atrial chamber size, aortic root size and related secondary measures (LV ejection fraction and fractional wall shortening) in a well characterized population based cohort. We will use a staged two-step analysis that includes both population-based case-control methods and family-based association tests.
Title	**Cardiovascular Disease and Estrogen Action**
Principal Investigator	David Housman, PhD (email: dhousman@mit.edu)
Abstract	The goal of our SCOR project in Ischemic Heart Disease is to investigate the genetic contribution to atherosclerotic cardiovascular disease (CVD) status of polymorphisms of the estrogen receptors and important genes regulated by these ligand-activated transcription factors. Initially we will carry out an association study using genetic variation in four genes estrogen receptor alpha (ER alpha), estrogen receptor beta (ER beta), aromatase (the enzyme responsible for local tissue conversion of testosterone to 17-beta estradiol), and SRC-1 (the prototypical estrogen receptor transcriptional coactivator). We will study the frequency of each variant in relation to specific phenotypic parameters of cardiovascular function (ECHO, blood pressure, lipid, hormone, and vascular reactivity measures and CVD outcomes) in 1,821 unrelated men and women from the Framingham Offspring Study. Genotyping will be

	carried out using fluorescence polarization, restriction fragment analysis, allele specific oligo hybridization or microsatellite analysis. For each marker allele and genotype, frequencies will be calculated and evaluated for Hardy-Weinberg equilibrium. The association between a marker and outcome measures will be evaluated using analysis of covariance regression models (continuous outcome measures) and logistic regression models (dichotomous outcome measures). Haplotype analysis will be performed for closely spaced markers within a gene. All analyses will be carried out separately for men and women with adjustment for potential confounders. These studies will permit an evaluation of the relationship between genotype for key genes involved in estrogen response and cardiovascular function. Furthermore, because this population has been examined longitudinally, cardiovascular parameters measured on the same individual both pre- and postmenopausally can be compared, supporting the study of associations between genotype and postmenopausal changes in cardiovascular parameters. Where we obtain statistically significant results we will attempt to confirm these using an independent set of 1,400 individuals, composed of families from the Framingham Heart Study.
Title	**Genetics of Vascular Stiffness**
Principal Investigator	Thomas Quertermous, MD (email: tomq1@stanford.edu)
Abstract	The prognostic significance of elevated systolic and diastolic blood pressure (SBP, DBP) in the pathogenesis of CVD has been established unequivocally by the Framingham Heart Study (FHS) and others. Based on BPs obtained in 10,000 FHS participants from two generations we find strong evidence that BP is heritable (heritability of SBP and PP of 0.594 and 0.54 respectively), indicating that this clinically important phenotype is genetically determined. The FHS now has over 5000 DNA specimens in two generations of subjects and we have completed a 390 marker autosomal genome scan in 1800 study participants. We propose to utilize an integrated set of noninvasive vascular measurements from pulse wave recordings of the carotid, femoral, brachial and radial arteries to define vascular phenotypes in 2700 FHS subjects (mean age 61, range 31-89; 52% female). We will assess the heritability of these phenotypes. For phenotypes that demonstrate significant heritability we will assess linkage using existing genome scan results. Significant linkages will be used to guide candidate gene research. In parallel with these studies we will examine the association of candidate genes that are expressed in the vascular wall for which defined SNPs have been identified in Projects 1 and 2 of the Stanford Reynolds Center.