University of Missouri, Kansas City
Theme: Identifying the genes that put women at risk for osteoporosis

Center Director: Hong-Wen Deng, Ph.D.
Professor, Franklin D. Dickson/Missouri Endowed Chair
Director, Orthopedic Research
Director, Genetics Core
Department of Orthopedic Surgery and Basic Medical Sciences
M3-C03
2411 Holmes Street
Kansas City, Missouri 64108

Email: dengh@umkc.edu
Phone: 816-235-5172

Center Abstract

Osteoporosis is the most prevalent metabolic bone disease responsible for a major public health problem. Osteoporosis is mainly characterized by low bone mineral density (BMD). In general, women have lower BMD and higher risk of osteoporosis than men. Most BMD variation is determined by genetic factors with heritability greater than 60%. However, the specific genes involved are largely unknown. Our studies and the studies of others have demonstrated that some osteoporosis risk genes/genomic regions are gender specific.

The GOAL of this SCOR is primarily to identify osteoporosis risk genes and their functional aspects in females and, secondarily, to assess the female specificity of these identified genes/functions in male samples. In addition, we will also perform in-depth molecular and cellular functional studies for specific mechanisms and confirmation of the risk genes identified, by studying two novel genes we discovered recently.

This SCOR will pioneer a comprehensive and novel approach in bone genetics by investigating osteoporosis at the genome-, transcriptome-, and proteome-wide levels simultaneously. We will use the samples largely recruited or archived for targeted recruitment and adopt state-of-the-art technologies proved successful in our recent pilot studies. This qenomic convergence approach will pinpoint and consolidate the most significant genes identified in each of the individual projects. The genes identified will be subject to replication studies within and across populations by ourselves and our collaborators. All the genes identified in the genomic convergence approach will be subject to in-depth functional studies for confirmation and functional mechanisms as exemplified in Stage 2 of Project 2 of this SCOR.

This SCOR is composed of three projects, all aimed at identifying osteoporosis risk genes but from different genomic approaches. Project 1 is to perform a whole genome association scan using dense SNPs to identify those genes/regions that are associated with risk of osteoporosis. Project 2 is to perform a DMA microarray study to scan >40,000 known human genes and ESTs to identify those mRNAs and corresponding genes associated with osteoporosis. Project 3 is to perform proteomics studies to identify those proteins (and corresponding genes) associated with osteoporosis.

The SCOR has three cores: A) Administrative Core; B) Clinical Core; and C) Biostatistics and Bioinformatics Core. Each core serves all the three projects. For example, the Clinical Core recruits samples that are shared by Projects 2 and 3; provides support for clinical related issues (such as choice of important medical and environmental factors for co-variate analyses) and for human subject research issues in Project 1. Identifying genes and their functions for human BMD variation, especially for women, is important for 1) gaining insights into the fundamental molecular mechanisms underlying risk of osteoporosis, 2) discovering new pathways and targets for therapeutic cures; 3) identifying genetically susceptible individuals, so that future preventions and interventions can be targeted to and based on individuals' specific genotypes.

Project1. Genome Wide Scans for Female Osteoporosis Genes
Type: Basic

PI: Yongjun Liu, M.D., Ph.D.
Assistant Professor of Basic Medical Sciences
Department of Bioinformatics and Personalized Medicine
Department of Basic Medical Sciences
00253 MED M3-421
University of Missouri - Kansas City (UMKC)
Kansas City, Missouri

Email: liuyon@umkc.edu

Abstract

Osteoporosis is the most prevalent metabolic bone disease and a major public health problem mainly characterized by low bone mineral density (BMD). BMD has a heritability > 60%.The specific genes involved are argely unknown. Women have lower BMD and higher risk to osteoporosis than men. Our previous studies have demonstrated that some osteoporosis risk genes/genomic regions are gender specific.

The GOAL of this project is primarily to identify such osteoporosis genes for females and, secondarily, to assess the gender specificity of these identified genes in male samples. Potential none-genetic covariates and interactions will be assessed and significant ones will be adjusted for. Using unrelated Caucasian female samples that we have accumulated in the past 10 years, we propose to conduct a powerful genome wide association (WGA) scan for BMD genes important for females. Our earlier data obtained in whole genome linkage scans (WGLS) and meta-analyses, DMA micoarray and proteomics studies, and those to be obtained in Projects 2 and 3 of this SCOR will be used to guide focused analyses of this WGA.

The 300 most significant genes/genomic regions identified in the WGA will be followed for validation in 800 nuclear families (each with two parents and at least two offspring aged 25-45) that we have recruited by the support of R01AR050496. Those genes that remain significant after transmission disequilibrium test (TDT) in the female offspring (n=936) will be tested by TDT in the male offspring (n=714). Those genes that remain significant in the males are common for risk of osteoporosis in both sexes; otherwise, they are female specific.

Our hypothesis is: sex-specific genes for BMD variation can be detected with a powerful WGA and robust TDT when complemented by previous WGLS and our gene and/or protein expression studies in major bone cells. We will fulfill the following Specific Aims:

1) To perform a powerful WGA study using latest Affymetrix SNP chips for 400 healthy women with high and 400 osteoporotic women with low BMD (belonging to aged matched population top or bottom 20%, respectively);

2) To compare the results obtained from the WGA with WGLS and gene/protein expression data (including those to be obtained in Projects 2 and 3 of this SCOR) and other available data of in vivo and in vitro studies;

3) To evaluate the 300 most promising genes/genomic regions obtained through Specific Aims 1 and 2 using ~10,000 SNPs in the 800 nuclear families with robust association analyses for female offspring and subsequently in male offspring, to identify sex-common and female-specific BMD genes;

4) To evaluate the most promising markers obtained through Specific Aim 3 in other populations, including US Caucasians, Blacks, one Israel population, one Amish Jewish population in US, Mexican Americans and Han Chinese.

Identifying genes for human BMD variation, especially for women, is important for 1) gaining insights into the fundamental molecular mechanisms of risk to osteoporosis, 2) discovering new pathways and targets for therapeutic cures; 3) identifying genetically susceptible individuals, so that future preventions and interventions can be targeted to and based on individuals' specific genotypes.

Project 2: Genome Wide and Specific Gene Expression Study of Esteogenic Cells
Type: Clinical

PI: Yao-Zhong Liu, M.D., Ph.D.
Assistant Professor, School of Medicine, UMKC
Department of Medical Informatics
00253 MED M3-219
Kansas City, Missouri

Email:
Phone: 816-235-1891

Abstract

Osteoporosis is a major public health problem, especially in women. It is mainly characterized by low bone mineral density (BMD). Women have much lower BMD than men. Some BMD genes/genomic regions are sex-specific. Menopause is associated with rapid bone loss.

Bone marrow mesenchymal stem cells (BMMSCs) and peripheral blood monocytes (PBMs), are precursors for osteoblasts (bone formation cells) and osteoclasts (bone resorption cells), respectively.

The GOAL of this project is to identify genes that are differentially expressed (at mRNA levels) in BMMSCs and PBMs in females with low vs. high BMD and with menopausal status changes. Such genes are expected to be important for variation of female BMD and women health in general.

Our preliminary functional genomic studies of PBMs suggested HDC and RUNX1 (NOT the extensively studied RUNX2) genes to be important in determining BMD in humans.

Project 2 is built upon this stimulating lead to screen much larger and more powerful samples of both BMMSCs and 3BMs to confirm and extend our preliminary studies and to comprehensively screen genes potentially important for BMD. This project has two inherently related aspects or stages; research in Stage 2 is for exemplifying functional studies to follow the completion of research in Stage 1 and in Projects 1 and 3.

STAGE1 (Primary): Whole genome gene differential expression (WGGDE) study.

Hypothesis: Changes in the mRNA expression profiles in female BMMSCs and PBMs underlie mechanisms of female BMD variation and are associated with menopause.

Specific Goals: To identify genes differentially expressed in BMMSCs and PBMs in women: 1) with high vs. low BMD; 2) before and after menopause, and thus identify genes associated with female BMD and menopause. We will recruit 80 otherwise healthy females and 80 age-matched otherwise healthy males aged 50-55, stratified by discordant BMD values and menopausal status (for females). We will perform bone marrow aspiration and obtain peripheral blood samples. BMMSCs and PBMs will be isolated and total RNA extracted. Microarray profiling experiments and analyses will be performed on females for >40,000 known human genes and ESTs. Differentially expressed genes will be verified by real-time RT-PCR with female samples. These verified genes in females will be examined by real-time RT-PCR with male samples to examine their sex-specificity.

STAGE2 (Secondary): Functional studies of molecular mechanisms of candidate genes. As an exploratory example, we will present and perform in-depth functional studies to dissect the mechanisms through which HDC and RUNX1 genes regulate BMD. We hypothesize that HDC and RUNX1 genes are important for osteoclast differentiation and/or bone resorption and HDC is a mediator for RLJNX1 gene in regulating BMD.

The results, together with those from Projects 1 and Project 3, will powerfully and efficiently identify genes and some of their functions for female osteoporosis.

Identifying genes for human BMD variation, especially for women, is important for 1) gaining insights into the fundamental molecular mechanisms of risk to osteoporosis, 2) discovering new pathways and targets for therapeutic cures; 3) identifying genetically susceptible individuals (by designing diagnostic DMA chip), so that future preventions and interventions can be targeted to and based on individuals' specific genotypes.

Project 3: Proteome-wide Expression Study of Osteogenic Cells

PI: Hong-Wen Deng, Ph.D.
Professor, Franklin D. Dickson/Missouri Endowed Chair
Director, Orthopedic Research
Director, Genetics Core

Department of Orthopedic Surgery and Basic Medical Sciences
M3-C03
2411 Holmes Street
Kansas City, Missouri 64108

Email: dengh@umkc.edu
Phone: 816-235-5172

Abstract

Osteoporosis is a major public health problem, especially in women. It is mainly characterized by low bone mineral density (BMD). BMD has high heritability of > 60%. Women have much lower BMD than men. We and others demonstrated that some BMD genes/genomic regions are gender specific. Menopause is a most significant physiology event in female's life and is associated with female-specific rapid bone loss.

Bone marrow mesenchymal stem cells (BMMSCs) and peripheral blood monocytes (PBMs), are precursors for osteoblasts (bone formation cells) and osteoclasts (bone resorption cells), respectively.

Our hypothesis is that changes in the protein expression profiles in female BMMSCs and PBMs underlie mechanisms of female BMD variation and are associated with menopause.

Our major goals here are to identify proteins differentially expressed in BMMSCs and PBMs in women: 1) with high vs. low BMD; 2) before and after menopause, and thus identify proteins (and their genes) associated with female osteoporosis and menopause in BMMSCs and PBMs. The proteins identified significant in females will be examined in male samples.

Together with Project 2, we will recruit 80 otherwise healthy females and 80 age-matched otherwise healthy males aged 50-55, including 40 subjects with low and 40 with high BMD (age matched population bottom or top 20% respectively) for each sex, all Caucasian. Each female BMD group includes 20 pre- and 20 age matched post-menopausal women. We will take fresh bone marrow (for which we have had extensive published research experience) and peripheral blood samples from each subject. BMMSCs and PBMs will be isolated and equally divided into two aliquots, one for Project 2 and one for Project 3. In this Project 3, we will extract the total proteins from aliquots of isolated BMMSCs and PBMs. Proteomic profiling experiments and analyses will be performed on Females using MD-nano-LC-MS/MS. Significant differentially expressed proteins identified will be verified by Western blotting with female samples for their importance in females. Significant proteins verified in females will be examined by Western blotting with male samples for sex-specificity.

The results obtained in Caucasians will be cross checked for ethnic generality/specificity in Chinese samples. The molecular and cellular functional studies of the identified proteins and their genes will be pursued as exemplified in Project 2 of this SCOR.

The major results (particularly those obtained from PBMs) of this study may be used to design customary diagnostic protein antibody chips and/or protein markers for prognosis of female osteoporosis. In particular, the results will be used to provide some functional evidence, when combined with the DMA polymorphism data in Project 1 and DMA microarray data in

Project 2 of this SCOR to powerfully and efficiently identify genes and some of their functions for female osteoporosis.

CORES

Core A: Clinical Core

PI: Hong-Wen Deng, Ph.D.
Professor, Franklin D. Dickson/Missouri Endowed Chair
Director, Orthopedic Research
Director, Genetics Core
Department of Orthopedic Surgery and Basic Medical Sciences
M3-C03
2411 Holmes Street
Kansas City, Missouri 64108

Email: dengh@umkc.edu
Phone: 816-235-5172

The planners of this proposal intend to establish and sustain a Specialized Center of Interdisciplinary Research (SCOR), aimed at identification of genes/proteins and their functions important for female osteoporosis. A smoothly functioning SCOR will require strong and capable leadership, a source of unity, interaction and direction, effective coordination of effort and efficient use and management of personnel and resources. These are the responsibilities of the Administrative Core.

The Administrative Core will be led by the SCOR Director, Hong-Wen Deng, Ph.D., and the Co-Directors, Robert R. Recker, M.D., and Lynda Bonewald, Ph.D. The Core Director and Co-Directors are experienced organizational leaders, managers of personnel and resources, and/or clinical investigators. They will form an executive committee to work closely with Internal and External Advisory Boards, and Pi's and Co-Pi's of Projects 1-3 and Directors and Co-Directors of the Clinical Core and the Biostatistics and Bioinformatics Core. They will monitor and evaluate progress of the proposed research and providing ongoing quality control and scientific review, discussion and advice. The LEADERSHIP GOALS of the Administrative Core include:

o Delivering competent and timely administrative services, including scheduling meetings, assuring efficient communications, handling correspondence, arranging travel, business, and personnel matters, etc.

o Coordinating the efforts of SCOR projects, cores, and personnel; coordination will include interactive planning and problem-solving across projects.

o Strengthening collaborative relationships with the Internal and External Advisory Boards and with scientific and medical colleagues from other departments of the participating universities and from other institutions.

o Extending the benefits of SCOR participation to junior faculty, students, and postdoctoral fellows.

o Coordinating scientific lectures and interactive presentations locally and at national and international levels.

o Providing staff support in the form of budgetary support and review, preparation of grant reports, writing of communications and manuscripts, and other supportive activities.

o Assuring ongoing compliance with leadership expectations, governmental policies, and institutional directives by means of a plan for project oversight.

o Coordinating implementation of the SCOR and other NIH funded projects of the SCOR investigators, so that all are implemented efficiently and all benefit maximally from each other.

Core B: Clinical Core

PI: Robert R. Recker, M.D., MAC.P., F.A.C.E.
Professor of Medicine
Chief, Section of Endocrinology
Director, Osteoporosis Research Center
Associate Dean of Research, School of Medicine
Clinical Professor of Periodontics
Creighton University
Omaha, Nebraska

Email:
Phone:

Abstract

This application for a Specialized Center of Interdisciplinary Research (SCOR), aims at identification of female osteoporosis risk genes using the genomic convergence approach. It will integrate efforts of the Orthopedic Research Program and Genetics Core (Dr. Deng), and the Bone Biology Program (Dr. Lynda Bonewald), at UMKC, and the Osteoporosis Research Center (ORC) at Creighton University (Dr. Recker).

The ORC is 2.5 hr drive from UMKC. The Clinical Core, located in the ORC at Creighton is described herein. The overall objective Core is to recruit 160 human subjects during four years to support work in Projects 2 and 3.

Steps in recruitment are: search the ORC archive; make telephone contact; schedule candidates who pass the telephone screen; if eligible, complete clinical examination and collect specimens.

Steps in specimen collection are: perform phlebotomy to obtain 130 ml of peripheral blood; schedule for bone marrow aspiration; perform 10 ml bone marrow aspiration; transport the specimens immediately to the laboratory Dr. Lundberg (co-l of Project 2) for cell isolation at Boys Town National Research Hospital (contiguous with Creighton University Medical Center, where the ORC is located).

The Creighton ORC has a long history of successful recruitment to clinical research studies. We will recruit 40 subjects during each of the Years 1-4. The cohort will include 80 healthy Caucasian female subjects at age 50-55, half with high, and half with low BMD at the spine or hip (top or bottom 20% in agematched population). Each group will be half pre- and half post-menopausal. We will also recruit 80 agematched healthy males with high or low BMD (top or bottom 20%). 80 age matched Caucasian males with discordant BMD values (40 with high and 40 with low BMD values) will also be recruited. Case and control subjects (e.g., those with high or low BMD) will be closely matched for age. The subjects will be recruited from our archive of ~28,000 persons who have all signed consent to allow us to contact them for future research opportunities. This large database will be used to locate candidates for recruitment into this study.

Core C: Biostatistics and Bioinformatics Core

PI: Kui Zhang, Ph.D.
Associate Professor of Statistical Genetics and Basic Medical Sciences
Department of Bioinformatics and Personalized Medicine
Department of Basic Medical Sciences
University of Missouri - Kansas City (UMKC)
Kansas City, MO

This SCOR proposes a novel inter-disciplinary genomic convergence approach that integrates results from Genetic Epidemiology, Functional Genomics, and Proteomics in Projects 1-3 to identify female osteoporosis genes and their functions. In addition, future molecular and functional studies aimed at confirming the genes to identified and thus pinpointing specific causal mutations are exemplified in Stage 2 of Project 2 of this SCOR by using two significant genes (RUNX1 and HOC) we identified in our earlier genetic epidemiology and genomics/functional genomics studies. The approach and each component project involve heavily data management and analyses. It is necessary to set up a Biostatistics and Bioinformatics Core to serve all the three projects efficiently and economically. The Core will essentially serve as a resource and foster exchange and collaboration for all individual projects in the SCOR.

The Biostatistics and Bioinformatics Core has, but are not limited to, the following specific aims:

To collaborate with project investigators on the experimental design issues and the design of questionnaires and forms for efficient data acquisition, entry, tracking, retrieval and transfer, etc.

To implement (and develop if necessary) efficient and high quality data entry/management database system for all individual projects and to build quality control measures into those systems.

To work with project investigators on an ongoing basis to ensure that the requirements of each protocol are satisfied for data acquisition.

To monitor the emergence of and evaluate new methods and programs for data management and analyses.

To work with project investigators on choosing and correct usage of the available updated and most appropriate software for analyses.

To oversee and conduct the statistical analyses of all data generated from this SCOR project.

To work with project investigators on interpretation of the analysis results and summarizing the results for publications.

To work with project investigators on custom software design and development tailored to the special need of this SCOR project should the need arise.

To provide biostatistics and bioinformatics support for all types of gene mapping, genomics and functional genomics platforms to be used in this SCOR.

* To develop new statistical methods and computational algorithms should the need arise for individual projects and the whole SCOR.