PROTOCOL

Trans-omics Integration of Multi-omics Studies for Male Osteoporosis

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STUDY SUMMARY

Title	Trans-omics integration of multi-omics studies for male osteoporosis
Sponsor	National Institute on Aging
NIH Grant Number	5U19AG055373
Study Design	Cross-sectional study
Study Duration	5 years
Principal Investigator	Hong-Wen Deng, Ph.D. Professor Chief, Division of Biomedical Informatics and Genomics John. W. Deming Department of Medicine Center for Biomedical Informatics and Genomics School of Medicine Tulane University Email: hdeng2@tulane.edu
Co-Principal Investigator	Hui Shen, Ph.D. Professor Division of Biomedical Informatics and Genomics John. W. Deming Department of Medicine Associate Director, Center for Biomedical Informatics and Genomics School of Medicine Tulane University Email: hshen3@tulane.edu
Study Center	Tulane Center for Biomedical Informatics and Genomics (CBG) at Tulane University School of Medicine.
Objectives	1) gaining comprehensive insights into the fundamental molecular and environmental mechanisms underlying risk of osteoporosis. 2) discovering novel pathways and druggable targets for therapeutic cures. 3) identifying (epi-)genetically susceptible individuals, so that future preventions and interventions can be targeted to and based on individuals' specific (epi-)genotypes.
Number of Subjects	1,055
Inclusion Criteria	1) Caucasian or African American males 2) Aged 20-50 years 3) Willing to visit the Center for Bioinformatics and Genomics' clinical recruitment sites for obtaining objective clinical measurements, donation of 120 ml blood, and collection of a small amount of stool sample.

	4) Willing to release de-identified genetic information derived from this study into a scientific database, and share it with other researchers for use in future studies of health/medical/biomedical purposes. 5) No antibiotic usage in the past 3 months. 6) No gastroenteritis in the past 3 months. 7) No major surgeries involving hospitalization in the past 3 months. 8) No intercontinental travel in the past 3 months.

1. INTRODUCTION

Osteoporosis is mainly characterized by low bone mineral density (BMD) and its risk later in life can be most powerfully predicted by peak BMD achieved at ages 20-40[1, 2]. Although women have a higher osteoporosis risk, men suffer much higher morbidity and mortality rates following osteoporotic fractures[3, 4]. The (epi-)genetic factors underlying e majority of the BMD heritability (>85%) (especially those sex-specific ones) are largely unknown mainly due to the limitation of the technology and approaches used[5, 6]. Studies focusing on male osteoporosis are rare[7]. Our General Hypothesis is that: while individual omics studies (genome /transcriptome /epigenome) are useful, integrative trans-omics analyses of multi-omics data will be much more productive and more powerful, in not only identifying novel risk (epi-)genes/variants, but also most importantly illuminating their functions in vivo in humans. The Overall Goal of this U19 program project (PPG) is to most comprehensively identify/characterize (epi-) genes/environmental factors and their functional mechanisms for male osteoporosis risk. We will pioneer a comprehensive and novel approach by investigating osteoporosis risk factors simultaneously at the genome- (DNA), transcriptome- (mRNA and miRNA), and epigenome- (DNA methylation) levels in males, while considering environmental factors. We will assess sex/ethnicity/population specificity of the identified (epi-)genes. Furthermore, we will perform in-depth functional studies for detailed molecular mechanisms and functional confirmation of specific novel osteoporosis susceptibility genes/variants to be discovered. Particularly, in addition to the state-of-the-art analyses of individual omics data, we will go beyond the horizon to develop/apply innovative analytical approaches to characterize interactions within and across different omics (such as interactions for miRNA-methylation, and regulation of mRNA by DNA variants and by miRNA/methylation). We will innovatively integrate such interactions across various -omics to identify/characterize (epi-)genetic variants for male osteoporosis.

Identifying (epi)genes/environmental factors and their in vivo functional mechanisms for human BMD variation, especially for men, is necessary and important for 1) gaining comprehensive insights into the fundamental molecular and environmental mechanisms underlying risk of osteoporosis, 2) discovering novel pathways and druggable targets for therapeutic cures; 3) identifying (epi-)genetically susceptible individuals, so that future preventions and interventions can be targeted to and based on individuals' specific (epi-)genotypes. This PPG is expected to break new ground for its innovation, which serves as a pioneering example to stimulate similar studies of other complex diseases.

2. OBJECTIVES

a. Recruitment: In total, 700 Caucasian and 400 African-American males with age between 20-50 years, will be recruited from the subjects participating in the ongoing Louisiana Osteoporosis Study (LOS) in four years. The BMD value will be ascertained by Dual Energy X-ray Absorptiometry (DXA).

b. Phenotyping: includes measuring or ascertaining BMD and bone quality, body composition (body fat mass, lean mass, and percentage fat mass), blood pressure, and other related traits such as weight, height, age, race, lifestyle factors (e.g., exercise, alcohol consumption, smoking, etc.), and medical and family history that may be relevant to osteoporosis.

c. Collect 120 ml blood from each of the 1100 subjects, which will be used for the PBMs isolation (with 110ml of blood) and for measuring serum levels of vitamin D [25(OH)D] (with 10ml of blood).

d. Extract DNA and RNA from the PBMs. The extracted DNA and RNA will be stored in a -80°C freezer, which will be used for downstream methylation and gene expression analyses to attain the goal as specified above.

e. Collect a small amount of stool sample from each subject, which will be used for DNA extraction. The extracted DNAs will be stored in a -80°C freezer, which will be used for subsequent whole genome metagenomic short-gun sequencing.

3. STUDY POPULATION

We will recruit 700 Caucasian males and 400 African-American males with age between 20-50 years. Finally, we recruited 655 Caucasian males and 398 African-American males aged between 20-51 years old for this study.

3.1 The Recruitment Strategy

We have been implementing the Louisiana Osteoporosis Study (LOS) (largely supported by Tulane University) at our clinical site since 2011. The study aims to build a population-based cohort (NOT ascertained through targeted outreach to osteoporosis related clinics) to serve as a large sample pool and database supporting our NIH-funded projects (e.g., U19AG055373, P50AR055081, R01AR050496, R01AR059781) for aging research. Thus far, the LOS has accumulated >17,000 subjects.

Our database manager will first screen the LOS database based on the selection criteria of this study (see below). The clinical coordinators will then initiate the contact with the eligible subjects by direct conversation when the subject is visiting our clinical sites for other projects (e.g., LOS), or by mails, emails, text message, and/or phone calls. In addition, subjects who read about the study on our clinical recruitment website (http://tulane.edu/publichealth/bio/clinical-recruitment.cfm) may contact our clinical coordinator by phone or email for further information. Meanwhile, flyers will be sent to the potential participants in the surrounding area and posted on websites, such as Craigslist, social media, and our website. Postcards will be mail to the potential subjects. On average, $5/participant will be given to the referrer once he/she refers **at least two** participant who successfully enrolls and completes the MM study procedures. Since our clinic facility only has $10 and $25 gift cards, referrers will not receive a gift card until they refer at least two participants. They receive a $10 Walmart gift card for every 2 participants recruited or a $25 Walmart gift card for every 5 participants recruited. Each participant referred can only count once. Each participant referred can only count once. There is no limit to the compensation amount. During their conversation, phone call or email communication, the clinical coordinator will briefly introduce the study to the subjects and ask if they are interested in this study. If the subject is interested in participating in the study, a visit will be scheduled to our clinical recruitment site (11th floor, 1440 Canal Street, New Orleans, LA 70112).

Subjects who need more time for deciding on participation will be allowed to bring the consent form home and be asked for their willingness to be re-contacted by our research staff regarding their final decisions.

One subject is supposed to participate in this study once, and our staff will be well trained to avoid duplicative enrollment adopting the following strategies: 1) Current enrollment list of all previous participants are available to our front desk and we will check full name and birthdate to avoid duplicative enrollment. 2) We will check each subject's driver's license to make sure self-reported identification information (full name and birth date) are reliable. However, duplicative enrollment may still occur due to staff's negligence, mistakes, etc. For duplicative enrollment, we will delete all data and specimen samples collected from the second enrollment. The first enrollment data will be retained for research usage.

3.2 Inclusion and Exclusion Criteria

Individuals must meet the following inclusion criteria to be eligible to participate in the study:

1) Caucasian or African American males

2) Aged 20-50 years

3) Willing to visit the Center for Bioinformatics and Genomics' clinical recruitment sites for obtaining objective clinical measurements, donation of 120 ml blood, and collection of a small amount of stool sample.

4) Willing to release de-identified genetic information derived from this study into a scientific database and share it with other researchers for use in future studies of health/medical/biomedical purposes.

5) No antibiotic usage in the past 3 months.

6) No gastroenteritis in the past 3 months.

7) No major surgeries involving hospitalization in the past 3 months.

8) No intercontinental travel in the past 3 months.

We will adopt the following exclusion criteria to minimize non-genetic influence on bone mass variation, so as to empirically enhance the chance to detect individual genetic factors for bone mass. The same criteria are being used in our ongoing LOS study.

1) Serious residuals from cerebral vascular disease;

3) Diabetes mellitus, except for those controlled under medication;

4) Chronic renal failure;

5) Chronic liver failure;

6) Significant chronic lung disease;

7) Alcohol abuses as defined by those who drink alcohol regularly and cannot control themselves and get drunk at least once a week;

8) Chronic obstructive pulmonary disease (COPD);

9) Corticosteroid therapy at therapeutic levels for more than 6 months duration;

10) Treatment with anticonvulsant therapy for more than 6 months duration;

11) Other metabolic or inherited bone diseases including hyper- or hypoparathyroidism, Paget's disease, osteomalacia, osteogenisis imperfecta, and hypochondrogenesis;

12) Rheumatoid arthritis, except for minor cases that involve only hand joint and wrist;

13) Chronic gastrointestinal disease including celiac disease, postygastrectomy, Crohn's disease, ulcerative colitis, liver transplant, and cirrhosis;

14). Upper or lower limb loss or disability;

We will not exclude people with a diagnosis of idiopathic osteoporosis, or those on calcium and/or vitamin D supplements. However, information collected for calcium and/or vitamin D supplements may be used as co-variates in analyses.

Given that blood monocytes are also essential component of the immune system, we will adopt the following additional exclusion criteria in order to minimize the effect of diseases or conditions, which may potentially lead to monocyte gene/protein expression changes:

1) Autoimmune or autoimmune-related diseases: systemic lupus erythematosus, multiple sclerosis, Graves disease, Hashimoto's thyroiditis, myasthenia gravis, Addison's disease, dermatomyositis, Sjogren's syndrome, Reiter's syndrome.

2) Immune-deficiency conditions: HIV infection, severe malnutrition, splenectomy, ataxia-telangiectasia, DiGeorge syndrome, Chediak-Higashi syndrome, job syndrome, leukocyte adhesion defects, panhypogammaglobulinemia, selective deficiency of IgA, combined immunodeficiency disease, Wiscott-Aldrich syndrome.

3) Haematopoietic and lymphoreticular malignancies: leukaemias, lymphomas (Hodgkin's disease, non-Hodgkin's disease), myeloma, Waldenström's macroglobulinaemia, heavy chain disease, leukemic reticuloendotheliosis, mastocytosis, malignant histiocytosis.

4) Other diseases: influenza (within one week of recruitment), active periods of asthma.

Subjects will only provide verbal confirmation of their HIV statuses for determination of study eligibility. HIV testing will NOT be performed on their blood samples.

4. PROCEDURES

The blood collection will be performed at our clinical recruitment site (11th floor, 1440 Canal Street, New Orleans, LA 70112). The clinical visits will be coordinated by the clinical coordinators for clinical measurements and experimental procedures. The clinical coordinators will also arrange for immediate transport of blood samples to the PI Dr. Deng's laboratories on the 3rd floor at Tulane JBJ building in New Orleans for downstream cell isolation experiments.

4.1 Questionnaire survey

For each study subject, information on age, gender, racial/ethnic background, medical history, family history, alcohol use, diet habits, smoking history, etc. will be assessed by a Louisiana Osteoporosis Study Questionnaire, Metagenomic Study Supplementary Questionnaire and a Food Frequency Questionnaire.

4.2 BMD and body composition measurement by DXA

If the subject had his previous DXA scan within 3 months, then no DXA scan will be administered to this subject in this visit. The subject's BMD and body composition data from the previous DXA scan will be retrieved from the LOS database.

If it has been greater than 3 months since the subject's last DXA scan, we will need to re-measure the subject's bone density.

We will measure BMD at the forearm of non-dominant hand, spine, left hip (or right hip if the left hip has metal implant), and whole body. Before obtaining DXA measurements, we will explain to the subject the position for each scan, referring to the guide photographs showing positions for accurate measuring. The subject will have learned before the visit to wear a non-metal, bone-scan-friendly outfit, without metal fasteners or zippers. The subject will need to remove any nylons/hosiery, as well as any metal fasteners. In the event a subject arrives without such clothing, disposable gowns and footwear will be available.

For spine BMD, the quantitative phenotype will be the combined BMD of the L_{1-4} vertebrae. For hip BMD, it will be the combined BMD of the femoral neck, trochanter, and intertrochanteric region. For each subject, BMD (g/cm^2) of the lumbar spine, hip, forearm, and total body will be measured by the DXA machine housed in our clinical recruitment sites. The DXA machine to be used in this study is the Hologic Discovery A system (Hologic Inc., Bedford, MA, USA). The machine will be calibrated daily. Software and hardware will be constantly kept up-to-date during the study. All DXA reports will be scrutinized for artifacts and confounding features such as significant scoliosis and osteophyte formation in the spine. Those reports with significant confounding features will be excluded from the analysis.

The Hologic Discovery A system DXA machine also performs body composition analysis, reporting body fat mass and lean mass in grams. We will calculate percentage fat mass as the ratio of fat mass divided by body weight (the sum of fat mass, lean mass, and bone mass). Similarly, percentage lean mass can be calculated as the ratio of lean mass divided by body weight.

4.3 Volumetric BMD and bone quality measurement by QCT

To have a more comprehensive bone health assessment, we will also measure volumetric BMD (vBMD), including cortical and trabecular bone components, at the proximal femur for the selected subjects, by QCT scan with GE Discovery* CT750 HD system at Tulane University Department of Radiology.

Furthermore, we will conduct finite element analysis (FEA) to estimate bone strength based the QCT data obtained. FEA is an engineering method that uses mathematical models to define how a structure or material will react to stress when loaded. FEA was recently applied to estimate bone strength by incorporating information about the geometry and density/distribution embedded in QCT bone scans and the loading conditions known to cause fractures.

4.4 Blood collection via phlebotomy

Blood (120 ml) will be drawn by trained research staff. Samples will be temporarily stored in room temperature and then quickly transferred to Dr. Deng's laboratories at New Orleans for PBMs isolation.

If for some reason we are UNABLE to collect the full amount of 120ml blood from the subject, then the subject will still receive up to $100 Walmart/Amazon gift cards for the visit. At that time, we will ask the subject to return on a different day so that we may try again to collect his blood. The subject will receive another $50 Walmart/Amazon gift card on the returning visit.

4.5 Stool sample collection

The study subjects will be given a self-collection kit to collect a small amount (~0.5 gram, approximately the size of a green pea) of stool sample at our clinical site (if possible) or at home. If the subjects choose to collect the stool sample at home, we will also provide him a mailing package and ask the subjects to mail the stool samples collected in the kit back to us within 7 days after their clinical visit.

If a subject chooses to collect the stool samples at home, then he will receive up to $100 (for clinical

measurements and 120 ml blood donation) Walmart/Amazon gift card after the completion of his visit to our clinical site. We will ask him to mail the stool samples collected in the self-collection kit back to us in 7 days (we will pay the mailing fee). The subject will receive another $50 Walmart/Amazon gift card after we receive the stool samples. If we are not able to extract DNA from a subject's stool sample due to insufficient amount of stool sample, absence of reserving liquid, etc., we will ask the subject to donate an additional stool sample upon his agreement. The subject will receive another $25 Walmart/Amazon gift card once we receive another stool sample.

For subjects who choose to collect stool samples at home, we will remind them sending back their stool samples by sending emails and/or text messages to them on the 2nd and 4th (if not receiving their stool samples by then) day after their clinic visit. If we don't receive their stool sample by the 7th day after their clinic visit, we will send the reminder email and/or text message to the subjects once a week for additional 5 weeks (or until the stool sample are received).

4.6 Grip strength

To assess subjects' physical function, grip strength (kg) will be measured by a dynamometer for the dominant hand by a trained staff.

Note: More than one clinic visit/visits may be needed to complete the "Procedures". The additional visit/visits may result from the following reasons: 1) subjects would like to complete different procedures at different time, such as collecting stool samples at home, instead of in the clinic facility; 2) the quantitative computed tomography (QCT) scan will be conducted at Tulane University Department of Radiology. Based on the availability of study subjects and available spots of Tulane University Department of Radiology, the date for QCT scan might differ from the date of the visit to our clinical recruitment site; and/or 3) if we are UNABLE to collect the full amount of 120 ml blood from a subject, the subject will be asked to return on a different day so that we may try again to collect his blood. We have decided to collect additional information from our currently enrolled participant to consider the effect of external factors on the gut microbiota. Therefore, currently enrolled participants who agreed to be further re-contacted will be re-contacted by our research staff for collecting the information that our research needs. The additional information includes:

1. Whether the participant had taken antibiotics in 3 months of the enrollment date.

2. Whether the participant had gastroenteritis in 3 months of the enrollment date.

3. Whether the participant had major surgery involving hospitalization in 3 months of the enrollment date.

4. Whether the participant had traveled intercontinentally in 3 months of the enrollment date.

4.7 Wet Lab Experimental Procedures

The 10ml of collected blood from each subject will be used for measuring serum levels of vitamin D [25(OH)D].

The rest 110ml of blood samples from each subject will be used for PBM isolation using the LymphoprepTM (Greiner Bio-One, Monroe, NC) and Monocyte Isolation Kit II (Miltenyi Biotec Inc., Auburn, CA) following the manufacturer's instruction. DNA and total RNA will be extracted from PBMs by using the AllPrep DNA/RNA/Protein Mini Kit (Qiagen, Valencia, CA) following the

manufacturer's instruction. The extracted DNAs and total RNAs will be stored in a -80°C freezer, which will be used for downstream assays.

Whole genome DNA methylation profiles will be assessed by whole genome bisulfite sequencing or other comparable approaches. Targeted DNA methylation analysis for specific genomic regions will be assessed by Sequenom EpiTYPER methylation assays or other comparable approaches. The mRNA expression levels of selected candidate genes will be assessed by real-time RT-PCR. Molecular and cellular functional assays will be conducted on cultured monocyte cell lines.

DNA will be extracted from the stool samples using the MoBio PowerFecal DNA Isolation Kit (MoBio, Solana Beach, CA) following manufacturer's instructions. The extracted DNAs will be stored in a -80°C freezer, which will be used for subsequent whole genome metagenomic short-gun sequencing.

4.8 Anonymize QCT Data

To anonymize DICOM data, we generated an in-house Python program that built up by “Pydocim” library, which is a pure Python package for working with DICOM files such as medical images, reports, and radiotherapy objects. The following DICOM tags and data elements have been removed (HIPAA Privacy Rule) from our QCT data, and each patient ID was replaced by random project ID. DICOM Tags includes “PN”, “SH”, “DA”, “TM”, and “LO”. Please refer more DICOM tags detail on “DICOM Library”. The information that has been removed includes names, telephone numbers, electronic mail addresses, etc. All anonymized QCT data will be stored in external hard drives and delivered to University of California, Irvine for finite element analysis (FEA).

5. RESEARCH SUBJECT PROTECTION

5.1 Risks

Obtaining blood via phlebotomy can cause pain, bleeding, bruising, and on rare occasions, fainting, and infection or inflammation (redness and swelling) at the site of the needle stick. Some subjects may experience temporary discomfort and anxiety.

DXA measurements will expose the subject to low levels of radiation. The level of radiation for the DXA is about 2.4 microsieverts [μSv] per scan of the hip. Radiation dose in QCT is 200-400 μSv per scan. The amount of radiation exposure is considerably lower than that of other X-ray based examinations for osteoporosis, such as radiographs of the spine (700 – 2,000 μSv), or the annual natural background radiation (~3,100 μSv). This exposure is well within acceptable limits for healthy volunteers.

Another potential risk of this study is the possible loss of privacy or breach of confidentiality.

To reduce risk from venipuncture, our clinical research staff will follow closely the study protocol and venipuncture will be performed in the appropriate clinical setting. Specially, we will follow the standard procedure for phlebotomy (disinfection of the local site using antiseptic before the procedure and stoppage of bleeding using medical cotton and a band-aid after the procedure). Before and after the phlebotomy, we will monitor the subject's blood pressure and pulse. The subject will be allowed to leave only after his blood pressure and pulse is stable and there is no bleeding in the site of phlebotomy.

To minimize radiation exposure, QCT bone densitometry will not be used for subjects who have had another radiological procedure that includes the introduction of high-density contrast material

(barium, iodine, thorotrast, thorium) or radio-opaque catheters and tubes within 3 months. We will ask such participants come back for the QCT bone densitometry after 3 months. In addition, DXA and QCT examinations will be performed by trained professional research staff with competent technical skills.

We will take specific measures to reduce the risk of breach of confidentiality. Any records, specimens, and data generated in the course of the study will be labeled with de-identified project ID numbers. The record linking the subjects and the project IDs will be kept in locked, fireproof file cabinets (for hardcopy documents) and password-protected computer files, which will be either accessible (for the one subject specifically recruited for this project) only to the PI and the database manager, or will not be accessed at all (for all the other samples) for this project. All information collected from subjects for this study will be kept strictly confidential and will not be released to any third parties (e.g., health insurance carriers, employer, family members, etc.). Laboratory results will not be divulged to anyone other than authorized research personnel. Laboratory records will be kept in locked hard-copy files, or password-protected computer files, only accessible to authorized research personnel.

The study results will be presented at scientific meetings without divulging identities of subjects.

5.2 Benefits

There will be no direct benefits to subjects for participating in this research, but the knowledge gained from the study may benefit society in general. For example, results of this study may lead to the eventual development of therapy used to prevent or reduce the risk of developing osteoporosis. This could significantly reduce pain, morbidity, and mortality in millions of patients. It could also save the United States and other countries billions in health care dollars.

Also, we will distribute an educational brochure to all participants for them to learn what causes osteoporosis, how to prevent it, and how to treat it. In addition, all subjects will have the opportunity to have an evaluation of bone and the related health at no charge, including a bone density and quality scan, results of which may be highly beneficial to subjects. Should any abnormal conditions be found, subjects will be notified and encouraged to visit their primary care physicians.

5.3 Remuneration

The subjects will be provided with free validated parking in LaSalle Parking Garage, which is ~5 min walking distance to our clinical site.

After the subject's clinical data and biological sample (blood and stool) collection is completed, each subject will receive Walmart/Amazon gift cards up to $150.00 as stipend to cover expenses associated with the visit and biological sample collection.

If a subject is not qualified for this study, the subject will be excluded from further clinical procedures and not included in this study. Subjects excluded will still receive a $25 Walmart/Amazon gift card.

A subject will receive a $50 Walmart/Amazon gift card after completing the questionnaire survey, clinical measurements, and donating the full amount of 120ml blood.

If for some reason we are UNABLE to collect the full amount of 120 ml blood from a subject, $50 Walmart/Amazon gift card will not be given to the participant until the participant return on a different day so that we may try again to collect his blood. The subject will receive $50 Walmart/Amazon gift

card on the returning visit, no matter whether we can obtain 120 ml blood or not.

If the subjects choose to collect the stool samples at home, then he will receive $50 Walmart/Amazon gift card after the completion of his visit to our clinical site (completing the questionnaire survey, clinical measurement, DXA scan if needed, and donating full amount of 120ml blood). At that time, we will provide the subject a mailing package and ask the subject to mail the stool samples collected in the self-collection kit back to us in 7 days. The subject will receive two $50 Walmart/Amazon gift cards after completing the rest of the study procedures, including QCT scan and stool sample donation.

For subjects who have previously participated in this study and donated 70ml blood, but not stool samples, we will invite them back to obtain new consent for donating additional 50ml blood and stool samples. In the returned visit, the subject will be instructed to complete two questionnaires. These questionnaires are the Food Frequency Questionnaire and the supplemental questionnaire. In addition to the blood sample (if a subject has donated 70ml blood in previous visit, the participants will only donate an additional 50ml blood to reach the total of 120ml blood), a small amount of stool donation will be collected from subjects either at the clinical site at the time of the visit, or at home. A subject will receive $50 Walmart/Amazon gift card after completing the two questionnaires and donating blood, and another $50 Walmart/Amazon gift card after we receive the stool sample.

If for some reason we are UNABLE to isolate peripheral blood monocytes (PBMs) from the blood, we will contact the subject to return on a different day so that we will try again to collect another 120ml blood. The subject will receive another $50 gift card if they donate their 120ml blood on the returning visit.

5.4 Academic or Extra Credit

NA.

5.5 Costs

There will be no costs to the subject for participating in this research study.

5.6 Alternatives

The alternative is that the subjects do not have to participate in the research.

5.7 Consent process and documentation

A written informed consent will be obtained in our clinical recruitment site. A subject will be provided an Informed Consent Form and be assisted in understanding the terms in the form by the clinical coordinators of this study. If the subject agrees with all the terms in the form, he may sign the form and join the study immediately.

However, a waiting period is allowed between informing the perspective subject and obtaining consent. For example, the form can be brought home by the subject to allow them more time to consider the decision in joining this study.

This study will only recruit English-speaking subjects who can understand independently all the English language in the consent form except for some medical or scientific terms (that will be explained by the recruiters to the subjects). At the end of the consent form, a special question will

be asked to the subject, which requires a "Yes" answer for the consent form to be effective. The question will read "Do you understand in full all the content in this consent form?".

Toward the end of the consent form, a special term for voluntary participation will be presented to the subject, which reads "You do not have to be in this study if you do not want to. If you agree to be in the study, but later change your mind, you may drop out at any time. There are no penalties or consequences of any kind if you decide that you do not want to participate. You do not have to answer any question that you do not want to answer."

The informed consent will be signed and dated by the subject and the clinical coordinator obtaining the consent. A copy of the signed Informed Consent Form will be provided to the subject for his own record. To ensure appropriate documentation, when consent documents are turned in by a subject, the clinical coordinator who administers the consent documents will go through each page of the signed consent documents to ensure that the subject's initials, signatures, and dates are obtained at all applicable locations with appropriate format. In addition, at the end of the visit, a clinical staff (may or may not be the same clinical staff who administers the consent documents) will again check the consent documents to ensure complete and appropriate documentation.

5.8 Qualifications of the investigators

Dr. Hong-Wen Deng is the PI of this study. He is a Professor and chief of Division of Biomedical Informatics and Genomics, Dept. of Medicine, Tulane School of Medicine (SOM) and the founding Director of the Tulane Center for Biomedical Informatics and Genomics (CBG). He is a geneticist with extensive experience and productivity in molecular genetics, genetic epidemiology, functional genomics, proteomics, epigenomics, statistical and computational genetics, and bone biology.

Dr. Hui Shen is the Co-PI and the faculty Project Manager of this study. He is the Associate Director of the Tulane CBG and a Professor in the Division of Biomedical Informatics and Genomics, Department of Medicine, Tulane SOM. He is a molecular geneticist with advanced experience in molecular genetics and genomics of complex diseases.

Dr. Melanie Ehrlich is a Co-I of this study. She is a Professor of Biochemistry and Human Genetics in the Tulane Hayward Human Genetics Center. She has been studying human epigenetics since the early 1980's and her lab has been at the forefront of research on the biological importance of human DNA methylation.

Dr. Michael Serou is a Co-I of this study. He is an Assistant Professor and the Director of Musculoskeletal Imaging in the Department of Radiology of Tulane University. He has broad experience in medical imaging, with fellowship training in musculoskeletal radiology, and has served as an imaging consultant in surgical and orthopedic research studies.

5.9 Inclusion of Tulane students and employees

Tulane students and employees will be enrolled into this study if they volunteer to do so. No coercion and/or undue influence will be provided to this population. Their decision to participate, decline, or withdraw will have no effect on their grades at, status at, or future relations with Tulane University. Their privacy and confidentiality will be strictly protected as detailed above and in our data monitoring plan.

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