Background

- Osteoporosis is a major public health problem in US, a disorder that increases with age, in both women and men.

<table>
<thead>
<tr>
<th>category</th>
<th>2002*</th>
<th>2010</th>
<th>2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>women-OP</td>
<td>7.8</td>
<td>9.1</td>
<td>10.5</td>
</tr>
<tr>
<td>women-low BMD</td>
<td>21.8</td>
<td>26.0</td>
<td>30.4</td>
</tr>
<tr>
<td>men-OP</td>
<td>2.3</td>
<td>2.8</td>
<td>3.3</td>
</tr>
<tr>
<td>men-low BMD</td>
<td>11.8</td>
<td>14.4</td>
<td>17.1</td>
</tr>
</tbody>
</table>

- It affects all ethnicity/racial groups
  - 30% white woman over 50 y.o.
  - 16% Mexican-American women
  - 10% African-American women

- Projected annual costs
  - $25.3B by 2025 and >$50B by 2040 total direct cost
    - >400k hospitalizations
    - 2.5M physician visits
    - >180k nursing home admissions

*In millions per year*
Annual Incidence of Common Health Conditions in Women

- Osteoporotic Vertebral Fracture: 560,000
- Osteoporotic Hip Fracture: 200,000
- Heart Attack: 210,000
- Stroke: 371,000
- Breast Cancer: 211,300
- Uterine Cancer: 52,300
- Ovarian Cancer: 25,400

Clinical Consequence of Osteoporosis

- Out of more than 2.0M fractures attributable to osteoporosis, there are more than 329,000 hip fractures each year
  - 550k vertebral fx
  - 400k wrist fx
  - 810k other fx
- Can lead to morbidity, disability, loss of independence and potentially death
- Genetic, co-morbid and environmental (lifestyle) factors contribute to risk
Survival Rates After Fractures

Adapted from Cooper C et al. *Am J Epidemiol*. 1993;137:1001-05

- Vertebral Fracture (Relative Survival = 0.81)
- Hip Fracture (Relative Survival = 0.82)
The Promise of Genomics for Osteoporosis

- Predictive of risk for disease
  - Low bone mineral density
  - Biomarkers
- Predictive of more aggressive disease
- Predictive of risk for fracture
- Predictive of treatment response
- Predictive of risk for adverse events associated with treatment
Case Study

- 60 y.o. post-menopausal white woman
  - Significant menopausal symptoms necessitated HT for 5 yrs
  - Fractured wrist after slipping on ice
  - HT discontinued 2 years ago
  - Daily total calcium intake 1000 mg with 800 units vit D
  - Family history of low bone mass
    - Mother on alendronate for 10 years
  - Weight 63.5 kg; Height 165.1 cm

- Is she at significant risk for osteoporosis and fracture?

- Should she consider pharmacologic treatment?
Risk Factors for Low Bone Mass

- age; ethnicity; gender
- cigarette smoking; excessive alcohol
- sedentary lifestyle
- decreased lifelong estrogen exposure
- low calcium intake
- family history
- medications

*Positive predictive value of clinical risk factors for BMD is low*
DXA: A Radiographic Biomarker of Fracture Risk

- Imaging biomarker of osteoporosis and fracture risk
- Interpretation
  - T score > -1.0 normal bone mass
  - T score < -2.5 osteoporosis
  - 1 sd decrease in BMD doubles fracture risk
- Clinical utility
  - define low bone mass
  - estimate relative fracture risk
  - monitor response to intervention

Her LS T-score is -1.6 and FN T-score is -2.0
Delineating Genetic Factors Contributing To Osteoporosis

- 60-85% inter-individual variability of BMD genetically determined
  - Heritability persists into 8th decade
- Maternal History of hip fracture increases risk
  - RR 1.48 (1.03, 2.11) (SOF)
  - 1.75 (1.17, 2.63) (meta-analysis)
- Twin studies show heritability of hip fracture 0.48 (0.28-0.57)
- Heritability related to age at fracture
  - < 69 yrs 0.68 (0.41, 0.78)
  - 70-79 yrs 0.47 (0.04, 0.62)
  - >80 yrs 0.03 (0.01, 0.11)
Putative Genetic Associations for BMD

- 15 genes reasonably considered as osteoporosis susceptibility genes
  - vitamin D pathway (*VDR*)
  - estrogen endocrine pathway (*ESR1*, *ESR2*)
  - Wnt-β catenin pathway (*LRP5*, *LRP4*, *SOST*, *GRP177*)
  - OPG/RANK-L pathway (*OPG*, *RANK*, *RANKL*)
  - Type 1 Collagen (*COLIA1*)
  - Bone remodeling-osteopontin (*SPP1*)
  - Others (Integrin- *ITGA1*; *OSTERIX*; *SOX6*)
- Additional 40 genes are considered as promising
Vitamin D Endocrine Pathway

- VDR earliest studied gene for PMO
  - *Fokl (C→T in exon 2)*
    - C more responsive to 1,25 vit D
  - *Cdx2 (G→A in promoter)*
    - A associated with reduced risk for vertebral fracture
  - *BsmI* associated with rates of falls
- DBP
  - Associated with low BMD
Estrogen Endocrine Pathway

- **ESR1**
  - *XbaI* (XX) associated with higher BMD
  - *XbaI* decreased fracture risk independent of BMD

- **ESR2**
  - Consistent effect on BMD
  - May interact with ESRI and IGF1

- **Cytochrome P450**
  - CYP19A1 (aromatase)
    - Age-dependent effect on BMD
Pathways Involved in Bone Remodeling
Bone Remodeling Pathways

- Wnt/β catenin signaling pathway
  - LRP5
    - Loss-of-function mutations-osteoporosis-psuedoglioma syndrome whereas activating mutations-hyperostosis and osteoporosis
    - Modest effect of common variants and BMD
  - SOST (sclerostin)
    - Loss-of-function-sclerosteosis
    - Decreased BMD associated with SNPs in promotor

- RANKL/RANK/OPG
  - OPG
    - GWAS demonstrates 2 SNPs in LD with OPG associated with LS and FN BMD and assocaited with OPG levels
  - RANKL
    - 3 SNPs in high LD associated with FN strength
Genome-wide Association Studies (GWAS) of Osteoporosis

• GWAS involves rapidly scanning markers across the complete sets of DNA to find genetic variations associated with a particular disease
  – Unconstrained by prior hypotheses
  – Unprecedented risk for false positives

• Requirements for GWAS
  – Selection of large numbers of subjects with disease or trait
    • Controls should be selected from same population as cases
  – Quality control of genotyping
  – Statistical tests pass quality threshold
    • Reduce false positive rate by Bonferoni correction
  – Replication in an independent sample
QQ Plots for Bone Mineral Density

A. Hip Bone Mineral Density

B. Spine Bone Mineral Density

- Red dots: Uncorrected $-\log_{10} P$ value
- Blue diamonds: Corrected $-\log_{10} P$ value

- X-axis: Expected $-\log_{10} P$ value
- Y-axis: Observed $-\log_{10} P$ value

Legend:
- Uncorrected $-\log_{10} P$ value
- Corrected $-\log_{10} P$ value
Manhattan Plot: Association of SNPs and Specific Trait

*Genome-wide association is set at $5 \times 10^{-8}$*
Novel SNPs Identified by GWAS

- Validated a number of candidate genes
  - *LRP5, ESR1, SOST, OPG, RANKL* and *RANK*

- Novel genes
  - *SP7 (OSTERIX)*
    - Associated with spine BMD-transcription factor essential for OB activity
  - *SOX6*
    - Bivariate association with BMI and BMD
  - *ADAMSTS18* and *TGFBR3*
    - Associated with BMD in Caucasians, Africans (Tobago) and Chinese-implicated in OB differentiation and remodeling
  - *IL21R*
    - Consistent association with FN BMD-negatively correlated with destruction of cartilage and bone
Effect Size of GWAS-identified Genetic Variants for Skeletal Traits is Small

<table>
<thead>
<tr>
<th>Author</th>
<th>Trait</th>
<th>Chromosome Location</th>
<th>Gene</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liu</td>
<td>Bone size</td>
<td>2q33.1</td>
<td>PLCL1</td>
<td>5 cm² ↑ size</td>
</tr>
<tr>
<td>Richards</td>
<td>BMD</td>
<td>11q13.2 8q24.12</td>
<td>TNFRSF11B LRP5</td>
<td>0.13 sd ↓ 0.09 sd ↓</td>
</tr>
<tr>
<td>Styrkarsdottir</td>
<td>BMD (LS)</td>
<td>1p36.12 8q24.12 6q25.1 13q14.11 6q25.1 18q21.33</td>
<td>ZBTB4 OPG ESR1 RANK-L ESR1-C6orf97 RANK</td>
<td>0.14 sd ↓ 0.09 sd ↓ 0.08 sd ↓ 0.10 sd ↓ 0.08 sd ↓ 0.07 sd ↓</td>
</tr>
</tbody>
</table>
Genetic Effect Modified by Number of Risk Alleles

- Single genetic variant may explain 2-3% variation in BMD
  - Gene-gene and gene-environment needed to fully elucidate risk
  - Effects could be additive or reduced depending on individual and interactive genetic effects

Richards et al. Lancet 2008; 371: 1505
Genetic Effect Modified by Environmental Factors

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Cases</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ever smoker</td>
<td>N=117</td>
<td>N=92</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>85.5%</td>
<td>81.5%</td>
<td>1.00</td>
</tr>
<tr>
<td>CT</td>
<td>14.5%</td>
<td>18.5%</td>
<td>2.78 (1.10-7.03)</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>N=176</td>
<td>N=217</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>81.3%</td>
<td>81.1%</td>
<td>1.00</td>
</tr>
<tr>
<td>CT</td>
<td>18.8%</td>
<td>18.9%</td>
<td>0.99 (0.52-1.88)</td>
</tr>
</tbody>
</table>

Giampietro et al. Osteoporosis Int 2010; 21: 467-477
BMD is Imperfect Predictor of Fracture Risk

- Rate per 100 Person Years
- % of NORA Population
- % of Total Fractures

BMD categories:
- > +1.0
- to 0.5
- to 0.0
- to -0.5
- to -1.0
- to -1.5
- to -2.0
- to -2.5
- to -3.0
- to -3.5
- < -3.5

Population distribution:
- 0 to 25
- 0 to 15
- 0 to 10
- 0 to 5
- 0 to 2.5
- 0 to 1.5
- 0 to 1

Fractures distribution:
- 0 to 2.5
- 0 to 2
- 0 to 1.5
- 0 to 1
- 0 to 0.5
- 0 to 0
- 0 to -0.5
- 0 to -1
- 0 to -1.5
- 0 to -2
- 0 to -2.5
- 0 to -3
- 0 to -3.5
Factors Contributing To Fracture Risk

Bone Size
- Cortical Thickness
- Trabecular Bone volume
- Mineralization

Bone Density
- Macroarchitecture
- Matrix composition
- Microdamage repair
- Crystal Structure
- Bone turnover
- Microarchitecture

Bone Quality
- Frailty
- Muscle strength
- Vision & cognition
- Neurologic function
- Co-morbidities

Fall Risk

TRAUMA
- Environmental Factors

Fracture
Predicting More Aggressive Disease: 5 yr Hip Fracture Risk

<table>
<thead>
<tr>
<th>Factor</th>
<th>OR</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>1.13</td>
<td>1.11 - 1.15</td>
</tr>
<tr>
<td>Poor health</td>
<td>2.38</td>
<td>1.66 - 3.40</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>1.11</td>
<td>1.07 - 1.16</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.98</td>
<td>0.98 - 0.99</td>
</tr>
<tr>
<td>Hx fracture</td>
<td>1.72</td>
<td>1.41 - 2.10</td>
</tr>
<tr>
<td>Parental hip fx</td>
<td>1.50</td>
<td>1.20 - 1.87</td>
</tr>
<tr>
<td>Black vs white</td>
<td>0.41</td>
<td>0.24 - 0.70</td>
</tr>
<tr>
<td>Physical activity</td>
<td>1.64</td>
<td>1.24 - 2.17</td>
</tr>
<tr>
<td>Smoking</td>
<td>2.33</td>
<td>1.71 - 3.18</td>
</tr>
<tr>
<td>Steroid use</td>
<td>1.94</td>
<td>1.16 - 3.25</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.74</td>
<td>1.17 - 2.60</td>
</tr>
</tbody>
</table>
FRAX Questionnaire for the Estimation of Fracture Risk

Country: US (Caucasian) Name / ID:

Questionnaire:

1. Age (between 40-90 years) or Date of birth
   Age: 67
   Date of birth: Y: M: D:

2. Sex
   - Male
   - Female

3. Weight (kg)
   49.89

4. Height (cm)
   160.02

5. Previous fracture
   - No
   - Yes

6. Parent fractured hip
   - No
   - Yes

7. Current smoking
   - No
   - Yes

8. Glucocorticoids
   - No
   - Yes

9. Rheumatoid arthritis
   - No
   - Yes

10. Secondary osteoporosis
    - No
    - Yes

11. Alcohol 3 or more units per day
    - No
    - Yes

12. Femoral neck BMD (g/cm²)
    - Hologic
    - T-score: -2.1

BMI: 19.5
The ten year probability of fracture (%)
with BMD
- Major osteoporotic: 36
- Hip fracture: 4.7

Weight Conversion:
- pound: 110
  - 110 pound = 49.89 kg

Height Conversion:
- inch: 63
  - 63 inch = 160.02 cm
Can Genomics Also Help Predict Fracture Risk?

- **VDR**
  - Nuclear transcription factor regulating gene expression through vit D response elements
  - Associated with BMD, fractures and falls

- **DBP**
  - Maintains calcium by binding and transporting D and acts as monocyte activating factor to mediate OC
  - Association seen only with interaction with other genes or environment (calcium intake)

---

Interaction between Vitamin D Receptor and D Binding Protein for Fracture Risk

<table>
<thead>
<tr>
<th></th>
<th>VDR Non-carrier</th>
<th>Heterozygous</th>
<th>Homozygous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total*</td>
<td>1</td>
<td>1.11</td>
<td>1.27</td>
</tr>
<tr>
<td>DBP Non-carrier</td>
<td>1</td>
<td>0.93</td>
<td>1.12</td>
</tr>
<tr>
<td>DBP Carrier**</td>
<td>1</td>
<td>1.14</td>
<td>1.32</td>
</tr>
<tr>
<td>DBP Heterozygous</td>
<td>1</td>
<td>1.17</td>
<td>1.20</td>
</tr>
<tr>
<td>DBP Homozygous</td>
<td>1</td>
<td>1.12</td>
<td>1.25</td>
</tr>
</tbody>
</table>

* *p=0.01 **p=0.008

Calcif Tis Int 2009; 85: 85-93
**COL1A and Risk for Fracture**

### Univariate Analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Any Fracture</th>
<th>Hip Fracture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (+5 yrs)</td>
<td>1.38</td>
<td>1.43</td>
</tr>
<tr>
<td>FN BMD (-0.12 g/cm²)</td>
<td>1.80</td>
<td>2.44</td>
</tr>
<tr>
<td>Past fx</td>
<td>2.72</td>
<td>2.01</td>
</tr>
<tr>
<td>Fall in last 12 months</td>
<td>1.24</td>
<td>1.30</td>
</tr>
<tr>
<td>Col1A1 (TT vs GG/GT)</td>
<td>1.91</td>
<td>6.42</td>
</tr>
</tbody>
</table>

- Adding Col1A1 into prediction models
  - Reclassified 13% of women
  - Improved classification of non-fracture cases
- Potentially add to better focus of treatment interventions for women at high risk for fracture
Case Study: Applying Genomic Strategies to Assess Risk

- 60-year-old white woman
  - FN BMD T-score of –2.0
  - One prior fracture
  - One fall in the last 12 month
  - **FRAX:** 2.3% 10 year risk for hip fracture

- Genotyping done
  - Col1A1 TT genotype
  - Newly adjusted risk score for hip fracture with genotype included: 12%
Case study: Next Steps

- Additional lab studies
  - 25(OH) vitamin D 20 ng/ml

- Treatment recommendations
  - Calcium supplementation increased by 500 mg daily
  - Vitamin D intake increased to 1200 units daily
  - Alendronate initiated at a dose of 70 mg weekly
25(OH) vitamin D as Biomarker of Hip Fracture

Quartiles 25(OH) vit D

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Odds Ratio Hip Fracture</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;19.04</td>
<td>1.2</td>
</tr>
<tr>
<td>19.04-24.09</td>
<td>1.0</td>
</tr>
<tr>
<td>24.08-28.29</td>
<td>0.65</td>
</tr>
<tr>
<td>&gt;28.29</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Cut points 25(OH) vit D

<table>
<thead>
<tr>
<th>Cut point</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;15</td>
<td>1.2</td>
</tr>
<tr>
<td>15 - &lt;30</td>
<td>1.0</td>
</tr>
<tr>
<td>≥30</td>
<td>0.6</td>
</tr>
</tbody>
</table>

+ Base=matched on age, ethnicity, blood draw date; MV adj for age, BMI, parental hx of fracture, hx of fracture, smoking, alcohol and total calcium intake.
Factors Contributing to Calcium and Vitamin D Deficiency in Older Adults

- Lack of adequate sunlight
  - Diminished vitamin D persistence in elderly
- Consumption of diet low in fortified foods
- Lack of dietary calcium
- High phytate diet decreasing GI calcium absorption
- ? Specific genotypes associated with insufficiency
Genomics Predict Likelihood for Being Vitamin D Insufficient

Vitamin D Insufficiency
According to Quartile of Genotype Score for Vitamin D Transport

OR for vit D < 75 nmol

Quartile of GC genotype score

p = 2.3 x 10^{-48}
Vitamin D Variants: Predicting CaD and HT Response

**CaD and Vit D SNP**
- CaD interaction with CYP2R1 borderline significant (p=0.07)
- Treatment response with CaD 2 alleles CYP2R1 OR for hip fracture with CaD (compared to placebo) is 0.60 (0.33, 1.10)

<table>
<thead>
<tr>
<th>“Beneficial” alleles on Hip Fracture with CaD Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td># of alleles</td>
</tr>
<tr>
<td>0-1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>≥ 4</td>
</tr>
</tbody>
</table>

**HT and Vit D SNP**
- Significant E+P interaction with SNPs related to GC on reduction in hip fracture risk

<table>
<thead>
<tr>
<th># minor alleles</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.60</td>
<td>0.40-0.90</td>
</tr>
<tr>
<td>1</td>
<td>1.18</td>
<td>0.74-1.88</td>
</tr>
<tr>
<td>2</td>
<td>1.16</td>
<td>0.44-3.02</td>
</tr>
</tbody>
</table>

- In combined analyses, the RR (95%CI) associated with 0 and 1 or 2 MA was 0.55 (0.42, 0.78) and 1.14 (0.82, 1.59) (p=0.003)
Case Study: Pharmacogenomics

- Genotyping showed GC genotype score of 3
- Explanation for low D despite 800 units intake daily
  - Will need higher dosing to achieve sufficiency
- Also likely to explain lack of effectiveness of HT in reducing risk for fracture
Additional Promise for Genomics

- Prediction of Adverse Effects with Treatment
  - Four SNPs mapped within the cytochrome P450-2C gene associated with ONJ

- New targets for drug development
  - Denosumab
  - Sclerostin
Summary

- Osteoporosis is a major public health problem for both men and women
  - Significant morbidity and mortality with fracture
- From public health perspective it would be valuable to focus interventions at highest risk (Personalized, predictive health)
  - Define individuals at highest risk
  - Identify most effective treatment
  - Minimize risk for adverse effects
- Genomics can begin to define new pathways that might be leveraged to design new therapies
  - Functional characterization of truly causative genes will provide new mechanistic insights