The genetics of osteoporosis

A paradigm for genetic studies of a complex disease in the last 3 decades

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Changes in bone mass

![Graph showing changes in bone mass over age for males and females](chart.png)
Definition

Osteoporosis is defined by the World Health Organization (WHO) in women as a bone mineral density 2.5 standard deviations (T-score) below peak bone mass (20-year-old healthy female average) as measured by DXA.
Osteoporosis

risk factors:
* Gender
* Age
* Early menopause in women
* Amenorrhea
* Low testosterone levels in men
* Low calcium intake
* Race and ethnicity
* Small or thin body frame
* Excessive alcohol use
* Smoking
* Inadequate physical exercise
* Certain endocrine disorders
* Chronic diseases of the lungs, kidneys, stomach, and intestines
* Prolonged use of medicines like steroids, antacids, anticonvulsants
Heritability

Bone mineral density
46 – 84 %
  hip: 73 %
  spine: 66 %

Bone size
  hip: 69 %
  spine: 60 %

Hip axis length
  62 %
Genetic research of osteoporosis

1980: Genetic studies on osteoporosis as a quantitative trait are relevant

Standard approach

association studies

but no - large cohorts with detailed phenotypical data

- no data on polymorphisms in human genome

- no techniques for high throughput genotyping
How to identify genes for complex traits

Identification of genes for relevant monogenic conditions
1. functional candidate gene approach
2. positional cloning of relevant monogenic conditions

Association studies
3. candidate genes
4. genome wide association studies

1980 1990 2000 2010
1. Functional candidate gene approach

Collagen genes

causative for conditions with decreased bone mineral density and brittleness of bone

Chu et al. Nature 1983
Internal deletion in a collagen gene in a perinatal lethal form of osteogenesis imperfecta.
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2. Positional cloning of relevant monogenic conditions

1984: map of 400 RFLPs
Sclerosing bone dysplasias

International working group on the classification and nosology of constitutional disorders of bone (Martigny, 2005)

About 40 different clinical entities with increased bone density
Increased bone formation
Increased bone formation

Van Buchem disease

Hyperostosis corticalis generalisata

- enlargement of the jaw
- thickening of the skull
  -> Nerve encroachment
    • facial nerve palsy
    • hearing loss
Van Buchem disease

Incidence: very low

- 25-30 patients worldwide

- Small village in The Netherlands

11 patients
Ethnic isolate

- Island until 1941
- Geographically, religiously and professionally isolated
- In 1637: 151 inhabitants
- Currently 16,000 inhabitants
- Most inhabitants related to each other
Dutch van Buchem family
Differential diagnosis

Sclerosteosis

- gigantism
- more severe character
- hand malformations
Gene identification

2758 bp

220 bp 422 bp

220 bp 422 bp

ATG

TAG

5'

3'

AAGgtat

acagACG

Senegalese sclerosteosis mutation

Brazilian sclerosteosis mutation

American sclerosteosis mutation

\[ \text{Senegalese sclerosteosis mutation} \]

\[ \text{Brazilian sclerosteosis mutation} \]

\[ \text{American sclerosteosis mutation} \]

\[ \text{Patient} \]

\[ \text{Normal} \]

\[ \text{C AAA GG TTT GGGG T G} \]

\[ \text{C AAA GG TTT TGGG T G} \]

\[ \text{C AAA GG TTT GC GG T G} \]

\[ \text{T GG T GG T G A CC T A} \]

\[ \text{T GG T GG C G A CC T A} \]

SOST
Increased bone formation

Van Buchem disease (*SOST*)

Sclerosteosis (*SOST*)
Endosteal hyperostosis
High Bone Mass-phenotype

• 2 families
  • Johnson et al. 1997
  • Boyden et al. 2002
• Cortical thickening of the long bones
• Phenotypical differences:
  mandible
torus palatinus
• Same LRP5 mutation (G171V)
LDL-Receptor-Related protein 5 (LRP5)
Osteoporosis pseudoglioma syndrome

- Autosomal recessive
- Juvenile osteoporosis
- Congenital blindness
Mutations in LRP5 gene

Osteoporosis-pseudoglioma syndrome (OPPS)
Increased bone formation

Van Buchem disease \((SOST)\)
Sclerosteosis \((SOST)\)
Endosteal hyperostosis \((LRP5)\)
Aut dom osteosclerosis \((LRP5)\)
“Van Buchem” \((LRP5)\)
LRP5

T253I

A242T

D111Y

G171R

G171V

A214T

A214V
Sclerostin-LRP5

A

LRP5/6

Wnt

Fz

Extracellular

Cytoplasm

B-catenine

TCF

Nucleus

B

LRP5/6

Wnt

Sclerostin

Fz

Extracellular

Cytoplasm

β-catenine degradation

TCF

Nucleus
Genetic variation within SOST and LRP5 genes

- Sclerosteosis
- Van Buchem disease
- Carriers Van Buchem disease / Sclerosteosis
- Healthy state
- High bone mass phenotypes
- Ideopathic osteoporosis
- Carriers OPPG
- OPPG

BMD

Loss-of-function mutations SOST

Gain-of-function mutations LRP5

Loss-of-function mutations LRP5
Genetic research of osteoporosis

1980: Genetic studies on osteoporosis as a quantitative trait are relevant

Standard approach - association studies
but no - large cohorts with detailed phenotypical data
- no data on polymorphisms in human genome
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Since 1990s: all three problems were getting solved slowly
How to identify genes for complex traits

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1994: osteoporosis gene!

Prediction of bone density from vitamin D receptor alleles

Nigel A. Morrison, Jian Cheng Qi, Akifumi Tokita, Paul J. Kelly, Linda Crofts, Tuan V. Nguyen, Philip N. Sambrook & John A. Eisman

Bone and Mineral Research Division, Garvan Institute of Medical Research, St Vincent’s Hospital, Sydney, New South Wales 2010, Australia
1994: osteoporosis gene!

250 healthy Caucasian twins (Australia)

BMD measurements at different sites

75% of genetic effect on bone density explained
Erratum

“We re-examined the original samples and found that in a proportion of these twins the genotype on new DNA differed from the earlier DNA samples.”

It seems most likely that the misclassifications arose from misgenotyping of DNA samples between extraction and PCR analysis.

1307 citations
## Association studies

<table>
<thead>
<tr>
<th>Condition</th>
<th>Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteopetrosis</td>
<td>Carbonic anhydrase II</td>
</tr>
<tr>
<td></td>
<td>H+ATPase/CLCN7/GL</td>
</tr>
<tr>
<td>Pycnodysostosis</td>
<td>Cathepsin K</td>
</tr>
<tr>
<td>Camurati-Engelmann</td>
<td>TGFB1</td>
</tr>
<tr>
<td>Van Buchem/Sclerosteosis</td>
<td>SOST</td>
</tr>
<tr>
<td>High Bone Mass</td>
<td>LRP5</td>
</tr>
<tr>
<td>Familial Expansile osteolysis</td>
<td>RANK</td>
</tr>
<tr>
<td>Paget’s disease</td>
<td>OPG/SQSTM1</td>
</tr>
</tbody>
</table>
Prospective meta-analyses of osteoporosis candidate genes

“GENOMOS” QLK6-CT-2002-02629
(Genetic Markers for Osteoporosis)
EU FP5 sponsored, 3 mio euro, 2003-2007

Total number of subjects: 21,040
14,399 women
5,587 men
4,575 fractures

Participants:
AG Uitterlinden, Netherlands
SH Ralston, United Kingdom
BL Langdahl, Denmark
ML Brandi, Italy
J Reeve, United Kingdom
A Carey, United Kingdom
W Van Hul, Belgium
JPA Ioannidis, Greece
A Diez-Perez, Spain

Coordinating Centre: Department of Internal Medicine, Erasmus MC, Rotterdam (AG Uitterlinden)
Genetic variation within SOST and LRP5 genes
How to identify genes for complex traits

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EU-FP7 project: GEFOS (start March 2008)

Number of subjects:
GENOMOS: >150,000
GWA: 40,000

- = GENOMOS study population
- = idem + GWA
- = idem, under negotiation / in development
One single study has insufficient power to identify genome-wide significant signals.

- Rotterdam Study

N=5000

Rivadeneira et al., ASBMR sept 2008
As sample size increases genome-wide significant signals become gradually evident.

N = 6200

Rivadeneira et al., ASBMR sept 2008
Four novel loci exceed GWS threshold, many others are close

- **1p36**
- **MHC**
- **C6orf10**
- **OPG**
- **LRP5**
- **RANK-L**

**Novel:** Chr 1p, 7p, 7q and 12p

**LUMBAR SPINE BMD**

- **N=18500**
- Rotterdam Study
- ERF Study
- Twins UK
- deCODE Genetics
- Framingham Study

Rivadeneira et al., ASBMR sept 2008
Recent meta-analysis of GWAS

- 140 research teams
- 130,000 individuals
- 56 genes for BMD
- 14 genes for fracture risk
Genetic architecture of BMD

Polymorphisms with subtle effects

~95% of variance

Rare Mutations with severe effects

Common

Recent reports:
GWAS: Lancet, NEJM
GENOMOS: JAMA (LRP5)

BMD

Trait Population Frequency

Low

High

LRP5 COL1A1 Etc.

LRP5 SOST CICN7 Etc.

Recent reports:
GWAS: Lancet, NEJM
GENOMOS: JAMA (LRP5)
Conclusions

• Study of large cohorts is essential
  => Importance of worldwide collaborations
• Genes involved in monogenic disease often role in complex forms
• GWAS: new loci and genes
• Only low percentage of phenotypical variation explained by currently identified loci
• Role for CNVs or rare variants?
• Complete picture: putative applicability of these data