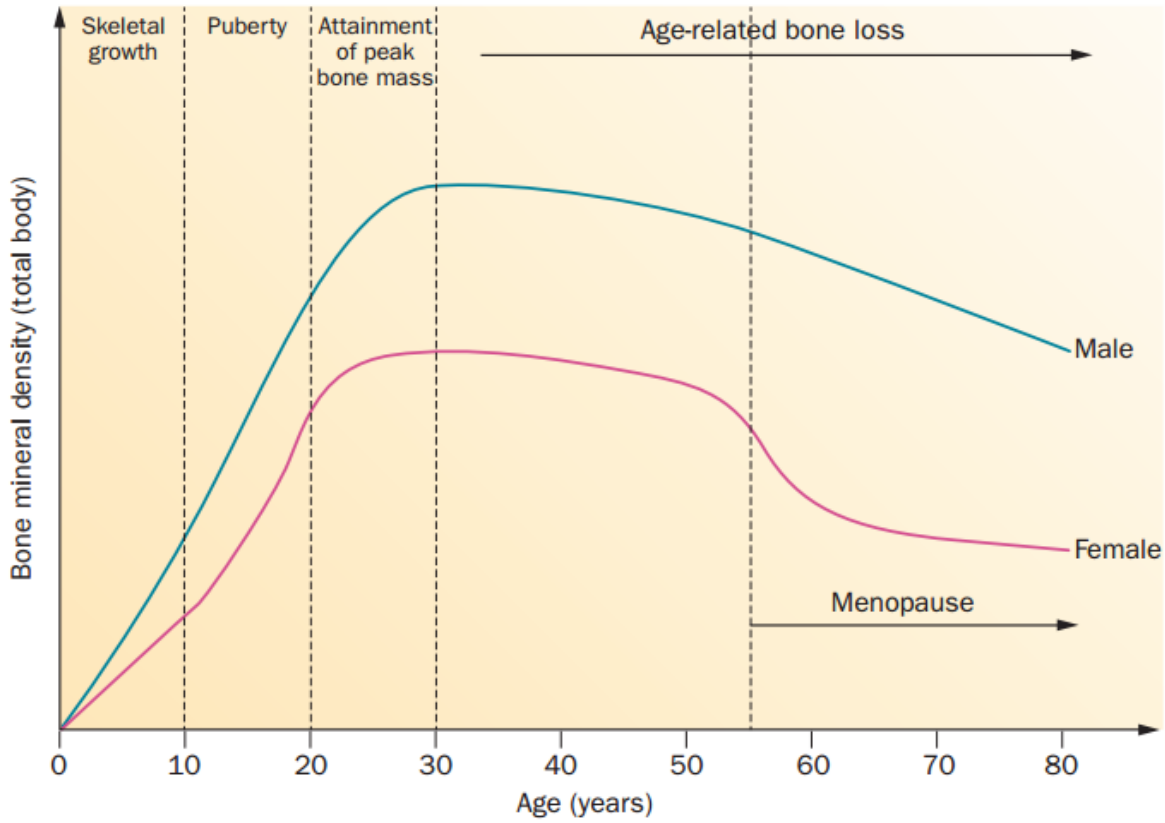
The genetics of osteoporosis

A paradigm for genetic studies of a complex disease in the last 4 decades

Wim Van Hul
Center of Medical Genetics
University of Antwerp

Bone mass

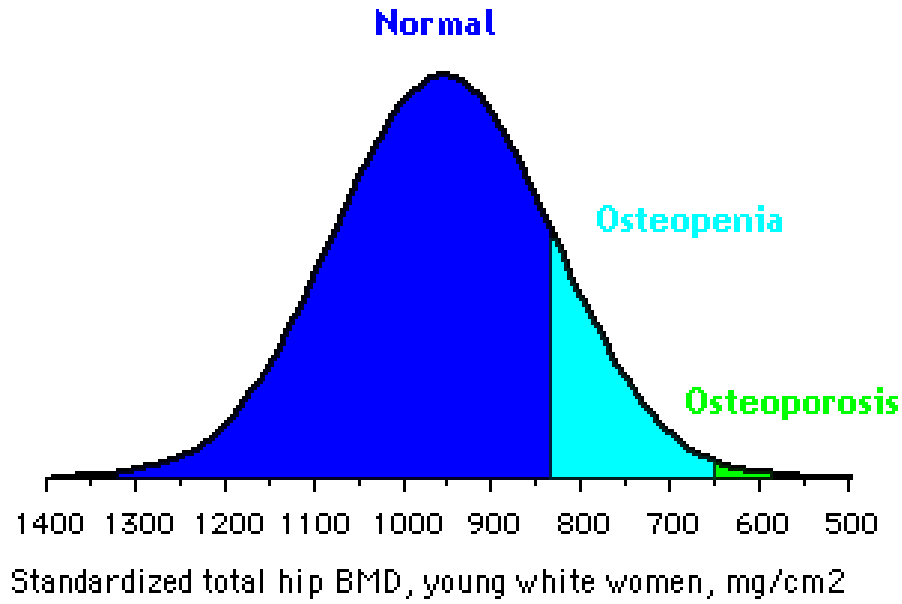


Hendrickx et al. Nature Rev Reumat, 2015

Osteoporosis

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



Bone mass

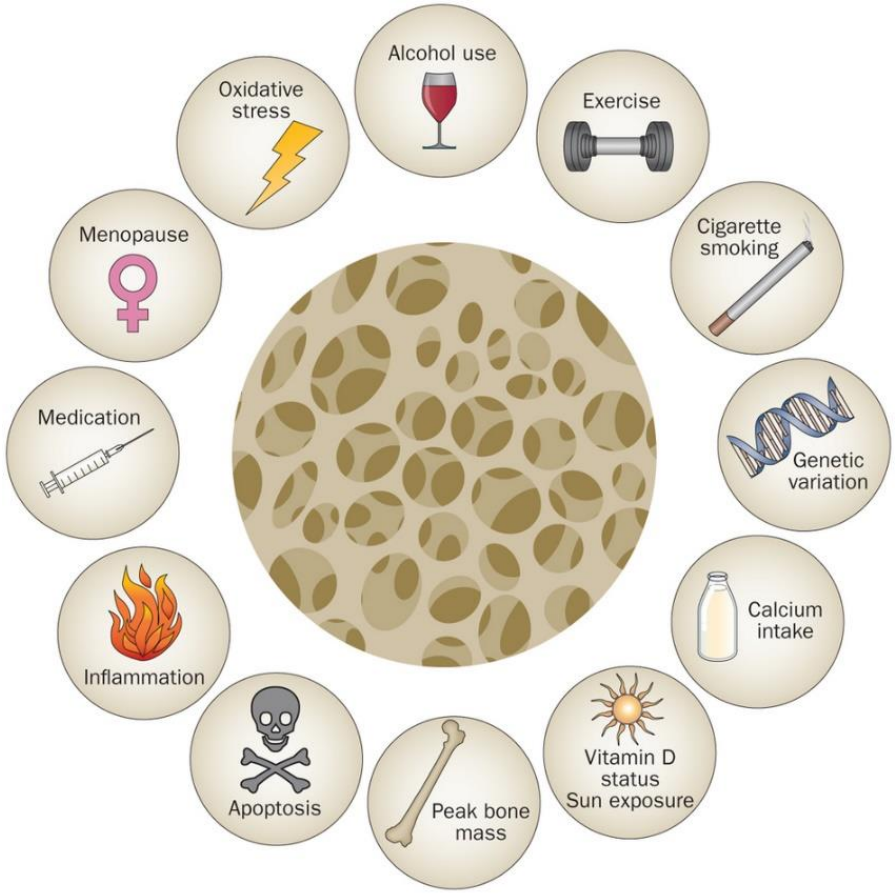
Life Style Factors
- Exercise
- Alcohol consumption
- Cigarette smoking
- Diet
- Sun exposure
- Medication

Aging-related Factors
- Oxidative stress
- Inflammation
- Apoptosis
- Menopause

20-50%

Genetic Variation

50-80%



Hendrickx *et al.*, Nature Reviews Rheumatology (2015)

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

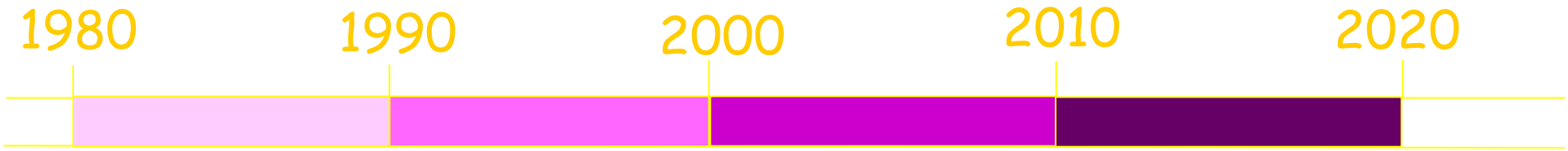
Identification of genes for relevant monogenic conditions

1. **functional candidate gene approach**
2. **positional cloning of relevant monogenic conditions**

Association studies

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

Next generation sequencing



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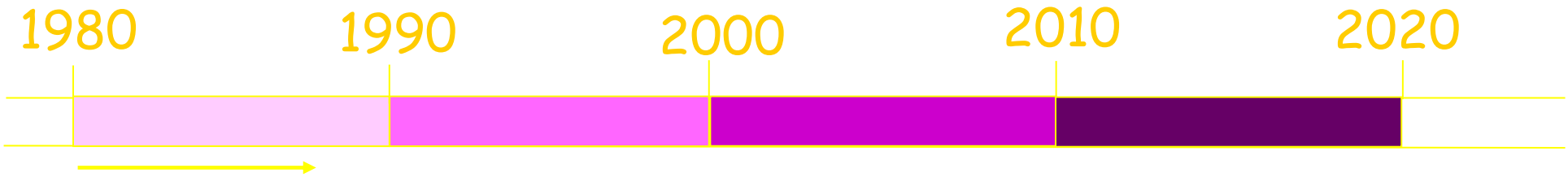

1. Functional candidate gene approach

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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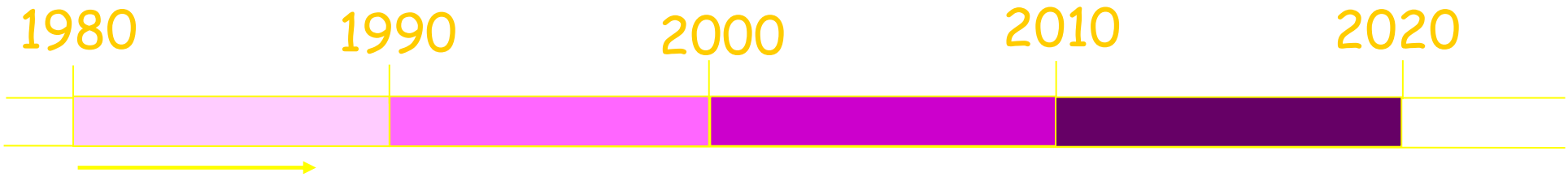

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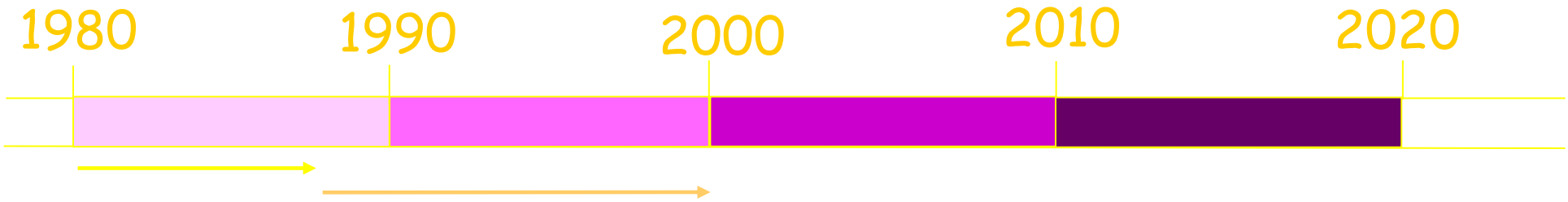
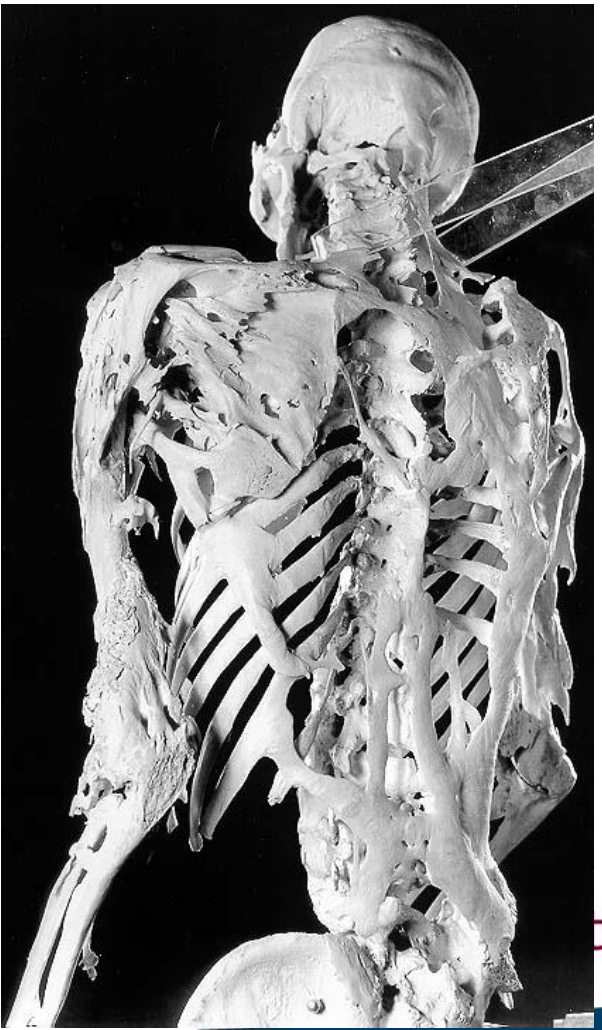


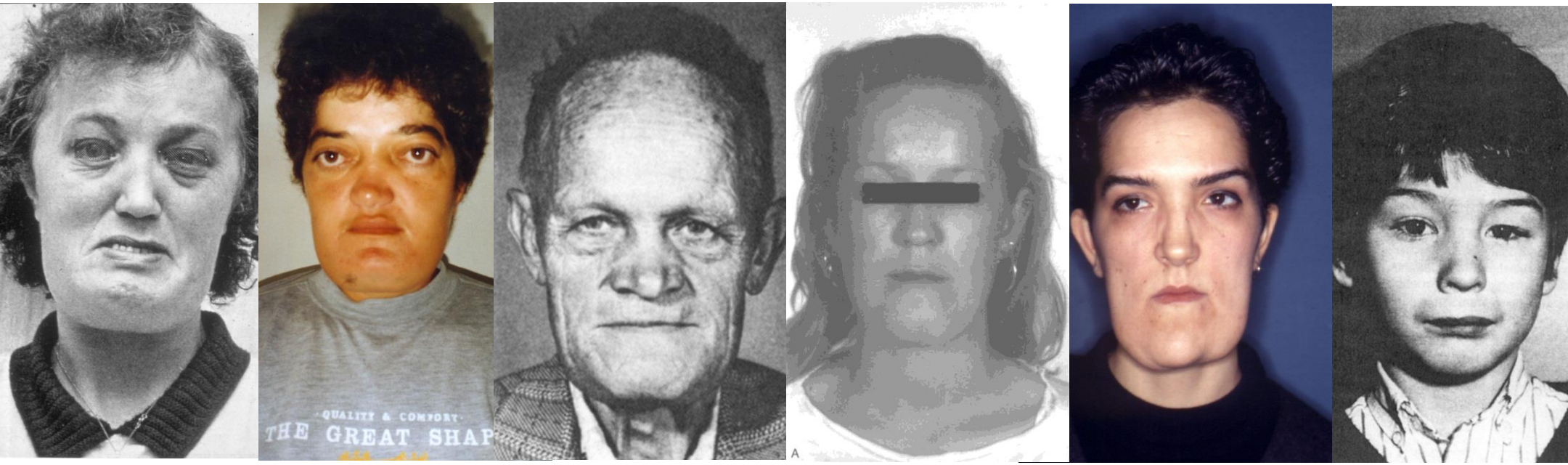
Sclerosing bone dysplasias



International working group on the classification and nosology of constitutional disorders of bone
(Unger et al., 2023)

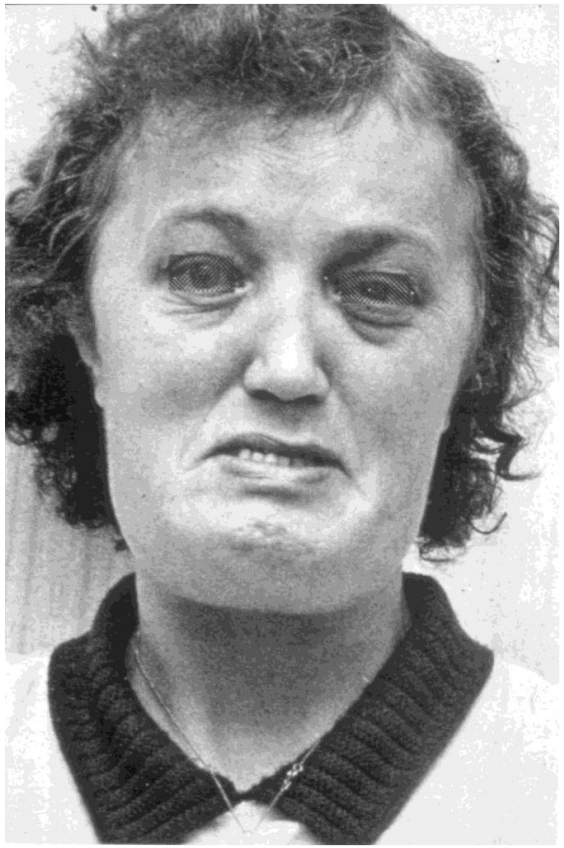
About 40 different clinical entities with increased bone density

Sclerosing bone dysplasias

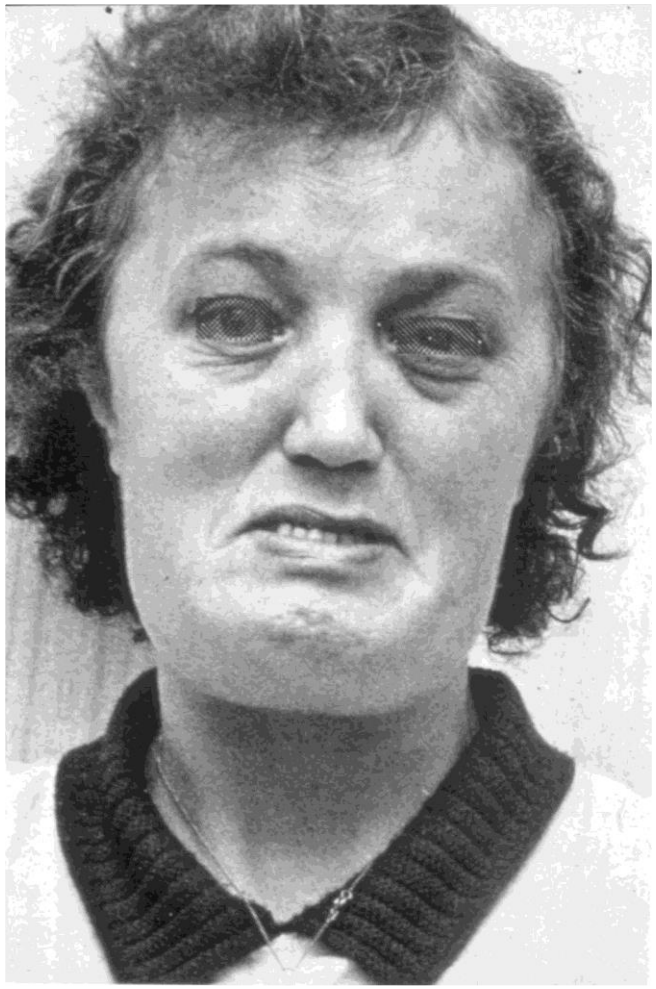


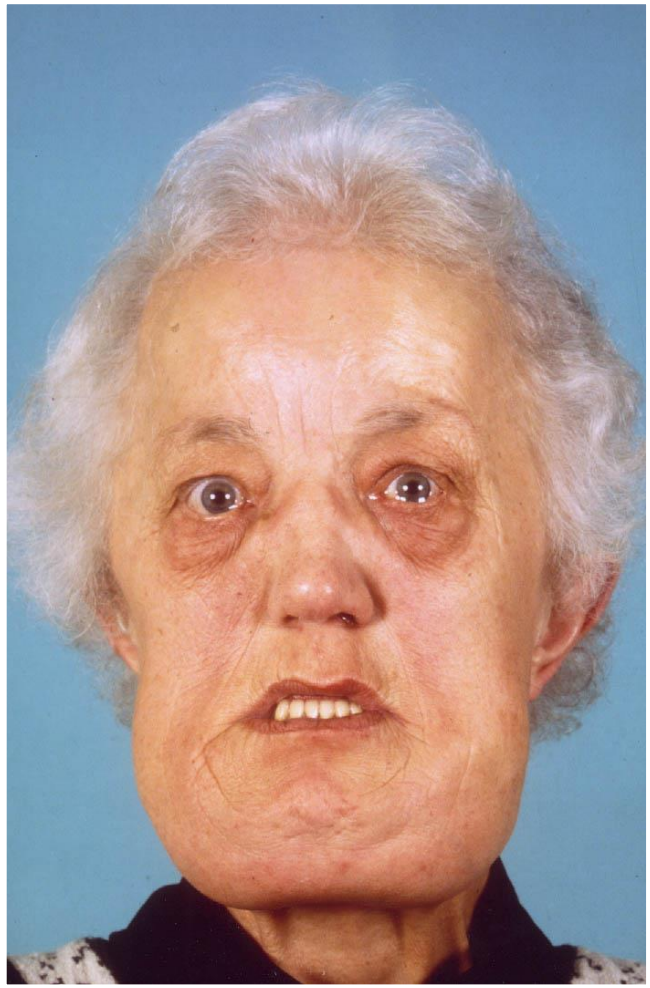
Van Buchem disease

Hyperostosis corticalis generalisata



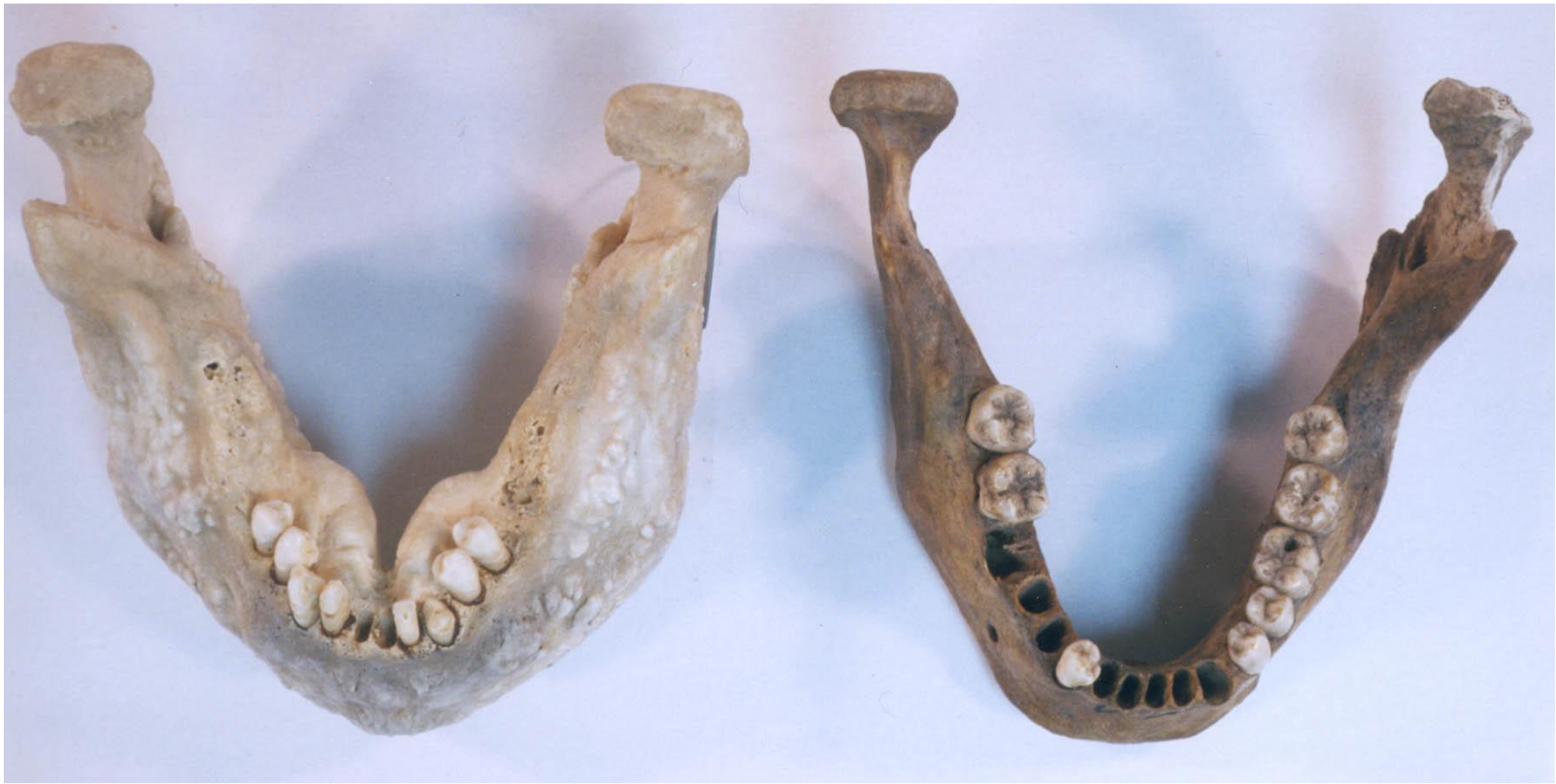
- enlargement of the jaw
- thickening of the skull
  - > Nerve encroachment
    - facial nerve palsy
    - hearing loss

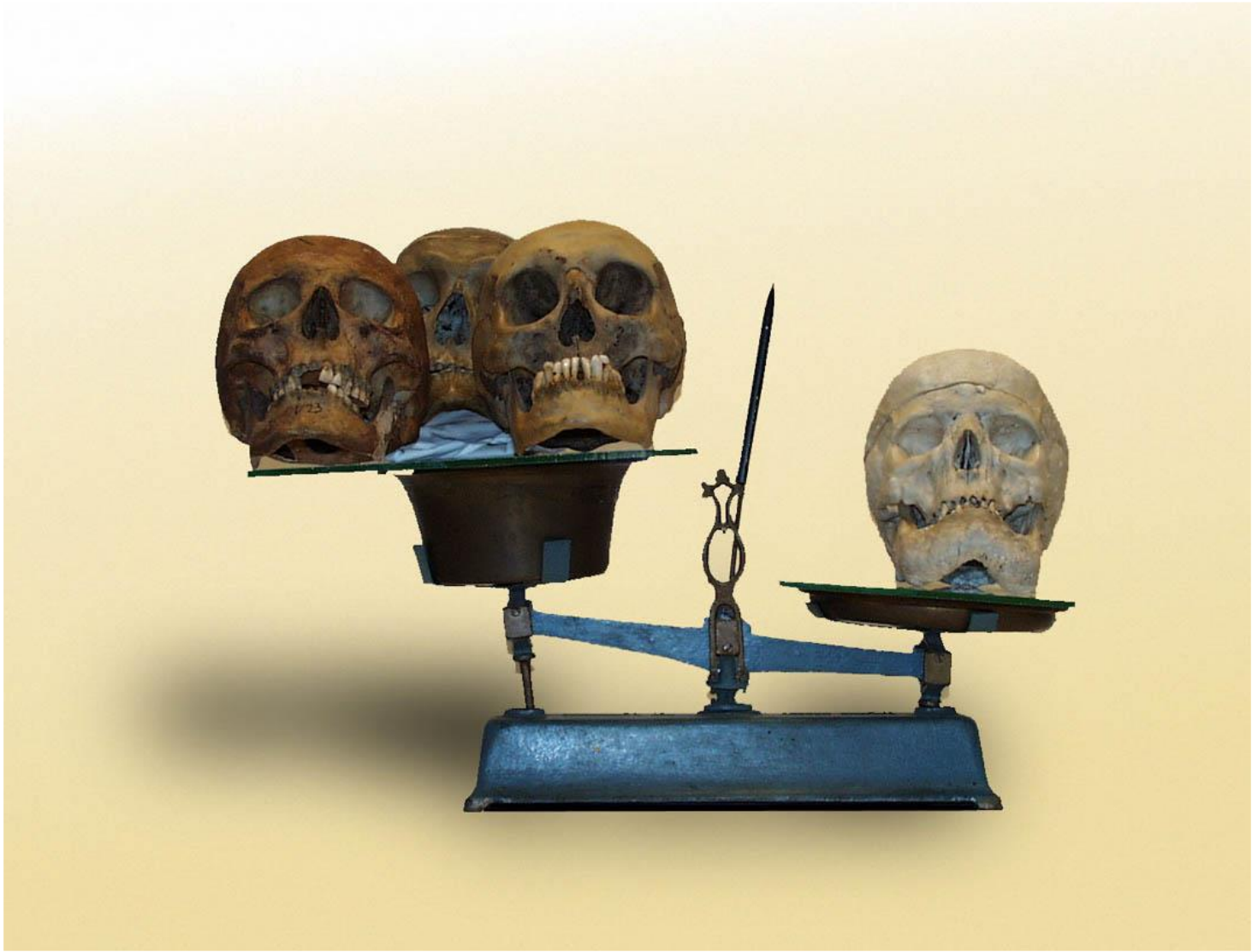




van Buchem patient

Control





Van Buchem disease

Incidence : very low

- 25-30 patients worldwide
- small village in The Netherlands

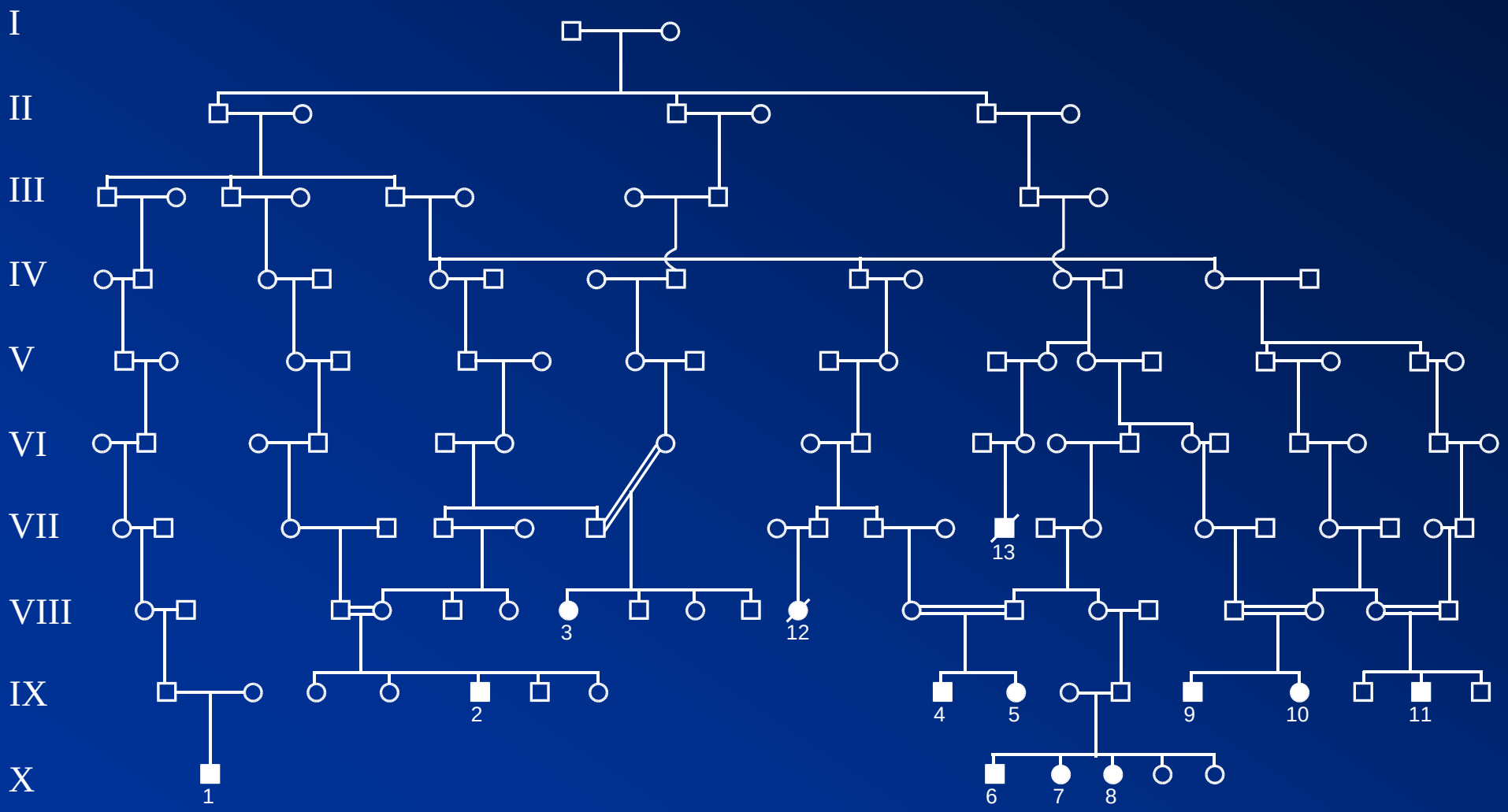
11 patients

overstroming 1916

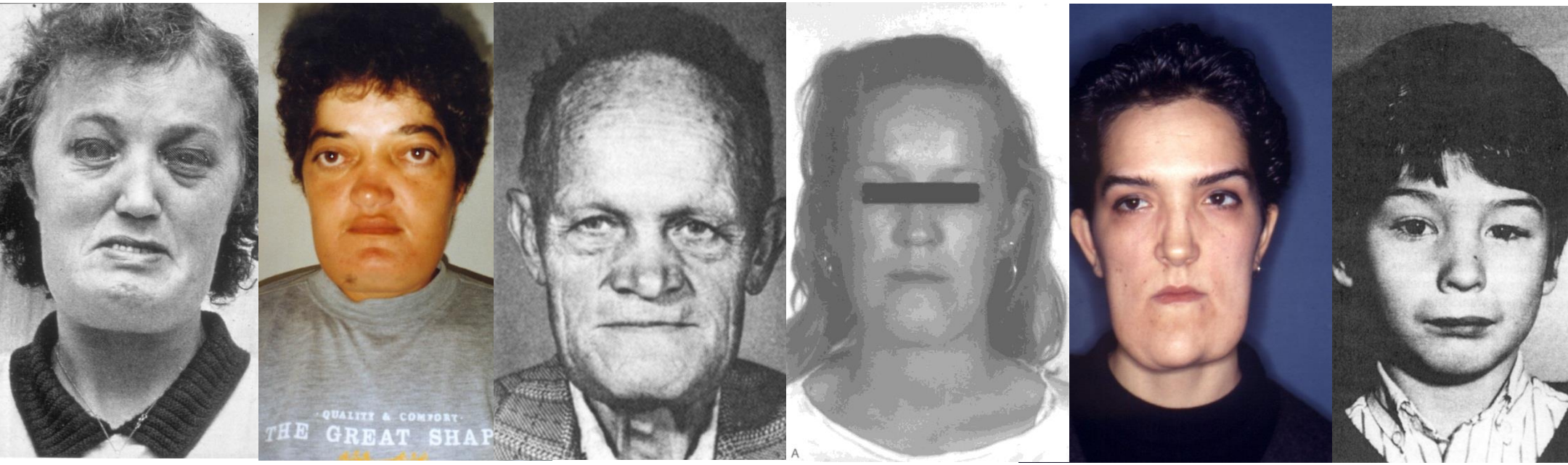
Ethnic isolate

- Island until 1941
- Geographically, religiously and professionally isolated
- In 1637: 151 inhabitants
- Currently 16.000 inhabitants
- Most inhabitants related to each other

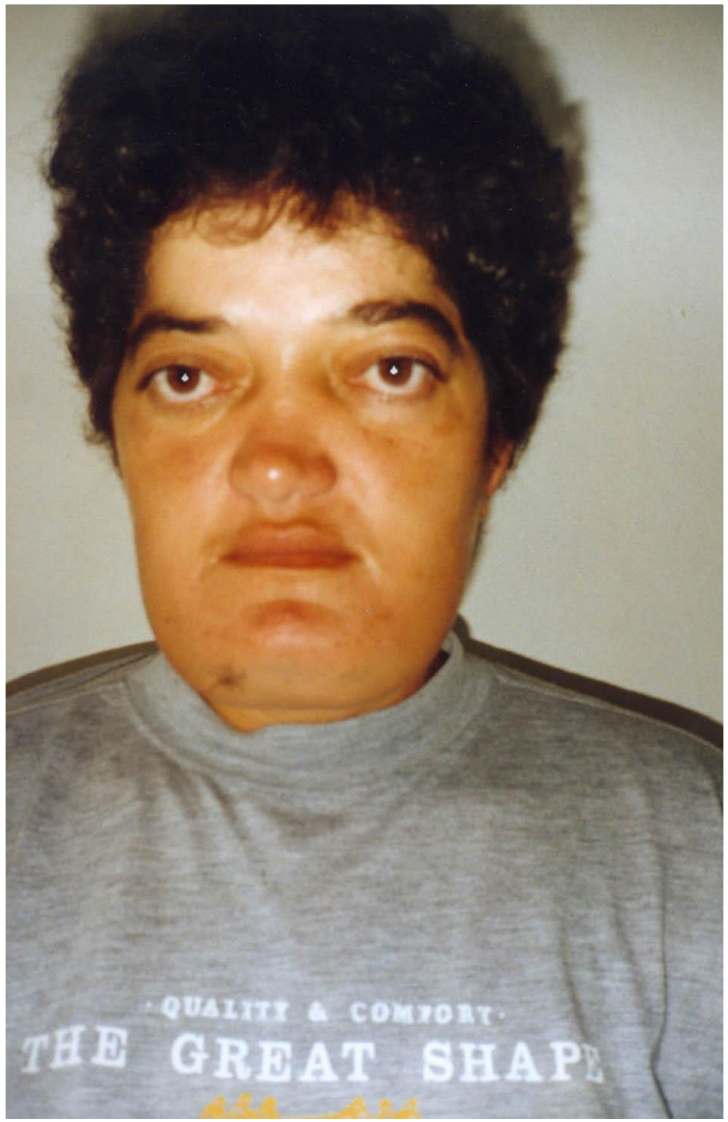
Dutch van Buchem family

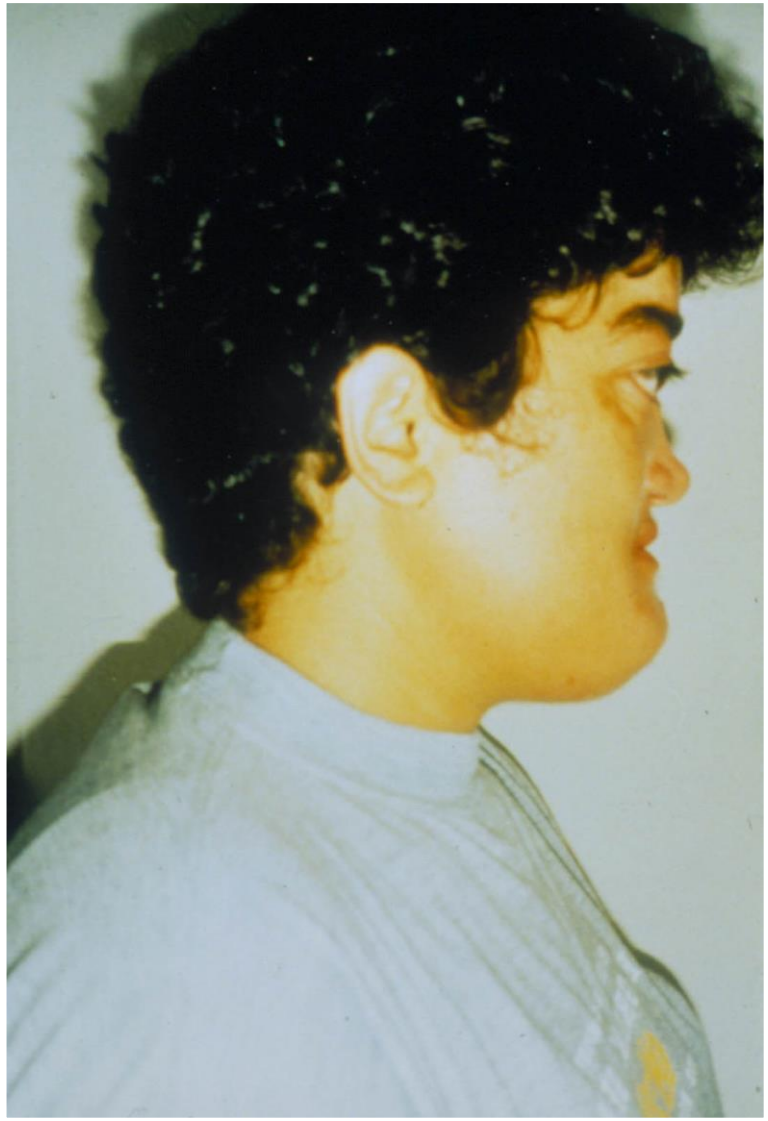


Sclerosing bone dysplasias



Van Buchem disease


QUALITY & COMFORT
THE GREAT SHAP



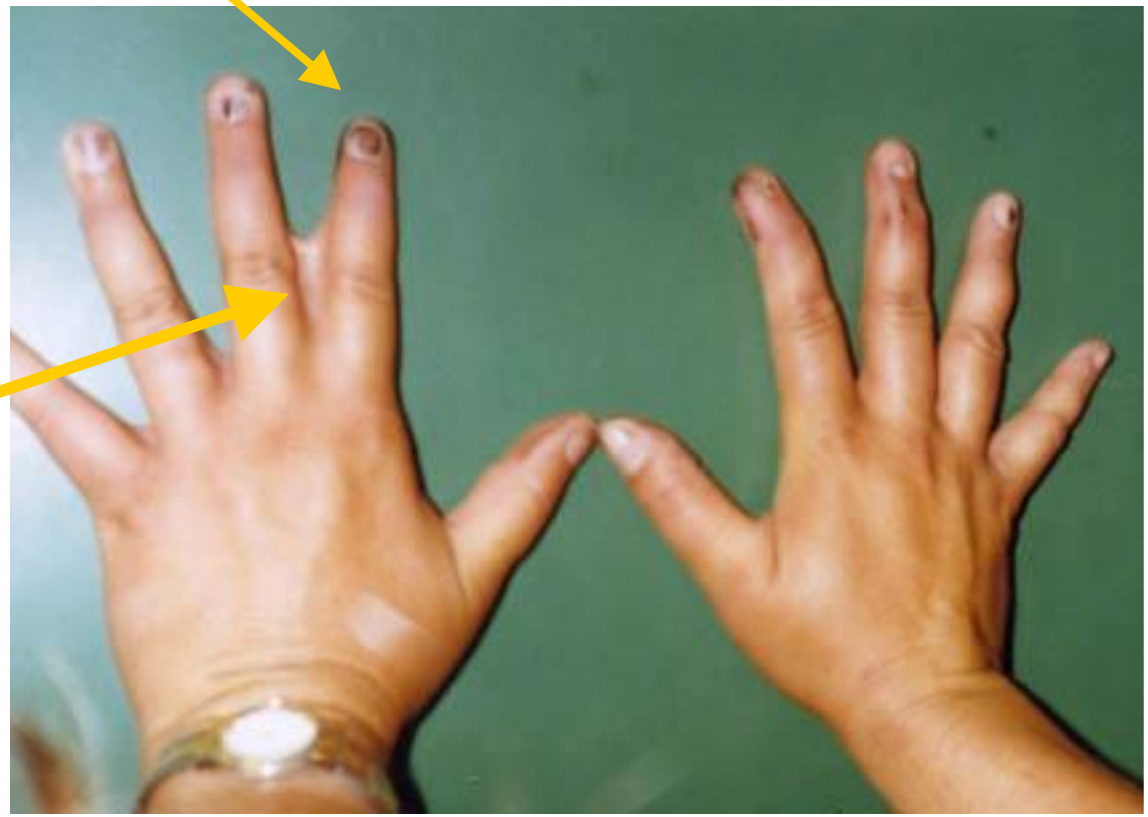
Differential diagnosis

Sclerosteosis

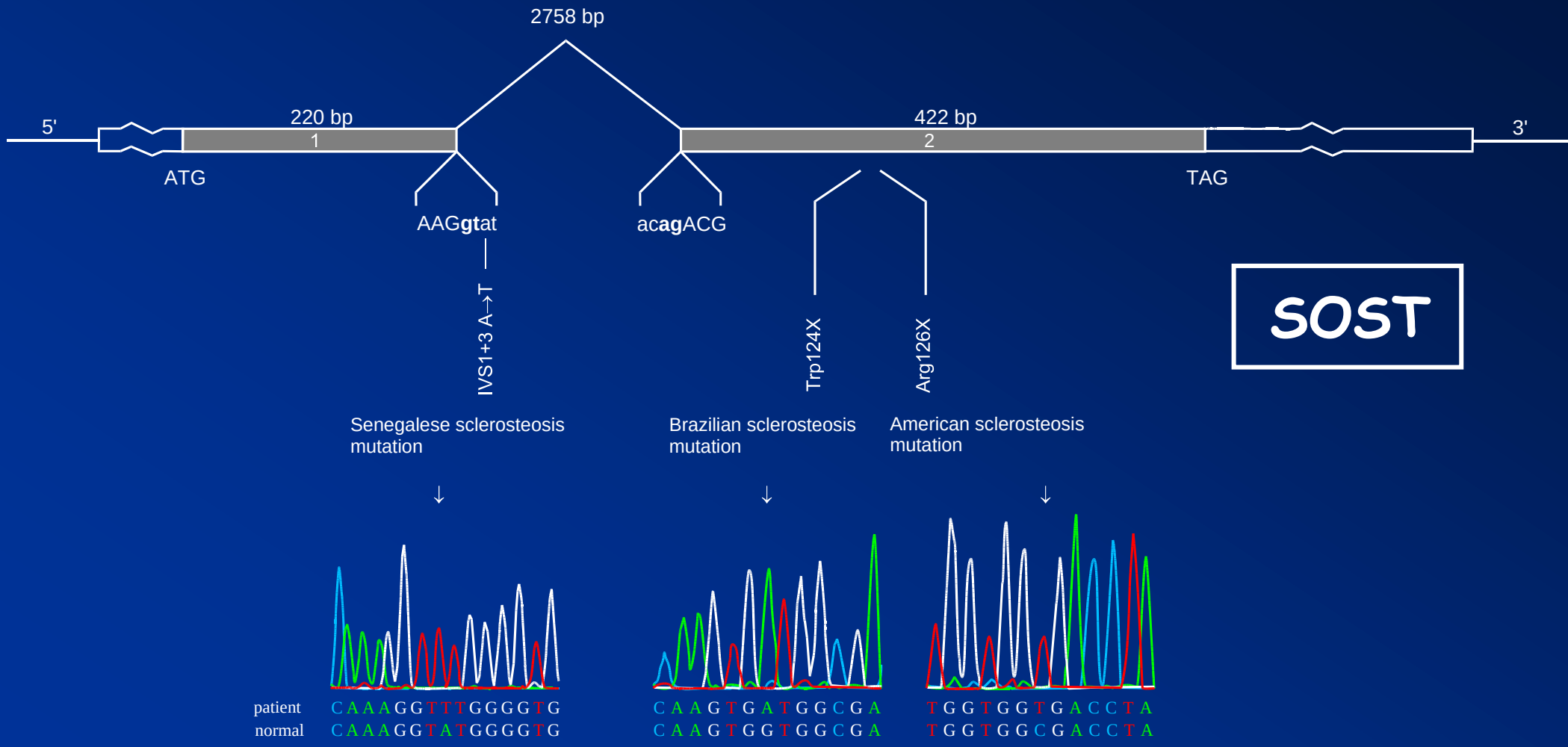
- gigantism
- more severe character
- hand malformations

nail dysplasia

syndactyly



Gene identification



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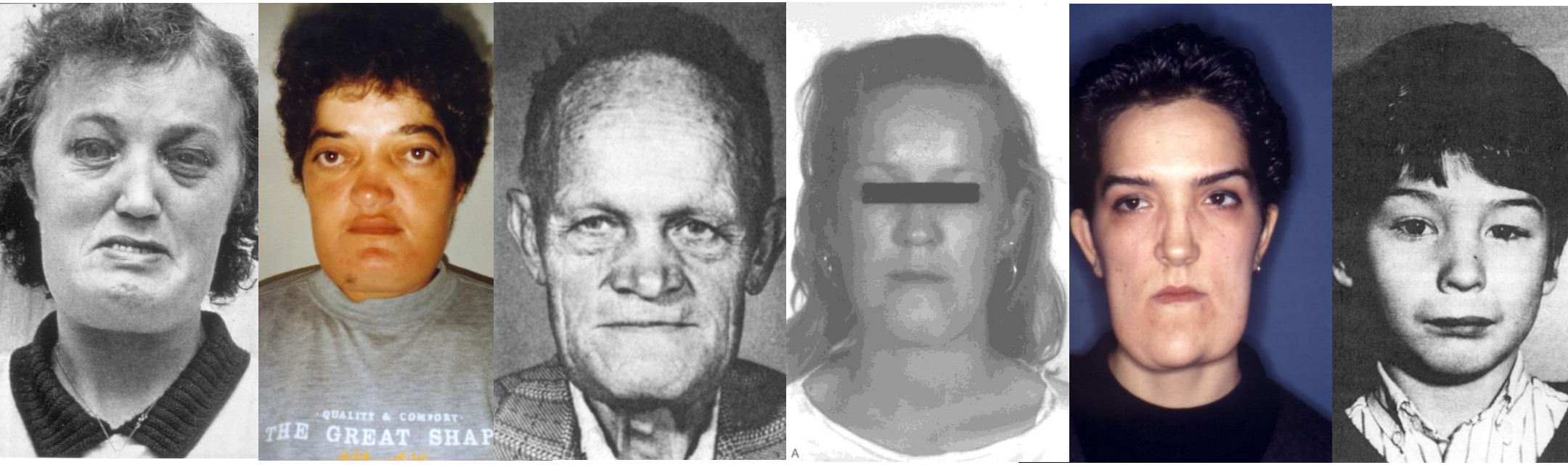
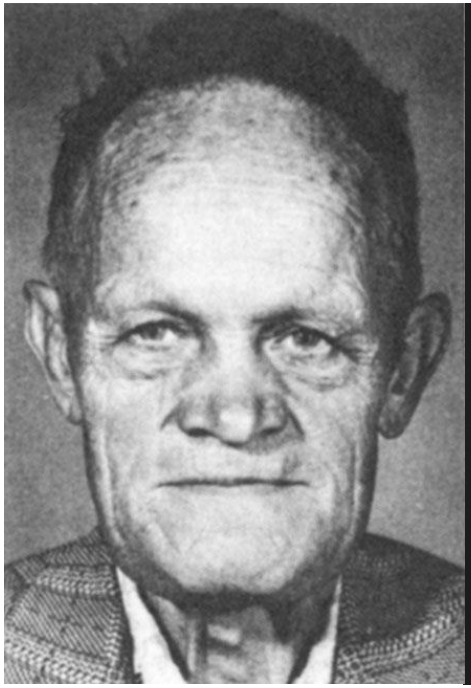
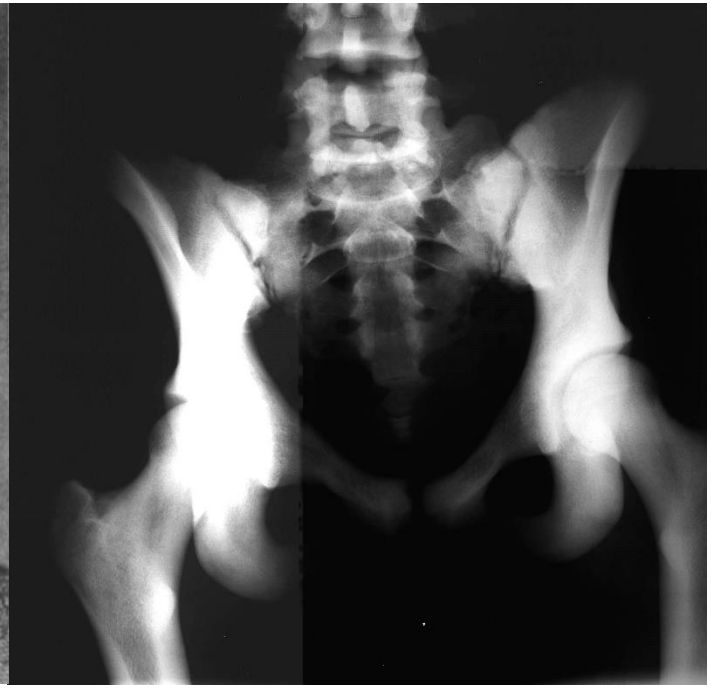


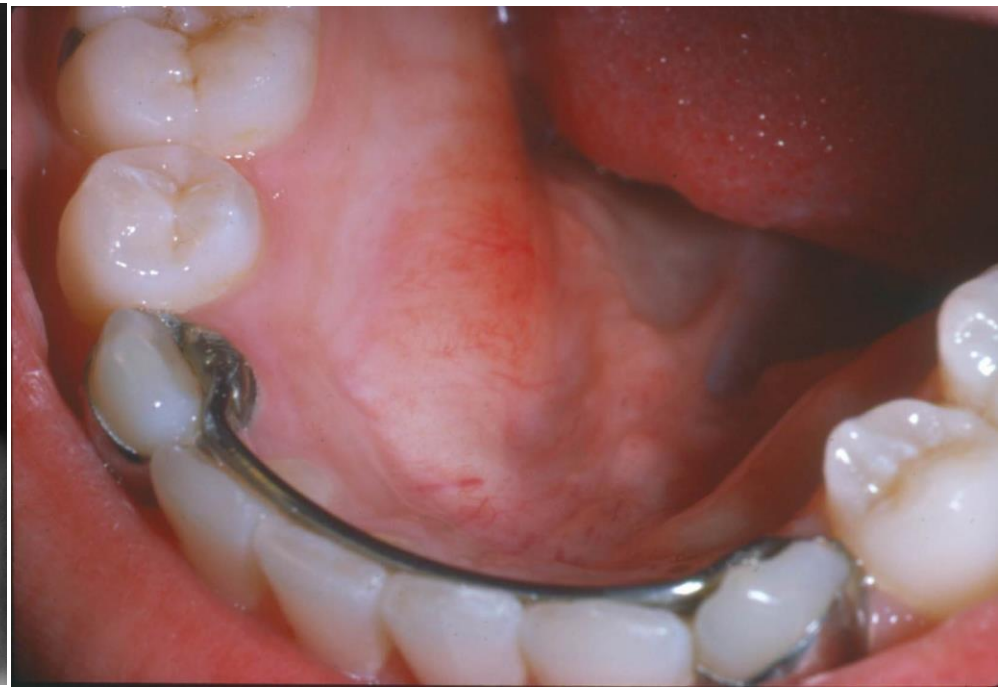
Van Buchem disease (*SOST*)

Sclerosteosis (*SOST*)

Endosteal hyperostosis



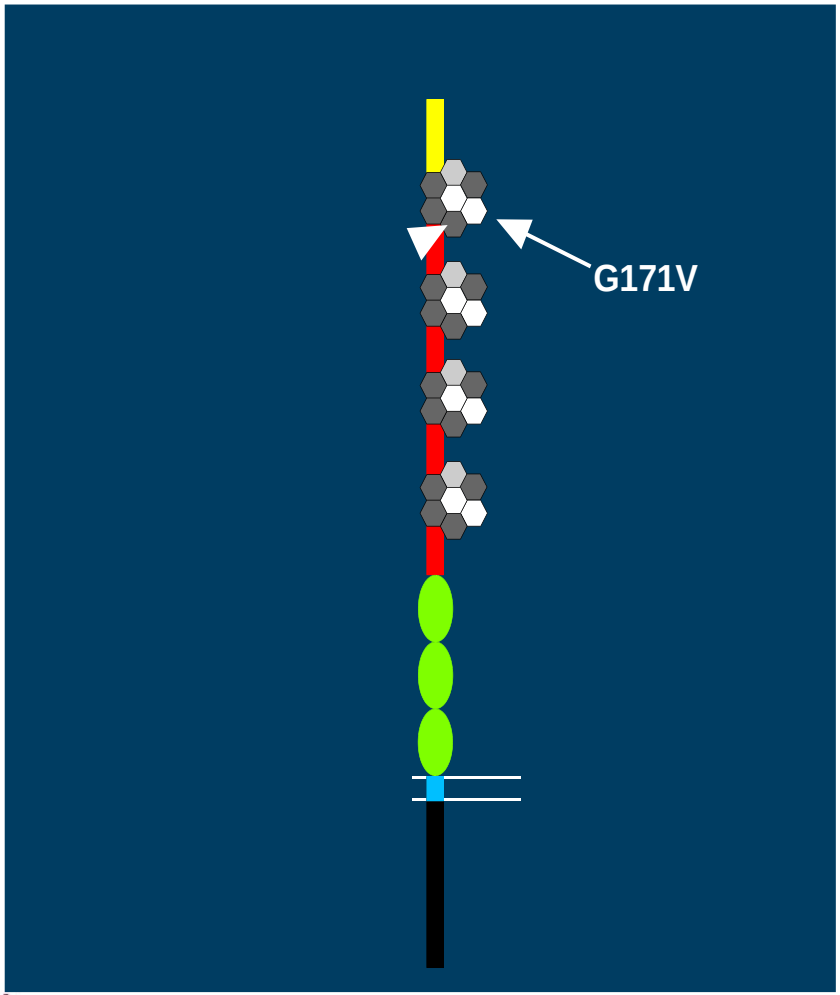




High Bone Mass-phenotype

- 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)



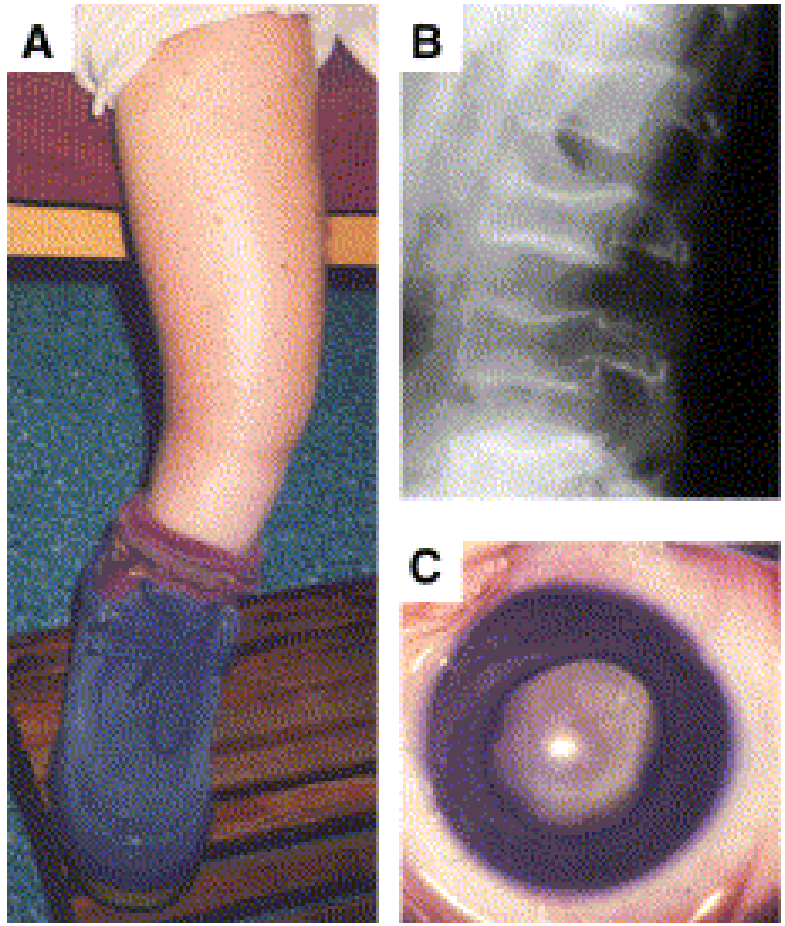
Van Wesenbeeck et al., 2003

Osteoporosis pseudoglioma syndrome

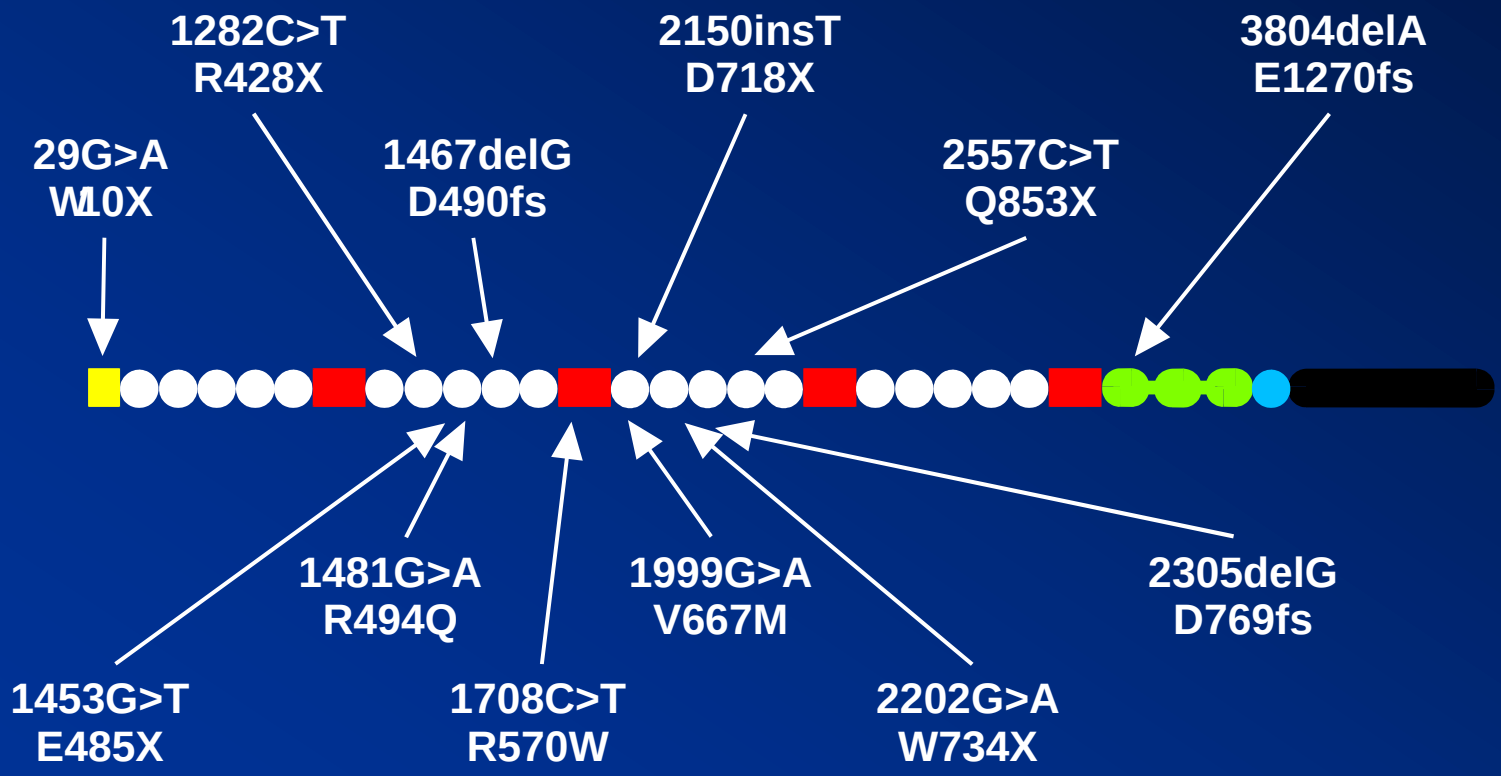
-Autosomal recessive

-Juvenile osteoporosis

-Congenital blindness

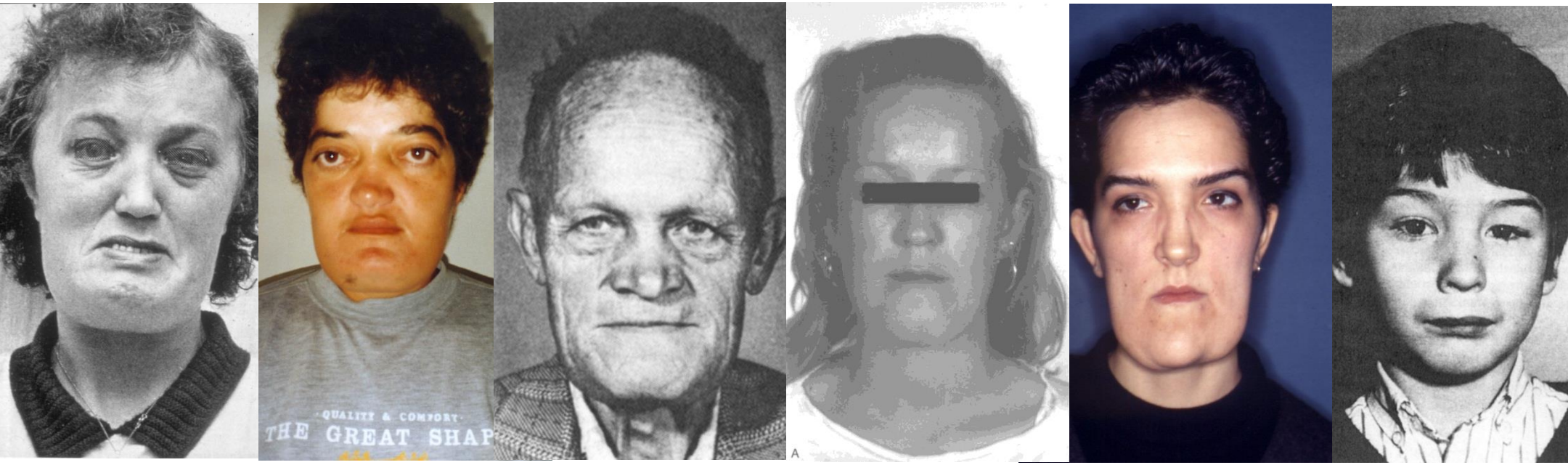


Mutations in LRP5 gene



Osteoporosis-pseudoglioma syndrome (OPPS)

Sclerosing bone dysplasias



Van Buchem disease (*SOST*)

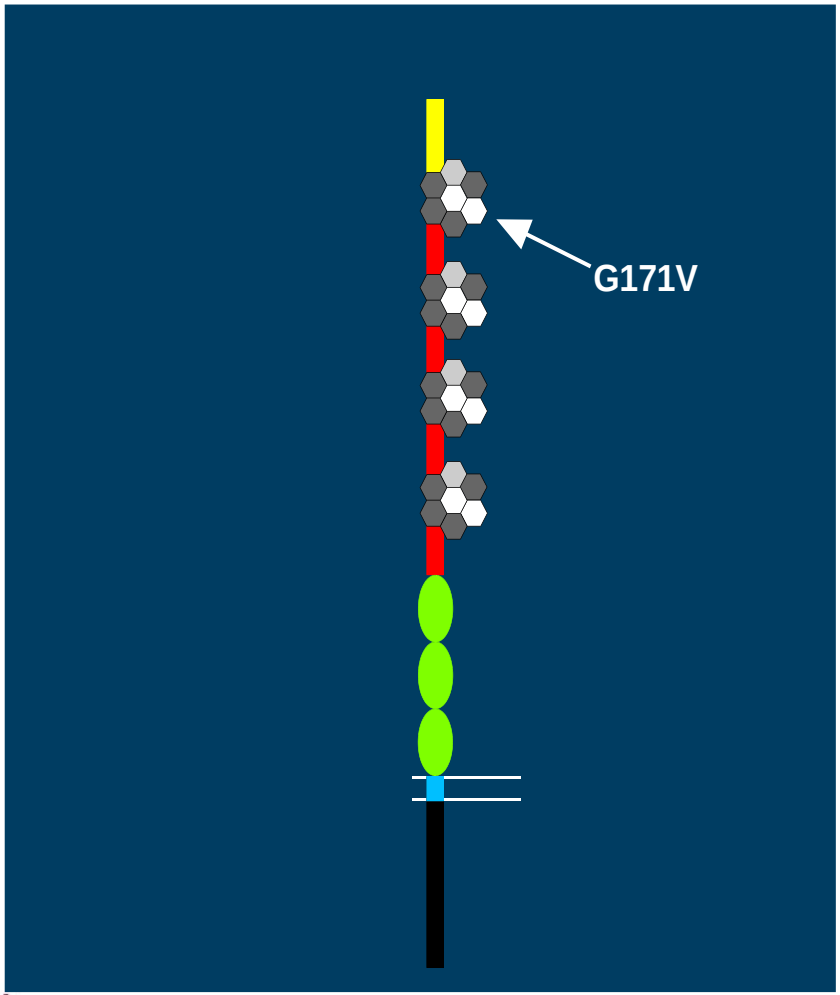
Sclerosteosis (*SOST*)

Endosteal hyperostosis (*LRP5*)

Aut dom osteosclerosis (*LRP5*)

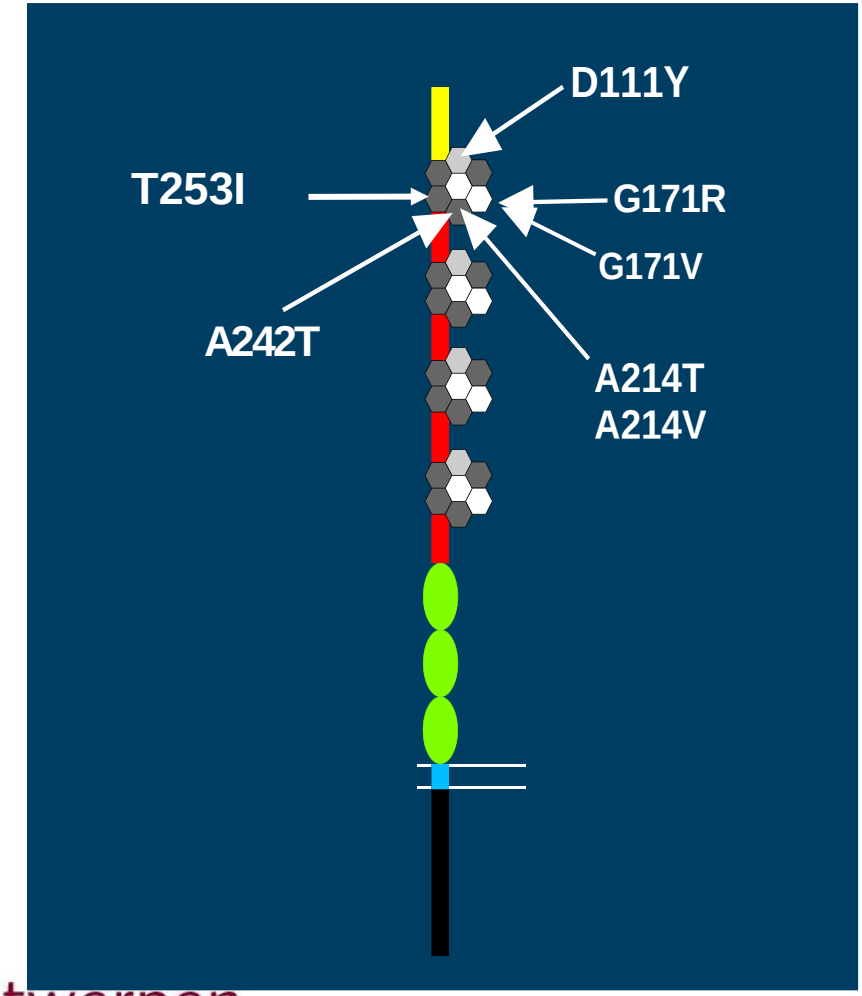
"Van Buchem" (*LRP5*)

LDL-Receptor-Related protein 5 (LRP5)



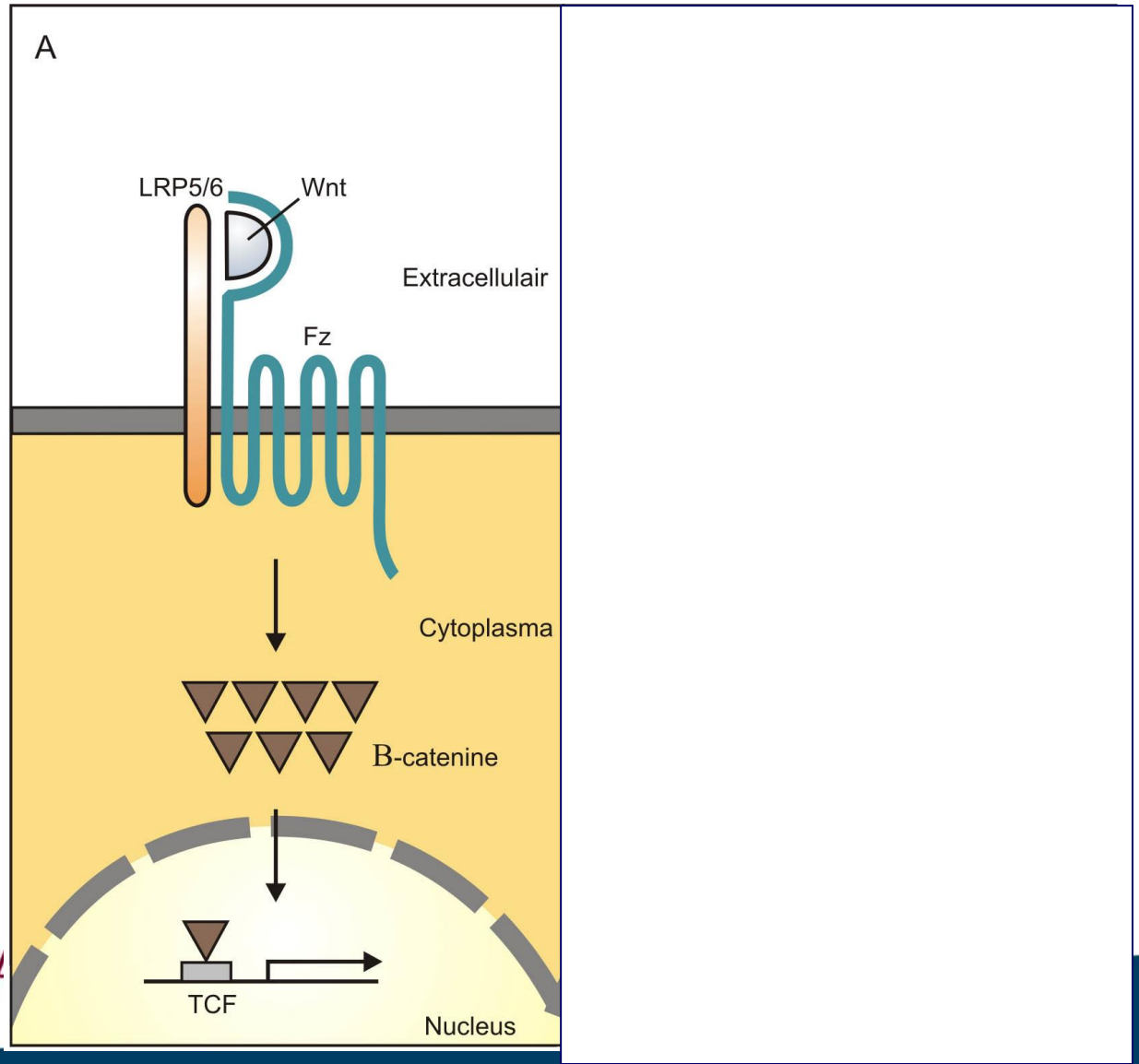
Van Wesenbeeck et al., 2003

LDL-Receptor-Related protein 5 (LRP5)

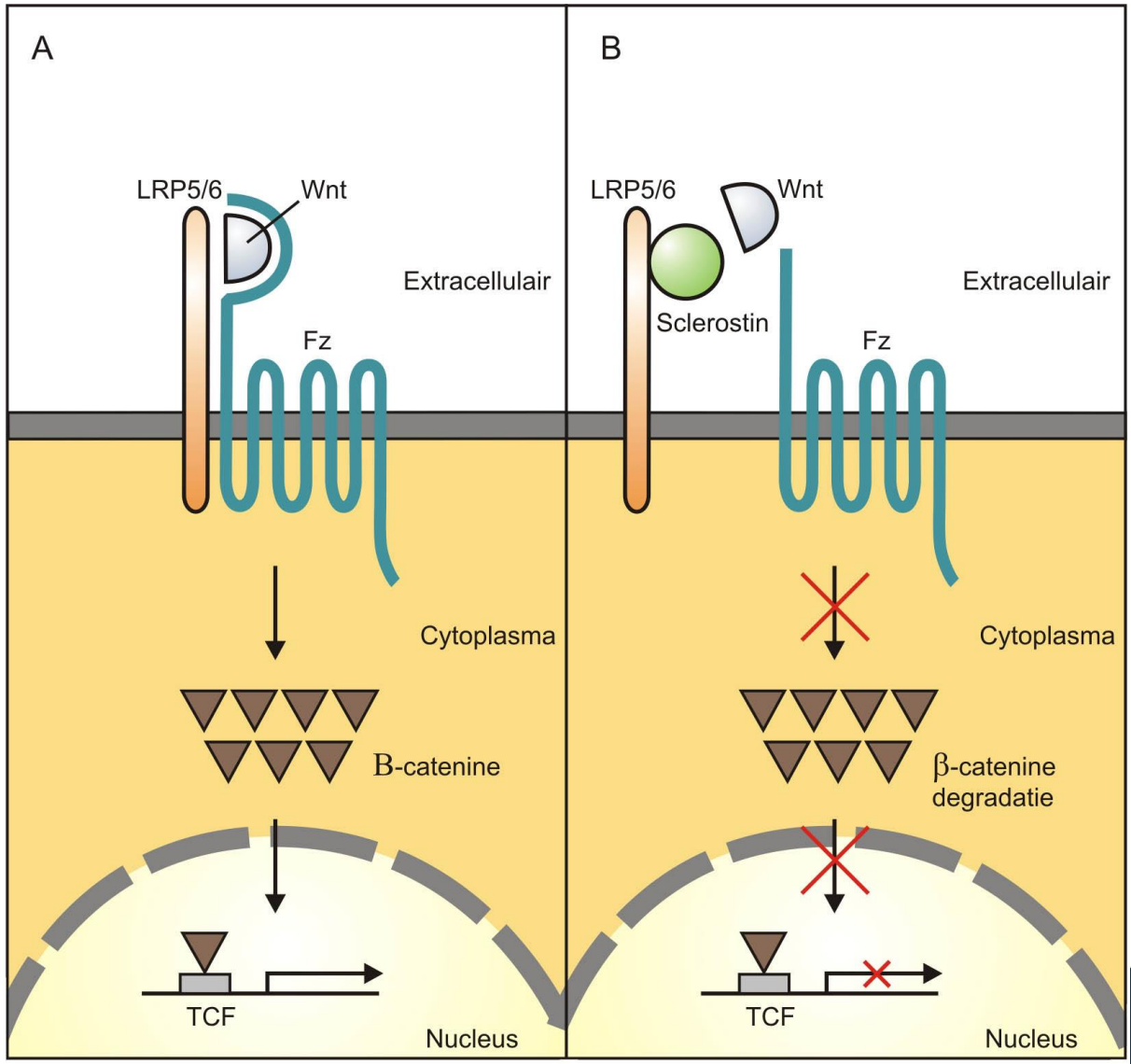


Van Wesenbeeck et al., 2003

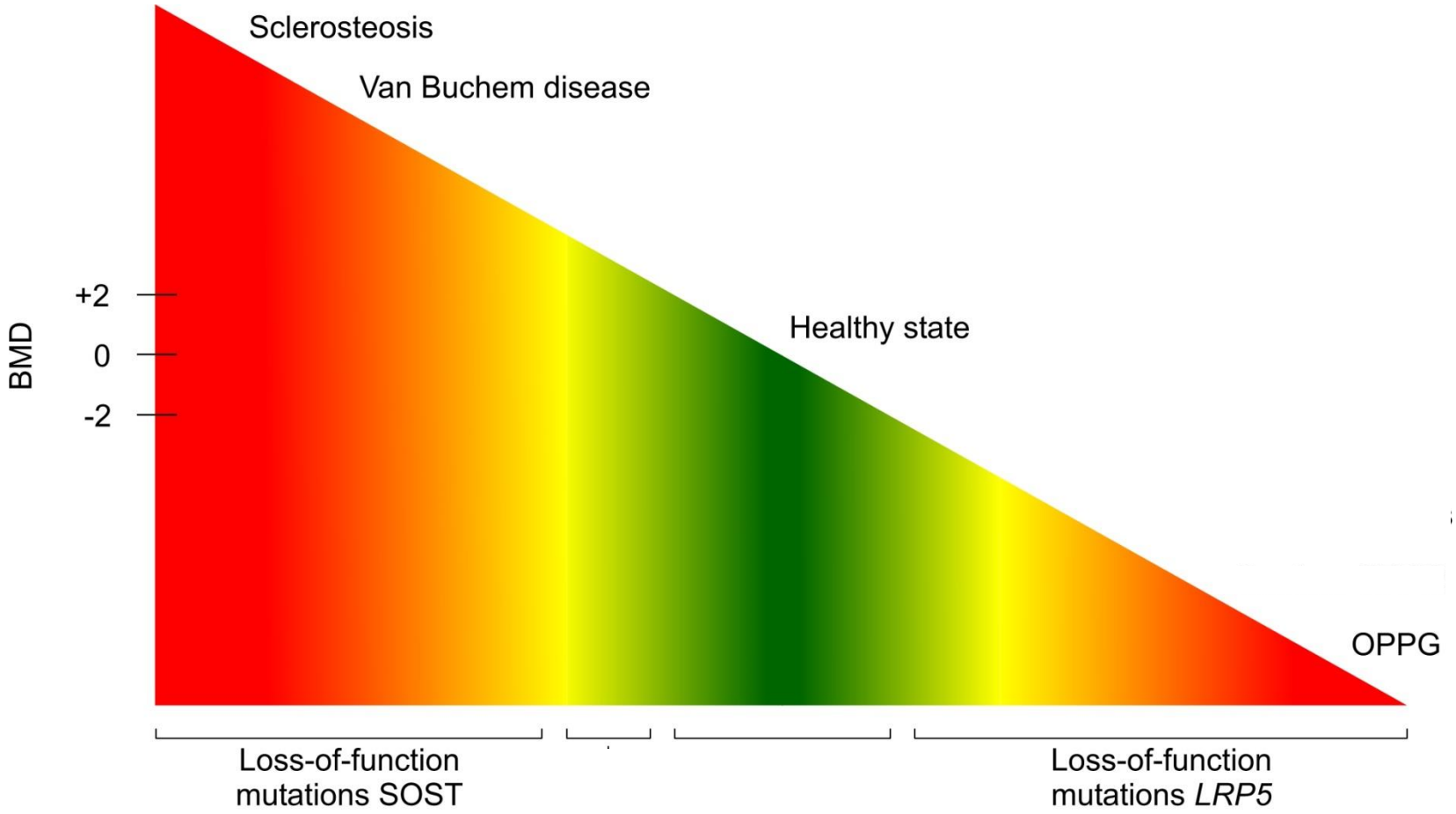
Canonical Wnt signaling



Sclerostin-LRP5



Genetic variation within SOST and LRP5 genes



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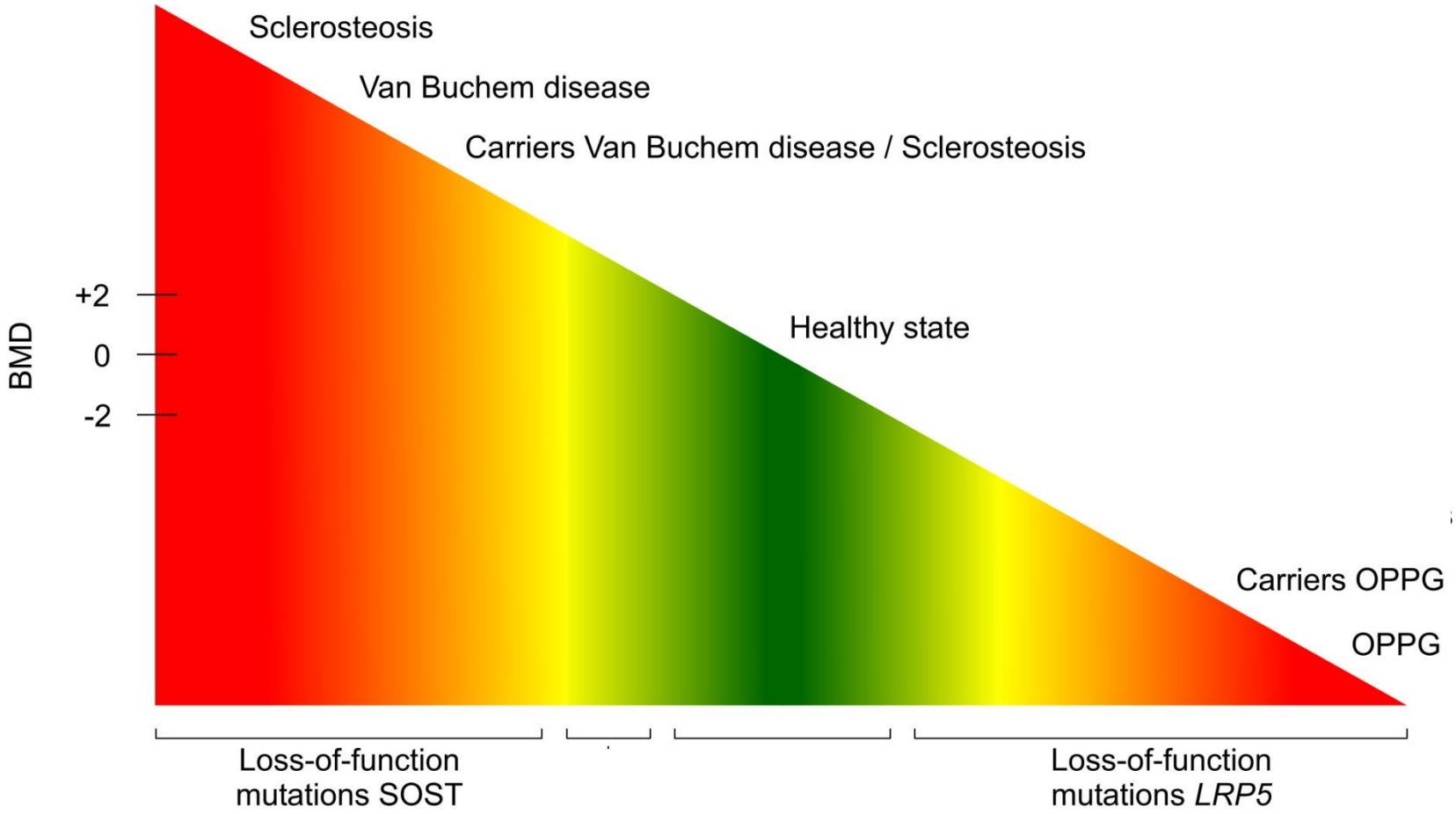

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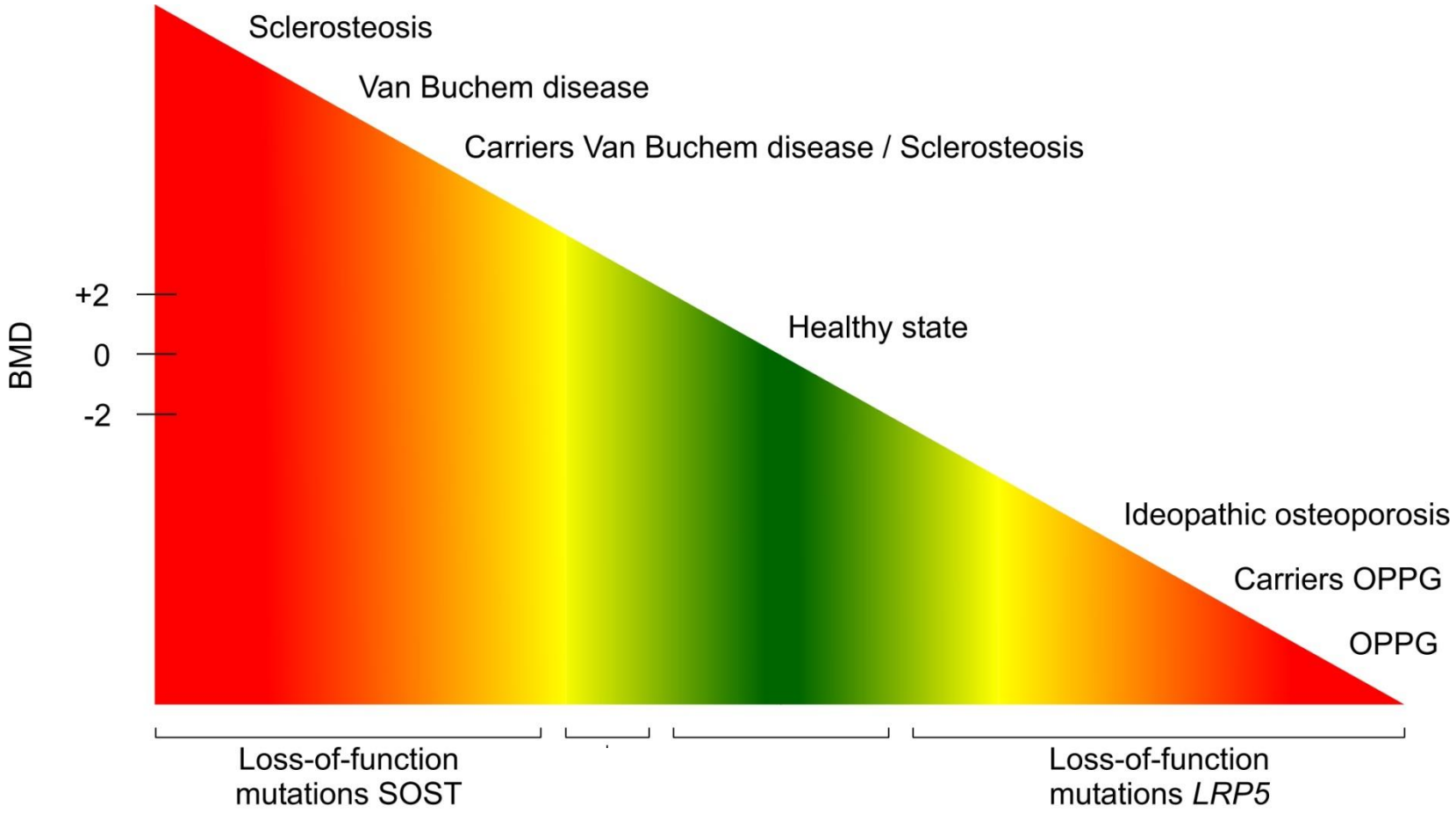

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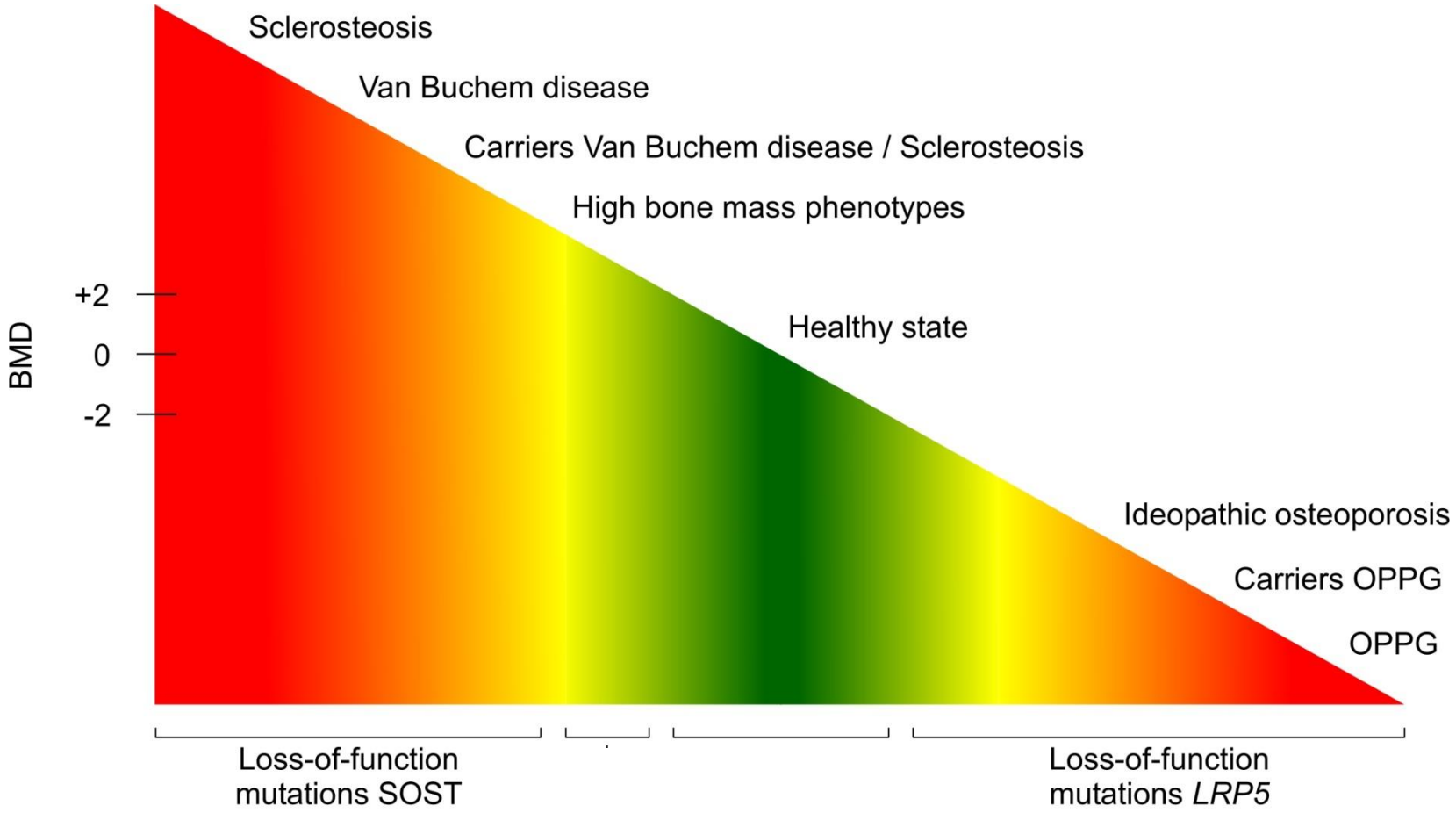

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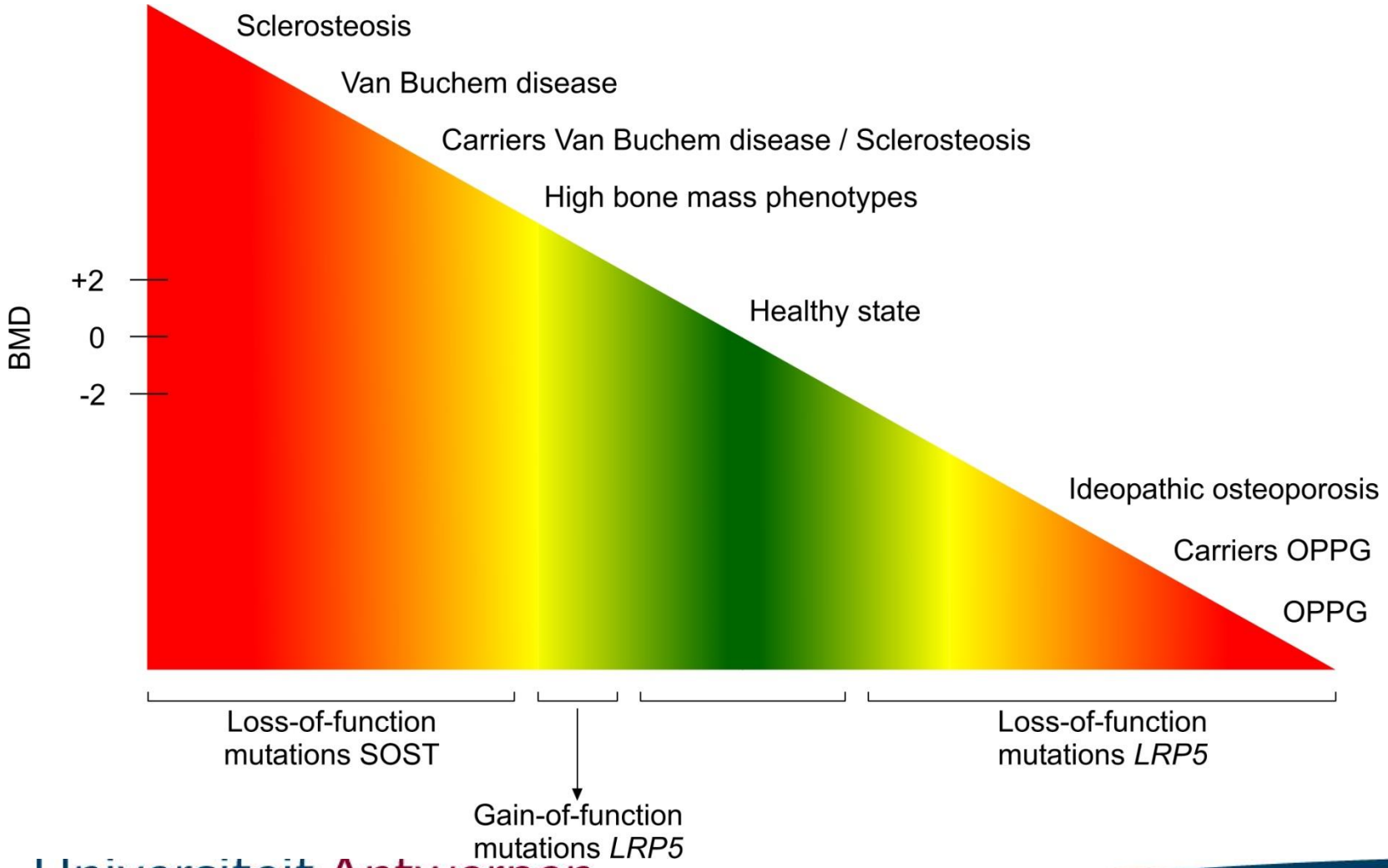

Genetic research of osteoporosis

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

Since 1990s: all three problems were getting solved slowly

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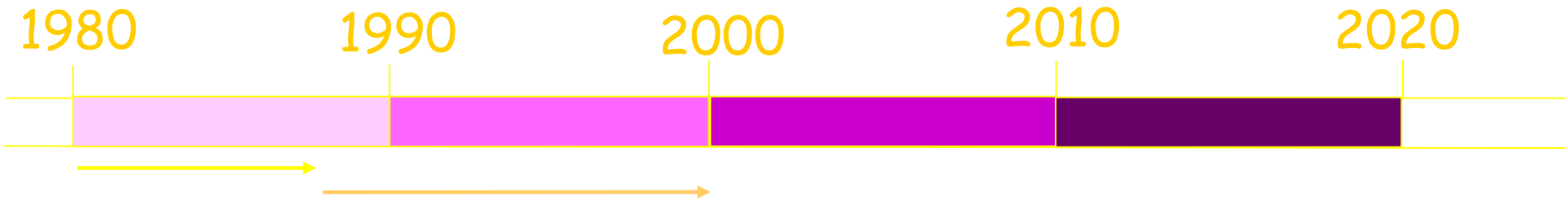

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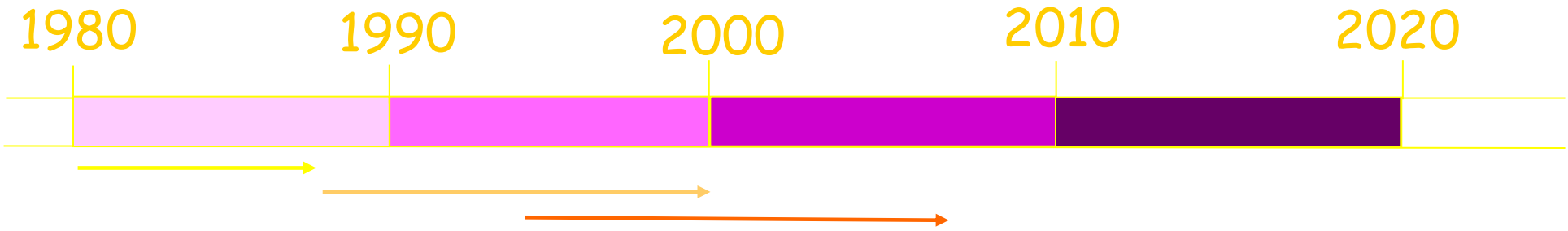
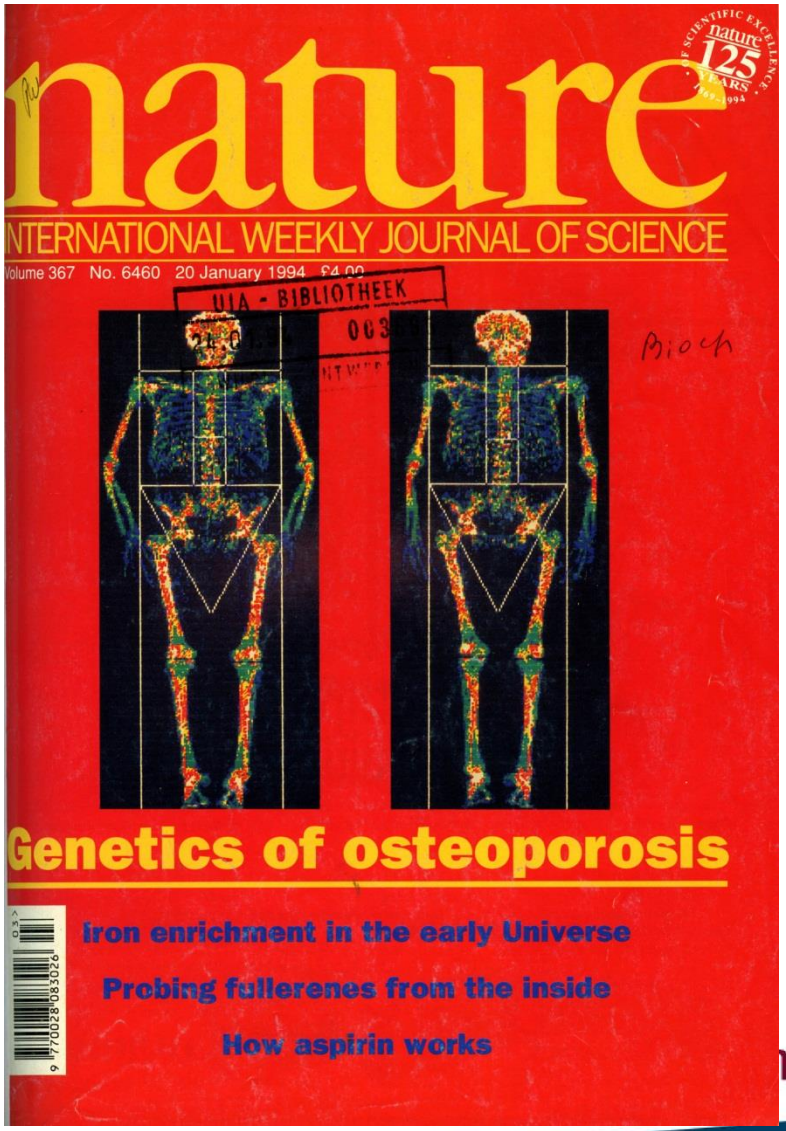


1994: osteoporosis gene!



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

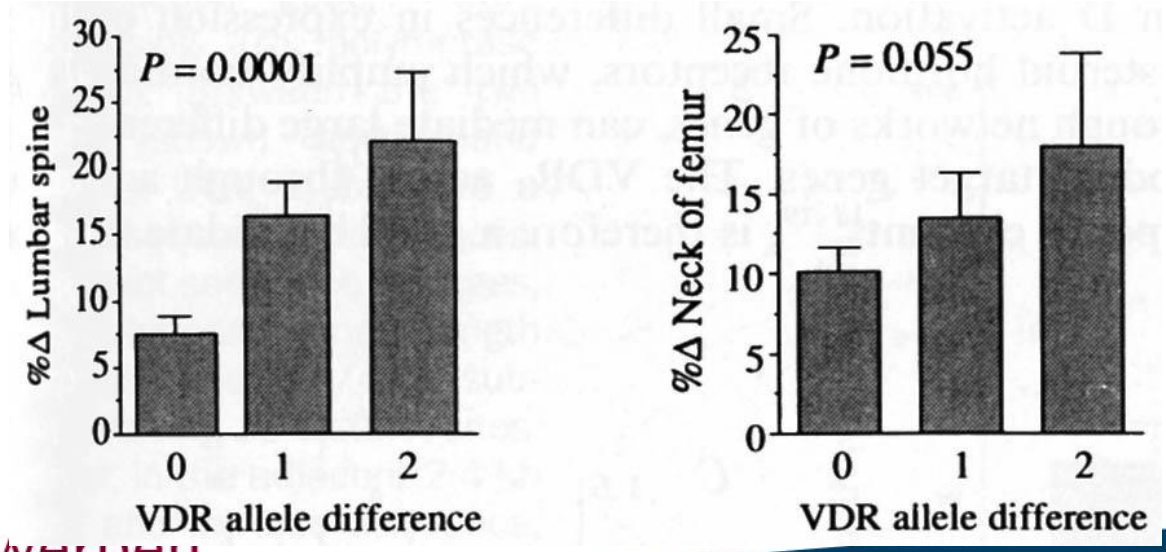
Bone and Mineral Research Division,
Garvan Institute of Medical Research, St Vincent's Hospital,
Sydney, New South Wales 2010, Australia

1994: osteoporosis gene!

250 healthy Caucasian twins (Australia)

BMD measurements at different sites

75% of genetic effect on bone density explained



1997

Nature 387: 106 (1997)

Erratum

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

"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.

1667 citations

Association studies

- Osteopetrosis	Carbonic anhydrase II
	H+ATPase/CLCN7/GL
- Pycnodysostosis	Cathepsin K
- Camurati-Engelmann	TGFB1
- Van Buchem/Sclerosteosis	SOST
- High Bone Mass	LRP5
- Familial Expansile osteolysis	RANK
- Paget's disease	OPG/SQSTM1

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Prospective meta-analyses of osteoporosis candidate genes

"GENOMOS" QLK6-CT-2002-02629

(Genetic Markers for Osteoporosis)

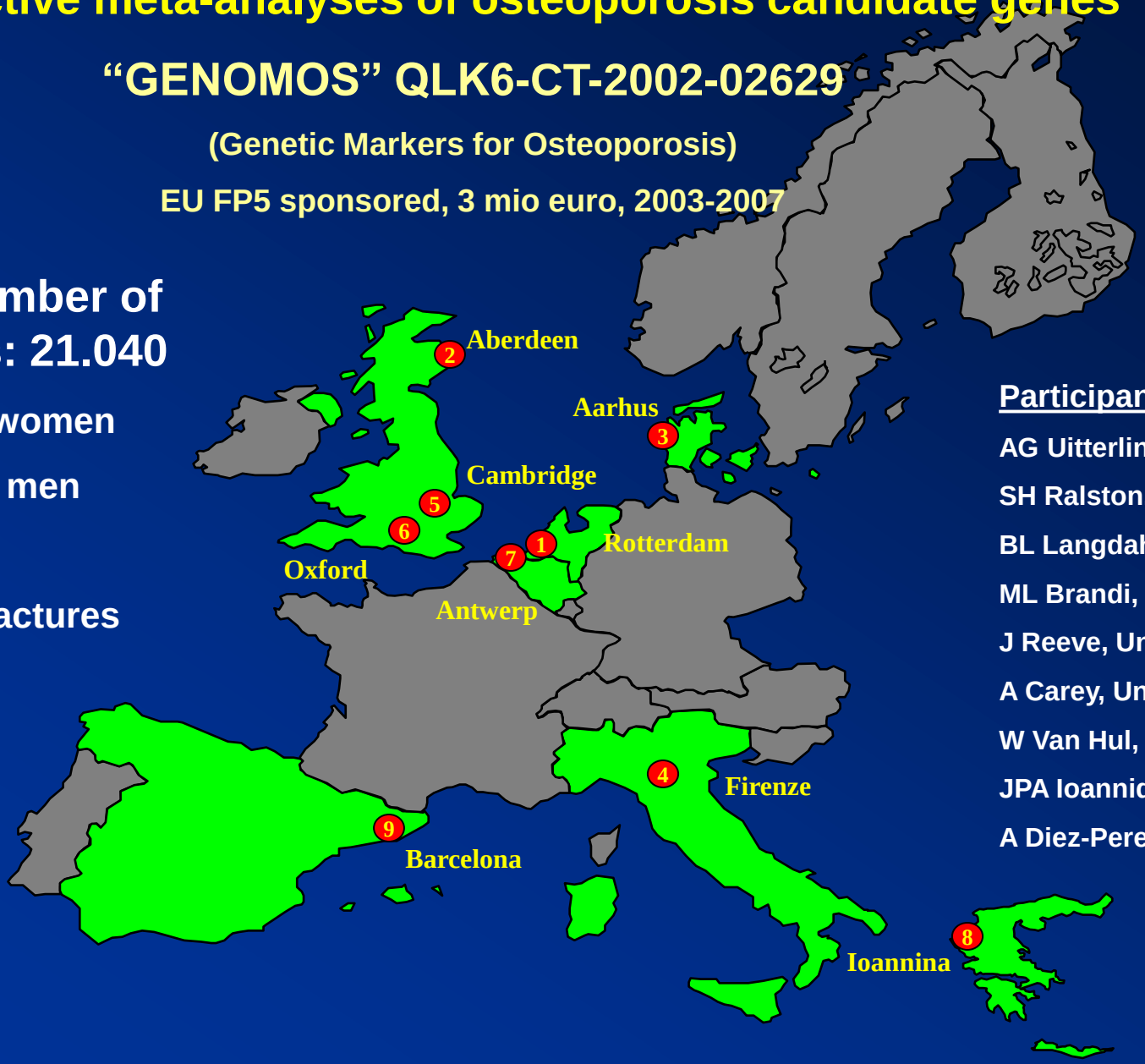
EU FP5 sponsored, 3 mio euro, 2003-2007

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)

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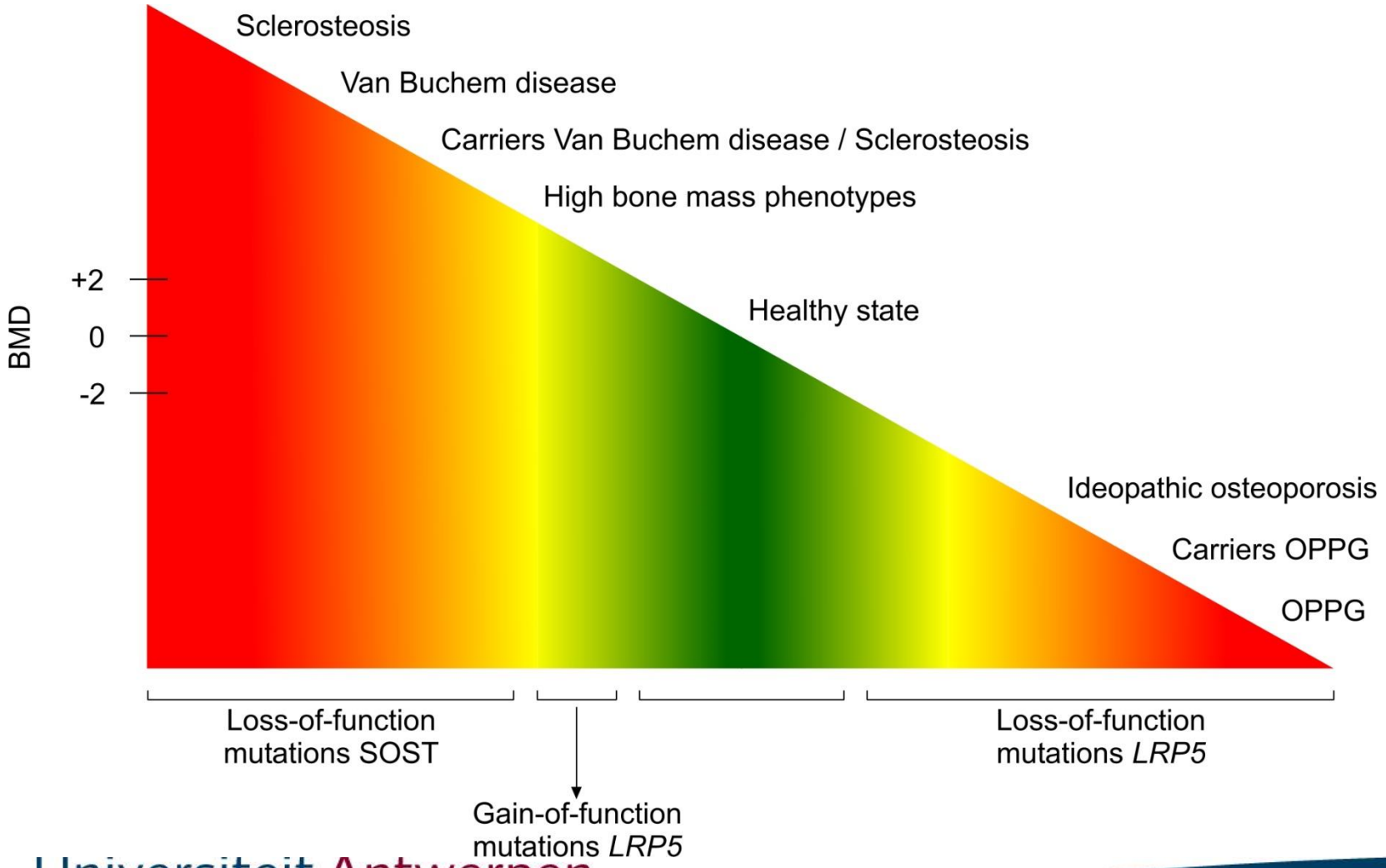

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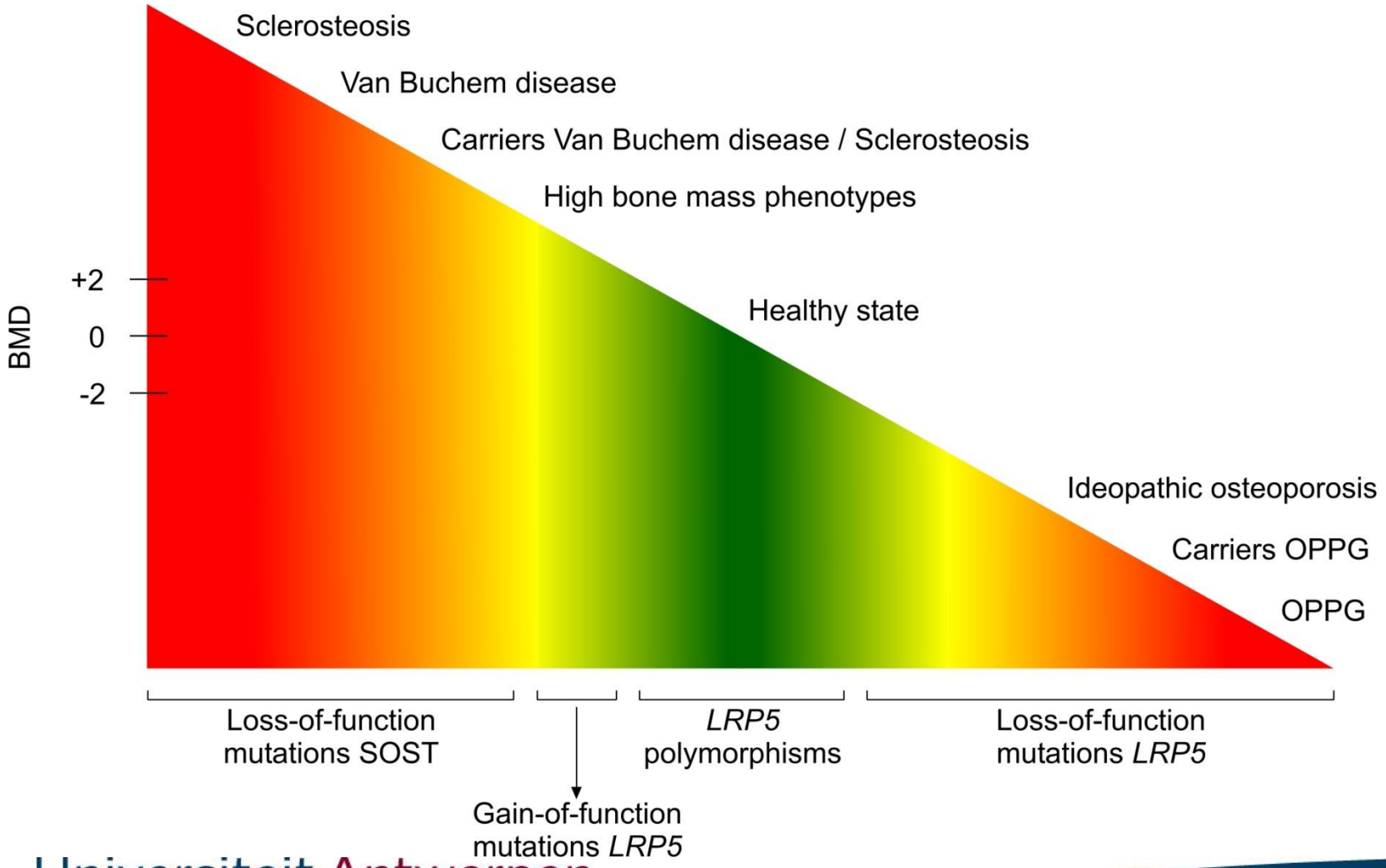

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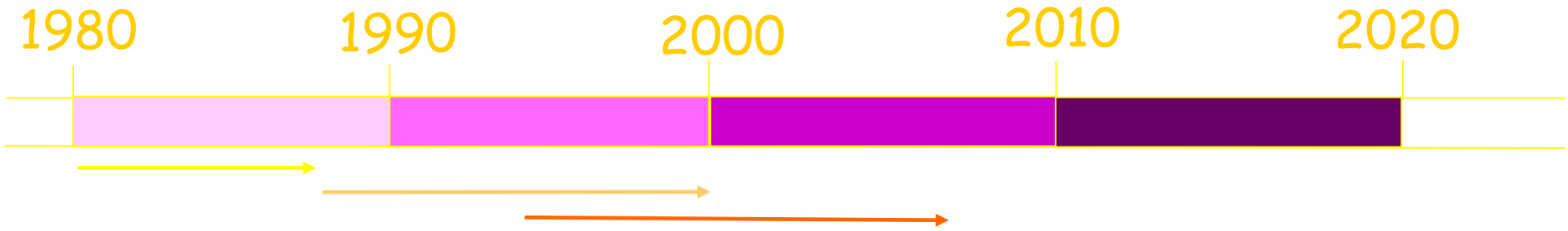

How to identify genes for complex traits

Identification of genes for relevant monogenic conditions

1. functional candidate gene approach
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Association studies

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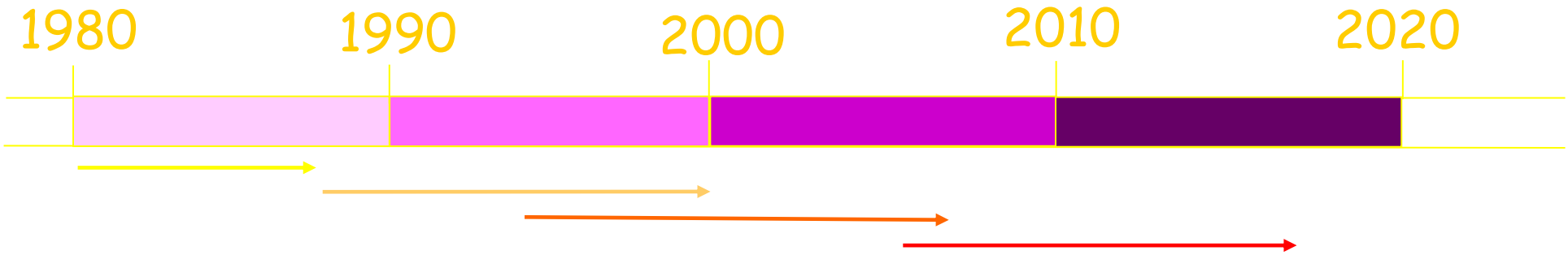
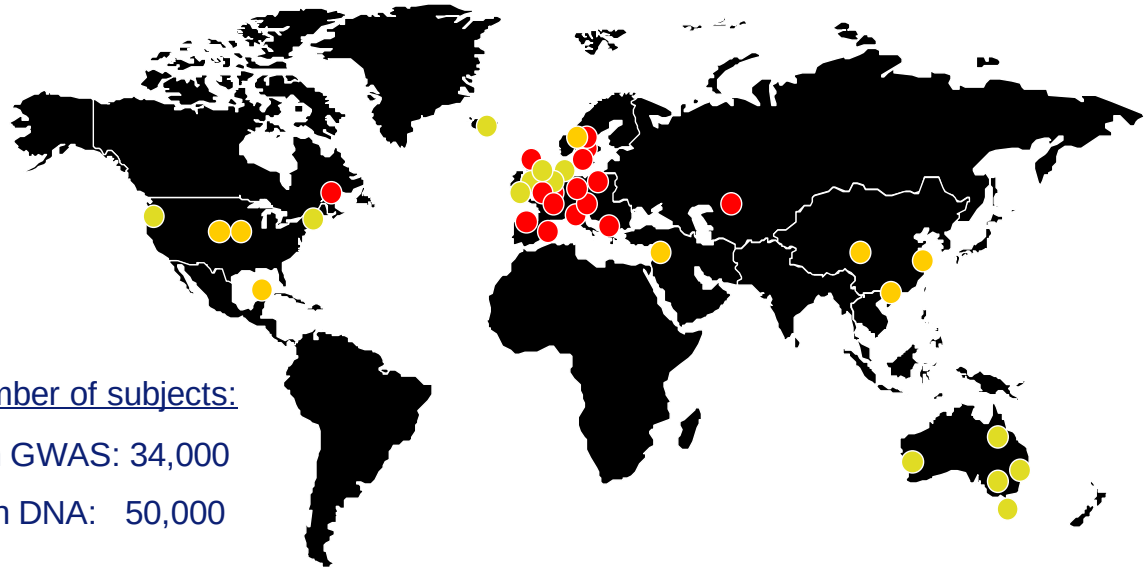


Genomos and Gefos consortium

AIM: Identification of novel genetic determinants of osteoporosis and fracture traits using a hypothesis-free approach

Genome-wide association studies (GWAS)



Number of subjects:

with GWAS: 34,000

With DNA: 50,000

● = GENOMOS study population

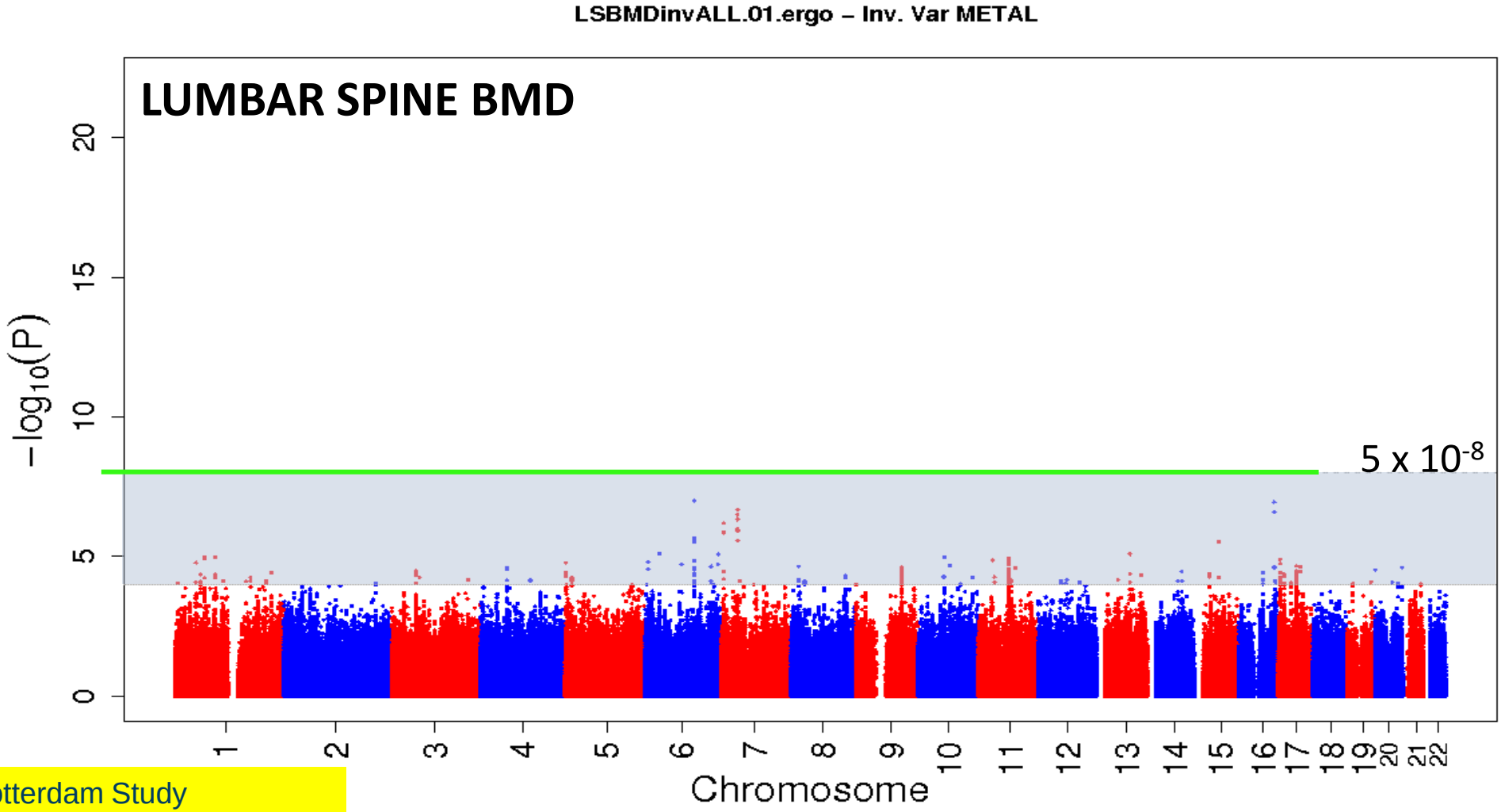
● = idem + GWAS

● = idem, under negotiation / in development

www.gefos.org

www.genomos.eu

One single study has insufficient power to identify genome-wide significant signals



- Rotterdam Study

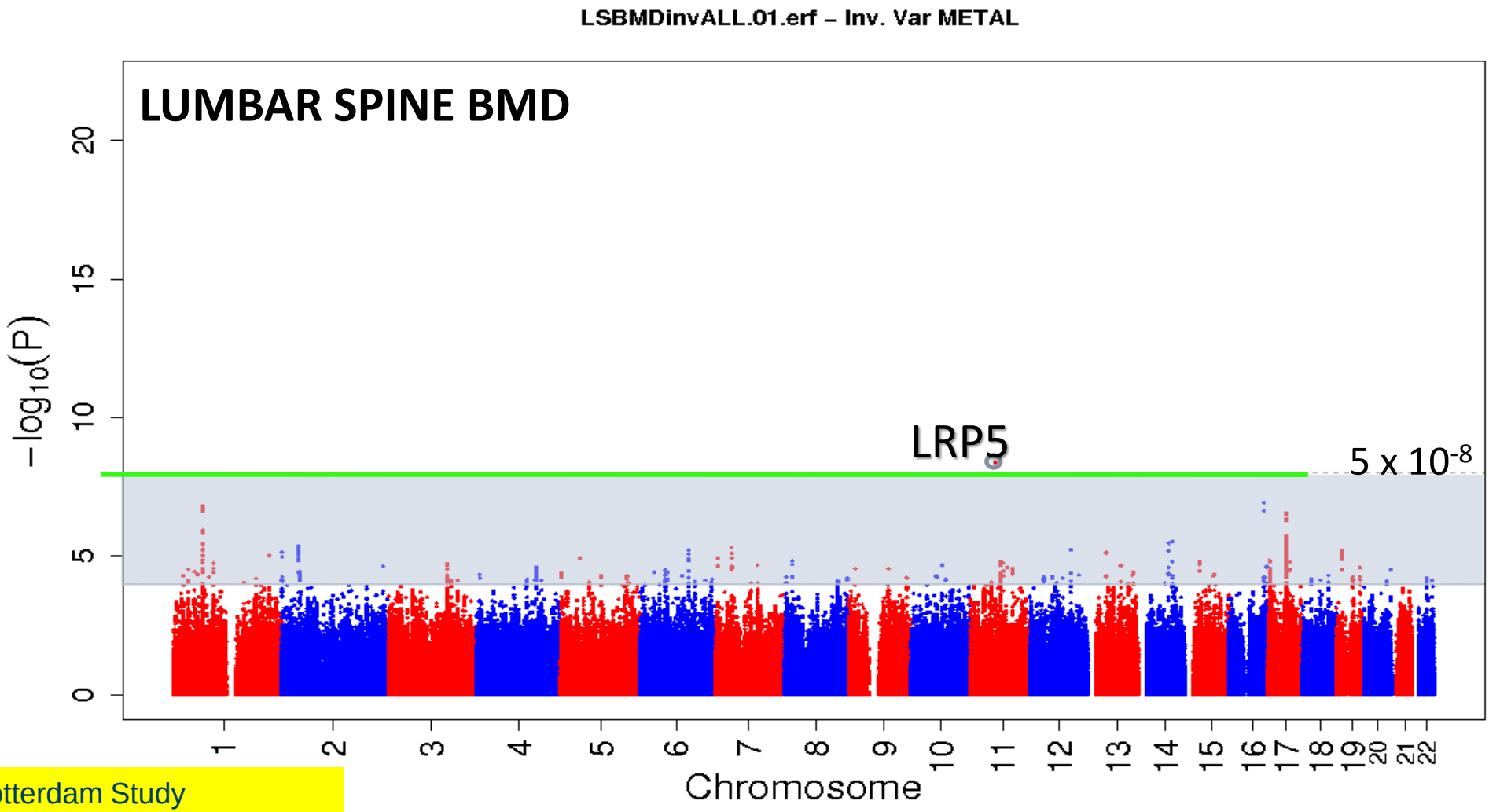
Universiteit Antwerpen

N=5000

Rivadeneira et al., ASBMR sept 2008

GEFOS

As sample size increases genome-wide significant signals become gradually evident



- Rotterdam Study
- ERF Study

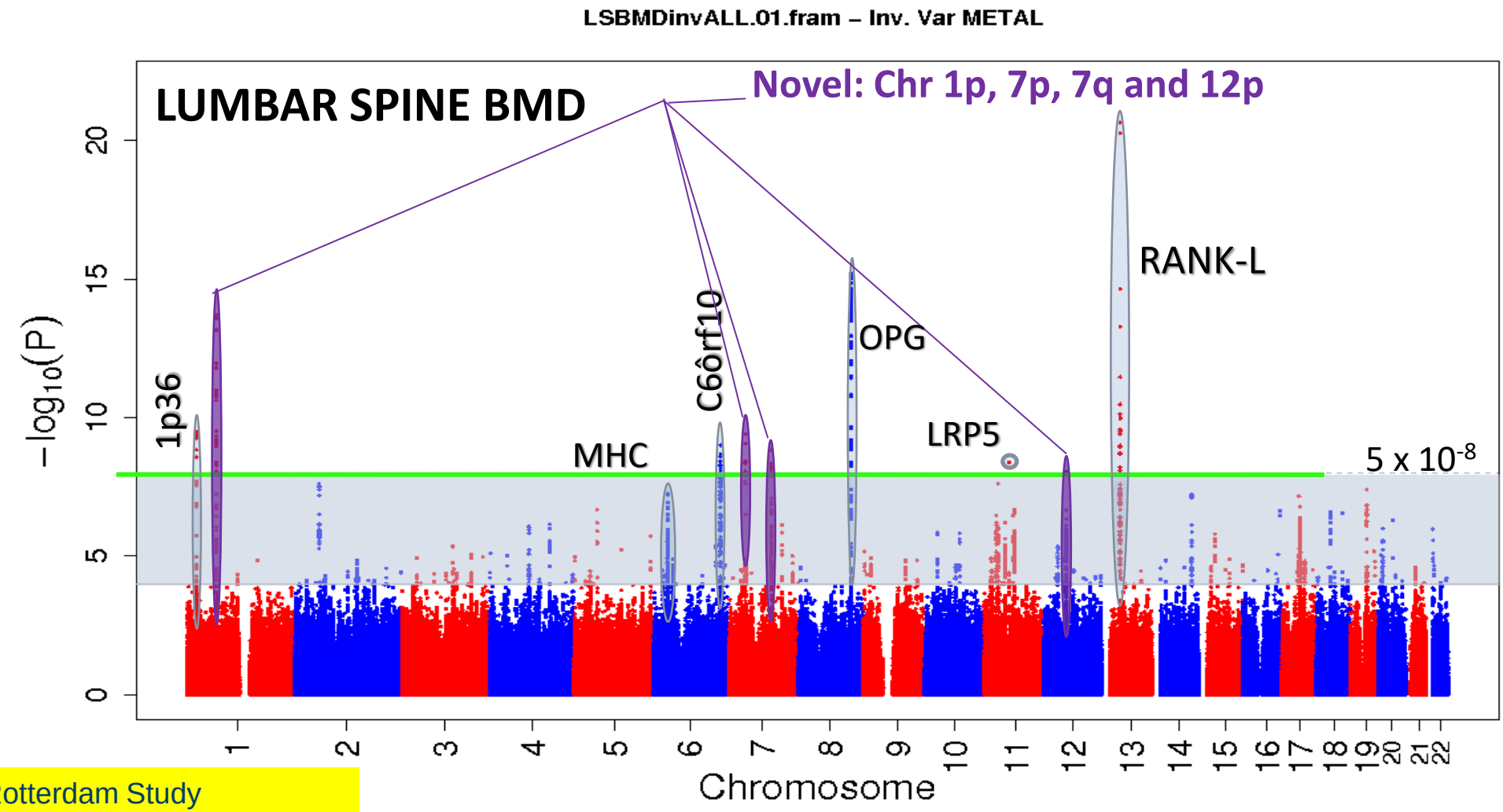
N=6200

Rivadeneira et al., ASBMR sept 2008

Universiteit Antwerpen

GEFOS

Four novel loci exceed GWS threshold, many others are close



- Rotterdam Study
- ERF Study
- Twins UK
- deCODE Genetics
- Framingham Study

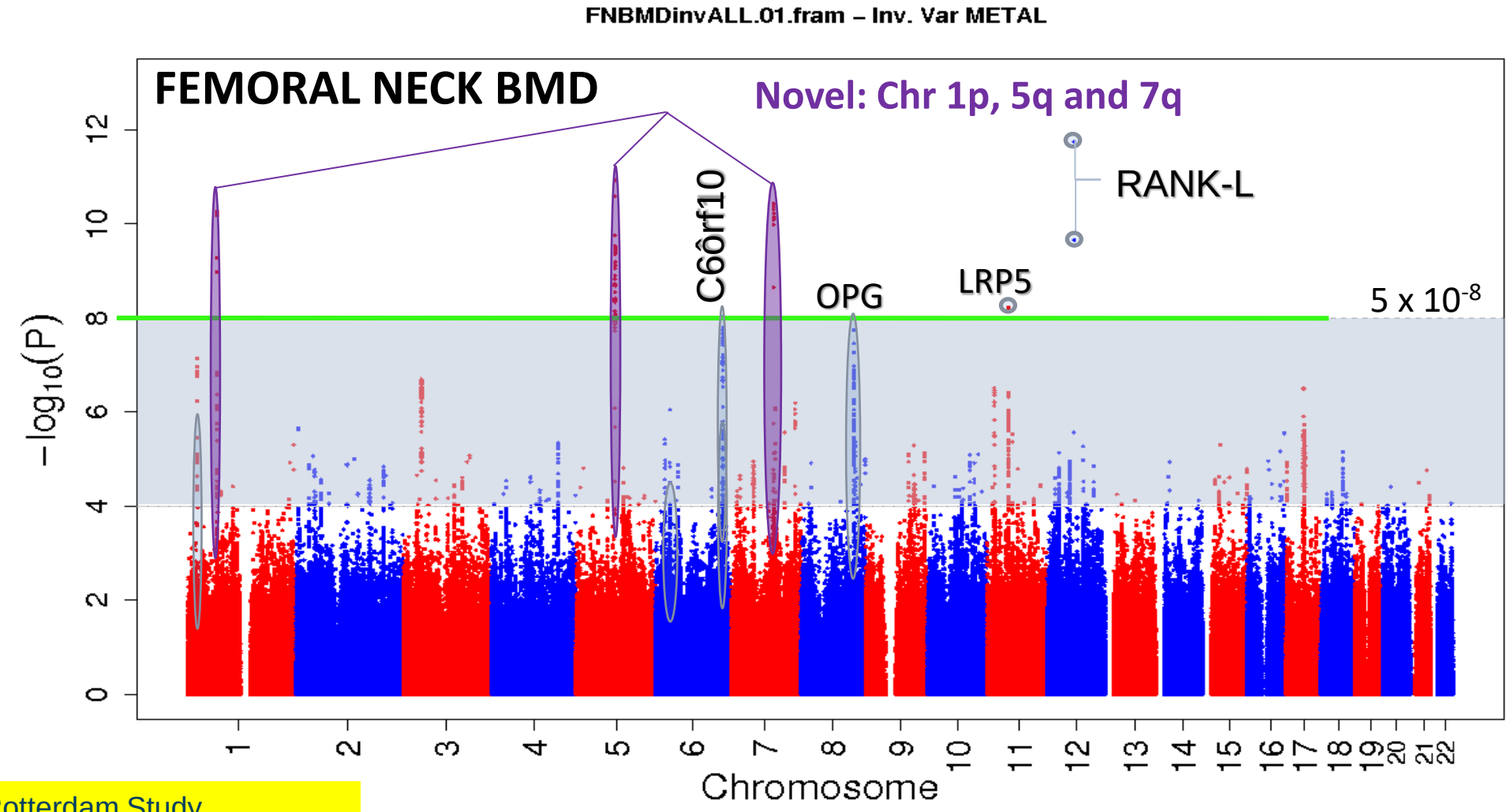
ntwerpen

N=18500

Rivadeneira et al., ASBMR sept 2008

GEFOS

Three novel loci exceed GWS threshold, many others are close



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Rivadeneira et al., ASBMR sept 2008

GEFOS

Genomos and Gefos consortium

Estrada et al. Nature genetics, 2012

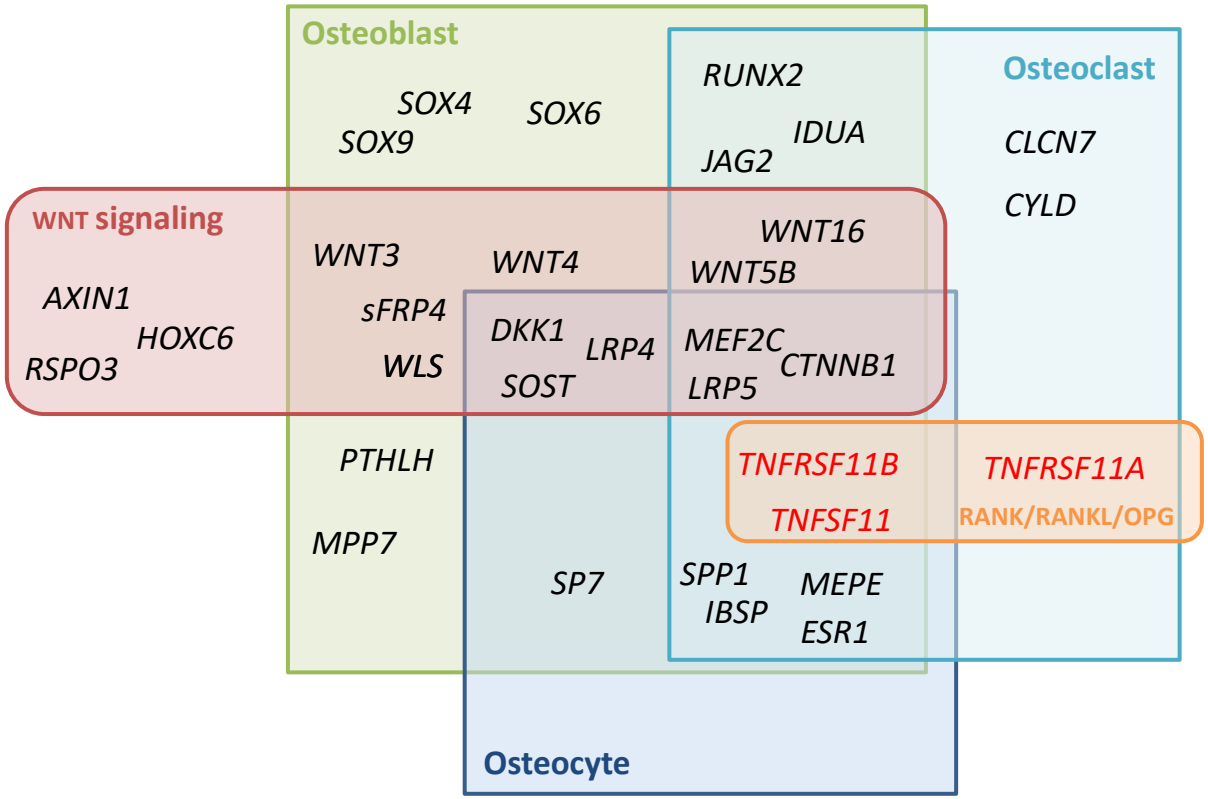
Largest meta-analysis for bone mineral density

- 17 GWAS studies (33000 individuals)

- 96 top SNPs : Replication : 51000 individuals

=> 56 bone mineral density loci

BMD-associated genes



Hendrickx et al. Nature Rev Reumat, 2015

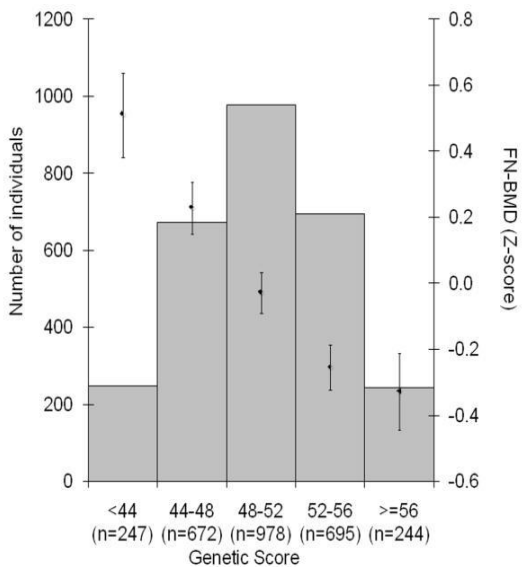
56 loci for BMD

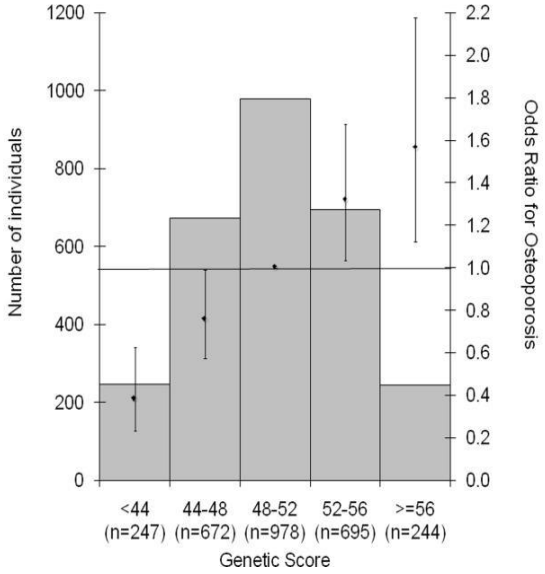
Estrada et al. Nature genetics, 2012

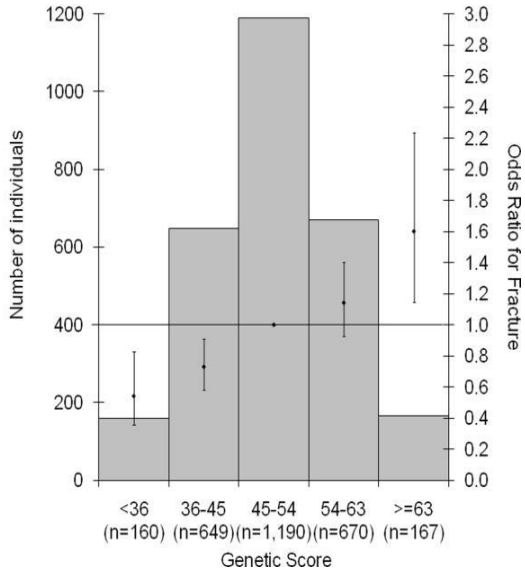
14 loci for osteoporotic fractures

- smaller sample size
- clinically heterogeneous collection
- risk variants: site specific

Genetic testing

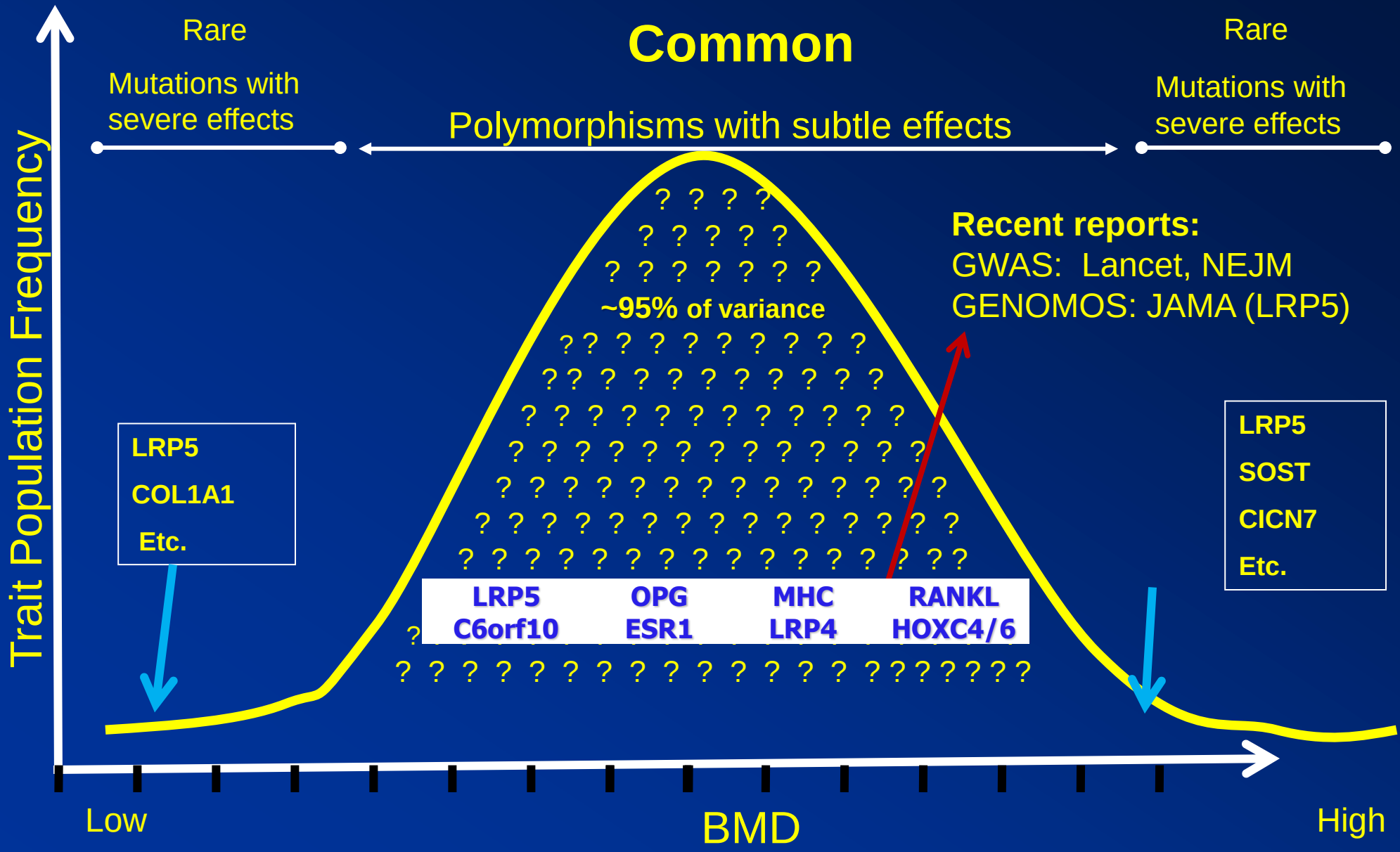






Estrada et al, *Nat Genet*, 2012

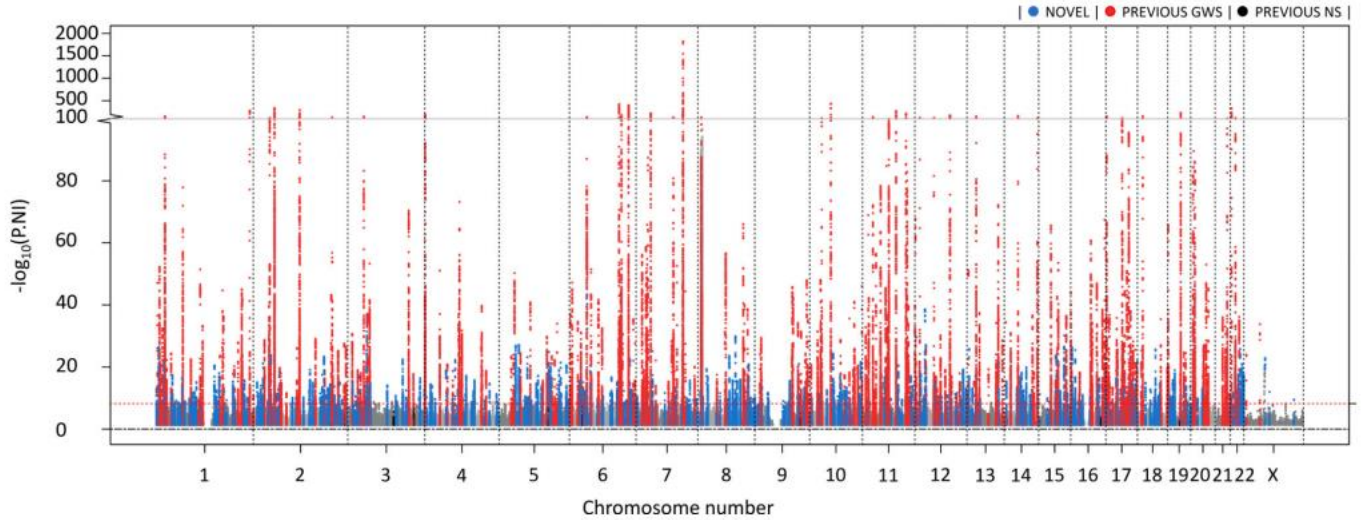
Genetic architecture of BMD



BMD-associated genes

426,824 individuals from the UK Biobank
1103 independent signals at 513 genetic loci
20.3% genetic variance

Morris JA, et al. An atlas of genetic influences on osteoporosis in humans and mice.
Nat Genet. 2019; 51(2): 258–266.



BMD: Missing heritability

- Larger samples to detect variants with smaller effects
- Copy number variations
- Additional variants: rare variants

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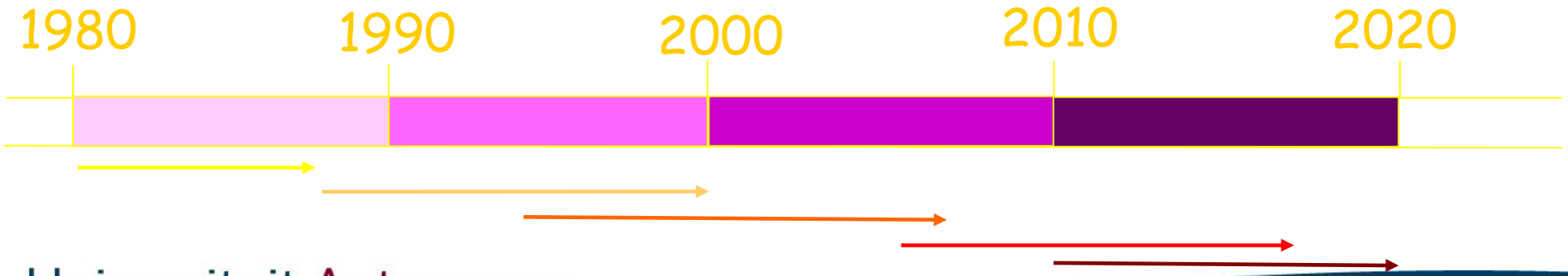


Rare variants

Whole genome sequencing by Decode, Iceland (Nature, 2013)

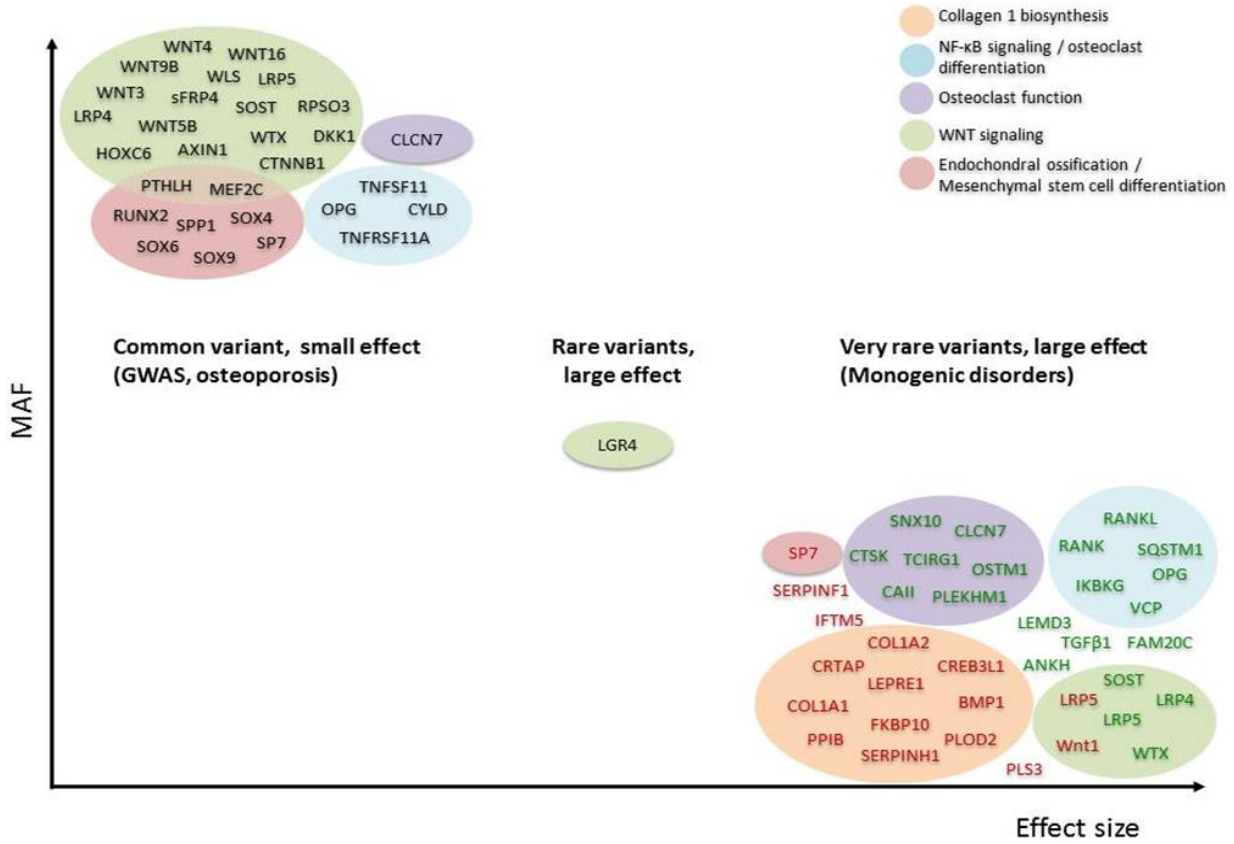
- Rare nonsense mutation in Leucine-rich-repeat-containing G-protein-coupled receptor 4 (LGR4): receptor for R-spondins

Rare variants

Whole genome sequencing by Decode, Iceland (Nature, 2013)

- Rare nonsense mutation in Leucine-rich-repeat-containing G-protein-coupled receptor 4 (LGR4): receptor for R-spondins
- Strong association with BMD and fractures (OR of 3.12)
- Frequency around 0.15%
- Specific for Icelandic population, 400 years ago

Genetic architecture of bone mass



Boudin et al., Mol Cell Endocrinol. 2015

Conclusions

- Genes causing monogenic diseases also relevant for complex diseases
- Identified genes interesting drug targets

  Romosozumab : antibody against sclerostin

  (Evenity, Amgen and UCB)

  Blockbuster!!!
- GWAS: Study of large cohorts is essential

  => Importance of worldwide collaborations
- Only low percentage of phenotypical variation explained by currently identified loci

  Clinical relevance genetic test still limited