The genetics of osteoporosis

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

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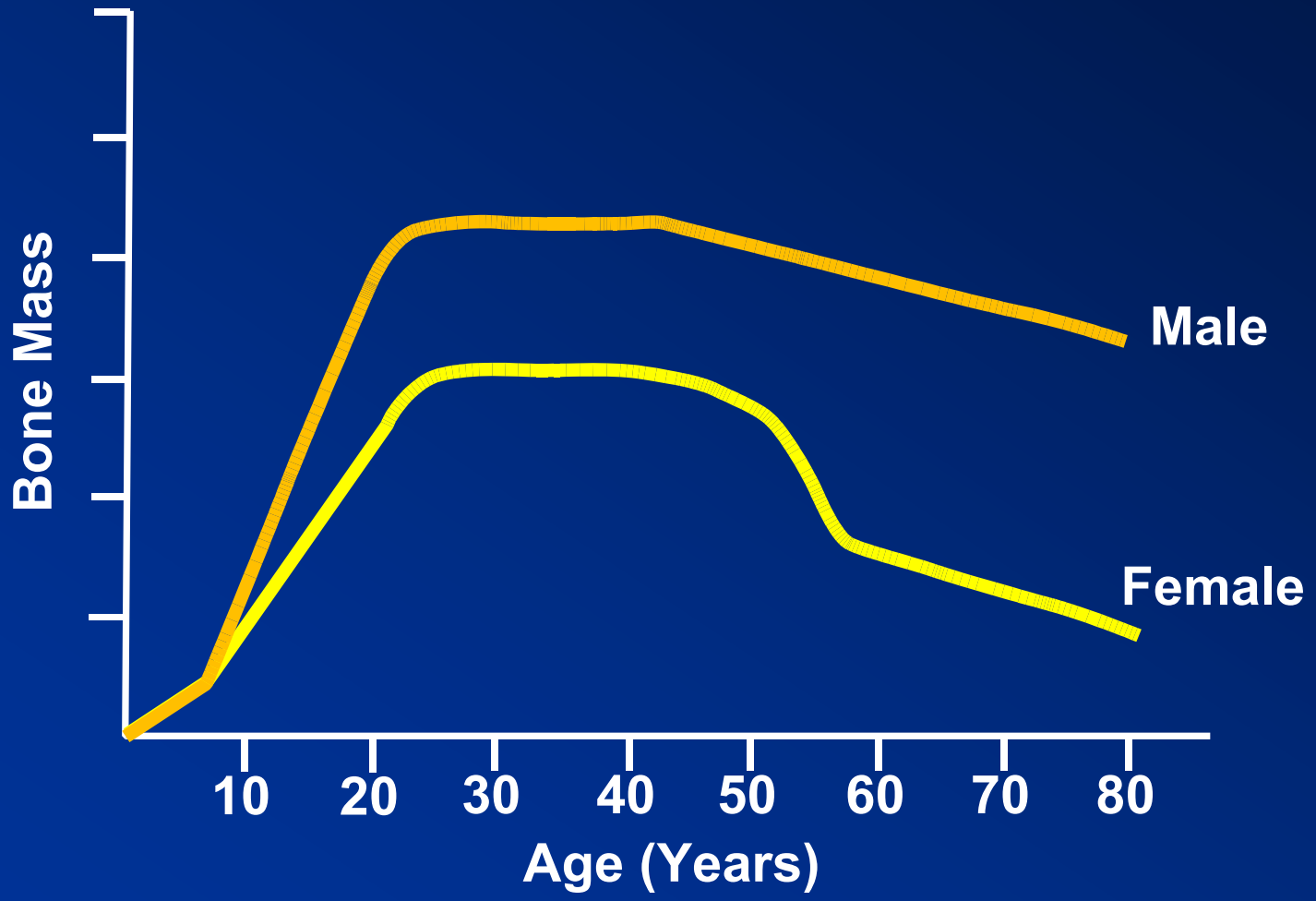
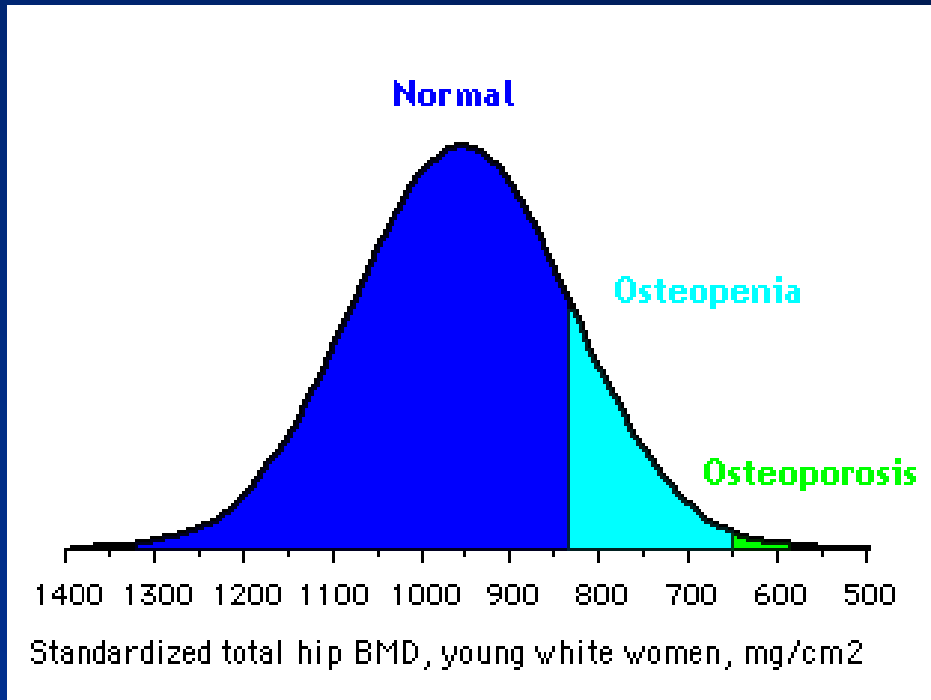
Changes in bone mass



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

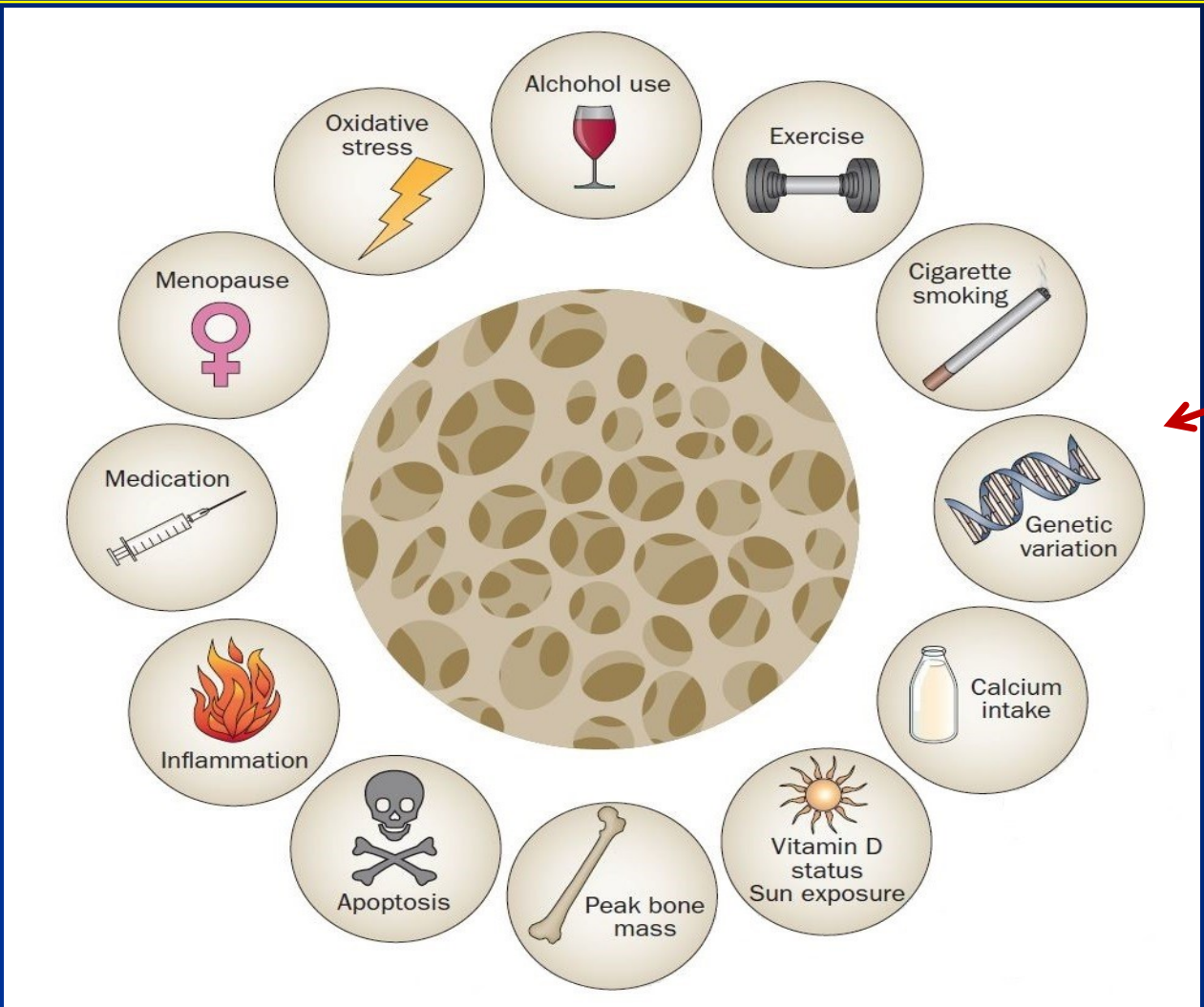


Osteoporosis

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

Changes in bone mass



Hendrickx et al. Nature Rev Reumat, 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

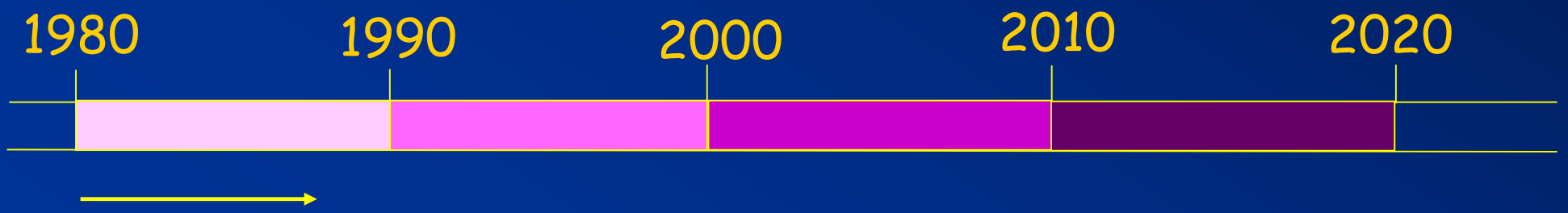
Identification of genes for relevant monogenic conditions

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



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.

How to identify genes for complex traits

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Association studies

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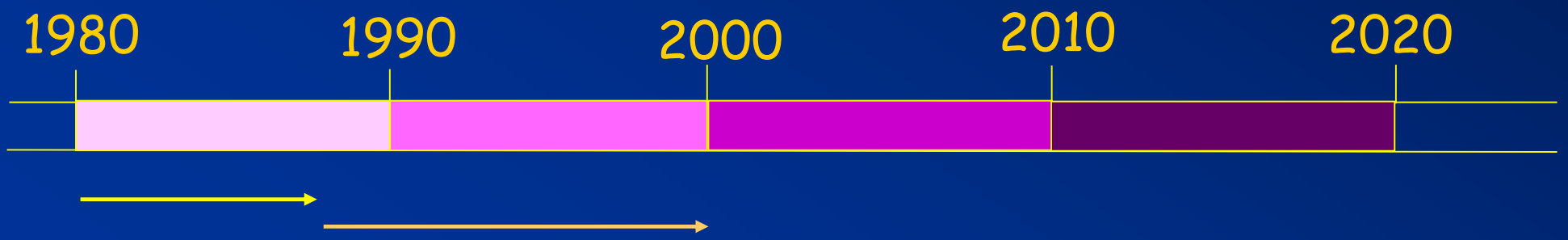
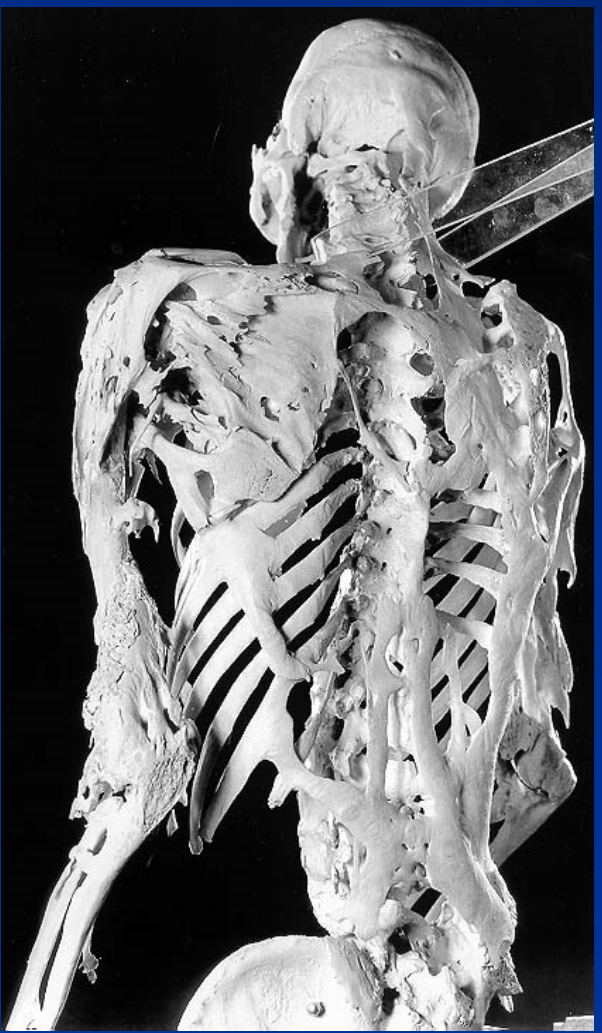


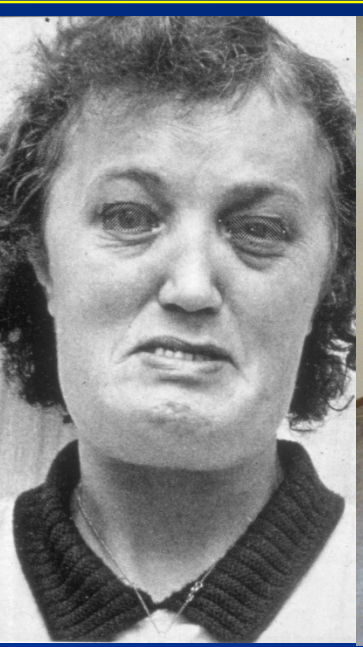
Sclerosing bone dysplasias

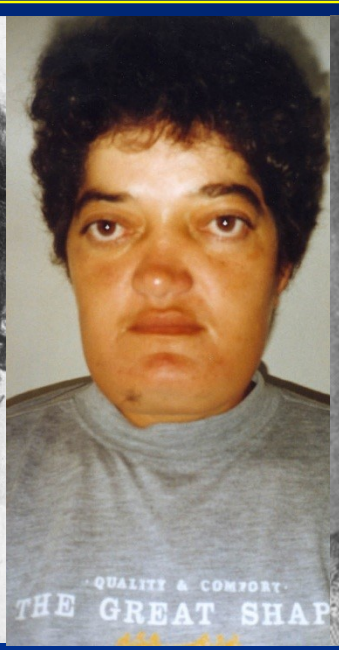


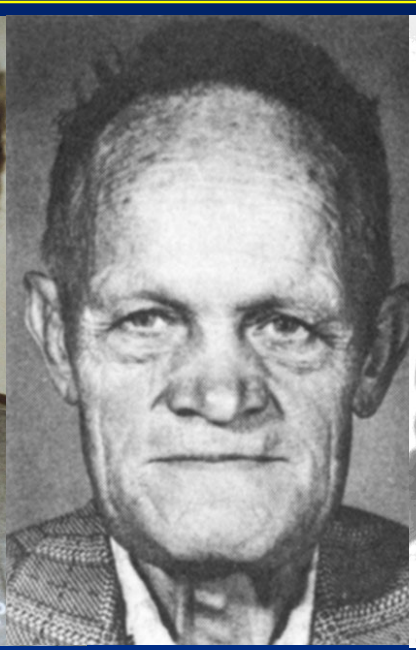
International working group on the classification and nosology of constitutional disorders of bone
(Martigny, 2005)

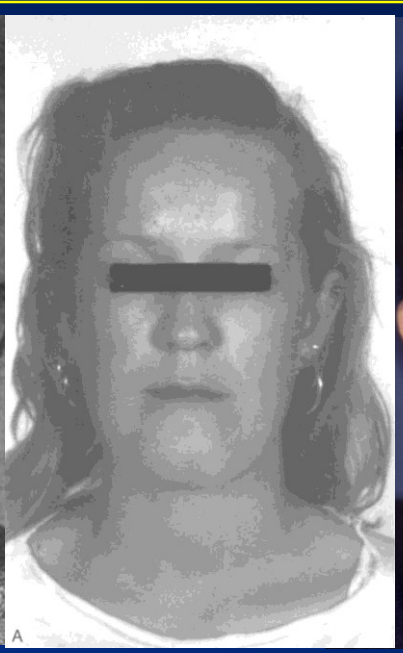
About 40 different clinical entities with increased bone density

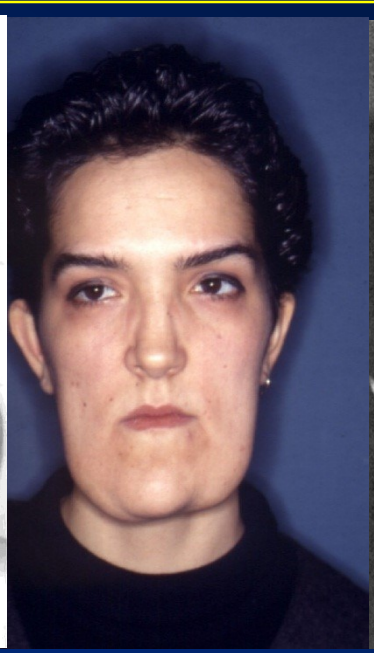
Increased bone formation

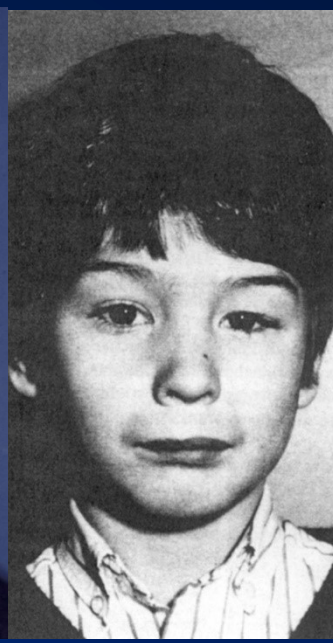








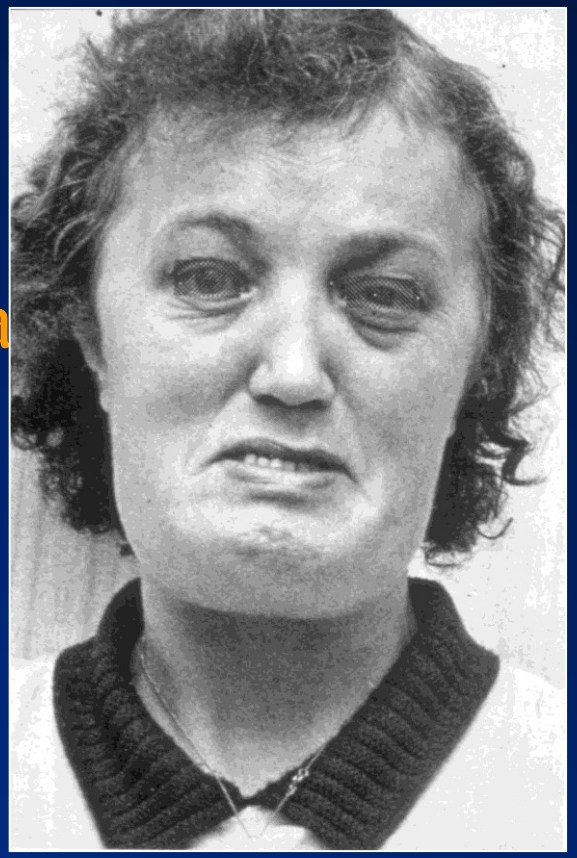




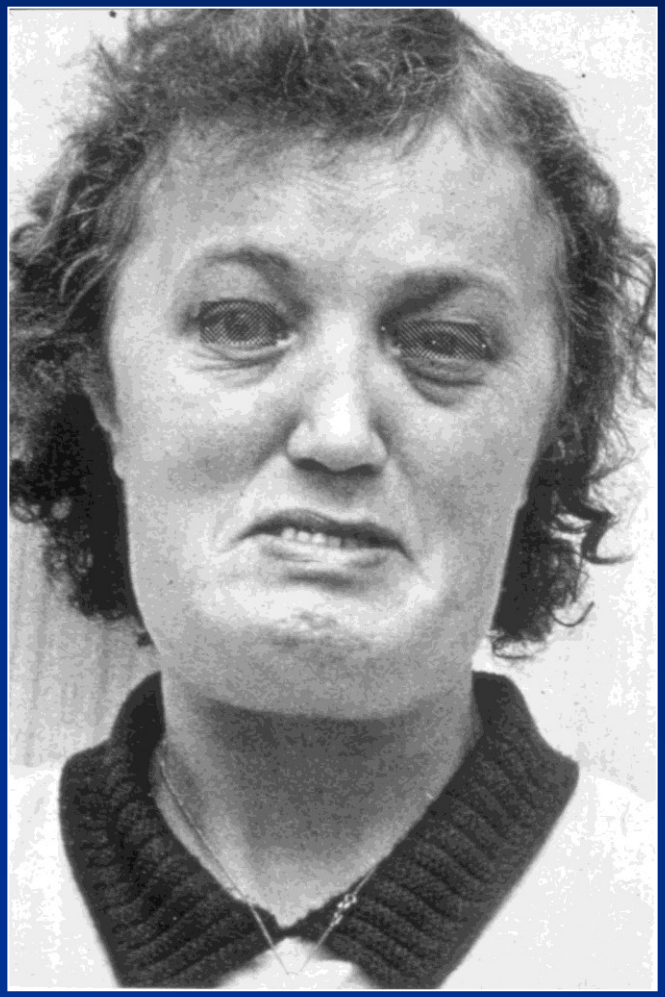
Increased bone formation

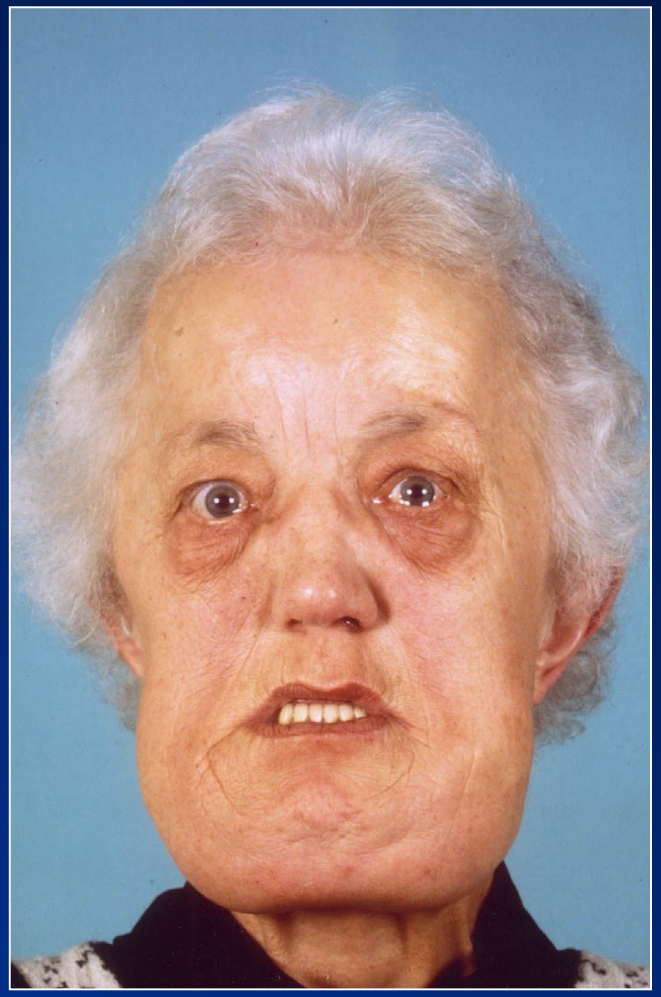
Van Buchem disease

Hyperostosis corticalis generalisata



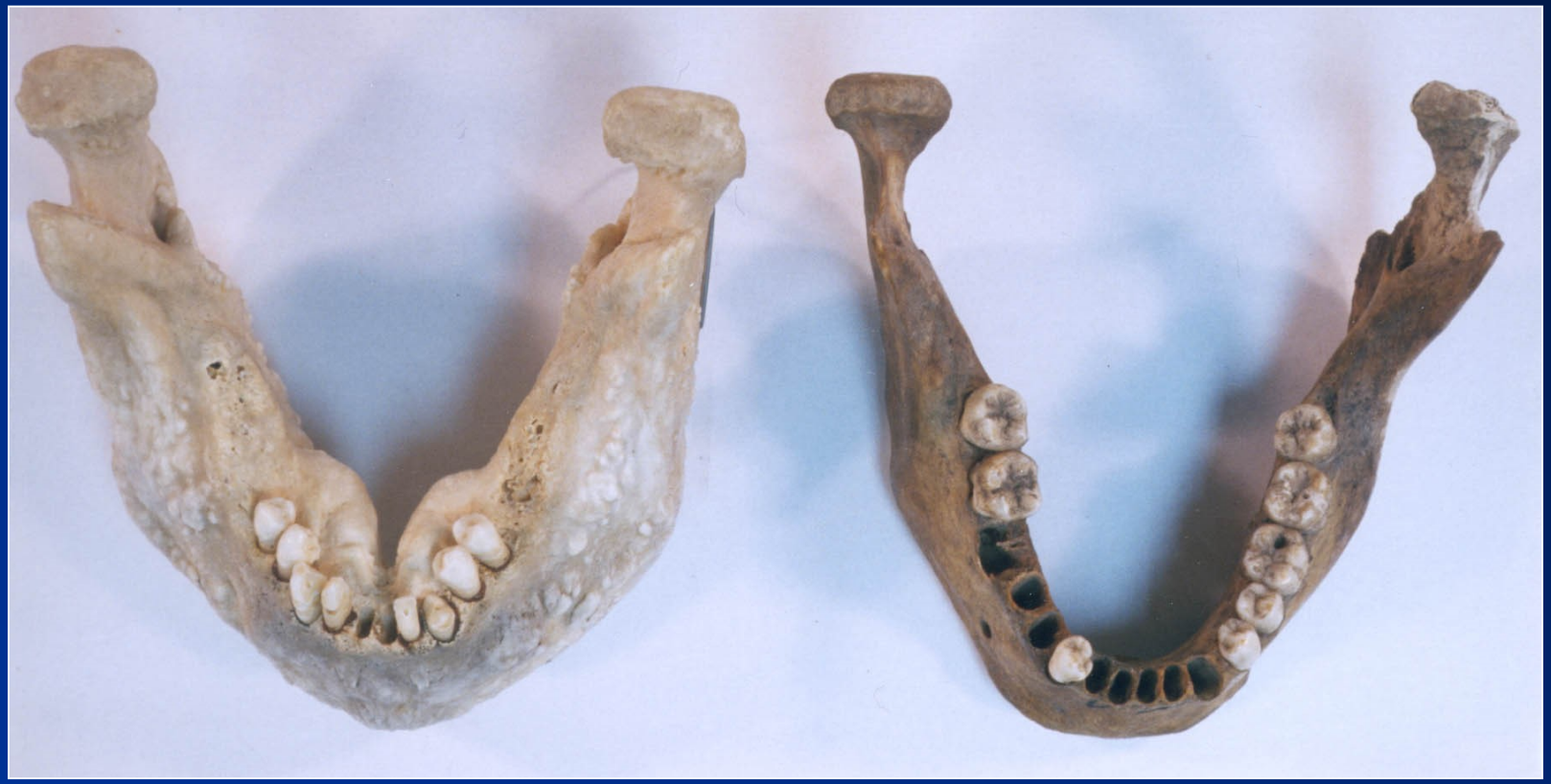
- enlargement of the jaw
- thickening of the skull
  - –> Nerve encroachment
    - facial nerve palsy
    - hearing loss





van Buchem patient

Control



Van Buchem disease

Incidence : very low

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

11 patients

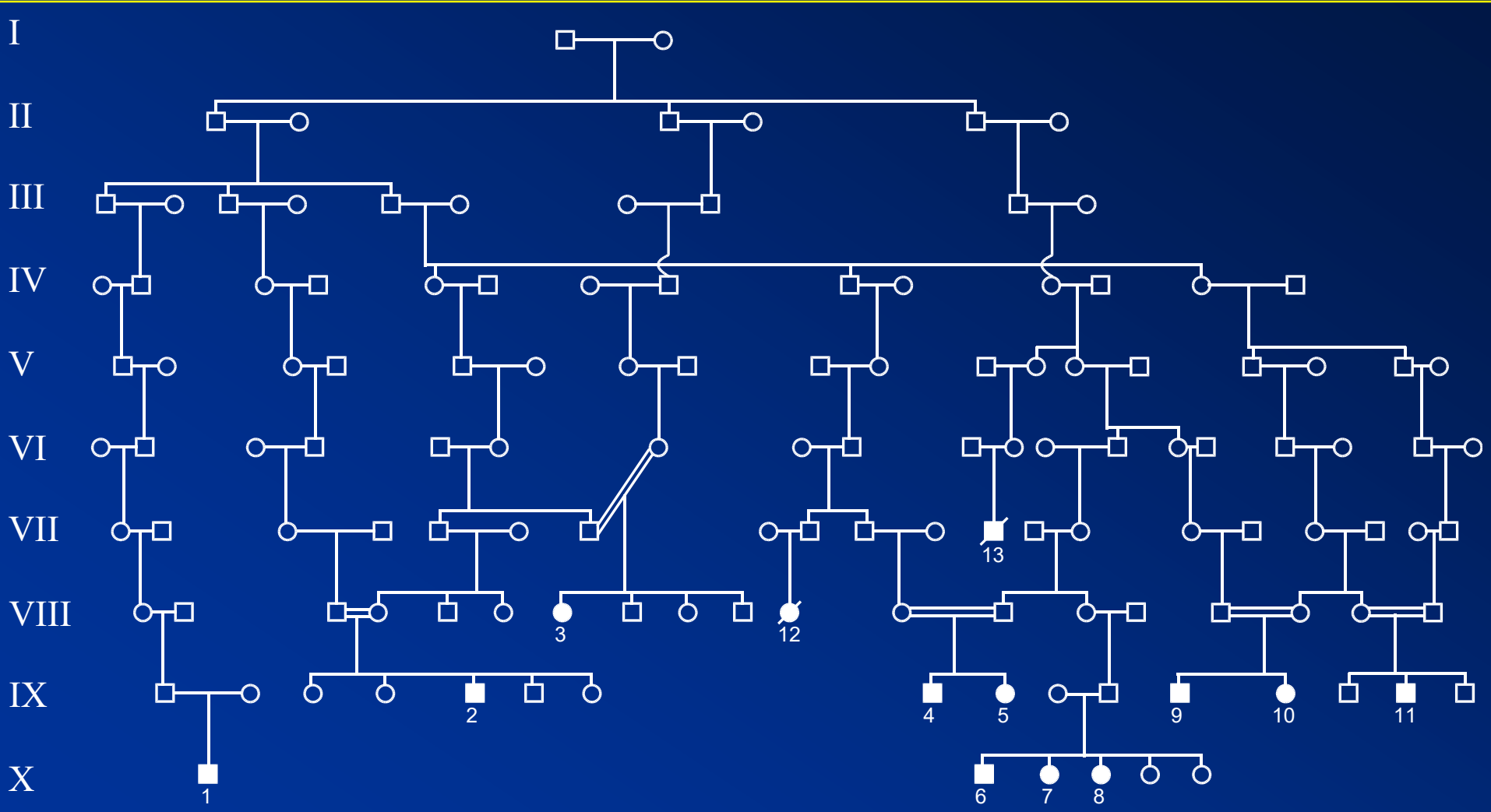

Noordzee
Waddenzee
IJsselmeer
Noordzeekanaal
Vecht
Eem
IJssel
Vecht
1980


Noordzee
Harlingen
Medemblik
Zuiderzee
Zwolle
Amsterdam
Amersfoort
overstroming 1916

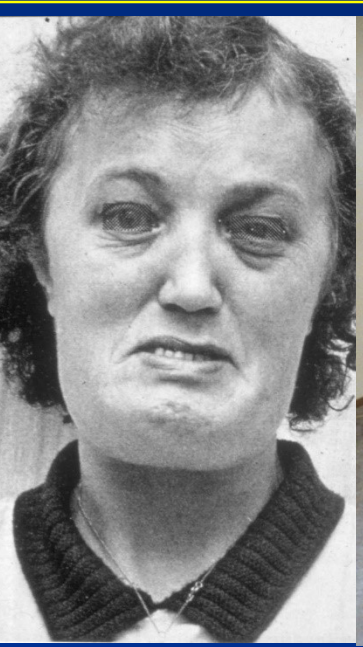
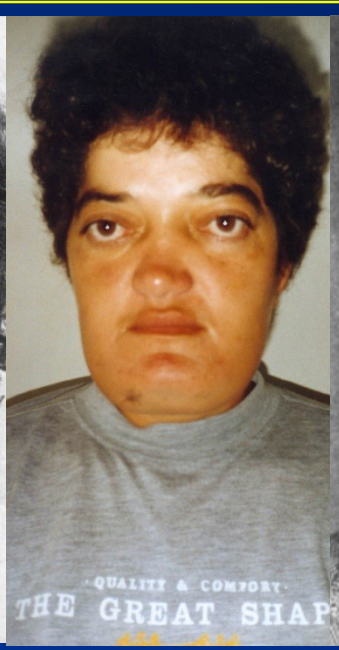
Ethnic isolate

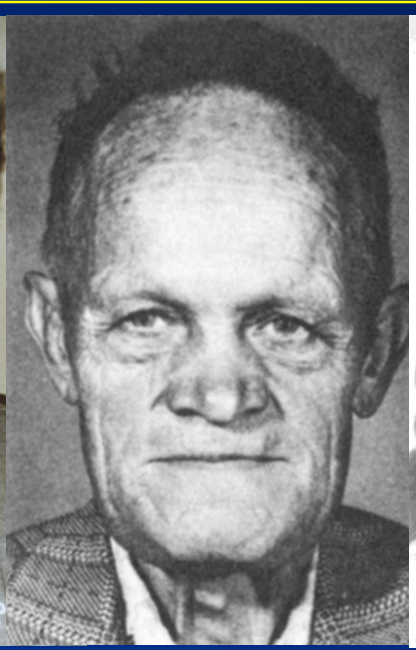
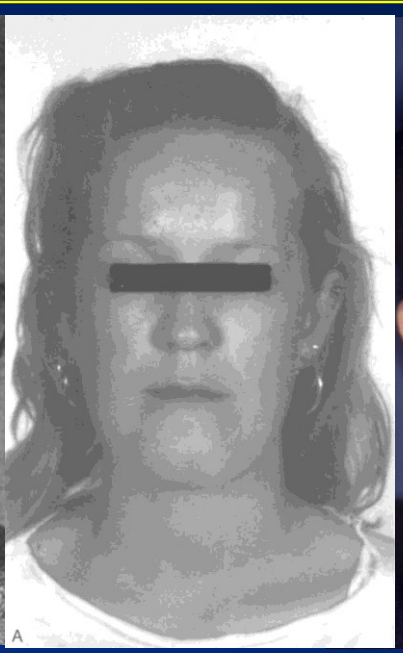
- Island until 1941
- Geographically, religiously and professionally isolated
- In 1637: 151 inhabitants
- Currently 16.000 inhabitants
- Most inhabitants related to each other

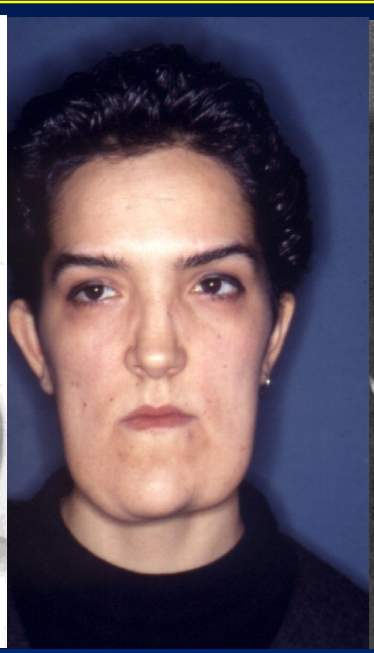
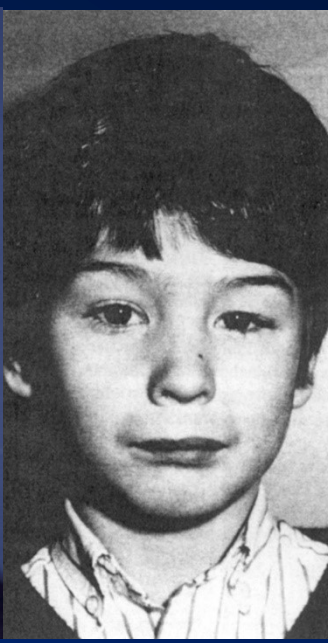
Dutch van Buchem family



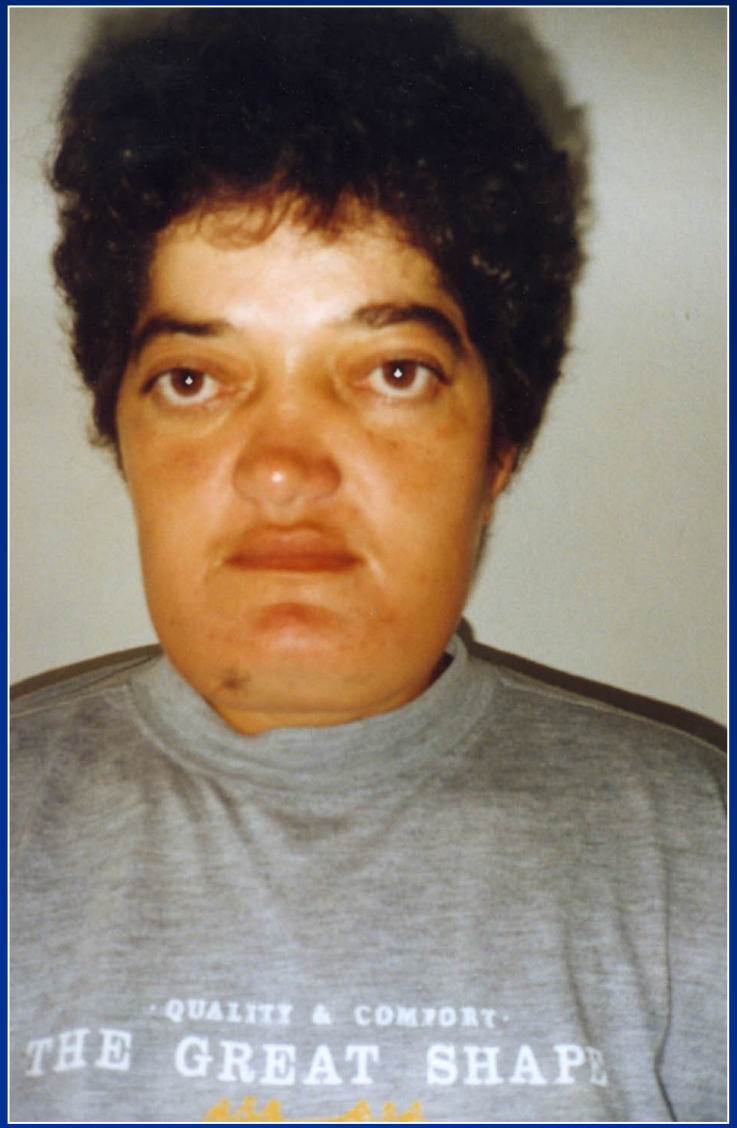
Increased bone formation

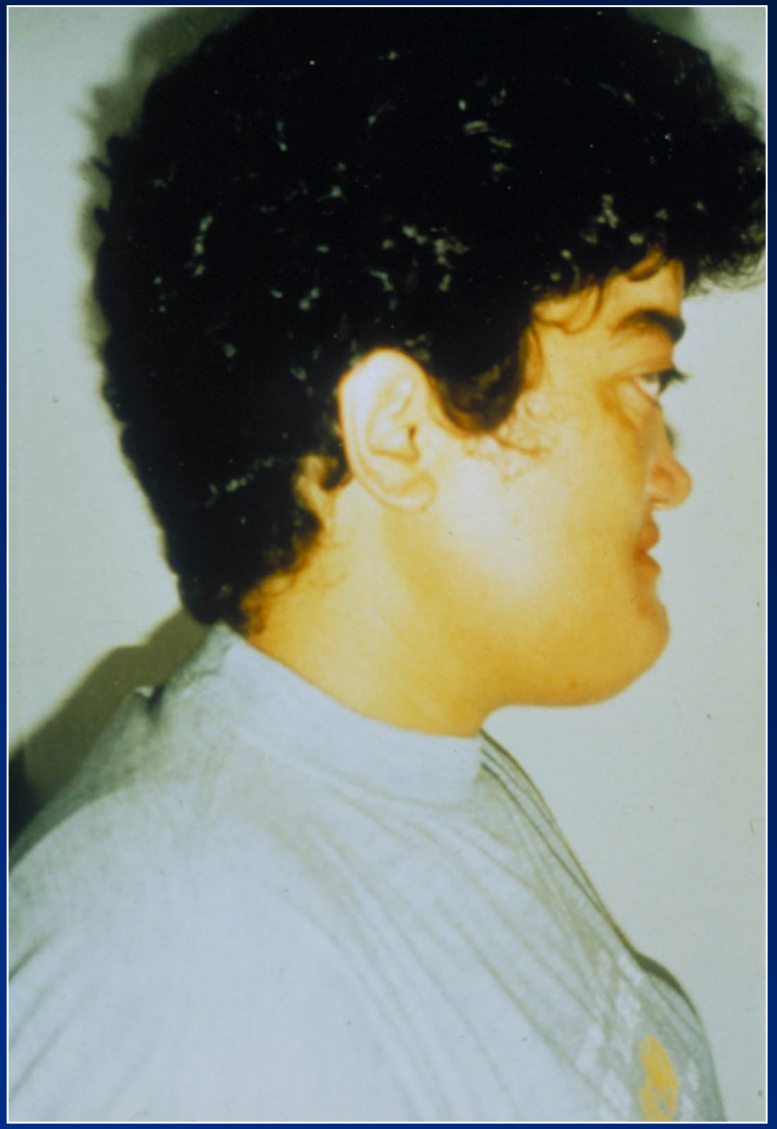



Van Buchem disease


·QUALITY & COMFORT·
THE GREAT SHAPE

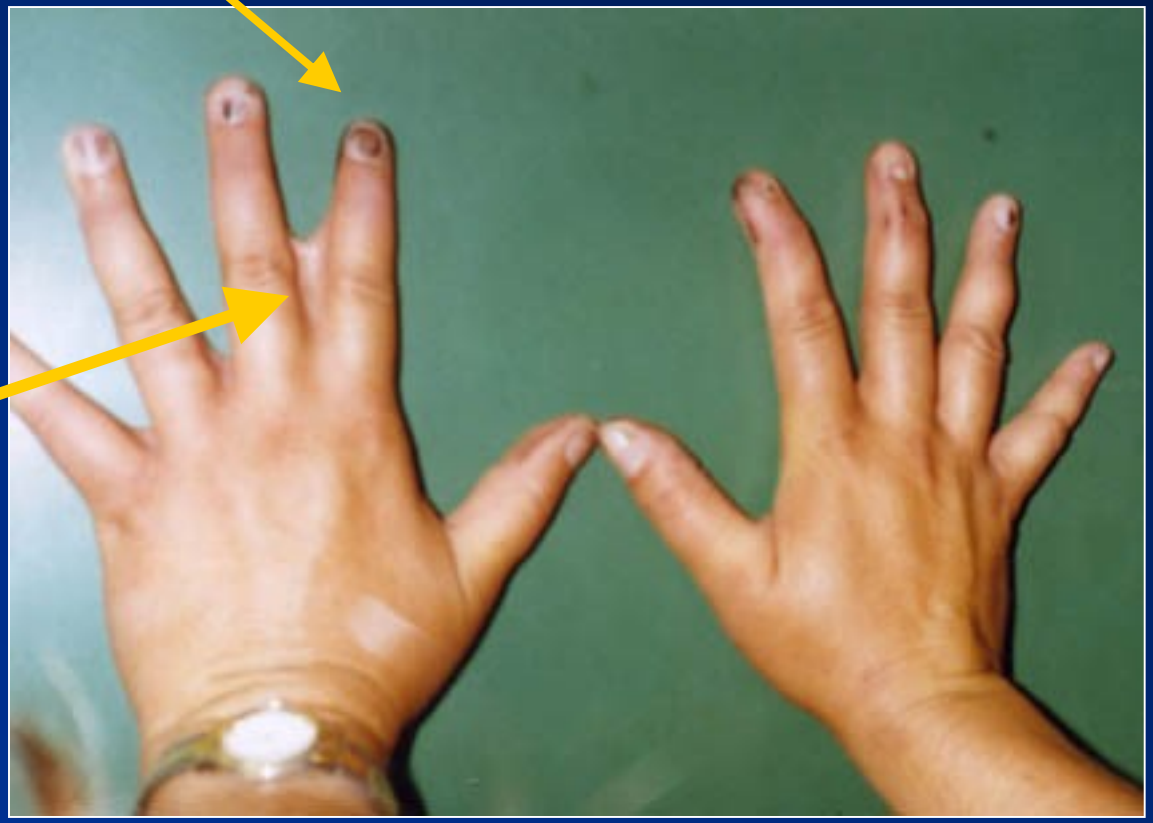


Differential diagnosis

Sclerosteosis

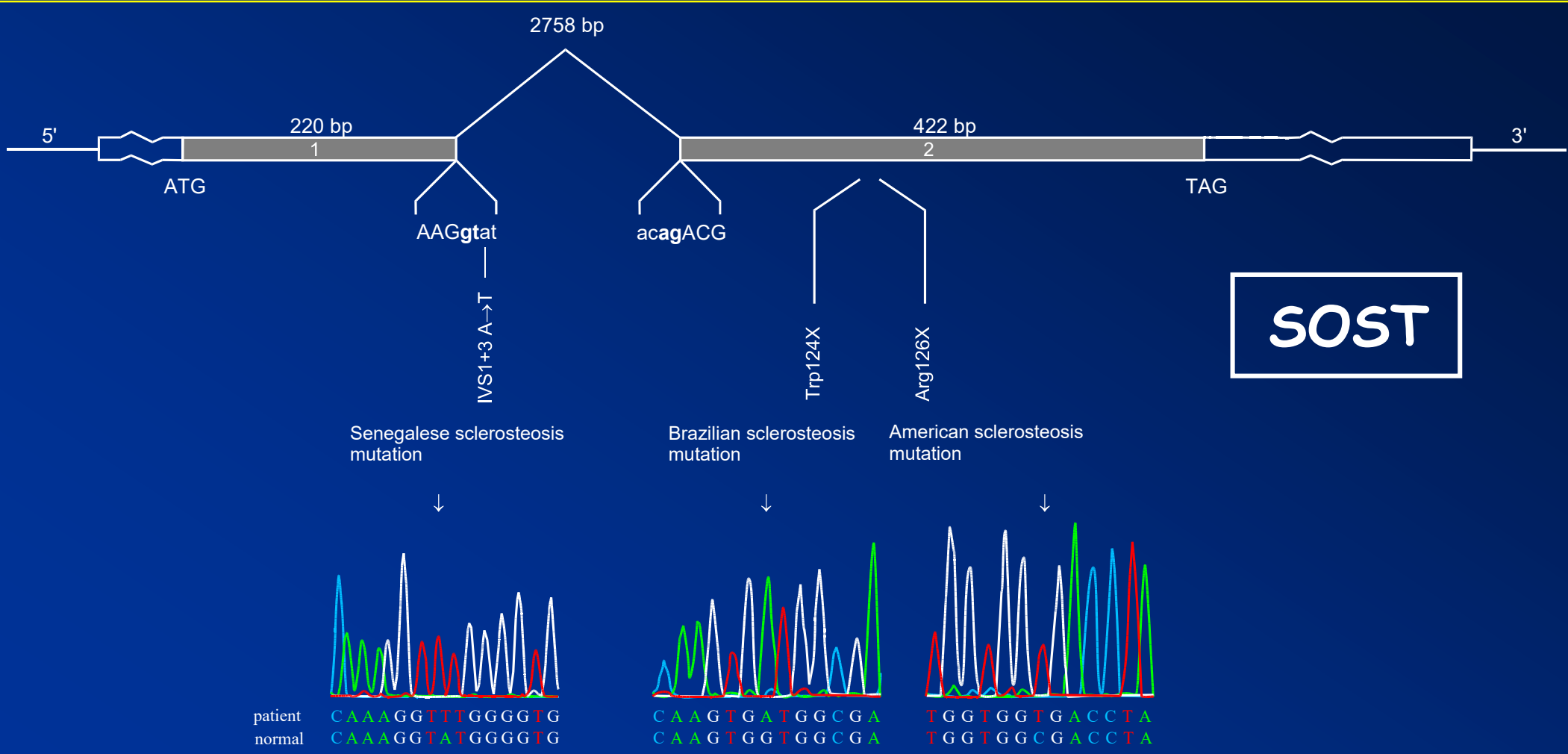
- gigantism
- more severe character
- hand malformations

nail dysplasia

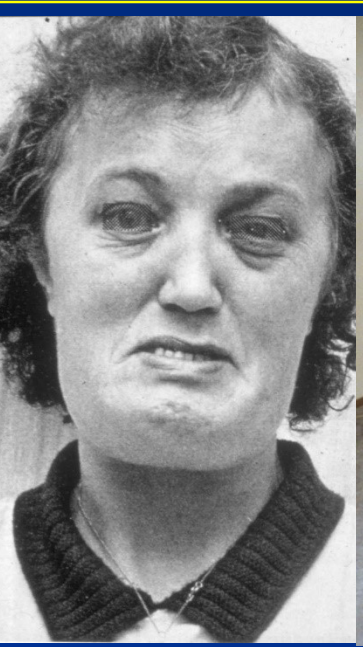
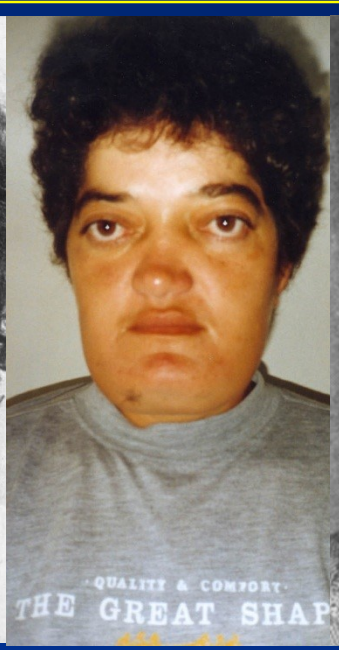


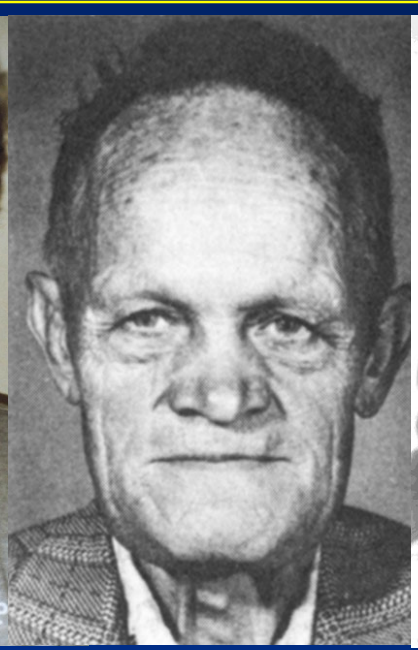
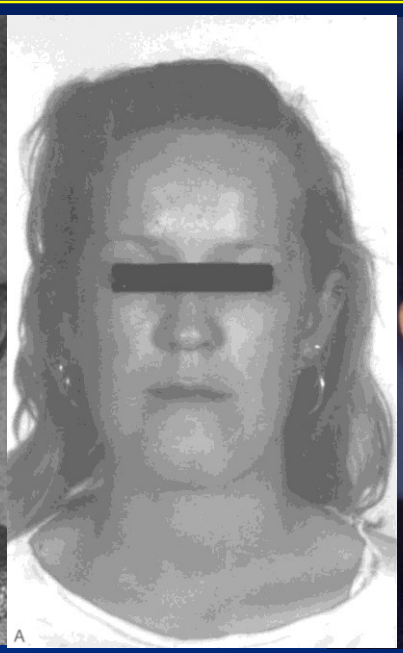
syndactyly

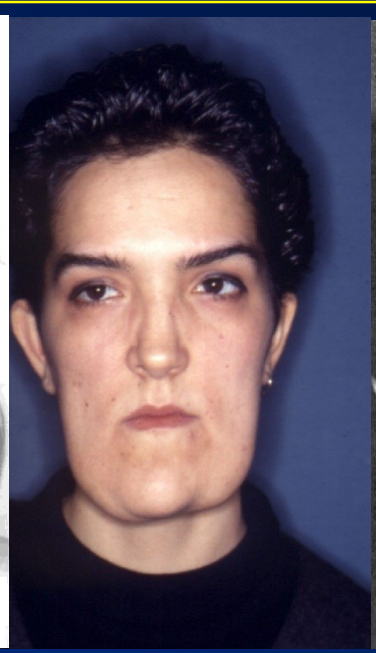
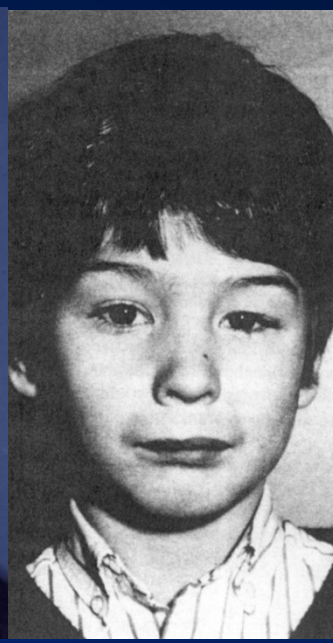
Gene identification



Increased bone formation

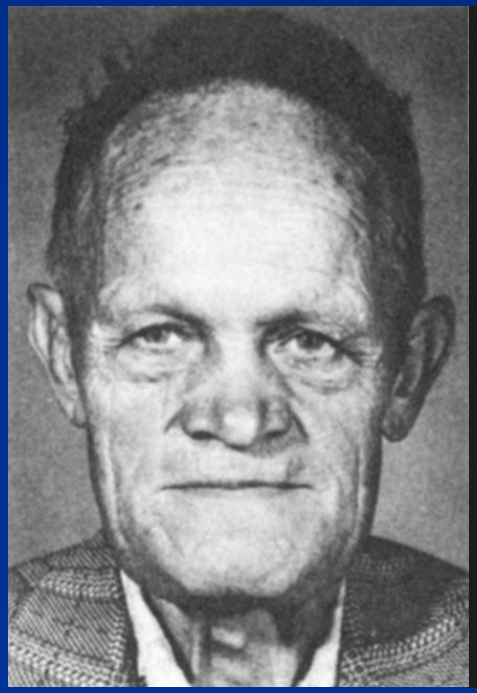



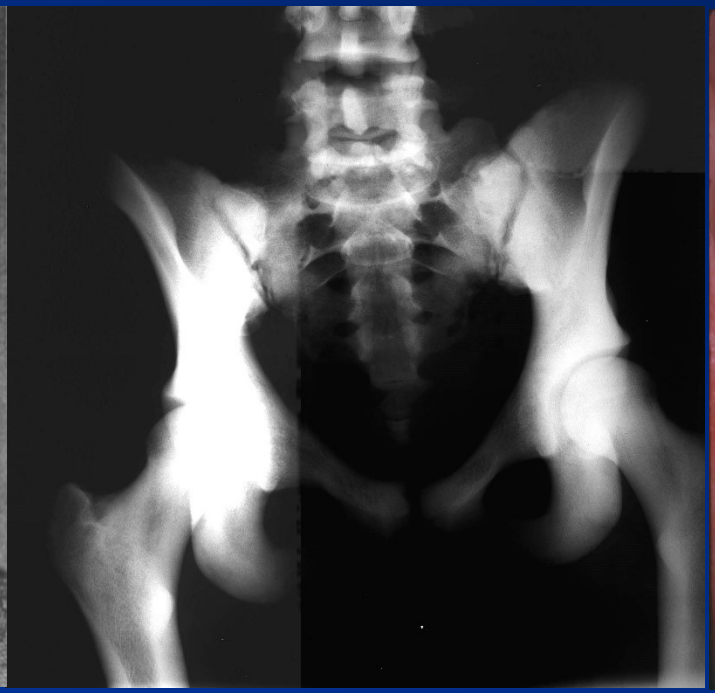



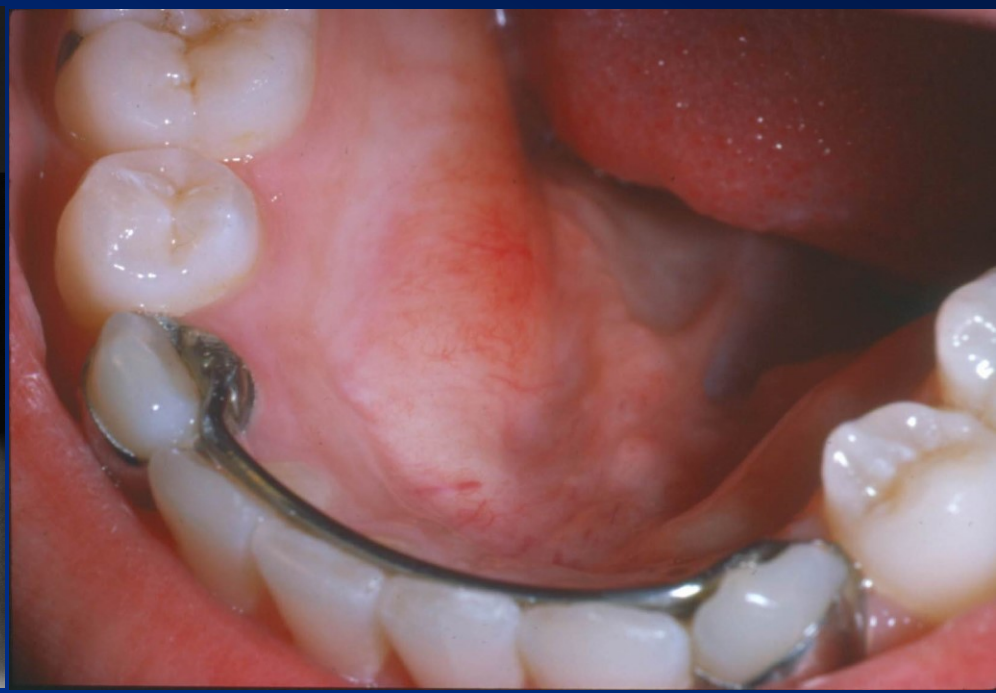
Van Buchem disease (*SOST*)

Sclerosteosis (*SOST*)

Endosteal hyperostosis



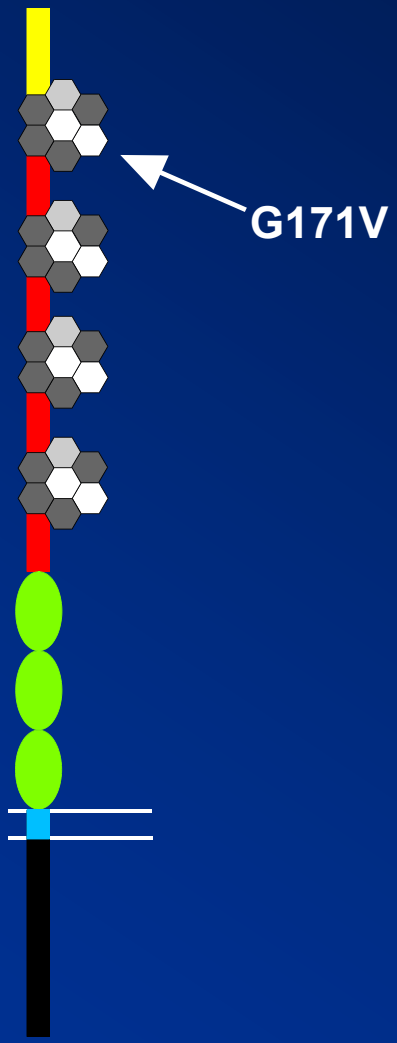




High Bone Mass-phenotype

- 2 families
  - Johnson *et al*. 1997
  - Boyden *et al*. 2002
- Cortical thickening of the long bones
- Same *LRP5* mutation (G171V)

LDL-Receptor-Related protein 5 (LRP5)

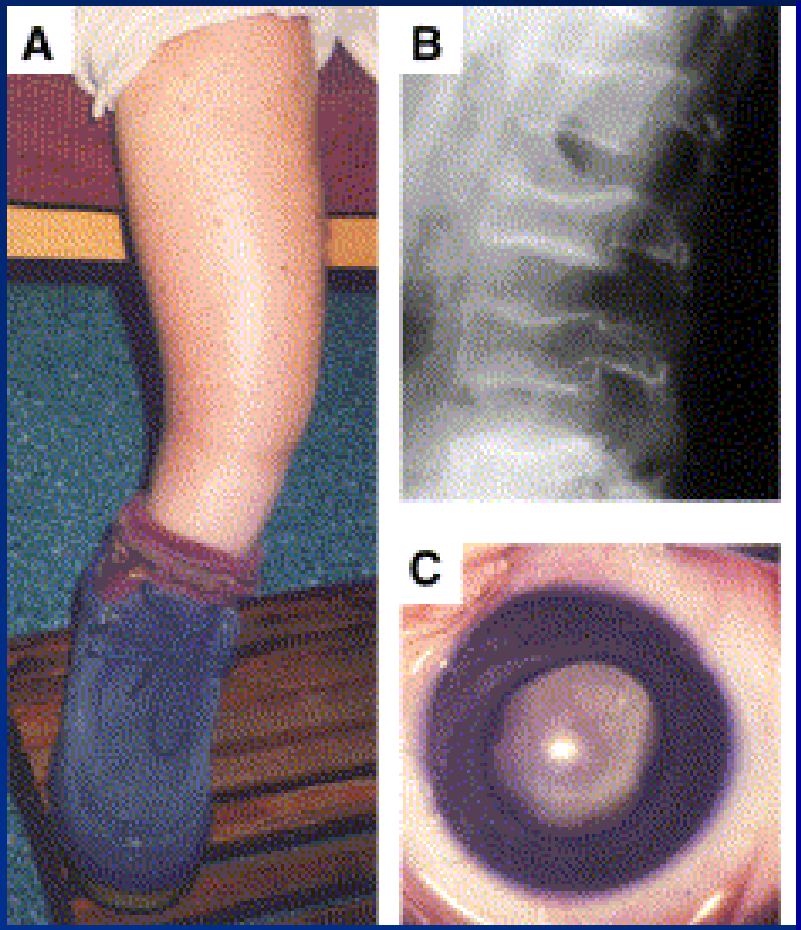


Osteoporosis pseudoglioma syndrome

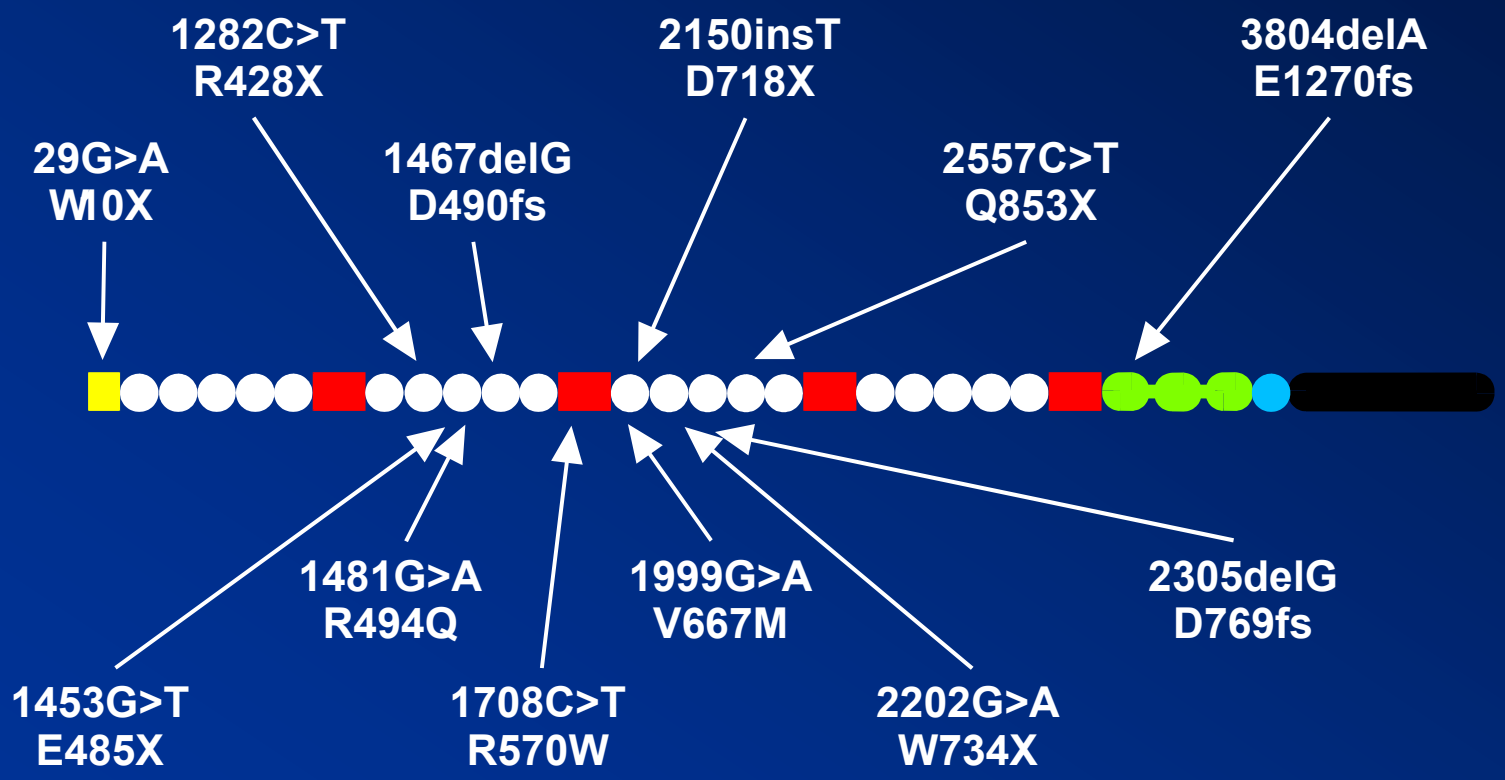
-Autosomal recessive

-Juvenile osteoporosis

-Congenital blindness

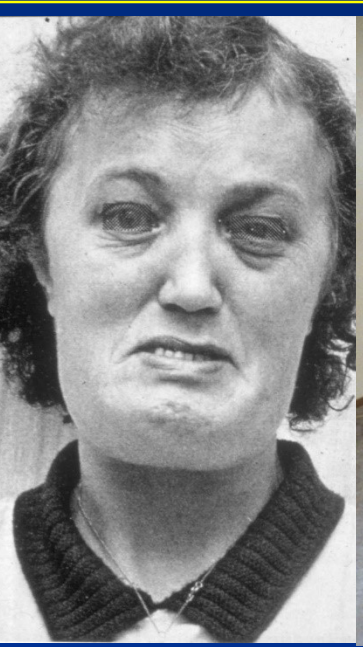
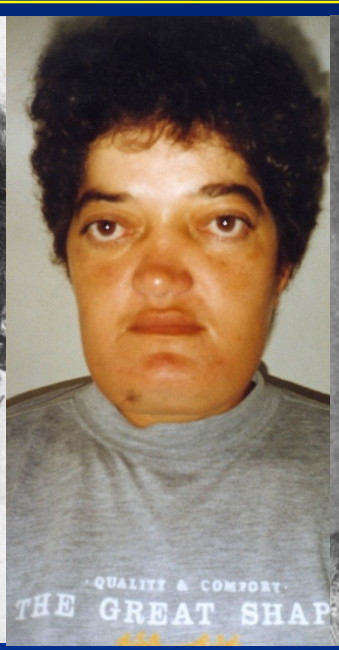


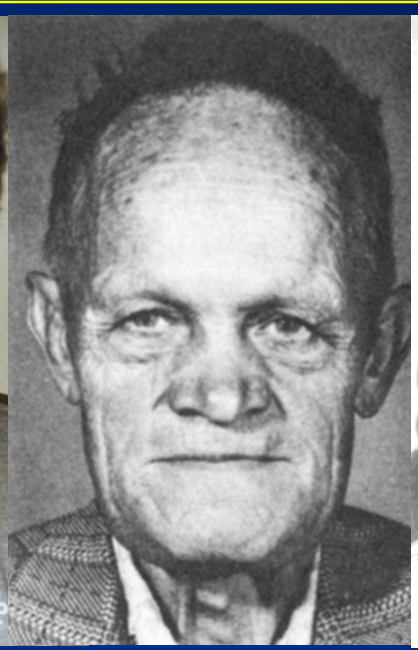
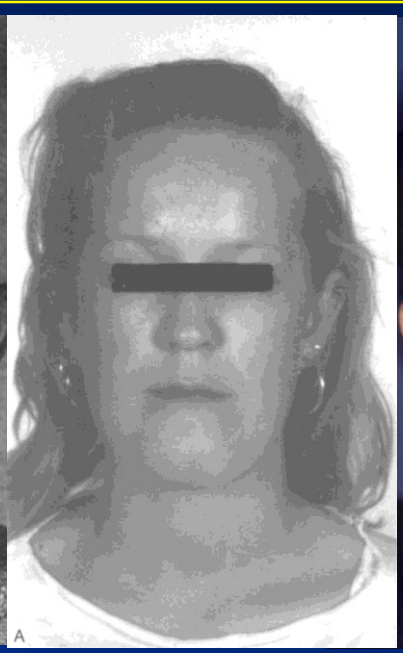
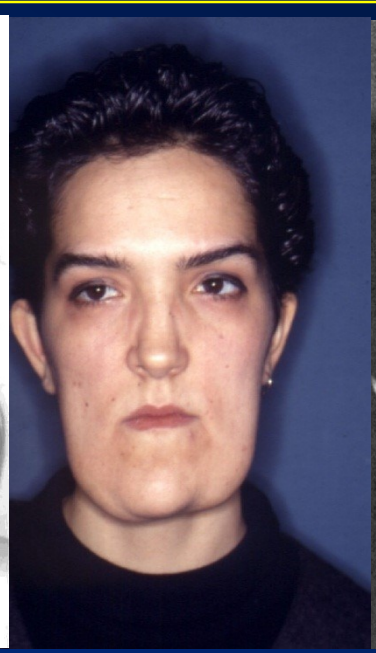
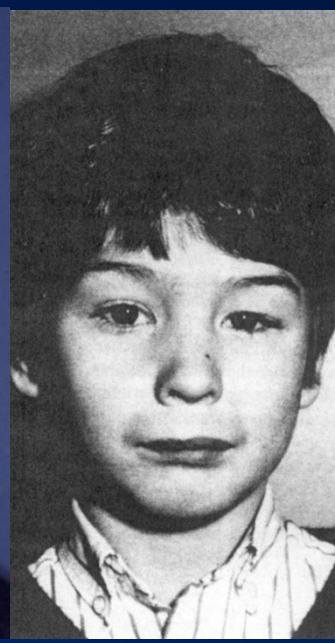
Mutations in LRP5 gene



Osteoporosis-pseudoglioma syndrome (OPPS)

Increased bone formation

Van Buchem disease (*SOST*)

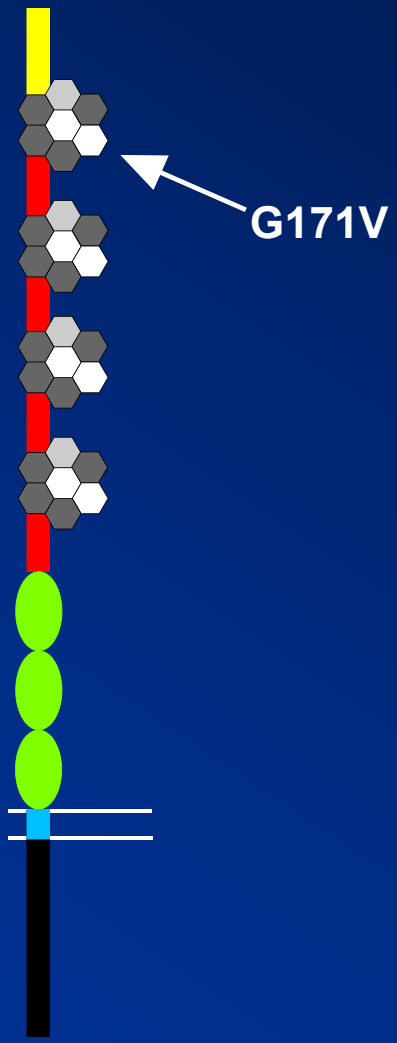
Sclerosteosis (*SOST*)

Endosteal hyperostosis (*LRP5*)

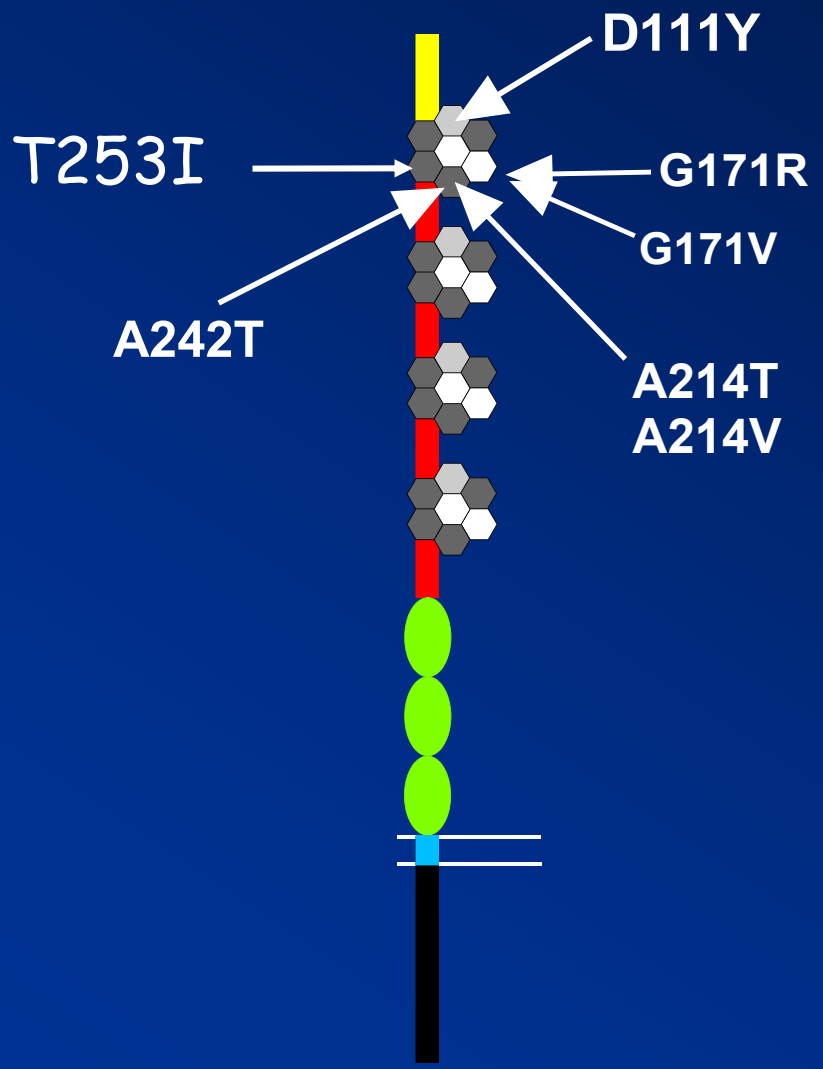
Aut dom osteosclerosis (*LRP5*)

"Van Buchem" (*LRP5*)

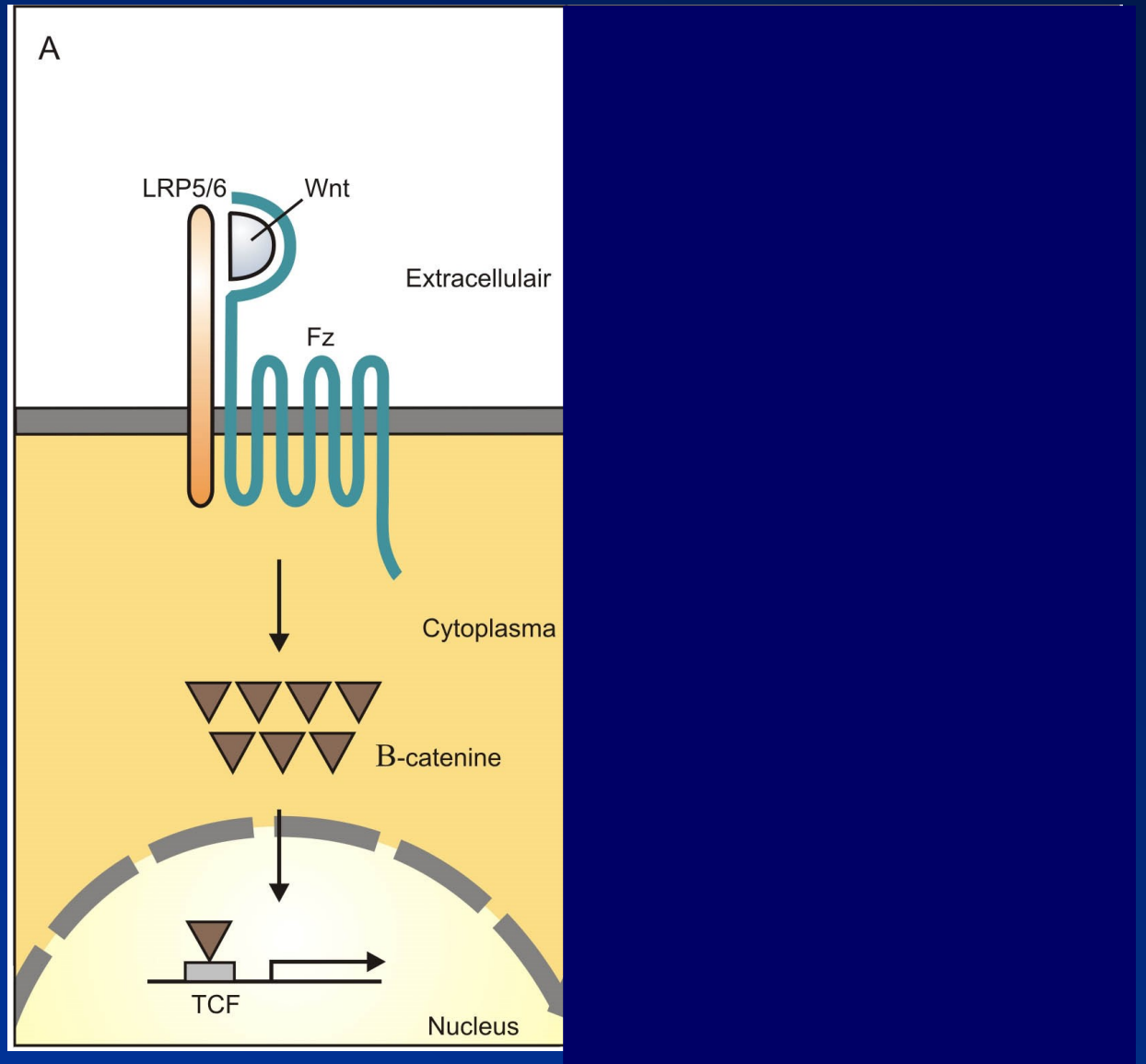
LRP5



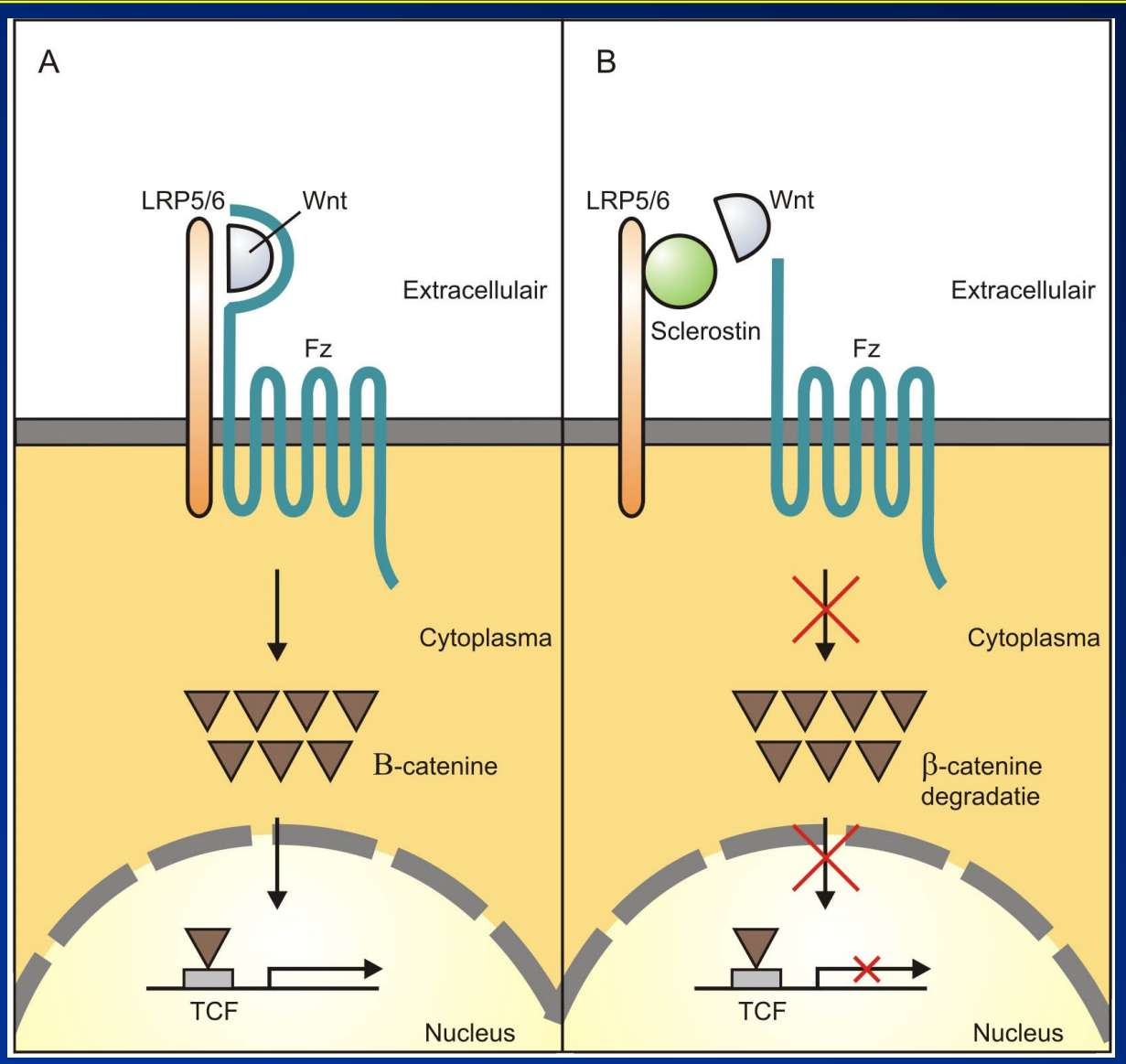
LRP5



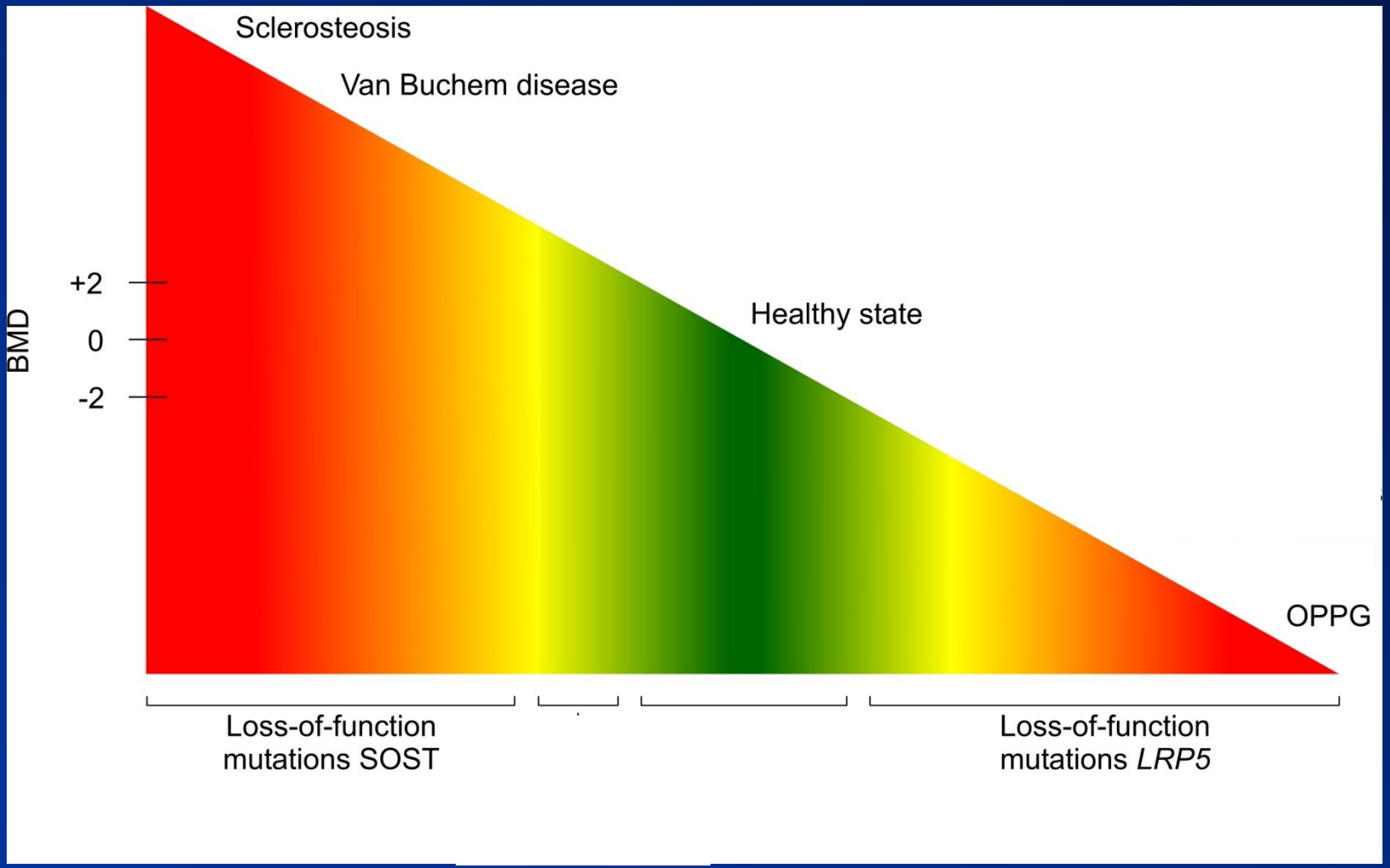
Canonical Wnt signaling



Sclerostin-LRP5



Genetic variation within SOST and LRP5 genes



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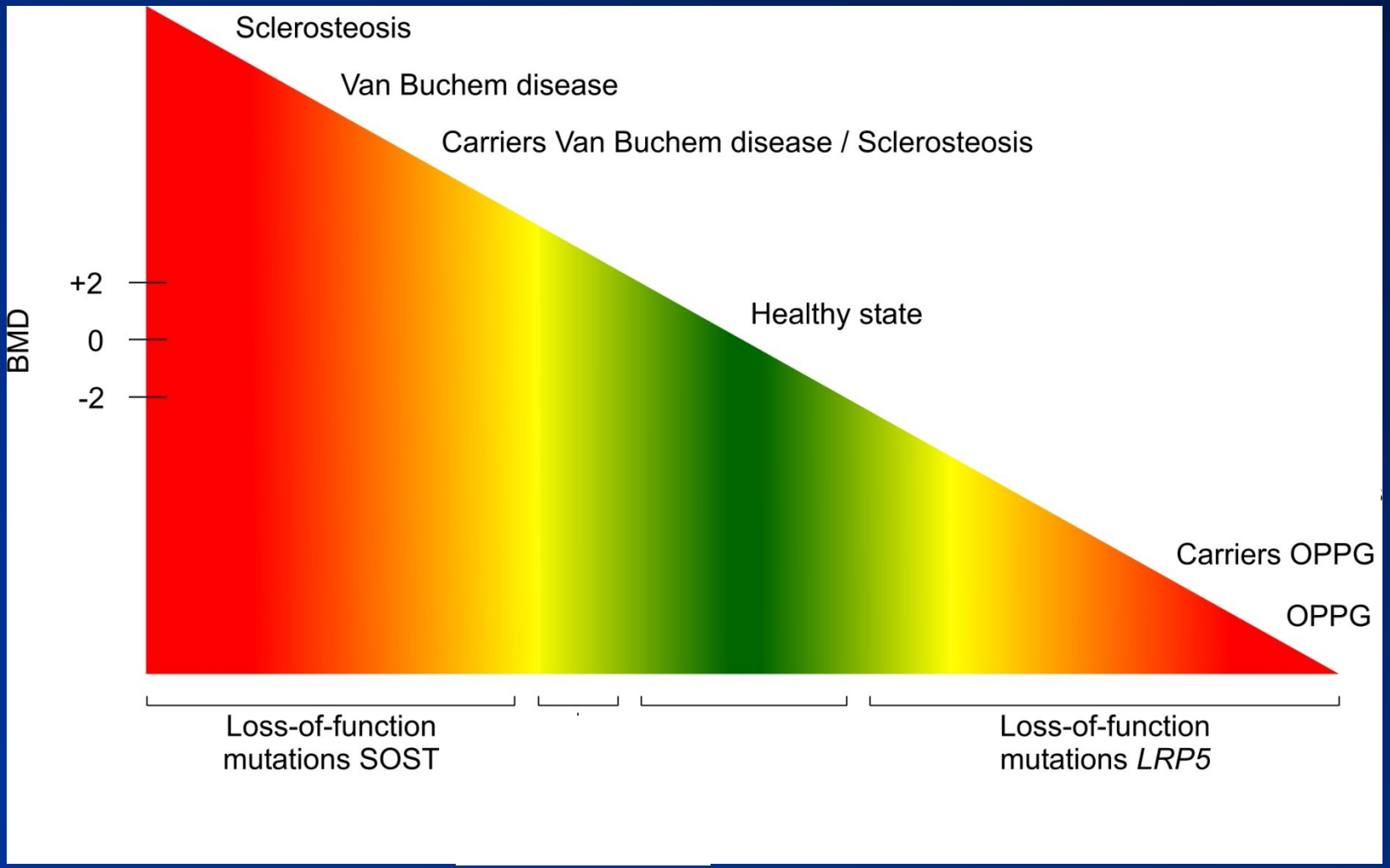

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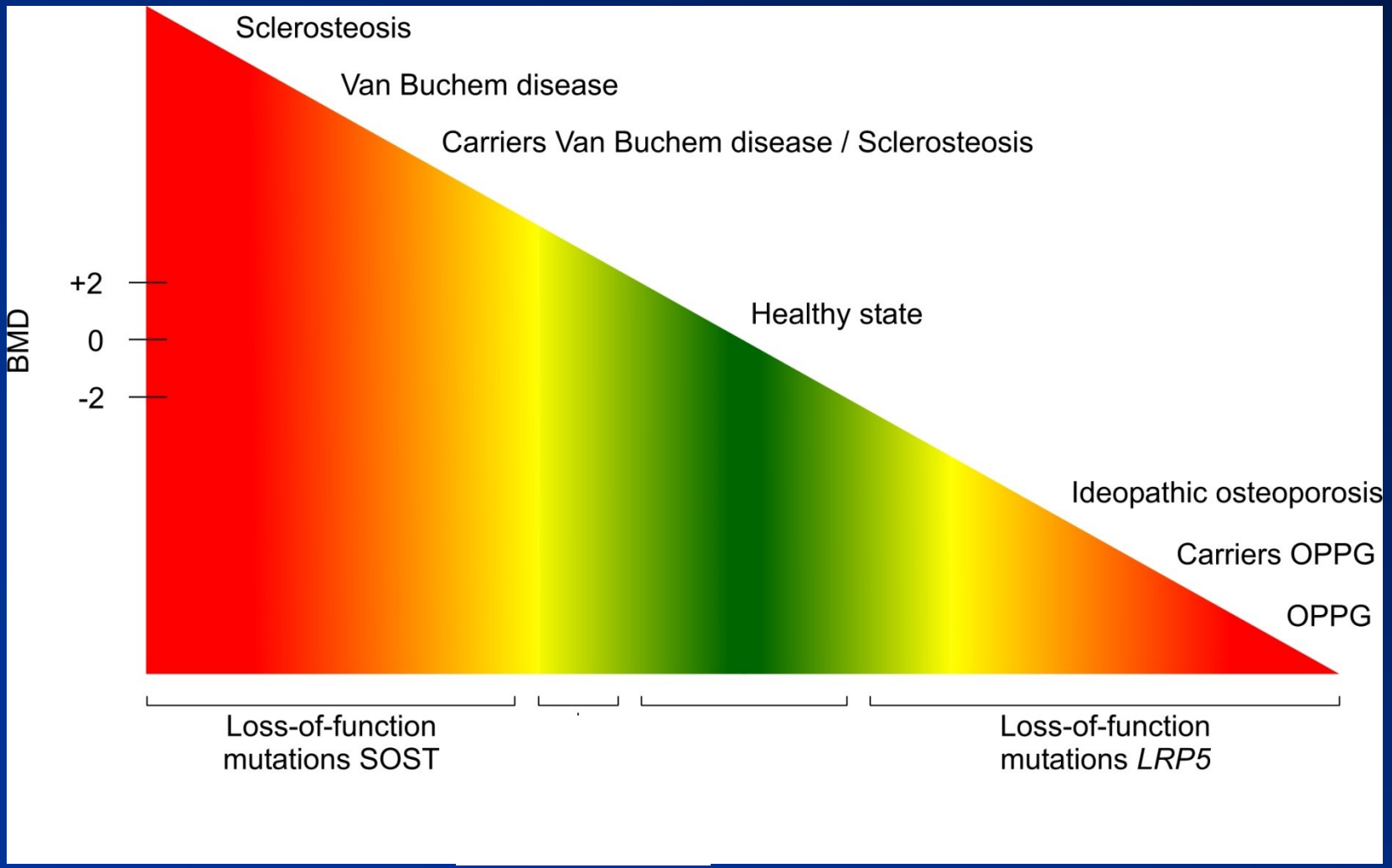

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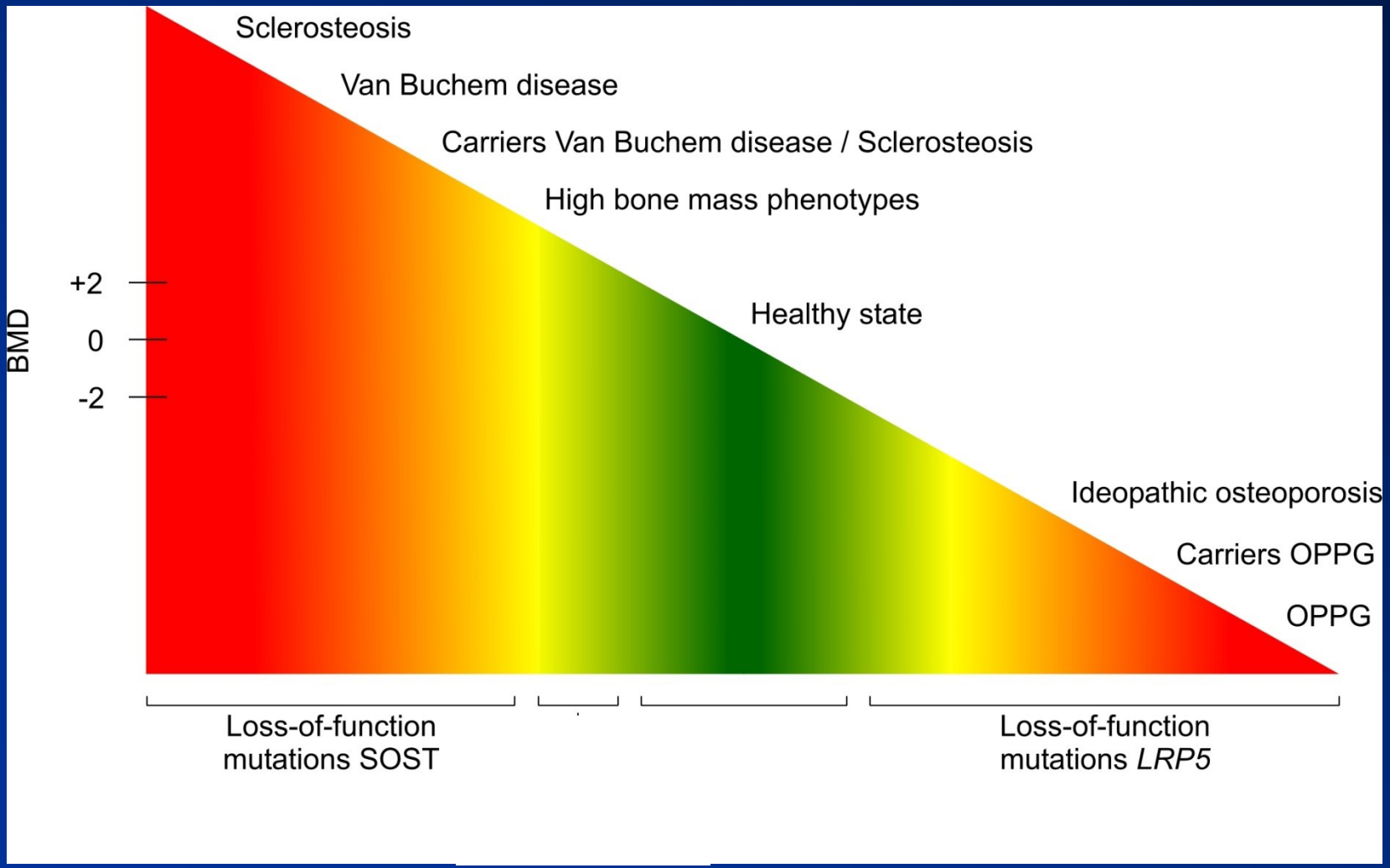

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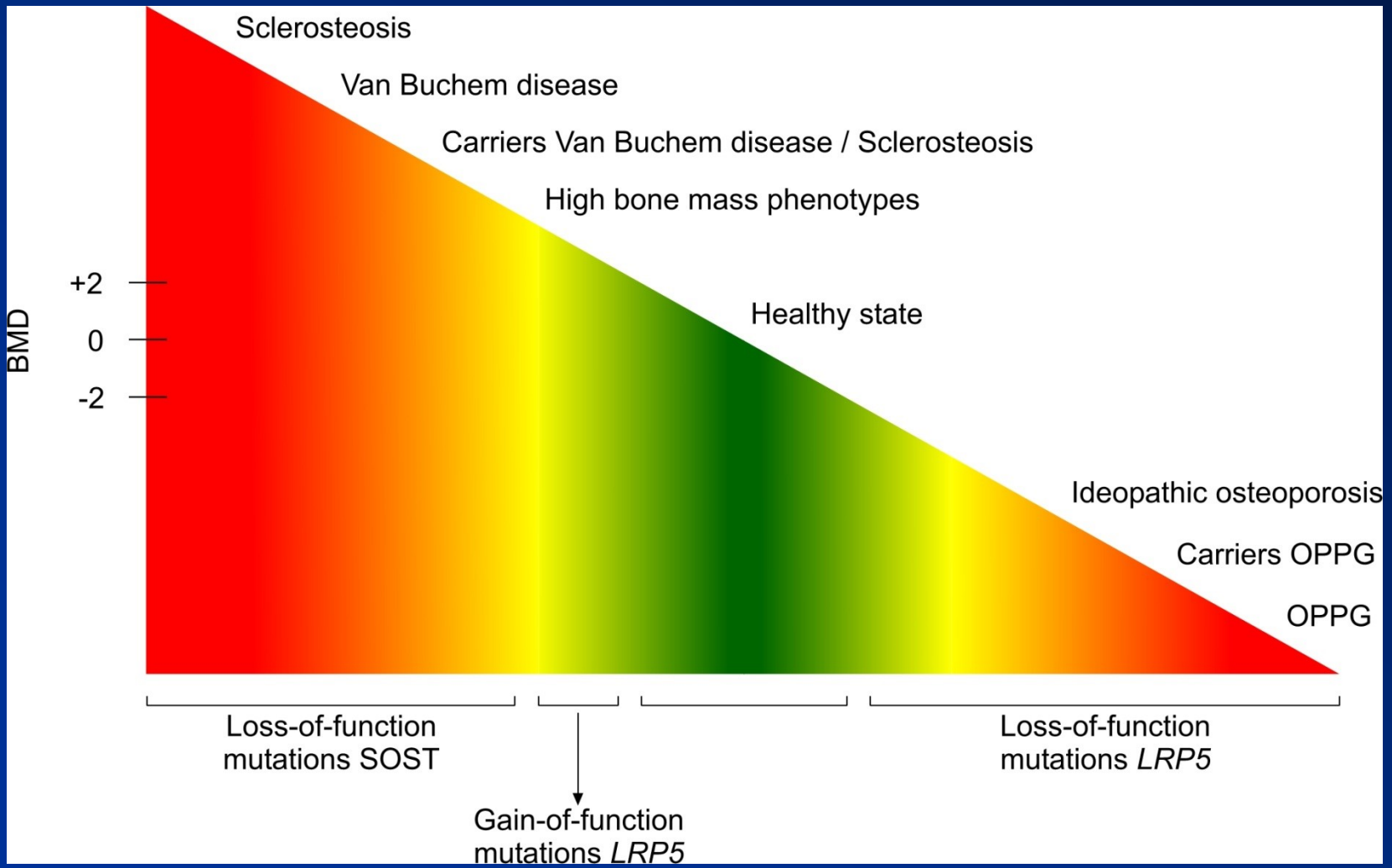

Genetic research of osteoporosis

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

How to identify genes for complex traits

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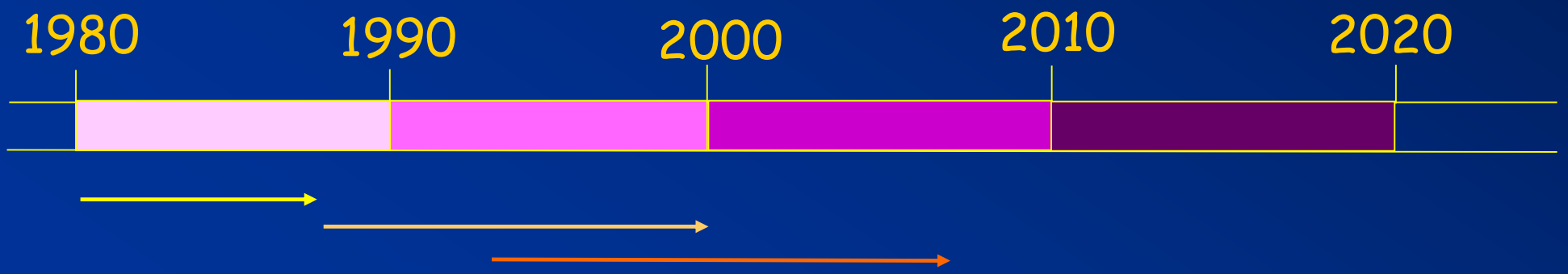
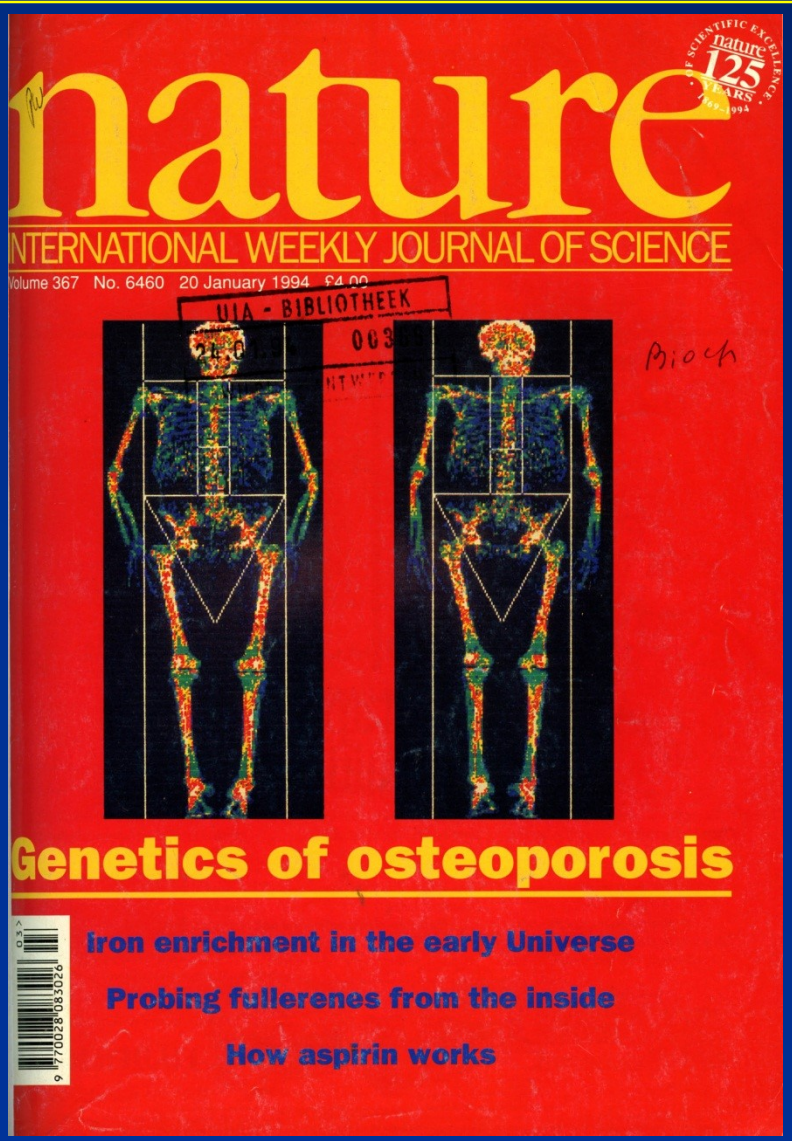


1994: osteoporosis gene!



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

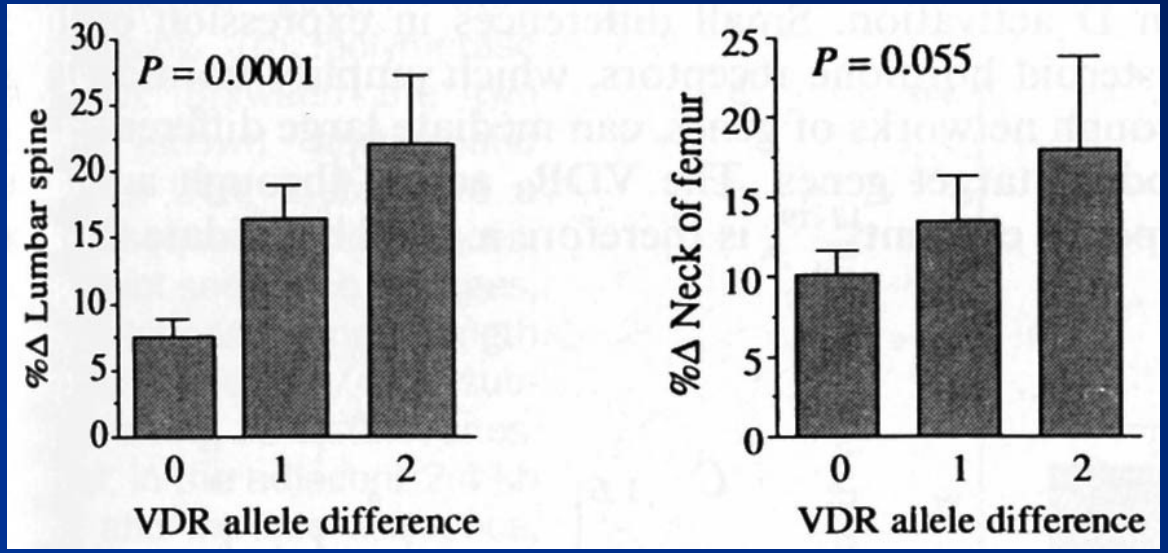
Bone and Mineral Research Division,
Garvan Institute of Medical Research, St Vincent's Hospital,
Sydney, New South Wales 2010, Australia

1994: osteoporosis gene!

250 healthy Caucasian twins (Australia)

BMD measurements at different sites

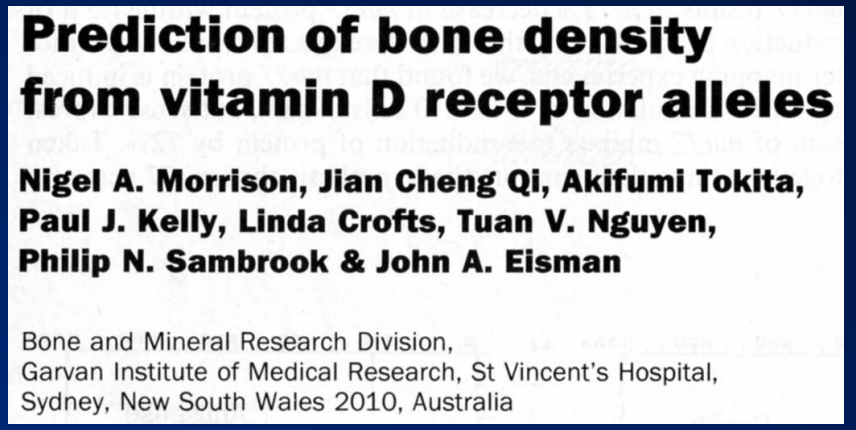
75% of genetic effect on bone density explained



1997

Nature 387: 106 (1997)

Erratum



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"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.

1588 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)

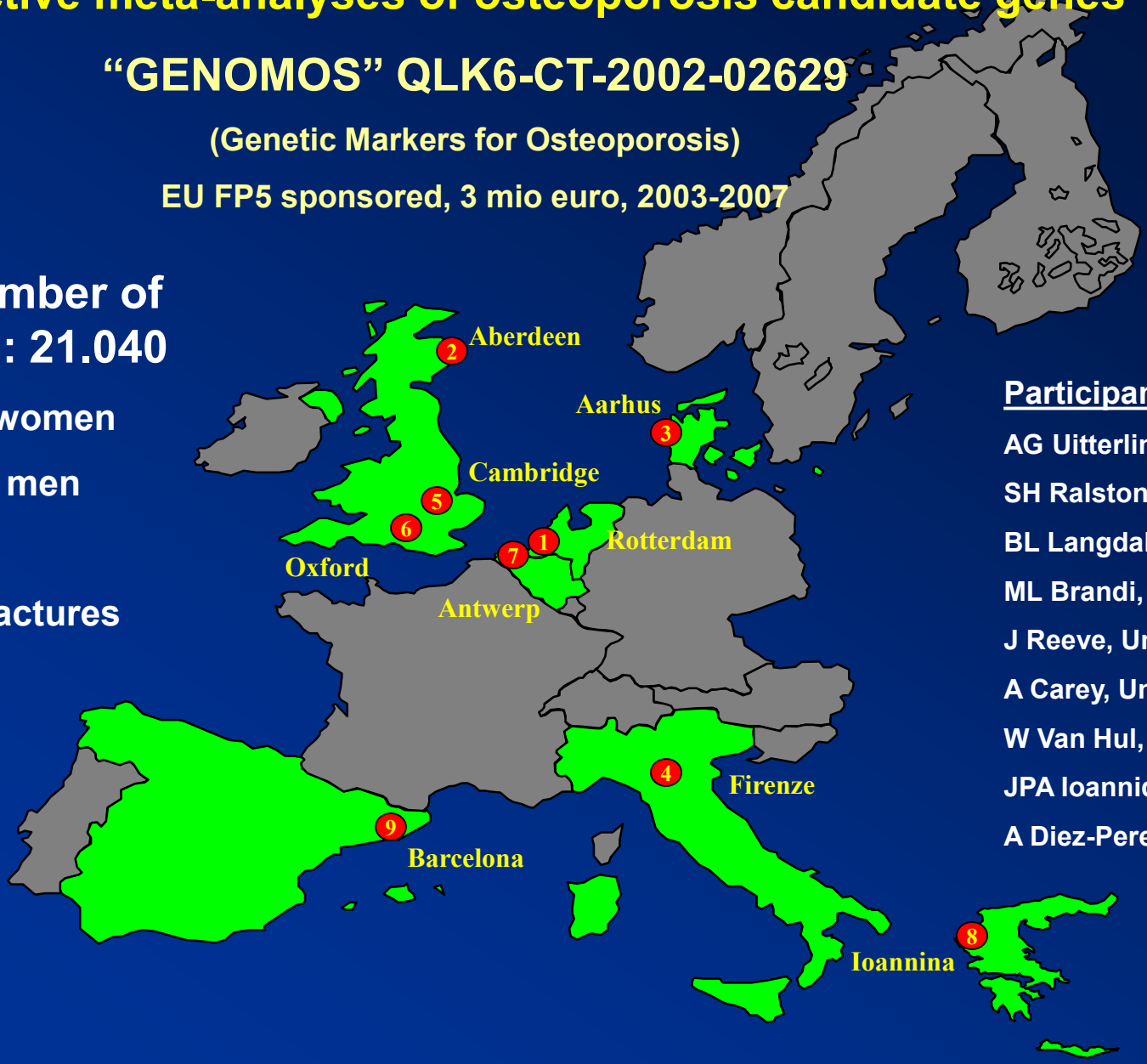
EU FP5 sponsored, 3 mio euro, 2003-2007

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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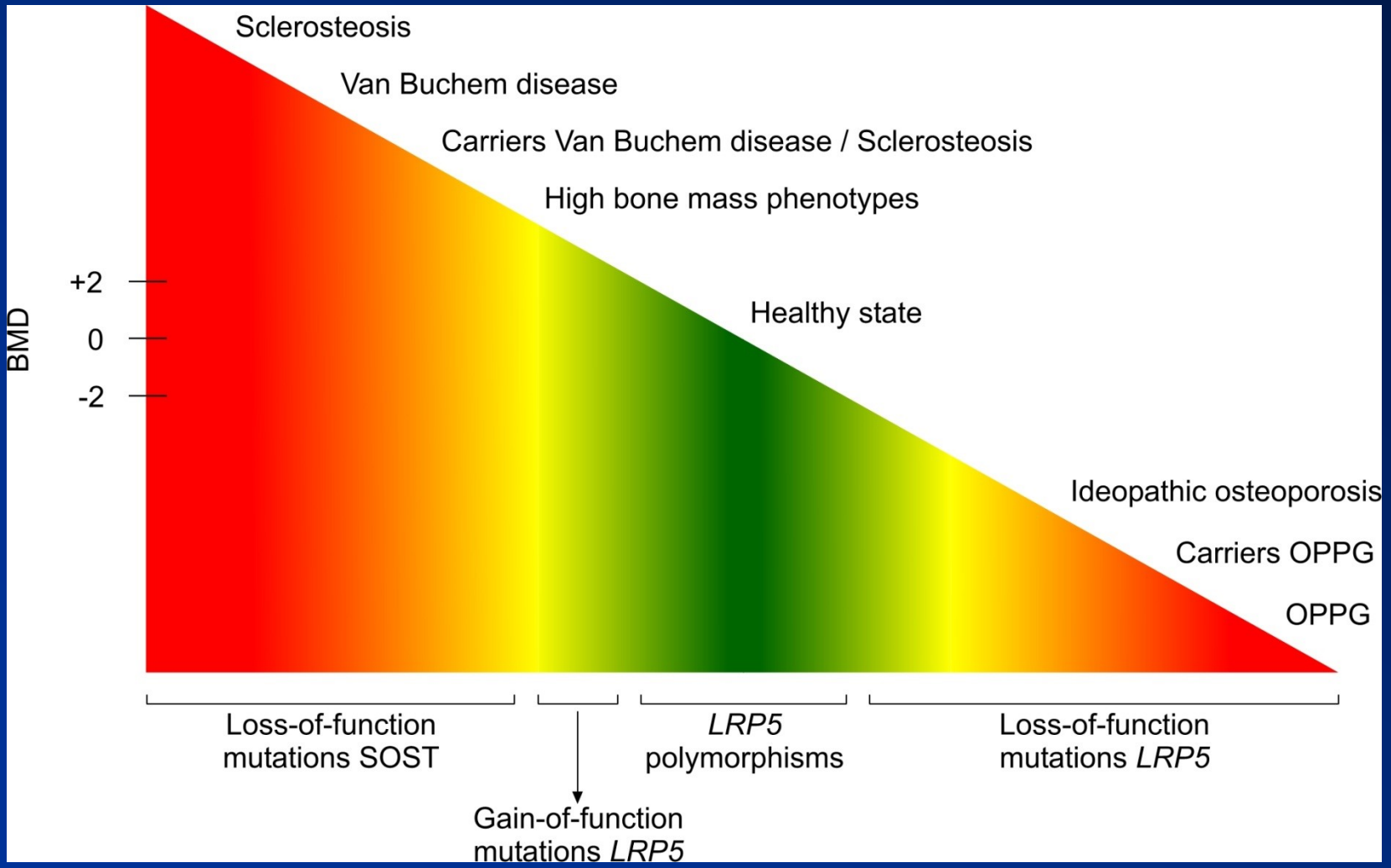

How to identify genes for complex traits

Identification of genes for relevant monogenic conditions

1. functional candidate gene approach
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Association studies

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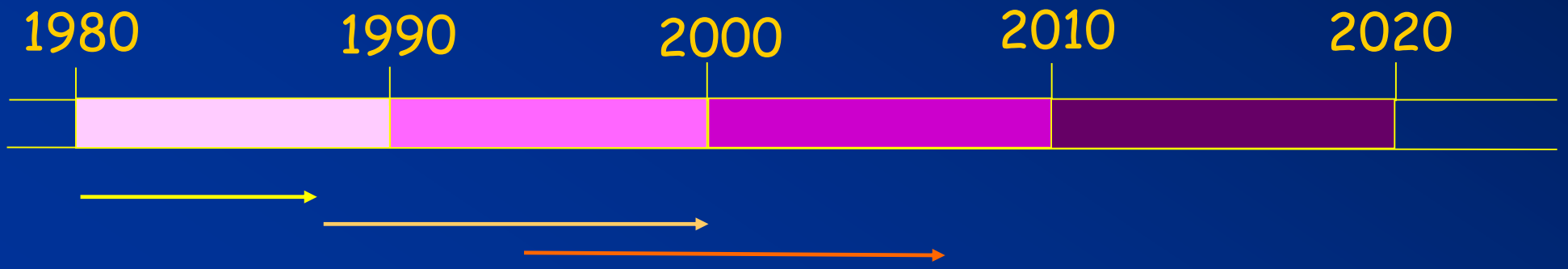

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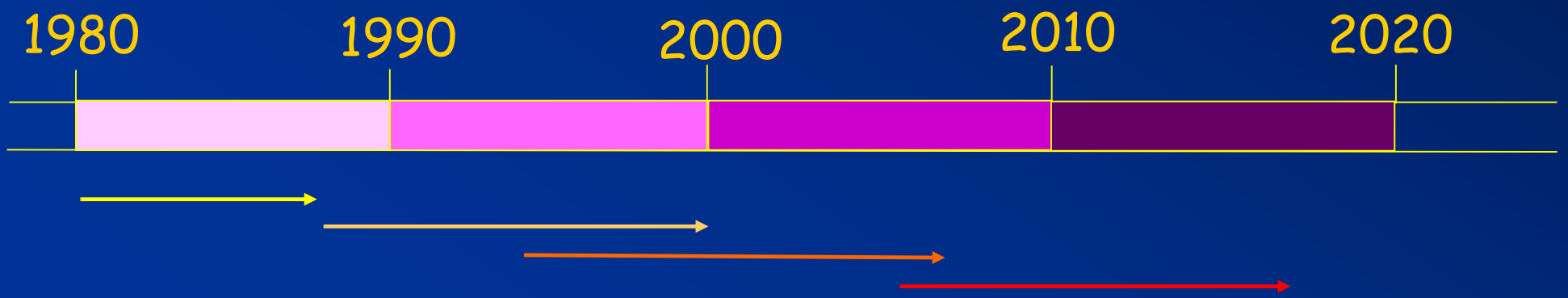
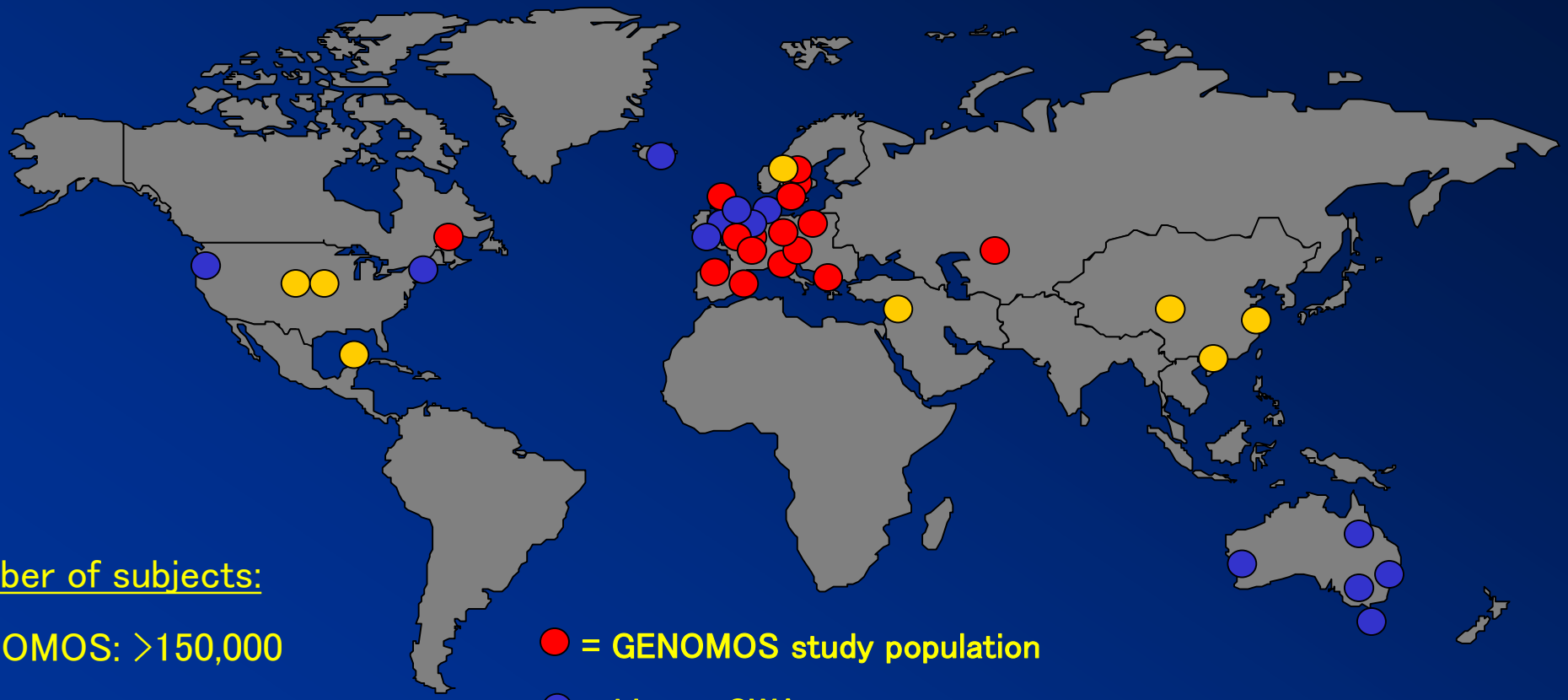


EU-FP7 project: GEFOS (start march 2008)



Number of subjects:

GENOMOS: >150,000

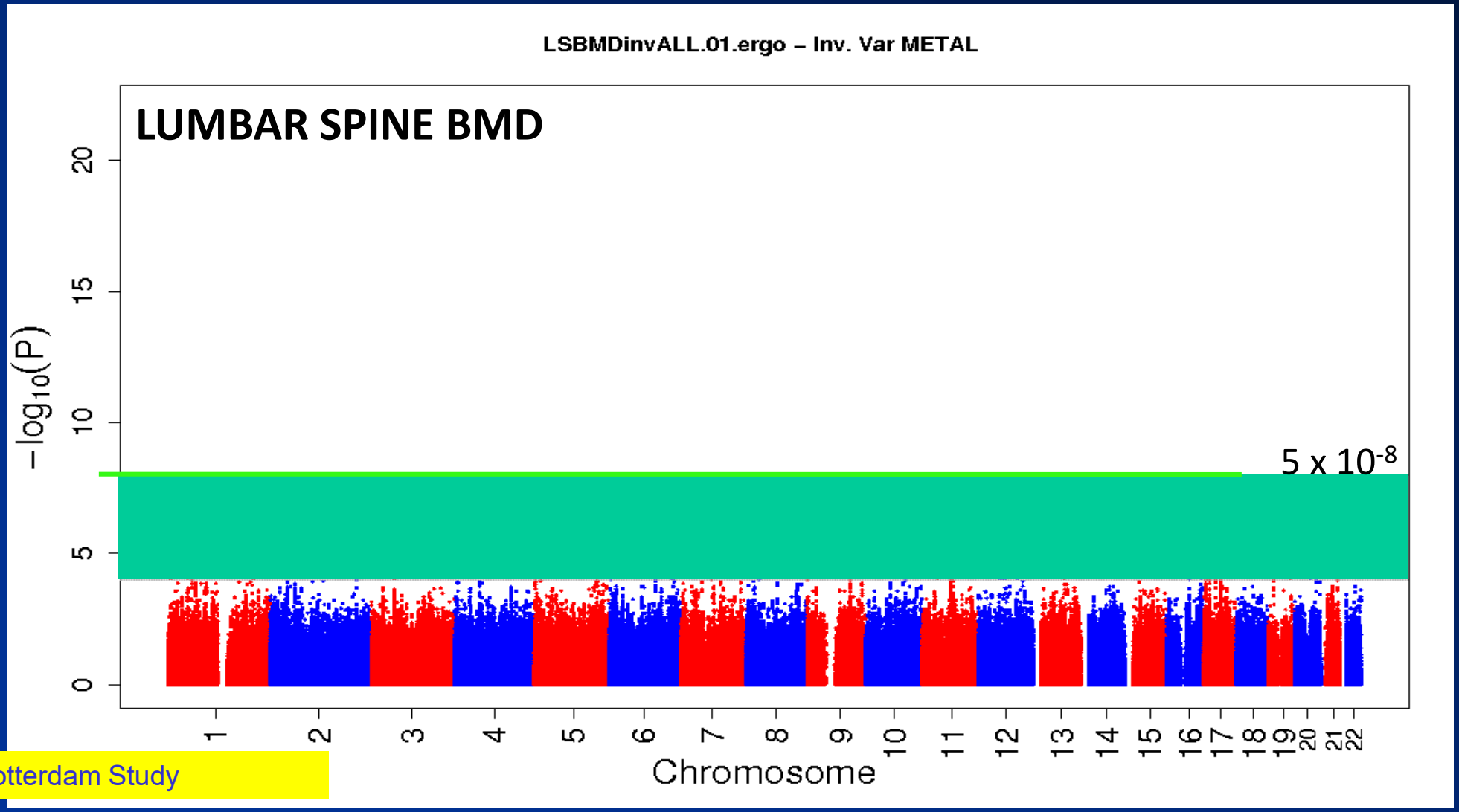
GWA: 40,000

● = GENOMOS study population

● = idem + GWA

● = idem, under negotiation / in development

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



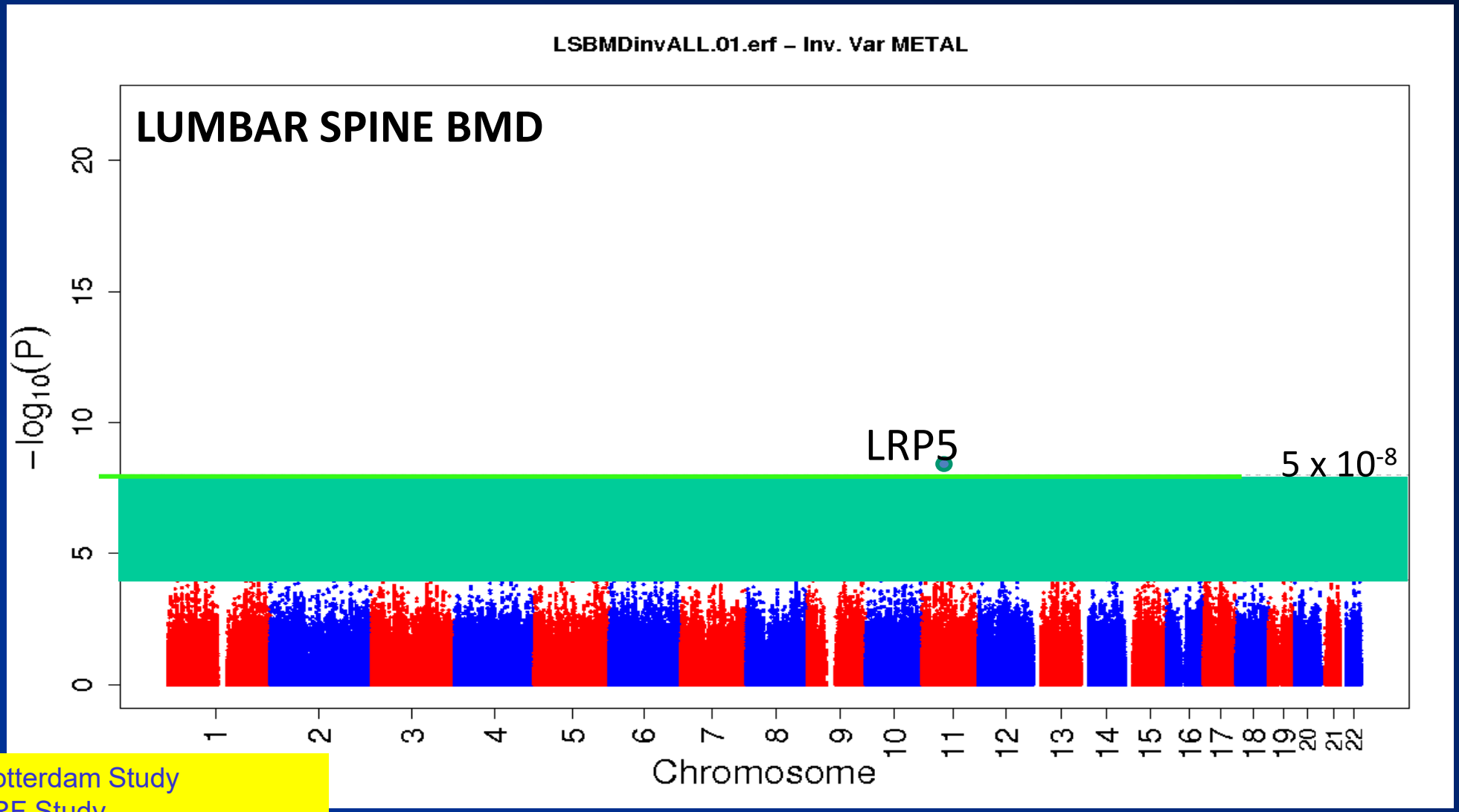
- Rotterdam Study

N=5000

Rivadeneira et al., ASBMR sept 2008

GEFOS

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



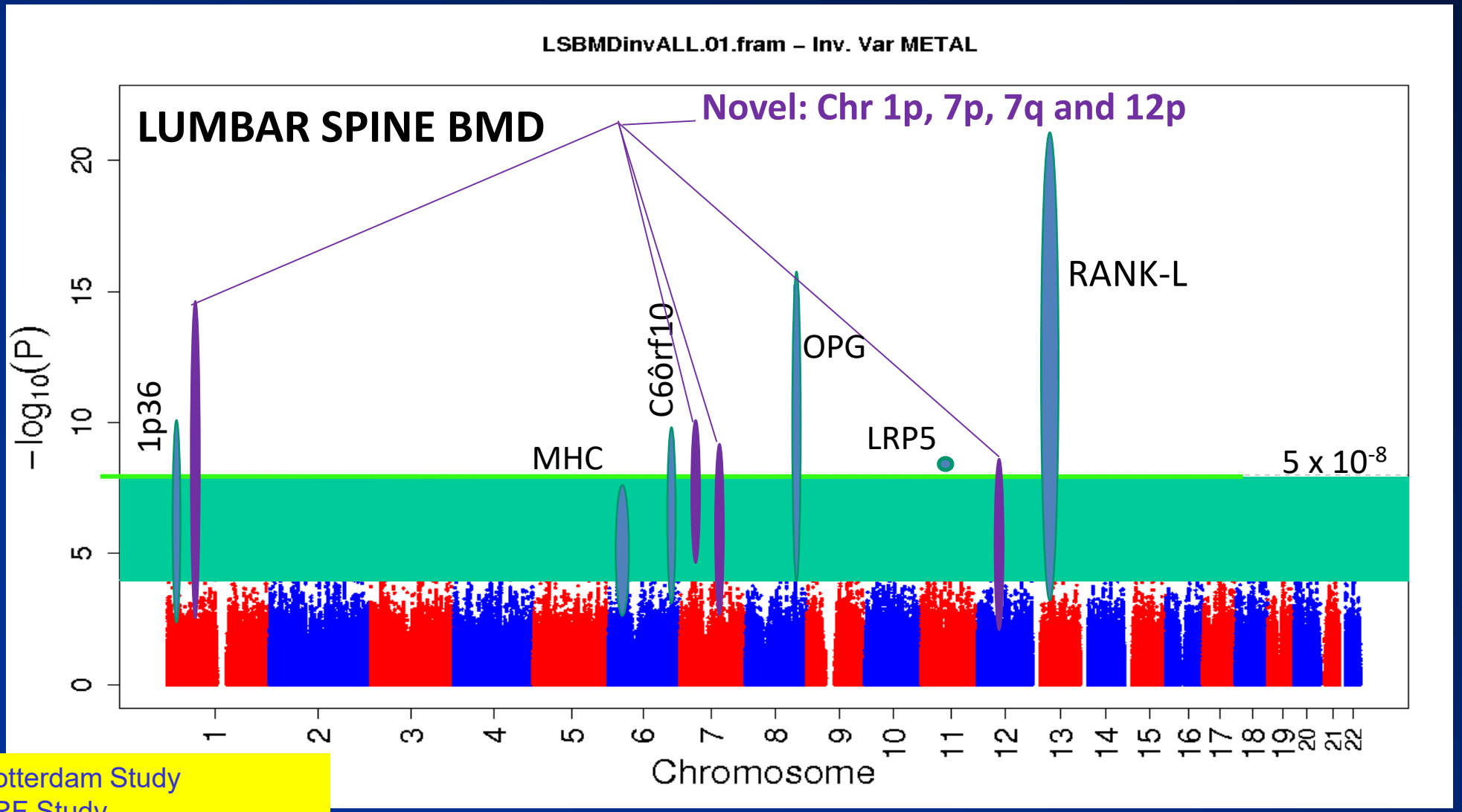
- Rotterdam Study
- ERF Study

N=6200

Rivadeneira et al., ASBMR sept 2008

GEFOS

Four novel loci exceed GWS threshold, many others are close



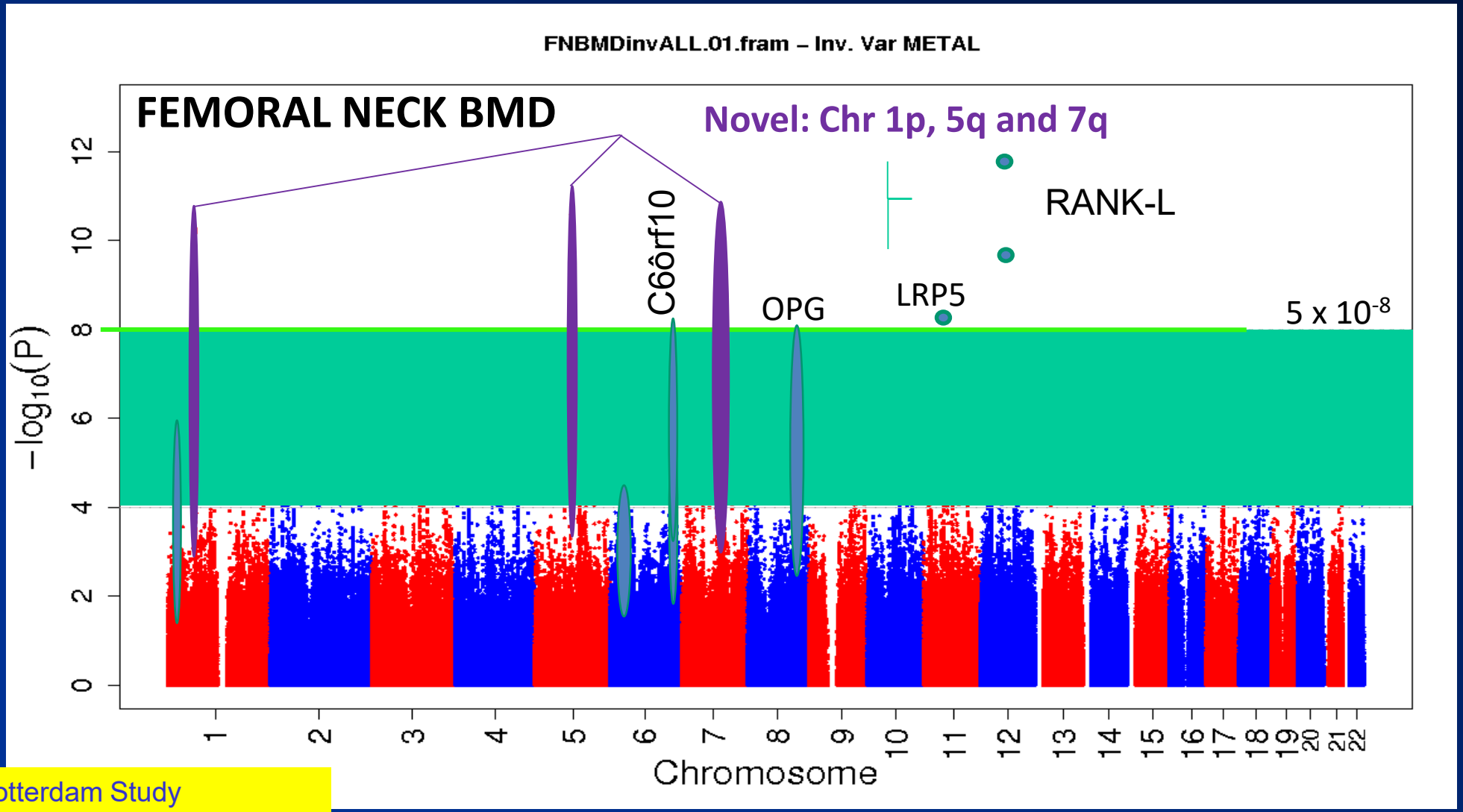
- Rotterdam Study
- ERF Study
- Twins UK
- deCODE Genetics
- Framingham Study

N=18500

Rivadeneira et al., ASBMR sept 2008

GEFOS

Three novel loci exceed GWS threshold, many others are close



- Rotterdam Study
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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

56 loci for BMD

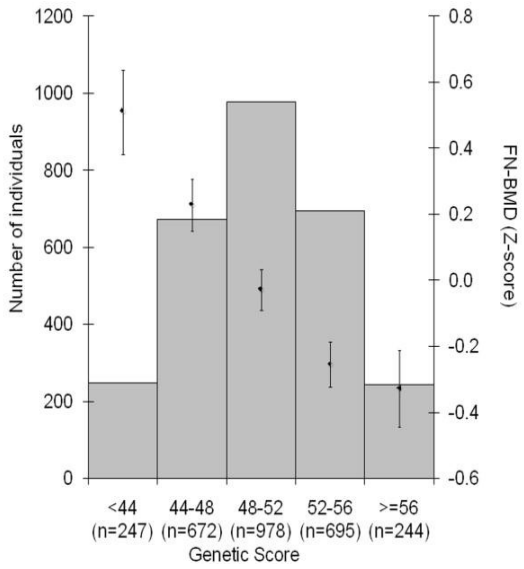
Estrada et al. Nature genetics, 2012

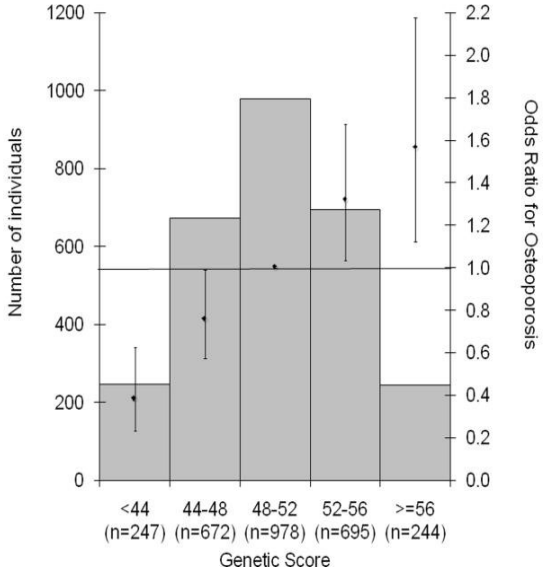
14 loci for osteoporotic fractures

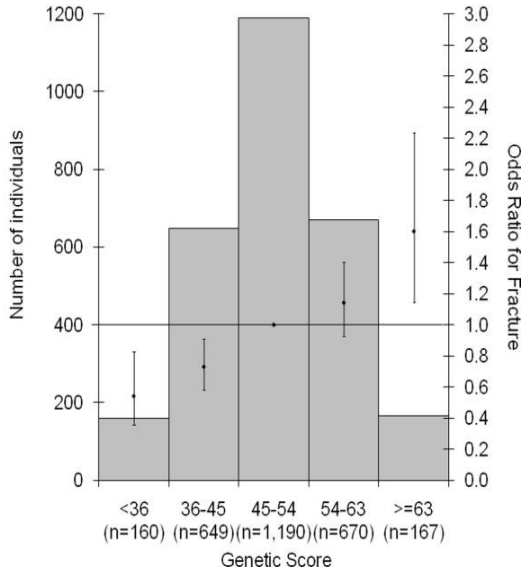
- smaller sample size
- clinically heterogeneous collection
- risk variants: - site specific
  - independent of BMD

Genetic variance explained less than 6%:
missing heritability

Genetic testing

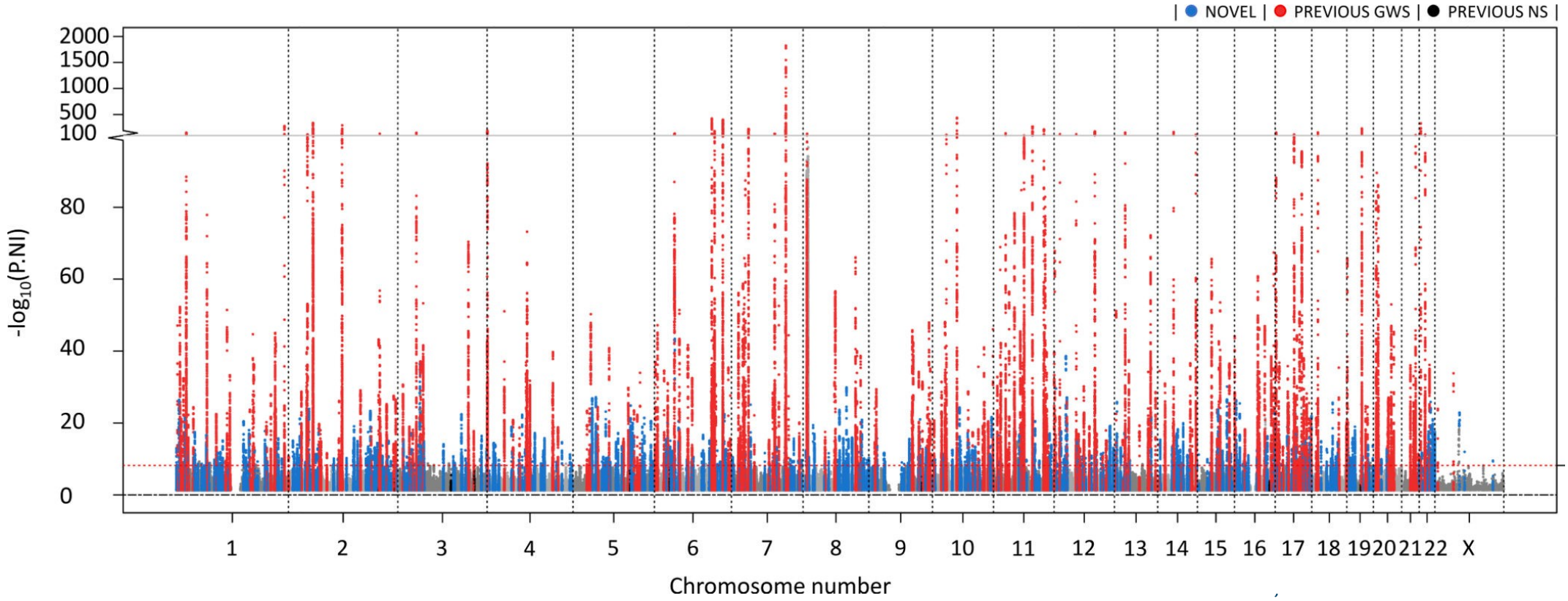






Estrada et al, *Nat Genet*, 2012

BMD-associated genes



BMD: Missing heritability

Genetic variance explained less than 6%

UK-biobank: BMD based on heel ultrasound

- Kemp et al. Nat Genet 2017

  403 SNP's from 376 loci

  13% of variance explained

- Kim. Plos One 2018

  About 400 000 individuals

  1362 SNP's from 899 loci

  17.4 fold increased risk for osteoporosis

  1.87 increased risk for fractures

  24.6% of variance explained

BMD: Missing heritability

- Larger samples to detect variants with smaller effects
- Copy number variations
- Long non-coding RNA and miRNA
- Epigenetic regulations
- Additional variants: rare variants

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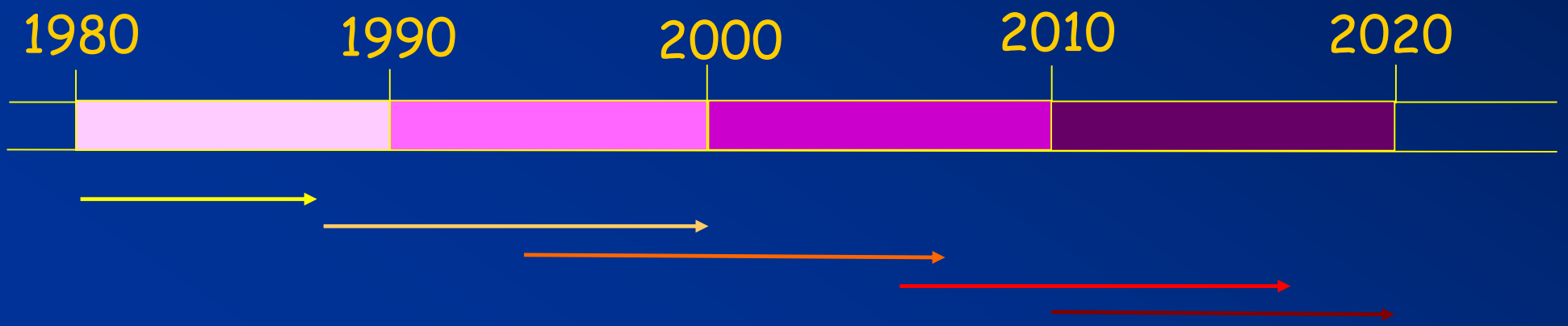


Rare variants

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

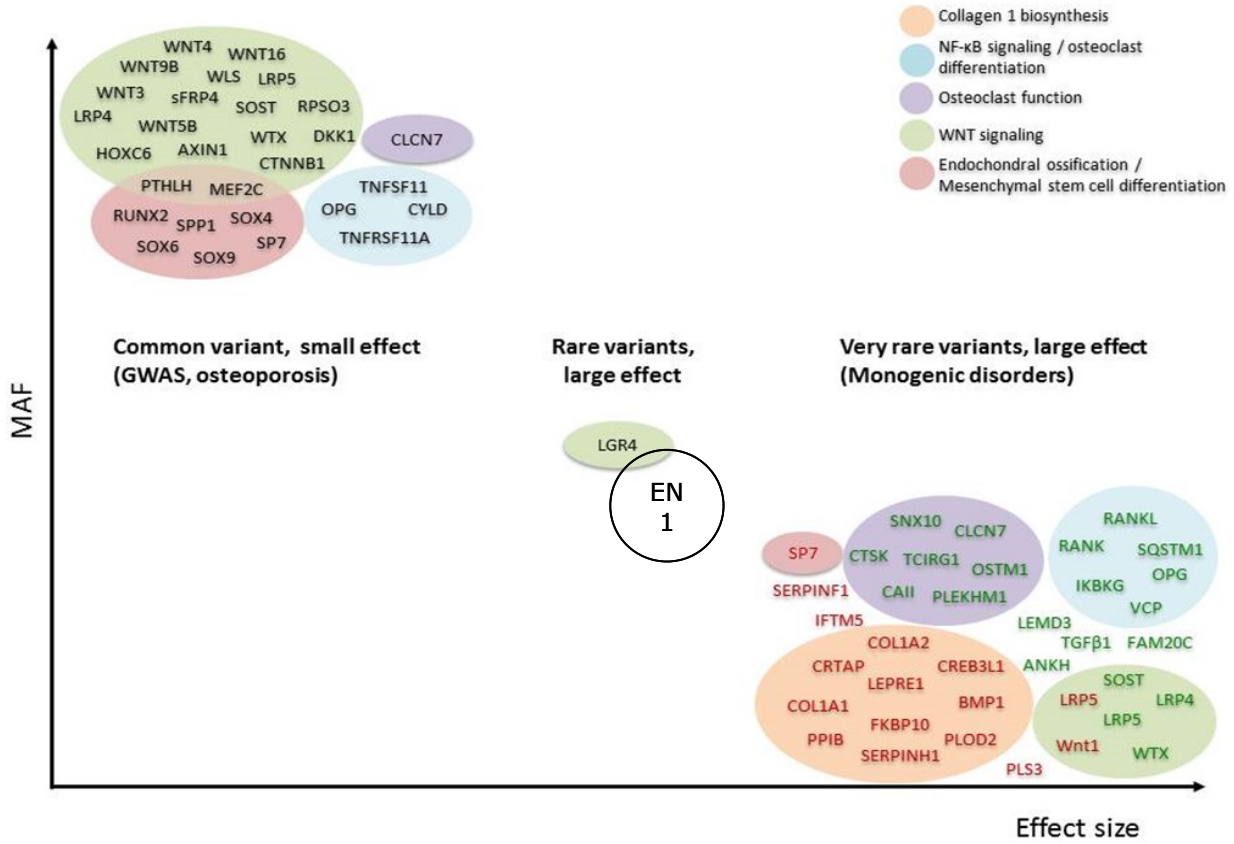
- Rare nonsense mutation in Leucine-rich-repeat-containing G-protein-coupled receptor 4 (LGR4): receptor for R-spondins

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 involved in monogenic disease often role in complex forms: therapeutic applications

Therapeutic applications

- Sclerostin encoded by SOST gene
- Inhibitor of bone formation
- Antibody against sclerostin in clinical trials
- Beginning of 2020: approval by FDA and EMA for treatment of patients with severe osteoporosis

Conclusions

- Genes involved in monogenic disease often role in complex forms: therapeutic applications
- Study of large cohorts is essential
  => Importance of worldwide collaborations
- 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