Identifying genetic factors underlying osteoporosis and fragility fractures in Malta

Chanelle Cilia[1], Melissa Marie Formosa[1], Josanne Vassallo[2] and Angela Xuereb Anastasi[1]

[1]Faculty of Health Sciences, University of Malta, Msida, Malta; [2]Diabetes and Endocrine Centre, Mater Dei Hospital, Msida, Malta

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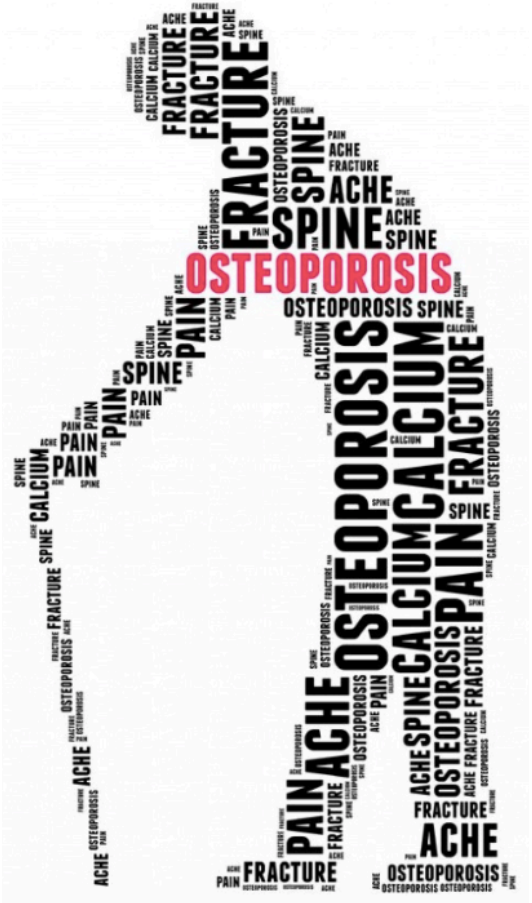
ENDEAVOUR
SCHOLARSHIPS SCHEME



Osteoporosis

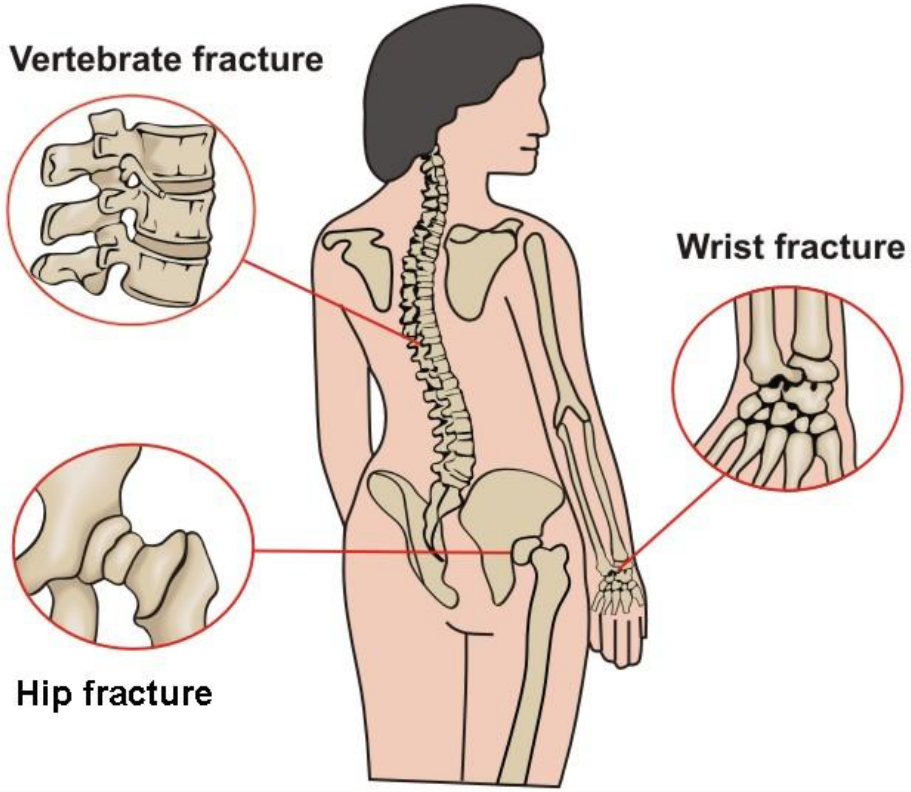
A **complex metabolic bone disease** characterised by

- *Reduced bone mineral density*
- *Deterioration of bone microarchitecture*

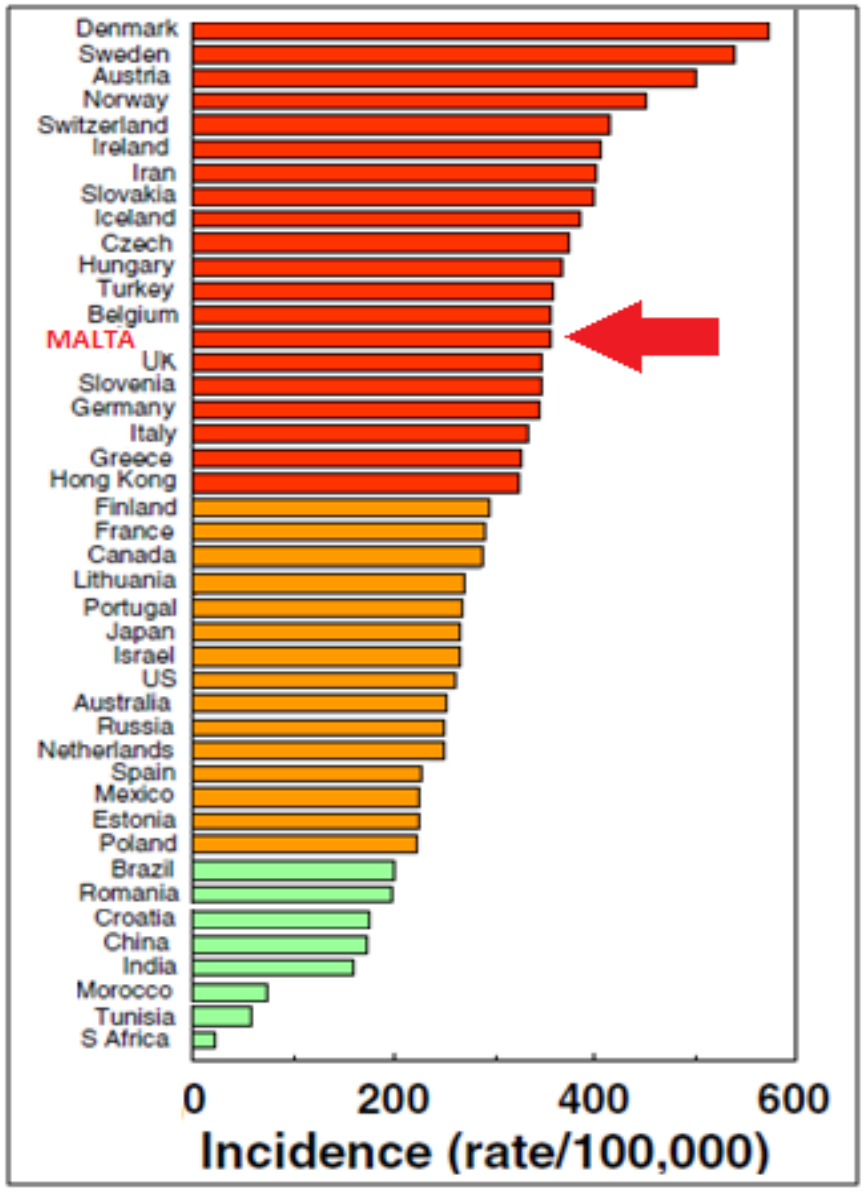
→ **Increased risk for fracture!**

Fragility fractures

- Osteoporosis is a '**silent**' & progressive disease
- Affected individuals do not show symptoms until the first fracture occurs



Osteoporosis & Fractures in MALTA



KEY

- HIGH-RISK**
- Medium-Risk
- Low-Risk

** *Osteoporosis in Malta*

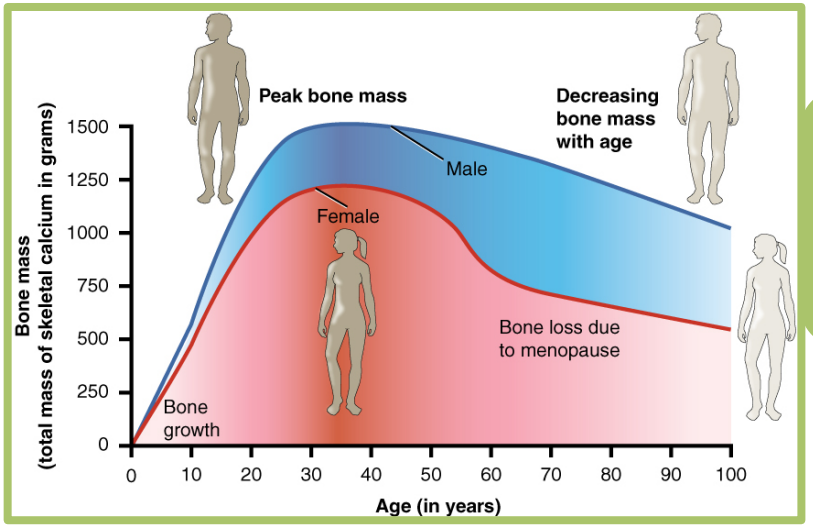
- **20% of Maltese women**
- **6% of Maltese men**

Fractures in Malta

- 2,600 fragility fractures sustained in 2010, costing 17 million euro
- 500 hip fractures amounting to 8 million euro
- 68% occurred in women

Svedbom et al (2013) *Arch. Osteoporos*

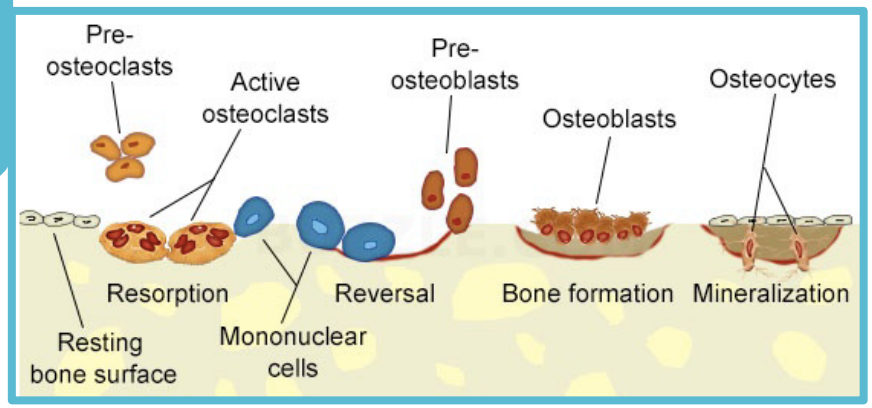
Pathophysiology of OP



Whether peak bone mass is reached

Age related bone loss

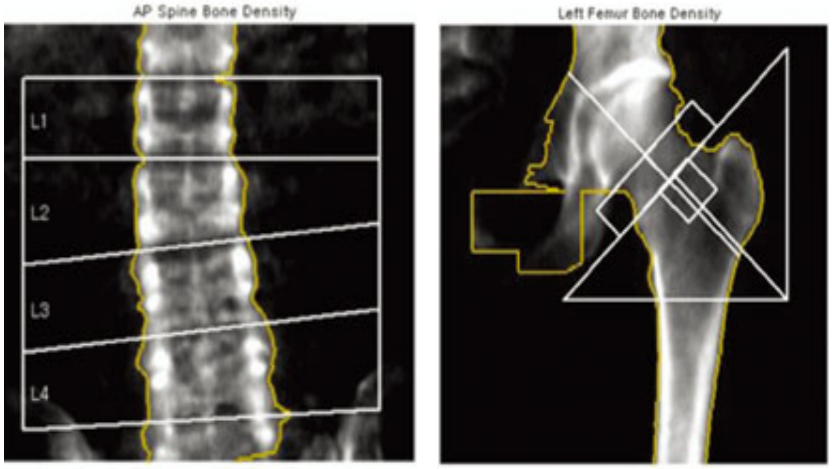
Imbalance in bone remodelling



Diagnosis of Osteoporosis

BMD measurement:

Dual-energy X-ray absorptiometry (DEXA)



T-score

Patient BMD compared BMD in healthy young adults matched for gender and ethnic group.

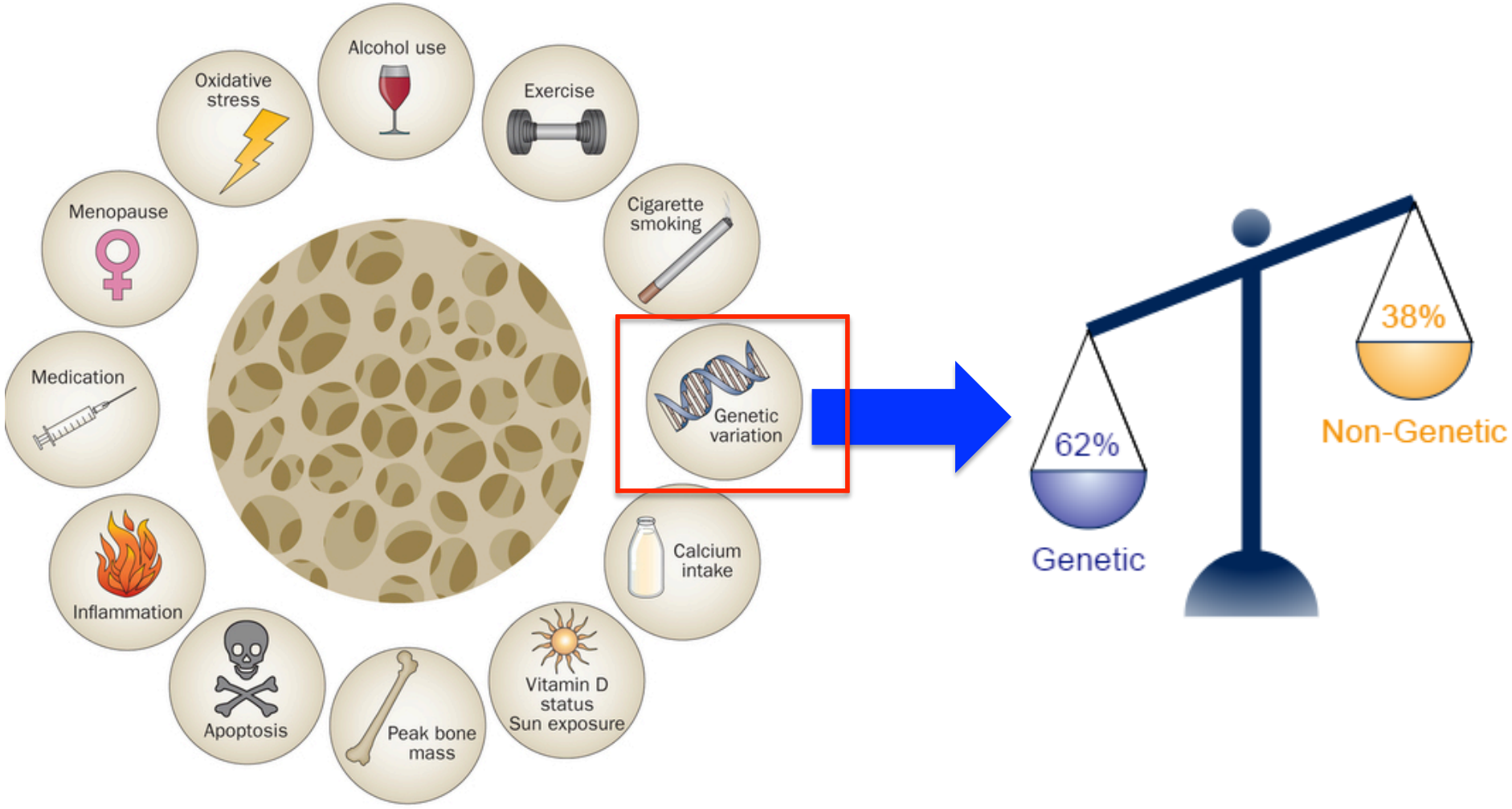
Normal Bone Mass (-1.0 and above)	Osteopenia (Between -1.0 and -2.5)	Osteoporosis (-2.5 and lower)

Z-score

Patient BMD compared to BMD of healthy normal subject matched for age, gender and ethnic group.

Normal Bone Mass (-1.0 and above)	Osteoporosis (-2.0 and lower)

Osteoporosis Risk Factors



Genetics of Osteoporosis

- OP is a polygenic disease where multiple gene variants each having a small effect contribute to an individual's increased susceptibility to the disease.

- OP has a **strong genetic component**
  - Heritability for BMD 50-85% (Hernandez-de Sosa *et al* (2014) *Calcif Tissue Int.)*
  - Heritability for fracture 25-68% (Michaelsson *et al*. (2005) *Arch Intern Med.)*

- Both **coding** and **low-frequency, non-coding** variants have been identified to be associated with BMD and fracture (Zheng *et al.* (2015) *Nature.)*

OP Genes Identified by Linkage Analysis ± NGS

Gene. 1998 Aug 17;216(1):103-11.

Cloning of a novel member of the low-density lipoprotein receptor family.

Hey PJ[1], Twells RC, Phillips MS, Yusuke Nakagawa, Brown SD, Kawaguchi Y, Cox R, Guochun Xie, Dugan V, Hammond H, Metzker ML, Todd JA, Hess JF.

Eur J Hum Genet. 2007 Jul;15(7):800-9. Epub 2007 Mar 21.

Linkage to chromosome 11p12 in two Maltese families with a highly penetrant form of osteoporosis.

Vidal C[1], Galea R, Brincat M, Anastasi AX.

N Engl J Med. 2013 Oct 17;369(16):1529-36. doi: 10.1056/NEJMoa1308223. Epub 2013 Oct 2.

PLS3 mutations in X-linked osteoporosis with fractures.

van Dijk FS[1], Zillikens MC, Micha D, Riessland M, Marcelis CL, de Die-Smulders CE, Milbradt J, Franken AA, Harsevoort AJ, Lichtenbelt KD, Pruijs HE, Rubio-Gozalbo ME, Zwertbroek R, Moutaouakil Y, Egthuijsen J, Hammerschmidt M, Bijman R, Semeins CM, Bakker AD, Everts V, Klein-Nulend J, Campos-Obando N, Hofman A, te Meerman GJ, Verkerk AJ, Uitterlinden AG, Maugeri A, Sistermans EA, Waisfisz Q, Meijers-Heijboer H, Wirth B, Simon ME, Pals G.

Effects of a synonymous variant in exon 9 of the *CD44* gene on pre-mRNA splicing in a family with osteoporosis

Christopher Vidal, Adela Cachia, Angela Xuereb-Anastasi
Edited by: M. Noda

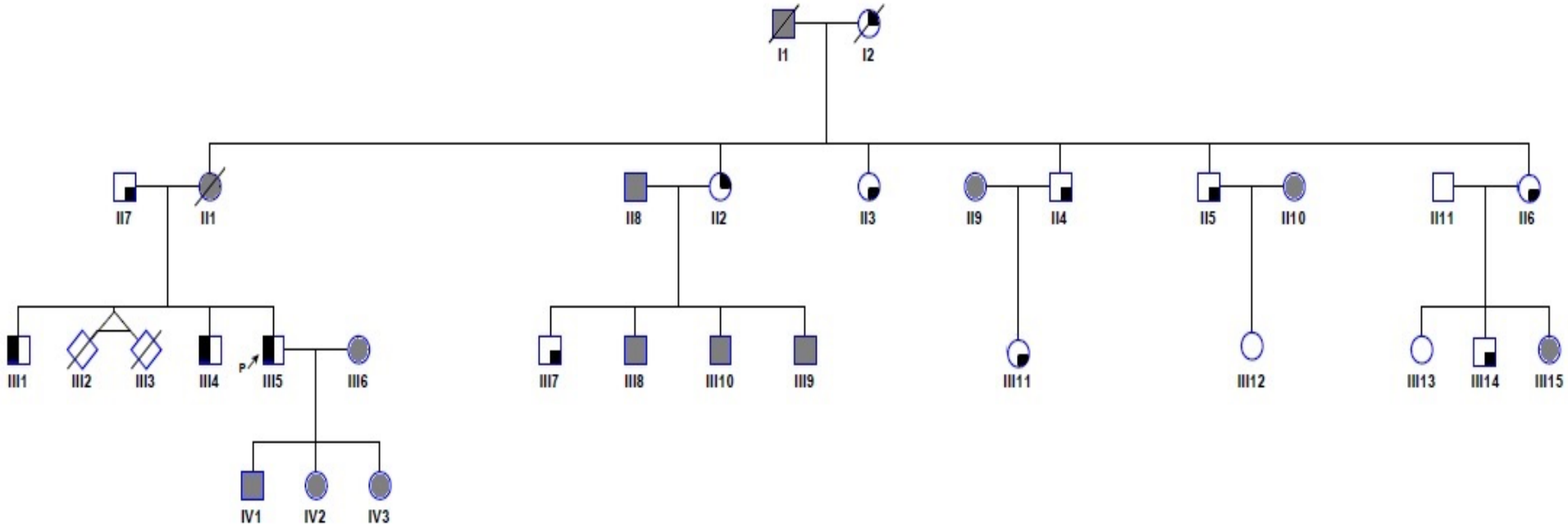
Study Objectives

An extended Maltese family having multiple affected members with osteoporosis and/or fragility fractures will be tested.

1. To identify the causal genes and gene variants in the recruited family, whether known or novel using next-generation sequencing.

2. Shortlisted variants will be replicated in a case-control collection of Maltese post-menopausal women to determine whether the variant affects the BMD and fracture risk at the population level.

The Research Family



Not affected = not osteoporotic, not osteopenic and no fracture history

Osteoporotic BMD + No fracture history

Osteopenic BMD + No fracture history

Fracture history

Unknown BMD

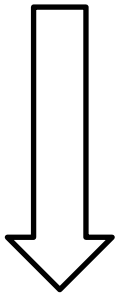
Proband

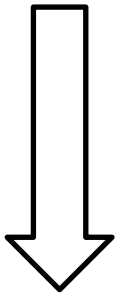
Recruitment process

1. Research Subject Consent	
2. Questionnaire	
3. Bone Density Scan	
4. Blood Collection	

Blood Collection

Collected blood vacutainers are separated





GENETICS

To be used for sequencing

- DNA extraction

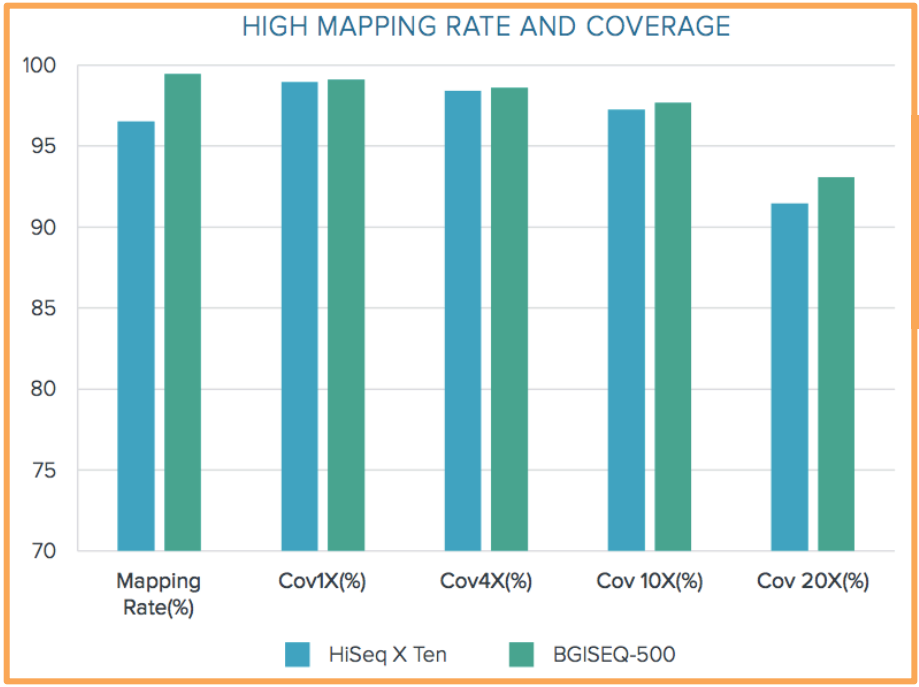
For storage purposes

- Aliquots
- RNA extraction

BIOCHEMISTRY

To exclude 2^{o} conditions

- Complete Blood Count + differential, ESR
- Folate, Vitamin B12
- Renal profile
- Vitamin D, Calcium, Magnesium
- Coeliac screen
- Hormone profile + Parathyroid Hormone
- Thyroid Function Tests
- Liver Function Tests
- HbA1c, Non-fasting glucose



Bar graph showing the mapping rate and sequencing coverage of the samples using BGISEQ-500 and Illumina HiSeq X Ten platform of 30X WGS.

	BGISEQ-500	HiSeq X Ten
Clean Reads	1001630550	732165210
Clean Bases (Mp)	100163	110083
Mapping Rate	99.47%	96.52%
Unique Rate	94.33%	85.14%
Duplicate Rate	1.77%	11.76%
Mismatch Rate	0.53%	0.56%
Average Sequencing Depth	33.02	31.57
Coverage	99.10%	98.95%
Coverage at least 4X	98.62%	98.43%
Coverage at least 10X	97.68%	97.24%
Coverage at least 20X	93.09%	91.45%

Table showing comparison of relevant data from BGISEQ-500 and Illumina HiSeq X Ten platforms. As a whole, BGISEQ-500 platform provides comparable results to the Illumina HiSeq X Ten platform.

BGISEQ-500 Project Workflow

100µl of sample containing 50ng/µl of DNA will be sent to BGI

SAMPLE PREPARATION

Sample QC

- DNA sheared = multiple fragments
- DNA end repair
- Adaptor ligation to fragments
- DNB formed by the RCA technique

LIBRARY PREPARATION

Library QC

- DNA loaded on a solid dual flow-cell
- Sequencing by combinatorial probe-anchor ligation sequencing
- Fluorescent probe attaches to DNA
- High resolution digital imaging
- Signalling colour = corresponding base

SEQUENCING

Data QC

Standard bioinformatics analysis

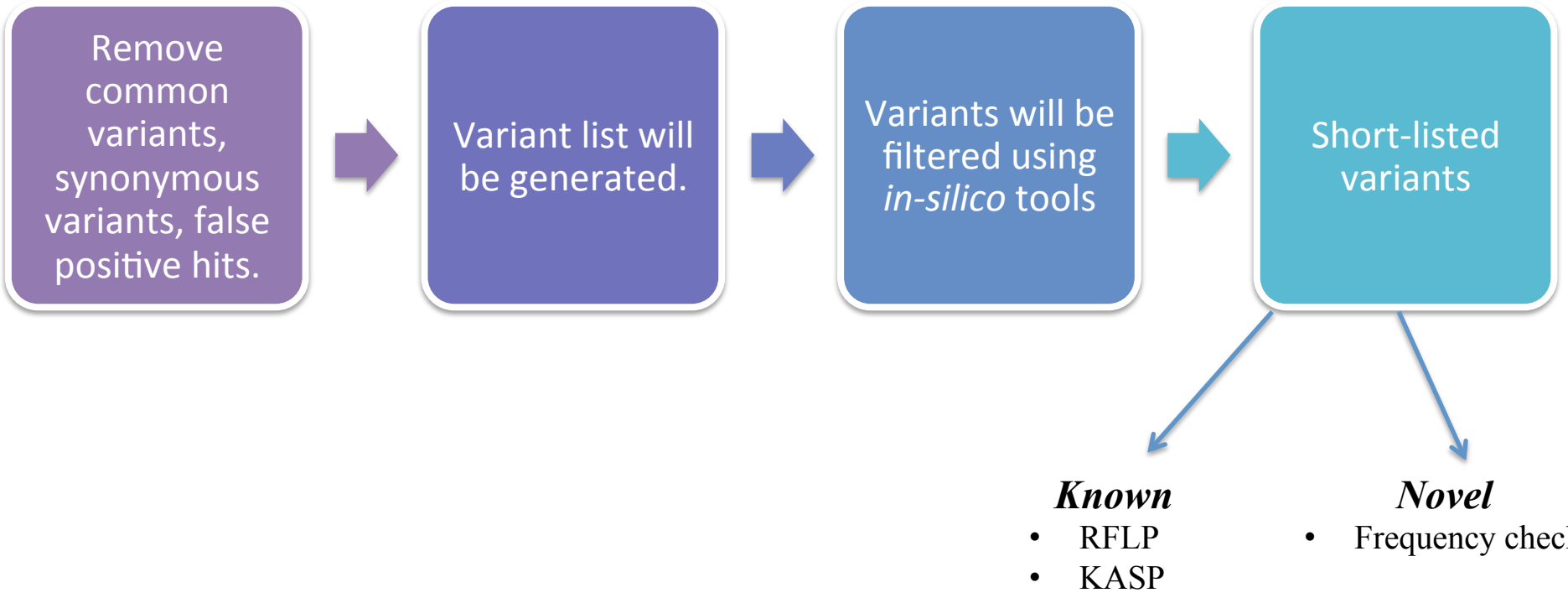
- **Filtering**
- **Alignment**
- **SNP** calling, annotation, validation, comparison, functionality and conservation prediction, statistics per functional element
- **InDel** calling, annotation, validation, comparison, statistics per functional element
- **CNV** calling and annotation
- **SV** calling and annotation

RAW DATA OUTPUT

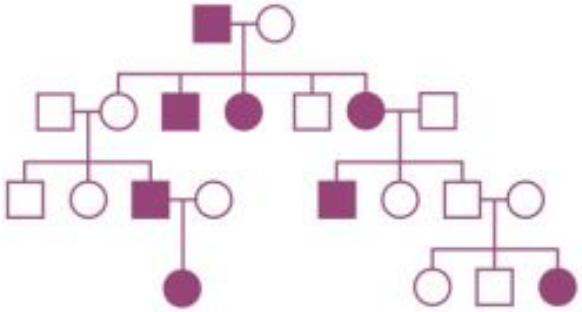
BIOINFORMATICS ANALYSIS

Bioinformatics:
Data filtering

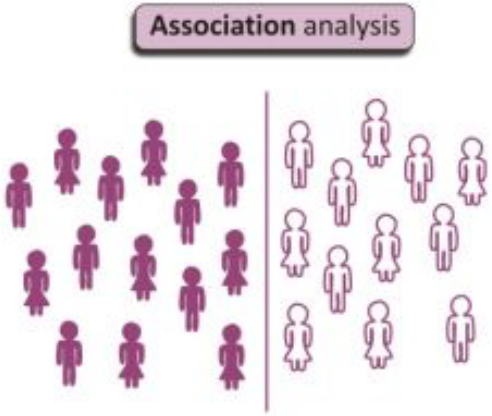
Data filtering will be performed to generate a list of variants linked to OP and fragility fractures.



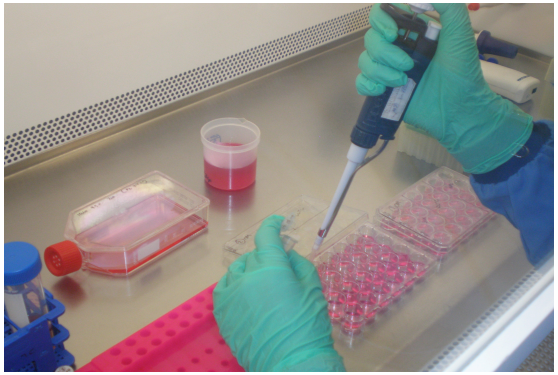
Future studies: Functional work



Testing in population



Functional testing of mutations



Testing of new genes

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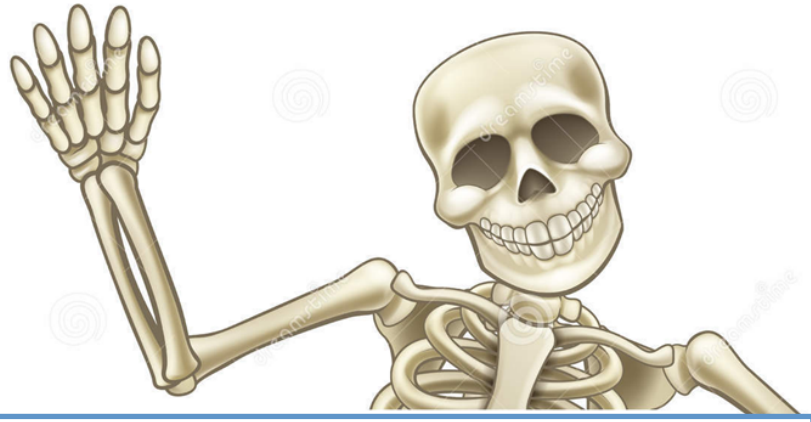
SPTAR MATER DEI



Thank you for your attention!