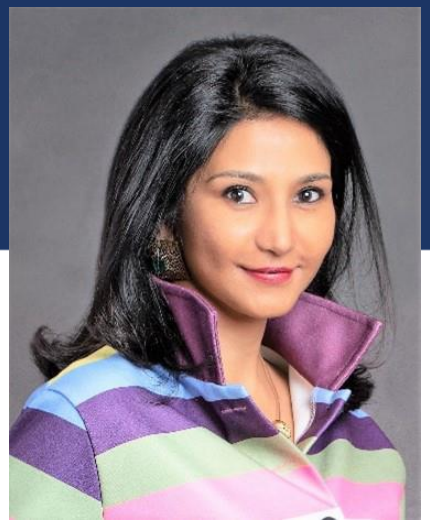
BoneCast

Special Edition

Practical and Clinical Approach to Skeletal Rare Disorders with Illustrative Cases

Dr Manju Chandran



IOF International Osteoporosis Foundation

A PRACTICAL AND CLINICAL APPROACH TO SKELETAL RARE DISORDERS

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IOF
International Osteoporosis Foundation

Below the feet of the Dancing Lord ----- Does he dance upon an Endocrine Mystery?

We have been missing Rare Bone Disorders since the dawn of time!

Endocrinology and the arts at the feet of the dancing Lord: Parathyroid hormone resistance in an Indian icon
Seshadri KG et al. IJEM 2014

Disclosures pertaining to this talk: None

WHAT IS A RARE DISEASE?

- **EU and CANADA: A disease is rare when its prevalence is </= 1 person in 2000**
- **USA: A disease is rare if it affects no more than 200,000 people at a given time**
- **JAPAN: A disease is rare if it affects no more than 50,000 people**
- **AUSTRALIA: A disease is rare if it affects </= 1:10,000 people**

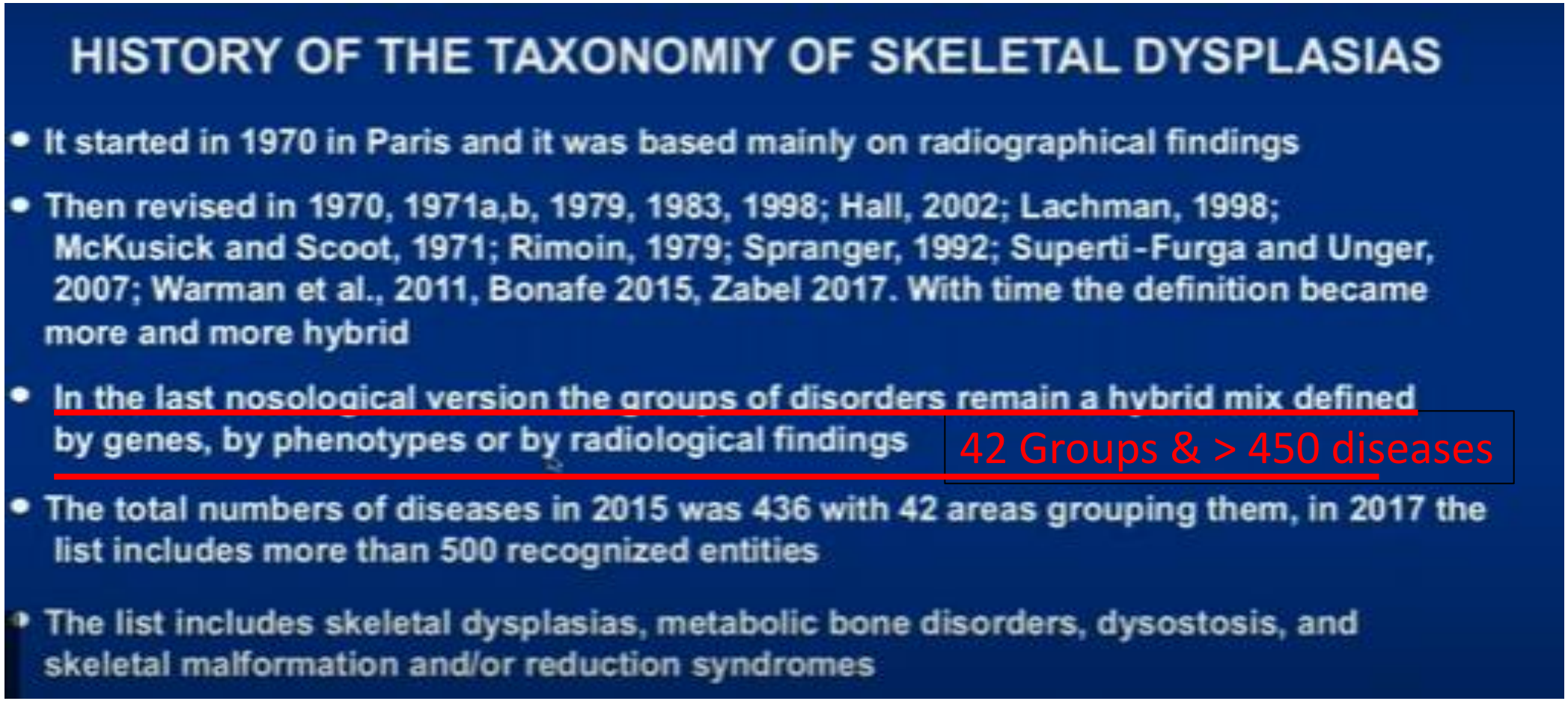
Rare Bone Diseases: The Numbers

- 461 rare skeletal disorders currently (High number when you consider that there are only 6000-8000 rare diseases known to mankind
- 80-90% of cases are genetic and in 1:3 cases, the genetic cause is unknown

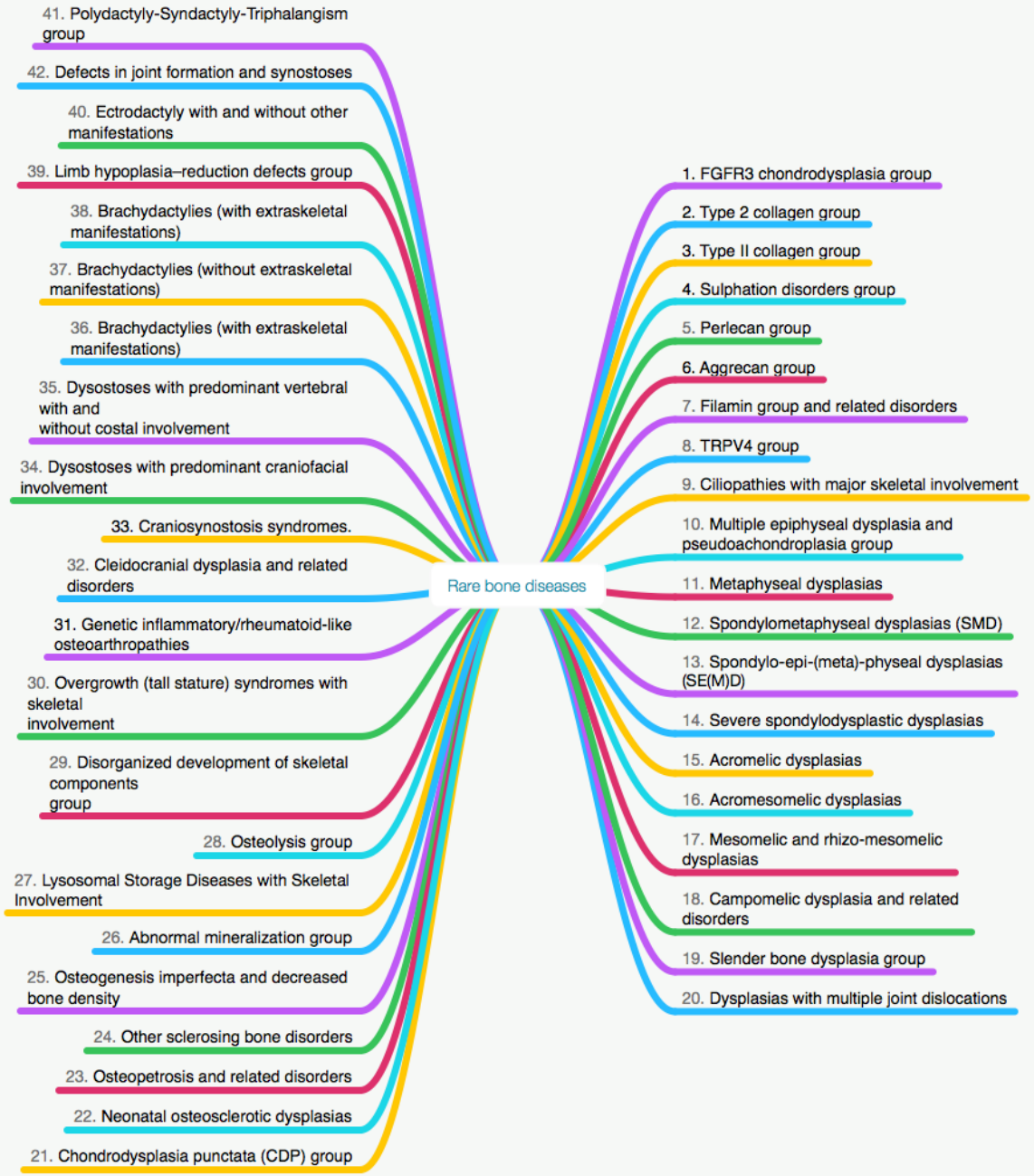
WHY IS IT IMPORTANT TO DIAGNOSE?

- Aids Prognostication and monitoring – inform about life expectancy, complications expected
- To Avoid harmful therapies/ unnecessary testing
- For Planning family, family screening
- Inform about Potential novel therapies
- Facilitate connection to patient support groups

Taxonomy of rare bone diseases



A taxonomy is an orderly system of nomenclature that abides by set rules and procedures. Disease taxonomies vary in their adherence to this principle. Some aspects, such as the chapters in the International Classification of Diseases, follow an anatomical or body-system orientation, and others are based on etiology and pathogenesis



456 different conditions placed in 40 groups.
Of these, 316 were associated with one or more of 226 different genes

Group/Name of Disorder	Inheritance
1. FGFR3 chondrodysplasia group	
Thanatophoric dysplasia type 1 (TD1)	AD
Thanatophoric dysplasia type 2 (TD2)	AD
Severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN)	AD
Achondroplasia	AD
Hypochondroplasia	AD
Camptodactyly, tall stature and hearing loss syndrome (CATSHL)	AD
Hypochondroplasia–like dysplasia(s)	AD, SP
See also group 33 for craniosynostoses syndromes linked to *FGFR3* mutations, as well as LADD syndrome in group 41 for another *FGFR3*-related phenotype	

Bonafe AJMG 2017

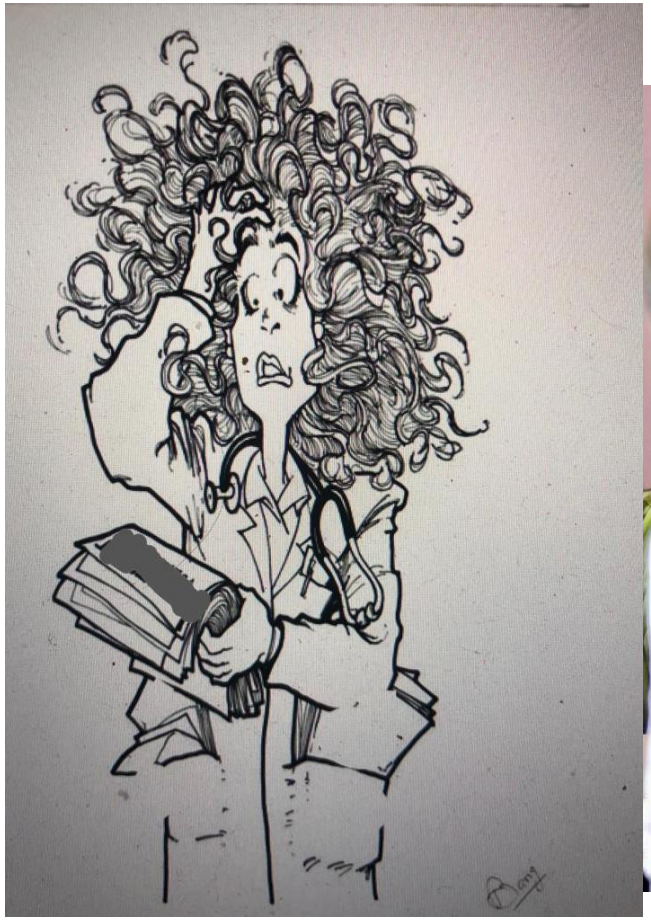
Taxonomy of Rare Genetic Metabolic Bone Disorders

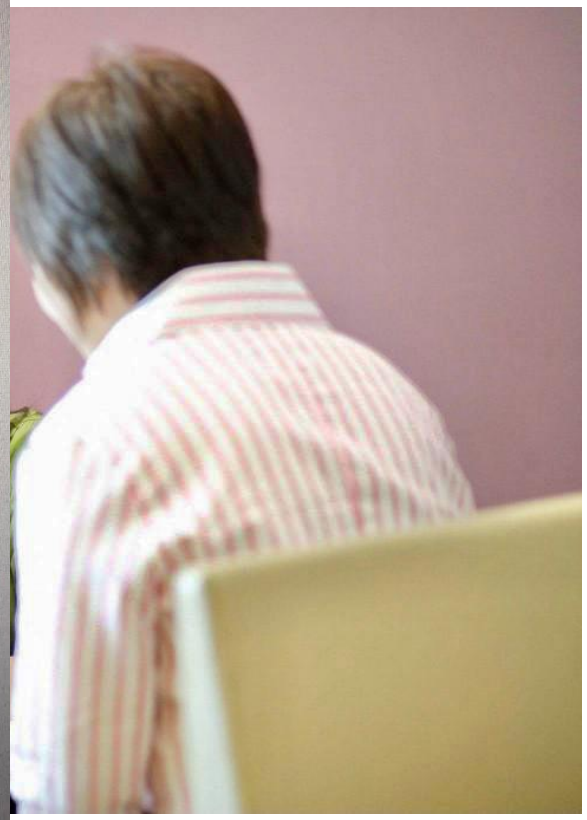
Altered osteoclast/osteoblast or osteocyte activity9	Altered Bone matrix proteins	Mutated bone microenvironment regulators	Deranged Calciotropic hormone activity
•Low Bone Resorption •High Bone Resorption •High Bone Formation •Decreased Bone Formation	•Disorders in Collagen Metabolism •Disorders of Alkaline Phosphatase	•Disorders of the Rank/Rankl/OPG System •Disorders of the Glycosylphosphatidylinositol Biosynthetic Pathway •Disorders of the LRP5 •Disorders of the Bone Morphogenetic Protein Receptor (BMPPR)	•Parathyroid Hormone Excess or Deficiency •Abnormal Parathyroid Hormone Receptor Signaling •Disorders of Vitamin D Metabolism & Action •Disorders of Phosphate Homeostasis

Masi L, Agnusdei D, Bilezikian, J et al. Taxonomy of rare genetic metabolic bone disorders. International Osteoporosis Foundation (IOF). 2015.https://www.iofbonehealth.org/osteoporosis-musculoskeletal-disorders/skeletal-rare-disorders

Online Atlas, prepared by the IOF Working Group on Skeletal Rare Diseases, classifies more than 90 rare genetic metabolic bone disorders according to their metabolic pathogenesis, outlined in four subcategories

What about in the Clinic???????---



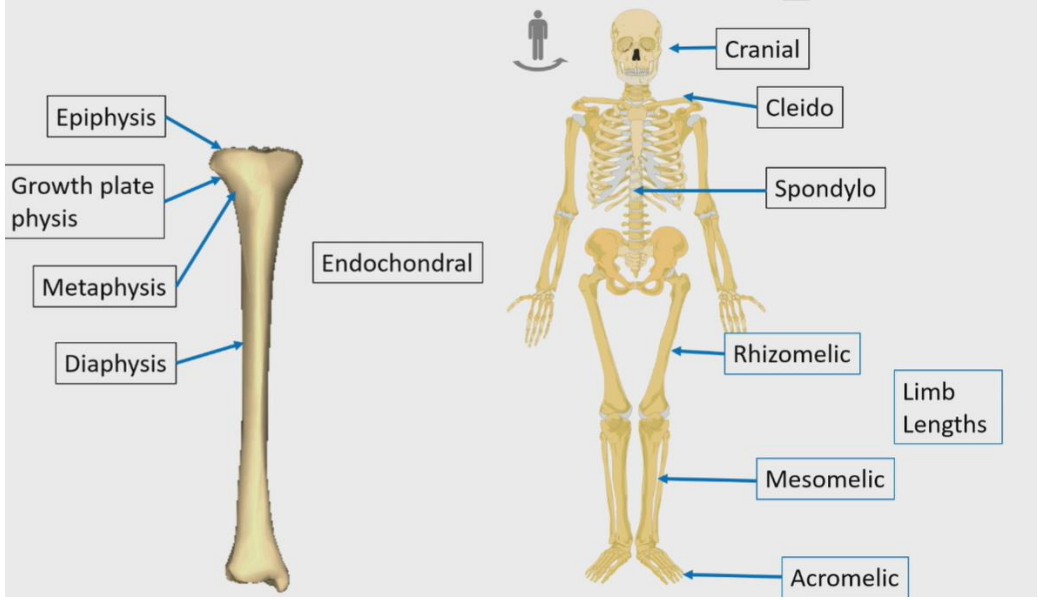


Definition of some terms

- **Dysplasia: Symmetrical Involvement of the Skeleton with more generalized involvement**

(eg: Cranio diaphyseal dysplasia: Cranium and diaphysis of bones are involved)

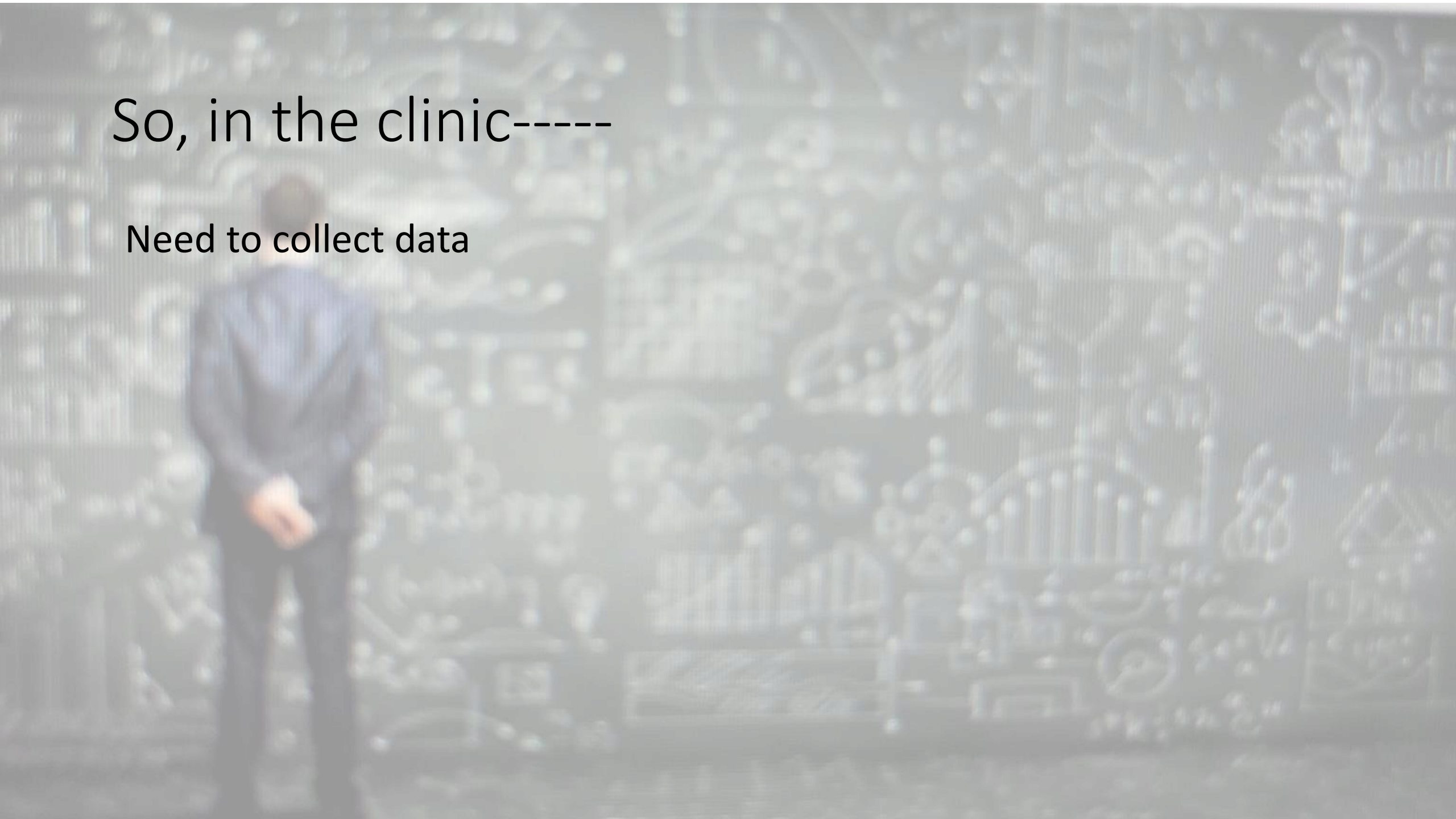
- **Dysostosis: Not symmetrical, specific bone involvement**
- **Synostosis: Fusion of adjacent bones**
- **Hyperostosis: Cortical Bone thickening**
- **Osteosclerosis: Trabecular bone thickening**
- **Endochondral: Coming from cartilage**



* Terminology that was derived from the past when RBDs were defined by the radiologist

So, in the clinic-----

Need to collect data



HISTORY

- **Ethnicity**
- Medication Use
- Fractures: Typical, Atypical, Stress
- Bone pain, muscle weakness
- Difficulty walking
- Joint pain, Osteoarthritis
- Hearing loss
- Dental history – Premature tooth loss, h/o dental abscesses
- Vision impairment
- Kidney stones
- Puberty: Delayed, Precocious

Family History: H/o Consanguinity

PHYSICAL EXAMINATION

- Height, Proportions
- Deformities: Facial, Limb and Trunk
- Eyes: Cataract, Blue Sclerae,
- Oral Cavity: Torus Palatinus, Grooved Palate, Dentition– shape of teeth
- Skin: Neavi, Café Au Lait Spots
- Localized lumps
- Range of movement
  - Hypermobility

Laboratory studies and others

- Calcium, Phosphate, Alkaline Phosphatase, PTH, 25OHD, 1,25OHD
- Renal Function
- Bone turnover markers
- Full blood count
- Urine PO4, Urine Calcium, Urine for protein, AAs, Urine for electrolytes

Next Level of Investigations

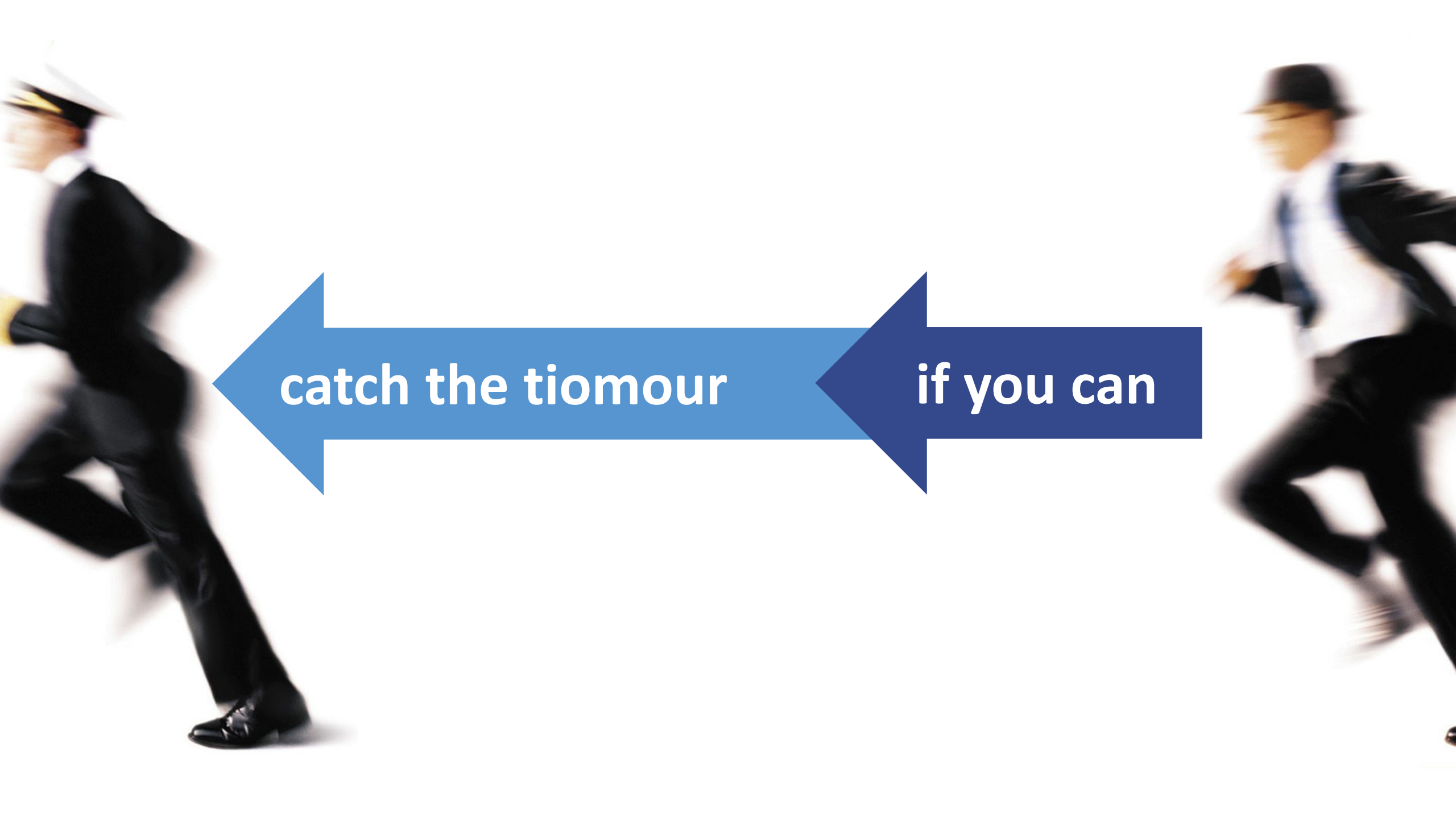
- Myeloma panel
- Serum Tryptase
- Hep C
- FGF23

Radiology: Xrays, Bone Scan, Functional Imaging

DXA Scan

? Bone Biopsy and Histomorphometry

Genetic Analysis

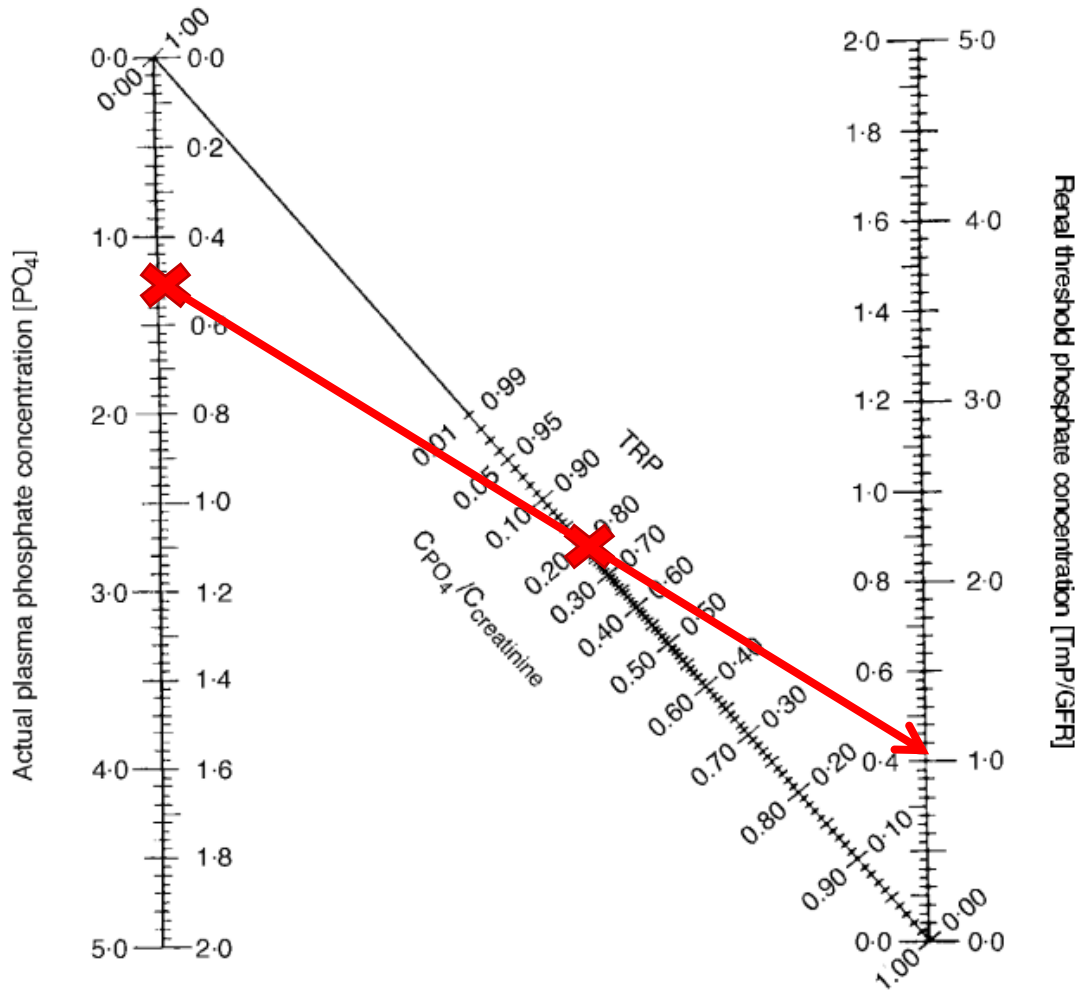

catch the tiomour
if you can

Case 1

- **60 year old Chinese male**
- **Non smoker/ no alcohol**
- **No family history of fractures or bone disorders**
- **2013**
  - **Recurrent non-traumatic rib fractures since 2010**
  - **CXR showed mild fibrosis in left lung base, hence referred to respiratory medicine**
  - **Referred to endocrinology for abnormal bone scan – osteoblastic lesions involving multiple areas, aetiology likely metabolic rather than metastatic**
- Right ankle pain for 2 weeks and left hip pain for 1 week
- X-rays revealed
  - Left femur shaft insufficiency fracture
  - Right distal tibial insufficiency fracture

Summary of Investigations

Investigation	Result	Reference Range
Calcium	2.10 mmol/L	2.09 – 2.46 mmol/L
24 hour urine calcium	1.2 mmol/day	0.82 – 6.74 mmol/day
Phosphate	**0.51** mmol/L (normal in 2010)	0.94 – 1.5 mmol/L
24 hour urine phosphate	15.33 mmol/day	6.5 – 32.3 mmol/day
iPTH	**11** pmol/L	0.9 – 6.2 pmol/L
25 hydroxyvitamin D	24.7 mcg/L	
Alkaline phosphatase	**372** U/L	39-99 U/L
Urine dipstick	No glycosuria, No albuminuria	
Myeloma panel	Negative	



TmP/GFR=0.41

Walton and Bijvoet. Lancet 1975

PHOSPHOPENIA (HYPOPHOSPHATAEMIA)

Renal Wasting

Phosphatonin Mediated

- XLH (PHEX gene inactivating mutation)
- ADHR (FGF23- Gain of fn mutation)
- ARHR

ARHR Type 1 (Inactivating mutations of DMP1 (Dentin Matrix Protein 1). DMP 1 expressed in osteocytes may suppress FGF23 prodn from bone). May present as a sclerosing bone dysplasia with hyperostosis and dense vert bodies

ARHR Type 2 (ENPP1 (Ectonucleotide pyrophosphatase /phosphodiesterase enzyme involved in generation of mineralization inhibitor pyrophosphate) loss of fn mutation->Generalized arterial calcification of infancy (GACI). Those who survive → ARHR 2)

ARHR Type 3 (FAM 20 C (Family with sequence similarity 20, member C (also known as DMP4)- phosphorylation by FAM20C normally regulates cleavage of FGF23) mutation-Raine's syndrome. Formerly believed to be a lethal osteosclerotic bone dysplasia but survivors into adulthood seen with features similar to XLH.

Cerebral calcifications, distinctive facial features

Perilacunar osteomalacia on bone Bx (also seen in XLH) , Facial features)

- FD (post-zygotic somatic GNAS mutation with local over-production of FGF23. In FD –MAS: associated café au lait macules, precocious puberty, endocrine hyperfn)
- Scimmelpenning-Feuerstein-Mims syndrome (also has café-au-lait spots and bone lesions)
- Osteoglophonic Dysplasia (FGFR1 mutation): rhizomelic dwarfism, craniosynsotosis, impacted teeth, multiple non-ossifying bone lesions.
- Opsismodyplasia (INPPL1 mutation): spondylo(epi)chondrodysplasia with delayed skeletal maturation
- Neurofibromatosis 1 (increased FGF23 prodn)
- Linear Sebaceous nevus syndrome (increased FGF23 prodn: Neuroectodermal disorder with seizures, development defects, cutaneous lesions)
- Jansen metaphyseal chondrodyplasia (due to activating PTH receptor 1 mutation. Severe short stature, brachycephaly, micrognathia, hypertelorism, clinodactyly)
- TIO (Increased FGF23 prodn)
- Iron Carboxymaltose (the specific CHO moiety interferes with degradation of FGF23)
- Massive blood transfusion: by increasing FGF23
- Hypophosphatemia after renal Tx (due to prolonged excessive FGF23 acquired during CKD): gradually resolves over time)
- Hypophosphatemic Rickets and Hyperparathyroidism (due to translocation of the Klotho promoter gene)

Non Phosphatonin Mediated

- PHPT (*FGF23 may be elevated in PHPT)
- Proximal RTA : Isolated or Fanconi's (when Proximal RTA (decreased ability to reclaim filtered HCO3) is asso with generalized dysfn of proximal tubule, it is called Fanconis which may be congenital or acquired.

Acquired Proximal RTA/Fanconis

1) MM, Sjogrens (in Sjogren's hypopo4emia may precede sicca symptoms)- Both MM and Sjogren's may be associated with isolated Prox RTA or Fanconi's

2) Adefovir, Tenofovir* associated (due to inhibition of DNA polymerase after these agents enter renal tubular cells. DNA polymerase is needed for mitochondrial DNA replication. This inhibition->ATP depletion -> alteration of membrane transporters-> urinary solute loss including K, PO4, AA, Uric aci

3) Rapamycin (mTOR inhibitor given post renal TX) associated

4) Na Valproate,

5) Ifosamide

Congenital Fanconi or Fanconis phenotype

1) Dent's disease: Mutation in CLCN5 gene (75%) or due to OCRL1 gene mutations. XL Recessive. Primary defect in the cells of the proximal tubule results in a phenotype of Prox RTA with hypercalciuria (due to bone resorption sec to c/c acidosis), nephrocalcinosis, renal failure, LMW proteinuria, AA uria, phosphaturia. But no HCO3 uria. Usually manifests in childhood

2) Nephropathic Cystinosis (mutation of Cystinosin (CTNS) →Fanconi's syndrome

- Very rarely Distal RTA
- HHRH (Hereditary hypophosphatemic rickets with Hypercalciuria)*: Inactivating homozygous mutations of the SLC34A3 gene which codes for NaPi2c (sodium dependent phosphate cotransporter 2c). In HHRH, unlike in XLH, appropriate increase in 1,25 D-> hyperabs of Ca++->hypercalciuria and nephrolithiasis. oral phosphate supplementation alone is enough to Rx the phosphate wasting and in fact if alphacalcidiol or Calcitriol is given as in XLH, ADHR, ARHR etc, it may worsen hypercalciuria.
- Nephrolithiasis Osteoporosis (NPHLOP) Syndromes 1 and 2: Heterozygous mutations in SCL34A1 and SCL9A3R1 respectively. Both HHRH and NPHLOP are asso with low bone density
- Fanconi Bickel Syndrome (Glycogen storage disorder due to defect in glucose transporter presents in neonatal agewith hepatomegaly, fasting hypo,, tubular nephropathy with glycosuria, phosphaturia, stunted growth)
- Rarely due to hypophosphatasia
- Vitamin D dependent Rickets Type 1A (mutation in 1alpha Ohylase (CYP27B1 gene), Type 1 B (mutation in 25 Ohylase (CYP2R1 gene) and 2A (1,25 D receptor mutation).

Decreased Absorption

- Severe Vitamin D Deficiency
- PO4 binders
- Antacids (Alumunium, Mg etc) by binding PO4 and forming insoluble salts
- Severe protracted diarrhoea /Malabsorption disorders such as Celiac disease etc

*HHRH, NPHLOP, Tenofovir etc may also have high FGF23 levels. Reason is unclear

Rickets and OM are more common with Proximal RTA (Type 2 RTA). But can very rarely occur with Distal RTA (Type 1 RTA) also

Transcellular Shifts

- DKA Rx with IV Insulin
- Refeeding Syndrome
- Respiratory Alkalosis as in salicylate poisoning
- HBS (After PTX for PHPT /SHPT, Aggressive Rx of Graves's disease ets
- Rx with BSPs→ decreased bone resorption, also secondary HPT from hypocalcemia
- Sepsis

Mannitol can cause a psuedohypophosphatemia due to an assay artifact but it may also have a phosphaturic effect

Post-hepatic resection hypophosphatemia: Cause unclear. Is transient

Hypophosphatemic osteomalacia due to cadmium exposure in silver industry

Psuedohypophosphatemia

Seen with

Mannitol

Monoclonal paraprotein (can cause psuedo**hyper**phosphatemia also)

High dose liposomal amphotericin B

Hyperbilirubinemia, Niacin

Renal Phosphate Wasting

Tumour Induced Osteomalacia

✓ Previously normal phosphate

☝ Definitive diagnosis is identification of tumour, and remission of syndrome following tumour resection

Autosomal Dominant Hypophosphataemic Rickets

? ADHR may present in adulthood

☝ FGF23 mutation analysis can be performed for definitive diagnosis

XLH – X-linked Hypophosphataemic Rickets

✘ Presents in early childhood, with slow growth and reluctance to weight bear

✘ Previously normal phosphate in 2010

Fanconi Syndrome

✘ No relevant drug hx

✘ Absence of proteinuria, glucosuria and normal myeloma panel makes MM diagnosis unlikely

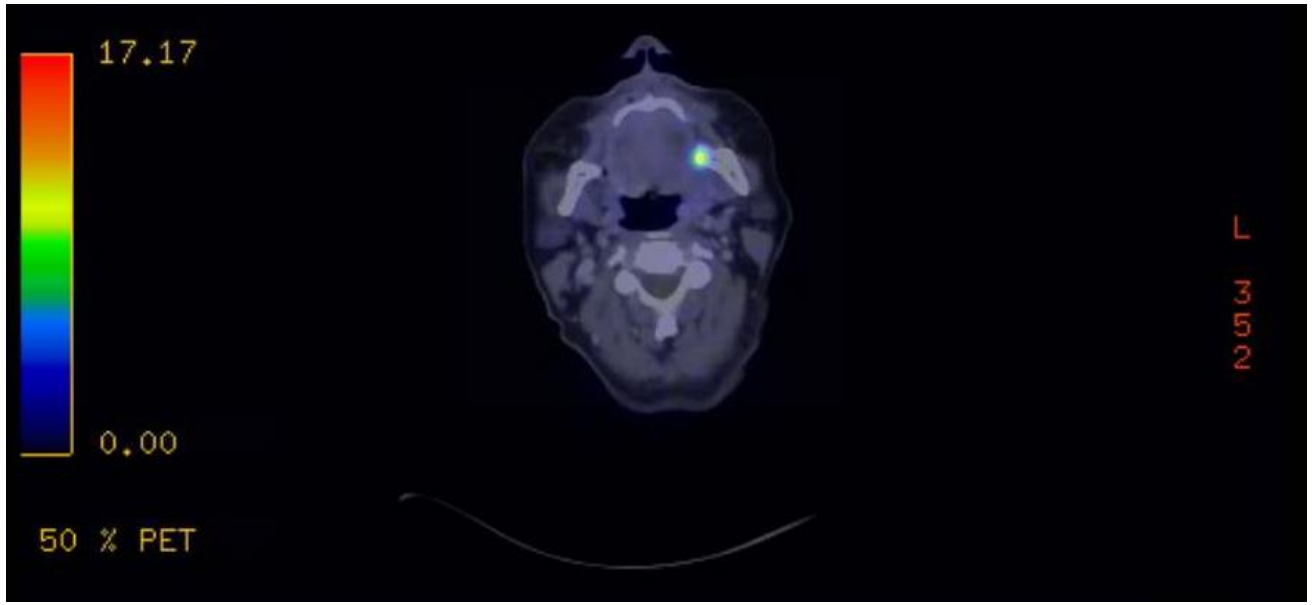
☝ *When Fanconi syndrome is suspected, screen for urine protein, glucose, uric acid + myeloma panel*

Further Investigations

Investigation	Result	Reference Range
FGF-23	250 RU/ml	< 180 RU/ml
1,25 dihydroxyvitamin D	20 pg/ml	18-64 pg/ml

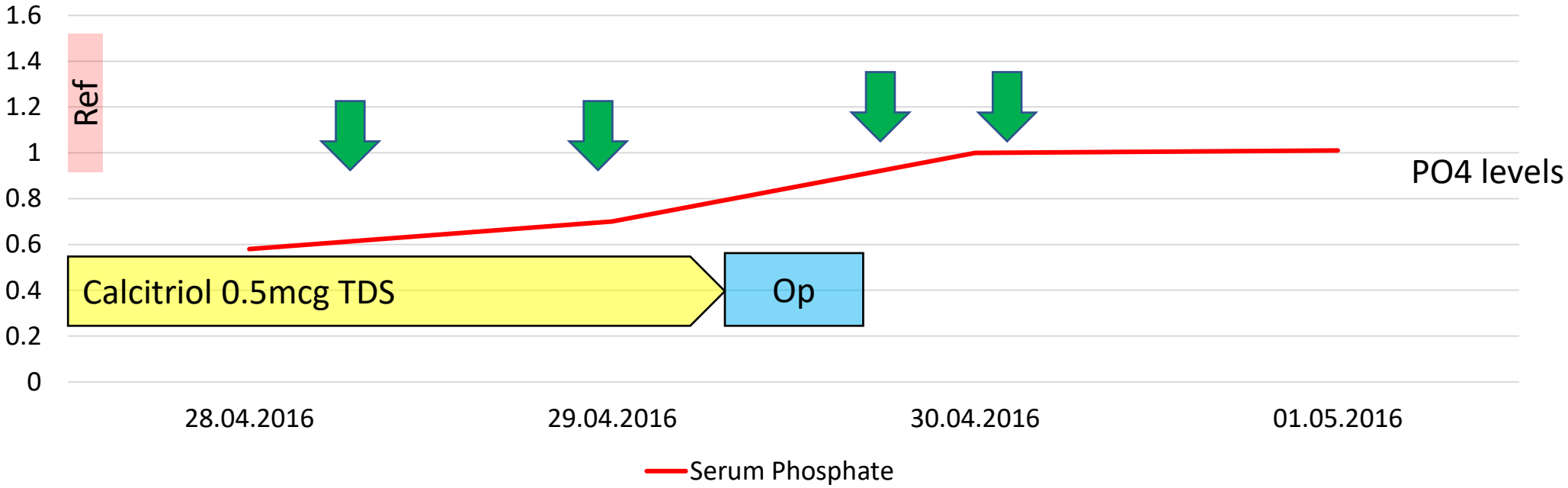
Imaging

- Parathyroid Sestamibi scan – no hyperfunctioning lesion
- Ga68 Dotapeptide scan - intense tracer avid focus in the ramus of left hemimandible with associated sclerosis, suspicious for a small mesenchymal tumour



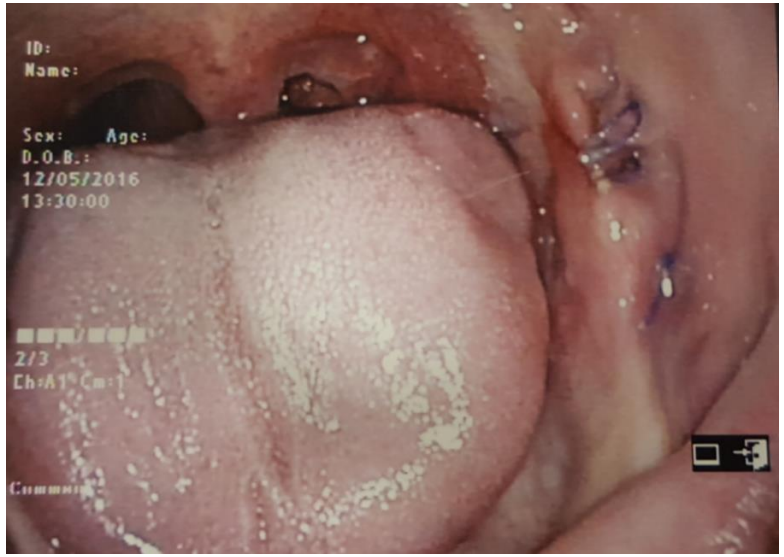
Resection of Tumour

- Underwent left marginal mandibulectomy on 29th April 2016
- Intra-op: 1cm bony lesion identified at retromolar trigone



Progress

- **Histology**
  - **Phosphaturic mesenchymal tumour, mixed connective tissue variant**
- **Biochemistry**
  - **Discharged without need for calcitriol and fleet's phosphosoda**
  - **Calcium and phosphate at follow-up on 20th May normal**
  - **TmP/GFR = 1.41**
- **Symptoms**
  - **No more bony aches and pains**

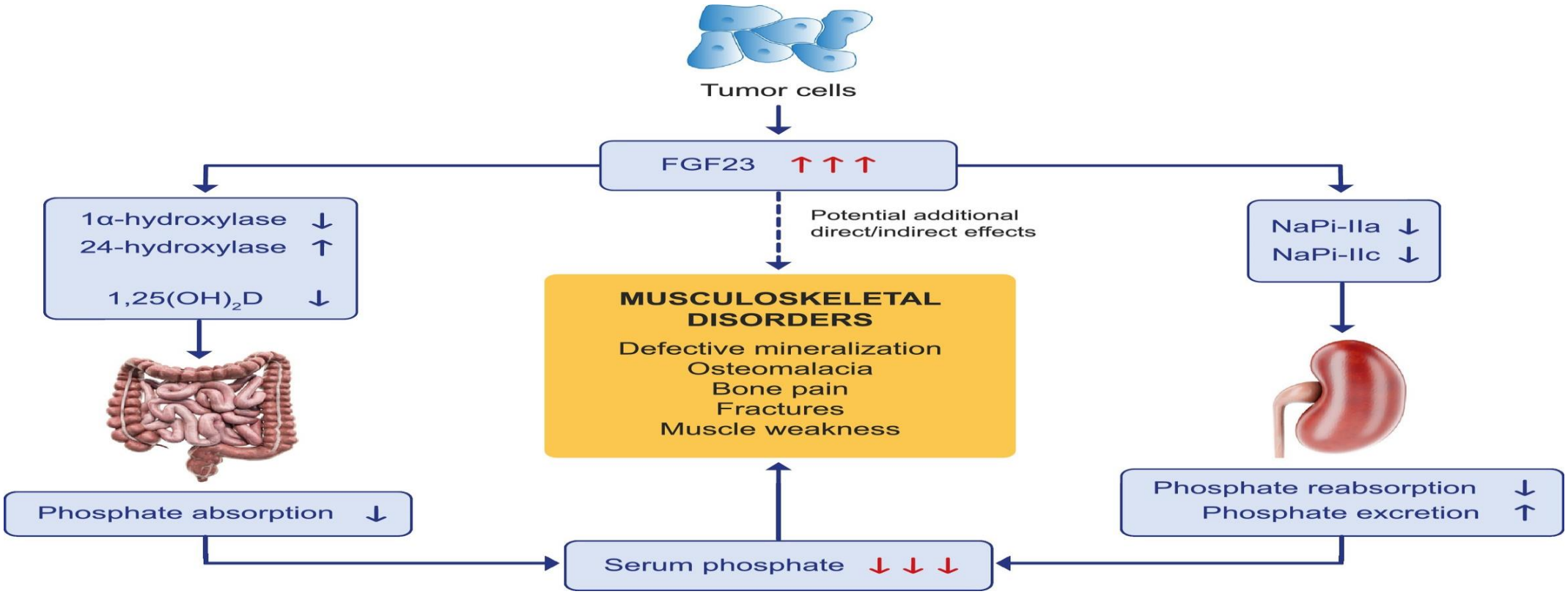


Oncogenic Osteomalacia

- Rare paraneoplastic syndrome caused by PO4 wasting
- Due to excess levels of tumour-secreted phosphatonins –FGF-23 most well characterized
- Vast majority of tumours are classified pathologically as phosphaturic mesenchymal tumours (PMTs) of the mixed connective tissue variant
- PMTs typically benign and singular, but may be multifocal, malignant or metastatic.
- Rarely, secondary TIO presents as a paraneoplastic syndrome in association with prostate, ovarian, lung cancer etc
- **Average age of presentation = 4^{th}-5^{th} decade. First and predominant symptom experienced is bone pain**
- **Average time to diagnosis = 5 years**

More precise nomenclature :Oncogenic Osteomalacia – umbrella term encompassing all benign and malignant neoplastic causes of FGF23 excess. TIO or more specifically Phosphaturic Mesenchymal Tumour- IO should be reserved for the syndrome associated with PMTs

Florenzano P------ Collins MT. CTI (2021)108:128-142



Typical laboratory profile

Normal serum calcium

Low serum phosphate

High ALP

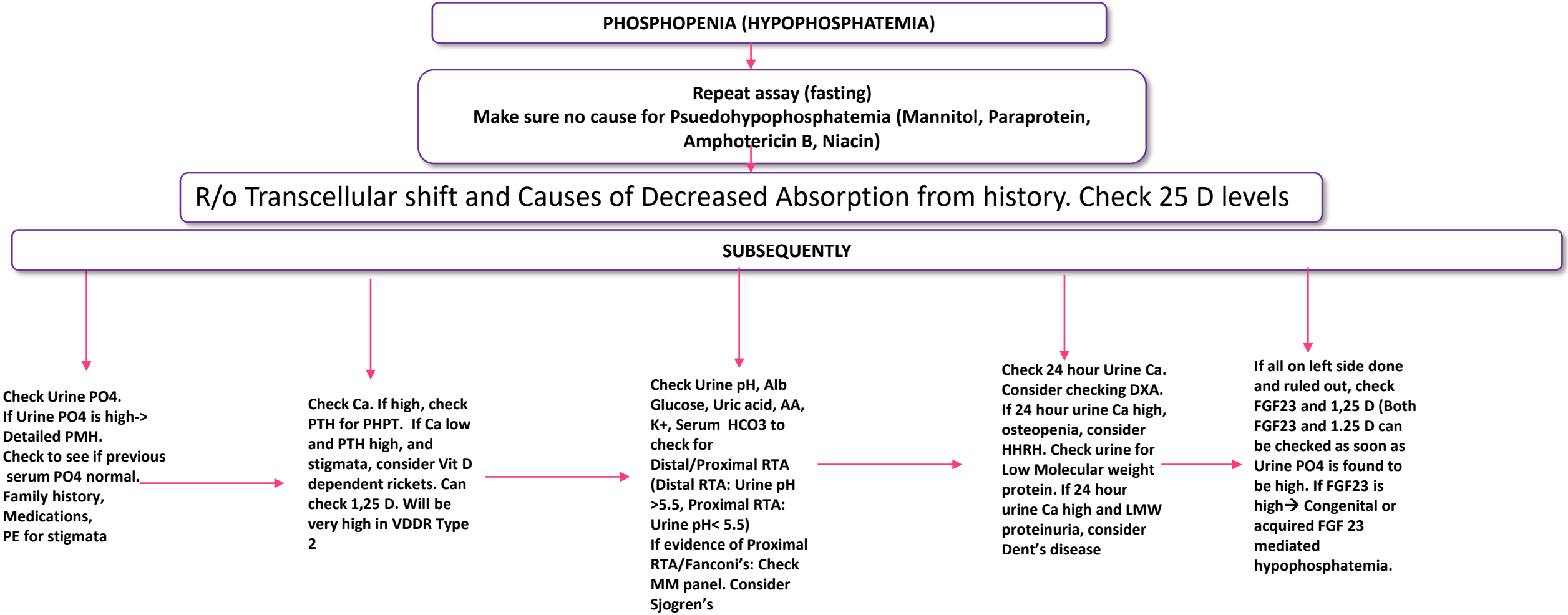
Low 1,25 dihydroxyvitamin D

Normal or mildly elevated PTH

Elevated FGF23

Brandi ML et al. Bone 2021

My personal approach to Evaluation of Hypophosphatemia



BIOCHEMICAL EVALUATION OF PHOSPHATE WASTING DISORDERS

Calculations

Phosphate clearance to Creatinine Clearance Ratio (Cp)

$$Cp = \frac{Urine\ Phosphate}{Serum\ Phosphate} \times \frac{Serum\ Creatinine}{Urine\ Creatinine}$$

*Convenient, can be calculated from a random, simultaneous sample of blood and urine (2nd void)

Tubular Reabsorption of Phosphate (TRP): When serum PO4 NL, NL TRP =85-95%

$$TRP = 1 - \frac{Cp}{Ccr} = 1 - \frac{Serum\ creatinine}{Urine\ creatinine} \times \frac{Urine\ phosphate}{Serum\ phosphate}$$

Maximum tubular reabsorption of phosphate to GFR (TmP/GFR) . Reference range for adults: 0.8-1.35 mmol/L.

TmP/GFR can be determined using either the Walton and Bijvoet nomogram or be calculated using the Kenny and Glen Algorithm or a web based calculator.

Payne. Ann Clin Biochem 1998

Measuring FGF 23

- **FGF23-diurnal variation. Therefore- early morning sample, store on ice and send for immediate centrifugation since it is an unstable protein and susceptible to decay into several fragments**
- **Fasting has little influence**
- **Principle of sandwich ELIZA recognizing 2 distinct epitopes**
- **Either detects the intact (full length) form (Intact Assay) or the Intact AND C-terminal fragments (C terminal assay) (depending on whether the assay recognizes the epitopes within the N and C-terminal domains flanking the cleavage point, or only the epitopes within the C-terminal portion**
- **3 ELIZA immunoassays for intact FGF 23 (Immutopics, Kainos, Millipore).**
- **1 automated intact (Diasorin)**
- **Intact assay reports in pg/ml or pmol/l or ng/L**
- **1 ELIZA Immunoassay for c-terminal FGF23 : Immutopics. Reported in Relative Units (RU)/ml**
- **C-terminal concentrations higher in children than in adults**
- **Intact concentrations same in children and adults**
- **C terminal concentrations lower in serum than in EDTA plasma samples**
- **Intact concentrations same in serum and in EDTA plasma samples with the exception of Diasorin Intact assay for which serum conc is less than EDTA and therefore EDTA plasma sample is preferred since more stability at room temperature**
- **Reference ranges available for the Diasorin Intact in adults (22.7-99.3 ng/L) and for the Immunotopics C-terminal assay for children**
- **In treated patients with an unclear diagnosis, if necessary to measure FGF-23, need to stop PO4 supplements at least 2 weeks before (coz PO4 supplements increase FGF23 levels)**
- **Burosumab causes analytical interference with certain FGF23 assays. Burosumab being an antibody to FGF23**

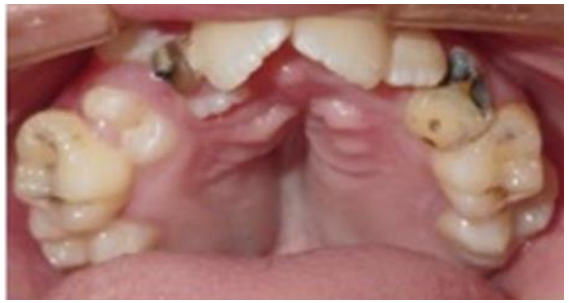
“Dense” Skeleton in the Closet

Case 2

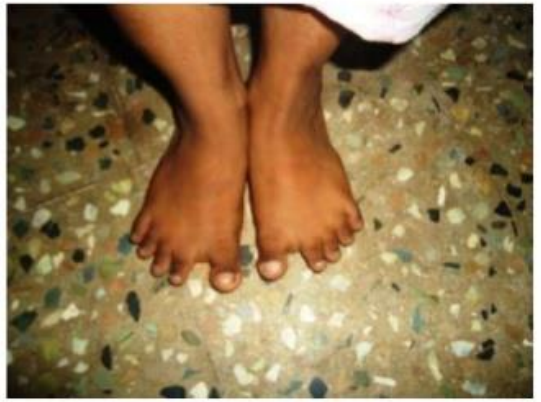
- 22 year-old female recently immigrated to Singaporefrom India. New Right femur fracture
- Spontaneous left femur mid-shaft incomplete fracture about 18 months ago,
- Treated with focal ultrasound for one month after orthopedic rodding to stimulate fracture healing, with slow callus formation on x-rays
- Multiple fractures of humerus, wrist, tibia, during early and late childhood
- No previous oral or IV bisphosphonates, or other anti-resorptive therapy

- Physical examination notable for:

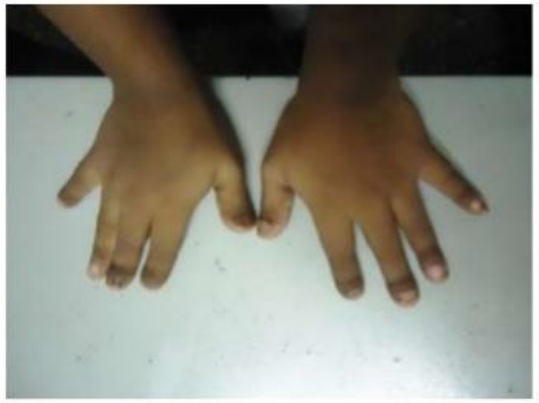
Dysmorphic facies with frontal bossing
Large skull, deformed mandible
High Arched grooved palate
Short Stubby toes and fingers



Short stubby toes

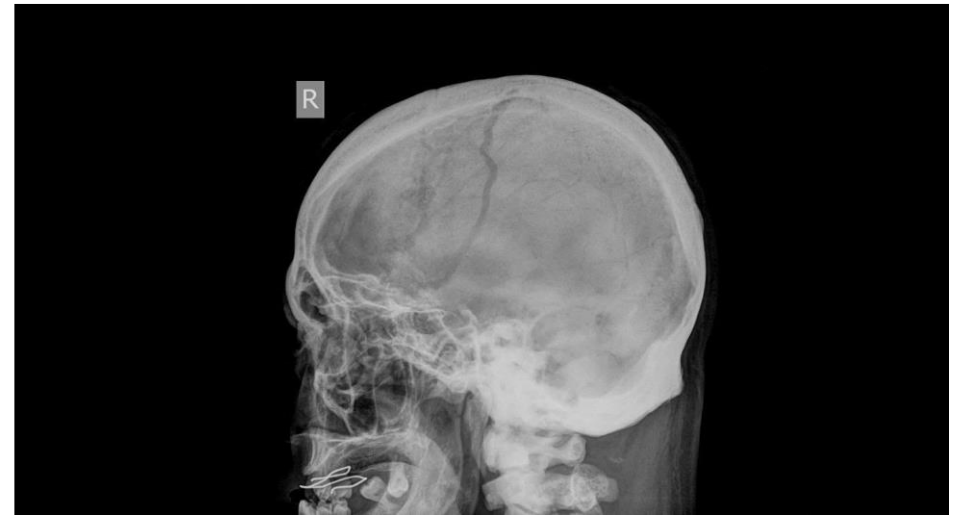


Short stubby fingers



DXA

- Left Neck of femur 0.954 gm/cm2
  2.3 SD above mean peak BMD (T-score)
  4.6 SD above the reference value for the patient's age (Z-score)
- Left Total Hip 1.227 gm/cm2
  3.7 SD above mean peak BMD (T-score)
  4.9 SD above the reference value for the patient's age (Z-score)
- Lumbar spine(L1-L4) 1.776 gm/cm2
  6.8 SD above mean peak BMD (T-score)
  6.9 SD above the reference value for the patient's age (Z-score)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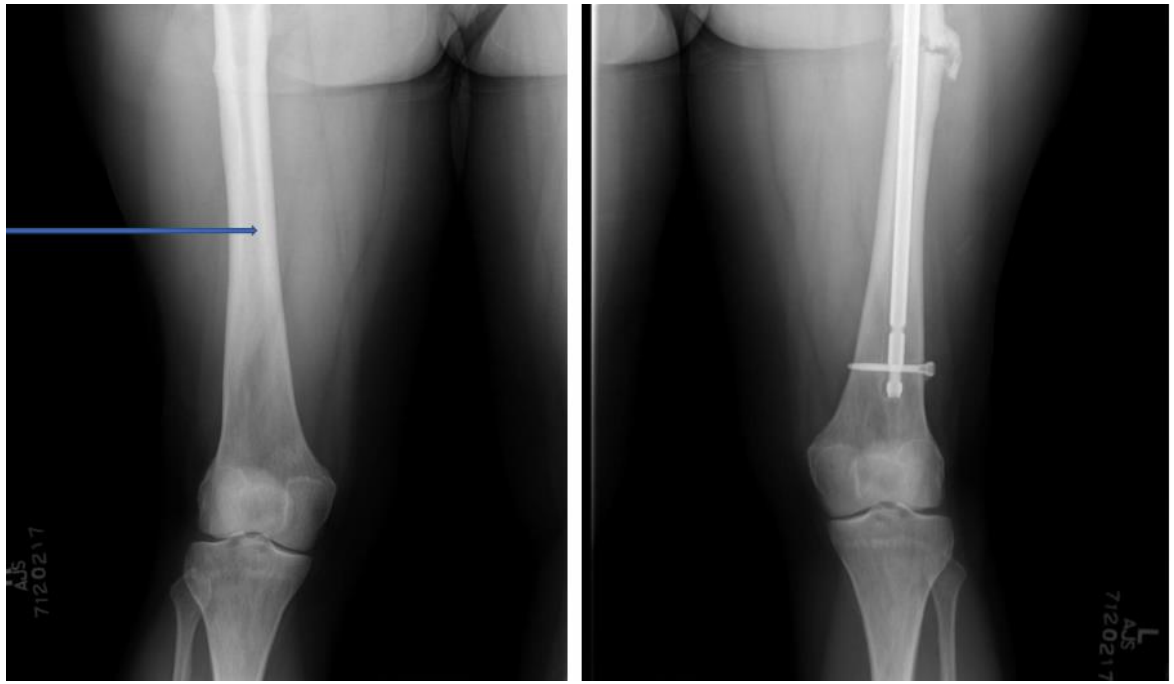

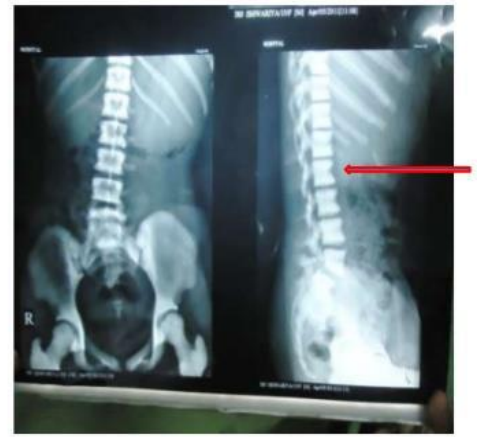
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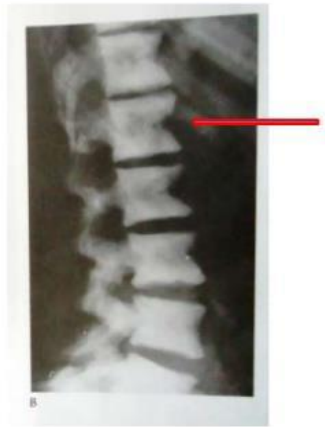


HIGH BMD

Acquired Causes of High BMD

Artefactual Acquired Causes of High BMD

DISH, Ankylosing Spondylitis, Vertebral fractures, Vascular Calcifications, Gall Stones, Renal Calculi, Gluteal Silicone Implants, Vertebroplasty and Kyphoplasty

True Acquired Causes of High BMD

Localized Acquired: Primary Malignancies: eg: Osteoblastoma, Secondary Malignancies: eg; Secondary metastasis from eg Prostate Cancer

Generalized Acquired:

Fluorosis, SAPHO Syndrome, Renal Osteodystrophy, Hepatitis C Associated Osteosclerosis, Myelofibrosis, Mastocytosis

Inherited Causes of High BMD

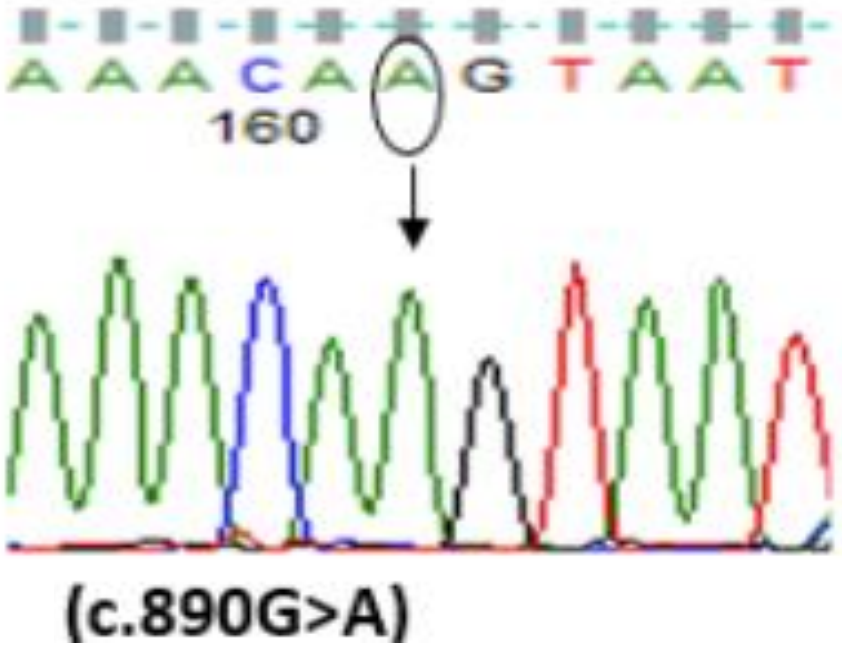
Due to Reduced Bone Resorption	Due to Increased Bone Formation	Disturbed Balance between Formation and Resorption	Unexplained HBM
Osteopetrosis Pycnodysostosis Osteopoikilosis and Buscke Ollendorf Syndrome Melorheostosis	Sclerosteosis Van Buchem Disease LRP5 HBM LRP4 HBM (loss of function mutation) (Sclerosteosis Type 2): has a sclerosteosis phenotype LRP6 HBM SMAD 9 Mutations Craniometaphyseal Dysplasia	Camurati Engelman Disease Ghosal Dermatodiaphyseal Syndrome Osteomesopycnosis Osteopathia Striata with cranial sclerosis	

- Only child of parents of a consanguinous marriage. Both parents apparently normal and average height
- Regular periods
- No other significant medical history
- No current medications
- Vital signs: Ht 144 cm (4’5”), Wt 49.0 kg, BP 135/82 mm Hg, P 96, reg
- Physical Exam:
  - Skin: normal elasticity
  - Eyes: hypertelorism with mild proptosis
  - Extremities: shortened fingers and toes
  - Gait: antalgic due to right mid-thigh pain

  Labs:

  Normal CBC with differential, Cr, Ca, PO4, iPTH

  Serum fasting CTX : 120 pcg/ml (Normal premenopausal range: 104-1008)



Direct sequencing of CTSK gene: Missense mutation of Exon 7
cDNA change c.890G>A with protein change p.Ser297Asn
CTSK gene has 8 exons encoding 329 amino acids,

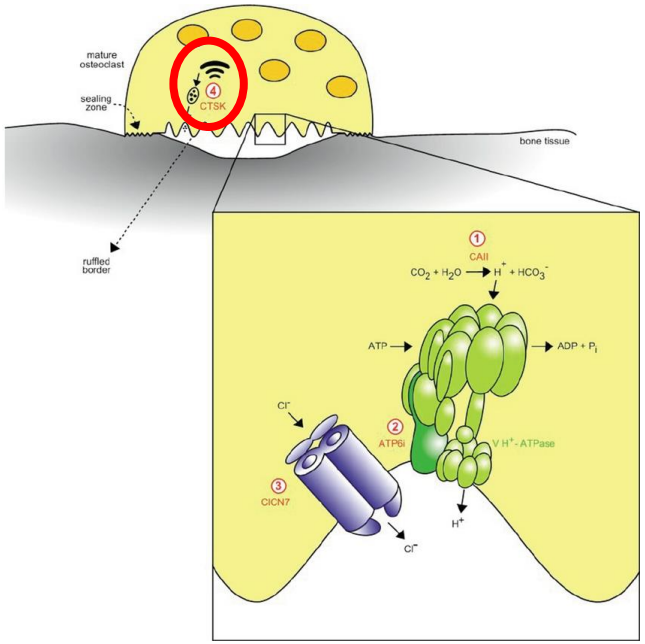
Pyknodysostosis

- **Very rare autosomal recessive form of sclerosing bone dysplasia due to cathepsin K deficiency resulting from *CTSK* gene mutations on 1q21**

Cathepsin K is a lysosomal cysteine proteinase that is expressed in osteoclasts and is required for the degradation of collagen.

- **First described 1962 by Maroteaux and Lamy**
- **Greek “pycnos” = dense**
- **Osteoclasts not able to degrade bone matrix collagens type I and II**
- **Increased bone matrix mineralization, with increased BMD**
- **Increased fragility fractures**

Decreased elasticity of bone and impaired repair capabilities → fractures



Pyknodysostosis	Osteopetrosis
Short stature **Autosomal recessive**	**Normal stature ;** **Autosomal dominant and recessive forms**
Open fontanelles and sutures present ; Frontal bossing present	Absent; absent
Blue sclera present occasionally ; Wormian bones present ;	Absent absent
Obtuse mandibular angle -present Terminal phalanx atrophy-present	Absent absent
Absent (rare)	Anemia ,hepatosplenomegaly common
Absent;	Bone within bone appearance
Hypolasia /aplasia of clavicles –present	Absent

Autosomal recessive OP usually results in fetal demise. Autosomal dominant OP : onset usually in adolescence or adulthood. Medullary involvement→anemia

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The term osteopetrosis is sometimes used in a general sense to describe the condition that results as a consequence of failure of osteoclast function during growth

Case 3

“ Hey Manju!, Is there something wrong with our Vitamin D assay? I have this patient who I am working up for recurrent nephrolithiasis. Her 25 (OH)D level has come back twice as very high at 136 ng/ml. She is not on any Vitamin D supplements. Her serum calcium is also upper limit of normal and she is not on any Calcium supplements.

She is 32 years old. She has a h/o recurrent kidney stones since young

Some family history also positive for kidney stones”

Labs

Serum Adjusted for Albumin Calcium: 2.65 mmol/l (2.3-2.6)

Serum iPTH: 1 pmol/l (0.9-6.2), PTHrP: Normal

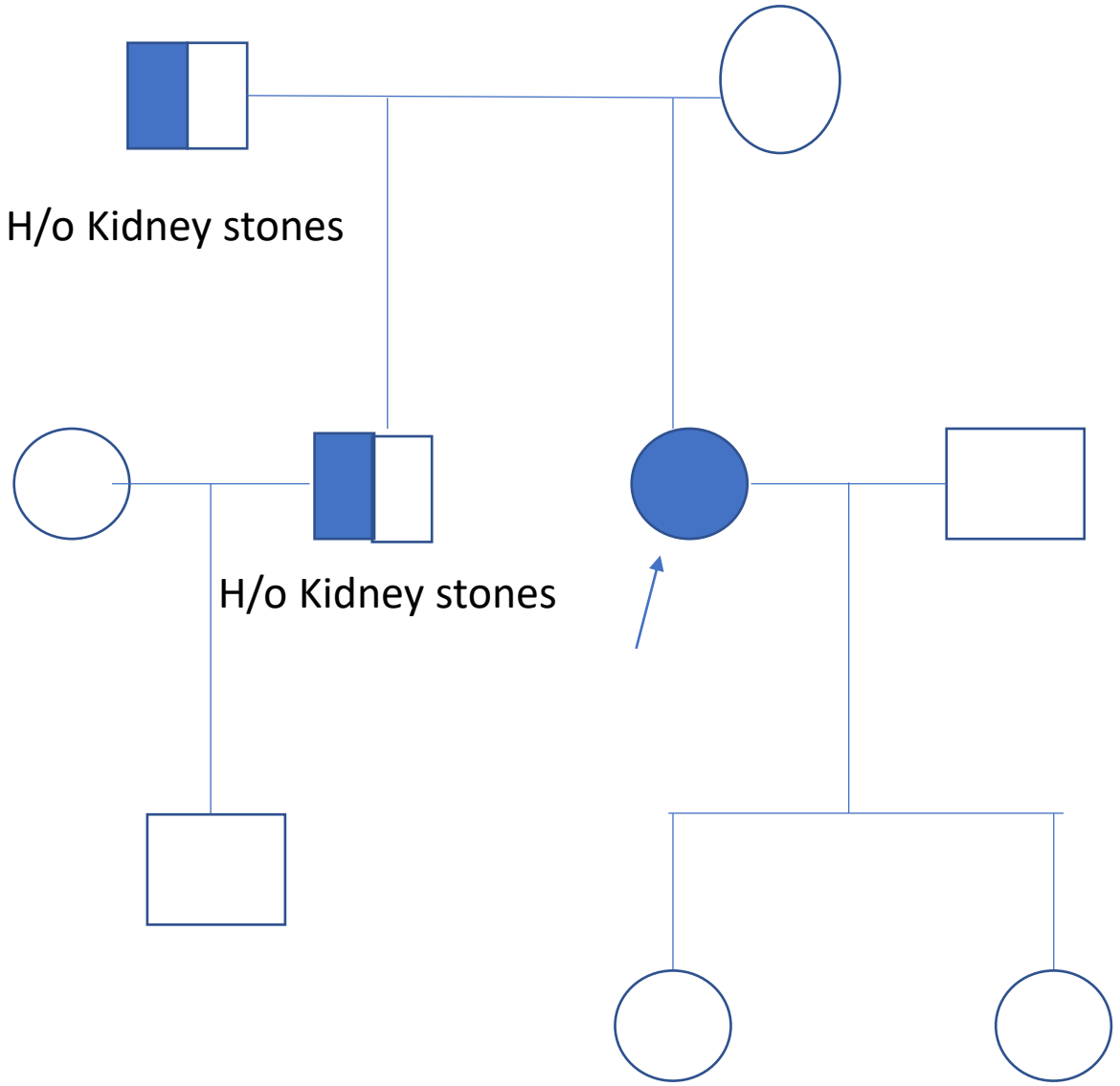
25(OH)D3: Repeated twice (Tandem Mass Spect): 136 ng/ml and 150 ng/ml

Cross checked on Diasorin RIA at another lab: Also 149 ng/ml

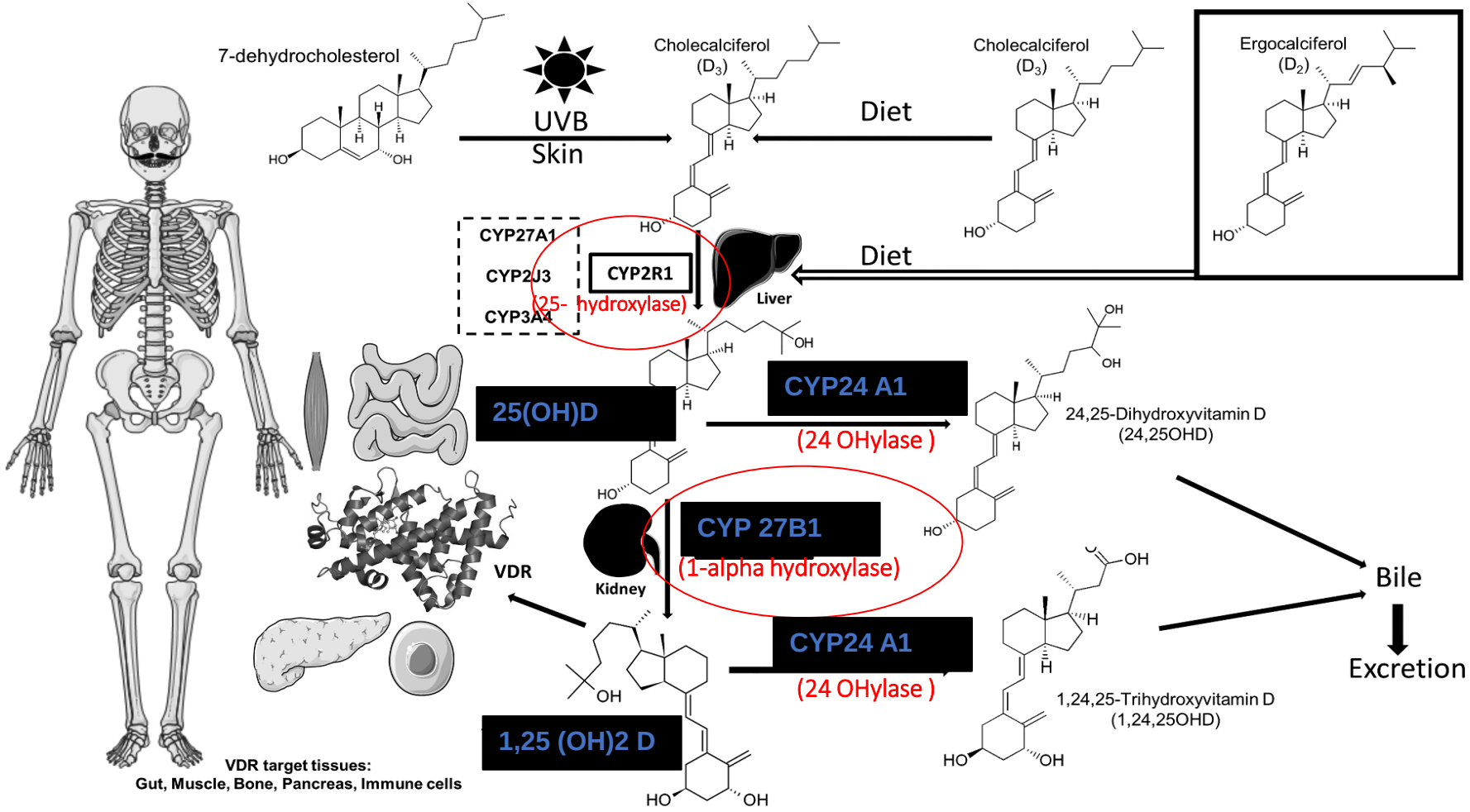
24 hour urine Calcium: 8.6 mmol/day (upto 6.5 mmol/day. 24 hour urine Na is lowish (definitely not high)

1,25 D (Sent out to Mayo Clinic): 140 pg/ml/ 337 pmol/l (18-64/ 43-154)

No evidence of Sarcoidosis or TB or other granulomatous diseases clinically or radiologically. Serum ACE levels normal


H/o Kidney stones
H/o Kidney stones

VITAMIN D METABOLISM



Vitamin D is an inactive secosteroid prehormone that must undergo two consecutive hydroxylation steps to activate the VDR and thus exert its biological effects. The active hormone, 1,25OHD, is produced by sequential 25-hydroxylation in the liver and 1α-hydroxylation in the kidney. The tightly regulated and rate-limiting step of 1,25OHD biosynthesis is mediated by the mitochondrial CYP27B1 enzyme. The principal vitamin D-inactivating enzyme is the mitochondrial CYP24A1, which 24-hydroxylates both 25OHD to 24,25-dihydroxyvitamin D (24,25OHD) and 1,25OHD to 1,24,25-trihydroxyvitamin D (1,24,25OHD), mainly in the kidney

- **25 D:24,25 dihydroxy Vitamin D ratio: 120**

Genetic analysis revealed a compound heterozygous CYP24A1 mutation p.R223*). The CYP24A1 mutation, p.R223*
was also found heterozygously in other family members the history of nephrolithiasis.

Ratios in the 25-80 range can be seen in patients with low vitamin D levels or heterozygous CYP24A1 mutations

Cyp24A1

- Enzyme that inactivates 25(OH)D to 24,25-dihydroxyvitamin D
- Also inactivates 1,25(OH)2D to 1,24,25(OH)3-vitamin D

- Homozygous or heterozygous loss of function mutations can cause variable clinical presentations, caused by increased 1,25 dihydroxyvitamin D and consequent hypercalcemia and hypercalciuria
  - Idiopathic infantile hypercalcemia
  - Adult onset nephrocalcinosis
  - Nephrolithiasis
  - Renal insufficiency
  - Recurrent pancreatitis

Vitamin D Metabolism

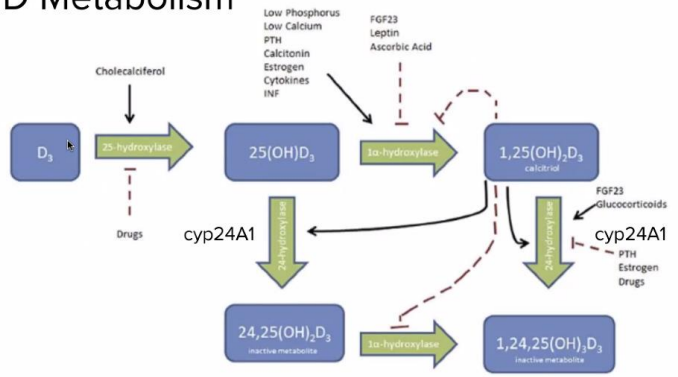


Fig. 1. Vitamin D metabolism. PTH, parathyroid hormone; INF, interferon; OH, hydroxyl; FGF, fibroblast growth factor.

Peiris et al. 2017. Cyp24A1 Mutations. *Nephrology and Dialysis*

Cyp24A1

- Genetic mutation should be considered if:
  - Hypercalciuria
  - Hypercalcemia
  - Elevated 1,25 dihydroxyvitamin D
  - Suppressed PTH
- There is a dose-dependent relationship between mutant alleles and phenotype. No genotype-phenotype association has been demonstrated.
- Heterozygotes typically have a less severe presentation than homozygotes, less severe calciuria and less nephrolithiasis.

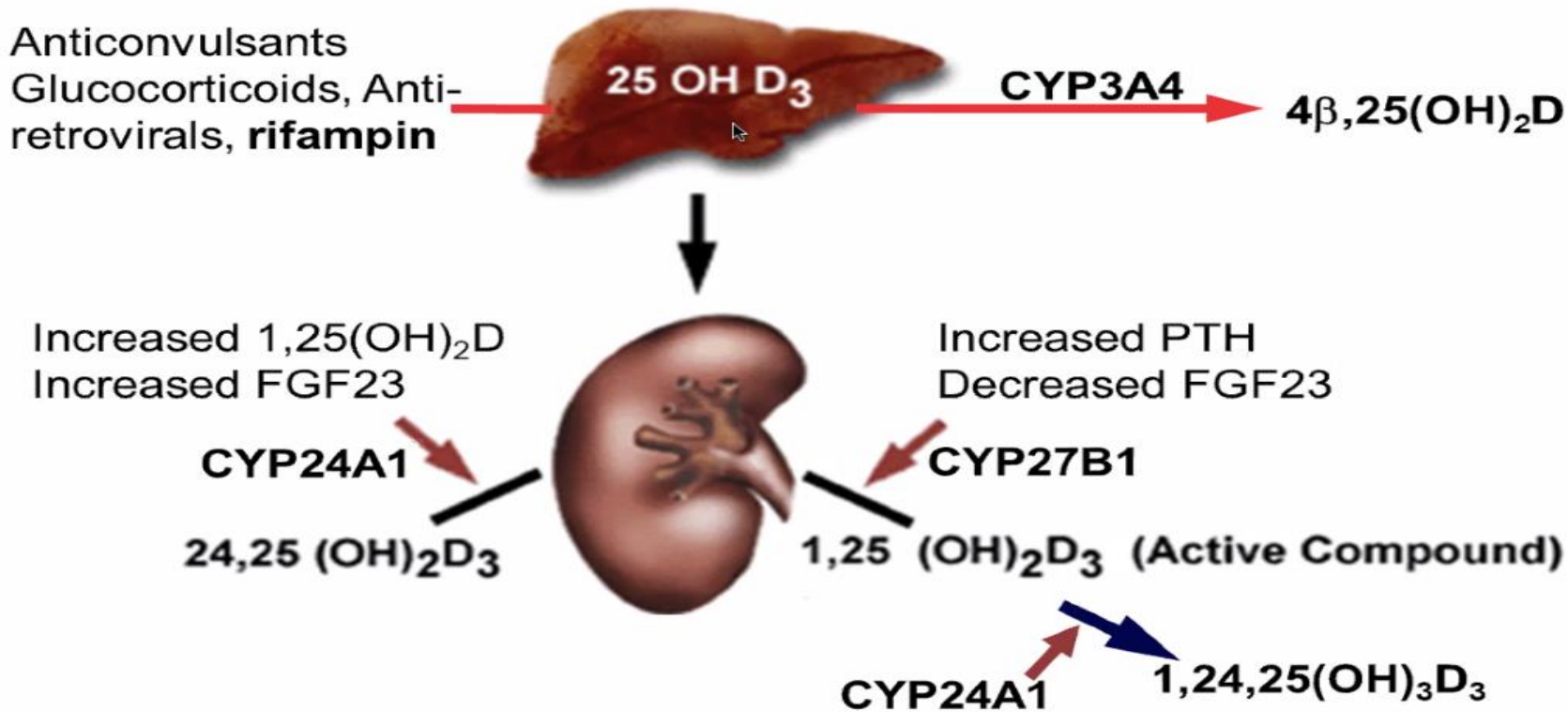
- Hydration, Avoid Vitamin D and Calcium supplements
- Thiazide diuretics sometimes used for the hypercalciuria
- Ketoconazole has been tried (utilizing its effect as a cytochrome P450 inhibitor –including its effect on 25 hydroxylase and 1 alpha hydroxylase to decrease production of 1,25 (OH)2D. Given thrice daily. Marked SE profile.
- Corticosteroids not recommended to reduce intestinal calcium absorption in the setting of hypercalcemia due to CYP24 A1 mutation. Its therapeutic effect is mediated primarily through inhibtion of CYP27B1 enzyme

JCEM THE JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM

CYP3A4 Induction by Rifampin: An Alternative Pathway for Vitamin D Inactivation in Patients With *CYP24A1* Mutations

Colin Patrick Hawkes,[1,2] Dong Li,[3] Hakon Hakonarson,[3] Kevin E. Meyers,[4,5] Kenneth E. Thummel,[6] and Michael A. Levine[1,5]

Metabolism of Vitamin D Metabolites



- Rifampin induces CYP3A4 and catalyzes hydroxylation of 1,25 dihydroxy vitamin D to inactive metabolite 1,23, 25 (OH)3 D3
- Given once daily

A final word: Genomic approach to diagnose SRDs

- First generation sequencing (Sanger sequencing) –can only sequence one region of interest per reaction with a max length of 1000bp
- Second Generation Sequencing –Next Generation Sequencing (NGS)

Whole Genome Sequencing (WGS): Uses genomic DNA containing exomic (coding) , intronic, promoter and intergenic regions. Can produce roughly 90GB of data(!) ; time consuming, leads to detection of tremendous number of variants

Whole Exome Sequencing: Uses only the Exomic part of the DNA which represents approximately 15-20% of the whole genome. Captures only the coding DNA which dramatically reduces both time and cost of sequencing

A variation of WES is the multiple gene sequencing panel, also known as targeted –exome sequencing or NGS panel. Uses a similar protocol as for WES, except that it only target genes chosen specifically because of their association with the disease

Falardeau F, Camurri MV, Campeau PM. Bone 102 (2017)5-14

Resources and Registries for SRDs

Review > Nat Rev Endocrinol. 2022 Jun;18(6):366-384. doi: 10.1038/s41574-022-00662-x. Epub 2022 Apr 28.

Interdisciplinary management of FGF23–related phosphate wasting syndromes: a Consensus Statement on the evaluation, diagnosis and care of patients with X–linked hypophosphataemia

Andrea Trombetti [1 2], Nasser Al-Daghri [3], Maria Luisa Brandi [4], Jorge B Cannata-Andía [5 6 7 8], Etienne Cavalier [9], Manju Chandran [10], Catherine Chaussain [11 12], Lucia Cipullo [13], Cyrus Cooper [14 15 16], Dieter Haffner [17], Pol Harvengt [18], Nicholas C Harvey [14 15], Muhammad Kassim Javaid [16], Famida Jiwa [19], John A Kanis [20 21], Andrea Laslop [22], Michaël R Laurent [23], Agnès Linglart [24 25], Andréa Marques [26 27], Gabriel T Mindler [28 29], Salvatore Minisola [30], María Concepción Prieto Yerro [31], Mario Miguel Rosa [32], Lothar Seefried [33], Mila Vlaskovska [34], María Belén Zanchetta [35], René Rizzoli [36]

1) European Registry for Rare Bone and Mineral Conditions (EuRR-Bone):https://eurr-bone.com/ It collaborates with

a) EuRRECa project (European Registries for Rare Endocrine Conditions

and

b) European Reference Network on Rare Bone Disorders: (ERN BOND https://ernbond.eu/

(ERN BOND is a collaborative network of 25 expert centres, located in 9 different European countries with additional16 Affiliated Partners to increase the accessibility of the ERNs to patients from non represented EU countries)

2) Osteogenesis Imperfecta Federation of Europe (OIFE)

3) US based Rare Diseases Registry Program (RaDaR): https://registries.ncats.nih.gov/

4) **International Osteoporosis Foundation** **https://www.osteoporosis.foundation/educational-hub/topic/skeletal-rare-disorders**

5) www.rbdsummit.com

6) AP Consensus Guidelines on XLH (In development)

Rare diseases are rare, but rare disease patients are numerous

THANK YOU

On behalf of IOF, we thank you for your participation in this webinar

BoneCast

Special Edition Webinar

IOF

International Osteoporosis Foundation

Our vision is a world without fragility fractures,
in which healthy mobility is a reality for all.

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