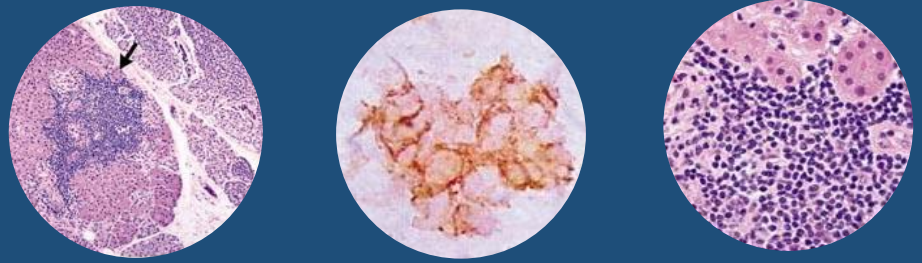
Acquired hypocalciuric hypercalcemia due to autoantibodies against the calcium-sensing receptor

J. Carl Pallais, M.D., M.P.H., Olga Kifor, M.D., Yi-Bin Chen, M.D., David Slovik, M.D., and Edward M. Brown, M.D.



Presented by: Mathew Hindi, Diana Lin, Arielle Gatbonton, and Katherine Duncan

But, we must first understand calcium homeostasis and the players involved…

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Lecture Material Recap - Calcium in the body

The normal ranges of calcium in the circulation are 8.8-10.6 mg/dl.

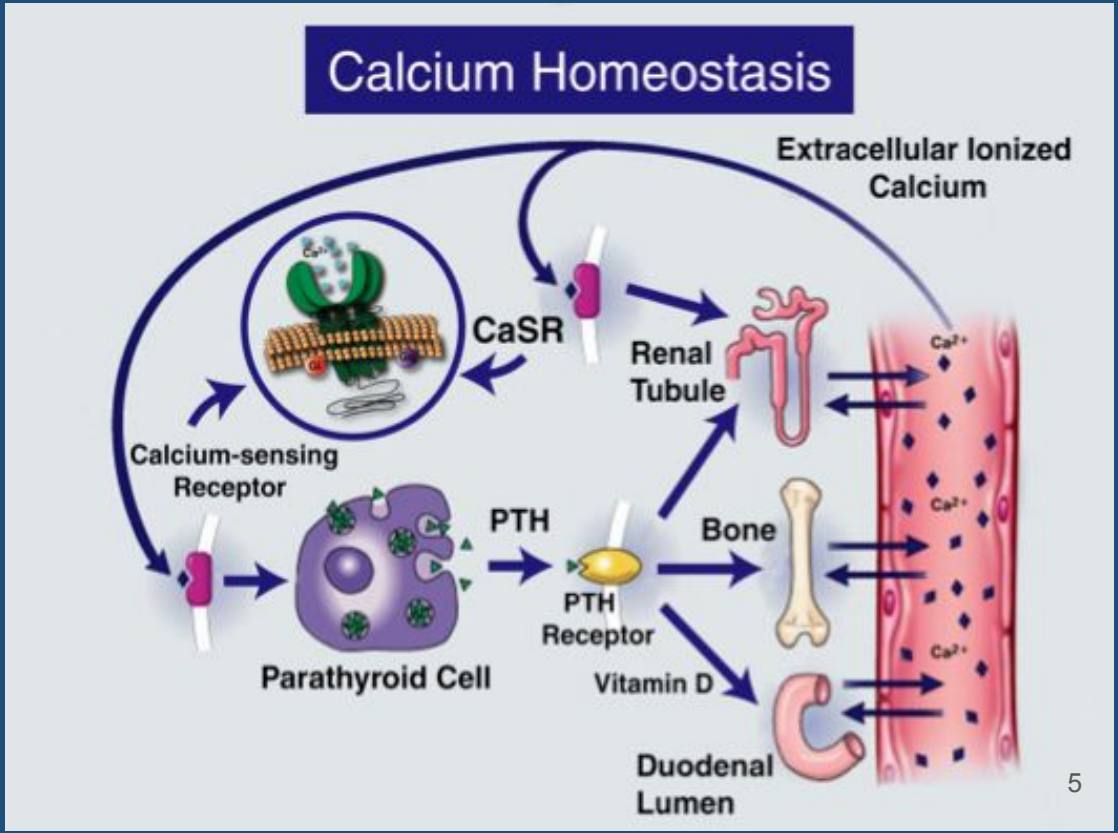
Majority (55%) of Calcium is bound to proteins such as albumin or globulins or complexed to ions such as bicarbonate and phosphate while about 45% of the calcium is ionized (biologically active).

The change, as well as the absolute serum calcium concentration, impacts symptom development.

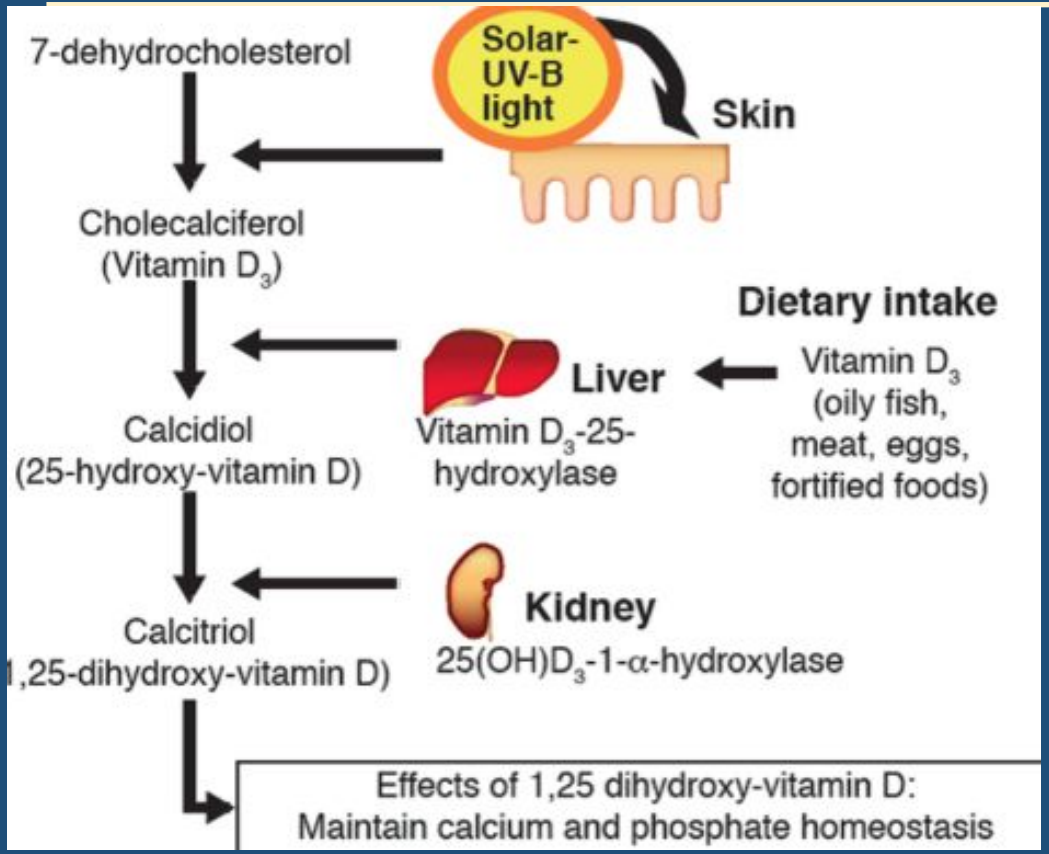
Background: Calcium Homeostasis Review

Complex homeostatic system involving the interplay of the bones, kidneys and intestine that keeps extracellular calcium in a narrow range. Regulated by 2 major players: Vit D and polypeptide hormone PTH.

PTH is major player with respect to skeleton, in the kidney, PTH acts on the distal tubule to reabsorb calcium (fine tuning role) and at proximal tubule to activate 1-alpha hydroxylase (Vit D production). At level of the gut, Vit D is the major player and brings Ca2+ and phosphate ion across.

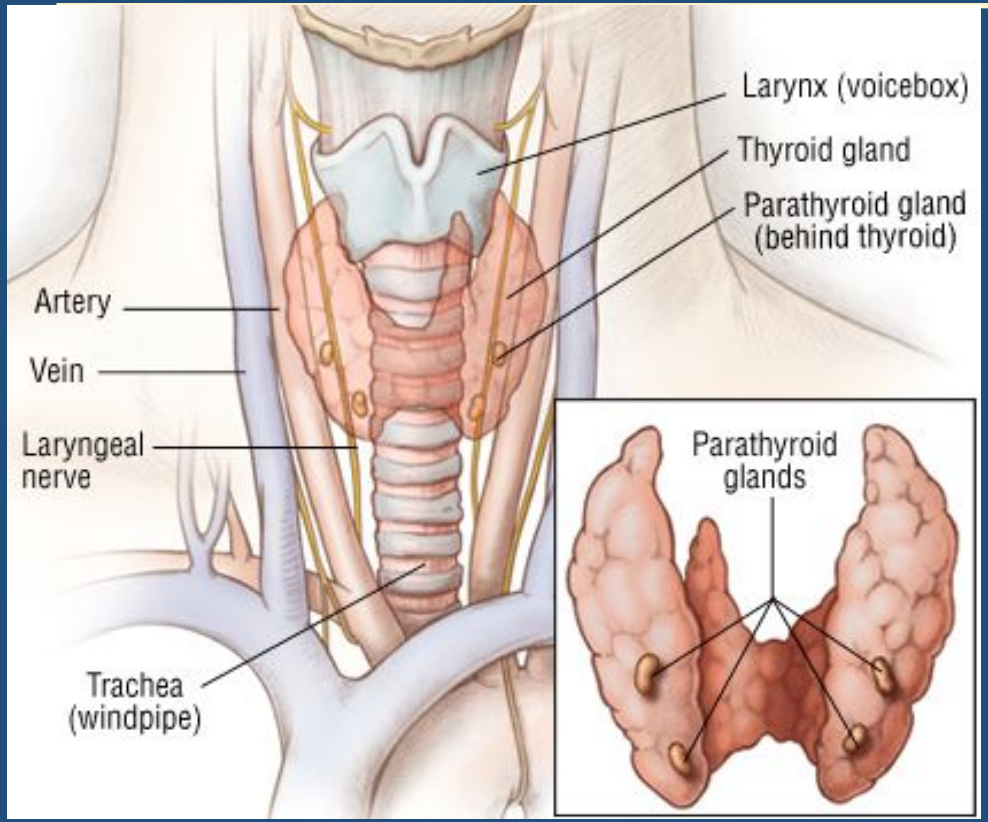


Parathyroid glands and PTH



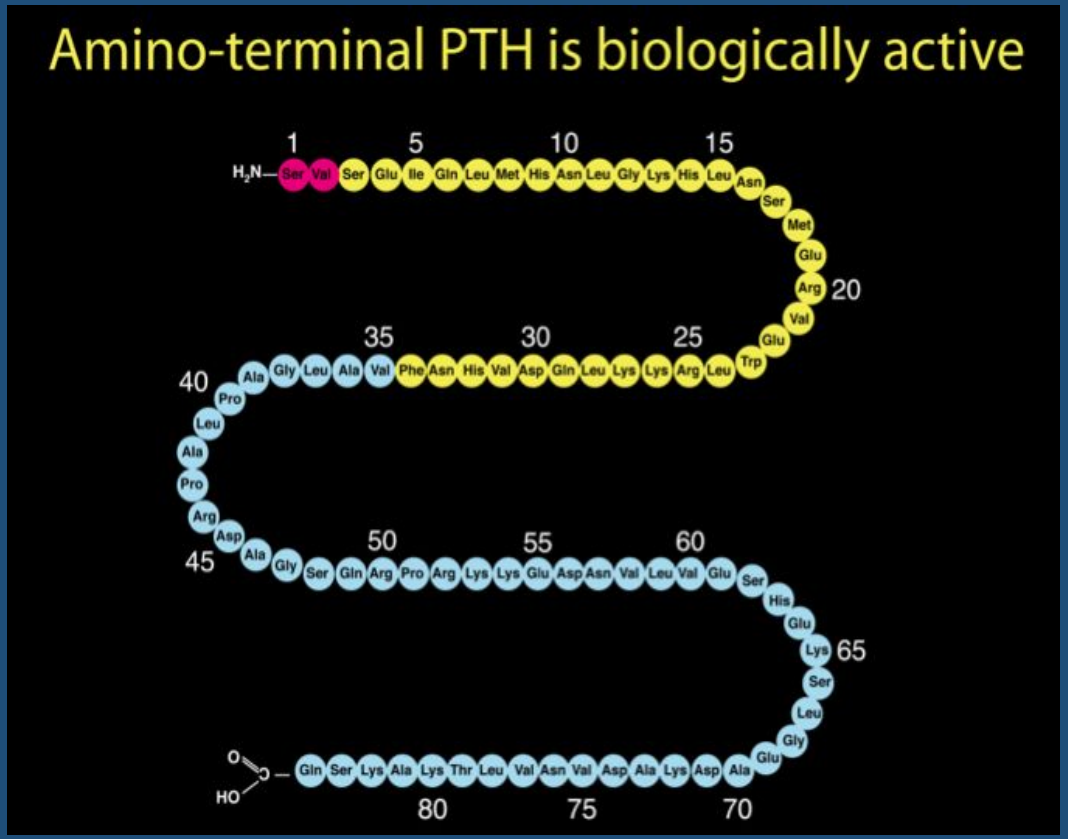
The parathyroid glands can detect a drop in ECF Ca2+ and release PTH. PTH has a role in the proximal tubule in the production of Vitamin D from its precursor (makes sure 1-alpha-hydroxylase is activated). 1,25-DihydroxyVitD3 is major player in the gut to bring Ca2+ across. An increase in ECF Ca2+ can signal through negative feedback to the parathyroid glands to shut off PTH release.

Parathyroid glands and PTH



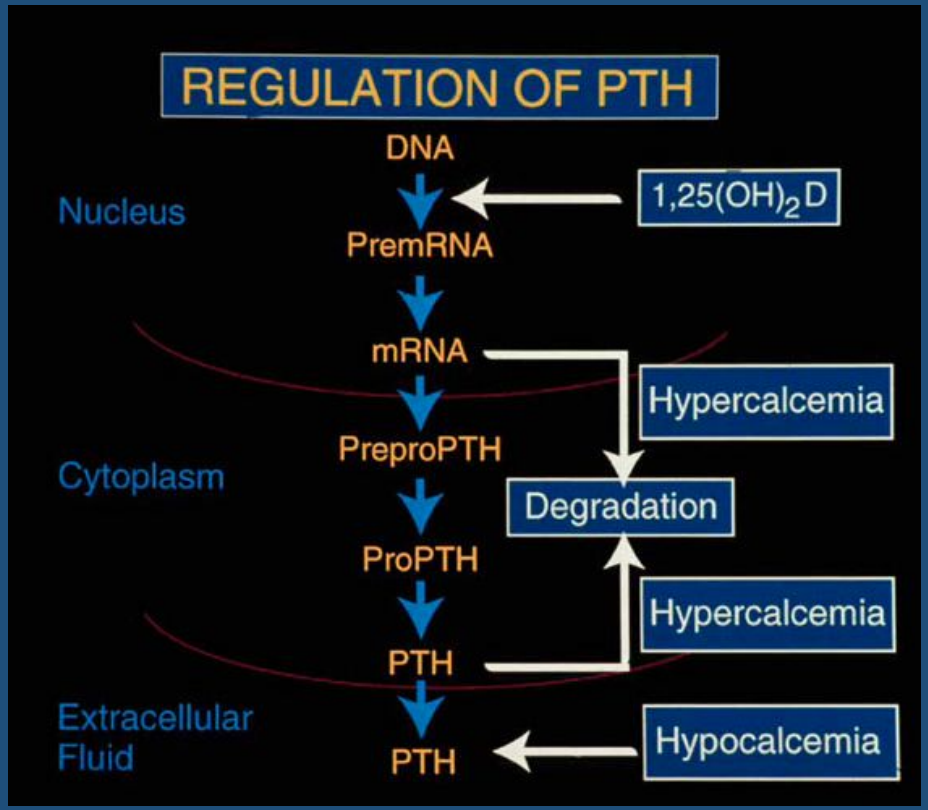
Parathyroid glands are small endocrine glands found in the neck. Humans have 2 pairs of these glands (4 glands total) that lie in anterior-cervical region. These glands are responsible for producing parathyroid hormone (PTH).

Parathyroid hormone (PTH)



Composed of 84 amino-acids as a single-chain polypeptide. For most of the known biological activities of PTH, the amino terminus (active end) 1-34 AAs is the only region required for effective hormone-receptor interactions. The first 2 AAs on the N terminus (Serine and Valine) are very critical when coupled with the PTH receptor and these help activate the signalling pathway. Without these 2 amino acids then you have an inhibitor (a blocker). Amino terminal of PTH (transient exposure – short-term exposure) can be used to treat osteoporosis as can promote bone-producing effects of PTH.

Parathyroid hormone (PTH) regulation

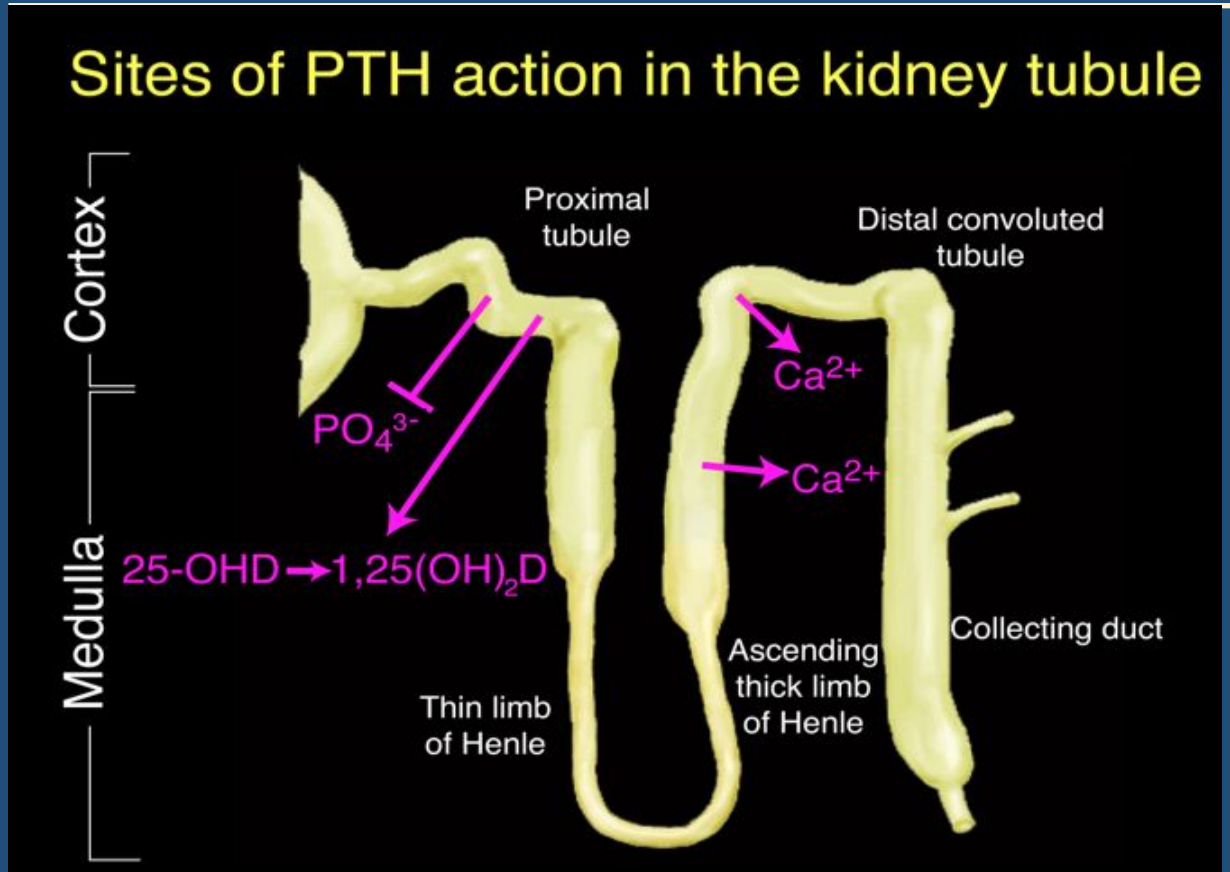


1,25-dihydroxyVitD is an inhibitor of the gene.

Premessenger RNA (PremRNA) processed to become mRNA and this encodes preproPTH (precursor). Pre is a tag at amino terminus and this gets PTH inserted into ER and thus can be trafficked to later on be packaged into granules.

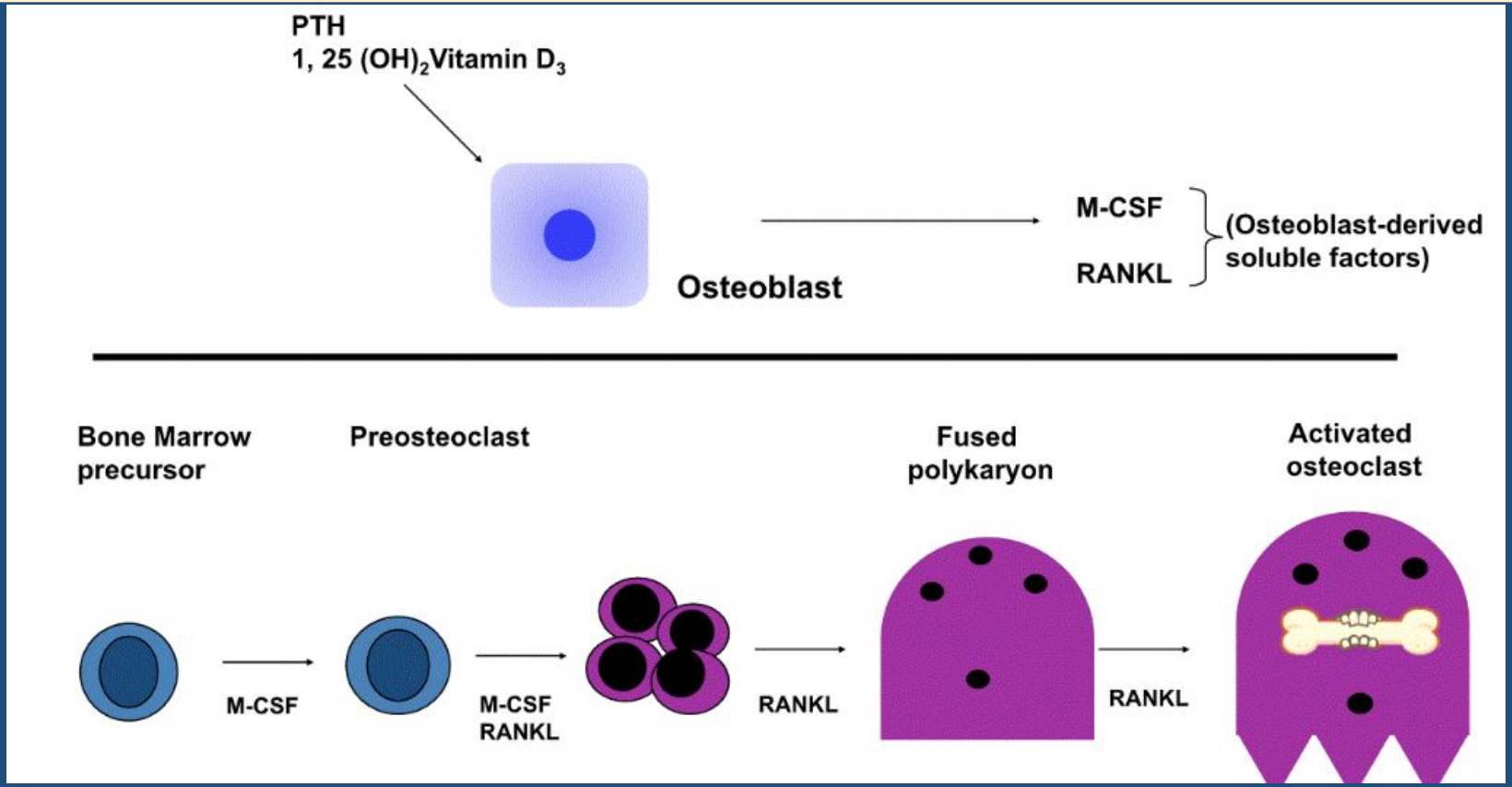
If have hypocalcemia then stable mRNA and make lots of PTH. If hyper- or normocalcemia then mRNA and PTH targeted and turned over.

Parathyroid hormone (PTH) action



PTH increases the activity of 1-α-hydroxylase enzyme, which converts 25-hydroxycholecalciferol, the major circulating form of inactive vitamin D, into 1,25-dihydroxycholecalciferol, the active form of vitamin D, in the kidney. PO4(3-) reabsorption inhibited to keep calcium homeostasis. TAL and DCT have Ca2+ fine tuning - PTH role.

Parathyroid hormone (PTH) action



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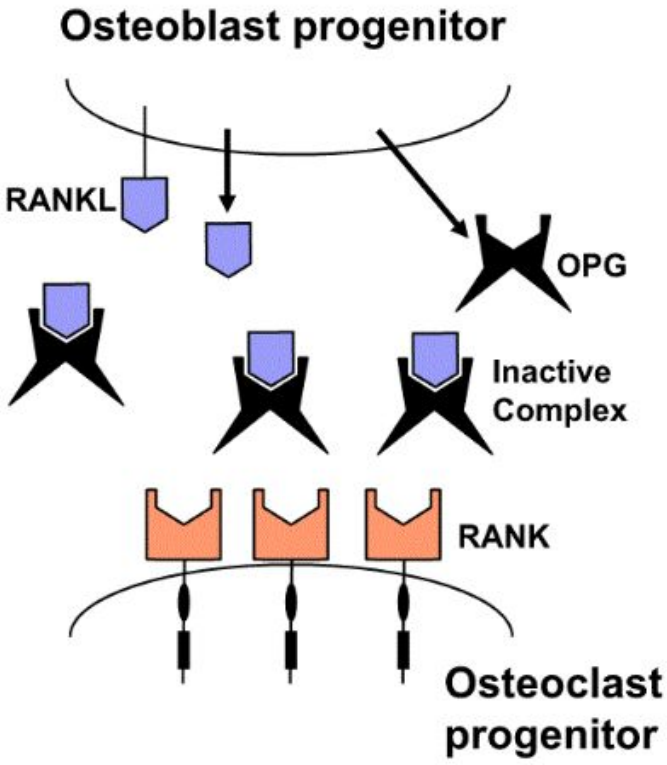
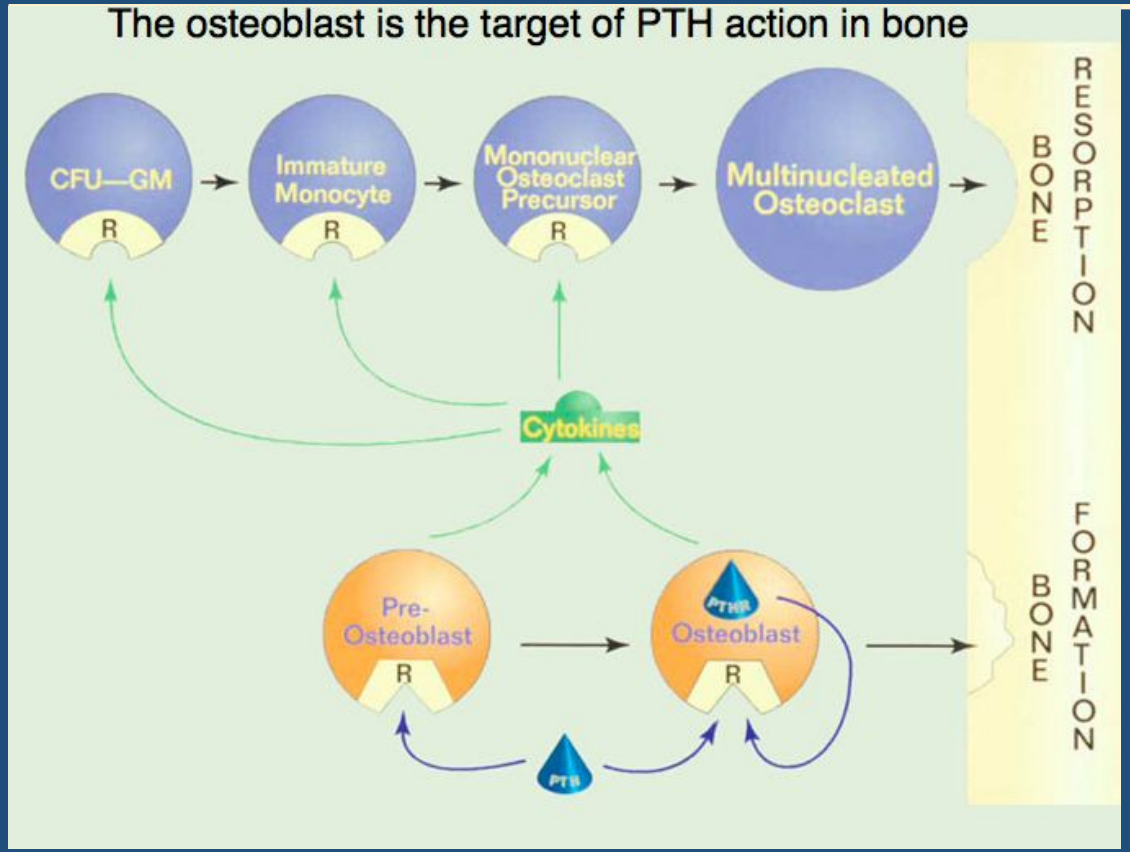


RANKL binds to RANK on cells of the myeloid lineage and is a key factor for osteoclast differentiation and activation. OPG is secreted mainly by cells of the osteoblast lineage and is a decoy receptor that is an inhibitor of osteoclast formation by preventing RANKL binding to RANK and thus OPG prevents RANK-mediated NF-kB activation.

Parathyroid hormone (PTH) action



Receptors for PTH are on pre-osteoblasts and osteoblasts and then communicate to osteoclasts to stimulate them. Critical cytokines are RANKL and osteoprotegerin.

PTH receptors and CaSR

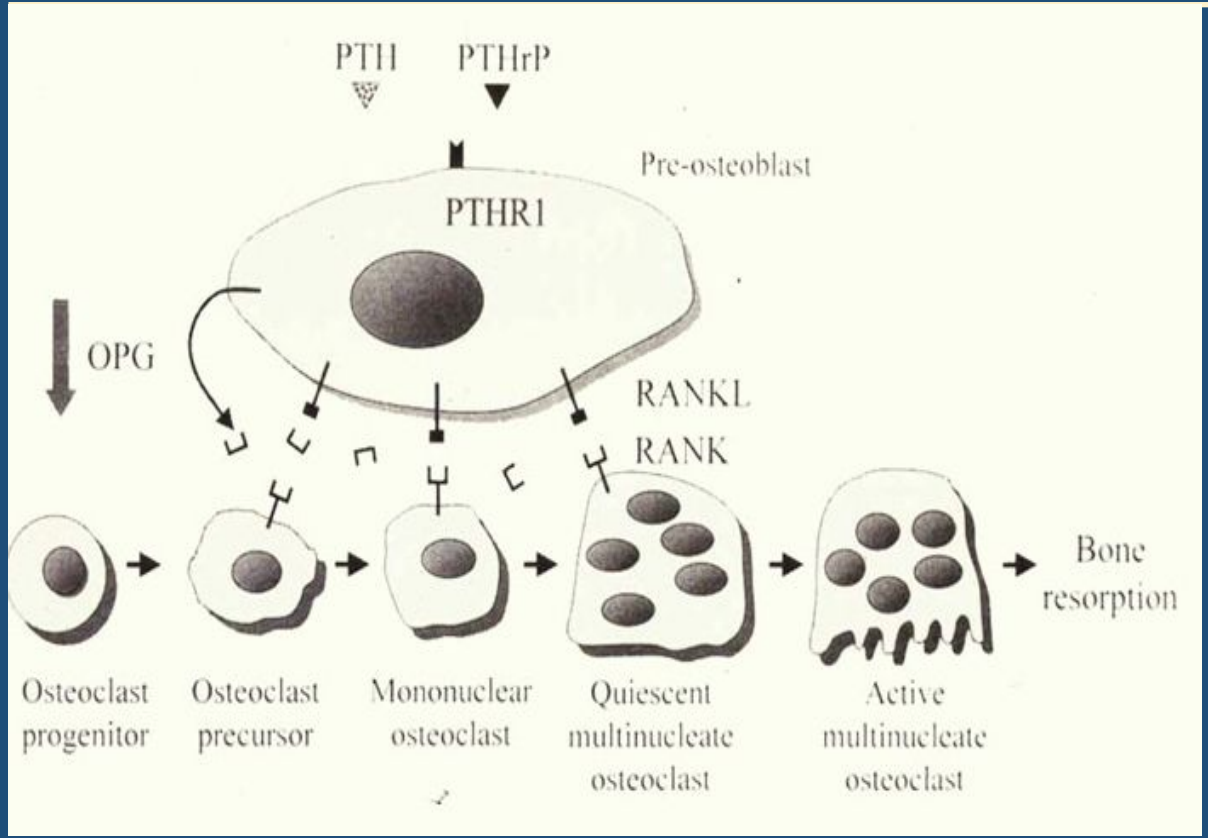
The release of PTH is inhibited in response to elevations in plasma calcium concentrations and activation of the calcium receptor.

Two parathyroid hormone receptors: PTH1R and PTH2R. They are members of the GPCR family of transmembrane proteins

- PTH1R is expressed in bone and kidney and regulates calcium homeostasis through adenylate cyclase activation and phospholipase C through G proteins.
- PTH2R is mainly found in the CNS, pancreas, testis, and placenta.

The calcium-sensing receptor (CaSR) is a Class C GPCR which senses extracellular calcium levels. Is mainly found in the parathyroid gland and the renal tubules. In the parathyroid gland, it regulates the release of PTH to regulate calcium homeostasis. In the kidney it inhibits the calcium and other ion reabsorption.

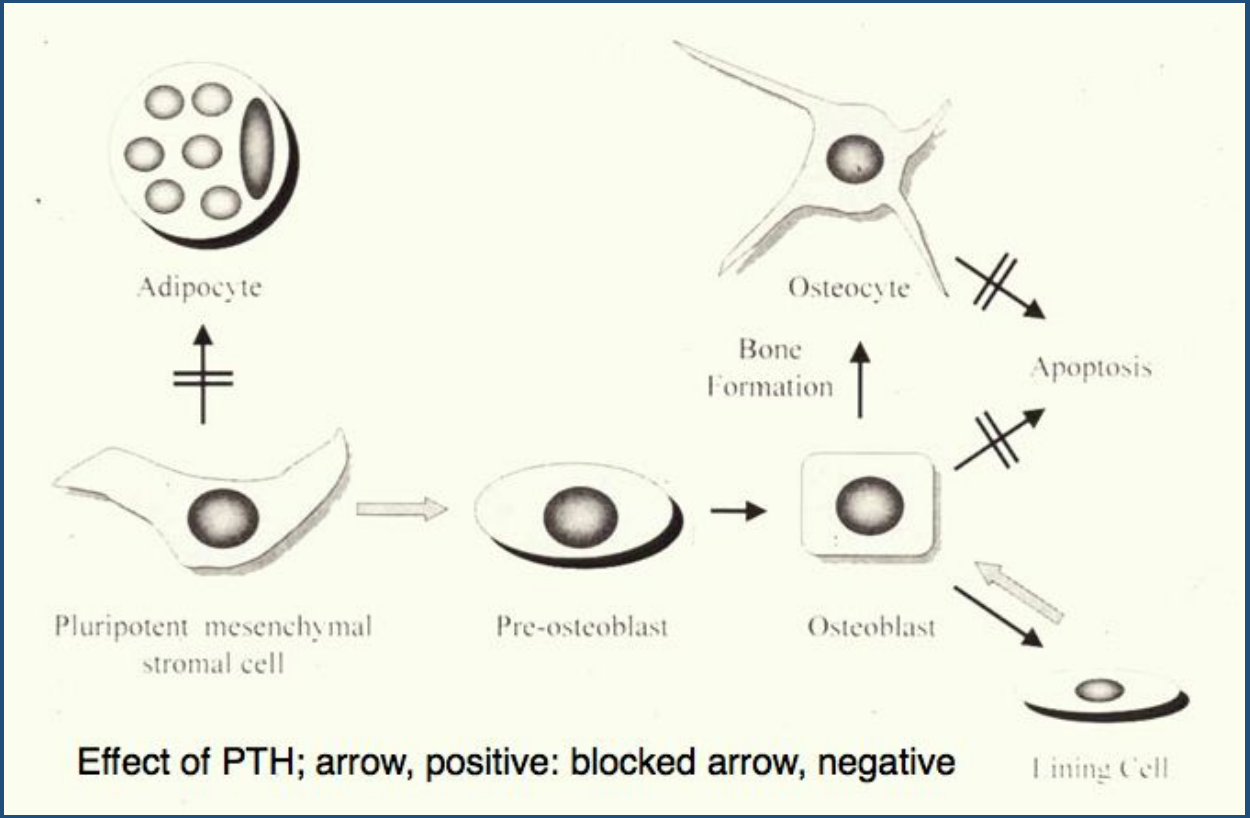
Parathyroid hormone (PTH) action



Relative amount of RANKL and osteoprotegerin dictate fate of osteoclastogenesis. If have more RANKL then more osteoclast activity. If more osteoprotegerin then osteoclastogenesis kept in check.

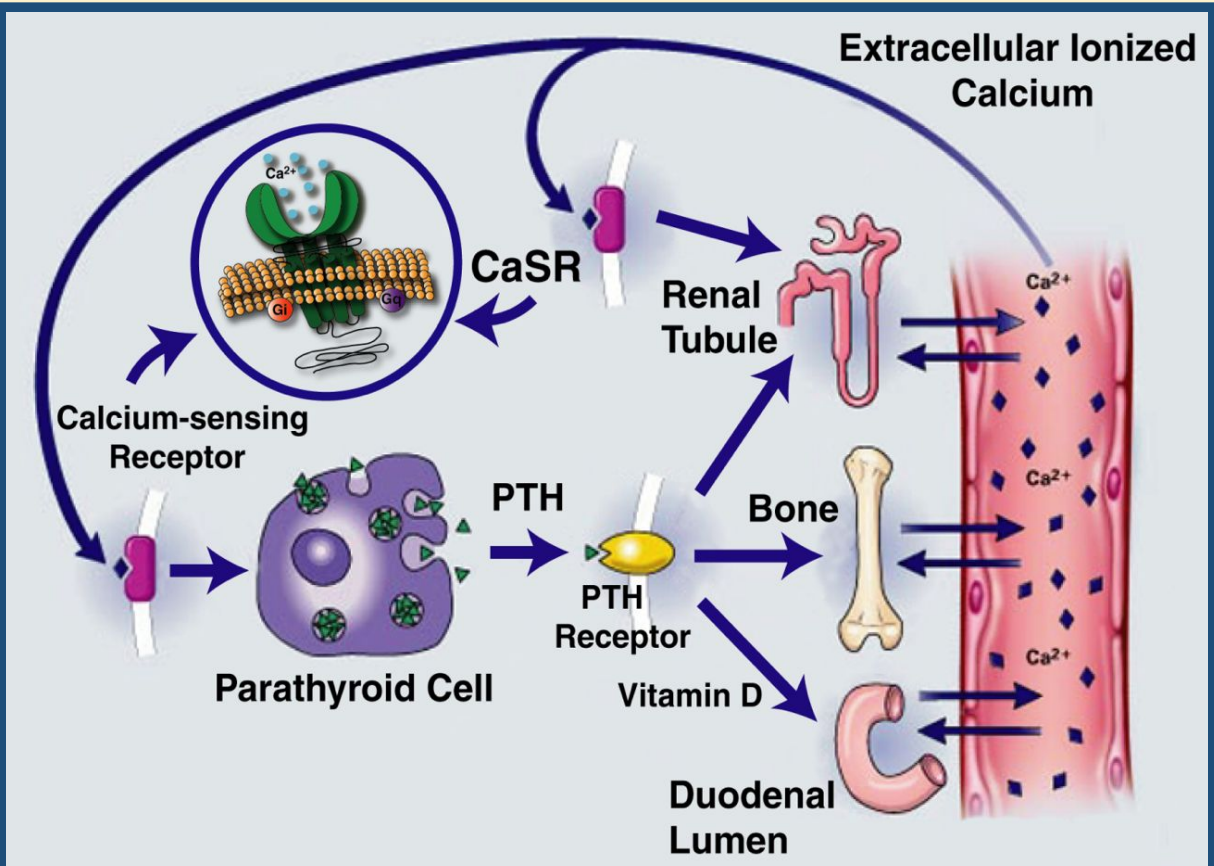
Under continuous exposure of PTH you favor reduction in OPG/RANKL ratio. If intermittent exposure then increase OPG/RANKL ratio and thus less osteoclastogenesis and encourage other effects of PTH.

Parathyroid hormone (PTH) action

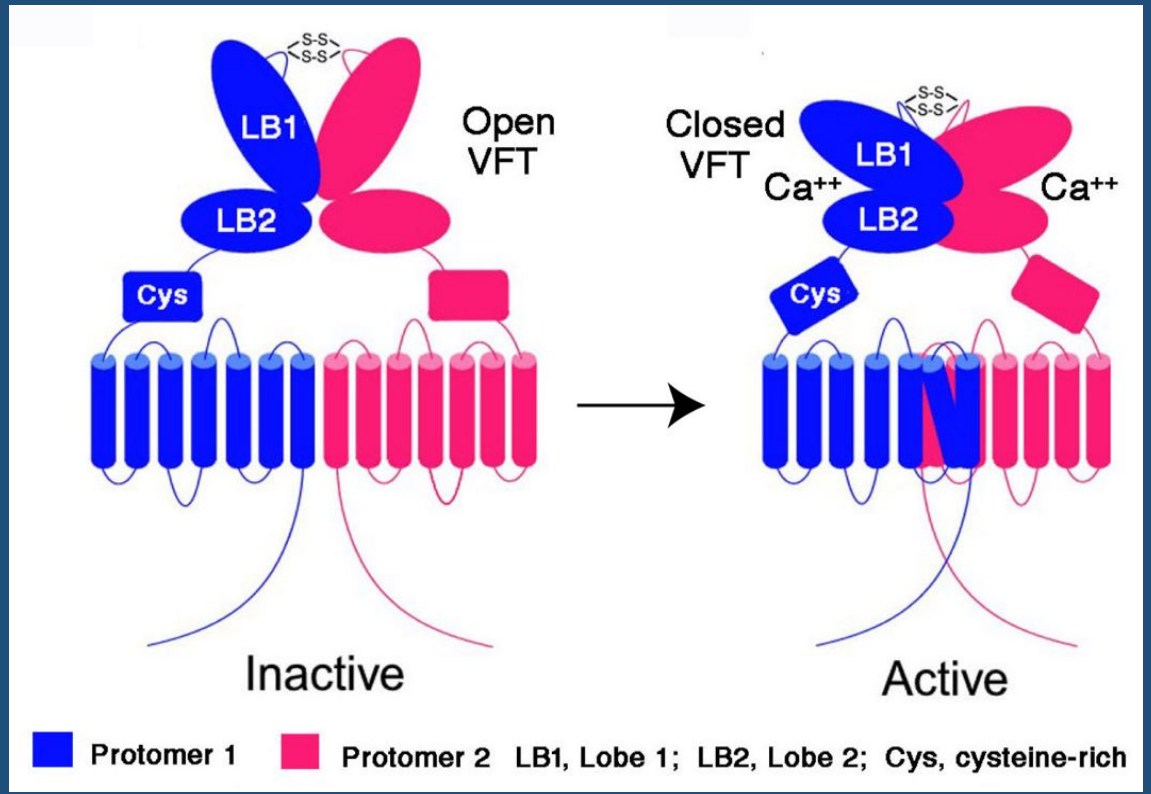


Pluripotent Mesenchymal stromal cell can differentiate into an osteoblast or other cells such as muscle cells and adipocyte. Low intermittent levels of PTH shut down pathway to other cells and stimulate the pre-osteoblast and then osteoblast and this produces collagen and lays down matrix. It then becomes locked into the matrix and becomes an osteocyte. Osteocytes are mechanosensory and can respond to PTH as well. PTH inhibits apoptosis of osteoblasts and promotes osteoblast differentiation and osteocyte production and promotes differentiation to a quiescent lining cell.

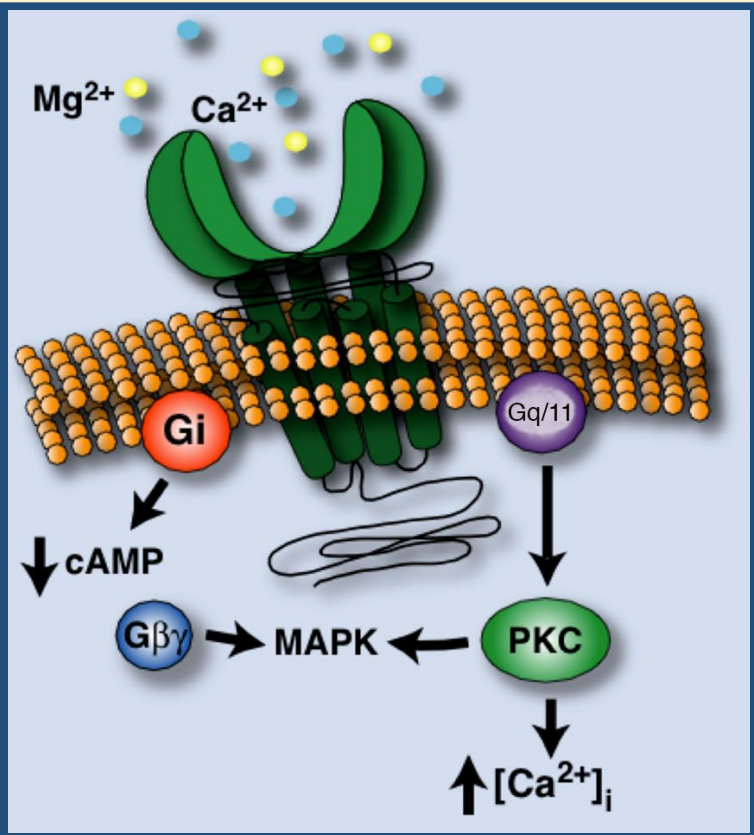
Calcium-sensing Receptor - CaSR



CaSR and its Venus flytrap (VFT) domain

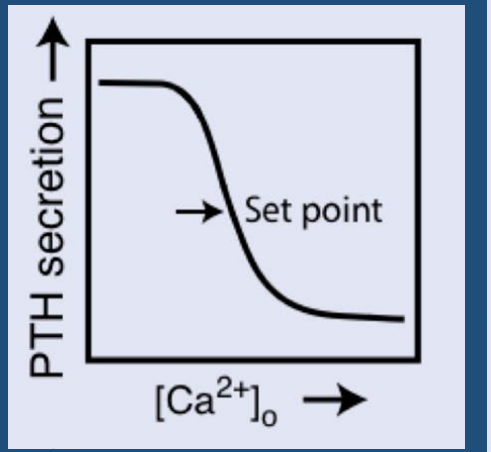


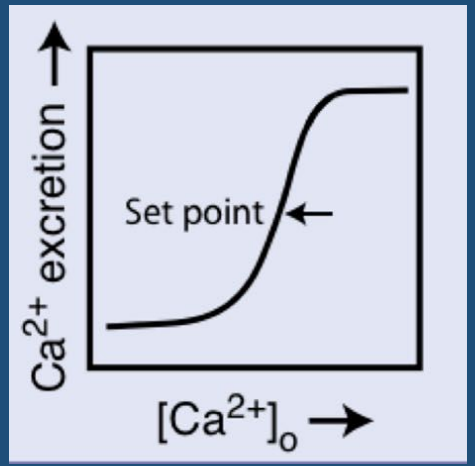
CaSR signalling

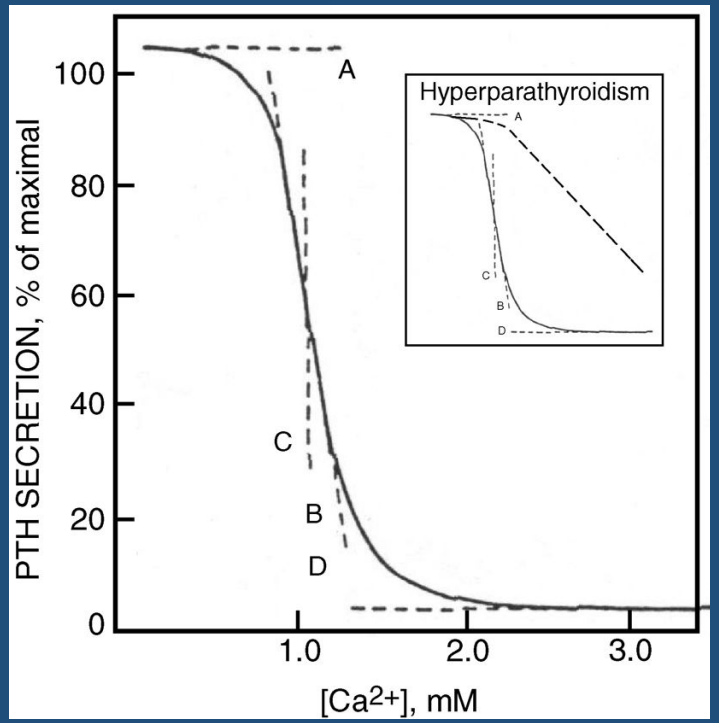


Calcium set point

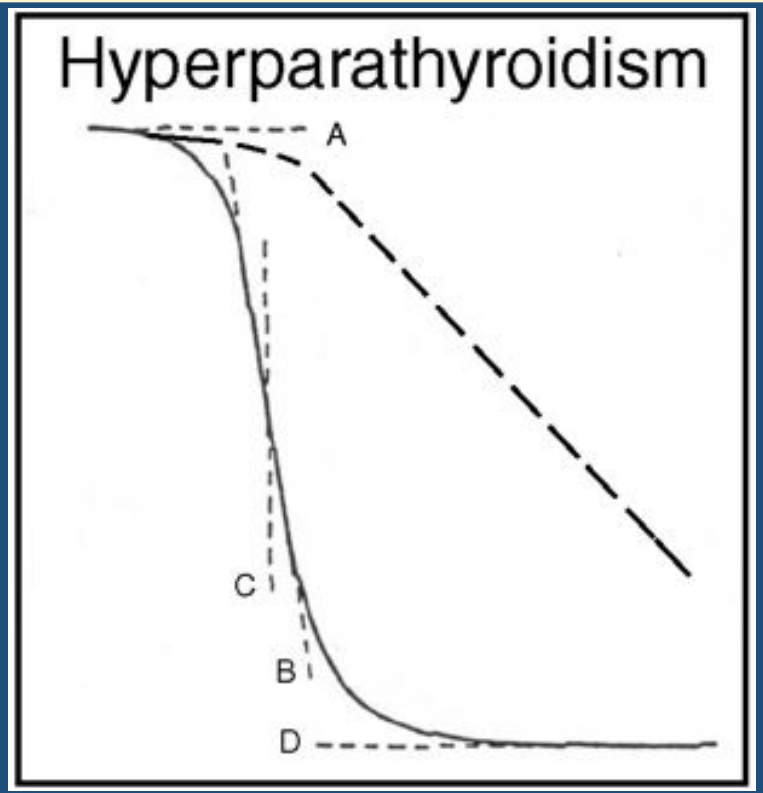
- Calcium set point: the calcium concentration required to reduce PTH to halfway between maximum and minimal levels
  - “Sensitivity” of the parathyroid glands to calcium concentration



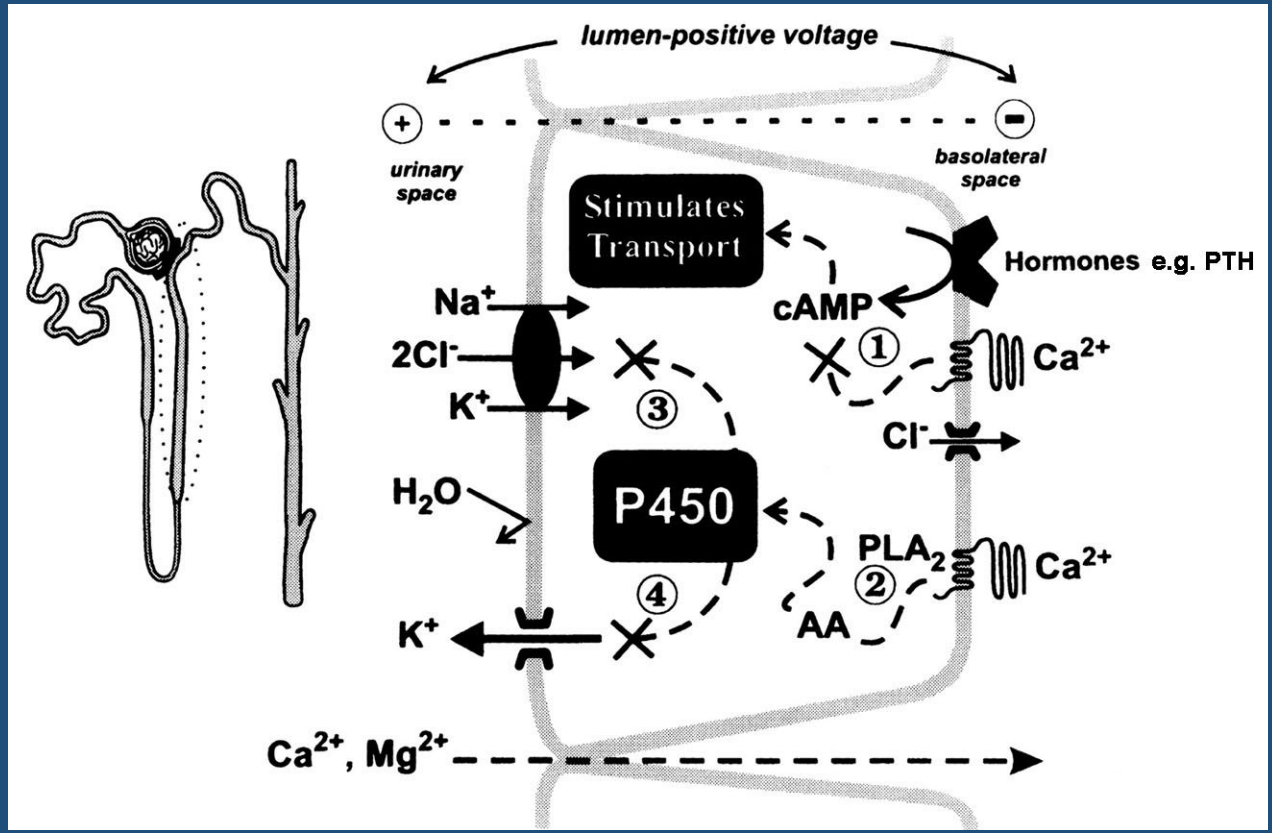




PTH secretion and extracellular calcium



CaSR and ion transport in renal cTAL



Inactivating mutations in CaSR

- Familial Hypocalciuric Hypercalcemia (FHH)
  - Heterozygous
- Neonatal Severe Hyperparathyroidism (NSHPT)
  - Homozygous

Familial Hypocalciuric Hypercalcemia (FHH)

- Benign, as it is a heterozygous inactivating mutation of CaSR
  - Inactivating mutation, meaning CaSR is not inhibiting NKCC2 in the renal tubule
- Mild hypocalciuria and hypermagnesemia
  - Voltage gradient in place allows for the reabsorption of magnesium and calcium ions paracellularly
  - Slight increase in levels of calcium in the blood
  - Lower levels of calcium in the urine (which does not reflect the elevation in the blood)
  - Higher levels of magnesium in the blood
- PTH is inappropriately normal
  - In normal range, but does not respond to elevation in calcium like it's supposed to
- Parathyroidectomy is NOT an effective solution to normalizing the hypercalcemia
  - Since calcium reabsorption at the renal tubule does not have to involve PTH, parathyroidectomy is ineffective at that location
- Anyone could be carrying this mutation and not know it

Neonatal Severe Hyperparathyroidism (NSHPT)

- Is a homozygous mutation
- Marked hypercalcemia
- Marked increase in PTH
- Neurodevelopmental deficits if untreated
  - CaSR is also expressed in the brain
- Treatment: Parathyroidectomy
  - open the neck and remove the parathyroid glands (enlarged, hyperplastic)
- Less severe forms now documented
  - Maintained life long on vitamin D and calcium
  - Live a relatively well life

Acquired hypocalciuric hypercalcemia due to autoantibodies against the calcium-sensing receptor

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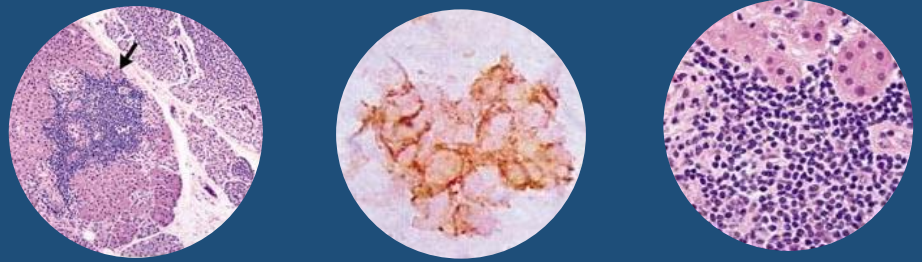


Presented by: Mathew Hindi, Diana Lin, Arielle Gatbonton, and Katherine Duncan

Goal of the paper

To investigate the novel mechanism of acquired hypocalciuric hypercalcemia due to IgG4 subclass autoantibodies against the calcium sensing receptor (CaSR) without glandular destruction (lack of complement activation) - can explain autoimmune hyperparathyroidism.

Researchers want to test this hypothesis.

Summary

- Previously, two families had features of FHH, who also had autoantibodies directed against the CaSR
- Patient has acquired form of hypocalciuric hypercalcemia with a history of multiple autoimmune processes
  - Hypercalcemia
  - Elevated parathyroid hormone levels
    - Responsive to administration of glucocorticoids (used for bullous pemphigoid)
- Subsequent testing showed patient’s disorder was due to the presence of IgG4 antibodies against calcium-sensing receptor

Case study - 66-year old woman

- Spring 2003
  - Physical Examination: patient was cachectic
    - Weight: 34.5 kg
    - Height: 155 cm
    - Two blisters on right wrist
    - Well-healed scar at the base of neck anteriorly
    - Mild abdominal discomfort on palpation

Case study - 66-year old woman

- Spring 2003
  - Laboratory Analysis: normal electrolyte levels
    - *BUN:* 18 mg/dl (6.4 mM)
    - *Creatinine:* 0.8 mg/dl (70.7µM)
    - *Magnesium:* **elevated** at 23 mg/dl (0.9 mM)
    - *Serum calcium:* **elevated** at 13.4 mg/dl (3.4 mM)
      - **Normal range:** 8.5 to 10.5 mg/dl (2.1 to 2.6 mM)
    - *Serum ionized calcium:* **elevated** at 1.77 mM
      - **Normal range:** 1.14 to 1.30 mM
    - *Phosphate:* **low** at 2.1 mg/dl (0.7 mM)
      - **Normal range:** 2.6 to 4.5 mg/dl (0.8 to 1.5 mM)
    - *PTH:* **elevated** at 81 to 128 pg/ml
      - **Normal range:** 10 to 60 pg/ml
    - *25-hydroxyvitamin D:* 13 ng/ml
      - **Normal range:** 8.9 to 46.7 ng/ml
    - *1,25-dihydroxyvitamin D:* 32 pg/ml
      - **Normal range:** 6 to 62 pg/ml

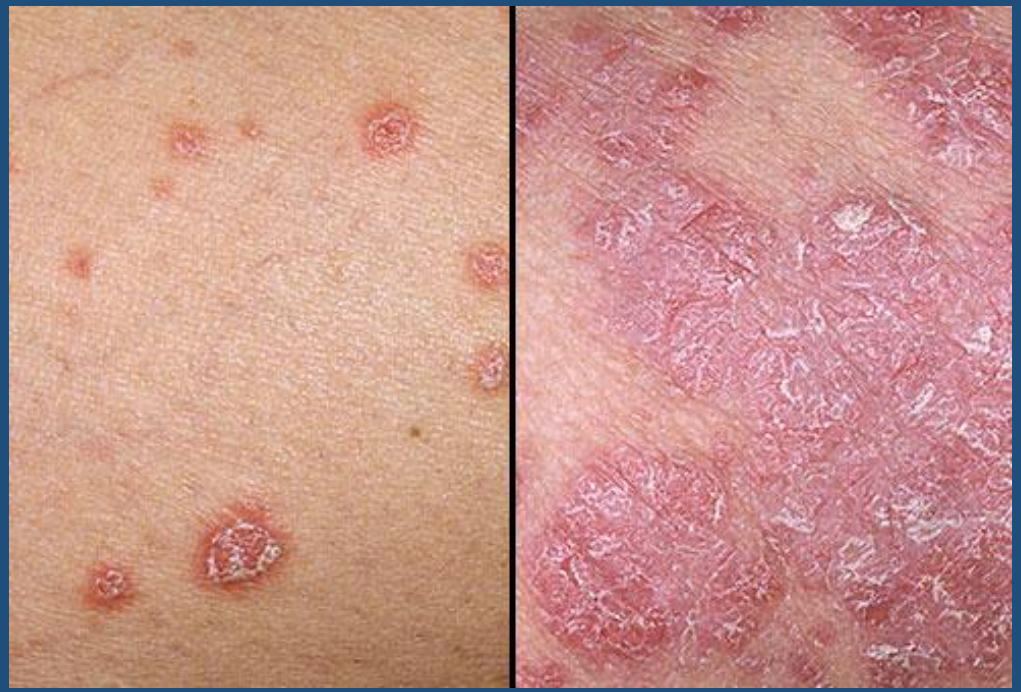
Case study - 66-year old woman

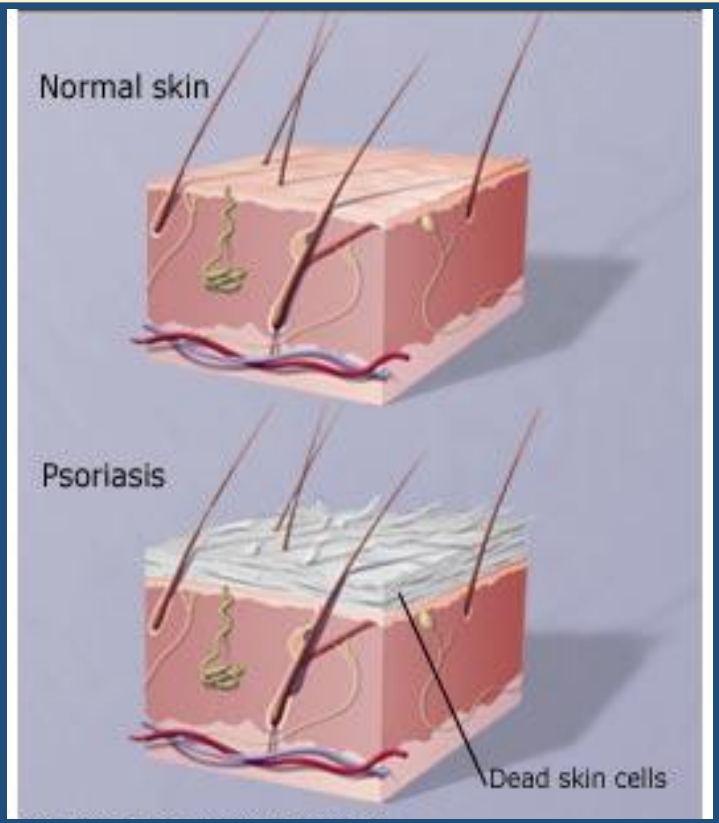
- Medical records: strong immune dysregulation
  - Adult-onset asthma
  - Coomb's-positive hemagglutination
  - Rheumatoid arthritis
  - Psoriasis
  - Uveitis
  - Autoimmune hypophysitis
    - Enhanced thickening of pituitary stalk
    - Central diabetes insipidus
    - Central hypothyroidism
    - Maintenance therapy: desmopressin and thyroxine

- Family History: no disorders of calcium metabolism
  - *Mother:* Raynaud's phenomenon - arterial spasms
  - *Maternal cousin:* Scleroderma

Case study - 66-year old woman

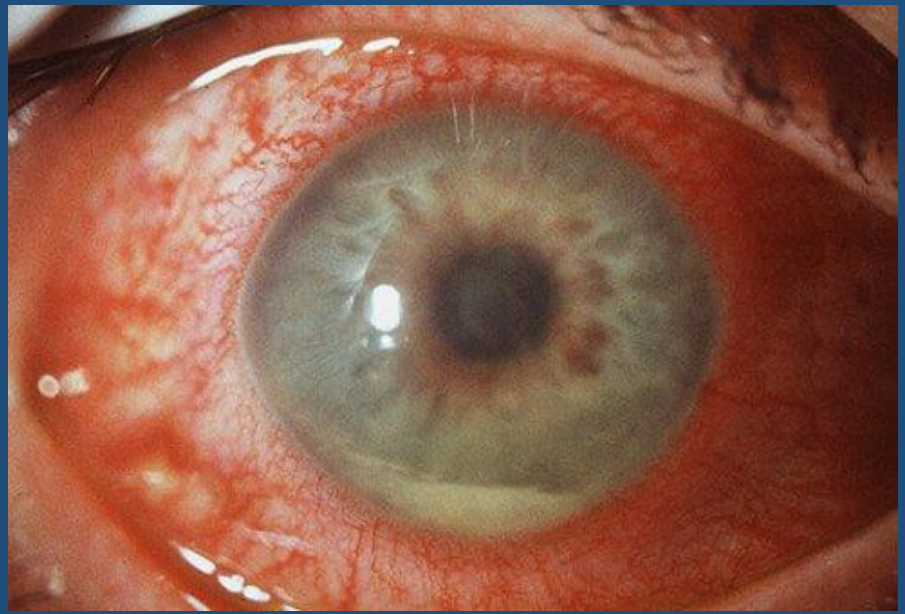
- Psoriasis

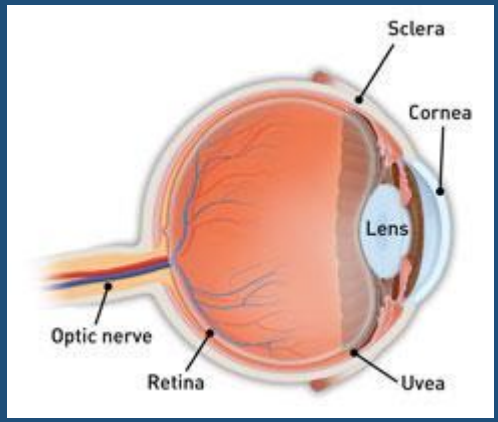


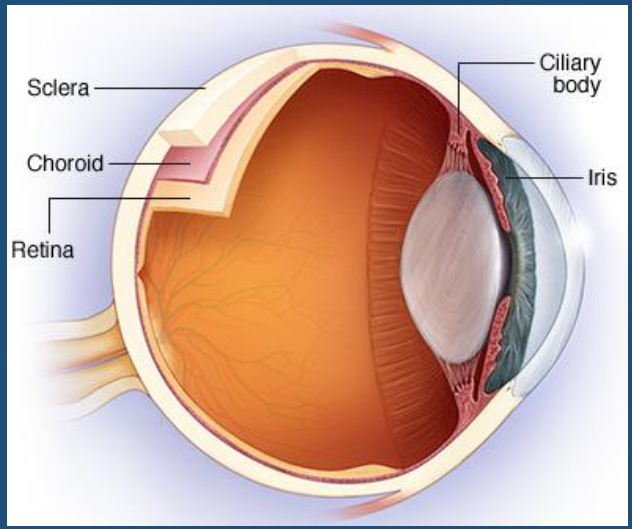


Case study - 66-year old woman

- Uveitis







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- Family History: no disorders of calcium metabolism
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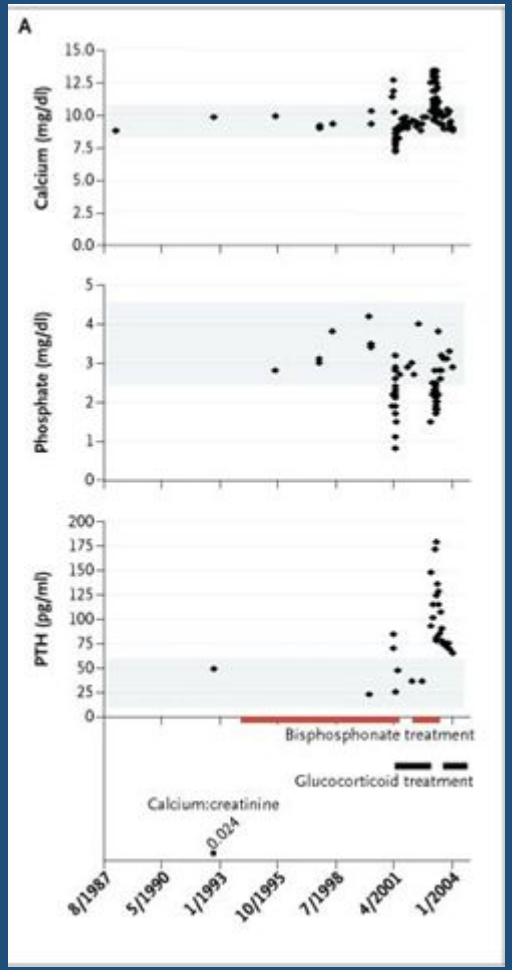
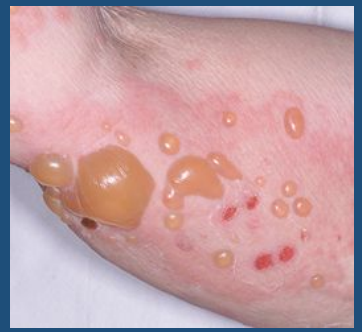
Case study - 66-year old woman

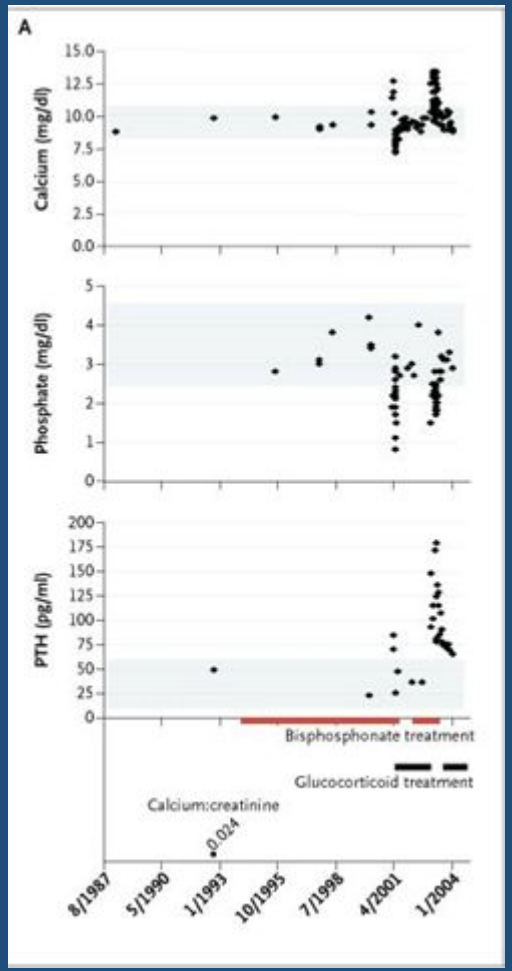
- October 1992
  - Osteoporosis evaluation (at 55 years of age)
    - *Serum calcium:* **normal** at 9.8 mg/dl (2.4 mM)
    - *PTH:* **normal** at 49 pg/ml
    - *Calcium excretion*: **normal** at 220 mg/24 h
    - *Calcium/Creatinine clearance ratio:* **normal** at 0.024
      - **FHH** when < 0.010



Case study - 66-year old woman

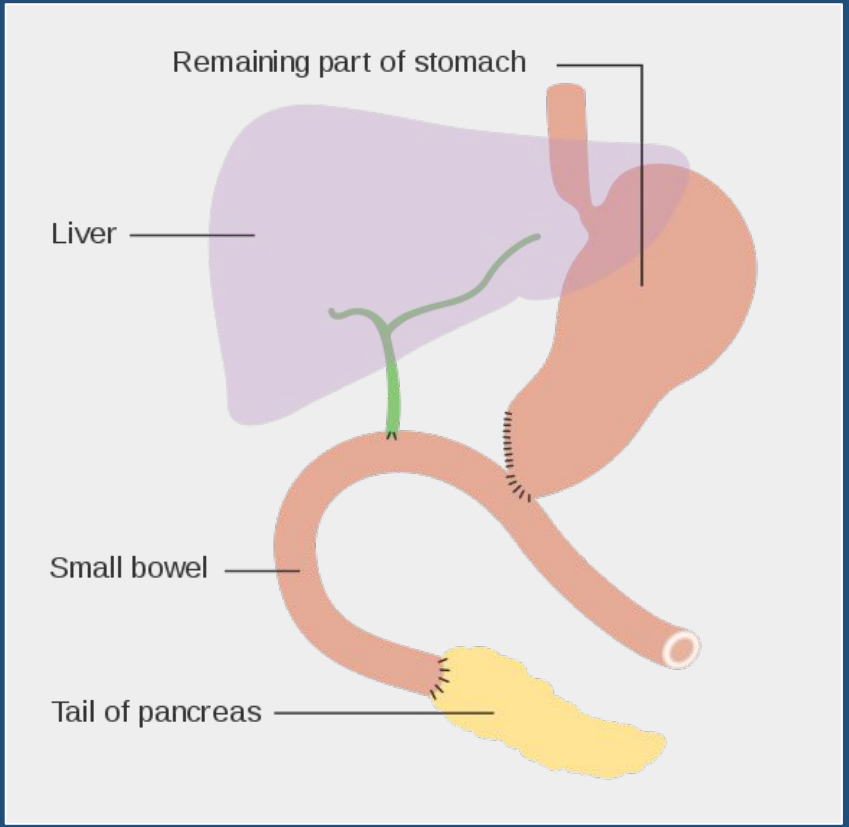
- 1995
  - Diagnosis: bullous pemphigoid (at 58 years of age)
    - Symptoms: tense bullae covered approximately 85% of her body's surface area
    - Treatment: intermittent treatment of varying doses of glucocorticoids
- April 2001
  - Diagnosis: repeated flares of pemphigoid
  - Treatment: daily treatment of glucocorticoids
    - Mycophenolate mofetil added to control pemphigoids





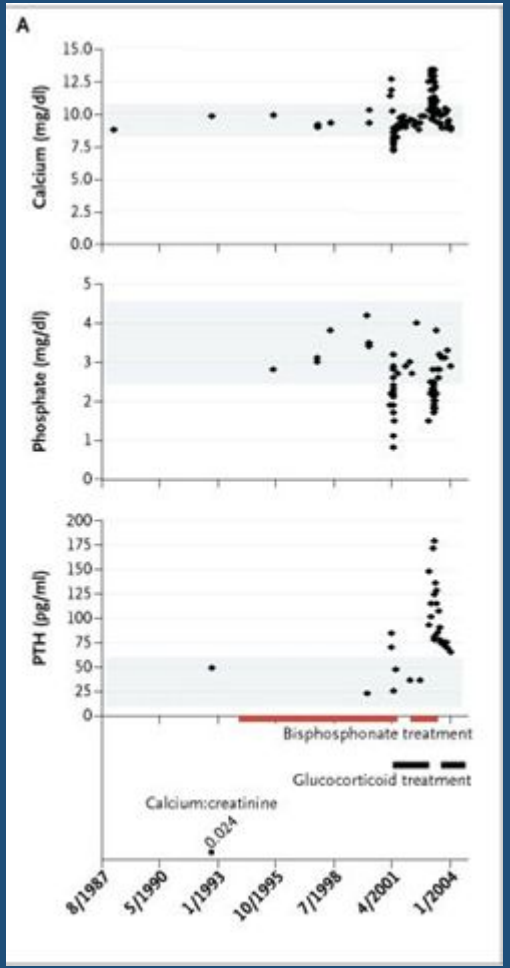
Case study - 66-year old woman

- Spring 2001
  - Diagnosis: 3-cm mass at the head of her pancreas
    - Treatment: Whipple procedure to remove
    - Pathology: Extensive fibrosis and lymphoplasmacytic pancreatitis
      - Sclerosing pancreatitis



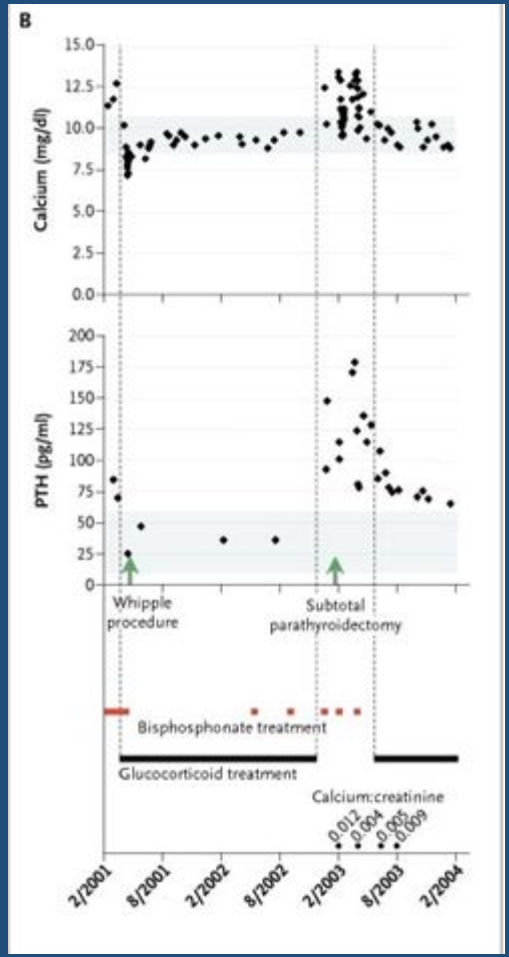
Case study - 66-year old woman

- March 2001
  - Patient had first episode of clinical hyperparathyroidism while undergoing the evaluation of the mass in her pancreas
    - Symptoms:
      - Hypercalcemia
        - *Serum calcium*: **elevated** at 12.7 mg/ml (3.2 mM)
      - Hypophosphatemia
        - *Phosphate:* **low** at 2.2 mg/dl (0.7 mM)
      - Hyperparathyroidism
        - *PTH:* **elevated** at 70 pg/ml
  - Diagnosis: acquired hypocalciuric hypercalcemia
    - Values in were normal since 1992



Case study - 66-year old woman

- February 2003
  - *Admitted into the hospital: Fatigue*
  - *Calcium Excretion*: **low** at 65 mg/24h
  - *Calcium/Creatinine clearance ratio:* **low** at 0.012
  - *Serum Calcium: 13.4 mg/dl* **elevated**
  - *PTH levels: 115 pg/ml* **elevated**
    - Treatment: IV hydration and pamidronate
- No evidence parathyroid adenoma
  - Severe calcium elevations
    - Removed 3.5 /4 parathyroid glands
- Microscopical evaluation
  - Patches of lymphocytic infiltration
- April 2003
  - Admitted to hospital with fatigue and PTH-mediated hypercalcemia



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- April 2003
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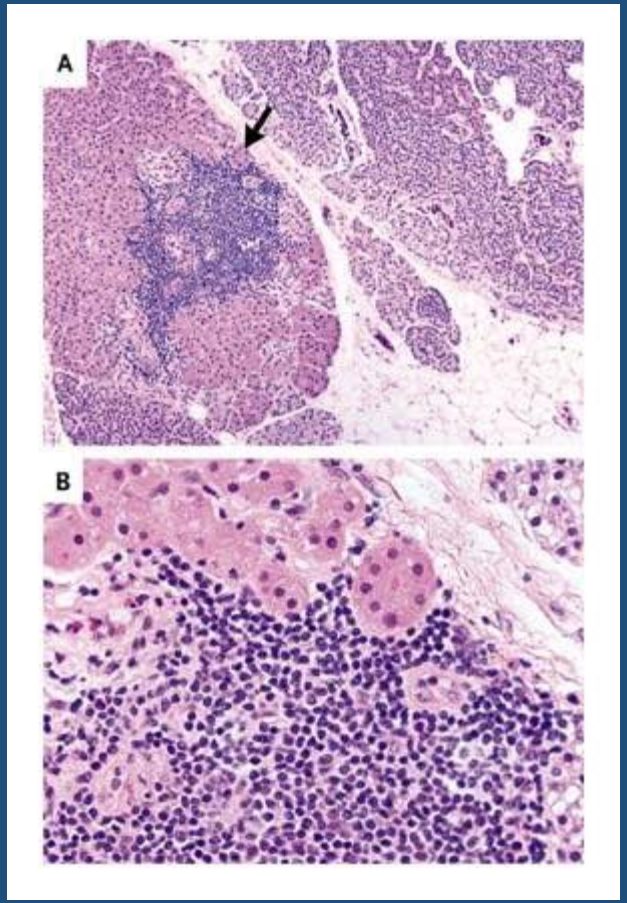


Method & Results

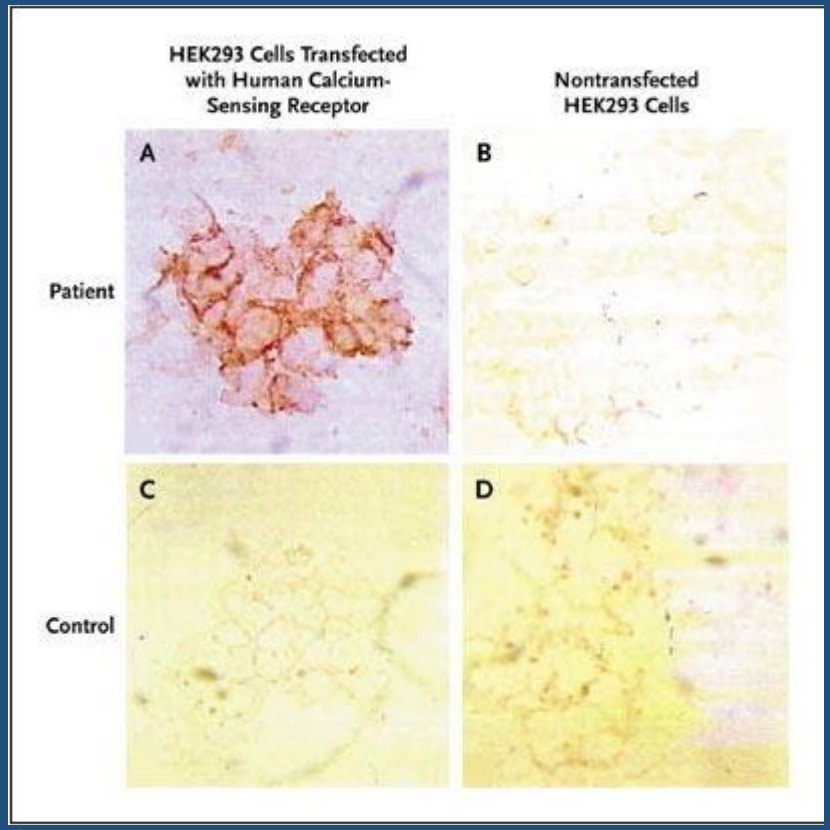
Human Embryonic-Kidney-Cell line (HEK293) cells

Peroxidase-conjugated goat polyclonal antihuman antibody (IgG-gamma chain)

Immunosorbent Assays - Peptide 4637, 4641, LRG

Sheep Monoclonal antihuman antibodies

*Triplicate measurements



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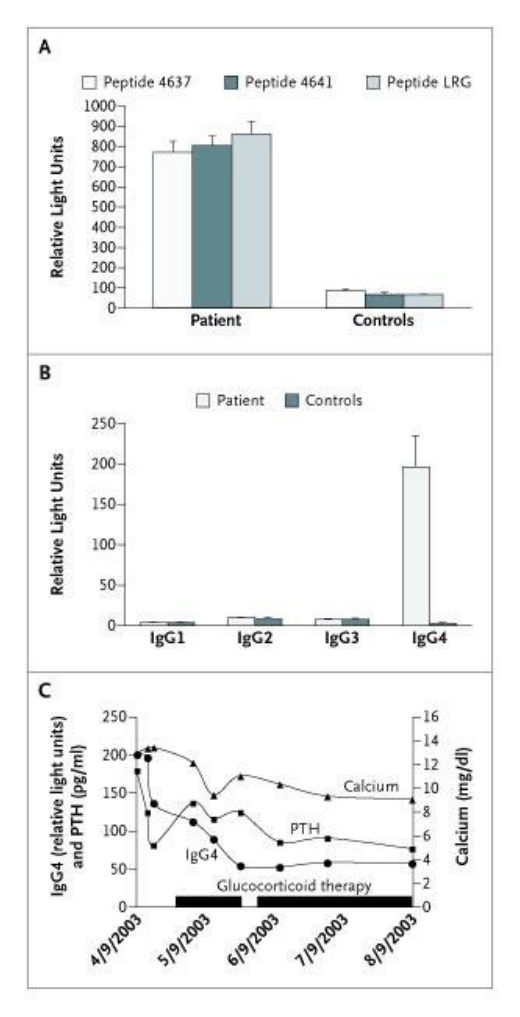
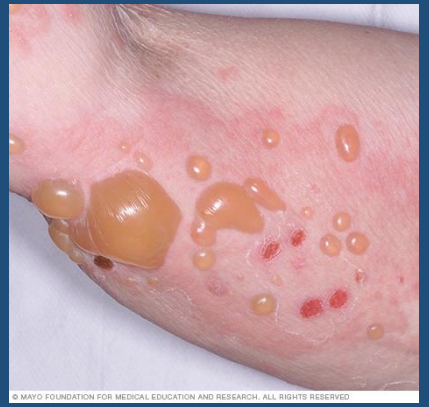


Methods

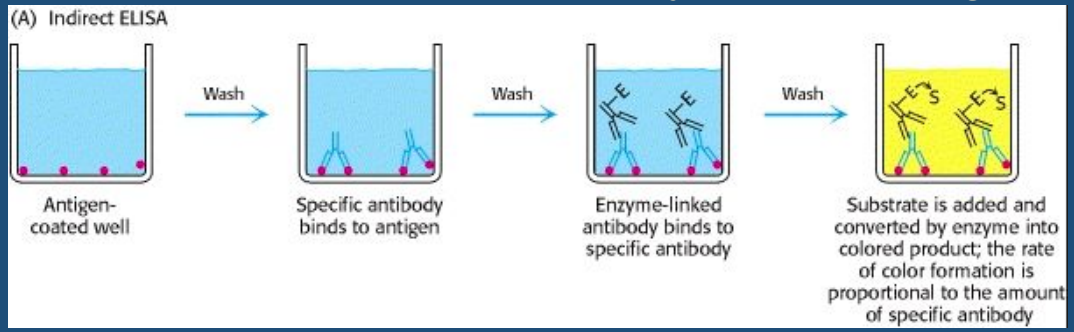
- Enzyme-linked immunosorbent assays (ELISA)



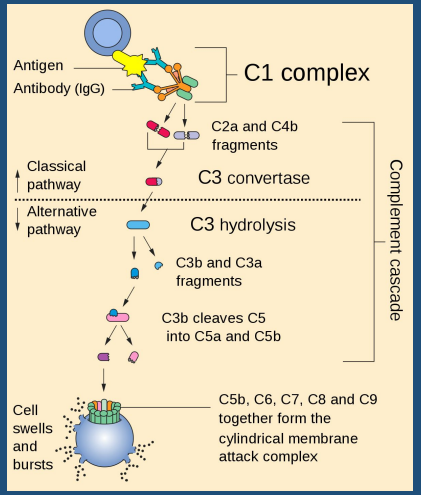
Sclerosing pancreatitis + Bullous pemphigoid

Associated with IgG4 autoantibodies

- Detecting bound autoantibodies → Antibody specific for IgG4 chain



Why No Parathyroid-cell Destruction?



Clin Exp Immunol. 1986 May; 64(2): 415–422. PMCID: PMC1542347

Inhibition of complement activation by IgG4 antibodies.

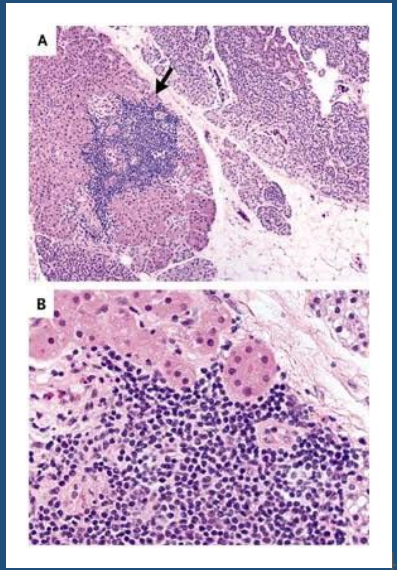
J S van der Zee, P van Swieten, and R C Aalberse

van der Zee, J. S., van Swieten, P., Aalberse, R. C. (1986) Inhibition of complement activation by IgG4 antibodies. Clin Exp Immunol. 64(2): 415–422

- Patients with hypoparathyroidism → Parathyroid-cell destruction
- Damage thought to be caused by complement fixation
- IgG4 inhibits complement
- Patient → No destruction of Parathyroid-cells
- Bullous pemphigoid + sclerosing pancreatitis → IgG4

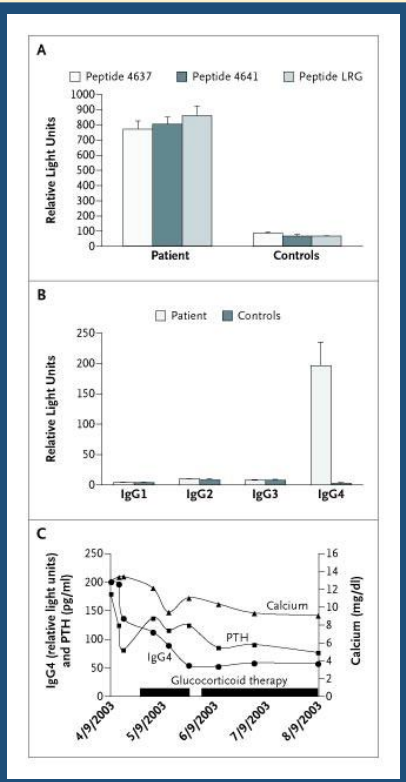
Main Conclusions of the Study

- *Acquired* nature of hypocalciuric hypercalcemia determined by comparison to 1995 serum sample
- Hyperparathyroidism directly correlated with autoantibody titers
- Glucocorticoids effective in
  -reducing antibody titers
  -normalizing serum calcium levels
  -lowering PTH levels
- Risks of parathyroidectomy → glucocorticoids instead



Limitations

- Small sample size - only one case
- Peroxidase-conjugated goat polyclonal antihuman antibody specific for the IgG-γ chain - why?
- Only glucocorticoid treatment



Alternative Treatments

Use of calcimimenic drugs to sensitize PTH glands to extracellular Ca^{2+}

For those with autoimmune hypoparathyroidism → treatment by blocking complement pathway? (ex. C1 inhibitor)