Calcium-Sensing Receptor (CaSR), a GPCR, Signalling Pathway Disorders: New Insights Enable Repurposing of Drugs for Treatment

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49th Annual Meeting of the Israel Endocrine Society
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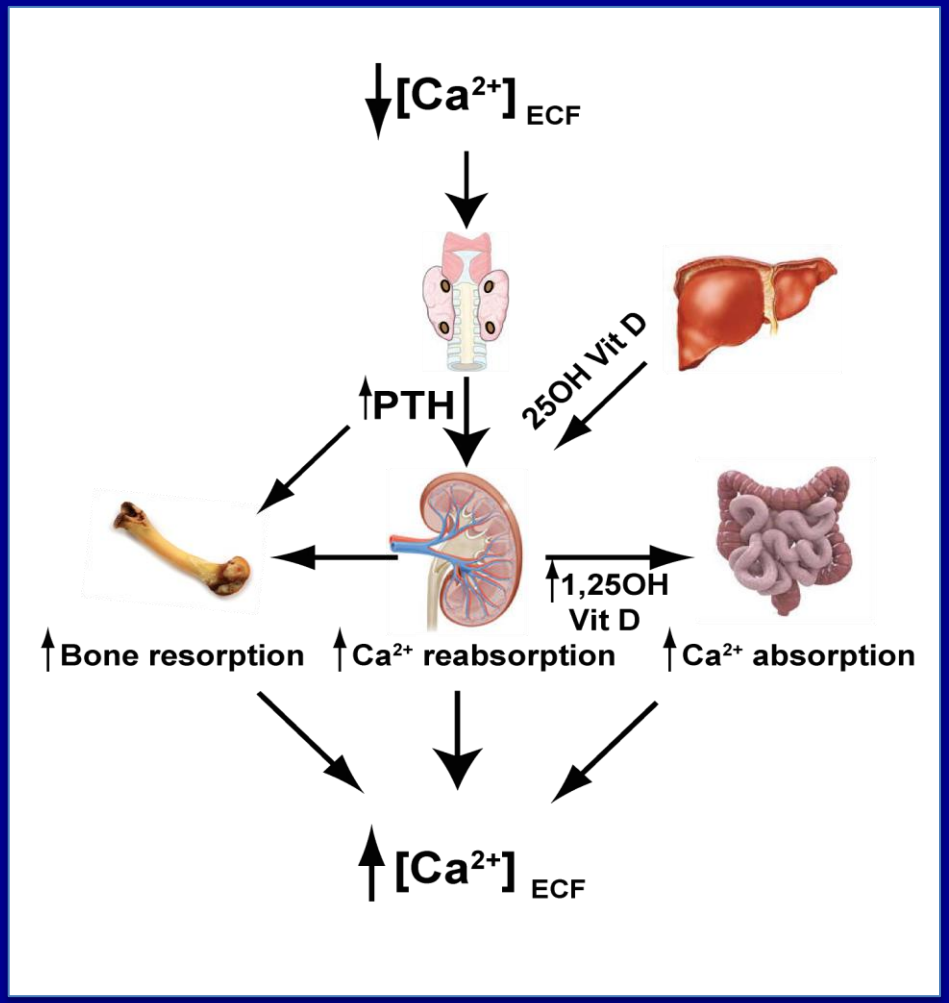
Plenary 1, 29-30th April 2018

Calcium regulation by Parathyroid - Kidney - Bone - Gut axis



Thakker R V (2015): Cecil Goldman Textbook of Medicine

- Parathyroids secrete PTH in response to hypocalcaemia
- PTH regulates ECF calcium (Ca^{2+}) , directly or indirectly via vitamin D, bone resorption, kidney reabsorption and gut absorption

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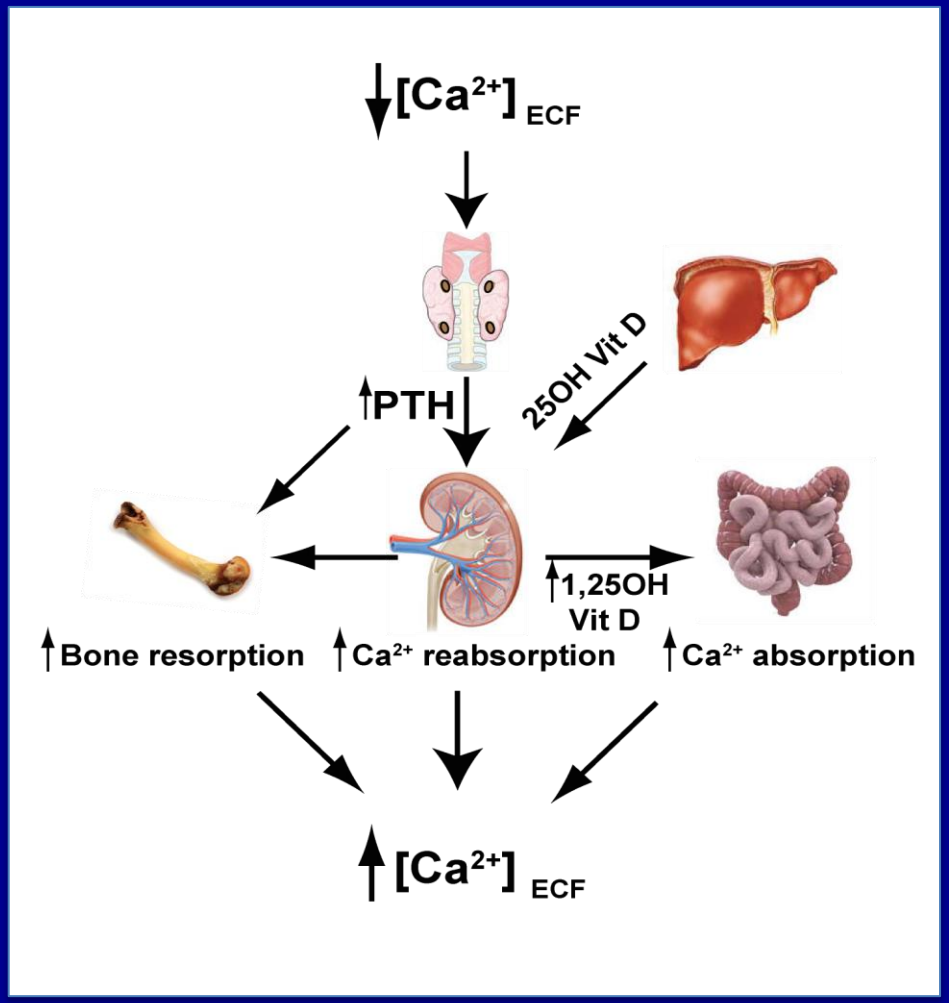

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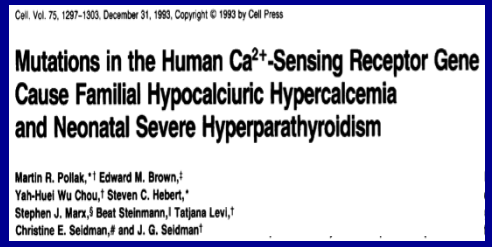
- Parathyroids secrete PTH in response to hypocalcaemia
- PTH regulates ECF calcium (Ca^{2+}), directly or indirectly via vitamin D, bone resorption, kidney reabsorption and gut absorption
- How is the ECF Ca^{2+} sensed?

ECF Ca^{2+} is detected by a Calcium-Sensing Receptor (CaSR), a GPCR: CaSR mutations lead Hyper- and Hypo - calcaemic Disorders

Cloning and characterization of an extracellular Ca^{2+}-sensing receptor from bovine parathyroid

Edward M. Brown*, Gerardo Gamba†‡, Daniela Riccardi†, Michael Lombardi†, Robert Butters*, Olga Kifor*, Adam Sun†‡, Matthias A. Hediger†, Jonathan Lytton† & Steven C. Hebert†

Nature 1993



Cell, Vol. 75, 1297–1303, December 31, 1993, Copyright © 1993 by Cell Press

Mutations in the Human Ca^{2+}-Sensing Receptor Gene Cause Familial Hypocalciuric Hypercalcemia and Neonatal Severe Hyperparathyroidism

Martin R. Pollak,*† Edward M. Brown,‡ Yah-Huei Wu Chou,† Steven C. Hebert,* Stephen J. Marx,§ Beat Steinmann,‖ Tatjana Levi,† Christine E. Seidman,# and J. G. Seidman†

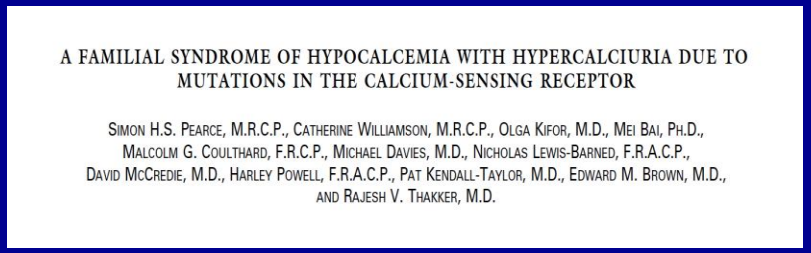
Cell 1993

Calcium-sensing Receptor Mutations in Familial Benign Hypercalcemia and Neonatal Hyperparathyroidism

S. H. S. Pearce,* D. Trump,* C. Wooding,* G. M. Besser,‡ S. L. Chew,‡ D. B. Grant,§ D. A. Heath,‖ I. A. Hughes,¶ C. R. Paterson,** M. P. Whyte,‡‡ and R. V. Thakker*

**MRC Molecular Endocrinology Group, Royal Postgraduate Medical School, London, W12 0NN, United Kingdom; ‡Department of Endocrinology, St. Bartholomew's Hospital, London, United Kingdom; §Institute of Child Health, London, United Kingdom; ‖Department of Medicine, Selly Oak Hospital, Birmingham, United Kingdom; ¶Department of Paediatrics, Addenbrookes Hospital, Cambridge, United Kingdom; **Department of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee, United Kingdom; and ‡‡Metabolic Research Unit, Shriners Hospital for Crippled Children, St. Louis, Missouri*

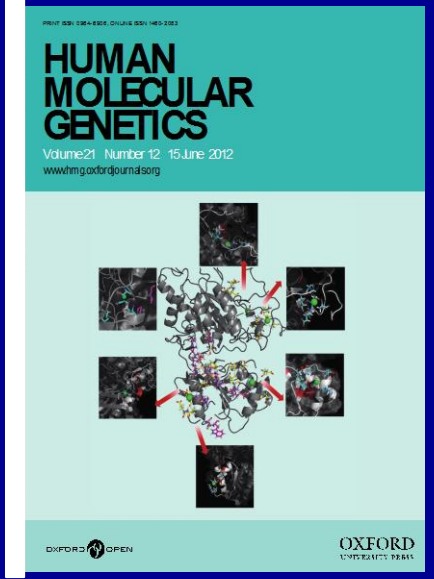
J Clin Invest 1995, 1996, 1997



A FAMILIAL SYNDROME OF HYPOCALCEMIA WITH HYPERCALCIURIA DUE TO MUTATIONS IN THE CALCIUM-SENSING RECEPTOR

Simon H.S. Pearce, M.R.C.P., Catherine Williamson, M.R.C.P., Olga Kifor, M.D., Mei Bai, Ph.D., Malcolm G. Coulthard, F.R.C.P., Michael Davies, M.D., Nicholas Lewis-Barned, F.R.A.C.P., David McCredie, M.D., Harley Powell, F.R.A.C.P., Pat Kendall-Taylor, M.D., Edward M. Brown, M.D., and Rajesh V. Thakker, M.D.

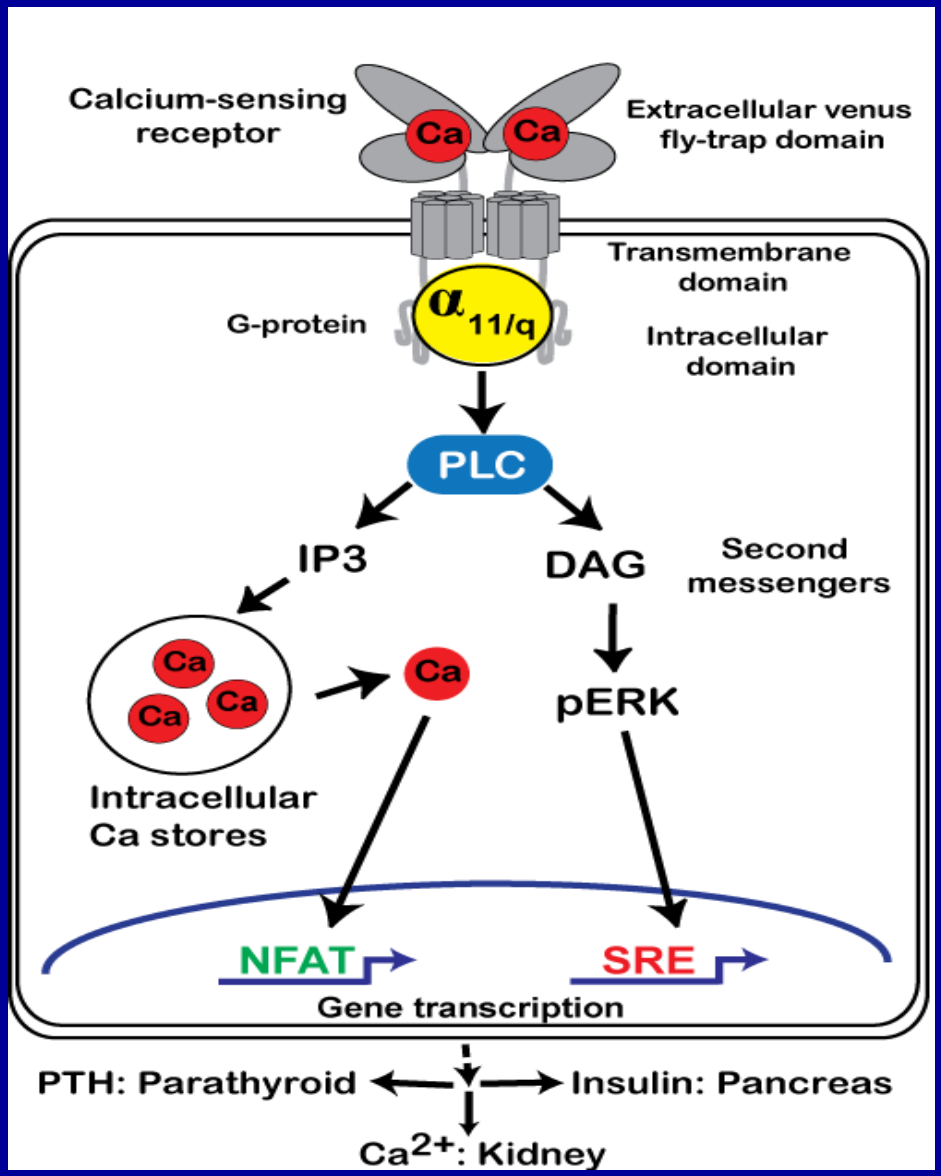
New Engl J Med 1996



Hum Mol Genet 2012

Calcium-Sensing Receptor (CaSR) – a GPCR

- GPCR with 3 domains : ECD (VFTD), 7 TDMs, and an ICD; forms dimers
- Ligands are cations e.g. Ca^{2+}
- Signals via Gα-proteins G11/q, G12/13, Gi/o and Gs, and different pathways e.g. IP_3 pathway to increase Ca_i^{2+} and decrease PTH expression and secretion
- Pivotal role Ca homeostasis
- Widely expressed, including parathyroids and kidneys
- Calcitropic and non-calcitropic roles (kidney, CNS, eye, lung, & cancers - breast, prostate and colon)



Brown et al. Nat 1993, Brown and Macleod Phys Rev 2001

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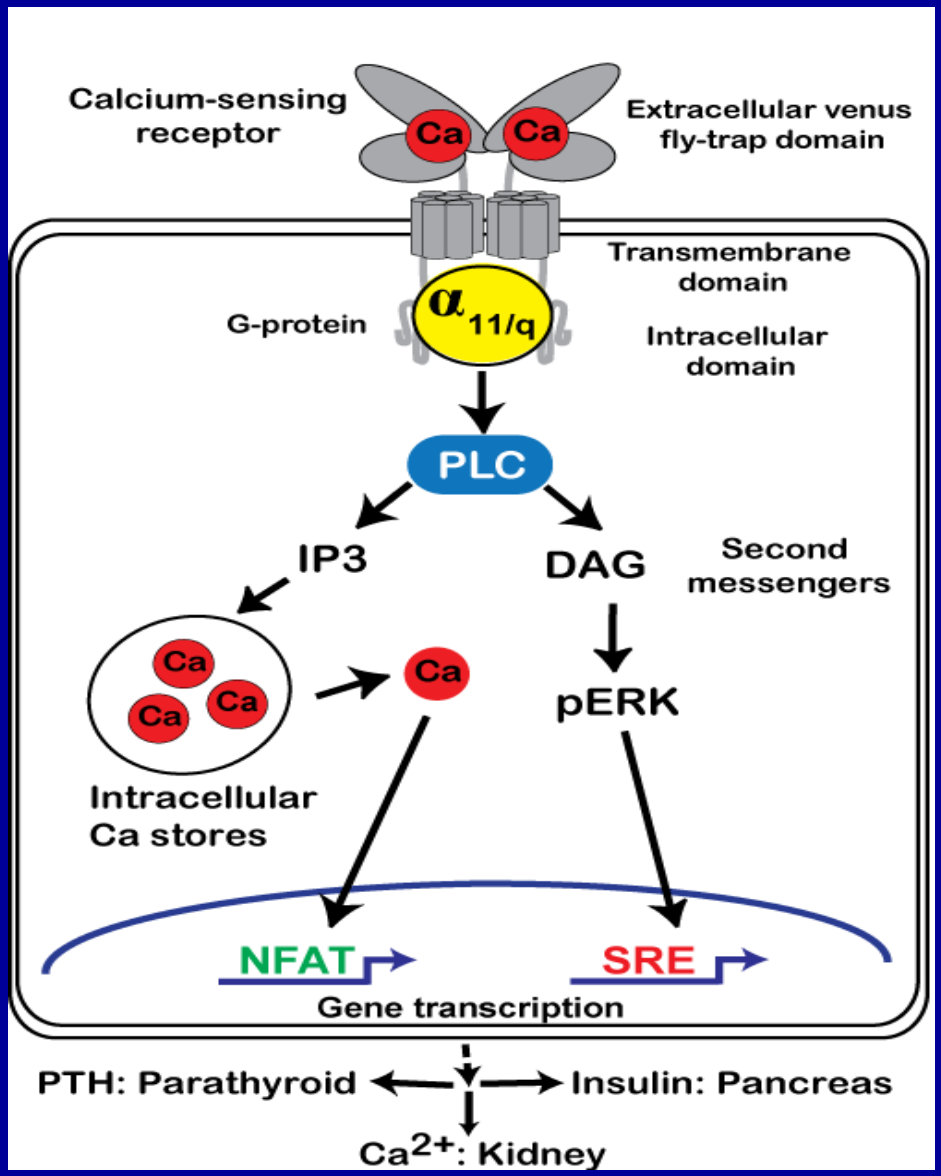

Brown et al. Nat 1993, Brown and Macleod Phys Rev 2001

Calcium sensing receptor (CaSR) and its Seven Calcitropic Disorders

	Clinical Phenotype	Serum Ca^{2+}	Urinary Ca^{2+}
1	Familial benign hypocalciuric hypercalcaemia (FBHH) ^	↑	↓
2/3	Adult and Neonatal severe primary hyperparathyroidism (NSHPT) ^*	↑	↑↓
4	Autosomal dominant hypercalciuric hypocalcaemia (ADHH) ^	↓	↑
5	Bartter syndrome Type V ^	↓	↑
6	Autoimmune hypocalciuric hypercalcaemia (AHH)	↑	↓
7	Autoimmune hypoparathyroidism (AH)	↓	?

*CaSR Mutations : ^ = +/- ; * = -/-*

Thakker Cell Calcium 2004

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*CaSR Mutations : ^ = +/- ; * = -/-*

Thakker Cell Calcium 2004

Familial Benign Hypocalciuric Hypercalcaemia (FBHH)

- **Raised serum calcium, reduced urinary calcium excretion (Ca Cl: Cr Cl <0.01)**
- **Autosomal dominant inheritance**
- **Due to loss of function CaSR heterozygous mutations; and over 250 reported mutations**
- **Results in an altered set-point of CaSR with a right-ward shift in dose-response curve.**
- **Some FBHH patients may not have a family history, and CaSR mutations may arise *de novo.***
- **Patients may develop pancreatitis and/or chondrocalcinosis**

Hannan et al Hum Mol Genet (2012)

Calcium-Sensing Receptor (CaSR) Mutations cause Hypercalcaemic and Hypocalcaemic Disorders

- ***Loss-of-function*** **CaSR mutations cause Familial Hypocalciuric Hypercalacemia (FHH) and Neonatal Hyperparathyroidism (NHPT), a life threatening hypercalcaemic disorder, with bone demineralisation, fractures, hypotonia and respiratory distress.**

NHPT, 3 weeks old, Pre PTX ***NHPT, 4.5 months old, Post PTX***

- ***Gain-of-function*** **CaSR mutations cause Autosomal Dominant Hypocalcaemia (ADH) with Hypercalciuria**

J Clin Invest 1995, 1996,1997; NEJM 1996

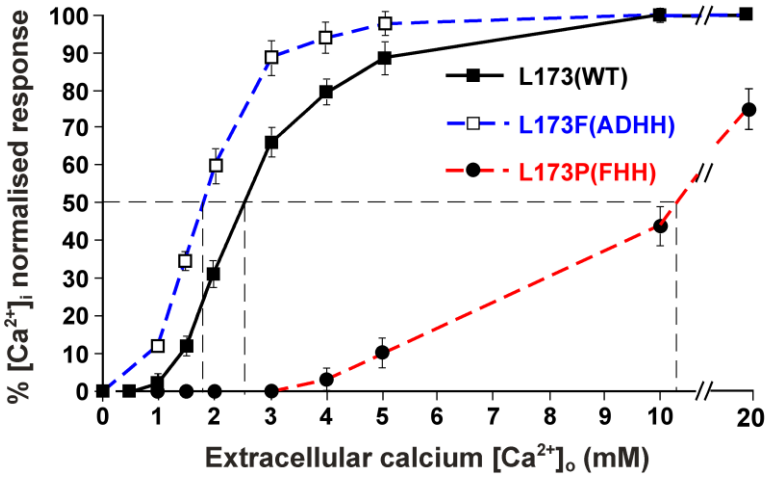
Autosomal dominant hypercalciuric hypocalcaemia (ADHH) – Due to CaSR Gain of Function Mutations

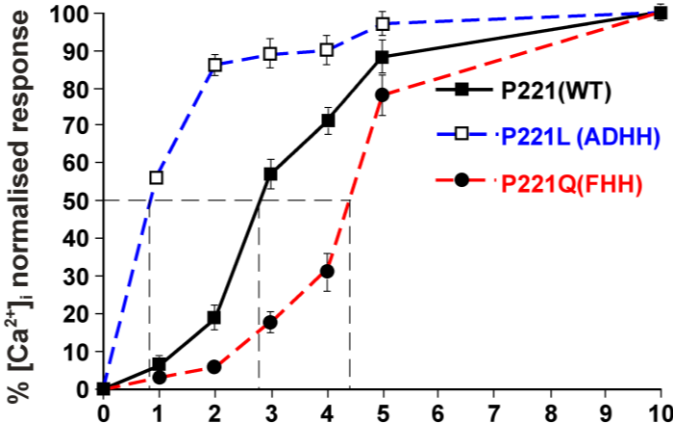
- "Hypoparathyroidism" diagnosed in patients, on basis of low serum calcium and serum PTH in the "normal" range, a combination consistent with CaR set-point abnormality.
- Due to gain of function CaSR heterozygous mutations.
- Results in an altered set-point of CaSR with a left-ward shift in dose -response curve.
- ADHH needs to be distinguished from hypoparathyroidism, as ADHH patients may have a more benign hypocalcaemia that does not require treatment with vitamin D, which may cause with polyuria, polydipsia, nephrocalcinosis and renal failure.
- ADDH patients with severe CaSR activating mutations may develop hypokalaemic metabolic alkalosis, salt wasting and secondary hyperaldosteronism - Bartter syndrome type V

Pearce et al NEJM 1996; Hannan et al HMG, 2012

Mutations of same CaSR residues can cause FHH and ADH, due to loss- or gain- of function (right- and left- ward shifts) : Switch Residues of VFTD - roles of salt bridges at dimer interface

Functional analysis of FHH and ADH mutations of Leu173 and Pro221





L173 and P221 are near R172-D215 salt bridges, which mediate agonist-induced changes at the dimer interface

Hannan et al HMG 2012; Zhang et al. Sci. Adv 2016; Geng et al. eLife 2016; Gorvin et al 2018 Sci Signaling

Drugs - CaSR allosteric modulators

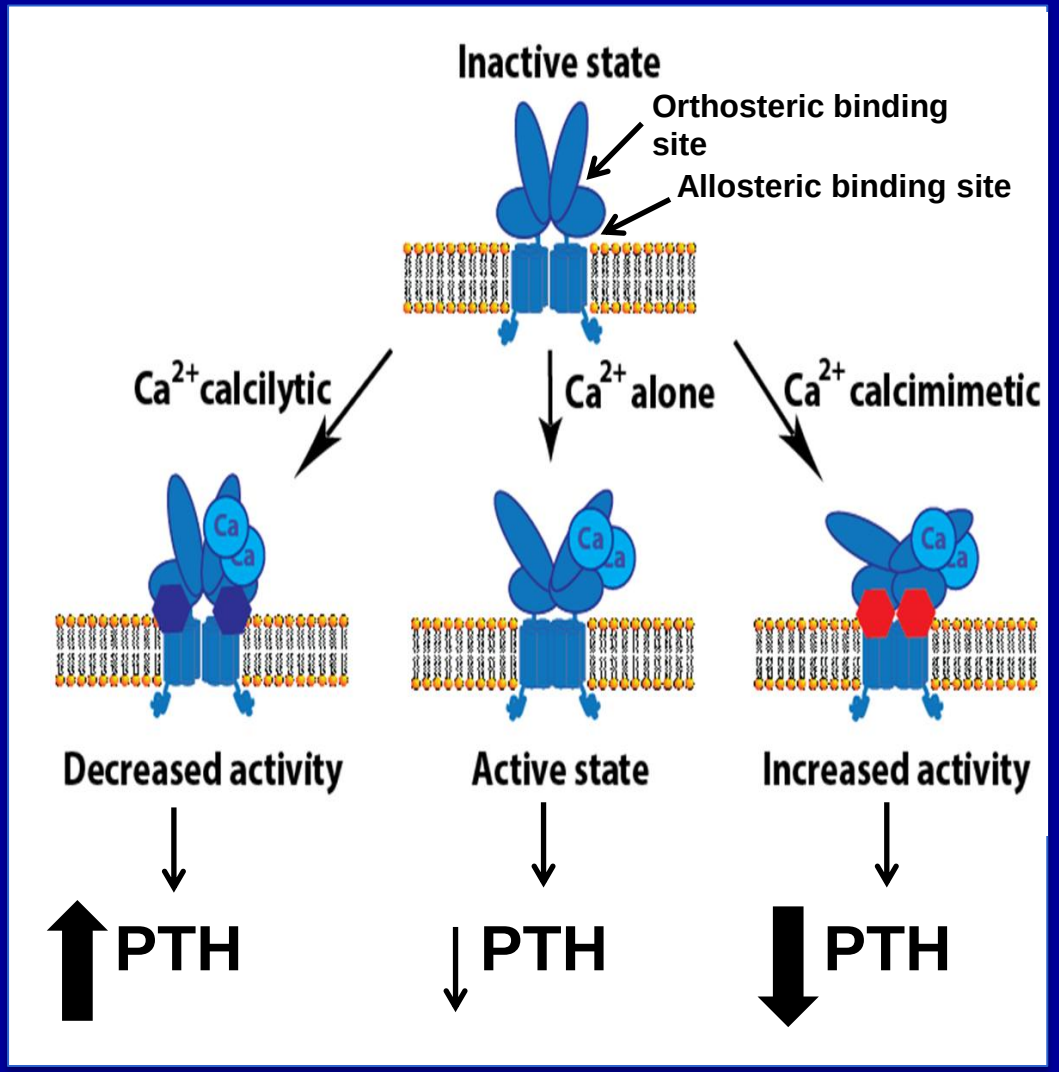
- CaSR allosteric modulators bind to the extracellular aspect of the transmembrane domain
- 2 Classes:

Positive - Calcimimetics e.g. *Cinacalcet*

- Decrease PTH and plasma calcium
- Therapy for secondary hyperparathyroidism and parathyroid carcinoma

Negative - Calcilytics e.g. *NPS2143*

- Increase PTH and plasma calcium
- Potential therapy for hypocalcaemia of ADH(H)



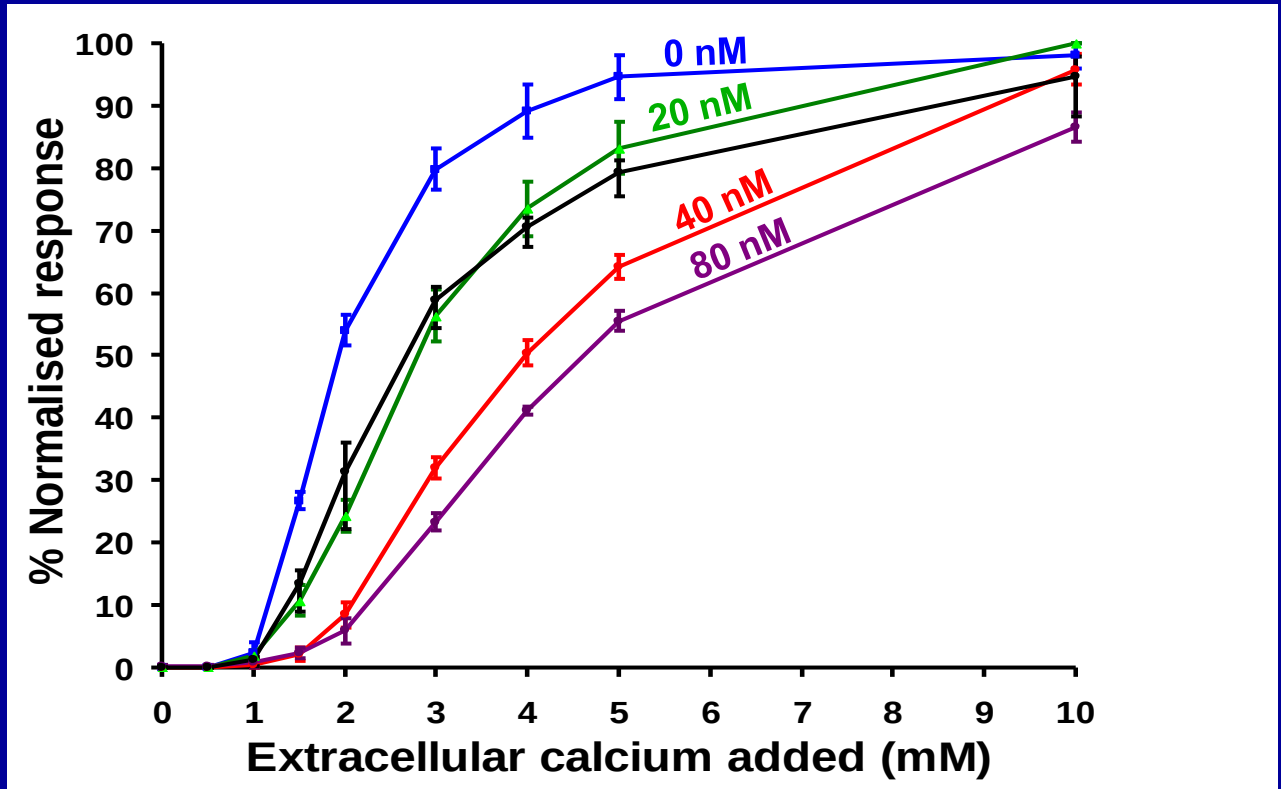
Gowen et al. J Clin Invest 2000, Goodman et al. J Am Soc Nephrol 2002, Hannan et al Endocrinology 2015

Nuf mouse is a model for autosomal dominant hypocalcaemia with hypercalciuria

Features	ADHH	*Nuf* mouse model
Hypocalcaemia	✓	✓
Hyperphosphataemia	✓	✓
Hypomagnesaemia	✓	✓
Low/normal PTH	✓	✓
Hypercalciuria	✓	✓
Ectopic calcification	✓	✓
Raised BMD	✓	?
Cataracts	?	✓
Activating CaSR mutation	✓, various	✓, Leu723Gln

Pearce et al. NEJM 1996, Hough et al. PNAS 2004

In vitro studies: *NPS 2143* corrects gain of function due to Leu723Gln CaSR mutation, occurring in Mouse Model, *Nuf*, for ADH

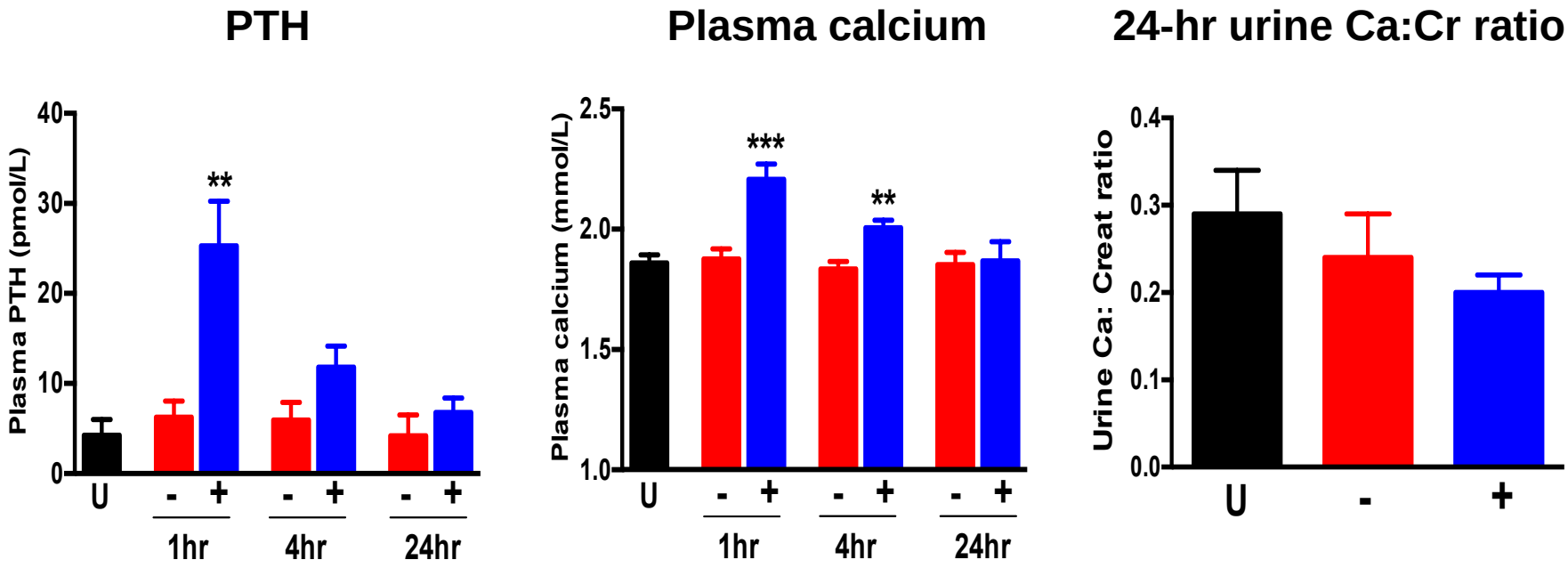


	EC_{50}	SEM	n	*p* value
L723 (WT)	2.53	± 0.14	4	-
Q723	1.94	± 0.07	4	< 0.01
Q723 + 20nM NPS2143	2.79	± 0.19	4	0.33
Q723 + 40nM NPS2143	3.97	± 0.10	4	< 0.01
Q723 + 80nM NPS2143	4.56	± 0.09	4	< 0.01

Hannan et al. Endocrinology 2015

NPS2143 increases plasma PTH and calcium without causing hypercalciuria in *Nuf* mice with an activating CaSR mutation

Single dose of calcilytic drug (NPS2143) given to *Nuf* mice by intraperitoneal injection



Untreated heterozygous-affected *Nuf* (*Nuf*/+) mice
Nuf/+ mice given drug-vehicle only
Nuf/+ mice given 30 mg/kg of NPS2143
n = 4-14 mice per study group

**p<0.01, ***p<0.001

Hough et al PNAS 2004, Hannan et al. Endocrinology 2015

CaSR Mutations found in ≅65% of Familial Hypocalciuric Hypercalcemia (FHH) Patients– Genetic Heterogeneity

FHH is genetically heterogeneous with 3 defined types - FHH1, FHH2 and FHH3 - with chromosomal locations of 3q21.1, 19p and 19q13.3, respectively.

- **FHH1 is caused by loss-of-function mutations of the calcium-sensing receptor (*CaSR*) GPCR.**
- **FHH2 is caused by a mutation of G protein alpha 11 (*GNA11*)**
- **FHH3 – Due to Adaptor protein 2 sigma 2 subunit , AP2σ2,(AP2S1) mutations**

Nesbit et al New Engl J Med 2013; Nesbit et al. Nature Genetics 2013

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Hypothesis Driven

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Mutations Affecting G-Protein Subunit α_{11} in Hypercalcemia and Hypocalcemia

M. Andrew Nesbit, Ph.D., Fadil M. Hannan, D.Phil., F.R.C.Path., Sarah A. Howles, B.M., B.Ch., Valerie N. Babinsky, M.Sc., Rosie A. Head, M.A., Treena Cranston, B.Sc., Dip.R.C.Path., Nigel Rust, M.Phil., Maurine R. Hobbs, Ph.D., Hunter Heath III, M.D., and Rajesh V. Thakker, M.D.

Hypothesis Generating

LETTERS

nature genetics

Mutations in *AP2S1* cause familial hypocalciuric hypercalcemia type 3

M Andrew Nesbit[1], Fadil M Hannan[1], Sarah A Howles[1], Anita A C Reed[1], Treena Cranston[2], Clare E Thakker[1], Lorna Gregory[3], Andrew J Rimmer[3], Nigel Rust[4], Una Graham[5], Patrick J Morrison[6], Steven J Hunter[5], Michael P Whyte[7], Gil McVean[3], David Buck[3] & Rajesh V Thakker[1]

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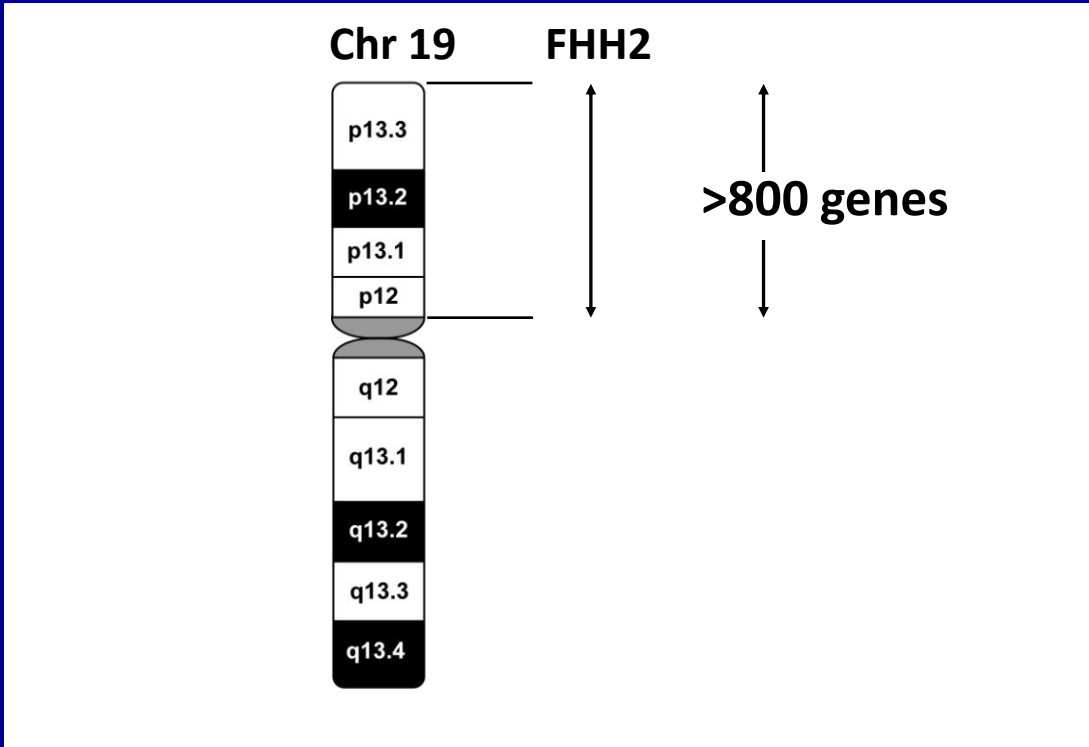
Nesbit et al New Engl J Med 2013; Nesbit et al. Nature Genetics 2013

Familial Hypocalciuric Hypercalcemia Type 2 (FHH2)

- FHH2 reported in 1 kindred from USA
- Affected members of FHH2 have non-progressive mild hypercalcemia not associated with the classical manifestations of primary hyperparathyroidism such as hyperparathyroid bone disease or renal calculi



Prioritised candidate gene analysis undertaken to identify the causative gene for FHH2

Heath et al., AJHG, 1993

Hypothesis-Driven : $G\alpha_{11}$ mutations cause FHH2

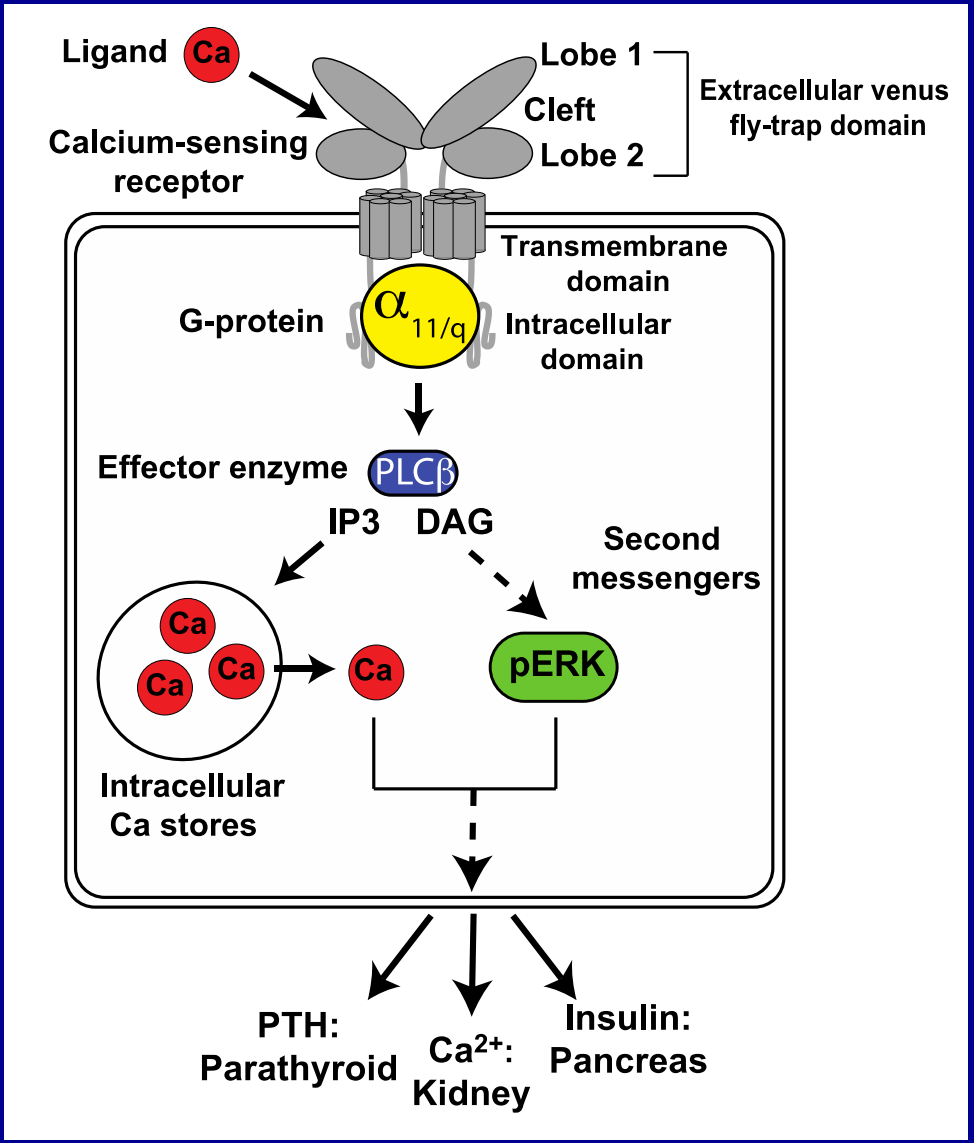
Mutations of the guanine nucleotide-binding protein, $G\alpha_{11}$ may cause FHH2 for the following reasons:

1. ***GNA11,*** **encoding $G\alpha_{11,}$ is located on chromosome 19p13.3 – the location of FHH2**

1. **CaSR signals through $G\alpha_q$ and $G\alpha_{11}$ to PLC**

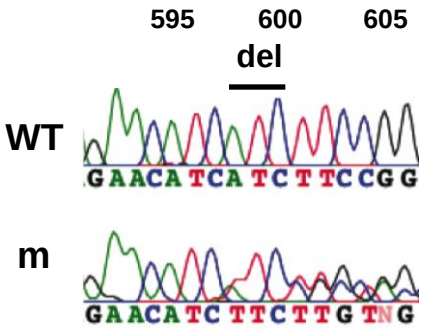
2. **Mice harbouring parathyroid-specific deletions of $G\alpha_{11}$ and $G\alpha_q$, develop hypercalcaemia**

FHH patients without CaSR mutations investigated for $G\alpha_{11}$ mutations.



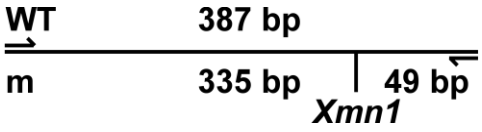
Nesbit et al. New Engl J Med 2013

GNA11 Ile200del mutation identified in the FHH2 kindred



Codon	197	198	199	200	201
WT			Ile	Ile	Phe
Amino	Glu	Asn			
m			**Ile**	**Phe**	**Arg**
WT			ATC	ATC	TTC
Triplet	GAG	AAC			
m			**ATC**	**TTC**	**CGG**

Xmn1

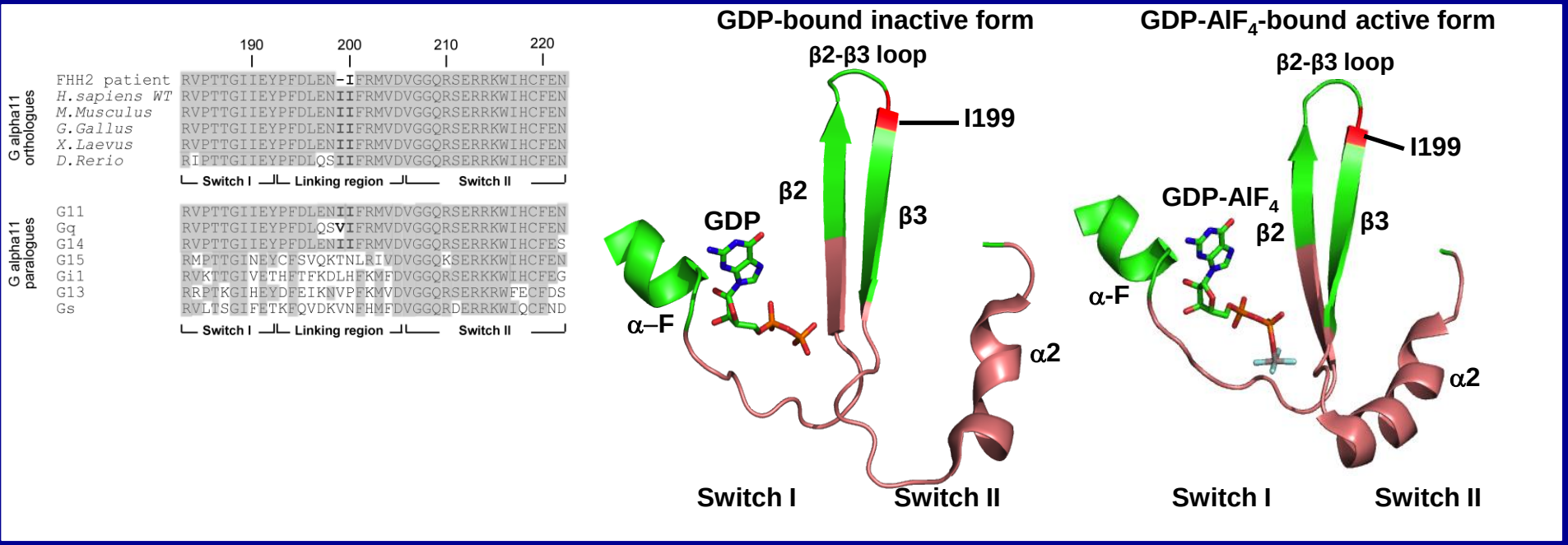


— WT
— m

Nesbit et al. New Engl J Med 2013

Predicted effects, based on 3-D Structural Analysis, of *GNA11* Mutation

Ile199/200 - conserved in all vertebrate $G\alpha_{11}$ subunit orthologues
- located within a 13 amino acid region whose length is conserved amongst $G\alpha_{11}$ orthologues and human paralogues

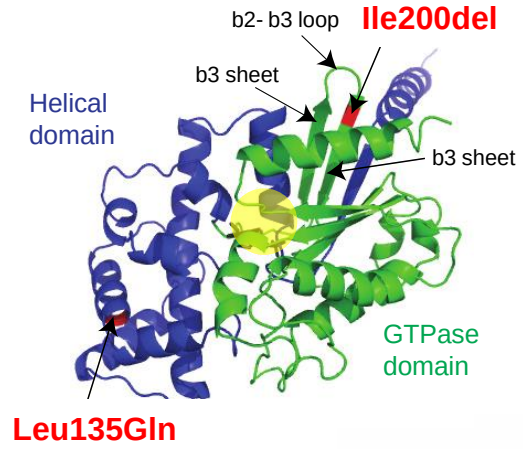


Ile199/200 - Sits in a loop linking the β2 and β3 strands that forms part of the interface at which Gα subunits interact with GPCRs
- Has a role in GPCR-mediated GDP release and G-protein activation.

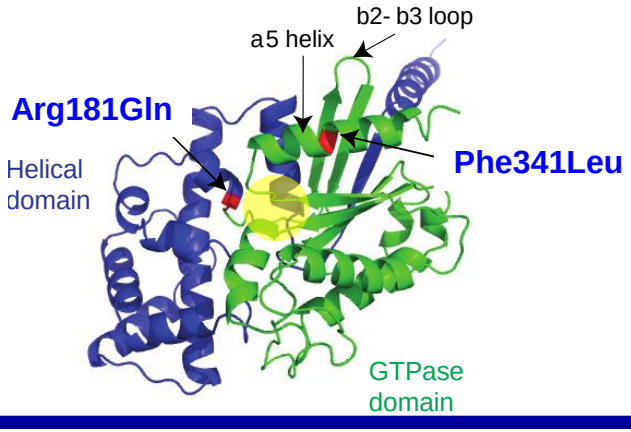
Nesbit et al. New Engl J Med 2013

GNA11 loss- and gain-of-function mutations lead to FHH2 and ADH2, respectively (*Nesbit et al. New Engl J Med 2013*)

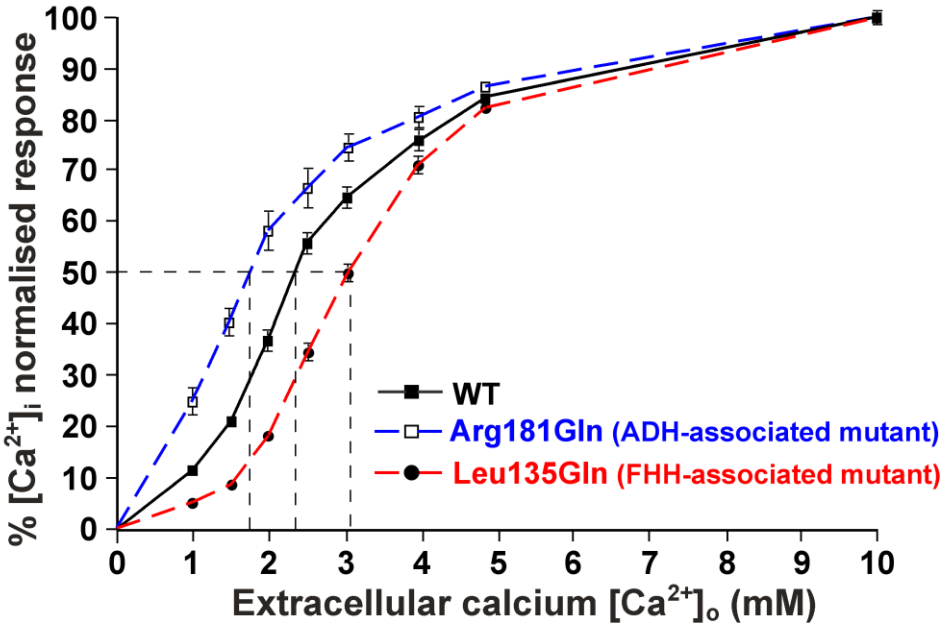
FHH2-associated *GNA11* mutations



ADH2-associated *GNA11* mutations



GNA11 mutations may cause loss- or gain of CaSR signal transduction

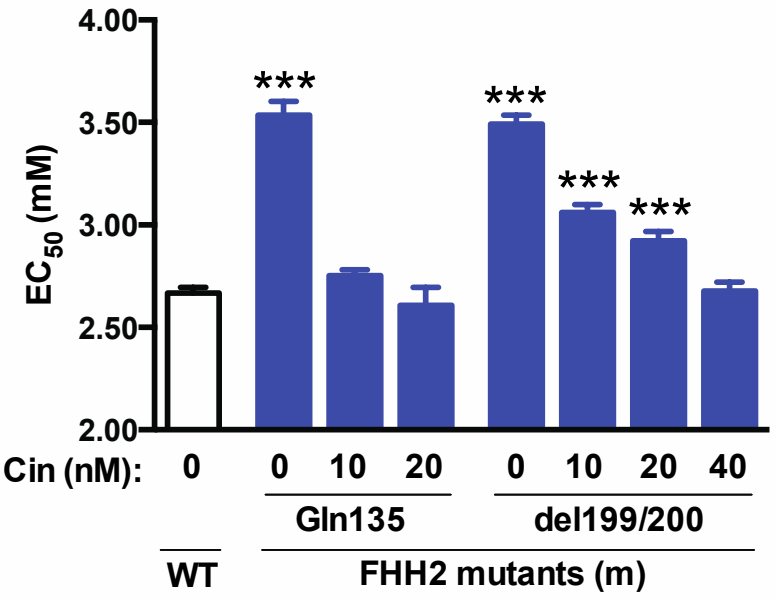


	EC_{50}	95% CI	*p* value
Wild-type (WT)	2.35	2.31-2.39	-
Arg181Gln (mutant)	1.68	1.59-1.78	<0.0001
Leu135Gln (mutant)	3.12	3.05-3.19	<0.0001

GNA11 mutations also reported In 2 families with hypoparathyroidism (Mannstadt et al NEJM 2013)

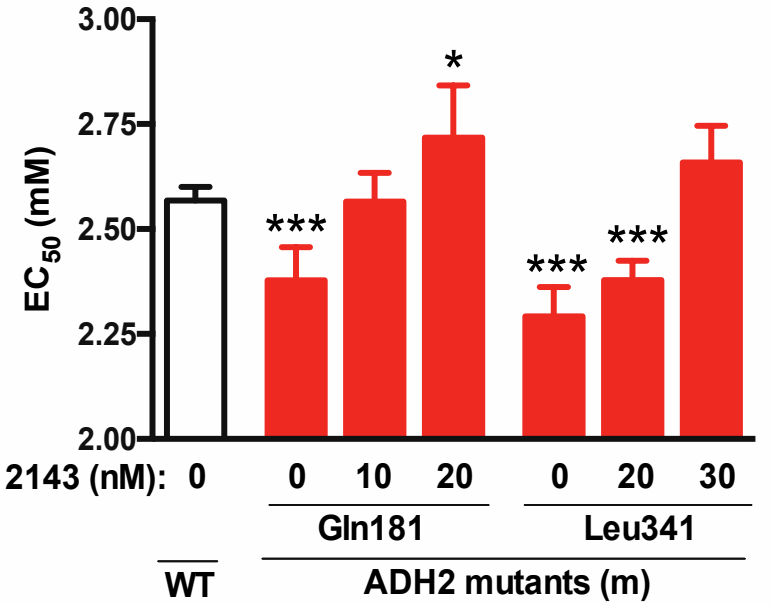
In Vitro: Cinacalcet (a calcimimetic) and NPS-2143 (a calcilytic) rectify $Ca2+_i$ responses of FHH2- and ADH2- causing $G\alpha_{11}$ mutant proteins, respectively

Effect cinacalcet on the EC_{50} values of FHH2 $G\alpha_{11}$ mutants



***p<0.0001 compared to WT

Effect NPS-2143 on the EC_{50} values of ADH2 $G\alpha_{11}$ mutants



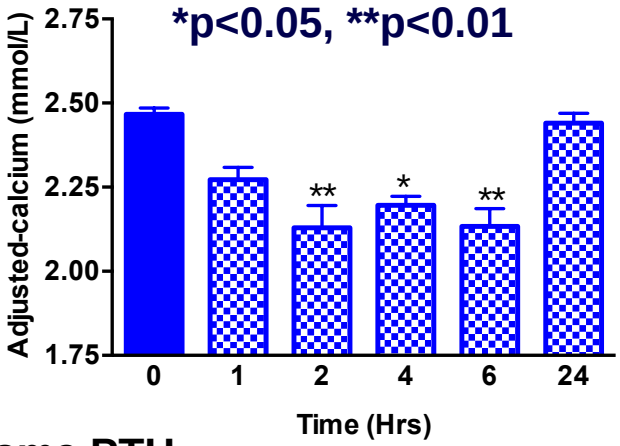
***p<0.0001 compared to WT

Babinsky, Hannan, Gorvin et al J Biol Chem 2016

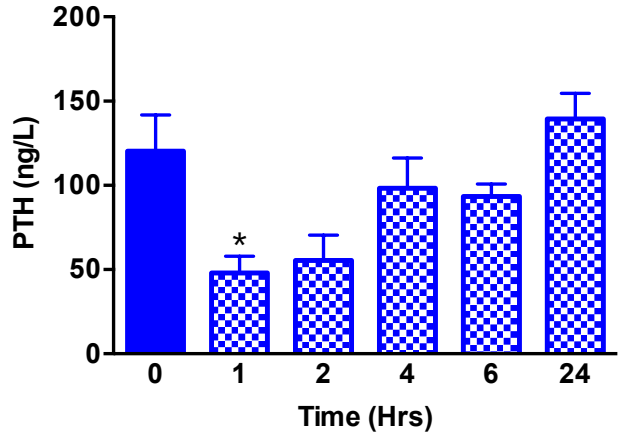
In vivo : Cinacalcet and NPS-2143 rectify the Hypercalcaemia and Hypocalcaemia of FHH2 - and ADH2 - mouse models, respectively.

FHH2- Het (+/*195G*) mice

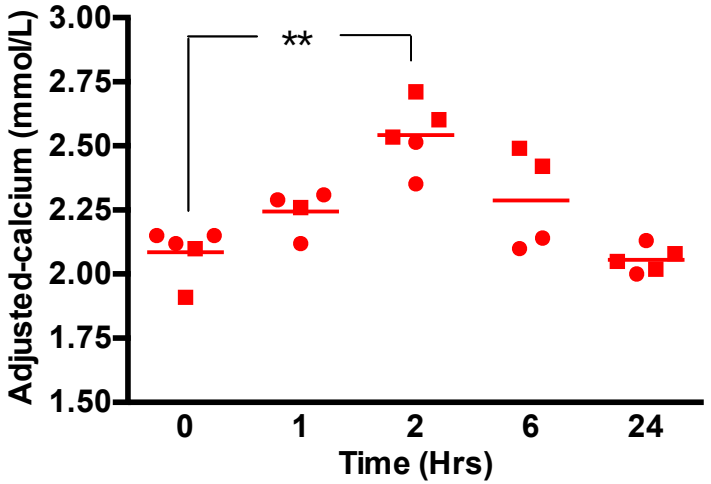
Plasma albumin-adjusted calcium:

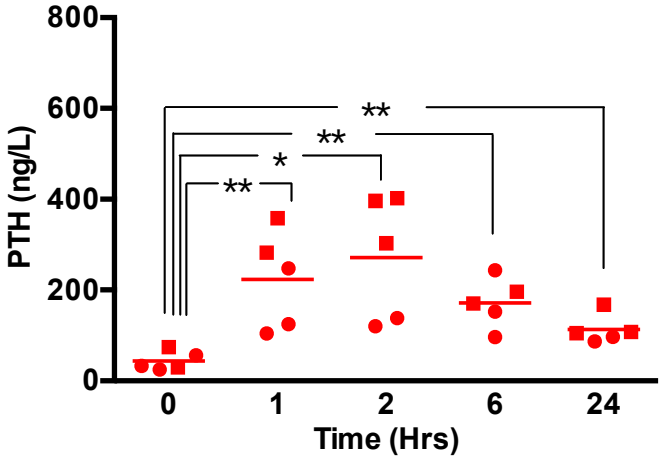


Plasma PTH:



ADH2- Het (+/*62V*) mice



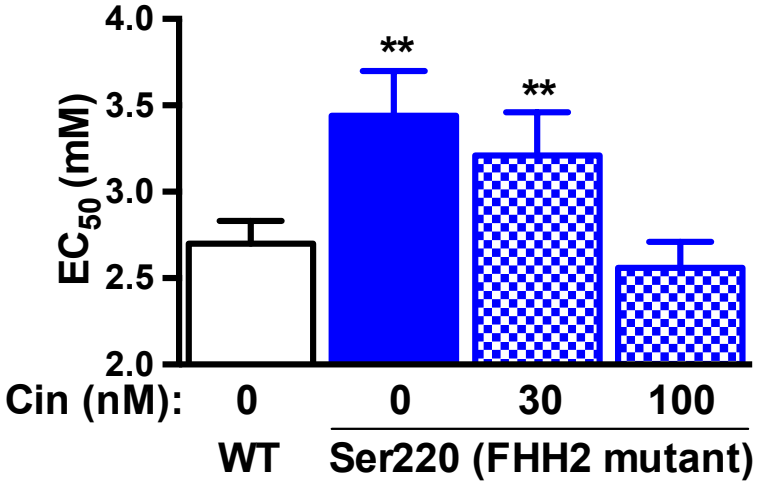


Gorvin et al JCI Insight, 2017;

Howles et al JCI Insight, 2017

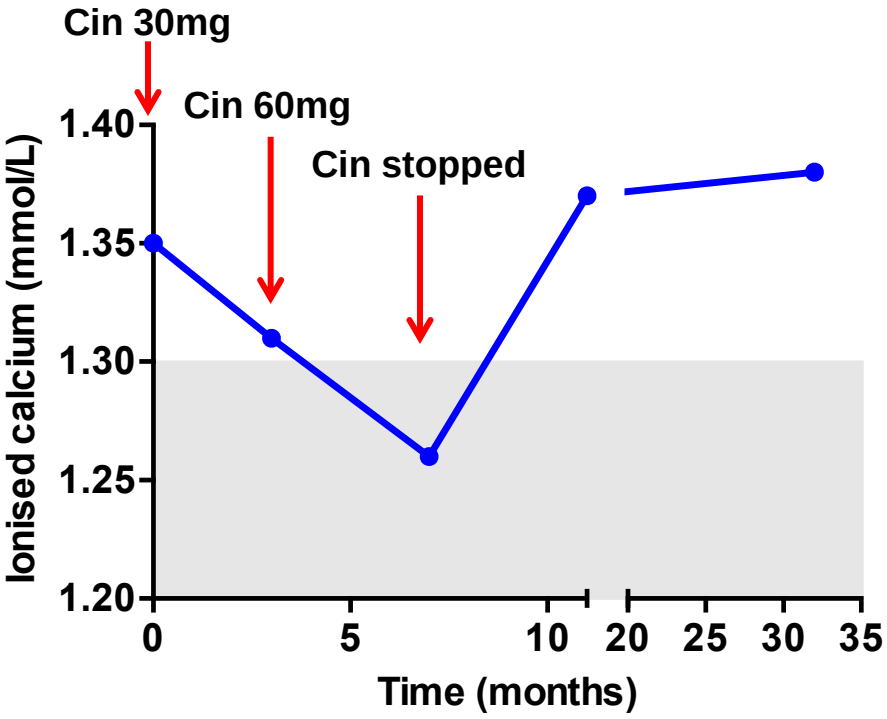
In FHH2 patient with Phe220Ser $G\alpha_{11}$ mutation : Cinacalcet (Cin) Rectifies Hypercalcaemia

In vitro **effects of Cin on Ca^{2+}_i EC_{50} values of Ser220 $G\alpha_{11}$ mutant**



**p<0.01 compared to WT

In vivo **effects of Cin on serum ionised calcium in FHH2 patient**



Gorvin et al, JBMR, 2017

CaSR Mutations found in ≅65% of Familial Hypocalciuric Hypercalcemia (FHH) Patients– Genetic Heterogeneity

FHH is genetically heterogeneous with 3 defined types - FHH1, FHH2 and FHH3 - with chromosomal locations of 3q21.1, 19p and 19q13.3, respectively.

- **FHH1 is caused by loss-of-function mutations of the calcium-sensing receptor (*CaSR*) GPCR.**
- **FHH2 is caused by a mutation of G protein alpha 11 (*GNA11*)**
- **FHH3 – Due to Adaptor protein 2 sigma 2 subunit , AP2σ2,(AP2S1) mutations**

Nesbit et al New Engl J Med 2013; Nesbit et al. Nature Genetics 2013

Hypothesis Driven

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Mutations Affecting G-Protein Subunit α_{11} in Hypercalcemia and Hypocalcemia

M. Andrew Nesbit, Ph.D., Fadil M. Hannan, D.Phil., F.R.C.Path., Sarah A. Howles, B.M., B.Ch., Valerie N. Babinsky, M.Sc., Rosie A. Head, M.A., Treena Cranston, B.Sc., Dip.R.C.Path., Nigel Rust, M.Phil., Maurine R. Hobbs, Ph.D., Hunter Heath III, M.D., and Rajesh V. Thakker, M.D.

Hypothesis Generating

LETTERS

nature genetics

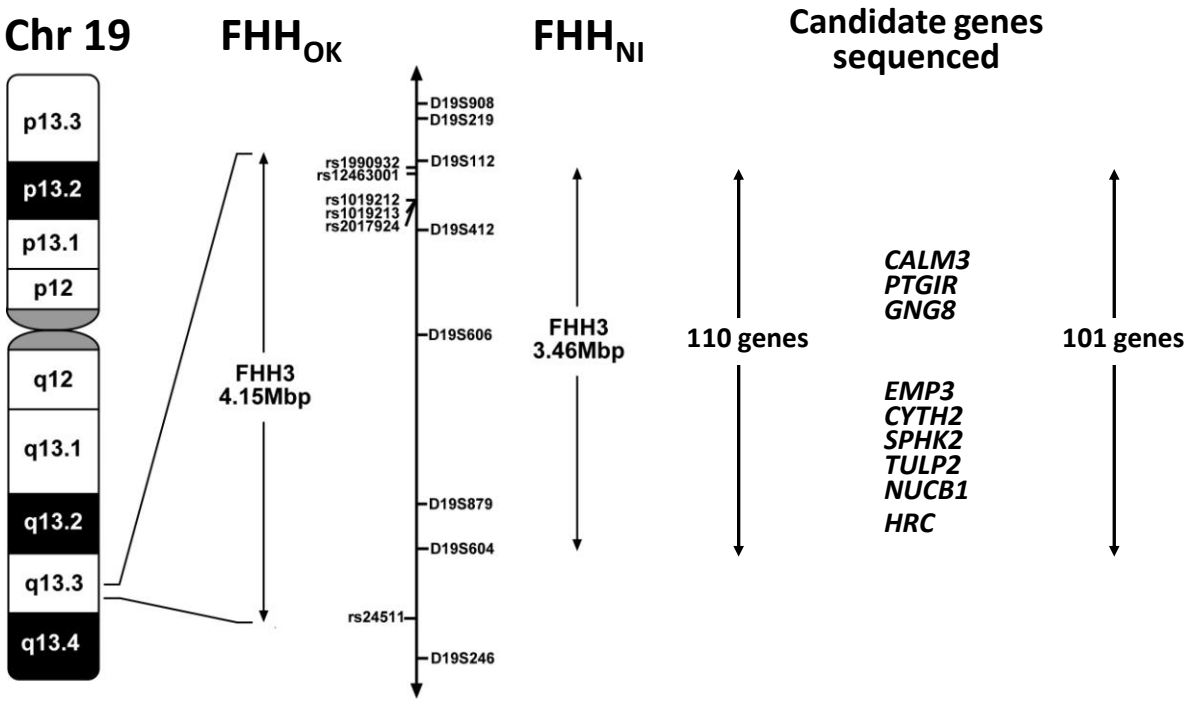
Mutations in *AP2S1* cause familial hypocalciuric hypercalcemia type 3

M Andrew Nesbit[1], Fadil M Hannan[1], Sarah A Howles[1], Anita A C Reed[1], Treena Cranston[2], Clare E Thakker[1], Lorna Gregory[3], Andrew J Rimmer[3], Nigel Rust[4], Una Graham[5], Patrick J Morrison[6], Steven J Hunter[5], Michael P Whyte[7], Gil McVean[3], David Buck[3] & Rajesh V Thakker[1]

Nesbit et al. New England Journal Medicine 2013; Nesbit et al. Nature Genetics 2013

Familial Hypocalciuric Hypercalcemia Type 3 (FHH3) – Hypothesis Generating Approach Used

- FHH3 is located on chromosome 19q13.3

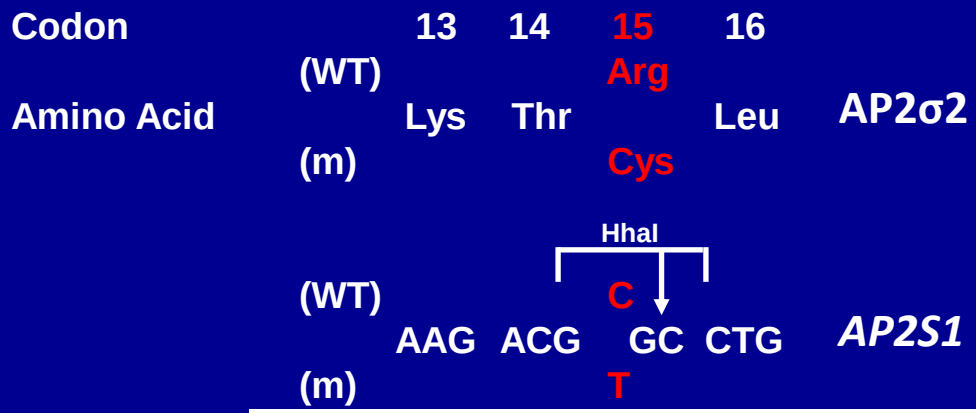


Causative gene *AP2S1* for FHH3 identified by whole exome sequencing – a method that enables exons to be captured and enriched from genomic DNA, thereby facilitating high-throughput DNA sequence analysis

C to T transition in *AP2S1*, resulting in missense mutation of highly conserved Arg15 residue to Cys15 in AP2σ

McMurtry et al 1992; Lloyd et al 1999; Nesbit et al, 2013

Co-Segregation of *AP2S1* Mutation, Arg15Cys, due to C to T transition in FHH3 Kindred



DNA sequence analysis of *AP2S1* in 50 additional unrelated FHH patients without *CaSR* mutations revealed occurrence of 11 missense heterozygous mutations *all involving Arg15 residue* (x4Arg15Cys; x3 Arg15His; x4 Arg15Leu)

Nesbit et al. Nature Genetics 2013

Hypothesis for the role of AP2σ2 in CaSR signalling.

Adaptor Protein 2 (AP2) – a heterotetrameric protein complex of α, β, μ, and σ subunits.

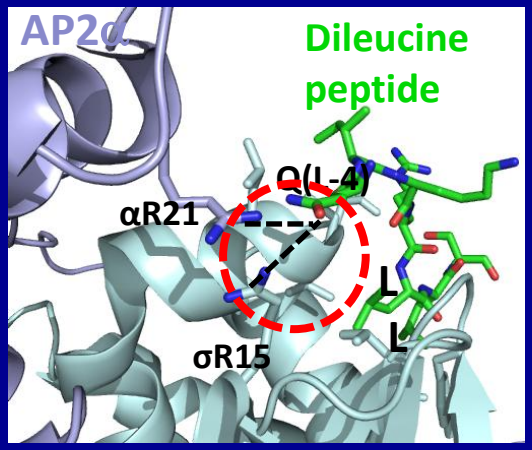
AP2 has a pivotal role in clathrin-mediated endocytosis

σ subunit (Arg15) binds to -dileucine motifs in cargo proteins.

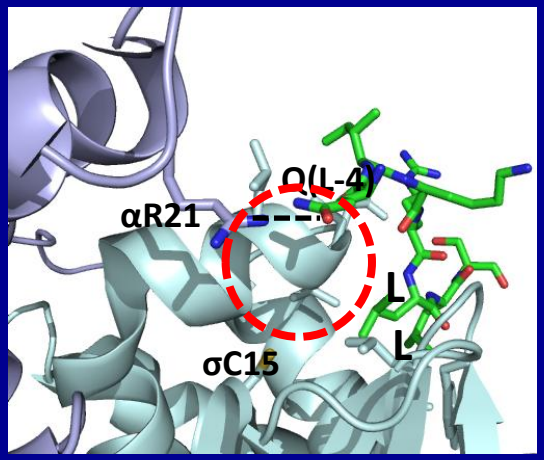
Nesbit, Hannan, Howles et al. Nat. Gen. 2013

FHH3: Exome Sequencing Reveals Adaptor Protein 2 Sigma (σ) Subunit Mutations

WT Arg15 AP2σ2

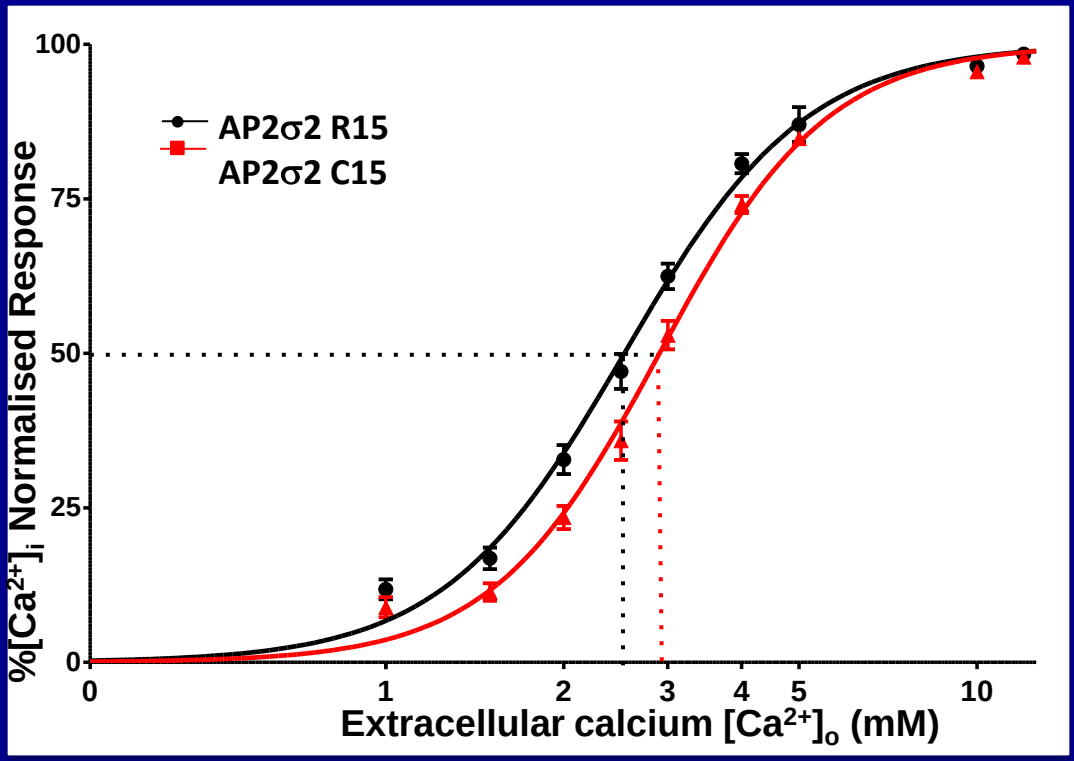


Mutant Cys15 AP2σ2



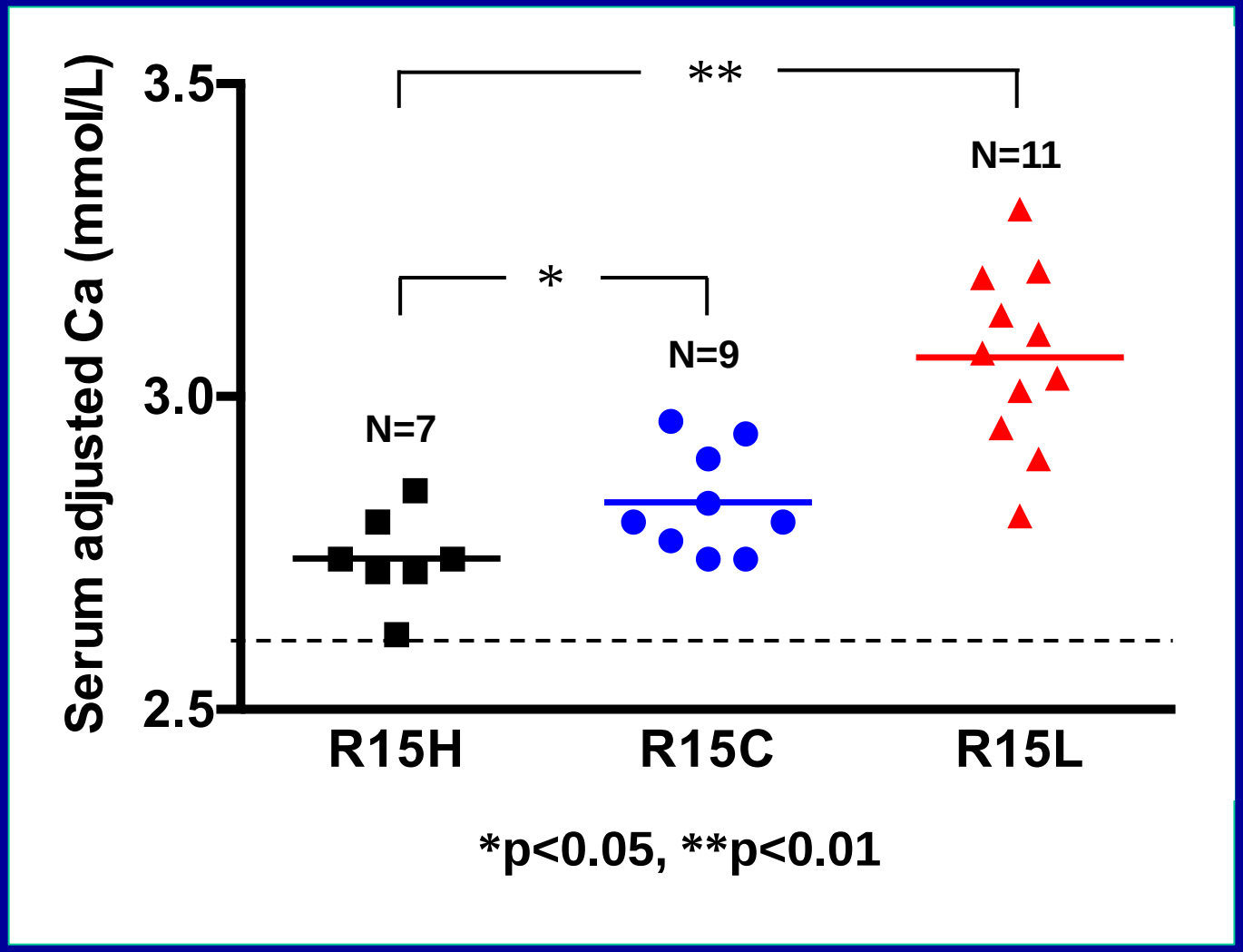
AP2σ2 mutations (R15C, R15H and R15L) found in FHH3 patients result in loss of key polar contact with dileucine motif on CaSR

- AP2, a heterotetrameric protein complex of α, β, μ, and σ subunits has pivotal role in clathrin-mediated endocytosis
- Mutations AP2σ2 mutations (R15C, R15H and R15L) alter sensitivity of CaSR expressing cells, and disrupt CaSR internalisation



Nesbit et al. Nature Genetics 2013

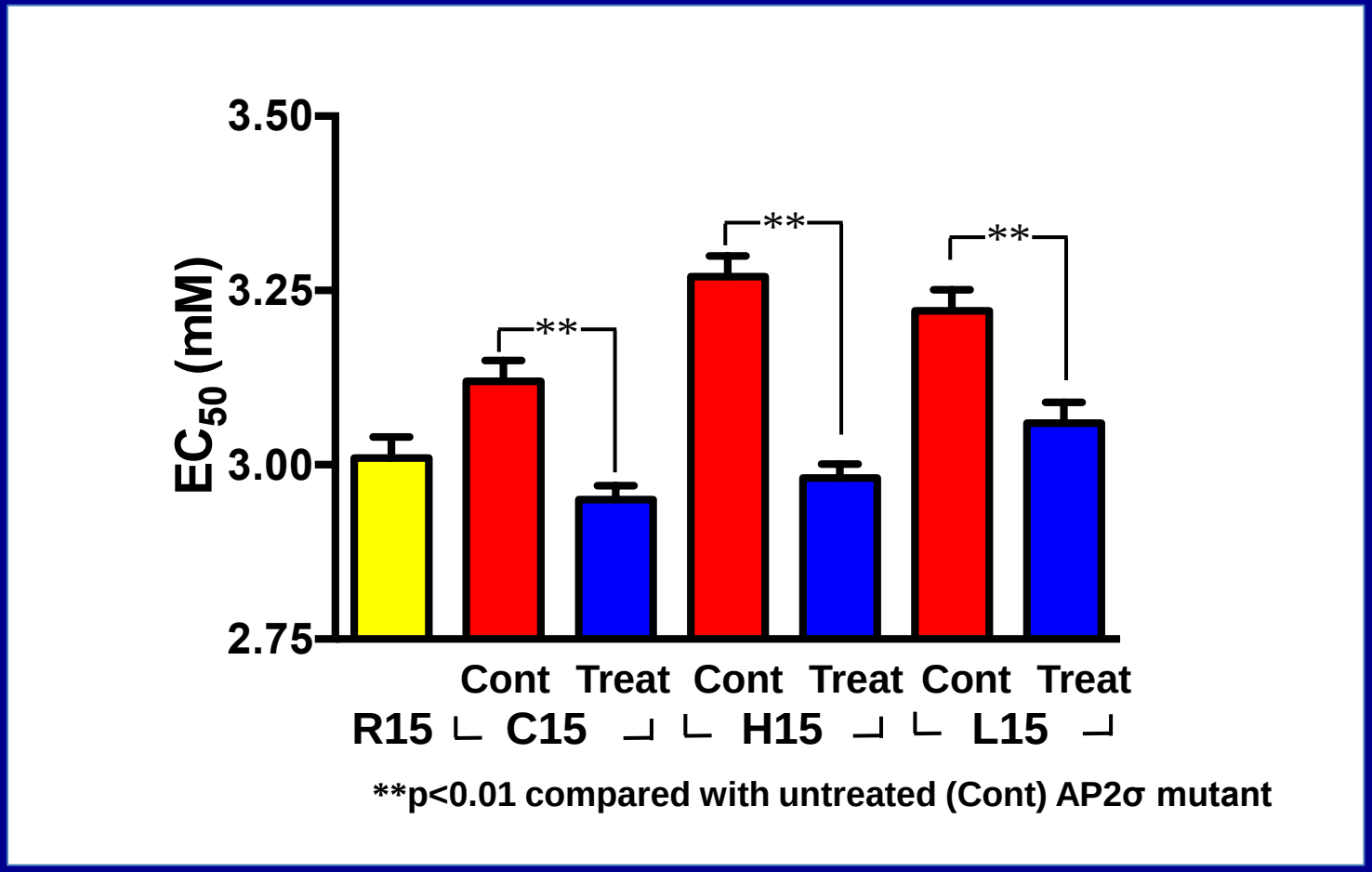
Genotype-phenotype correlation at the R15 residue – Serum calcium



FHH3 probands harbouring R15L AP2σ2 mutation have the highest serum calcium concentrations

Hannan et al. Hum Mol Genet, 2015

Cinacalcet improves CaSR signal transduction in HEK-CaSR cells expressing all three FHH3-causing AP2σ mutations



10 nM of Cinacalcet normalises EC_{50} values of cells expressing R15C, R15H or R15L AP2σ mutants

Howles et al. NEJM, 2016

Cinacalcet lowers serum calcium concentrations in FHH3 patients

Serum calcium

Serum phosphate

Serum PTH

Howles et al. NEJM, 2016

Mechanisms of CaSR Signalling and Trafficking: Role of FHH3 - associated AP2σ Mutants (R15C, R15H and R15L)

- ***Paradox*: AP2σ mutants ↓CaSR endocytosis and trafficking, thereby ↑CaSR at plasma membrane (PM), yet ↓signalling**

- ***Hypothesis*: CaSR signals through canonical (PM) and non-canonical (via endosomes (EE)) pathways**

- ***Signalling*:**
  - **Normal = PM + EE**
  - **Mutant AP2σs = PM only**

Gorvin et al 2018
Cell Reports

Summary – CaSR Pathway Disorders

- FHH and ADH are genetically heterogeneous disorders
- Manipulations by calcimimetic and calcilytic drugs can rectify the abnormalities for patient benefit

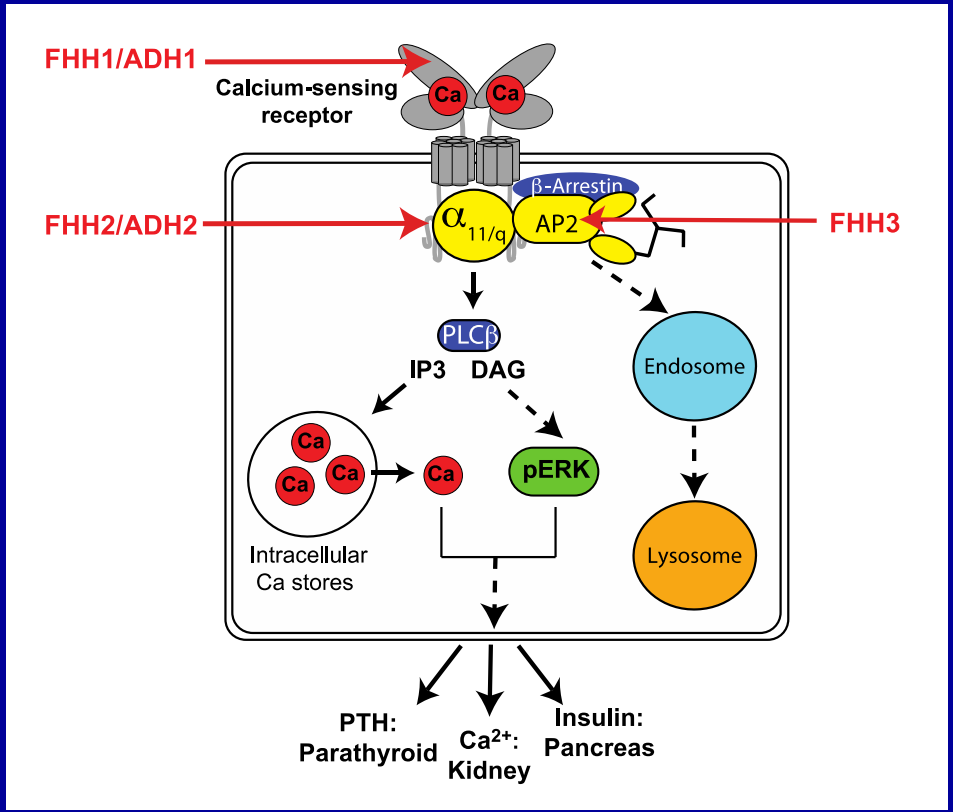
Signalling

FHH1 & ADH1

• Loss- and gain-of-function mutations of the CaSR gene on 3q21.1

FHH2 & ADH2

• Loss- and gain-of-function mutations of the G protein alpha 11 gene (GNA11) on 19p



Trafficking

FHH3

• Loss-of-function mutations of the adaptor protein 2 sigma subunit (AP2S1) gene on 19q13.3

Hannan et al HMG 2012, Nesbit et al Nat Genet 2013, Nesbit at al NEJM 2013, Howles et al NEJM 2016, Babinsky et al JBC 2016, Gorvin et JBMR 2017

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