

## Abstract

We report a kindred with Familial Benign Hypocalciuric Hypercalcaemia (FBHH) due to a mutation which is listed in the "Professional" version of the Human Genome Mutation Database (HGMD-pro)<sup>1</sup> as causing Neonatal Severe Hyperparathyroidism (NHPT) in the homozygote<sup>2</sup>. It not listed as causing FBHH in the heterozygote.

## Background

Familial Benign Hypocalciuric Hypercalcaemia is a benign autosomal dominant condition characterised by elevated serum calcium, inappropriately high parathyroid hormone (PTH) and low urine calcium. It is a genetically heterogeneous disorder but the majority of cases (type 1 FBHH\*) can be shown to be due to inactivating mutations in the Calcium Sensing Receptor (CASR). This is a guanine nucleotide-binding-protein (G-protein) coupled receptor that signals through the G-protein subunit  $\alpha_{11}$  ( $G\alpha_{11}$ ). Patients who are homozygous for inactivating CASR mutations present with Neonatal Severe Hyperparathyroidism (NHPT) which, in contrast to FBHH, is characterised by severe hypercalcaemia and early death. 3 sub types of FBHH are recognized. Type 1 (see\* above) is the most common . Type 2 is due to mutations effecting  $G\alpha_{11}$  which result in loss of function<sup>3</sup>, while type 3 is due to adaptor-related protein complex 2, sigma 1 subunit (AP2S1) mutations which result in altered CASR endocytosis<sup>4</sup>. In FBHH, mutations result in the CASR being less sensitive to serum calcium so that the "set point" for serum calcium is reset at a higher value, leading to hypercalcaemia and increased PTH secretion (PTH secretion is controlled via the CASR). Although FBHH is a benign condition it can be confused with primary hyperparathyroidism because the two are similar in terms of biochemistry. It is important therefor to confirm the diagnosis to avoid unnecessary parathyroidectomy.

## Kindred

The proband was a 62 year old male. During investigations for a possible Transient Ischaemic Attack, he was found to have an adjusted calcium of 2.80 mmol/l and a PTH of 11.7 pmol/l. He was asymptomatic from his hypercalcaemia and had no history of osteoporosis or renal stones. We were able to obtain samples from 2 other members of his kindred, namely his 65 year old sister and his 40 year old son.

Relevant biochemical results were:

	Proband	Sister	Son
Adj Ca (mmol/l)	2.80	2.89	3.08
Phosphate (mmol/l)	0.87	0.93	0.80
Alk phos (U/l)	78	72	117
PTH (pmol/l)	11.7	6.5	6.4
25-OH-Vit D (nmol/l)	108	60	ne*
Urine Ca (mmol/l)	<0.5	<0.5	0.6

\* not estimated

Reference ranges: Adj Ca 2.10-2.60. Phosphate 0.70-1.40. Alk Phos 30-130. PTH 1.8-7.7. 25-OH-Vit D 25-170. Lower limit of detection for urine calcium 0.5.

## DNA seqencing

DNA was extracted from whole blood using the Maxwell 16 system (Promega Corp. Madison, WI) with the Maxwell 16 Blood DNA Purification Kit (AS1010). Amplification of CASR coding exons 2 to 7 was performed in 10 amplicons under standard PCR conditions: 30 ng genomic DNA in a total volume of 10 mL, 1,5 mM MgCl GoTaq Buffer system (M792B - Promega Corp. Madison, WI) and GoTaq DNA Polymerase (M830C - Promega Corp. Madison, WI). Cycle sequencing 32 rounds with the following settings: 30 sec @ 95°C, 30 sec. @ 66°C annealing temperature and 50 sec. @ 72°C. Amplicons were subsequently sequenced with the ABI Big Dye Terminator 3.1 kit as recommended by the manufacturer (Applied Biosystems, Foster City, CA). The ethanol precipitation method was used as recommended.

## DNA sequencing (contd)

Sequencing reactions were separated on a Spectrumedix 9614 Capillary Electrophoresis system (Transgenomics Inc, Omaha, NE). Datafiles were processed by use of the Basespectrum software (Transgenomics Inc, Omaha, NE) and files were basecalled and analysed in SeqMan Pro from the DNA Star Lasergene 10 Suite (DNASStar Inc. Madison, WI)

## Results of DNA sequencing

DNA sequencing of all exons and exon-intron boundaries revealed a c.1745G>A mutation in all 3 kindred members. This results in a p.C582Y missense variation. (i.e. Cysteine to Tyrosine change at amino acid 582 of the CASR).

## Discussion

Although only 3 members of the kindred were available for testing, biochemical and clinical findings are in keeping with FBHH. While the mutation is not listed in HGMD-pro<sup>1</sup> as causing FBHH, the fact that the mutation is listed as causing NHPT<sup>2</sup> in the homozygote is in keeping with other mutations known to be causative for FBHH in the heterozygote. HGMD-pro<sup>1</sup> is the gold standard resource for comprehensive up to date data on published human inherited disease mutations. It therefore facilitates the assessment of whether an observed mutation is novel or has been adequately described previously. The mutation described is a missense mutation of the CASR, which means that the kindred described here are an example of type 1 FBHH.

## References

1. <http://www.hgmd.cf.ac.uk/docs/register.html>
2. Pearce et al. 1995. J Clin Inv. 96:2683-2692
3. Nesbit et al. 2013. N Engl J Med. 368(26): 2476-2486.
4. Nesbit et al 2013. Nat Genet. 2013;45(1): 93-97.