

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Au AYM, McDonald K, Gill A, Sywak M, Diamond T, Conigrave AD, Clifton-Bligh RJ. *PTH* mutation with primary hyperparathyroidism and undetectable intact PTH. *N Engl J Med* 2008;359:1184-6.

## Supplement

### Supplement, Table 1

Serum and urinary biochemistry and bone histomorphometry data.

	Measurement	Normal range
<i>Serum</i>		
Calcium (mg/dl)	11.8	8.8-10.2
Albumin (g/l)	48	37-46
Phosphate (mg/dl)	2.79	2.42-4.43
PTH (pg/ml)	<3	3-50
1,25-dihydroxyvitamin D (pmol/liter)	82, 208	38-162
Alkaline phosphatase (U/liter)	159	41-119
Osteocalcin (ng/ml)	27.7	3.7-10
PTHrP (pmol/liter)	<0.6	1.2-4.3
ACE activity (nmol/ml/min)	70	42-134
<i>Urine</i>		
Calcium (mg/d)	456.4	50-300
Urinary deoxypyridinoline (nmol/mmol of creatinine)	7.5	3.0-7.4
Urinary NTX (nmol/mmol of creatinine)	110	26-124
Nephrogenous cAMP (nmol/100 ml)	1.10	<1.92

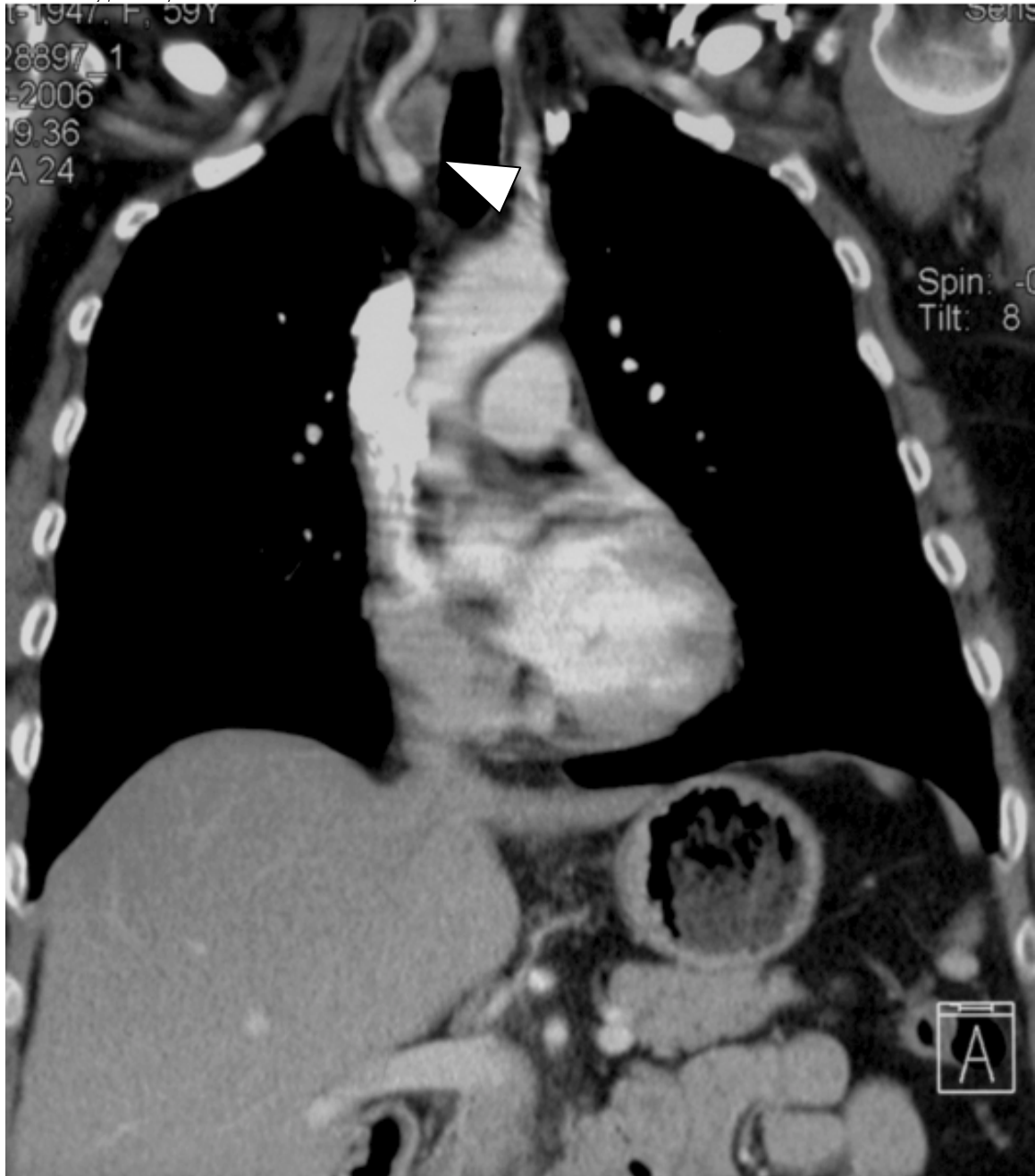
GFR)		
<i>Bone histomorphometry</i>		
Trabecular bone area (%)	13.6	22-28
Cortical width (mm)	1.18	0.65-1.2
Osteoid area (%)	8.6	1.5-3.3
Osteoid surface (%)	36.4	8.9-14.9
Active resorption surface (%)	3.2	0.8-1.9
Double label surface (%)	12.6	5.2-11.5
Mineral apposition rate (mm/d)	0.63	0.65-0.80
Bone formation rate (mm <sup>3</sup> /mm <sup>2</sup> /d)	0.08	0.04-0.09
Osteoid seam (µm)	16.9	8.1-14.2
Mineralization lag time (d)	80	10.5-17.8

To convert the value for serum calcium to millimoles per liter, multiply by 0.25; to convert the value for serum phosphate to millimoles per liter, multiply by 0.3229; to convert the value for urinary calcium excretion to millimoles per day, multiply by 0.025.

ACE, angiotensin converting enzyme; cAMP, cyclic adenosine monophosphate; NTX, N-terminal telopeptides of type 1 collagen; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related protein.

**Supplementary Fig. 1**

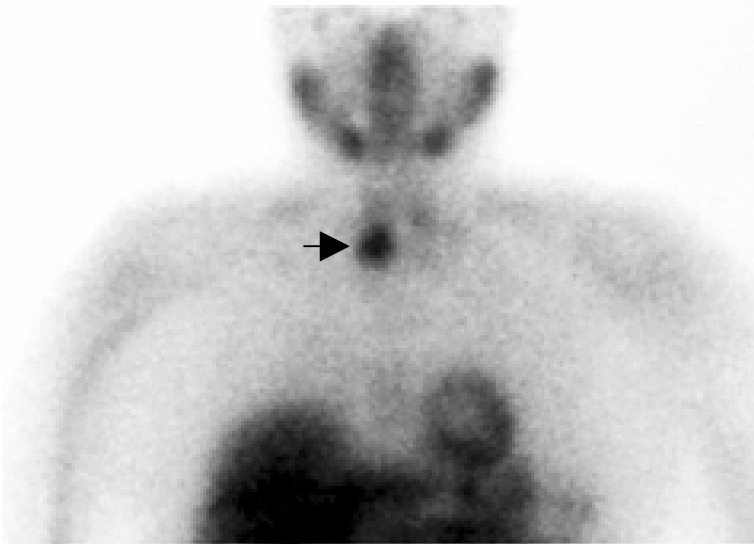
Coronal CT image of lower neck and thorax. A soft tissue mass inferior to the lower pole of the right thyroid lobe is shown by an *arrowhead*.



## Supplementary Fig 2

Scan of the neck and thorax at 15 minutes and 3.5 h after injection of Technetium-99m Sestamibi. A focus of persisting uptake in the right side of the neck is *shown by an arrow at each timepoint.*

### CHEST 15 MINS PI



### CHEST 3.5 HRS PI



## Supplementary Table 2

Differentially expressed genes in the parathyroid tumor compared with normal parathyroid from the same patient, as assessed by microarray. Genes are denoted by their symbol, encoding proteins named in the adjacent column, and are listed in rank order of significance. The M-value shown in the right-hand column refers to the log<sub>2</sub>-fold change of gene expression; positive values represent upregulated genes and negative values are downregulated genes relative to the normal parathyroid control. The patient provided written informed consent for the use of her tissues (tumor, biopsied normal parathyroid gland tissue, peripheral blood) for these studies. The study was approved by the Local Human Research and Ethics Committee.

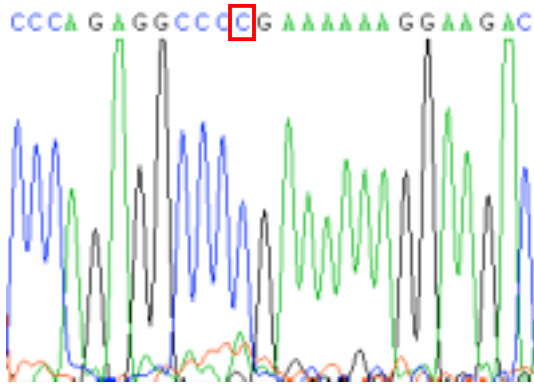
Rank	Symbol	Name	M
1	PVALB	parvalbumin	9.519226
2	ADIPOQ	adiponectin, C1Q and collagen domain containing	-9.07367
3	MGST1	microsomal glutathione S-transferase 1	-8.61872
4	PCK1	phosphoenolpyruvate carboxykinase 1 (soluble)	-8.49412
5	RBP4	retinol binding protein 4, plasma	-8.22232
6	GCM2	glial cells missing homolog 2 (Drosophila)	8.065604
7	PLIN	Perilipin	-8.00998

8	UCP1	uncoupling protein 1 (mitochondrial, proton carrier)	-7.98776
9	KCNJ16	potassium inwardly-rectifying channel, subfamily J, member 16	7.985853
10	CDO1	cysteine dioxygenase, type I	-7.95533
<b>12</b>	<b>PTH</b>	<b>parathyroid hormone</b>	<b>7.497016</b>
13	VDR	vitamin D (1,25- dihydroxyvitamin D3) receptor	7.494854
84	CaSR	calcium-sensing receptor (hypocalciuric hypercalcemia 1, severe neonatal hyperparathyroidism)	5.590949

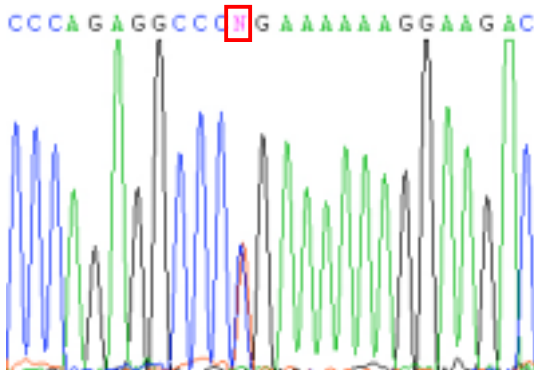
### Supplementary Fig. 3

Nucleotides 235 to 267 in exon 3 of the *PTH* gene. Sequence of normal control DNA is shown in the *top* panel. Sequencing of DNA isolated from peripheral blood leukocytes shows a heterozygous pattern for the C→T change at position 247, corresponding to the R83X mutation (*boxed, bottom*).

Normal



Peripheral Blood



### Supplementary Table 3

Measurement of N-terminal PTH 1-32 (Immutopics, CA) and intact PTH 1-84 (Immulite Bio-Mediq DPC, Victoria, Australia) during perfusion of ex vivo tumor parathyroid cells, whereby a saline solution is passed through tumor cells and then collected in timed aliquots. Briefly, 40,000- 100,000 cells were loaded onto the surface of a 1-ml bed volume of Bio-gel P-4 and then gently covered with a 1 ml bead volume of Sephadex G-25 in a small perfusion column. The column was suspended in a water bath at 37°C and perfused at 1.5 mL/min with control physiological saline, 125 mM NaCl, 4.0 mM KCl, 1.0 mM MgCl<sub>2</sub>, 0.8 mM NaH<sub>2</sub>PO<sub>4</sub>, 20 mM HEPES (NaOH, pH 7.4), 0.1 % D-glucose, 1 mg/ml bovine albumin, and 1.2-1.8 mM CaCl<sub>2</sub>. Routinely, three samples were collected during each treatment period, at two minute intervals, into tubes immersed in an ice bath, and the tubes were transferred to dry ice upon completion of each collection period. All samples were stored at -80°C until measurement of PTH using either a chemiluminescent assay for intact PTH (Immulite) or ELISA for N-terminal PTH (Immutopics, CA, #60-3600).

Extracellular ionized Ca <sup>2+</sup> (mg.dl <sup>-1</sup> )	Intact PTH (fg.min <sup>-1</sup> cell <sup>-1</sup> )	N-PTH (fg.min <sup>-1</sup> cell <sup>-1</sup> )
4.8	<0.05	0.62
5.6	<0.05	0.49
6.4	<0.05	0.53
7.2	<0.05	0.34
4.8	<0.05	0.70

To convert the values for calcium concentration to millimoles per liter, multiply by 0.25.

PTH, parathyroid hormone; N-PTH, N-terminal parathyroid hormone.