Supplemental information for Dasgupta et al. "**Mutations in *SLC34A3/NPT2c* are associated with kidney stones and nephrocalcinosis**"

Supplemental Results:

Full case reports of novel HHRH kindreds and IH cases

The index case of **kindred A** (Fig. 1A), A/IV-1 presented at $9^{2/12}$ years with leg pain when walking and progressive crura vara since age 1, which required surgical correction at age 5. She is the oldest of four children to Afghan parents, who are first-degree cousins. Her physical exam at $9^{1/12}$ years revealed a weight (W) above 97th percentile, height (H) between the 90th-97th percentile, severe crura vara, flared condyles, large hands and feet, prominent sternum, carious teeth, no enamel defects. Her pubertal development was Tanner breast 3. On presentation, her total calcium (Ca) and ionized Ca (iCa) were normal at 2.4 mmol/l (2.4-2.65) and 1.28 mmol/l (1.1-1.35), respectively, she was hypophosphatemic with a phosphate (P) level of 0.77 mmol/l (1.0-1.8), her alkaline phosphatase (ALP) was elevated to 725 IU/l (<300), parathyroid hormone (iPTH) was suppressed at 1.48 pmol/l (1.6-6.9), and 1,25$(OH)_2$D was at the upper limit of normal at 155 pmol/l (48-160) in the setting of low 25OHD of 55 nmol/l (62-200). Her magnesium (Mg) 0.67 mmol/l (0.73-1), creatinine (Crea) 39 μmol/l (33-68), uCa/Crea ratio 0.86 mol/mol (<0.85) were normal, while tubular reabsorption of phosphate (TRP) of 82% was inappropriately low in the context of hypophosphatemia (>90%). There was no glucosuria, proteinuria, or aminoaciduria. Intact fibroblast growth factor 23 (iFGF23) level was undetectable. On treatment with 20 mg/kg/d of elemental phosphorus, calcitriol 12 ng/kg/d her ALP improved to 585 IU/l, but she developed hypercalciuria; uCa/Crea was 1.28 mol/mol (<0.85) and her renal ultrasound revealed extensive nephrocalcinosis. After discontinuation of calcitriol while continuing treatment with 20 mg/kg/d of elemental phosphorus, her laboratory findings at age $10^{1/12}$ years improved to ALP 491 U/l, 1,25$(OH)_2$D 136 pmol/l and uCa/Crea 0.5 mol/mol. The proband's 7-year old brother, A/IV-2, had no clinical symptoms, but nephrocalcinosis was observed on renal ultrasound and ALP was elevated at 342 IU/l (<300). The $2^{4/12}$ year old sister, A/IV-3, presented with crura vara but had no other rachitic signs and renal ultrasound was normal. Her laboratory results showed an elevated ALP 492 (<300), suppressed iPTH of 1.06 pmol/l (1.6-6.9), and an elevated uCa/Crea ratio of 1.35 mol/mol (<1.00). There was no glucosuria, proteinuria, or aminoaciduria. Intact FGF23 was below the detection limit. The 1 year-old brother, A/IV-4, had a normal physical examination, normal renal ultrasound and his labs were normal with the exception of ALP that was elevated at 320 IU/l (<300). The father, A/III-1, and mother, A/III-2, were healthy and had normal renal ultrasounds.

Additional information on kindred A

A/IV-1, index case, age $10^{1/12}$ years: On treatment with sodium-phosphate 20 mg/kg/d, calcitriol 12 ng/kg/d ALP improved to 585 IU/l, and she developed hypercalciuria; uCa/Crea 1.28 mol/mol (<0.85). After discontinuation of calcitriol while continuing treatment with sodium phosphate 20 mg/kg/d, her laboratory findings at age improved further; ALP decreased to 491 U/l. On this therapy, 1,25$(OH)_2$D initially decreased to 136 pmol/l and uCa/Crea improved to 0.5 mol/mol, but subsequently 1,25$(OH)_2$D again increased 226 pmol/l at **$10^{4/12}$ years** and 284 pmol/l at **$10^{7/12}$ years**, presumably due to noncompliance with therapy. On therapy, P ranged from 0.65 to 1.15 mmol/l and TRP decreased further to 73.1%.

A/IV-2, brother, age 7 years: Additional laboratory findings include Ca 2.52 mmol/l (2.40-2.65), P 1.15 mmol/l (1.00-1.8), iCa 1.28 mmol/l (1.10-1.35), Crea 44 µmol/l, suppressed iPTH of 1.27 pmol/l (1.60-6.90), 25(OH)D 77 nmol/l, $1,25(OH)_2D$ 130 pmol/l, uCa/crea ratio 0.65 mol/mol (<0.85), TRP 95.3%.

A/IV-3, sister, age $2^{4/12}$ year: Additional clinical information: 75-90th percentile for W and 25-50th percentile for H, HC 10-25th percentile. Additional laboratory results were; Ca 2.41 mmol/l (2.4-2.65), ion. Ca 1.31 mmol/l, low P 0.87 mmol/l, Mg 0.75 mmol/l (0.73-1.0), Crea 24 µmol/l, 25(OH)D 40 nmol/l, $1,25(OH)_2D$ 99 pmol/l (48-160). ALP improved to 243 U/l under treatment with 20 mg P/kg/d and calcitriol 11.4 ng/kg/d, but her hypercalciuria persisted with a uCa/Crea ratio of 1.36 mol/mol (<1.00). Her hypercalciuria worsened to 2.2 mol/mol after accidentally adding 2500 IU cholecalciferol/day to her regimen, and 25(OH)D and $1,25(OH)_2D$ increased to 168 nmol/l and 255 pmol/l, respectively, as a result of which all vitamin D analogs were stopped.

A/IV-4, brother, age 1 year: Additional labs were Ca 2.39 mmol/l (2.4-2.65), P 1.26 mmol/l (1.00-1.8), a low ion. Ca of 0.93 mmol/l (1.1-1.35), elevated ALP at 320 IU/l (<300), Crea 31 µmol/l (20-65), iPTH 2.54 pmol/l (1.6-6.9), 25(OH)D 19 nmol/l, $1,25(OH)_2D$ 90 pmol/l, uCa/Crea 0.52 mol/mol (<1.27), TRP 92.5%.

A/III-1, father, age 34 years: H was 185.0 cm, additional labs were Ca 2.39 mmol/l (2.1-2.55), P 1.02 mmol/l (0.84-1.45), Crea 92 µmol/l (59-104), ALP 71 U/l (40-130), uCa/Crea 0.19 mol/mol (<0.62), TRP 89%, no glucosuria, proteinuria, or aminoaciduria, and his renal US was normal.

A/III-2, mother, age 29 years: H was 158.0 cm, her laboratory studies were: Ca 2.31 mmol/l (2.1-2.55), P 0.86 mmol/l (0.84-1.45), Crea 59 µmol/l (45-84), ALP 72 U/l (35-105), uCa/Crea 0.38 mol/mol (<0.62), TRP 91.4%, no glucosuria, proteinuria, or aminoaciduria.

The index case in **kindred B** (Fig. 1 B), B/II-2, the younger of two children from non-consanguineous Caucasian parents, presented at 11 years of age with an elevated ALP of 675 IU/L (reference range 50-350) incidentally found during investigation for vasovagal syncope. He previously fractured his left femur in a skateboard park at age 6 years, sustained a greenstick fracture of his left forearm at age 8 years, and a displaced left radial fracture at age 12 years while snowboarding. His P was slightly reduced at 0.94 mmol/L (1.20-1.74) and his 25(OH)D was 44 nmol/L (51-250). No further action was taken for a two-year period apart from monitoring his ALP, which fluctuated between 650-820 U/L until age 13 years when he was referred to one of us for investigation of his persistently elevated ALP, bone pain and recurrent fractures. Repeat blood tests at this time showed an ALP of 769 U/L despite a meanwhile improved 25(OH)D level of 71 nmol/L, but his serum P was low 0.90 mmol/L (1.20-1.74), $1,25(OH)_2D$ was elevated 419 pmol/L (60-158), while Ca, Crea, and iPTH levels were within normal limits. TmP/GFR of 0.98 mmol/L (0.77-1.29) and TRP 84% were inappropriate in the setting of hypophosphatemia. Calcium excretion was at the upper limit of normal with an uCa/Crea ratio of 0.507-0.706 mol/mol (<0.73) and 24hr Ca excretion was 4.8 mmol/day (1-7). His bone mineral densitometry showed a total hip BMD z-score of -3.6, spine BMD z-score of -1.6, and whole body bone mineral z-score of -6.1. On examination B/II-2 appeared well. He was not dysmorphic and had no birthmarks. He had been growing adequately; his latest height was 162.3cm (75th

percentile) with a weight of 53.9kg (77th percentile). He had normal sclerae and dentition, hypermobile fingers, hyperextensible knees, bilateral pes planus and calcaneovalgus. His spine was straight. General clinical examination was otherwise unremarkable, he was normotensive, and renal ultrasound was unremarkable. The parents (B/I-1 and I-2) and an older brother (B/II-1) were well and revealed normal biochemistries as shown in Figure 1B, with the exception of an elevated 1,25$(OH)_2$D level for father and brother of 218 and 172 pmol/L (60.00-158.00), respectively. There is no family history of renal stones.

The index case of **kindred C** (Fig. 1 C), C/III-1, presented at age 5 years, following an earlier history of nephrolithiasis at age 3, managed with low calcium intake and hydration. Upon referral to endocrinology, a more complete evaluation was performed revealing hypophosphatemia of 1 mmol/L (1.39-1.74). His 1,25$(OH)_2$D was also elevated at 343.20 pmol/L (52-163.80), as was his urine Ca/Crea ratio at 1.90 mol/mol (<0.66). TmP/GFR was 0.74, and TRP was low at 76%(>90%). His remaining biochemical findings were within normal limits: 25(OH)D 84.86 nmol/L (24.96-169.73), serum Ca 2.40 mmol/L (2.13-2.63), serum Crea 44.23 umol/L (17.63-52.88). His kidney stones required lithotripsy at age 4, since he had experienced mild back pain. He had had fracture of the radius and ulna after a fall but was otherwise healthy. Radiographs at that time revealed osteopenia and widened growth plates suggestive of "early rickets." His physical examination revealed a normal child at the 10th centile for both height and weight. A DXA scan revealed a lumbar spine BMD Z score of -1.9 (at age 5). His father, C/II-2, age 37, developed renal stones at age 32, and reported intermittent symptoms, but was otherwise healthy. His labs were: Ca 2.38 mmol/L (2.13-2.63), P 1.03 mmol/L (0.87-1.45), Crea 88.14 mmol/L (70.512-114.58), iPTH 12.94 pmol/L (5.30-34.99), 1,25$(OH)_2$D 135.20 pmol/L (52-163.80), Urine Ca/Crea 0.33 mol/mol (<0.66), TmP/GFR at 0.86 mmol/L (0.89-1.08), TRP of 85% (>90%). The mother, C/II-1, age 32 years was healthy. Her biochemical findings upon presentation were: Ca 2.45 mmol/L (2.13-2.63), P 1.13 mmol/L (0.87-1.45), Crea 70.51 mmol/L (52.88-96.95), iPTH 13.47 pmol/L (5.30-34.99), 1,25$(OH)_2$D 67.60 pmol/L (52-163.80), U-Ca/U-Crea 0.36 mol/mol (<0.66), TmP/GFR 1.08 mmol/L (0.89-1.08), and a TRP of 90% (>90%). His sister, C/III-2, 3 years of age, carried a diagnosis of juvenile rheumatoid arthritis, although this was mild. She had one episode of hematuria but no evidence of renal stones. Her biochemical findings upon presentation were: serum Ca 2.43 mmol/L (2.13-2.63), serum P 1.39 mmol/L (1.39-1.74), Crea 44.07 mmol/L (8.81-35.26), iPTH 11.03 pmol/L (5.30-34.99), 1,25$(OH)_2$ vitamin D of 122.20 pmol/L (52-163.8), urinary Ca/Crea of 0.46 mol/mol (<0.66), a TmP/GFR of 1.42, and a TRP of 92% (>90%). A younger sister (1 yr of age) was reportedly normal. A paternal grandmother had a history of kidney stones. No further information is available.

The index case of **kindred D** (Table 2 B), D/I-1, first presented at age 27 years 6 months to one of us. Her medical history started at 12 months of age when she developed bilateral genua valga and rickets, which required several years of bracing both lower extremities to prevent further valgus deformities. At age 11 she sustained a hairline fracture of the left elbow as well as a greenstick fracture of the right wrist. No laboratory studies are available from until age 16.6 years, when her uCa/Crea was elevated to 1.38 mol/mol (<0.74) and TRP was 88% (<90). At presentation age 27 she reportedly had asymptomatic hypercalciuria, P was reduced to 0.78 mmol/L (0.81-1.45) and 1,25$(OH)_2$ was elevated at 228 pmol/L (35-140). All other biochemical findings were normal: Ca 2.29 mmol/L (2.12-2.54), Crea 51 μmol/L (60-124), 25(OH)D 48.6 nmol/L (40-130), iPTH 0.74 pmol/L (1.06-5.83), ALP 240 (U/L) (50-276). Physical examination at age 27 showed a height of 157.5 cm, a slightly enlarged thyroid gland but was otherwise nor-

mal. There is no family history of rickets or any other bone disease. Follow up biochemical findings at age 41 off treatment with phosphate supplements showed hypophosphataemia 0.73 mmol/L (0.80-1.50) and low 25(OH)D of 72.2 nmol/L (80.0-200.0), hypercalciuria of 9.48 mmol/d (2.50-7.50), uCa/Crea 0.92 mol/mol (<0.45) and TRP 58%, while the remaining biochemical findings were normal: Ca of 2.51 mmol/L (2.10-2.55), iPTH 0.74 pmol/L (1.1-5.81), $25(OH)_2D$ 123 pmol/L (55-190), ALP 56 U/L (30-115). The proband's son, D/II-2, age 16 months, H 78.5 cm (just below 50^{th} percentile), W 9.1 kg (<3^{rd} percentile, but less than 1 standard deviation below) suffers from Poland Syndrome (acquired hypoplasia of the pectoralis muscle) but is otherwise asymptomatic with respect to skeletal development and there is no history of renal stones. His available biochemical findings include Ca 2.42 mmol/L (2.20-2.60), P 1.53 mmol/L (1.39-1.74), a low Crea of 12 µmol/L (20-60), ALP 194 U/L (40-390), a depressed 25(OH)D of 46.2 nmol/L (80-200), $1,25(OH)_2D$ 172 pmol/L (55-190). The proband's daughter, D/II-1 was likewise well until age 9 years and renal or bone malformations are absent, but further studies are unavailable.

The index case of **kindred E** (Table 2 B), E/II-2, presented at age 6 with kidney stones and nephrocalcinosis. At age $13^{1/12}$ years his H: 155 cm, W: 48 kg ($50\text{-}75^{th}$ percentile for weight and $25\text{-}50^{th}$ percentile for height) His u-Ca/Crea ratio was 1.03 mol/mol (<0.74) and $1,25(OH)_2D$ 437 pmol/L (26-156) were elevated, while the remaining biochemical findings included normal Ca 2.33 mmol/L (2.20-2.60), P 1.1 mmol/L (1.29-1.68), Crea 55 umol/L (35.26-61.70), 25(OH)D 119 nmol/L (25-125), iPTH 0.63 pmol/L (1.06-6.89), ALP 966 U/L (150-900), TmP/GFR 1.18 (0.77-1.29), and TRP was 107% (>90). Ultrasound and CT scan of his kidneys confirmed nephrocalcinosis, a 1.5 cm large cyst in the left kidney. On phosphate supplements (300-600 mg 5 times daily) he had no further episodes of kidney stones, and his DEXA-scan, which reportedly showed low bone density initially, but normalized 4 years after start with oral phosphate (Z-score -0.1 for spine and hip). At age 24 he developed gallstone pancreatitis, which resolved after cholecystectomy and papillotomy of the pancreatic duct. The proband's mother, E/I-1, age 53 years, H 174 cm, W 76.9 kg, has a history of kidney stones/nephrocalcinosis, rheumatoid arthritis, Sjögren Syndrome, hyperparathyroidism and thyrotoxicosis and underwent thyro-parathyroidectomy. Her biochemical findings were within normal limits including Ca 2.14 mmol/L (2.20-2.60), P 1.1 mmol/L (0.6-1.5), Crea 74 mmol/L (52.88-96.95), iPTH 1.27 pmol/L (1.06-6.89), ALP 240 U/L (50-276), u-Ca/Crea ratio 0.50 mol/mol (<0.45), 25(OH)D 62 nmol/L (25-125), $1,25(OH)_2D$ 104 pmol/L (26-156), TmP/GFR was 0.9 (0.77-1.29), and TRP was 90% (>90%). The proband's father, E/I-2, age 56 years, H 175 cm, W 80 kg, is healthy, with no renal abnormalities, bone pain, or fractures. Ca 2.22 mmol/L (2.20-2.60), P 0.8 mmol/L (0.6-1.5), Crea 103 U/L (70.51-114.58), iPTH 3.92 pmol/L (1.06-6.89), ALP 210 U/L (50-276), u-Ca/Crea ratio 0.11 mol/mol (<0.45). 25(OH)D 63 nmol/L (25-125), $1,25(OH)_2D$ 146 pmol/L (26-156), TmP/GFR was 0.7 (0.77-1.29), and TRP was 87% (>90%). The proband's sister, E/II-1, H 166.5 cm, W 59.2 kg, has malignant melanoma diagnosed age 24, no history of kidney stones, rickets, bone pain, or fractures. Her biochemical findings were normal Ca 2.58 mmol/L (2.20-2.60), P 1.0 mmol/L (0.6-1.5), Crea 72 mmol/L (52.88-96.95), iPTH 0.45 pmol/L (1.06-6.89), ALP 510 U/L (150-900), u-Ca/Crea 0.67 mol/mol (<0.74). 25(OH)D 99 nmol/L (25-125), $1,25(OH)_2D$ 119 pmol/L (26-156), TmP/GFR was 0.77 (0.77-1.29), and TRP was 77%(>90??%). The proband's paternal uncle has recurrent kidney stones. Also her paternal great-grandfather (deceased at 93 years) had kidney stones.

Case F presented at age 6 with pyelonephritis, subsequent evaluation showed hypercalciuria 6-10 mg/Kg/day, low P 1.11 mmol/L (1.16-1.91), but normal Ca 2.57 mmol/L (2.40-2.65), Crea 33.49 umol/L (17.63-44.07), PTH 1.38 pmol/L (1.60-6.90). Renal ultrasound showed grade 2-3 nephrocalcinosis. Therapy at the time consisted in oral potassium citrate (Shohl'ssolution). At age 6.7 her height was 118.4cm (P43), weight 20.7 kg (P28), at the time laboratory studies showed Crea 44.07 umol/L (17.63-44.07), P 1.23 mmol/L (1.16-1.91), S-Ca 2.6 mmol/l (2.40-2.65), 25(OH)D 24.25 nmol/L (62.4-199.68), 1,25(OH)2-D 174 pmol/L (62.4-223.6), PTH 0.4 pmol/L (1.60-6.90), u-Ca/Crea 1.51-1.56 mol/mol (<0.85), uOxalat/Crea 50mg/g (24-56), uCitrat/Crea 1905mg/g (>180). At this age hydrochlorothiazide 12.5 mg bid was added. Repeat laboratory studies showed iCa 1.26 mmol/L (1.15-1.33), Ca 2.5-2.6 mmol/L (2.40-2.65), P 1.09-1.29 mmol/L (1.16-1.91), u-Ca/Crea 1.11 mol/mol (<0.85), c-terminal FGF23 (cFGF23) 21-28 kRU/l (26-110), 25-OH-D 65 nmol/L (62.4-199.68), 1,25-OH2-D 209 pmol/L(62.4-223.6), PTH 0.74 pmol/L (1.60-6.90). Her mother was found to have hypercalciuria 4.7 mg/kg/d (<4) but no evidence was seen for nephrocalcinosis on ultrasound. Father and sister had no laboratory abnormalities and there was no family history for symptomatic nephrolithiasis. There is, however, a family history of osteoporosis in mother`s mother, mother`s aunt and their mother (mothers grandmother).

Case G presented age 11 with renal colic due to a right urethral-bladder junction stone. Her weight was 30.7kg (P21), height 142.5cm (P58). Subsequent ultrasonographic evaluation showed grade 2-3 nephrocalcinosis, Ca 2.63mmol/l (2.40-2.65), P 1.26 mmol/L (1.16-1.91), Crea 88.14 umol/L (35.26-61.69), suppressed iPTH <0.3 pmol/L (1.60-6.90), 25-OH-D 45 nmol/L (62.4-199.68), elevated 1,25-OH2-D 283 pmol/L (62.4-223.6) and uCa/Crea 0.86 (<0.85). A 24h-urine collection showed a Ca excretion of 3.8 mmol (1-7), uOxalate/Crea 33 mg/g (24-56), uCitrate/Crea 417 mg/g (>180). Treatment consisted of lithotripsy and pig-tail placement. Stone analysis showed calcium-oxalate (95% Weddellit, 5% Whewellit). Subsequently her renal function improved. Repeat laboratory analyses showedCa 2.6 mmol/l (2.40-2.65), P 1.22 mmol/L (1.16-1.91), Crea 70.5 umol/L (35.26-61.69). However, she continued to have elevated uCa/Crea 1.48 (<0.85) and low TmP/GFR = 1.17 mmol/L (1.22-1.61). Since she had low U-citrate 183mg/l, therapy with 8.8 mmol potassium citrate bid was started and laboratory studies three months later showed: Ca 2.6 mmol/l (2.40-2.65), P 1.19 mmol/L (1.16-1.91), Crea 70.5 umol/L (35.26-61.69), ALP 358 U/l (215-476), suppressed iPTH 0.63 pmol/L (1.60-6.90), cFGF23 was 28-33kRU/l (26-110), 25-OH-D 56.75 nmol/L (62.4-199.68), 1,25-OH2-D 153 pmol/L (62.4-223.6), uCa/Crea 1.44 (<0.85), uOxalate/Crea 28 mg/g (24-56), uCitrate/Crea 666 mg/g (>180), TmP/GFR = 1.04 mmol/L (1.22-1.61). Laboratory followup 12 months later showed: Ca 2.5mmol/l (2.40-2.65), P 1.19 mmol/L (1.16-1.91), Crea 79.3 umol/L (35.26-61.69), ALP 307 U/l (178-526), iPTH 1.47 pmol/L (1.60-6.90), 25-OH-D 33.75 nmol/L (62.4-199.68), 1,25-OH2-D 153 pmol/L (62.4-223.6), bone age was 11 ½ years and ultrasound showed unchanged nephrocalcinosis. Her family history was negative for nephrolithiasis. A maternal second degree cousin reportedly had vitamin D deficient rickets.

Nucleotide sequence analysis of the *SLC34A3*/NPT2c gene of kindreds C-E, and case F

Sequence analysis of five additional cases with HHRH or IH revealed previously described mutations in *SLC34A3*/NPT2c (see detailed case descriptions in Supplemental Results and Supplemental Fig. S-1). Three heterozygous sequence variations were found in the proband of kindred C (C/III-1): c.413C>T(p.S138F), c.1304delG, c.1579_81del(p.L527del). The frameshift mutation

c.1304delG was inherited from the father, while c.413C>T(p.S138F) was inherited from the mother. Both parents carry the deletion c.1579_81del(p.L527del); c.1579_81del(p.L527del) and c.413C>T(p.S138F) were previously described in an unrelated kindreds with HHRH by us [1] and c.413C>T(p.S138F) was subsequently annotated as a rare single nucleotide polymorphism (rs141734934, MAF 0.001 in 4352 chromosomes)) in dbSNP [2]. c.1304delG was also found in an unrelated individual [3] and introduces 45 novel amino acids followed by premature termination of the NPT2c protein after residue p.A434 (TPQTGCSAPCRSPSSTSSST-WPASCCGTWCLHCGCPSRWPGTSGW-Stop). Additional genotype information of the index cases C/II-1 includes a novel heterozygous single nucleotide polymorphism c.558G>A(p.Q186Q). Proband D/I-1 was found to carry a homozygous missense mutation, c.1402C>T (p.R468W), which was previously described in a compound heterozygous HHRH case [4]. Proband E/II-2 was found to be homozygous for a previously described mutation c.575C>T (p.S192L) [1, 4] that she inherited from her two parents. Case F was found to carry two known *SLC34A3*/NPT2c mutations: c.575C>T (p.S192L) [1, 4] and c. 1093+41_1094-15del (g.2615_2699del) [5].

Supplemental Figure S-1A: Clinical and biochemical characteristics of kindreds A, B

Supplemental Figure S-1B: Clinical and biochemical characteristics of kindreds C-G.

Laboratory findings in patients with hereditary hypophosphatemic rickets with hypercalciuria (HHRH) or idiopathic hypercalciuria (IH) caused by novel SLC34A3 mutations. All abnormal values are shown in bold. Circles denote females, squares denote men. Black symbols indicate affected individuals, who presented with hypophosphatemia, hypercalciuria, elevated $1,25(OH)_2$ vitamin D levels, and skeletal findings consistent with rickets, while gray symbols indicate affected individuals, who had one or more of the above biochemical abnormalities and nephrocalcinosis. Open symbols indicate healthy individuals. Abbreviations are as follows: ALP, alkaline phosphatase; uCa/Cr, urinary calcium/creatinine ratio; PTH; parathyroid hormone; TmP/GFR, maximum tubular phosphate reabsorption per glomerular filtrate; TRP, tubular phosphate reabsorption; $1,25(OH)_2D$, $1,25(OH)_2$ vitamin D; 25(OH)D, 25(OH) vitamin D. The normal range for TRP is >90% in the setting of hypophosphatemia.

Supplemental Figure S-2: Biochemical and renal phenotype of frequent *SLC34A3*/NPT2c mutations.

Legend: Representation of biochemical and renal phenotype (1=nephrocalcinosis, 2=stones, 3=nephrocalcinosis and stones) of individual mutations, which were observed in two or more individuals affecting one allele (het) or both alleles (hom). See Methods and legends of Fig. 2 and 3 for abbreviations, normalization and RVs of biochemical data and Table S-1 for the data table.

Supplemental Table S-1: *SLC34A3*/NPT2c mutations and nucleotide sequence variants of of the index cases of kindreds A and B, and of case G

Sample	Exon/ Intron	Flanking Sequence	Nomenclature	dbSNP/1000 genome	primers	Kit	AT °C	Band size (bp), detection

A/II-1	Intron 12	ccccctg-gaacccac(g/c)ctcgttcttcttgcc	Hom.c.1336-549G>C	rs28368709				
	Exon 13	TTCTTCAAC-CTGGCC(G/A)GCATC CTGCTGTGGT	Hom.c.1369G>A (p.G457S)	Novel missense mutation	52, 276	Ex.1	55	267, seq.
	Intron 13	GAcgggcagtt-gctg(a/c)gcagaccgccccacc	Hom.c.1800+14A>C	rs28591989				
B/II-2	5'UTR	ctcccgcccc(g/a)tgtctcctcc	Hom.c.1-804G>A	rs9777338				
	Intron 6	tgtgggtg-gaagggctgggc(tggggctgcagtgg-cagccccagcccgggc/del tggggctgcagtgg-cagccccagcccgggc)ccccc cacct	Het.c.560+27_561-38del (g.1440-1469del)	Known intronic deletion [6]	734-735	Ex.1	55	208+179 bp
	Intron 12	ccccctg-gaacccac(g/c)ctcgttcttcttgcc	Hom.c.1336-549G>C	rs28368709				
	Exon 13	ATCCAC-TTCTTC(TTC/ del-TTC)AACCTGGCC	Het.c.1357delTTC (p. F453del)	Novel microdeletion	41-276	Ex.1	55	571, seq.
	Exon 13	TGGCAGGGGG-CATGG(A/T)GCTGGC CGCTGTCGG	Hom.c.1538A>T (p.E513V)	rs28542318				
	3'UTR	Gcagtt-gctg(a/c)gcagaccgcc	Hom.c.1800+14A>C	rs28591989				
Case G		GTGGCTG-GACTGGT(C/delC)ATT GGCGTGCTGGTC	Het.c.367delC	Novel deletion				
		CTTTCAGCGGCT(C/T) GGCGGTGCACGG-GAT	Het. c.575C>T(p.S192L)	Known missense mutation [4].				

Supplemental Table S-2: Review of current and previously published HHRH kindreds.

Legend: Comprehensive table of all the available information collated from published and unpublished cases of HHRH and their families. Conversion into SI units is given where mass units where initially reported. Conversion factors were taken from www.endmemo.com/medical/unitconvert/, with the following exceptions: PTH conversion factor was taken from www.parathyroid-forum.co.uk/, and the TmP-GFR conversion factor was taken from www.mayomedicallaboratories.com/test-catalog/Clinical+and+interpretive/. For Ca/Crea, where the Mayo Clinic RVs (www.mayomedicallaboratories.com/test-catalog/Clinical+and+interpretive/) were used after conversion to SI using the factor 0.25/0.07626 (=3.278) was used to convert these to SI units where the paper presents the biochemical data in mass units. Where age-specific RVs were missing from the papers and for Braithwaite et al. [7], the Mayo Clinic age-adjusted RVs were used. For TmP/GFR, the RVs from the Alon & Hellerstein paper (1994) were used when not provided in the original publication. The daughter of index II-1 was subsequently genotyped [c.145C>T(p.Q49*)], and her biochemical lab values were included in the systematic meta-analysis [8].

Supplemental Table S-3: Clustal-W sequence alignment of novel *SLC34A3* mutations (c.1357delTTC (p.F453del); c.G1369A (p.G457S)).

c.1357delTTC (p.F453del)

c.G1369A (p.G457S)

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ENSP00000442397/1-599     YPLLLGSNIGT-TTTALLAALASPA--DRMLSALQVALIHFFFNLAG-ILLWYLVPALR-
ENSAMEP00000019187/1-641  YPLTLGSNIGT-TTTAILAALASPR--EKLSSSFQIALCHFFFNISGI-LLWYPVPCTR-
ENSACAP00000006271/1-622  YPLTLGSNMGT-TTTAILAALASPG--EKLASAFQIALCHFFFNLSGI-LLWYPLPCSR-
ENSOANP00000015354/1-519  YPLTLGSNIGT-TTTAILAALASPG--DKLASAFQVGRCHFFFNISGI-LLWYPLPCTR-
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ENSCAFP00000024164/1-694  YPLTLGSNIGT-TTTAILAALASPG--NTLRSSLQIALCHFFFNISGI-LLWYPIPFTR-
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ENSPTRP00000027486/1-690  YPLTLGSNIGT-TTTAILAALASPG--NALRSSLQIALCHFFFNISGI-LLWYPIPFTR-
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ENSDORP00000008855/1-593  XXXXXXXXXXX-XXXXXXXXXXXXX--XXXXXXXXVALIHFFFNLAGI-LLWYTVPVLR-
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ENSSHAP00000012725/1-215  ----------------------------------------------------------
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ENSSTOP00000012949/1-602  YPLFLGSNIGT-TTTALLAALASPA--DMLLFAVQVALIHFLFNLAG-ILLWYLVPILR-
ENSCJAP00000051496/1-446  YPLLLGSNIGT-TTTALLAALASPA--DRIFSALQV-----------------------
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ENSCAFP00000024163/1-639  YPLTLGSNIGT-TTTAILAALASPR--EKLSSAVQIALCHFFFNISGI-LLWYPVPCTR-
ENSPSIP00000018384/1-676  YPLTLGSNIGT-TTTAILAALASPG--STLKYSLQIALCHFFFNISGI-ILWYPIPFTR-
```

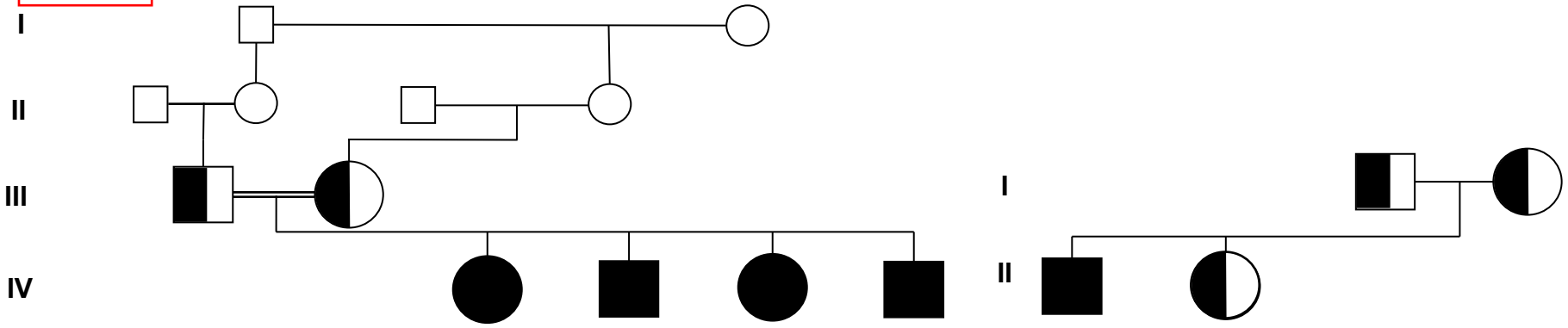
Supplemental Table S-4: List of primers

Primer	Orientation	Location	Sequence
22	Reverse	Intron 12	AGGAGGTCTCAAGGGAGGAGA
32	Forward	Intron 4	GAGGGCCAGCCAGGGACA
33	Reverse	Exon 13	TCCAGAGAATGGAGCCAGAC
46	Forward	Exon 12	CAGGGCTGACCCAGCATC
52	Forward	Intron 12	CATCCACTTCTTCTTCAACCTG
53	Reverse	Exon 13	TCCAGAGAATGGAGCCAGAC
57	Forward	Intron 5	GGGTGTCAGGCTGGCGGC
87	Reverse	Exon 7	AGCATGGTGGCTGCTAAGC
276	Reverse	Exon 13	CGCCGCTGCAGGACAGTAAC
734	Forward	Exon 6	GTGTCAACGTAGGCACATCC
735	Reverse	Exon 7	CAGTTGAAGATCCCGTGCAC

REFERENCES

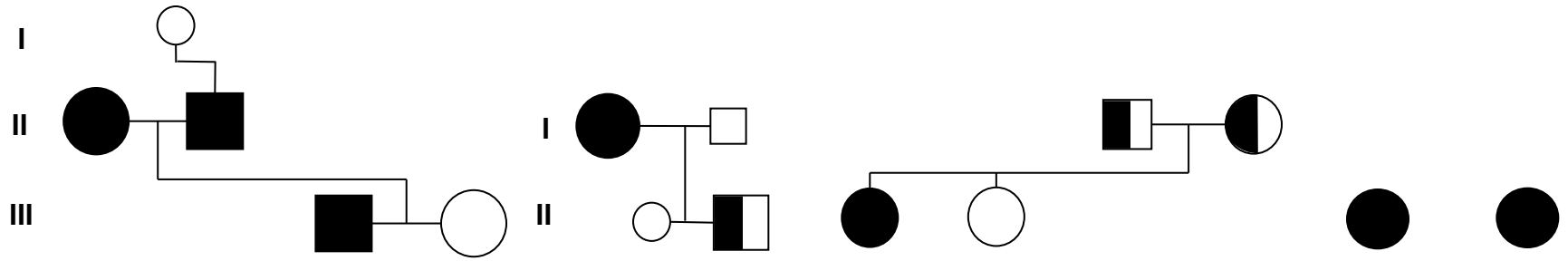
1. Bergwitz, C, Roslin, NM, Tieder, M, Loredo-Osti, JC, Bastepe, M, Abu-Zahra, H, Frappier, D, Burkett, K, Carpenter, TO, Anderson, D, Garabedian, M, Sermet, I, Fujiwara, TM, Morgan, K, Tenenhouse, HS, Juppner, H: SLC34A3 mutations in patients with hereditary hypophosphatemic rickets with hypercalciuria predict a key role for the sodium-phosphate cotransporter NaPi-IIc in maintaining phosphate homeostasis. *Am J Hum Genet,* 78**:** 179-192, 2006.
2. Wheeler, DL, Barrett, T, Benson, DA, Bryant, SH, Canese, K, Chetvernin, V, Church, DM, DiCuccio, M, Edgar, R, Federhen, S, Geer, LY, Helmberg, W, Kapustin, Y, Kenton, DL, Khovayko, O, Lipman, DJ, Madden, TL, Maglott, DR, Ostell, J, Pruitt, KD, Schuler, GD, Schriml, LM, Sequeira, E, Sherry, ST, Sirotkin, K, Souvorov, A, Starchenko, G, Suzek, TO, Tatusov, R, Tatusova, TA, Wagner, L, Yaschenko, E: Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res,* 34**:** D173-180, 2006.
3. Ichikawa, S, Tuchman, S, Padgett, LR, Gray, AK, Baluarte, HJ, Econs, MJ: Intronic deletions in the SLC34A3 gene: A cautionary tale for mutation analysis of hereditary hypophosphatemic rickets with hypercalciuria. *Bone,* in press, 2013.
4. Lorenz-Depiereux, B, Benet-Pages, A, Eckstein, G, Tenenbaum-Rakover, Y, Wagenstaller, J, Tiosano, D, Gershoni-Baruch, R, Albers, N, Lichtner, P, Schnabel, D, Hochberg, Z, Strom, TM: Hereditary Hypophosphatemic Rickets with Hypercalciuria Is Caused by Mutations in the Sodium-Phosphate Cotransporter Gene SLC34A3. *Am J Hum Genet,* 78**:** 193-201, 2006.
5. Ichikawa, S, Sorenson, AH, Imel, EA, Friedman, NE, Gertner, JM, Econs, MJ: Intronic Deletions in the SLC34A3 Gene Cause Hereditary Hypophosphatemic Rickets with Hypercalciuria. *J Clin Endocrinol Metab,* 91**:** 4022-4027, 2006.
6. Yu, Y, Sanderson, SR, Reyes, M, Sharma, A, Dunbar, N, Srivastava, T, Juppner, H, Bergwitz, C: Novel NaPi-IIc mutations causing HHRH and idiopathic hypercalciuria in several unrelated families: Long-term follow-up in one kindred. *Bone,* 50**:** 1100-1106, 2012.
7. Braithwaite, V, Pettifor, JM, Prentice, A: Novel SLC34A3 mutation causing hereditary hypophosphataemic rickets with hypercalciuria in a Gambian family. *Bone,* 53**:** 216-220, 2013.
8. Page, K, Bergwitz, C, Jaureguiberry, G, Harinarayan, CV, Insogna, K: A patient with hypophosphatemia, a femoral fracture, and recurrent kidney stones: report of a novel mutation in SLC34A3. *Endocr Pract,* 14**:** 869-874, 2008.

Fig. S-1A



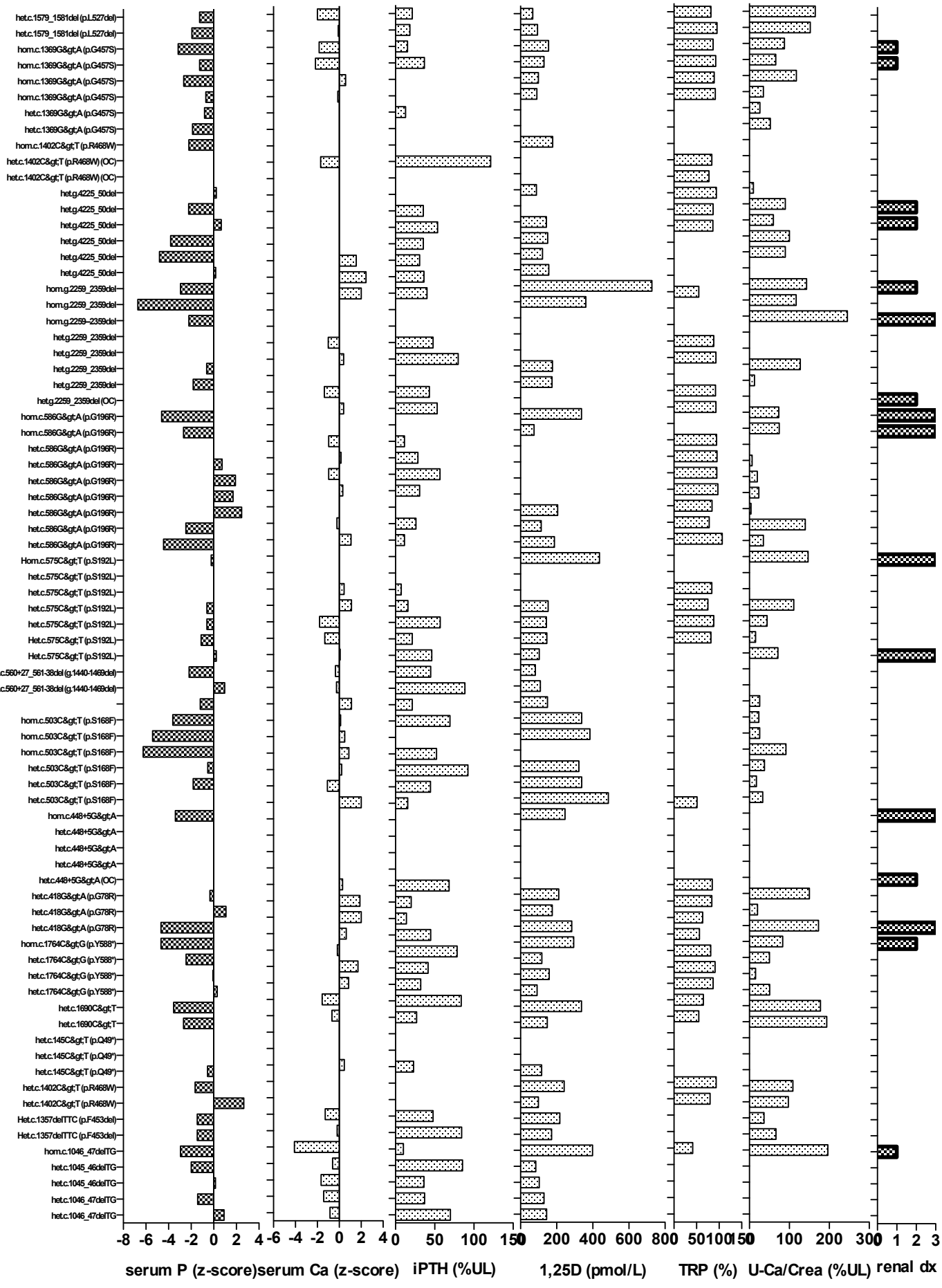
Kindred	A						B			
Individual **age**	A/III-1 34 y, 9 m	A/III-2 29 y, 2 m	A/IV-1 (index) 9 y, 1 m	A/IV-2 7 y	A/IV-3 2 y, 4 m	A/IV-4 1 y	B/II-2 (index) 13 y	B/II-1 15 y	B/I-1 adult	B/I-2 adult
ion. Ca (mmol/L)			1.28 (1.10-1.35)	1.28 (1.10-1.35)	1.31 (1.10-1.35)	**0.93** (1.10-1.35)				ND ND
serum calcium (mmol/L)	2.39 (2.10-2.55)	2.31 (2.10-2.55)	2.40 (2.40-2.65)	2.52 (2.40-2.65)	2.41 (2.40-2.65)	**2.39** (2.40-2.65)	2.40 (2.40-2.65)	2.42 (2.38-2.63)	2.35 (2.10-2.65)	2.34 (2.10-2.65)
serum phosphate (mmol/L)	1.02 (0.84-1.45)	0.86 (0.84-1.45)	**0.77** (1.00-1.80)	1.15 (1.00-1.80)	**0.87** (1.00-1.80)	1.26 (1.00-1.80)	**0.90** (1.20-1.74)	1.27 (1.20-1.74)	0.95 (0.80-1.90)	1.02 (0.80-1.90)
serum iPTH (pmol/L)			**1.48** (1.59-6.89)	**1.27** (1.59-6.89)	**1.06** (1.59-6.89)	2.55 (1.59-6.89)	1.70 (1.59-6.89)	3.30 (1.59-6.89)	5.90 (1.00-7.00)	6.20 (1.00-7.00)
serum 25(OH)D (nmol/L)			**55.00** (62.40-199.68)	77.00 (62.40-199.68)	**40.00** (62.40-199.68)	**19.00** (62.40-199.68)	71.00 (51.00-250.00)	78.00 (51.00-250.00)	73.00 (51.00-250.00)	51.00 (51.00-250.00)
serum 1,25(OH)$_2$D (pmol/L)			155.00 (48.00-160.00)	130.00 (48.00-160.00)	99.00 (48.00-160.00)	90.00 (48.00-160.00)	**419.00** (60.00-158.00)	**218.00** (60.00-158.00)	**172.00** (60.00-158.00)	149.00 (60.00-158.00)
serum creatinine (µmol/L)	92.00 (59.00-104.00)	59.00 (45.00-84.00)	39.00 (33.00-68.00)	44.00 (33.00-56.00)	24.00 (20.0-42.00)	31.00 (20.00-65.00)	60.00 (30.50-61.01)	**75.00** (38.13-68.63)	90.00 (61.01-99.14)	75.00 (61.01-99.14)
U-Ca/Crea (mol/mol)	0.19 (<0.62)	0.38 (<0.62)	**0.86** (<0.85)	0.65 (<0.85)	**1.35** (<1.00)	0.52 (<1.27)	0.71 (<0.73)	0.31 (<0.73)	0.30 (<0.45)	0.12 (<0.45)
TmP/GFR (mmol/L)							0.98 (0.77-1.29)			
TRP(%)	**89.00** (>90.00)	91.40 (>90.00)	**82.00** (>90.00)	95.30 (>90.00)	**87.00** (>90.00)	92.50 (>90.00)	**84.00** (>90.00)	**85.00** (>90.00)	**77.00** (>90.00)	89.00 (>90.00)
serum ALP (U/L)	71.00 (40.00-130.00)	72.00 (35.00-105.00)	**725.00** (86.00-300.00)	**342.00** (69.00-300.00)	**492.00** (104.00-300.00)	**320.00** (104.00-300.00)	**699.00** (182.00-587.00)	157.00 (138.00-511.00)	71.00 (50.00-350.00)	68.00 (50.00-350.00)
bone involvement	N	N	**Y**	N	**Y**	N	**Y**	N	N	N
renal involvement	N	N	**Y**	**Y**	N	N	N	N	N	N
c.1369G>A (p.G457S)	+			+	+	+		+		
c.560+27_561-38del		+	+		+	+	+		+	+
c.1357delTTC (p. F453del)							+	+	+	

Fig. S-1B



Kindred	C				D		E				Case F	Case G
Individual	C/II-1	C/II-2	C/III-1 (index)	C/III-2	D/I-1(index)	D/II-1	E/II-2 (index)	E/II-1	E/I-1	E/I-2	F/II-1	G/II-1
age	adult	adult	4 y, 11 m	3 y	27 y, 7 m	12 m	13 y	15 y	45 y	42 y	5 y, 9 m	10 y, 3 m
ion. Ca (mmol/L)							1.27 (1.15-1.33)					
Ca (mmol/L)	2.45 (2.13-2.63)	2.38 (2.13-2.63)	2.40 (2.13-2.63)	2.43 (2.13-2.63)		2.42 (2.20-2.60)	2.33 (2.2-2.6)	2.58 (2.2-2.6)	2.22 (2.2-2.6)	2.14 (2.2-2.6)	2.57 (2.4-2.65)	2.6 (2.4-2.65)
P (mmol/L)	1.13 (0.87-1.45)	1.03 (0.87-1.45)	**1.00** (1.39-1.74)	1.39 (1.39-1.74)	**0.775** (0.81-1.45)	1.59 (1.39-1.74)	**1.1** (1.29-1.68)	**1** (1.13-1.58)	**0.8** (0.81-1.45)	1.1 (0.81-1.45)	**1.11** (1.20-1.74)	1.26 (1.20-1.74)
PTH (pmol/L)	13.47 (5.30-34.99)	12.94 (5.30-34.99)		11.03 (5.30-34.99)	**0.74** (1.06-5.83)		**0.64** (1.06-6.89)	**0.46** (1.06-6.89)	3.92 (1.06-6.89)	1.27 (1.06-6.89)	**1.38** (1.59-6.89)	**0.32** (1.59-6.89)
25(OH)D (nmol/L)			84.86 (24.96-169.73)		48.6 (40-130)	70 (80-120)	119 (25-125)	99 (25-125)	63 (25-125)	62 (25-125)	**24.21** (62.4-199.68)	**44.928** (62.4-199.68)
1,25(OH)$_2$D (pmol/L)	67.60 (52.00-163.80)	135.20 (52.00-163.80)	**343.20** (52.00-163.80)	122.20 (52.00-163.80)	**178** (35-140)	172 (55-190)	**436.8** (26.00-156.00)	119.6 (26.00-156.00)	145.6 (26.00-156.00)	104 (26.00-156.00)	174.2 (62.4-223.6)	**283.4** (62.4-223.6)
Crea (µmol/L)					51 (60-124)	**12** (20-60)	**55** (30.50-53.38)	**72** (30.50-53.38)	**103** (45.76-83.89)	74 (61.01-99.14)	28.979 (15.25-38.13)	**76.26** (30.50-53.38)
Ca/Crea (mol/mol)	0.31 (<0.57)	0.28 (<0.57)	**1.64** (<1.00)	0.39 (<1.00)	**0.92** (<0.45)		**1.03** (<0.74)	0.67 (<0.74)	0.11 (<0.45)	**0.5** (<0.45)	**1.56** (<0.85)	**2.75** (<0.85)
TmP/GFR (mmol/L)	1.08 (0.89-1.08)	**0.86** (0.89-1.08)	0.74 ND	1.42 ND			1.18 (0.77-1.29)	0.77 (0.77-1.29)	**0.7** (0.77-1.29)	0.9 (0.77-1.29)		**1.08** (1.17-1.54)
TRP(%)	90.00 (>90.00)	**85.00** (>90.00)	**76.00** (>90.00)	92.00 (>90.00)	**88** (>90.00)		107 (>90.00)	77 (>90.00)	88 (>90.00)	82 (>90.00)		92 (>90.00)
ALP (U/L)					74 (39-117)	223 (40-390)	966 (150-900)	510 (150-900)	210 (50-276)	240 (50-276)		358 (215-476)
bone involvement	N	N	**Y**	N	**Y**	N	N	N	N	N	N	N
renal involvement	N	**Y**	**Y**	N	N	N	**Y**	N	N	**Y**	**Y**	**Y**
c.413C>T	+		+									
c.1304delG			+									
c.1579_81del		+		+			+	+				
c.575C>T				+					+		+	
g.2615_2699del										+		+
c.367delC											+	
c.1402C>T					+	+						+

Fig. S-2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het.c.1579_1581del (p.L527del)
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hom.c.1369G>A (p.G457S)
hom.c.1369G>A (p.G457S)
hom.c.1369G>A (p.G457S)
het.c.1369G>A (p.G457S)
het.c.1369G>A (p.G457S)
hom.c.1402C>T (p.R468W)
het.c.1402C>T (p.R468W) (OC)
het.c.1402C>T (p.R468W) (OC)
het.g.4225_50del
het.g.4225_50del
het.g.4225_50del
het.g.4225_50del
het.g.4225_50del
het.g.4225_50del
hom.g.2259_2359del
hom.g.2259_2359del
hom.g.2259_2359del
het.g.2259_2359del
het.g.2259_2359del
het.g.2259_2359del
het.g.2259_2359del
het.g.2259_2359del (OC)
hom.c.586G>A (p.G196R)
hom.c.586G>A (p.G196R)
het.c.586G>A (p.G196R)
het.c.586G>A (p.G196R)
het.c.586G>A (p.G196R)
het.c.586G>A (p.G196R)
het.c.586G>A (p.G196R)
het.c.586G>A (p.G196R)
het.c.586G>A (p.G196R)
Hom.c.575C>T (p.S192L)
het.c.575C>T (p.S192L)
het.c.575C>T (p.S192L)
het.c.575C>T (p.S192L)
het.c.575C>T (p.S192L)
Het.c.575C>T (p.S192L)
Het.c.575C>T (p.S192L)
het.c.560+27_561-38del (g.1440-1469del)
het.c.560+27_561-38del (g.1440-1469del)
hom.c.503C>T (p.S168F)
hom.c.503C>T (p.S168F)
hom.c.503C>T (p.S168F)
het.c.503C>T (p.S168F)
het.c.503C>T (p.S168F)
het.c.503C>T (p.S168F)
hom.c.448+5G>A
het.c.448+5G>A
het.c.448+5G>A
het.c.448+5G>A
het.c.448+5G>A (OC)
het.c.418G>A (p.G78R)
het.c.418G>A (p.G78R)
het.c.418G>A (p.G78R)
hom.c.1764C>G (p.Y588*)
het.c.1764C>G (p.Y588*)
het.c.1764C>G (p.Y588*)
het.c.1764C>G (p.Y588*)
het.c.1690C>T
het.c.1690C>T
het.c.145C>T (p.Q49*)
het.c.145C>T (p.Q49*)
het.c.145C>T (p.Q49*)
het.c.1402C>T (p.R468W)
het.c.1402C>T (p.R468W)
Het.c.1357delTTC (p.F453del)
Het.c.1357delTTC (p.F453del)
hom.c.1046_47delTG
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het.c.1045_46delTG
het.c.1046_47delTG
het.c.1046_47delTG
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serum P (z-score)
-6 -4 -2 0 2 4
serum Ca (z-score)
0 50 100 150
iPTH (%UL)
0 200 400 600 800
1,25D (pmol/L)
50 100 150
TRP (%)
0 100 200 300
U-Ca/Crea (%UL)
0 1 2 3
renal dx