SUPPLEMENTARY DATA

SUPPLEMENTARY METHODS

Patient-derived cells

Epstein-Barr virus transformed lymphoblastoid (EBVL) cell lines were generated from patient and control whole blood by the European Collection of Cell Cultures (Porton Down, UK) and maintained in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine, 100U/L penicillin and 100µg/mL streptomycin (all from Sigma-Aldrich). Dermal fibroblasts from patients and controls were cultured in Dulbecco's modified eagle medium (DMEM) containing 25mM glucose supplemented with 10% FBS, 2mM L-glutamine, 100U/l penicillin and 100µg/ml streptomycin (FBS-DMEM).

Delivery of shRNAs to 3T3-L1 preadipocytes

Freshly-seeded BOSC-HEK293 cells were transfected with 5µg plasmid (containing shRNAs) using 5µl polyethylenimine/µg DNA in 500µl OptiMEM serum-free medium (Invitrogen). After 24 hours, retrovirus-containing medium from the BOSC cells was applied to preconfluent 3T3-L1 preadipocytes with 4µl/ml polybrene (Millipore, Billerica, MA). Fresh medium was applied to BOSC cells and infection was repeated after 24 hours. After a further 24 hours, selection for shRNA-infected cells commenced by treatment with 4µg/ml puromycin.

SUPPLEMENTARY DATA

SUPPLEMENTARY RESULTS

Available anthropometric data and non endocrine clinical features are summarised in **Supplementary Table 4**. Biochemical and genetic data are provided in **Table 4**.

Summary of Endocrine Histories and Additional Molecular/Cellular Studies

Patient 1 (Patient 23 in Rauch *et al* (1)), on examination at 28 years, had characteristic features of MOPDII (Supplementary Table 4). As previously reported (1) compound heterozygous nonsense mutations in exon 28 (c.59992C>T, p.Q1998X) and exon 42 (c.9316C>T, p.Q3106X) were identified in *PCNT* (Supplementary Figure 2A). Quantitative real time PCR of cultured EBVLs revealed approximately 60 per cent reduction of *PCNT* mRNA expression compared to a panel of controls, that included healthy subjects, a patient with a proven insulin receptor mutation, and four patients with severe insulin resistance (IR), reduced adult height but no *PCNT* defects ("OPD-SIR") (Supplementary Figure 2B). Moreover, western blot analysis of cultured dermal fibroblasts revealed markedly reduced pericentrin expression in Patient 1 compared to a healthy control (Supplementary Figure 2C), a finding confirmed by immunofluorescence microscopy, which showed punctuate, juxtanuclear pericentrin in dermal fibroblasts from controls but not from Patient 1 (Supplementary Figure 2D). Patient fibroblasts also had a higher incidence of morphological abnormalities, consistent with previous reports (not shown) (1; 2). Despite proposed roles for pericentrin in primary cilia formation (3-5), we found no evidence of primary cilia defects in patient cells stained with acetylated tubulin (Supplementary Figure 3). This is consistent with the lack of typical ciliopathic features in MOPDII.

Patient 2 (Patient 20 in Rauch *et al* (1)) was examined in detail at 10.7 years, revealing characteristic features of MOPDII (Supplementary Table 4). As previously reported, sequencing of the *PCNT* gene revealed a homozygous nonsense mutation in exon 30 (T2128fsX2129, Supplementary Figure 4A) (1). Surprisingly, although the proband's mother was heterozygous for this mutation, her father was wild type. Microsatellite studies ruled out exogamy. High density SNP and copy number analysis using Affymetrix 6.0 arrays showed a region of loss of heterozygosity at the end of the long arm of chromosome 21 spanning 0.6 megabases and encompassing the *PCNT* gene in the proband, without evidence of copy number variation at the *PCNT* locus (Supplementary Figure 4B,C). Similar analysis of her father was normal. Collectively these studies suggest that the proband was homozygous for the maternally inherited truncating mutation in *PCNT* due to segmental maternal uniparental disomy mutation at the end of chromosome 21.

Patient 3 (previously unreported) had a clinical diagnosis of MOPDII with homozygous *PCNT* mutations (Table 4 and Supplementary Table 4). She showed neither clinical nor biochemical evidence of IR (Table 4).

Patients 4 & 12 (from Family 1 in Griffith *et al* (2)) are siblings from Saudi Arabia. **Patient 12** is a 13 year old girl originally diagnosed clinically with Seckel syndrome (Supplementary Table 4), and previously reported to have homozygous mutations in *PCNT* (2). Testing at 12 years old revealed fasting hyperglycemia of 153mg/dL, rising to 400mg/dL after a glucose challenge. At 13 years old fasting hyperinsulinemia of over 600pmol/L was accompanied by severe dyslipidemia (Table 4). Acanthosis nigricans was noted. Her 3 year old brother (**Patient 4**), although manifesting a similar degree of growth retardation and dysmorphic features, had neither clinical nor biochemical evidence of IR (Table 4).

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Patient 5 (from Family 3 in Griffith *et al* (2) is a 3 year old Moroccan boy diagnosed clinically with Seckel syndrome, and previously reported to have homozygous mutations in *PCNT* (2). At three years old fasting insulin was elevated at 161pmol/L without attendant hyperglycemia or dyslipidemia. No clinical evidence of IR was reported.

Patient 6 (previously unreported) is a 6 year old girl of Chilean origin with primordial dwarfism and characteristic dysmorphism of MOPDII (supplementary table 4). Compound heterozygous mutations in *PCNT* have been confirmed (Table 4). She had normal glucose tolerance, fasting and postprandial insulin levels, and lipid profile at 3 years old (Table 4).

Patient 7 (Patient 16 in Rauch *et al* (1)) had a clinical diagnosis of MOPDII with homozygous *PCNT* mutations. He showed no clinical evidence of IR at 4 years old, and his glucose tolerance was normal. His fasting insulin was elevated at 165pmol/l, however. Biochemical data at 4.5 years are shown in Table 4.

Patients 8 & 9 (previously unreported) are siblings of Palestinian descent whose parents are first cousins. **Patient 8** is an 8 year-old boy born at 29 weeks *via* Caesarean section. A clinical diagnosis of MOPDII was made at 1 year old, at the same time as his sister, with biallelic *PCNT* mutations confirmed later (Table 4). Growth hormone therapy was commenced at 1 year old but withdrawn after a left hemisphere stroke occurred at 3 years old. Truncal obesity and nuchal acanthosis nigricans were first documented at 7 years. On examination at 8.75 years there was central obesity, acanthosis nigricans and residual right hemiparesis. The patient was prepubertal. Treatment consisted of twice daily metformin (850mg). Fasting triglycerides was grossly elevated and HDL cholesterol low. All biochemical results at 8 years are shown in Table 4.

Patient 9 is the 10 year-old sister of patient 9, born at 32 weeks *via* Caesarean section. At 1 year growth hormone response to glucagon stimulation was normal. At 3 years a clinical diagnosis of MOPDII was made with documentation of classical features of MOPDII (Supplementary Table 4). Growth hormone therapy was used from 4 years old for 1 year. Truncal obesity and acanthosis nigricans around the neck were first documented at 8 years old. At 9 years a diagnosis of diabetes was made, with haemoglobin A1c (HbA1c) of 12%. Treatment with insulin and metformin was commenced but glucose levels remained over 270mg/dL, regardless of insulin dose. Insulin was subsequently stopped and metformin therapy continued. On examination at 10 years there was central obesity and evidence of early pubertal development (Tanner A0, P2, B3). Treatment consisted of twice daily metformin (250mg). Metabolic testing at 10 years showed elevated triglyceride and low HDL cholesterol. HbA1c was 7.8% but glucose remained over 270mg/dL. Further fasting biochemical results at 10 years are shown in Table 4.

Patient 10 (patient 18 in Rauch *et al* (1)) had a clinical diagnosis of MOPDII with homozygous *PCNT* mutations. She was commenced empirically on growth hormone therapy at 2.5 years due to severe growth retardation. At 5 years old fasting blood glucose was 85mg/dL and insulin 40.6pmol/l. At 5.5 years old fasting glucose was 106mg/dL, with a postprandial glucose of 173mg/dL. Growth hormone (GH) therapy was stopped, and after 1 month glycemia had normalised. GH was recommenced, however 3 months later symptomatic hyperglycemia developed and diabetes was diagnosed based on a random glucose of 339mg/dL and HbA1c 10.2%. Prominent nuchal acanthosis nigricans was noted. Subcutaneous insulin therapy was commenced at 1.2 IU/kg/day by multiple daily injection. At 6.3 years old HbA1c was 7.7%, total cholesterol 170mg/dL, triglyceride 79mg/dL and HDL cholesterol 89mg/dL. Continuous subcutaneous infusion of insulin was started at 6.6 years, and in the following 4 years HbA1c was maintained between 7 and 8.5%. At 10.5 years, on a total daily insulin dose of 1.8 IU/kg,

the HbA1c was 8.4%. Nuchal acanthosis nigricans persisted. Further results at 10.5 years are shown in Table 4.

Patient 11 is a 12 year-old female who was diagnosed neonatally with MOPD II (Supplementary Table 4). Between the ages of 11 and 16 months, she was treated with GH without any improvement in growth velocity. *PCNT* gene sequencing revealed compound heterozygous mutations. Truncal obesity was observed as early as 7 years old, while thelarche was noted at around 9 years old, and menarche was at age 10. At 10.8 years of age, patches of nuchal acanthosis nigricans were documented. At 12 years of age impaired fasting glucose and gross hyperinsulinemia were determined (Table 4), and diabetes was diagnosed on oral glucose tolerance testing, although HbA1c remained within the reference range.

Patient 12: see **Patient 4** above.

Patient 13 (Patient 6 in Rauch *et al* (1)) is a 14 year-old boy with clinical features of MOPDII as well as a homozygous genetic defect in *PCNT*, as previously reported (Supplementary Table 4) (1). He was noted to have a centripetal pattern of adiposity and diffuse acanthosis nigricans. Biochemical testing at 14 years old revealed severe hyperinsulinemia, impaired fasting glucose, and metabolic dyslipidemia (Table 4). HbA1c was 6.1%.

Patient 14 (Hall *et al* (6)) was diagnosed with MOPDII at 1 year old (Supplementary Table 4) and compound heterozygous mutations in *PCNT* were subsequently identified (Table 4). Provocative testing at 1 year old suggested GH deficiency and human GH therapy was initiated, lasting approximately 6 years, but with no impact on growth velocity. At 10 years there was evidence of puberty, as well as a macular hyperpigmentation over the trunk and extremities. In addition, several papular, reddish-brown wart-like lesions were noted bilaterally in the axillae, histologically confirmed as acanthotic epidermal hyperplasia. On examination at 14 years old there was truncal obesity and biochemical investigations revealed euglycemia with hyperinsulinemia (Table 4).

Patient 15 is a 14 year old female who was diagnosed with MOPDII at the age of 2 years, whereupon human GH therapy was begun, though no significant increases in growth velocity were noted. Her karyotype was normal, and *PCNT* mutations were later confirmed (Table 4). Thelarche was noted at around 8 years old, and although the precise time of onset of truncal obesity and acanthosis nigricans cannot be obtained from her records, both have been noted. At 14.5 years she is euglycemic but severely hyperinsulinemic.

Patient 16 (previously unreported) was referred for endocrinological evaluation at 8.9 years with severe growth retardation, first noted at 4 months' gestation. She was unresponsive to GH therapy (0.3 mg/kg/week) and treatment was stopped after two years. At 17.2 years she underwent further metabolic evaluation after acanthosis nigricans and a centripetal fat distribution were noted. There was fasting hyperglycemia, hyperinsulinemia and dyslipidemia (Table 4), and the HbA1c was 6%. Diabetes was diagnosed and metformin therapy commenced. Compliance was poor and the metabolic profile worsened (HbA1c 9%). There was no significant improvement with addition of rosiglitazone and at 21 years insulin therapy was commenced (2 units/kg/day in total). Diabetes was accompanied by hypertension renal insufficiency (blood urea nitrogen 34.2mg/dL, creatinine 1.52mg/dL). Myocardial infarction and multiple episodes of cerebral ischemia have contributed to debilitating cognitive and functional deficits. Neuroimaging revealed diffuse old and recent cerebral ischemic lesions, occlusion of the left medial cerebral artery and a supra-clinoidal arteropathy of the internal carotid arteries.

SUPPLEMENTARY DATA

Patient 17 (Patient 8 in Rauch et al (1)) is an 18 year-old woman with clinical features of MOPDII as well as a homozygous genetic defect in *PCNT*, as previously reported (Supplementary Table 4) (1). She had mild centripetal adiposity but no other clinical evidence of metabolic disease. Fasting glucose was normal and fasting insulin modestly elevated. Fasting lipids were all within the normal range (Table 4).

Patients 18 and **19** are Europid siblings of unrelated parents. **Patient 18** (patient 2 in Bober *et al* (7)) is currently 18 years old, and was diagnosed with MOPDII *in utero* due to growth retardation and MOPDII in his older sister. He was later confirmed to have compound heterozygous *PCNT* mutations (Table 4). Pubarche was reached at 14 years of age; it is believed that truncal obesity began at this time as well, though no precise date of onset can be determined. During his last clinical visit, at age 16, the patient was noted to have patches of acanthosis nigricans on his neck. He was never treated with GH. He had fasting hypoglycemia with elevated insulin levels (Table 4).

Patient 19 (Hall *et al* (6)), the sister of patient 17, is now 20 years old. She was diagnosed with MOPD II at 18 months, and was later found also to have compound heterozygous *PCNT* mutations. Menarche was at 16 years of age, at which time truncal obesity was noted. At present she has acanthosis nigricans on her neck. She was never treated with human GH, and is euglycemic with elevated insulin levels (Table 4).

Patients 20 and 21 (previously unreported) are siblings of Italian descent with a clinical diagnosis of MOPDII and homozygous *PCNT* mutations (supplementary table 4). **Patient 20** underwent surgical correction of cryptorchidism at 16 years old. Repeated urinary infections resulted in renal insufficiency. At 22 years old diabetes mellitus was diagnosed and treated with insulin, with poor subsequent glycemic control. Total cholesterol and triglyceride levels were elevated. Progressive renal dysfunction led to commencement of renal dialysis at 25 years old. On evaluation at 27 years, severe acanthosis nigricans was present on the neck, upper limbs, and in the submammary region. Biochemical results at 26 years are shown in Table 4. Death occurred at 28 years following intestinal bleeding.

Patient 21 was delivered at term, when IUGR was noted. Precocious puberty occurred at 7 years, with subsequent oligomenorrhoea. At 26 years old she suffered a stroke, and MRI revealed multiple aneurysms and haemorrhages in the frontal and temporal regions. Characteristic facial dysmorphism and skeletal abnormalities lead to a clinical diagnosis of MOPDII at 28 years old. In addition, acanthosis nigricans was noted on the neck and limb flexures, with relatively thick skin over the dorsum of the hands and hypertrichosis of the face and limbs. *PCNT* gene analysis revealed a previously described homozygous frameshift mutation (Table 4). Biochemical assessment at 28 years old revealed fasting hyperglycemia, hyperinsulinemia and hypertriglyceridemia (Table 4). Alanine aminotransferase (ALT) and gamma glutamyltransferase (γGT) were also elevated (66 U/L and 130 U/L, respectively), suggestive of non-alcoholic fatty liver disease.

SUPPLEMENTARY REFERENCES

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SUPPLEMENTARY DATA

Supplementary Table 1. Oligonucleotide sequences used for quantitative real-time PCR.

Gene	Sequence
Mouse genes	
AdipoQ	5'-CAGTGGATCTGACGACACCAA-3' (F)
	5'-TGGGCAGGATTAAGAGGAACA-3' (R)
	5'-[6FAM]GGGCTCCAGGATGCTACTGTTGCAAGC[TAMRA]-3' (Probe)
Fabp4	5'-CCTTTGTGGGAACCTGGAAGC-3' (F)
	5'-GTGGTCGACTTTCCATCCCAC-3' (R)
	5'-[6FAM]TGAAGAGCATCATAACCCTAGATGGCGG[TAMRA]-3' (Probe)
Glut4	5'-ACTCATTCTTGGACGGTTCCTC-3' (F)
	5'-CACCCCGAAGATGAGTGGG-3' (R)
	5'-[6FAM]TGGCGCCTACTCAGGGCTAACATCA[TAMRA]-3' (Probe)
Insr	5'-CAATGGGACCACTGTATGCATTCT-3' (F)
	5'-GTCCGGCACGTACACGAAGA-3' (R)
	5'-[6FAM]TGAGTACCTCAGTGCCAGTGATGTGTTTCC[TAMRA]-3' (Probe)
Pcnt	GCCACCAGATCCACTTGATCTT (F)
	GGCTTTACTGAGTTTCACCTGACA (R)
	AGCTTGGGCAGCCGAAGGTCCA (Probe)
Pparγ2	5'-GATGCACTGCCTATGAGCACTT-3' (F)
	5'-AGAGGTCCACAGAGCTGATTCC-3' (R)
	5'-[6FAM]AGAGATGCCATTCTGGCCCAC[TAMRA]-3' (Probe)
Ppia	5'-TTCCTCCTTTCACAGAATTATTCCA-3' (F)
	5'-CCCGCCAGTGCCATTATGG-3' (R)
	5'-[6FAM]ATTCATGTGCCAGGGTGGTGACTTTACAC[TAMRA]-3' (Probe)
Human genes	
36B4	5'-GCAGATCCGCATGTCCCTT-3' (F)
	5'-TGTTTTCCAGGTGCCCTCG-3' (R)
	5'-[6FAM]AGGCTGTGGTGCTGATG[TAMRA]-3' (Probe)
PCNT	GACAGATGCTGCGTAGAGACTTCA (F)
	GTTGCACCTTGTGCTCAGTTT (R)
	AGCCAAGAGCTTCCCTGGGTGCATCT (Probe)
	Hs00195774_m1 (Applied Biosystems)
PPIA	5'-ACGGCGAGCCCTTGG-3' (F)
	5'-TTTCTGCTGTCTTTGGGACCT-3' (R)
	5'-[6FAM] CGCGTCTCCTTTGAGCTGTTTGCA[TAMRA]-3' (Probe)

F, forward primer; R, reverse primer. All oligonucleotides were synthesised by Sigma-Aldrich.

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Supplementary Table 2. Antibodies used for western blotting and immunofluorescence.

Target Protein	Species	Company	Product Number
Primary antibodies			
Pericentrin	Rabbit	Abcam, Cambridge, UK	ab4888
Pericentrin	Mouse	BD Biosciences, San Jose, CA	611814
INSR	Rabbit	Santa Cruz Biotechnology, Santa Cruz, CA	sc711
pINSR	Rabbit	Invitrogen, Carlsbad, CA	44894G
AKT	Rabbit	Cell Signaling Technology, Beverly, MA	9272
pAKT	Rabbit	Cell Signaling Technology, Beverly, MA	9271S
ERK1/2	Rabbit	Abcam, Cambridge, UK	ab7942
pERK1/2	Rabbit	Cell Signaling Technology, Beverly, MA	9106S
Calnexin	Rabbit	Abcam, Cambridge, UK	ab13504
p85	Rabbit	Gift from Professor K Siddle	
Secondary antibodies			
Rabbit IgG (H+L) HRP-linked	Donkey	Pierce	31458
Rabbit IgG Alexa594 (HCA)	Goat	Invitrogen, Carlsbad, CA	A11037

SUPPLEMENTARY DATA

Supplementary Table 3. Metabolic evaluation of the extended family of Patient 1

Study ID	Relation to Proband	*PCNT* Genotype	Sex	Age (y)	Height (m)	Height SDS	Weight (kg)	BMI (kg/m^2)	BMI SDS	Fasting glucose (mg/dL)	Fasting insulin (pmol/L)	Triglycerides (mg/dL)	HDL (mg/dL)
III.9	Proband	p.Q1998X/ p.Q3106X	M	29	1.11	-9.8	28.7	23.3	N/A	72.0	1990	486	11.2
II.8	Father	p.Q3106X het	M	58	1.64	-1.9	68.3	25.4	1.4	93.7	62	NK	NK
II.9	Mother	p.Q1998X het	F	54	1.6	-0.6	66.6	26	1.4	77.5	26	NK	NK
III.10	Sister	p.Q1998X het	F	26	1.63	-0.14	65.5	24.7	0.76	82.9	24	123	52.9
III.11	Sister	NK	F	NK	NK	NK	NK	NK	NK	NK	NK	NK	NK
III.13	Brother	p.Q3106X het	M	18	1.76	-0.33	74.1	23.9	0.27	91.9	1	79	51.7
II.1	Paternal Uncle	Wild type	M	73	1.73	-0.77	94.1	31.4	2.21	95.5	30	106	66.4
II.2	Paternal Aunt	Wild type	F	72	1.54	-1.63	52.6	22.2	-0.06	86.5	6	61	86.5
III.1	Paternal Cousin	Wild type	F	43	1.66	0.36	59.6	21.6	-0.29	88.3	1	106	50.2
III.2	Paternal Cousin	Wild type	F	38	1.64	0.36	70.0	26.0	1.12	NK	NK	NK	NK
II.3	Paternal Aunt	Wild type	F	68	1.54	-1.63	65.0	27.4	1.46	90.1	18	79	68.3
II.4	Paternal Aunt	p.Q3106X het	F	62	1.57	-1.13	74.5	30.2	2.05	93.7	44	168	67.6
III.3	Paternal Cousin	p.Q3106X het	M	35	1.75	-0.47	82.8	27.0	1.22	102.7	4	176	59.1
III.4	Paternal Cousin	p.Q3106X het	M	33	1.72	-0.91	89.5	30.3	2.00	100.9	7	203	73.4
III.5	Paternal Cousin	p.Q3106X het	M	30	1.83	0.70	89.5	26.7	1.14	79.3	3	132	55.6
III.6	Paternal Cousin	p.Q3106X het	M	21	1.76	-0.33	81.4	26.3	1.03	93.7	2	115	49.8
II.7	Paternal Aunt	Wild type	F	60	1.63	-0.14	76.8	28.9	1.80	97.3	35	150	74.1
III.7	Paternal Cousin	Wild type	F	35	NK	NK	NK	NK	NK	NK	NK	NK	NK
III.8	Paternal Cousin	Wild type	F	30	NK	NK	NK	NK	NK	NK	NK	NK	NK
II.11	Maternal Uncle	p.Q1998X het	M	50	1.65	-1.94	71.8	26.4	1.06	90.1	3	186	51.0
III.14	Maternal Cousin	p.Q1998X het	M	20	1.69	-1.19	59.1	20.7	-0.37	75.7	0	53	40.9
III.15	Maternal Cousin	Wild type	F	16	1.59	-0.73	50.0	19.8	-0.34	NK	NK	NK	NK
III.16	Maternal Cousin	p.Q1998X het	F	16	1.55	-1.31	52.3	21.8	0.53	NK	NK	NK	NK
III.17	Maternal Cousin	Wild type	F	10	1.30	-1.28	28.2	16.7	0.18	NK	NK	NK	NK
II.12	Maternal Aunt	Wild type	F	48	1.54	-1.63	73.8	31.1	2.27	86.5	29	115	58.3
III.18	Maternal Cousin	Wild type	M	15	1.75	0.90	56.8	18.5	-0.31	NK	NK	NK	NK
III.19	Maternal Cousin	Wild type	M	12	1.52	0.18	37.2	16.1	-0.87	NK	NK	NK	NK
Fasting reference range										*68-100*	*<60*	*<177*	*>39*

SUPPLEMENTARY DATA

Supplementary Table 4. Clinical features of MOPDII in 21 patients with proven genetic defects in *PCNT*

Patient Number	Sex	Gestation (weeks)	Birth Weight /kg (SD)	Age	Height /cm (SDS)	Weight /kg (SDS)	OFC /cm (SDS)	Dysmorphism?	Skeletal or dental dysplasia?	Cerebro-vascular abnormality?	Other features	Reference
3	F	32	0.89 (-3.2)	1.6	52 (-9.52)	3.3 (-11.57)	33.5 (-11.9)	Yes	Yes	NK		Previously unreported
4*	M	33	1.1 (-3.0)	3	61 (-9.6)	5.4 (-10.2)	41 (-5.8)	Yes	No	Yes	Myopia, learning disability	Family 1, Griffith *et al* (2008)
5	M	35	0.87 (-5.5)	3	66 (-8.1)	5.3 (-10.6)	47 (-5.8)	Yes	No	NK	Learning disability	Family 4, Griffith *et al* (2008)
6	F	36	1.03 (-4.5)	2.75	61.5 (-8.9)	4.4 (-12.5)	36.8 (-11.0)	Yes	No	NK		Previously unreported
7	M	34	1.08 (-3.0)	4.5	60.2 (-10.5)	5.7 (-12.7)	NK	Yes	Yes	NK	Mild to moderate developmental delay	P16, Rauch *et al* (2008)
8†	M	29	0.96 (-1.3)	9	103 (-5.0)	21.3 (-1.8)	42 (-7.7)	Yes	Yes	NK		Previously unreported
9†	F	32	1.1 (-2.4)	10	105 (-5.3)	20.3 (-3.0)	42 (-9.3)	Yes	Yes	Yes		Previously unreported
10	F	40	1.55 (-4.6)	10.5	84.8 (-7.83)	8.6 (-3.61)	43 (-7.46)	Yes	Yes	No		P18, Rauch *et al* (2008)
11	F	37	1.4 (-3.8)	11	94.7 (-7.2)	16 (-5.5)	43.3 (-8.4)	Yes	Yes	Yes	Learning disability	Hall *et al* (2004)
2	F	36	1.28 (-3.3)	12	114 (-4.2)	25.3 (-1.9)	46.5 (-5.0)	Yes	Yes	NK	Epilepsy	P20, Rauch *et al* (2008)
12*	F	33	0.94 (-3.5)	13	96.5 (-8.5)	17.5 (-6.2)	43.5 (-8.4)	Yes	Yes	NK	5th finger clinodactyly	Family 1, Griffith *et al* (2008)
13	M	38	1.14 (-4.8)	12	84 (-8.5)	NK	42.8 (-7.0)	Yes	Yes	Yes	Severe developmental delay	P6, Rauch *et al* (2008)
14	F	35	1.049 (-4.0)	12	99 (-9.2)	13.5 (-9.8)	42.4 (-9.3)	Yes	Yes	Yes	Learning disability	Hall *et al* (2004)
15	F	36	1.33 (-3.5)	14	98 (-9.4)	20 (-6.5)	42 (-9.6)	Yes	Yes	Yes	Learning disability	Hall *et al* (2004)
16	F	34	1.82 (-1.0)	21	116 (-7.9)	NK	48.5 (-5.1)	Yes	Yes	Yes		Previously unreported

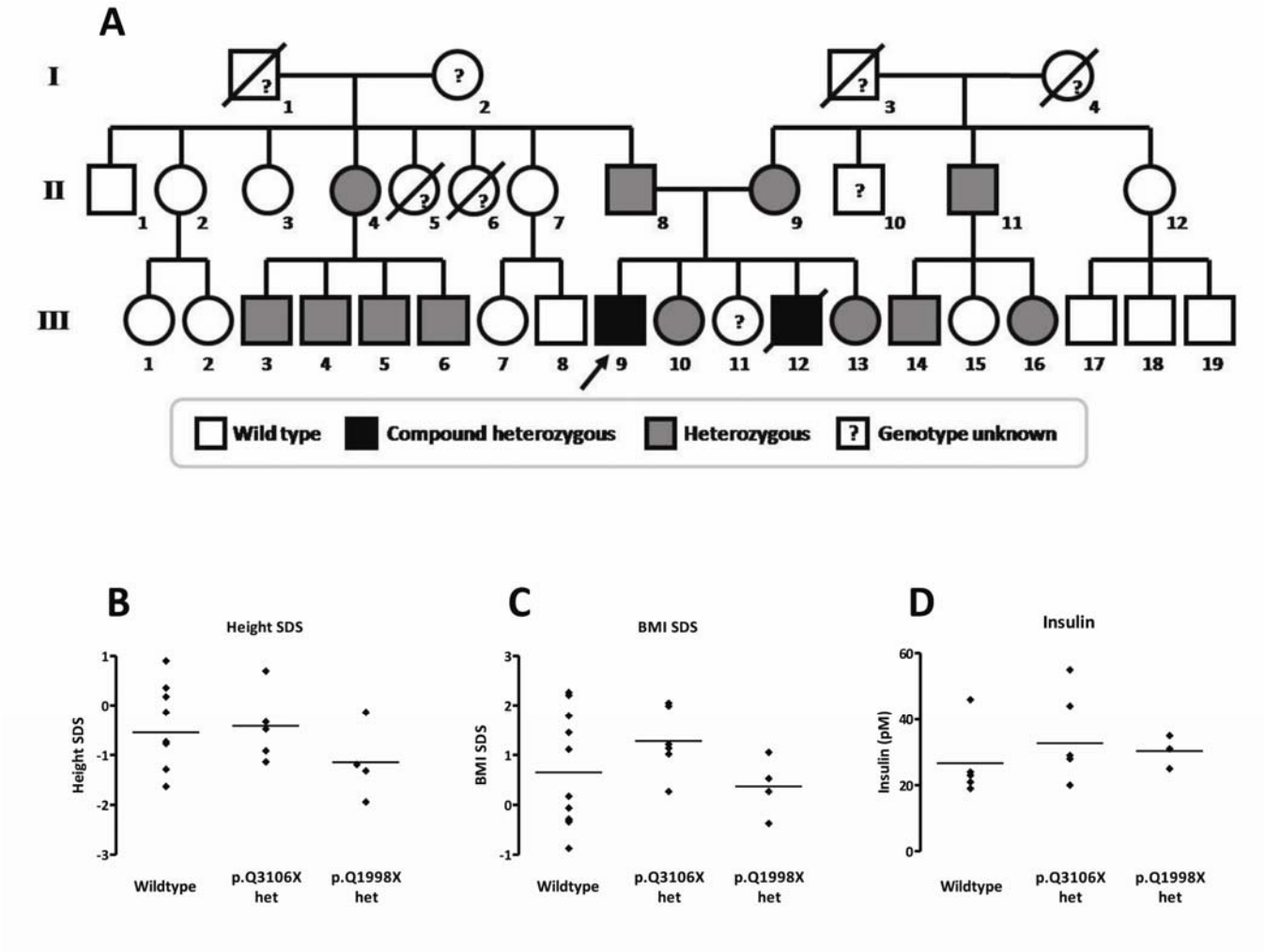
17	F	35	1.3 (-3.0)	18	88 (-12.5)	12.1 (-16.9)	39.5 (-11.5)	Yes	Yes	Yes	Mild to moderate developmental delay	P8, Rauch *et al* (2008)
18‡	M	34	1.02 (-3.6)	17	97.3 (-10.9)	14.5 (-14.9)	42.5 (-8.5)	Yes	Yes	Yes	Learning disability	Hall *et al* (2004)
19‡	F	33	0.794 (-4.0)	19	69 (-15.7)	8.5 (-25.2)	39.5 (-11.5)	Yes	Yes	Yes	Learning disability	Hall *et al* (2004)
20§	M	40	1.7 (-4.1)	27	123 (-8.1)	30 (-9.9)	48.5 (-5.1)	Yes	Yes	Yes		Previously unreported
21§	F	40	1.4 (-5.0)	28	116 (-7.9)	35 (-4.2)	48 (-5.4)	Yes	Yes	Yes		Previously unreported
1	M	38	1.9 (-3.2)	29	111 (-9.8)	28.7 (-10.6)	49.5 (-4.5)	Yes	Yes	No	5th finger clinodactyly	P23, Rauch *et al* (2008)

Abbreviations: NK, not known; OFC, occipitofrontal circumference; SDS, standard deviation score. *, †, ‡ and § indicate sibling pairs.

SUPPLEMENTARY DATA

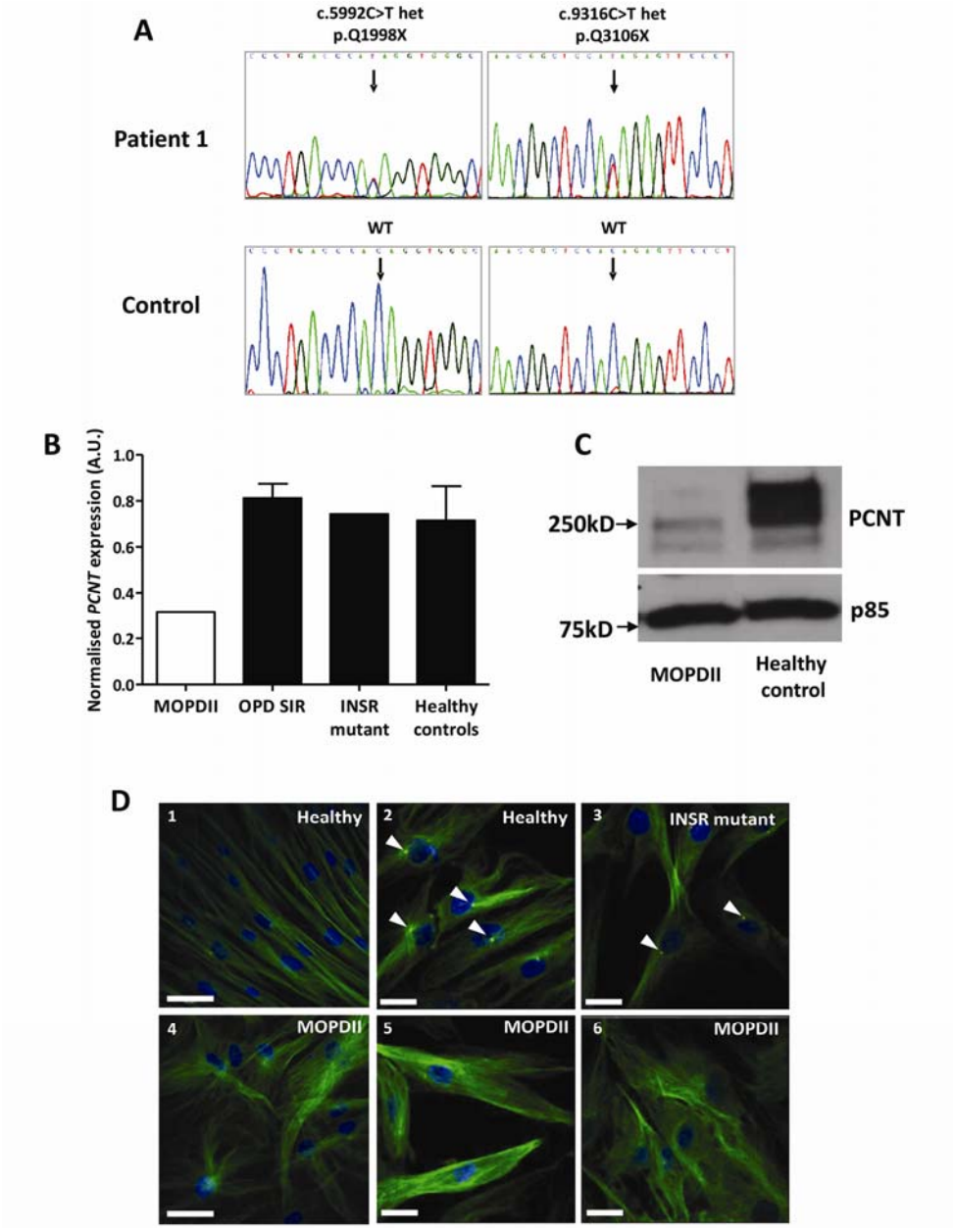
Supplementary Figure 1. Clinical and biochemical investigation of the extended family of Patient 1.

A: Extended pedigree showing *PCNT* genotypes (where confirmed by genetic testing Patient 1 (proband) indicated (arrow). *B:* Individual height standard deviation scores (SDS), body mass index SDS and fasting insulin levels in family members, grouped by genotype. Horizontal lines denote mean values. No significant differences were identified between genotype groups in any of three parameters.



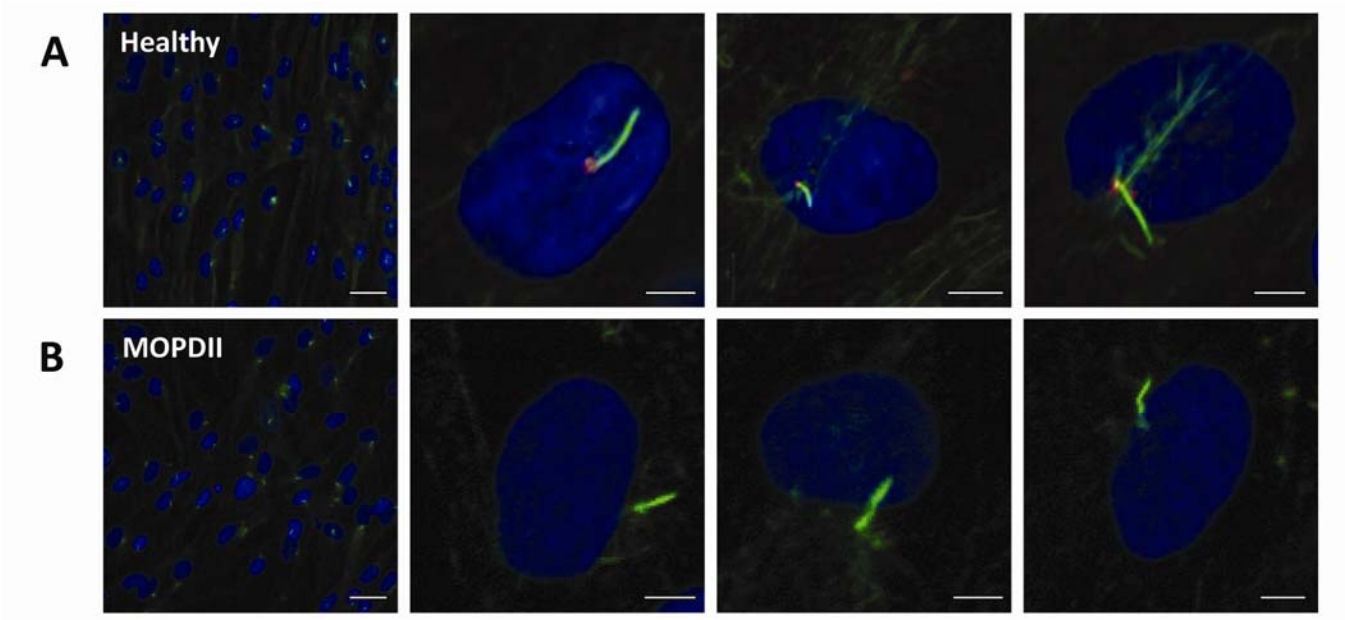
SUPPLEMENTARY DATA

Supplementary Figure 2. Reduced *PCNT* expression in Patient 1.
A: Direct sequencing of *PCNT* in Patient 1 and a healthy control. *B:* Quantitative real-time PCR analysis of *PCNT* mRNA expression in cultured EBV-transformed lymphoblastoid cells (EBVLs) derived from patient 1 (MOPDII, n=1, white bar), healthy subjects (n=3) and patients with either primordial dwarfism, IR and wild type *PCNT* (OPD-SIR, n=4) or severe IR resulting from insulin receptor mutations (INSR mutant, n=1). *C:* Western blot analysis of pericentrin expression in cultured dermal fibroblasts from Patient 1 and a healthy control. *D:* Coimmunostaining of cultured dermal fibroblasts from Patient 1 (MOPDII), a healthy control (Healthy) and a patient with an insulin receptor defect (INSR mutant) for pericentrin (red) and α-tubulin (green). Nuclear material stained with 4',6'-diamidino-2-phenylindole (DAPI, blue). Arrows indicate colocalisation of pericentrin and α-tubulin at the centrosome. Scale bars, 50μm (1 & 4) and 20μm (2, 3, 5 & 6).



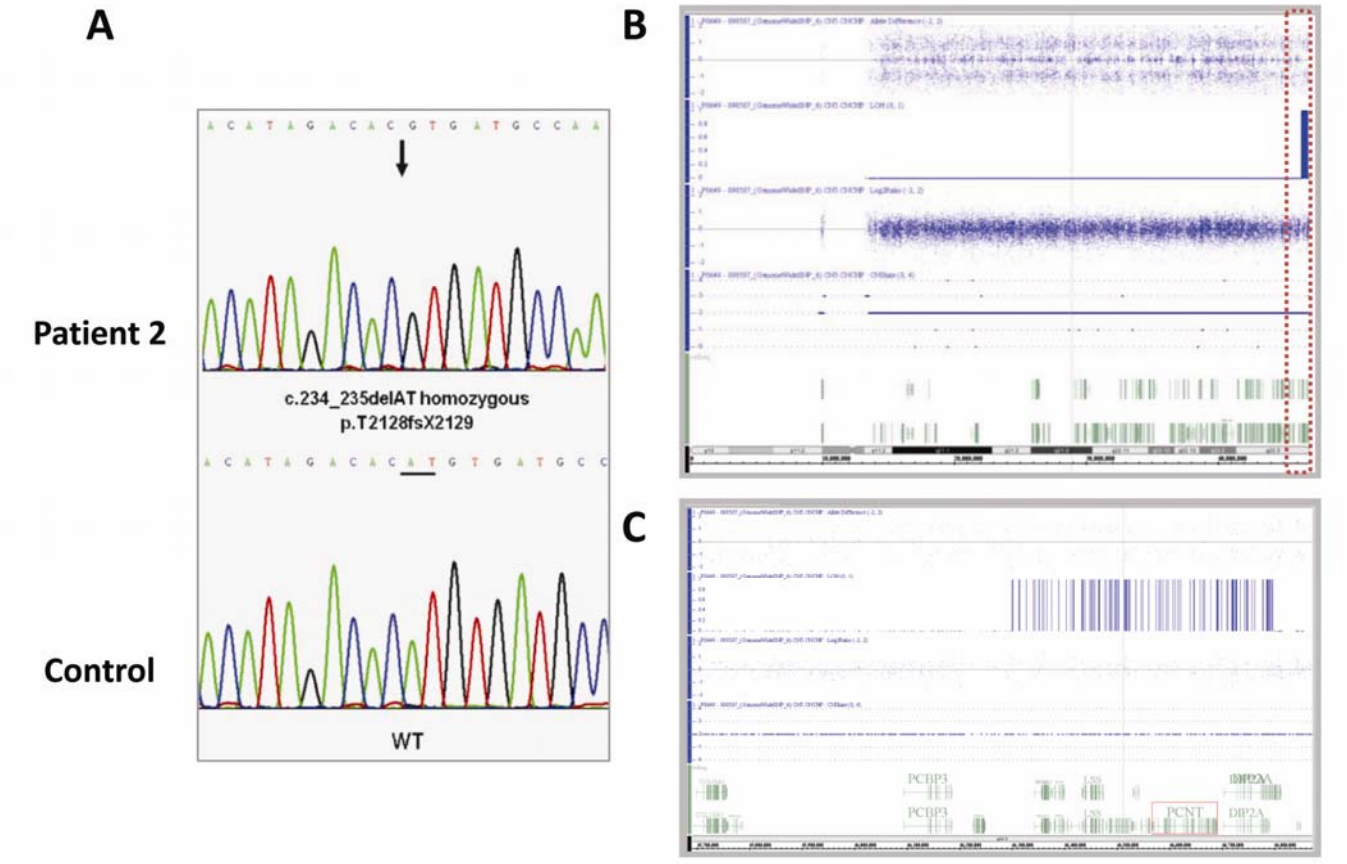
SUPPLEMENTARY DATA

Supplementary Figure 3. Normal primary cilia formation associated with genetic defects in *PCNT*. Cultured dermal fibroblasts from a healthy subject (*A*) and a patient with MODPII and a proven *PCNT* defect (*B*) coimmunostained for acetylated tubulin (green) and pericentrin (red). Nuclear material stained with DAPI (blue). Scale bars, 5μm (far left, 50μm).



SUPPLEMENTARY DATA

Supplementary Figure 4. Genetic analysis of *PCNT* in Patient 2.
A: Direct sequencing of the *PCNT* gene in Patient 2 and a healthy control. *B:* High density single nucleotide polymosphism (SNP) and copy number analysis in Patient 2 using Affymetrix 6.0 arrays showing a region of loss of heterozygosity at the end of the long arm of chromosome 21 encompassing *PCNT*. *C:* Region of homozygosity in detail.



SUPPLEMENTARY DATA

Supplementary Figure 5. Timecourse of adipocyte gene induction during differentiation of Pcnt-knockdown preadipocytes.

Transcript expression of mature adipocytes genes assessed by quantitative real-time PCR following hormonal induction of adipocyte differentiation. Black bars, luciferase knockdown; white/hatched bars, *Pcnt* knockdown. All data represent mean ± SEM (n=4).