Insulin resistance uncoupled from dyslipidemia due to C-terminal PIK3R1 mutations

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Supplemental Appendix

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

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Patient 2

Patient 2 (P2) is currently 14.5 years old. She was born at term to non-consanguineous parents of European ancestry. Pregnancy was complicated by an amniotic fluid leak and at 30 weeks of gestation intra-uterine growth restriction was noted. Feeding difficulties during infancy were attributed to gastro-oesophageal reflux. Weight remained at or below the 3rd percentile from infancy. Head circumference followed the 10th percentile and length was between the 3rd and 10th percentile. She had a left duplex kidney and recurrent urinary tract infections secondary to grade 4 urinary reflux, eventually requiring ureteric re-implantation surgery at 5 years. There was no history of nephrocalcinosis.

At two years old, dysmorphic features including a triangular face, wide mouth, thin upper lip, an “unusual” nose with prominent veins on the scalp, face and trunk were noted. The ears were prominent, posteriorly-placed and slightly low-set. There was reduced subcutaneous fat on the head, trunk and over the buttocks, but normal fat coverage on the legs and arms.

At six years old, facial dysmorphism was again noted. Investigation at this age revealed normal lipid and immunoglobulin profiles. At 10.5 years onset of puberty was noted with breast bud development.

At 13.5 years, the age at which the detailed biochemical evaluation is reported, she had narrowed facial features, deep set eyes, prognathism, a pointed chin and nose and over-crowded teeth in the upper and lower jaw. There was widespread acanthosis nigricans, most prominent in the nuchal and periumbilical regions and behind the ears. She was very lean with muscular arms and legs, and very little subcutaneous fat over the face and around her hands. Nails were concave. A deepening voice, hirsutism, and an enlarging laryngeal prominence were noted. Breast development had regressed, and primary amenorrhoea was reported. Pelvic ultrasonography showed numerous small, circumferential ovarian follicles. At 13.8 years, bone age was 15 years. Metformin was started.

No visual problems nor refractive error were reported, however ophthalmological assessment revealed Axenfeld-Rieger anomaly with iris hypoplasia, iridogoniodygenesis, and posterior embryotoxon (Supplemental Figure 5). On gonioscopy of the angle, iris strands associated with redundant peri-umbilical skin were detected. Ocular pressures and nerves were normal.

Her mother was fit and well. Her father was unavailable for assessment but was said to be 160 cm in height, to be of slight build and to have “fine features”, raising the unconfirmed possibility of autosomal dominant inheritance.

Patient 3

Patient 3 (P3) is currently 13 years old, is the second daughter of unrelated Italian parents, and has two healthy siblings. She was born at term after fetal growth was found to be subnormal in the last 2 months of gestation. Birth weight was 2200 g (-2.2 SDS). Facial dysmorphism was noted at
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birth, including ocular depression, a prominent nose and generalized paucity of adipose tissue. Subsequent psychomotor development was normal. Investigation including brain MRI, LMNA gene sequencing and fasting plasma insulin were said to be normal in infancy.

On endocrine evaluation at age 12 years, height was 144.1 (-1.1 SDS) and weight 28.7 kg (BMI 13.8). Despite well advanced puberty (Tanner stage 4), menarche had not occurred. On physical examination, generalized reduction of adipose tissue, a triangular face with small chin, thin upper lip, and prominent ears were noted. Skin was pale and dry with prominent veins. Severe acanthosis nigricans was present. She did not show joint hyperextensibility.

Patient 4

Patient 4 (P4) is a girl currently aged 14 years old, born at term to unrelated German parents. Intrauterine growth retardation had been observed ultrasonographically during the pregnancy. At birth facial dysmorphism was noted, and she subsequently exhibited poor linear growth, persisting between the 3rd and 10th centiles for height, however development was otherwise normal. At 8 years old she developed a squint and myopia was diagnosed, with raised intraocular pressure later detected at 11 years old. At 13.5 years old glycosuria was detected during an episode of abdominal pain. Further metabolic investigation at that age showed impaired glucose tolerance with extreme hyperinsulinemia during oral glucose tolerance testing.

At 14 years old menarche had not yet occurred although puberty was well established (Tanner A2 B2 P4). There was generalised paucity of subcutaneous adipose tissue, and facial dysmorphism featuring a triangular face with a prominent forehead, deep set eyes, large and low set ears, a narrow nose and dimpled chin. There was no acanthosis nigricans.

Patient 5

Patient 5 (P5) is currently 9 years old. He is the first son of unrelated Bulgarian parents, and was born following an uncomplicated pregnancy at term. He was admitted to the neonatal unit due to small size and poor feeding. Development progressed normally apart from mild speech and language delay, and delay in tooth eruption.

On examination he had fine facial features with prominent eyes and the appearance of megalocornea and ocular depression, although formal ophthalmology assessment was normal. He had a narrow nasal bridge, prominent columella, low set and prominent ears, wide mouth and extensive dental caries of deciduous teeth. He had normal, thick hair. He had generalized lipoatrophy with prominent veins particularly affecting his face and trunk and giving an ‘aged appearance’. His hands had wrinkled skin. There was no joint hypermobility. He also had a normal renal ultrasound examination and echocardiogram.
## SUPPLEMENTAL TABLES

| Gene   | Mutation | Chr | Position (GRCh37) | ref | alt |
|--------|----------|-----|-------------------|-----|-----|
| PIK3R1 | Y657X    | 5   | 67592155          | T   | A   |
| ABCB5  | S240F    | 7   | 20685419          | C   | T   |
| MYH10  | R690X    | 17  | 8424463           | G   | A   |

### Supplemental Table 1. De novo mutations found in Patient P1:

Chromosome positions are relative to NCBI build 37 and dbSNP identifiers to version 137. ref refers to the NCBI reference sequence allele, alt refers to the alternate allele. Variants were absent from the 1000 Genomes (release 2012-07-19, 1,092 genomes) – EUR (European ancestry), AMR (from the Americas), AFR (West African ancestry), ASI (East Asian ancestry); NHLBI Exome Variant Server (release ESP6500SI-V2, Apr-2013, 6,250 exomes) – EA (European American ancestry), AA (African American ancestry); 3,781 Genomes from the UK10K Cohorts group (UK10K Cohorts, release 2012-06-02) - all of European ancestry; 409 CoLaus Exomes (ODEX, release Dec-2010) – all of European ancestry and dbSNP (build 137).
Supplemental Table 2. Rare sequence variants identified in **PIK3R1**, **PIK3R2** and **PIK3CA** in 262 severely insulin resistant probands: Heterozygous, functional (non-synonymous) single nucleotide variants in **PIK3R1** (encoding p85α, p55α and p50α), **PIK3R2** (encoding p85β) and **PIK3CA** (encoding p110α) identified in 262 severely insulin resistant patients, including **PIK3R1** p.Arg649Trp (R649W, underlined). Where available, allele frequencies were obtained from the Exome Aggregation Consortium (ExAC) (1). Predicted effects of variants on protein function were determined using three online algorithms: SIFT Sequence (Database: UniRef90 2011 Apr, Median conservation of sequences 3.00), Polyphen-2 (v2.2.2) and PhD-SNP on Uniprot ID P27986 (algorithm-specific scores in parentheses) (2-4). Note that the R951L variant in **PIK3CA** did not co-segregate with insulin resistance in the family. The R409Q variant in **PIK3R1** has been previously reported in association with severe human insulin resistance (5).
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| Feature                              | P1 Y657X | P2 R649W | P3 R649W | P4 R649W | P5 R649W |
|--------------------------------------|----------|----------|----------|----------|----------|
| IUGR                                 | Yes      | Yes      | Yes      | Yes      | Yes      |
| Short Stature                        | Yes      | Yes      | Yes      | Yes      | Yes      |
| Hyperextensibility of Joints         | No       | Yes      | No       | No       | No       |
| Ocular Depression                    | Yes      | Yes      | Yes      | Yes      | Yes      |
| Rieger Anomaly (other ophthalmologic problems) | No       | Yes (Supp. Figure 6) | No | No (myopia from 8 years; raised intraocular pressure from 11 years) | No |
| Teething Delay                       | Not noted | No       | Not noted | Not noted | Yes      |
| Facial Dysmorphism                   | Yes      | Yes      | Yes      | Yes      | Yes      |
| Adipose Tissue                       | Normal   | Reduced  | Reduced  | Yes      | Not known |
| History of recurrent infections      | No       | No       | No       | No       | No       |
| IgG (NR 5.4-16.1 g/L)                | 13.7     | 9.2      | 7.7      | 8.0      | 6.6      |
| IgM (NR 0.5-1.9 g/L)                 | 1.1      | 1.4      | 1.1      | 0.5      | 1.0      |
| IgA (NR 0.8-2.8 g/L)                 | 1.6      | 2.1      | 1.4      | 1.2      | 1.3      |

Supplemental Table 3. Summary of clinical features of 5 probands with PIK3R1 mutations. NR = Normal Range.
**Gene_exon | F/R primer | Oligonucleotide sequence**

| PIK3R1_exon2 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R1_exon3 | F | TGTAABACGCGCGAGATCTCCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R1_exon4 | F | TGTAABACGCGCGGCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R1_exon5 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R1_exon6 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R1_exon7 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R1_exon8 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R1_exon9 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R1_exon10 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R1_exon11 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R1_exon12_13 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R1_exon13_14 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R1_exon15 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R1_exon16 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R2_exon2 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R2_exon3 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R2_exon4_5 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R2_exon6 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R2_exon7_8 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R2_exon9 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R2_exon10 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R2_exon11 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R2_exon12 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R2_exon13 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R2_exon14 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R2_exon15 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| PIK3R2_exon16 | F | TGTAABACGCGCGCCAGTTCTTTGTCCTGCTGTGGAAC |
| R | AGGAAACAGCTATGACCATAGCAGTACATCTCCCTGCAAG |
| Gene_exon   | F/R primer | Oligonucleotide sequence                           |
|------------|------------|--------------------------------------------------|
| PIK3CA_exon2A F | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon2B R | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon3 F | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon4 R | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon5 F | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon6 R | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon7 F | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon8 R | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon9 F | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon10 R | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon11 F | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon12 R | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon13 F | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon14 R | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon15 F | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon16 R | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon17 F | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon18 R | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon19 F | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon20 R | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |
| PIK3CA_exon21 F | TGTAAAACGACGGCCAGTTCTCAAGTCAAACTATGGAAGGAGGAGGAGAG | AGGAAAACAGCTATGACCATTTTCACAGTTGCTGAGATAAGGAGGAGGAGAG |

Supplemental Table 4. Primer sequences for Sanger sequencing of PI3K subunits.
Abbreviations: F, forward primer; R, reverse primer.
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| Gene  | Experiment | F/R primer | Oligonucleotide sequence | Length, bps | $T_m$, °C | G/C content, % |
|-------|------------|------------|--------------------------|-------------|-----------|---------------|
| PIK3R1 | Gene amplification | F | ATGAGTGCTGAGGGGTAC | 18 | 58.1 | 55.6 |
|       |            | R | TCATCGCCCTGCTGTT | 16 | 59.7 | 56.3 |
| PIK3R1 | Y657X SDM (for stable over-expression) | F | GTAAACAGGGCTGCTAAGCCTGCTCTGTAGTG | 32 | 82.2 | 53.1 |
|       |            | R | CACTACAGAGCAGGCTTAGCAGCCCTGTTTAC | 32 | 82.2 | 53.1 |
| PIK3R1 | Y657X SDM (for stable over-expression) | F | TACAGACAGGGCTAGCAGCCCTGTTTACTGC | 32 | 77.7 | 56.3 |
|       |            | R | GCAGTAAACAGGGCTGCTAGCAGCCCTGCTCTGTA | 32 | 82.2 | 53.1 |
| PIK3R1 | R649W SDM | F | CTGTATCTGCTCCACAGACAAGAAAAGTGCCAT | 35 | 76.5 | 45.7 |
|       |            | R | ATGGCACTTTTTCTTGCTGGGAGGAGCACAAGAACAG | 35 | 76.5 | 45.7 |
| ADIPOQ | D231A | F | GCTGATAATGACAATGCTCCACCTCACAGGC | 33 | 82.2 | 51.5 |
|       |            | R | GCCTGTAAAGGTTGGAGGCATTGCTATTACAGC | 33 | 82.2 | 51.5 |

Supplemental Table 5. Primer sequences for site-directed mutagenesis: Oligonucleotide primers for site-directed mutagenesis were designed according to the criteria listed in the Quikchange site-directed mutagenesis kit (Stratagene) and obtained from Sigma-Aldrich. Abbreviations: F, forward primer; R, reverse primer; bps, base pairs; $T_m$, melting temperature; G/C, guanine/cytosine; SDM, site-directed mutagenesis.
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| Gene Name | Species  | Catalogue Number | Sequence                  |
|-----------|----------|------------------|---------------------------|
| PIK3R1    | Human    | Hs00236128_m1    | N/A                       |
| PIK3R2    | Human    | Hs00178181_m1    | N/A                       |
| PIK3R3    | Human    | Hs01103591_m1    | N/A                       |
| 36B4      | Human    | N/A              | Forward: GCAGATCCGCATGTCCCTT
Reverse: TGTGTTCCAGGTGCCCTCG
Probe: [6FAM]AAGCTGTGGTGCTGATGG[TAMRA] |

Supplemental Table 6. PCR primers and probes used for quantitative real-time PCR: Pre-made primer/probe stocks were obtained from Applied Biosystems (catalogue numbers indicated). Human 36B4 was used as a housekeeping gene; individual forward/reverse primer and Taqman probe sequences are listed (generated by Sigma-Aldrich).
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| Target Protein       | Species | Application | Company                                           | Product ID | Dilution factor |
|----------------------|---------|-------------|---------------------------------------------------|------------|-----------------|
| AKT                  | Rabbit  | WB          | Cell Signalling Technology, Beverly, MA           | 9272       | 1000            |
| Calnexin             | Rabbit  | WB          | Abcam, Cambridge, UK                             | ab13504    | 1000            |
| ERK1/2               | Rabbit  | WB          | Abcam, Cambridge, UK                             | ab7942     | 1000            |
| α tubulin            | Mouse   | WB          | Abcam, Cambridge, UK                             | ab13303    | 1000            |
| β actin              | Rabbit  | WB          | Cell Signalling Technology, Beverly, MA          | 4967       | 1000            |
| HA-tag (C29F4)       | Rabbit  | WB/IP       | Cell Signalling Technology, Beverly, MA          | 3724       | 1000            |
| IRS1                 | Rabbit  | IP          | Cell Signalling Technology, Beverly, MA          | 2382       | N/A             |
| Myc-tag (4A6)        | Mouse   | WB          | Santa Cruz Biotechnology, Santa Cruz, CA         | 05-724     | 1000            |
| p110α                | Mouse   | WB          | BD Biosciences, San Jose, CA                     | 611398     | 1000            |
| p110β                | Rabbit  | WB          | Cell Signalling Technology, Beverly, MA          | 30115      | 1000            |
| p85α                 | Mouse   | WB          | Gift from Dr L. Foukas (University College London, UK) [Cell Sciences, Canton, MA] | [CMP021] | 1000            |
| p85β                 | Mouse   | WB          | Gift from Dr L. Foukas (University College London, UK) [Abcam, Cambridge, UK] | [ab28356] | 500             |
| Phospho-AKT (Ser473/474) | Rabbit  | WB          | Cell Signalling Technology, Beverly, MA          | 9271S      | 1000            |
| Phospho-AKT (Thr308) | Rabbit  | WB          | Cell Signalling Technology, Beverly MA           | 9275       | 1000            |
| Total AKT            | Mouse   | WB          | Cell Signalling Technology, Beverly, MA          | 2920       | 1000            |
| Phospho-p42/44 ERK1/2 (Thr202/Tyr204) (E10) | Rabbit  | WB          | Cell Signalling Technology, Beverly, MA          | 9106S      | 1000            |
| Phosphotyrosine (4G10 Platinum) | Mouse | IP          | Millipore, Billerica, MA                         | 05-1050    | N/A             |
| PTEN                 | Mouse   | WB/IP       | Santa Cruz Biotechnology, Santa Cruz, CA         | sc7974     | 1000            |
| Total p85 "lightening" | Rabbit  | WB          | Gift from Professor K Siddle (University of Cambridge, UK) | Ref (6) | 5000            |
| Rabbit IgG (H+L) HRP-linked | Donkey | WB          | Thermo-Scientific/Pierce, Waltham, MA             | 31458      | 5000            |
| Mouse IgG HRP-linked | Goat    | WB          | Cell Signalling Technology, Beverly, MA          | 7076       | 2500            |

Supplemental Table 7. Antibodies used for western blotting (WB) and immunoprecipitation (IP).
Supplemental Figure 1. Expression of type 1A PI3K regulatory subunit genes in EBVLs from Patient 1: mRNA expression of \textit{PIK3R1}, \textit{PIK3R2} and \textit{PIK3R3} in EBV-transformed lymphoblastoid cells (EBVLs) from Patient 1 (white bars) and three healthy control subjects (black bars), determined by quantitative real-time PCR. Data represent mean +/- SEM.
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Supplemental Figure 2. Interaction of mutant p85α with phosphotyrosine residues and p110 within a heterologous expression system: (A) Anti-HA immunoprecipitates of HEK293-T cells transiently overexpressing HA-tagged WT or mutant p85α and myc-tagged WT p110α subject to western blotting first against HA (upper panel) and subsequently against myc (lower panel, upper bands; lower bands represent residual signal from the anti-HA antibodies). (B) Western blot of HA-tagged p85α (upper panel) and total p85 (endogenous and recombinant; lower panel) in phosphotyrosine immunoprecipitates of lysates from HEK293-T cells transiently overexpressing HA-tagged wild type or mutant p85α and myc-tagged p110α (or empty vector), treated with PBS or 100nM insulin for 5 minutes. Representative blots shown from three independent experiments.
Supplemental Figure 3. AKT dephosphorylation in primary dermal fibroblasts: Western blot of phosphorylated AKT at serine 473/474 in dermal fibroblasts from P1 and two healthy controls (C1, 2) serum-starved overnight, stimulated with 10nM insulin for 5 minutes, washed twice in warm PBS then incubated at 37°C for the times indicated. Representative example of two independent experiments.
Supplemental Figure 4. PTEN expression and association with p85 in primary dermal fibroblasts: (A) Western blot of total p85 in anti-PTEN immunoprecipitates from dermal fibroblasts of P1 and two healthy controls (C1, C2). Lane marked “B” indicates beads only control, without PTEN antibody. (B) Western blot of PTEN in fibroblast lysates from P1 and two healthy controls (C1, C2).
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**Supplemental Figure 5: Axenfeld-Rieger anomaly in Patient 2:** (A) Normal eye for comparison. (B, C). Ocular appearance of P2 showing missing pigment layer and visible blood vessels, exposed sphincter muscle seen as a grey rim around the pupil, and the posterior embryotoxon seen as a faint grey rim around the edge of the cornea.
Severe human insulin resistance without dyslipidaemia caused by C terminal mutations in PIK3R1; Huang-Doran et al

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Insulin resistance uncoupled from dyslipidemia in humans with C-terminal mutations in PIK3R1

Full Unedited Gels
Fig 2c. Full-length and truncated p85α, p55α and p50, and full-length p85β, in EBV-transformed lymphoblastoid cells (EBVLs) from P1 and healthy controls (C1-4), assessed by western blotting for total p85 (p85α/β) or p85β alone.

1 = Positive control (HA-p85)
2 = C0158
3 = C0146
4 = P0621
5 = P0621
6 = C0085
7 = C0039
8 = C0212
Fig 2d. Representative western blot of p85α or p85β in dermal fibroblasts from P1 and healthy controls (C1, C2).

D

|       | C1 | P1 | C2 |
|-------|----|----|----|
| p85α  | 100| 75 |  |
| α tubulin | 100| 75 |  |
| p85β  | 100| 75 |  |
| α tubulin | 75 |   |   |

**Fibroblasts**

**Full unedited gels for Figure 2d**

**p85α**

**p85β**

**α tubulin**

**α tubulin**

ID: PI3K_080
Full unedited gels for Figure 2f

Fig 2f. Western blot of p110α after immunoprecipitation of total p85 in primary dermal fibroblast lysates.

Long exposure

Short exposure

WB: p110α

WB: p110α
Fig 2g. Western blot of p110β after immunoprecipitation of total p85 in primary dermal fibroblast lysates.
Fig 3a. Western blot of total p85 (p85α/β) in phosphotyrosine immunoprecipitates or total lysates from dermal fibroblasts from P1 and healthy controls (C1-3) treated with PBS or 100nM insulin. Representative blots with quantified data from three independent experiments normalised to mean baseline intensity. Lane order in image of lysate blot adjusted to reflect that of the immunoprecipitate blot.

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| Insulin | IP: pY | WB: p85α/β | Lysate WB: p85α |
|---------|--------|-------------|-----------------|
| -       | -      | -           | 75              |
| +       | +      | +           | 75              |

**A**
Fig 3b. Western blot of total p85 (p85α/β) in phospho-IRS1 immunoprecipitates from patient and control fibroblasts after treatment with PBS or insulin. Representative blot with quantified data from three independent experiments normalised to mean baseline intensity across control cell lines.

IP: IRS1
WB: total p85
Fig 3d. Western blot of phosphorylated AKT1/2 (Ser473/474) and ERK1/2 (Thr202/Tyr204) in dermal fibroblasts from P1 and healthy controls (C1,2) after stimulation with a range of insulin doses.
Fig 4a. Total p85 and p110α expression in preadipocyte lysates 72 hours after treatment with or without doxycycline to induce expression of WT or mutant p85α. β actin was assessed as a loading control.
Fig 4c. Insulin-induced phosphorylation of AKT at Ser473/474 and Thr308 in preadipocytes infected with wild type and mutant p85α lentivirus, with or without doxycycline treatment, after stimulation with 1, 10 or 100nM insulin for 10 minutes.

Full unedited gels for Figure 4c

![Gel images with bands for pAkt (Ser473), pAkt (Thr308), Total Akt, Total p85, and β-actin (Thr308)].
Full unedited gel for Figure 5c

Fig 5c. Representative western blots of adiponectin in serum from P1, insulin resistant controls with mutations in INSR or AKT2, and insulin sensitive controls (IS), after non-denaturing, non-reducing SDS-PAGE. Low (LMW), medium (MMW) and high (HMW) molecular weight adiponectin complexes indicated. F/M denotes female/male.
Fig 5d. Secretion of low molecular weight adiponectin complexes into the culture medium of HEK293-T cells transfected with 1μg or 2μg wild type (black) or mutant (white) ADIPOQ, or co-transfected with 1μg wild type and 1μg mutant ADIPOQ (grey), determined by non-denaturing, non-reducing SDS-PAGE followed by western blotting of adiponectin.
Supp Fig 2a. Anti-HA immunoprecipitates of HEK293-T cells transiently overexpressing HA-tagged WT or mutant p85α and myc-tagged WT p110α subject to western blotting first against HA (upper panel) and subsequently against myc (lower panel, upper bands; lower bands represent residual signal from the anti-HA antibodies).

1) WT p85 + WT p110, -ins
2) WT p85 + WT p110, +ins
3) Mutant p85 Y657X + WT p110, -ins
4) Mutant p85 Y657X + WT p110, +ins
5) Empty vector, -ins
6) Empty vector, +ins
7) Lysate + beads only
Supp Fig 2b. Western blot of HA-tagged p85α (upper panel) and total p85 (endogenous and recombinant; lower panel) in phosphotyrosine immunoprecipitates of lysates from HEK293-T cells transiently overexpressing HA-tagged wild type or mutant p85α and myc-tagged p110α (or empty vector), treated with PBS or 100nM insulin for 5 minutes. Representative blots shown from three independent experiments.
Supp Fig 3. AKT dephosphorylation in primary dermal fibroblasts: Western blot of phosphorylated AKT at serine 473/474 in dermal fibroblasts from P1 and two healthy controls (C1, 2) serum-starved overnight, stimulated with 10nM insulin for 5 minutes, washed twice in warm PBS then incubated at 37°C for the times indicated. Representative example of two independent experiments.
Supp Fig 4. PTEN expression and association with p85 in primary dermal fibroblasts: (A) Western blot of total p85 in anti-PTEN immunoprecipitates from dermal fibroblasts of P1 and two healthy controls (C1, C2). Lane marked “B” indicates beads only control, without PTEN antibody. (B) Western blot of PTEN in fibroblast lysates from P1 and two healthy controls (C1, C2).