

# PIK3R1 Mutations Cause Syndromic Insulin Resistance with Lipoatrophy

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Short stature, hyperextensibility of joints and/or inguinal hernia, ocular depression, Rieger anomaly, and teething delay (SHORT) syndrome is a developmental disorder with an unknown genetic cause and hallmarks that include insulin resistance and lack of subcutaneous fat. We ascertained two unrelated individuals with SHORT syndrome, hypothesized that the observed phenotype was most likely due to de novo mutations in the same gene, and performed whole-exome sequencing in the two probands and their unaffected parents. We then confirmed our initial observations in four other subjects with SHORT syndrome from three families, as well as 14 unrelated subjects presenting with syndromic insulin resistance and/or generalized lipoatrophy associated with dysmorphic features and growth retardation. Overall, we identified in nine affected individuals from eight families de novo or inherited *PIK3R1* mutations, including a mutational hotspot (c.1945C>T [p.Arg649Trp]) present in four families. *PIK3R1* encodes the p85 $\alpha$ , p55 $\alpha$ , and p50 $\alpha$  regulatory subunits of class IA phosphatidylinositol 3 kinases (PI3Ks), which are known to play a key role in insulin signaling. Functional data from fibroblasts derived from individuals with *PIK3R1* mutations showed severe insulin resistance for both proximal and distal PI3K-dependent signaling. Our findings extend the genetic causes of severe insulin-resistance syndromes and provide important information with respect to the function of *PIK3R1* in normal development and its role in human diseases, including growth delay, Rieger anomaly and other ocular affections, insulin resistance, diabetes, paucity of fat, and ovarian cysts.

Insulin resistance is closely associated with many common, complex, and multifactorial conditions, including obesity, type 2 diabetes, atherosclerosis, nonalcoholic fatty-liver disease, and polycystic-ovary syndrome. Rare monogenic forms of insulin resistance also exist, and identifying the genetic basis of such phenotypes provides unique opportunities for deciphering the multiple molecular mechanisms leading to insulin resistance.<sup>1</sup> SHORT syndrome (MIM 269880)—whose acronym stands for short stature, hyperextensibility of joints and/or inguinal hernia, ocular depression, Rieger anomaly, and teething delay—is a developmental disorder of unknown genetic

cause and is associated with generalized thin habitus despite normal food intake. Onset of insulin resistance and/or diabetes mellitus typically occurs in adolescence.<sup>2</sup> Although affected individuals frequently present with short stature and relative microcephaly when compared to unaffected family members, height is in the low-normal range in one-third of cases. Other features include characteristic facial dysmorphism, delayed bone age and gracile long bones, variable ocular anomalies, normal intellect or mild impairment with speech delay, frequent illnesses, and lack of subcutaneous fat with wrinkled skin and readily visible veins enhancing the progeroid appearance.

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Vertical and male-to-male transmission have been described, indicative of autosomal-dominant inheritance.<sup>2</sup> Mutations in *PITX2* (MIM 601542) and *BMP4* (MIM 112262) have been discussed as putatively causative, but no mutations were identified in subjects with typical SHORT syndrome.<sup>3,4</sup>

We ascertained two unrelated individuals with a strikingly similar clinical presentation consistent with SHORT syndrome (Table 1 and Figures 1A–1D). Given the absence of recurrence of the syndrome in both families, we hypothesized that the observed phenotype was most likely due to de novo mutations in the same gene and performed whole-exome sequencing in the two probands and their unaffected parents. We then confirmed our initial observations in four other subjects with SHORT syndrome from three families, as well as 14 unrelated subjects presenting with syndromic insulin resistance and/or generalized lipodystrophy associated with dysmorphic features and growth retardation. Written, informed consent was obtained from all subjects, legal representatives, and relatives before enrollment in the study. The research protocol was approved by the ethics committees at participating institutions. Individuals were diagnosed with SHORT syndrome on the basis of previously reported clinical features.<sup>2</sup>

For all study subjects, genomic DNA was extracted from blood according to standard procedures. Whole-exome capture and sequencing were performed from 5 µg of genomic DNA at the PerkinElmer DNA Sequencing Service. Exome capture was performed with the 51 Mb SureSelect Human All Exon V4 kit (Agilent). Generated libraries were sequenced on a HiSeq 2000 (Illumina) according to the manufacturer's recommendations for paired-end 100 bp reads. We generated over 4 Gb of mappable sequence per individual, resulting in a depth of coverage of at least ten reads for more than 85% of the targeted exome (Table S1, available online). Sequencing data were processed as previously described.<sup>5,6</sup> Raw single-nucleotide variants (SNVs) and indels were identified with the Genome Analysis Toolkit (GATK v.2.1-10)<sup>7</sup> Unified Genotyper tool with a variant quality score  $\geq 30$ . Variants were then filtered with the GATK Variant Filtration tool and the following hard-filtering expressions: "QD < 2.0," "ReadPosRankSum < -8.0," and "FS > 200.0." Filtered variants were annotated with SeattleSeq SNP Annotation (see Web Resources). As previously described,<sup>6</sup> we systematically identified candidate de novo events by focusing on protein-altering and splice-site DNA changes (1) absent in the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project Exome Variant Server (EVS; see Web Resources), (2) supported by at least two reads and  $\geq 20\%$  of total reads in the proband, and (3) absent in both parents (two variant reads or fewer and  $\leq 5\%$  of total reads) at base-pair positions covered by at least four reads in the entire trio. Candidate de novo changes were then manually inspected with the Integrative Genomics Viewer.<sup>8</sup> This analysis identified de novo mutations in *PIK3R1* (MIM 171833, RefSeq accession number

NM\_181523.2) in both affected individuals, P1 (c.1615\_1617delATT [p.Ile539del]) and P2 (c.1465G>A [p.Glu489Lys]) (Table S2). Both mutations were confirmed as de novo by standard PCR protocols with the use of custom intronic primers combined with capillary sequencing. *PIK3R1* encodes the p85 $\alpha$ , p55 $\alpha$ , and p50 $\alpha$  regulatory subunits of class IA phosphatidylinositol 3 kinases (PI3Ks), which are dimers composed of a p110 catalytic subunit and a p85 regulatory subunit. PI3K signaling is involved in multiple cellular functions, such as cell growth, proliferation, migration, metabolism, angiogenesis, survival, and apoptosis, and mutations in several of the core components of the PI3K pathway have been associated with some of the most prevalent human diseases, including diabetes and cancer.<sup>9</sup>

We screened *PIK3R1* for mutations in four individuals with SHORT syndrome from three additional families. The coding and flanking intronic regions of *PIK3R1* (RefSeq NM\_181523.2, NM\_181524.1, and NM\_181504.3) were amplified by standard PCR protocols (primer sequences are available upon request). Sequencing was performed with the ABI BigDye Terminator Cycle Sequencing kit (v.3.1) and an ABI 3130 Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions. Sequence traces were analyzed with Mutation Surveyor (v.4.0.7, SoftGenetics). This experiment identified a recurrent substitution (c.1945C>T [p.Arg649Trp]) in all four subjects (Table 2), and it proved to be de novo in the only simplex case for which parental DNA was available (P3, who was previously reported;<sup>10</sup> Figure 1E). The remaining three individuals with *PIK3R1* mutations included a simplex case (P4) and an affected woman and her terminated fetus (P5 [II-5 in Figure 1F] and P6 [III-2 in Figure 1F], Figures 1F–1H). The c.1945C>T mutation occurs within the context of a CpG dinucleotide, which might explain its recurrence. We then sequenced *PIK3R1* in a heterogeneous clinical group of 14 additional unrelated subjects presenting with severe insulin resistance and/or generalized lipodystrophy associated with dysmorphic features and growth retardation (Table S3). None of these cases had been previously diagnosed with SHORT syndrome, and none had mutations in *AGPAT2* (MIM 603100), *BSCL2* (MIM 606158), *LMNA* (MIM 150330), *PPARG* (MIM 601487), or *INSR* (MIM 147670). Capillary sequencing identified mutations in three of these subjects: the previously identified recurrent substitution (c.1945C>T [p.Arg649Trp]) in P7, a single-nucleotide insertion predicted to result in a prematurely truncated protein (c.1943dupT [p.Arg649Profs\*5]) in P8, and a substitution (c.1892G>A [p.Arg631Gln]) in P9 (Table 2). Although all three individuals had facial dysmorphism retrospectively consistent with SHORT syndrome, the limited available clinical data did not allow confirmation of the clinical diagnosis of SHORT syndrome (Table 1). Nucleotide-level conservation and the impact of amino acid substitutions were assessed with Genomic Evolutionary Rate Profiling (GERP),<sup>11</sup> Grantham matrix,<sup>12</sup> and

**Table 1. Clinical Features of Individuals with Mutations in *PIK3R1***

	Individual								
	P1	P2	P3 <sup>a</sup>	P4	P5	P6	P7	P8	P9
Sex	male	male	female	male	female	male	female	female	male
<b>Features of SHORT Syndrome Acronym</b>									
Measurements at birth									
Gestational age	34 weeks of gestation + 4 days	34 weeks of gestation	35.5 weeks of gestation	35 weeks of gestation	term	27 weeks of gestation	term	ND	prematurity
Length (cm)	42 (<3 <sup>rd</sup> per)	40 (<3 <sup>rd</sup> per)	43.5 (<3 <sup>rd</sup> per)	47 (25 <sup>th</sup> per)	52 (20 <sup>th</sup> per)	40 (50 <sup>th</sup> per)	NA	NA	NA
Weight (g)	1,460 (<3 <sup>rd</sup> per)	1,380 (<3 <sup>rd</sup> per)	1,830 (<3 <sup>rd</sup> per)	2,000 (3 <sup>rd</sup> per)	2,260 (<3 <sup>rd</sup> per)	1,400 (50 <sup>th</sup> per)	2,700 (3 <sup>rd</sup> per)	ND	950
OFC (cm)	29.5 (3 <sup>rd</sup> per)	28.5 (<3 <sup>rd</sup> per)	32 (20 <sup>th</sup> per)	33 (40 <sup>th</sup> per)	unknown	25 (10 <sup>th</sup> per)	ND	ND	ND
Measurements at last follow-up examination									
Age	7 years and 7 months	7 years and 8 months	19 years	20 months	40 years	ND	28 years	60 years	28 years
Length (cm)	109.7 (<3 <sup>rd</sup> per)	111.8 (<3 <sup>rd</sup> per)	146.5 (<3 <sup>rd</sup> per)	80.5 (40 <sup>th</sup> per)	160 (25 <sup>th</sup> per)	ND	145 (<3 <sup>rd</sup> per)	143 (<3 <sup>rd</sup> per)	155 (<3 <sup>rd</sup> per)
Weight (kg)	15 (<3 <sup>rd</sup> per)	15.5 (<3 <sup>rd</sup> per)	36 (<3 <sup>rd</sup> per)	8.76 (<3 <sup>rd</sup> per)	50 (<3 <sup>rd</sup> per)	ND	44.6 (<3 <sup>rd</sup> per)	35 (<3 <sup>rd</sup> per)	42 (<3 <sup>rd</sup> per)
OFC (cm)	47 (<3 <sup>rd</sup> per)	47 (<3 <sup>rd</sup> per)	unknown	47 (20 <sup>th</sup> per)	51 (<3 <sup>rd</sup> per)	ND	ND	ND	ND
BMI	12.5 (<3 <sup>rd</sup> per)	12.4 (<3 <sup>rd</sup> per)	16.8 (<3 <sup>rd</sup> per)	13.5 (<3 <sup>rd</sup> per)	19.5 (35 <sup>th</sup> per)	ND	21.2 (25 <sup>th</sup> per)	17.1 (3 <sup>rd</sup> per)	17.5 (3 <sup>rd</sup> per)
Hyperextensibility of joints	–	–	+	–	+	–	ND	ND	ND
Inguinal hernia	–	–	–	–	–	ND	–	–	–
Ocular depression	+	+	+	+	+	+	ND	+	–
Rieger anomaly	–	–	+	–	+	ND	–	–	–
Teeth delay	+	+	+	+ <sup>b</sup>	+	ND	+	ND	ND
Number of features of acronym	3/5	3/5	5/5	2/5	4/5	1/3	2/3	2/3	1/3
<b>Other Findings</b>									
Ophthalmological findings									
Hyperopia	+	+	+	+	–	ND	ND	ND	ND
Astigmatism	+	–	+	–	–	ND	+	ND	ND
Myopia	–	–	–	–	+	ND	ND	ND	ND
Esotropia	–	–	+	–	–	ND	ND	ND	ND
Lack of subcutaneous fat	+	+	+	+	+	ND	+	+	+
Insulin resistance	+	+	+	ND	+	ND	+	+	+
Diabetes	–	–	+	–	+	ND	+	+	+

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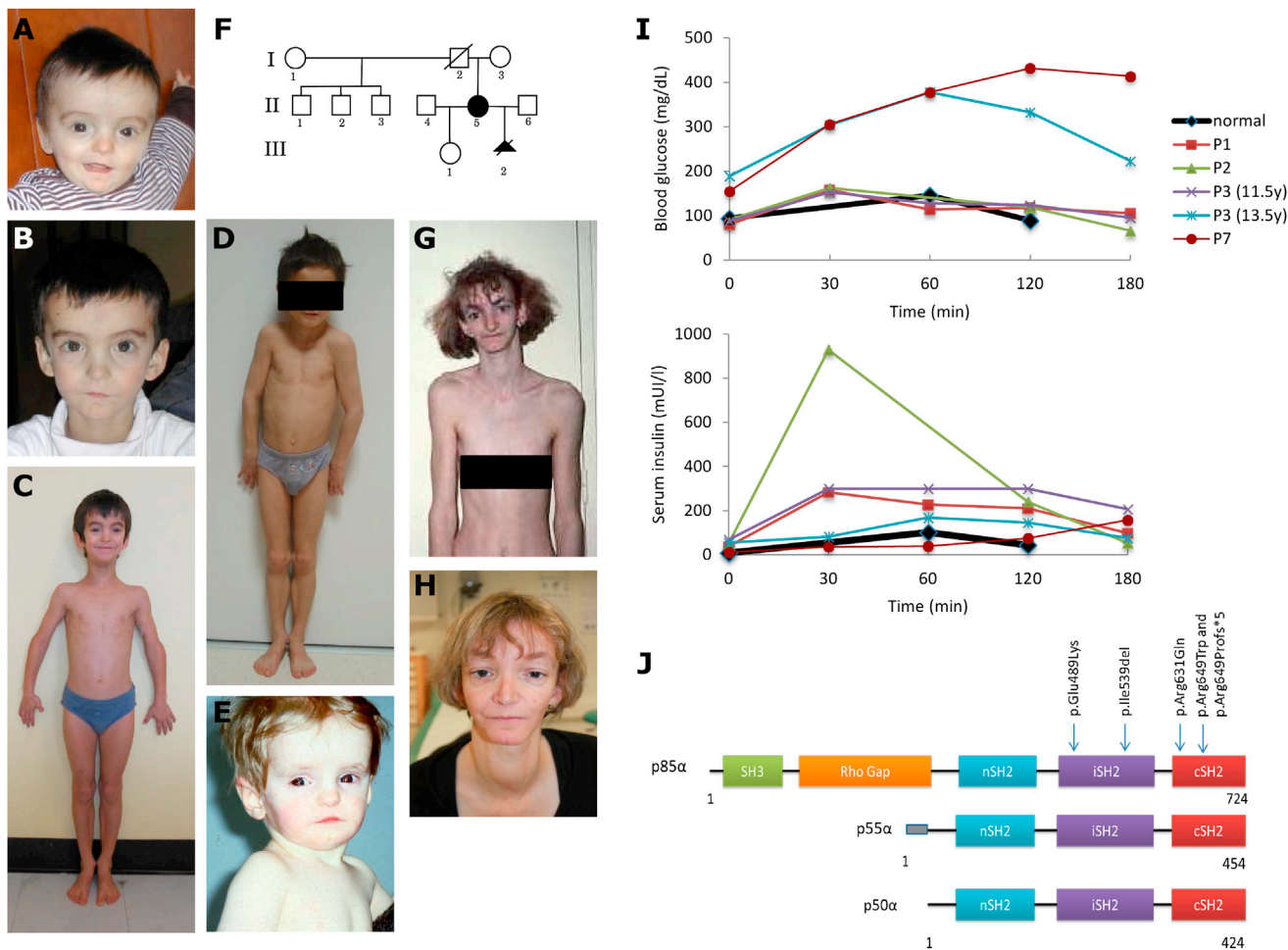
**Table 1. Continued**

	Individual								
	P1	P2	P3 <sup>a</sup>	P4	P5	P6	P7	P8	P9
Facial dysmorphism									
Triangular shape	+	+	+	+	+	+	ND	+	+
Prominent forehead	+	+	+	+	+	+	+	+	–
Hypoplastic or thin alae nasi	+	+	+	+	+	+	+	+	+
Small chin	+	+	+	+	+	+	+	+	+
Large low-set ears	+	+	+	+	+	+	+	+	+
Mild midface hypoplasia	+	+	+	+	+	+	ND	+	–
Micrognathia	+	+	+	+	+	+	+	+	+
Thin, wrinkled skin	+	+	–	+	–	ND	+	+	ND
Readily visible veins	+	+	–	+	–	ND	+	+	ND
Frequent illnesses	–	–	–	–	+	ND	ND	+	ND
Ovarian cysts	NA	NA	+	NA	+	NA	+	ND	NA
Mental development									
Normal	–	–	–	+	+	ND	+	+	–
Mild impairment and/or speech delay	+	+	+	–	–	ND	–	–	+
Radiographic findings									
Delayed bone age	+	+	+	ND	ND	ND	ND	ND	ND
Gracile long bones	–	–	+	ND	ND	ND	ND	ND	ND

Abbreviations are as follows: per, percentile; +, presence of a feature; –, absence of a feature; OFC, occipitofrontal circumference; BMI, body mass index; NA, not applicable; and ND, no data.

<sup>a</sup>Subject previously reported.

<sup>b</sup>No teeth at last follow-up examination.



**Figure 1. Appearance of Individuals with SHORT Syndrome, Test Results for Oral Glucose Tolerance, and Distribution of *PIK3R1* Mutations**

(A–H) Photos of subjects P1 (A–C), P2 (D), and P3 (E) and familial pedigree and photos of subject P5 (F–H). Subjects presented with a typical triangular facial shape, deep-set eyes, thin and hypoplastic alae nasi, a small chin, mild midfacial hypoplasia, and lipoatrophy. We obtained written consent to publish photographs of the subjects.

(I) Test results for oral glucose tolerance show hyperglycemia in subjects P3 (at 13.5 years of age) and P7 and increased serum insulin levels in all affected children (subjects P1, P2, and P3).

(J) Schematic representation of the three isoforms encoded by *PIK3R1*, their functional domains, and the alterations identified in individuals with SHORT syndrome.

PolyPhen-2 (with the HumVar-trained model)<sup>13</sup> scores. In addition to the fact that three of the identified missense *PIK3R1* mutation sites (encoding p.Ile539del, p.Glu489Lys, and p.Arg649Trp) were found to be de novo in one subject each, all mutation sites affect highly conserved nucleotides and are predicted to be possibly or probably damaging (Table 2). None of the five mutation sites were reported in the NHLBI EVS (Table S4), which consists of whole-exome-sequencing data from ~6,500 individuals (the EVS was accessed March 2013), dbSNP137 (see Web Resources), or the 1000 Genomes Project (see Web Resources). A single truncating mutation (c.901C>T [p.Arg301\*]), which was present in a heterozygous state in a single individual, was reported in the EVS data set. Contrary to the truncating mutation identified in the present study and predicted to affect all three subunits encoded by *PIK3R1* (i.e., p85 $\alpha$ , p55 $\alpha$ , and p50 $\alpha$ ), the muta-

tion encoding p.Arg301\* is predicted to affect only the p85 $\alpha$  subunit. We can speculate that disruption of all three *PIK3R1* subunits is required for the development of SHORT syndrome, which is consistent with a recent report of a complete loss of p85 $\alpha$  (but normal p55 $\alpha$  and p50 $\alpha$  expression) in an individual with absent B cells but normal growth, development, and fasting glucose and insulin levels.<sup>14</sup>

Class IA PI3Ks play a key role in insulin signaling through binding of phosphorylated insulin-receptor substrates, production of phosphatidylinositol-4,5-trisphosphate (PIP3), and subsequent activation of AKT serine-threonine kinase.<sup>15</sup> A test for oral glucose tolerance was performed according to the recommended procedures<sup>16</sup> and showed increased insulin levels in the three affected children tested (subjects P1, P2, and P3 [Figure 1I and Table S5]). Subjects P3 and P7 developed diabetes at

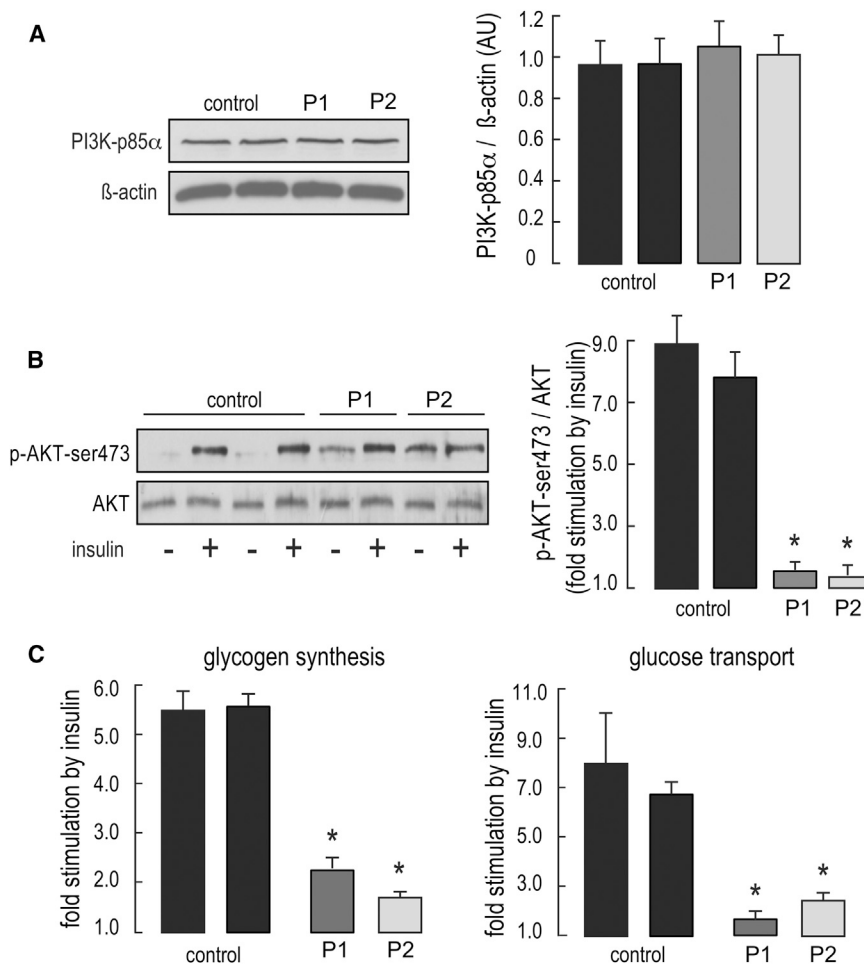
**Table 2. Summary of *PIK3R1* Mutations Identified in Nine Affected Individuals from Eight Families**

Individual	Mutation Coordinates (hg19)	Alleles (Ref/Alt)	cDNA Change	Amino Acid Change	Grantham Score	GERP Score	PolyPhen-2	Inheritance
P1	chr5: 67,591,018	AATT/A	c.1615_1617delATT	p.Ile539del	-	-	-	de novo
P2	chr5: 67,590,403	G/A	c.1465G>A	p.Glu489Lys	56	5.07	possibly damaging (0.67)	de novo
P3	chr5: 67,592,129	C/T	c.1945C>T	p.Arg649Trp	101	5.15	probably damaging (1.00)	de novo
P4	chr5: 67,592,129	C/T	c.1945C>T	p.Arg649Trp	101	5.15	probably damaging (1.00)	absent in mother, father not available
P5	chr5: 67,592,129	C/T	c.1945C>T	p.Arg649Trp	101	5.15	probably damaging (1.00)	absent in mother, father not available
P6	chr5: 67,592,129	C/T	c.1945C>T	p.Arg649Trp	101	5.15	probably damaging (1.00)	inherited from mother (p5)
P7	chr5: 67,592,129	C/T	c.1945C>T	p.Arg649Trp	101	5.15	probably damaging (1.00)	parents not available
P8	chr5: 67,592,127	T/TT	c.1943dupT	p.Arg649Profs*5	-	-	-	parents not available
P9	chr5: 67,592,076	G/A	c.1892G>A	p.Arg631Gln	43	5.15	probably damaging (0.94)	parents not available

Mutation nomenclature is based on RefSeq accession number NM\_181523.2. Abbreviations are as follows: Ref, reference allele; alt, alternative allele; and GERP, Genomic Evolutionary Rate Profiling.

13.5 and 12 years of age, respectively, which explains their hyperglycemia and why their levels of serum insulin were lower than those of other affected individuals. Interestingly, in contrast to other monogenic forms of insulin resistance and lipodystrophy, SHORT syndrome resulting from mutations in *PIK3R1* is not associated with severe hypertriglyceridemia (Table S5). Multiple lines of evidence indicate that the *PIK3R1* mutations identified here should result in decreased PI3K activity. In humans, loss of insulin receptor (*INSR*) causes Donohue (MIM 246200) and Rabson-Mendenhall (MIM 262190) syndromes, both of which share many features with SHORT syndrome, including growth restriction, extreme insulin resistance, paucity of body fat, dysmorphic features, inguinal hernia, wrinkled skin, ovarian cysts, and/or dysplastic teeth.<sup>17</sup> Mice carrying a heterozygous mutation abrogating p110 $\alpha$  kinase activity display small body size, insulin resistance, and glucose intolerance.<sup>18</sup> Interestingly, loss of AKT2 function is associated with severe insulin resistance, diabetes, and lipoatrophy in humans and mice,<sup>19,20</sup> but unlike in SHORT syndrome, hypertriglyceridemia and increased de novo lipogenesis are part of the phenotype.<sup>21</sup> Conversely, mutations in several PI3K-AKT-pathway-related genes result in increased PI3K signaling and cause severe brain and/or body overgrowth,<sup>5</sup> and an activating mutation in *AKT2* in humans was shown to cause severe hypoglycemia, weight gain, and somatic overgrowth,<sup>22</sup> which is the opposite phenotype to SHORT syndrome. To assess the impact of *PIK3R1* mutations, we evaluated the effects of short-term insulin stimulation on proximal (i.e., AKT, also known as protein kinase B) and distal (i.e., glycogen synthesis and glucose transport) PI3K-dependent insulin signaling in fibroblast cells derived from two individuals (P1 and P2) with *PIK3R1* mutations and two unaffected males. Skin fibroblasts were prepared according to standard procedures, as

described previously.<sup>23</sup> Protein levels of *PIK3R1* were evaluated by immunoblot on whole-cell lysates with antibodies to the p85 $\alpha$  subunit (sc-423 [Z-8], Santa Cruz Biotechnology). Serum-depleted cells were incubated for 10 (for AKT detection) or 30 min (for the glycogen synthesis and the glucose-transport assays) with or without insulin (100 nMole/l). Insulin stimulation was evaluated by immunoblot analysis on whole-cell lysates with antibodies to AKT and phospho-AKT-ser473 (SC 8312 and SC 7985, Santa Cruz Biotechnology). Glycogen synthesis was evaluated by the addition of <sup>14</sup>C-glucose (75 kBq/ml, PerkinElmer) to fibroblasts for 2 hr. Radiolabeled glycogen was then extracted and counted, as described previously.<sup>24</sup> Similarly, glucose uptake was evaluated with <sup>3</sup>H-2-deoxy-D-glucose (PerkinElmer). As shown in Figure 2, the studied *PIK3R1* mutations did not affect the protein levels of *PIK3R1* (Figure 2A). However, cells from both affected individuals displayed a markedly decreased effect of insulin on AKT activation, glycogen synthesis, and glucose uptake (by 70%–90%; Figures 2B and 2C), thus indicating severe insulin resistance for both proximal and distal PI3K-dependent signaling. These results might partly be explained by the increased levels of basal activity in cells from affected individuals (3- to 4-fold, 1.5- to 2-fold, and 1- to 2-fold increase for AKT activity, glycogen synthesis, and glucose uptake, respectively). This increased basal activity with further desensitization to insulin action has been described previously in other states of severe insulin resistance caused by mutations in *INSR*.<sup>24–26</sup> Although further metabolic investigations will be needed for precisely deciphering the underlying signaling defects, our observations are consistent with impaired insulin-stimulated pathways, which lead to a generalized insulin-resistance syndrome affecting glucose homeostasis, growth, adipogenesis, and lipogenesis (Figure 1, Table 1, and Table S5).



**Figure 2. Cellular Insulin Resistance in Subjects with *PIK3R1* Mutations**

(A and B) *PIK3R1* levels and short-term effects of insulin on proximal PI3K-dependent insulin signaling in fibroblasts from affected individuals (P1 and P2) and controls. *PIK3R1* levels (A) and the effect of insulin on AKT activation (B) were evaluated by immunoblotting on whole-cell lysates. Intensities were quantified by densitometry scanning. *PIK3R1* levels and AKT phosphorylation levels were normalized to  $\beta$ -actin and total AKT, respectively. Representative immunoblots are shown. The following abbreviation is used: p-AKT-ser473, phospho-AKT-ser473.

(C) Short-term effects of insulin on distal PI3K-dependent signaling. The effects of insulin on glycogen synthesis and glucose transport were evaluated by the incorporation of  $^{14}\text{C}$ -glucose into glycogen and the cellular uptake of  $^3\text{H}$ -2-deoxy-D-glucose, respectively. In fibroblasts from control subjects and individuals P1 and P2, basal glycogen synthesis was  $22.3 \pm 1.6$ ,  $54.6 \pm 6.0$ , and  $52.5 \pm 0.4$  pmoles of glucose incorporated into glycogen per mg protein per hour, respectively, and basal glucose transport was  $10.5 \pm 1.1$ ,  $23.2 \pm 2.4$ , and  $11.4 \pm 1.5$  pmole of cellular 2-deoxyglucose per mg protein per min, respectively. Results are expressed as means  $\pm$  SEM. Asterisks indicate a statistically significant difference compared to control cells ( $p < 0.05$ , Student's *t* test).

Mice lacking *Pik3r1* display enhanced insulin sensitivity and glucose tolerance,<sup>27</sup> whereas p85 overexpression is associated with insulin resistance in skeletal muscle,<sup>28</sup> thus indicating that p85 subunits act as negative regulators of PI3K signaling downstream of the insulin receptor. However, the mechanisms underlying negative regulation of insulin signaling by the PI3K regulatory subunits remain unclear.<sup>15</sup> Two of the mutations identified here are located in *PIK3R1*'s inter-*Src* homology 2 (iSH2) domain (Figure 1J), which is a major interacting domain with p110 catalytic subunits. This interaction regulates PI3K activity through the formation of active and inactive dimers.<sup>29</sup> Although further functional studies will be necessary for elucidating the exact mechanisms leading to SHORT syndrome, we can speculate that these mutations might affect the interaction between p85 regulatory subunits and p110 catalytic subunits and thus lead to decreased PI3K activity.

Other clinical SHORT syndrome features, such as bone demineralization, ovarian cysts, Rieger anomaly, and teething delay, are consistent with decreased PI3K-AKT signaling. Indeed, the PI3K-AKT pathway is critical for bone development, growth, and integrity and is a key regulator of osteoclastic bone resorption.<sup>30</sup> Mice lacking both *Akt1* and *Akt2* display a severe bone-deficiency phenotype, and *Akt1*<sup>-/-</sup> mice exhibit growth deficiency, along with

impaired bone growth early in life and mild osteopenia in adulthood.<sup>31</sup> Besides severe diabetes and lipoatrophy, loss of *Akt2* was shown to predispose mice to the formation of ovarian cysts.<sup>32</sup> Finally, haploinsufficiency of *PITX2*, which is a downstream phosphorylation target of *AKT2*,<sup>33</sup> causes Axenfeld-Rieger syndrome (MIM 602482), a condition that has significant clinical overlap with SHORT syndrome and is characterized by anterior-segment eye defects and umbilical and teeth anomalies.<sup>34</sup>

Because individuals with SHORT syndrome are usually born small for gestational age (SGA), which predisposes them to permanent short stature,<sup>35</sup> two of the subjects reported here (P1 and P2) were being treated by growth hormone (GH). This treatment was approved in 2001 and 2003 in the United States and Europe, respectively, for SGA children without catch-up growth after the age of 4 years. However, GH treatment is known to induce hyperinsulinemia and to decrease insulin sensitivity; this phenomenon might aggravate insulin resistance and accelerate the onset of diabetes in subjects with SHORT syndrome, particularly during puberty, when insulin resistance is observed in both normal and diabetic children.<sup>36</sup> Given the presence of diabetes in all adult *PIK3R1*-mutation-positive individuals presented here (Table 1), the well-known association between insulin resistance and

PI3K-pathway dysfunction, and the fact that GH-induced insulin resistance is rapidly reversible upon treatment cessation,<sup>37</sup> GH treatment was ceased in both subjects. This well illustrates the fact that genetic findings can have direct implications for care practice by enabling a more personalized clinical management.

In conclusion, our findings provide important information with respect to the function of *PIK3R1* in normal development and its role in human diseases, including growth delay, Rieger anomaly and other ocular affections, insulin resistance, diabetes, paucity of fat, and ovarian cysts. The relatively mild clinical presentation of several of the subjects reported here suggests that SHORT syndrome might be underdiagnosed and that mutations in *PIK3R1* might be a rare cause of apparently nonsyndromic insulin resistance in lean cases.

### Supplemental Data

Supplemental Data include five tables and can be found with this article online at <http://www.cell.com/AJHG>.

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### Web Resources

The URLs for data presented herein are as follows:

1000 Genomes Project, <http://www.1000genomes.org/>

dbSNP, <http://www.ncbi.nlm.nih.gov/projects/SNP/>

Dyscerne, <http://www.dyscerne.org/>

NHLBI Exome Sequencing Project (ESP) Exome Variant Server, <http://evs.gs.washington.edu/EVS/>

Online Mendelian Inheritance in Man (OMIM), <http://www.omim.org/>

SeattleSeq Annotation 137, <http://snp.gs.washington.edu/SeattleSeqAnnotation137/>

University of Burgundy Centre de Calcul, <https://haydn2005.u-bourgogne.fr/dsi-ccub/>

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