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An Activating Mutation of *AKT2* and Human Hypoglycemia

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Abstract

Pathological fasting hypoglycemia in humans is usually explained by excessive circulating insulin or insulin-like molecules, or by inborn errors of metabolism impairing liver glucose production. We studied three unrelated children with unexplained, recurrent and severe fasting hypoglycemia and asymmetrical overgrowth. All were found to carry the same *de novo* mutation, p.Glu17Lys, in the serine/threonine kinase AKT2, in two cases as heterozygotes and, in one case, in mosaic form. In heterologous cells, the mutant AKT2 was constitutively recruited to the plasma membrane, leading to insulin-independent activation of downstream signaling. This represents a novel mechanism of systemic metabolic disease, characterised by constitutive, cell-autonomous activation of signaling pathways normally controlled by insulin.

Insulin promotes energy storage and growth through effects on glucose, lipid and amino acid metabolism, cell division and apoptosis. These are mediated by a transmembrane tyrosine kinase receptor that phosphorylates Insulin Receptor Substrate (IRS) and other adaptor proteins to initiate signaling events including, critically, activation of AKT serine/threonine kinases. Murine studies suggest that the AKT2 isoform is most closely linked to insulin's metabolic effects(1). Consistent with this, a loss-of-function mutation in AKT2 produces severe insulin resistance in humans(2).

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Supporting Online Material Materials and Methods SOM Text Figs. S1-S3 Table S1

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Insulin hypersecretion from tumoral or genetically hyperactive pancreatic beta cells produces hypoglycemia, weight gain and accelerated early growth. We previously described a child with severe recurrent hypoglycemia from infancy and undetectable plasma insulin despite the classical biochemical profile of hyperinsulinism (i.e. low serum levels of ketone bodies and branched chain amino acids, and no elevation of free fatty acids). He also had an increased birth weight, and left-sided overgrowth(Figure 1A, Table S1)(3). We have subsequently studied two further patients with a similar metabolic profile and left-sided facial overgrowth (Table S1, Figure S1, supplementary online text). From around 6 months old each patient exhibited symptomatic severe hypoglycemia, including reduced consciousness and seizures, when fasted beyond 3 hours, requiring surgical implantation of enteral tubes for long-term overnight carbohydrate feeding.

All patients were born to unaffected, unrelated Europid parents. The sequence of genes encoding preproinsulin, insulin-like growth factors 1 and 2 and their receptors was normal, and no basal hyperphosphorylation of insulin receptors in fibroblasts was seen. Exome-wide sequencing of the index case was undertaken, and resulting variants were filtered to exclude those that were common or not predicted to alter amino acid sequences (4). This yielded 326 rare mutations including the heterozygous c.49G>A mutation in the *AKT2* gene, leading to substitution of glutamate 17 in the pleckstrin homology (PH) domain for lysine (Figure 1B-D). Sanger sequencing confirmed the heterozygous mutation in lymphocytes, left and right sided dermal fibroblasts from the proband but neither parent, consistent with a *de novo* germline mutation, and further detected the same mutation in the other two probands. One was heterozygous for the mutation and the other showed 20% mosaicism in lymphocyte and cheek epithelial DNA (4). No canonical insulin-responsive tissues were available to assess their mutation burden. None of their parents carried the mutation, which was also absent in 1,130 control genomes and exomes (470 Europid)(4).

Insulin-induced AKT activation requires type 1 phosphatidylinositol 3-kinase (PI3K), which generates phosphatidylinositol 3,4,5 trisphosphate (PIP_3) in the plasma membrane. The PH domain of AKT then docks with PIP_3, leading to its apposition to the stimulatory kinases PDK1 and mTORC2 and thus activation(5). Overexpression of AKT2 p.Glu17Lys in 3T3-L1 adipocytes produces non insulin-dependent membrane localisation of the GLUT4 glucose transporter(6). Consistent with this we found the mutant AKT2 to exhibit plasma membrane localization even in serum-starved HeLa cells (Fig. 1E), and to produce tonic nuclear exclusion of FoxO1 in 3T3-L1 cells (Fig. S2)(4).

Somatic AKT2 mutations have been described rarely in cancer, although not, to date, p.Glu17Lys (7). In contrast, the homologous p.Glu17Lys mutation in AKT1 is commonly found in cancer(8), and while this paper was in review somatic mosaicism for AKT1 p.Glu17Lys was reported to underlie the dramatic progressive skin and skeletal overgrowth of Proteus syndrome(9). The very different, mostly metabolic, phenotype of patients with germline or mosaic AKT2 p.Glu17Lys emphasizes the distinct functions of AKT1 and AKT2 in humans. However the accelerated growth seen in our patients suggests that AKT2, too, may have some growth promoting effect, although the observed asymmetry is unexplained. Proof of constitutive activation of a key element of insulin signal transduction affords the prospect of novel and rational treatment for the associated severe hypoglycemia in future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Summary

An activating mutation in AKT2 in humans produces severe insulin-independent hypoglycemia and asymmetric overgrowth

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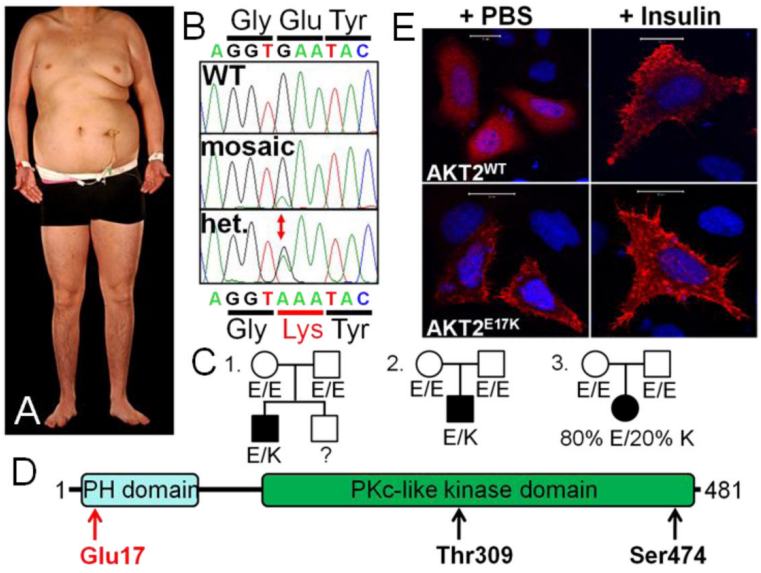

Figure 1. Identification of a *de novo activating AKT2* mutation

(A) Patient 1 showing increased left leg size, increased left truncal adipose tissue, and surgical feeding tube (**B**) c.49G>A/p.Glu17Lys mutation in the *AKT2* gene seen in either heterozygous or mosaic form. (**C**) Pedigrees of affected patients, showing *de novo* occurrence of the mutation in each case. (**D**) Domain structure of AKT2, showing location of Glu17 in the pleckstrin homology domain and the location of residues phosphorylated by PDK1 and mTORC2 during activation. (**E**) Plasma membrane recruitment of hemagglutinin-tagged AKT2 p.Glu17Lys but not wild type in serum starved HeLa cells treated with either phosphate buffered saline (PBS) or 100 nmol/l insulin. Nuclei are stained with DAPI. Scale bars = 20μm

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