Novel syndromes of hypoinsulinaemic, hypoketotic hypoglycaemia

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Introduction

Hypoglycaemia in infancy is commonly caused by hyperinsulinism and is characterised by low ketones, low free fatty acids and low branched-chain amino acids during hypoglycaemia, and a high glucose infusion rate to maintain euglycaemia.

Hypoketotic hypoglycaemia in the absence of detectible serum insulin can be caused by an activating mutation in serine-threonine kinase AKT2 (PKBβ) leading to hyperactivation of the AKT2-dependent arm of the insulin signalling cascade (1,2). These patients have a lower requirement for glucose and show left-sided hemihypertrophy.

Here we present a series of five further patients with hypoketotic hypoglycaemia and suppressed insulin but no activating mutation in AKT2, and outline our approach to identifying the underlying genetic and cellular causes.

Patient Group

Patient 1 case history

- born at 35 weeks of gestation. Birth weight 3.05kg (+0.98SD), length 48cm (+0.05SD), and head circumference 35cm (+1.10SD)
- non-consanguineous German parents
- bilateral renal masses noted during gestation (diagnosed as bilateral cystic nephropathy)

Hypoglycaemica (0.4mmol/l) noted at 2 days (Table 1)
- no urinary ketones during hypoglycaemia
- preserved mobilisable glucagon stores as evidenced by glucagon response
- short synacthen test confirmed normal adrenal function; steroids ineffective
- required parenteral glucose (10-19mg/kg/day) to maintain euglycaemia

Left adrenal mass noted post-natally on ultrasound
- surgical removal did not improve hypoglycaemia
- histology revealed a high-grade adrena; tumour with some normal parenchyma
- no evidence increased “big” IGF in tumour or blood

Left-sided hemihypertrophy was noted at age 2 and an MRI confirmed overgrowth of left-sided abdominal organs.

At 14 years old she had a fasting tolerance of around 5 hours and did not show any signs of developmental delay. Euglycaemia is currently maintained through frequent meals.

	Patient 1	Reference range in Euglycaemia
Blood glucose (mmol/l)	1.4	Fasting: 3.5-5.9
Insulin (pmol/l)	<10	0-60
Glucagon (ng/l)	379	50-200
Cortisol (nmol/l)	552	>550
βOHB (mmol/l)	0.0	<0.6
Free fatty acids (mmol/l)	0.37	<0.72
Valine (µmol/l)	98	139-474
Leucine (µmol/l)	39	85-169
Isoleucine (µmol/l)	15	31-105
Adrenaline (ng/l)	100	<140
Lactate (mmol/l)	0.61	0.5-2.2
ACTH (pmol/l)	5.72	2.2-13.3
pH	7.38	7.35-7.45
HCO_3^- (mEq/l)	22	20-29
Ketouria, organic aciduria	negative	

Table 1 – summary of biochemical tests confirming hypoketotic, hypoglycaemia hypoglycaemia in patient 1 after 180 minutes of fasting

Further patients

In addition to patient 1, four further patients have been identified with hypoketotic, hypoinsulinaemic hypoglycaemia. These show a variety of additional features. (Table 2)

ID	Fasting hypoglycaemia	Insulin during hypoglycamia	Low ketones	Overgrwth	Other features
P1	Yes	Undetectable	Yes	Left-sided	bilateral cystic nephrophathy, adrenal tumour
P2	Yes	Undetectable	Yes	none	none
P3	Yes	Undetectable	Yes	obesity	Obstructive sleep apnea, epilepsy
P4	Yes	Undetectable	Yes	Right-sided	Jejunal atresia, liver disease associated with parenteral feeding
P5	yes	Undetectable	Yes	Dysmorphic features	Muscle hypotonia, Hepatic fibrosis, Arnold-Chiari malformation

Table 2 – overview over biochemical, overgrowth and other clinical features in patients with hypoinsulinaemic hypoglycaemia in the absence of detectible serum insulin.

Excluding AKT2 mutations and hyperactivation

None of the patients carry the previously reported *AKT2* p.Glu17Lys mutation in blood nor, where available, tissue.

Primary dermal fibroblasts are available for patients 1 and 3. ELISA and a quantified sandwiched immunoassay did not show basal hyperphosphorlyation of AKT2 or downstream target proteins. (Fig 1)

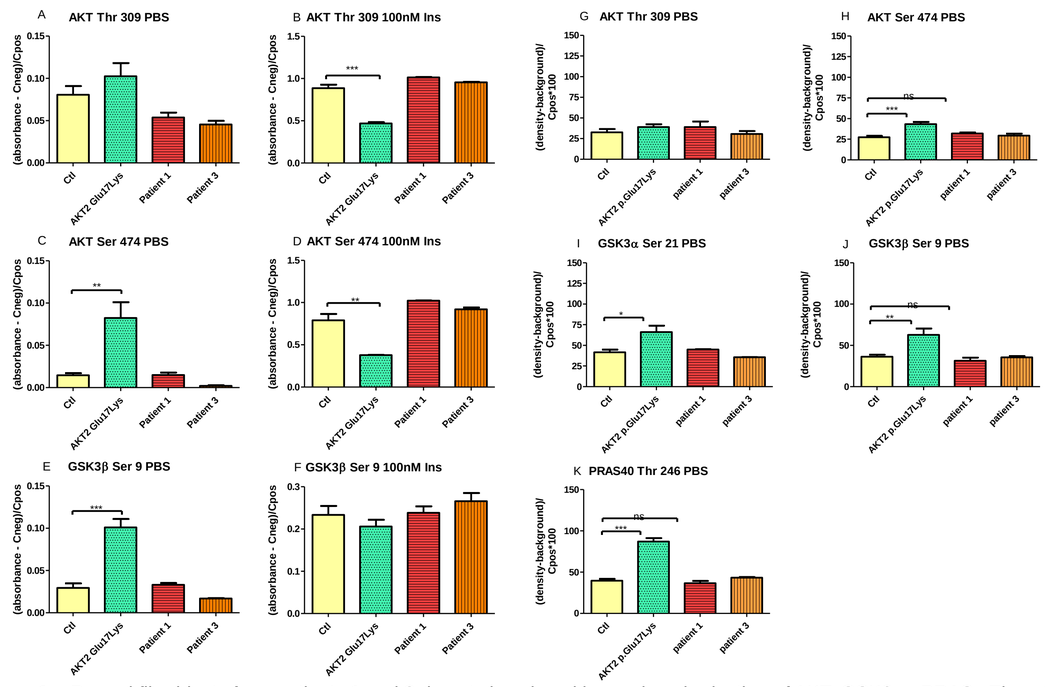

Figure 1 – Dermal fibroblasts from patients 1 and 3 do not show basal hyperphosphorlyation of AKT, GSK3 or PRAS. There is no blunted response to insulin in AKT2 phosphorlyation. A-F: ELISA G-K: quantified sandwich immunoassay
*: P ≤ 0.05; **: P ≤ 0.01; ***: P ≤ 0.001.

Analytic approach for novel syndromes

Further investigation is focused on identifying both the genetic and cellular mechanisms underlying these syndromes (Fig. 2). FOXO1 is a key transcriptional regulator in hepatic gluconeogenesis which is translocated into the nucleus during the fasted state. Permanent nuclear exclusion has been demonstrated in cells from patients with the *AKT2* p.Glu17Lys mutation.

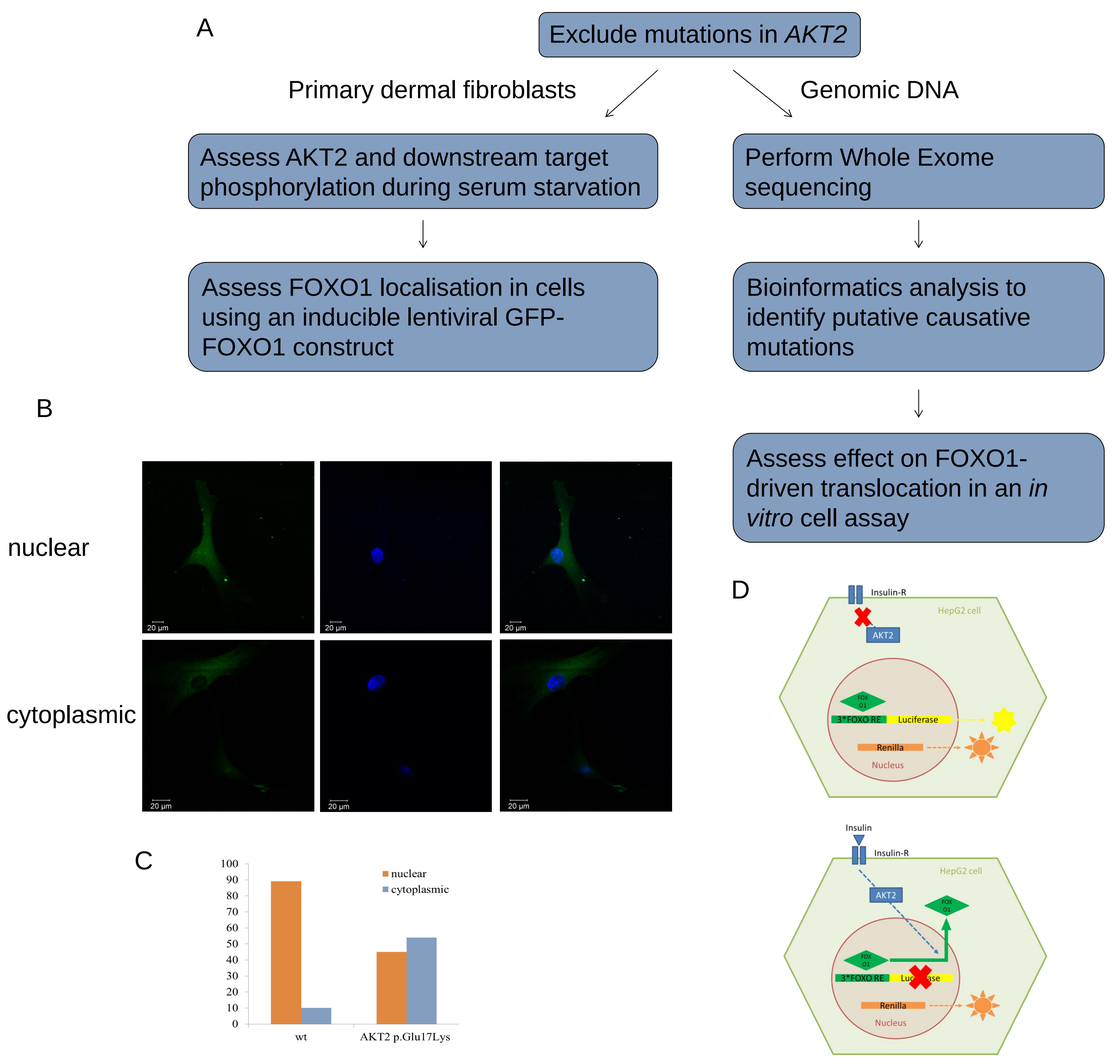

Figure 2 – Overview of approach taken to investigate these novel syndromes of hypoglycaemia
A) Schematic showing two-part approach with primary cell investigation and genetics
B) Examples of fibroblasts infected with a GFP-FOXO1 lentivirus in the serum starved state
C) Validation of lentiviral vector through blinded scoring of 100 cells
D) Diagram illustrating Dual Luciferase assay to investigate FOXO1 activity *in vitro*

Acknowledgments and references

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Conclusions

We present a group of five patients with a novel syndrome of hypoinsulinaemic, hypoketotic hypoglycaemia in the absence of a mutation in *AKT2.* The patients represent a biochemical phenocopy of the previously reported *AKT2* p.Glu17Lys patients but lack basal hyperphosphorlyation of AKT2 and downstream kinases. Furthermore, the patients presented have a varied group of additional clinical feature including asymmetric overgrowth, adrenal tumors, polycystic kidneys, jejunal atresia, muslce hypotonia and hepatic fibrosis. Further investigations are underway to determine the genetic causes and cellular mechanisms.