SUPPORTING ONLINE MATERIAL.

Materials and Methods:

Genetic studies. All exons and intron/exon boundaries of *AKT2* were screened for mutations using fluorescent single-stranded conformation polymorphism (SSCP) analysis and direct sequencing as previously described (*1*). SSCP primer sequences and conditions are described below. Family members were tested for the presence of the mutation by direct sequencing with dye termination cycle sequencing (BigDye v1.1, Applied Biosystems, Foster City, CA) on an ABI377. Genotyping of control subjects was outsourced to the MRC Geneservice (Babraham, Cambridge, UK).

AKT2 kinase activity. HA-tagged wild type AKT2 was subjected to site directed muatagenesis (Stratagene, La Jolla, CA) to create AKT2H274 and this was confirmed by direct sequencing. Chinese Hamster Ovary cells overexpressing the human insulin receptor (CHO-T cells) were transiently transfected with HA-tagged wild type AKT2 or AKT2H274 or empty vector. Following serum starvation for 16 hours, the cells were stimulated with 100nM insulin for 10 min then lysed. AKT2 was immunoprecipitated using an anti-HA antibody (Roche, Indianapolis, IN) and assayed for kinase activity using Crosstide as substrate (*2*). Identical immunoprecipitates were also subjected to western blotting for AKT expression with an anti-AKT antibody (Upstate Biotechnology). Results represent the mean±SEM of 4 experiments.

Effects of mutant AKT2 in hepatocytes. Immunofluorescence studies examining the effects of mutant and wild type AKT2 on FOXA2 translocation in HepG2 cells were undertaken as previously described (*3*). To assess FOXA2 transcriptional activity, we transfected HepG2 cells with pPCK1 promoter reporter construct, pCMV-β-Gal as internal reference and 125ng of expression vectors for wild type and/or mutant AKT1,

AKT2 and FOXA2 using the transfection reagent Fugene6 (Roche, Indianapolis, IN). Cells were grown for an additional 48 hours after transfection and luciferase activity was measured using the Luciferase Detection System (Promega, Madison, WI) essentially as previously described (*3*). Luciferase was normalized for transfection efficiency by correction for β-galactosidase activity. Transfection of HepG2 cells with vectors encoding wild type AKT2 or AKT2H274 followed by western blotting demonstrated equal expression of the two proteins for a given quantity of DNA transfected.

Analysis of mutant AKT2 in preadipocytes. Pools of murine 3T3-L1 preadipocytes stably expressing either wild type AKT2, AKT2H274 or empty vector were generated by transfecting cells using Lipofectamine Plus (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions then subjecting them to antibiotic selection using G418 at 1mg/ml 48 hours after transfection and maintaining them in the selection medium thereafter. For all experiments cells were plated in the absence of G418. Cells were induced to differentiate into adipocytes as in (*4*).

Hyperinsulinemic Euglycemic Clamp. A variable rate insulin infusion was administered between 1800h and 0800h to maintain euglycemia (5mmol/l). A two-step hyperinsulinemic (2.5 and 10mU/kg/min) clamp was then performed between 0800h and 1200h incorporating infusion of [6,6 $^{2}H_{2}$] glucose to allow calculation of peripheral glucose utilization (Rd) and endogenous glucose production (Ra) as previously described (*5*). Glucose infusion rate (mg/kg/min) was calculated during the final 30 minutes of each step of the clamp and compared with that of 47 non-Type 2 diabetic subjects. The control group consisted of 25 healthy individuals and 22 Type 1 diabetics with a median age of 21.6 years. (inter-quartile range 18.7-28.0) and median BMI of 22.5 kg/m^2 (inter-quartile

range 20.2-25.2). Ra and Rd were calculated in 25 of these non-Type 2 diabetic subjects with a median age of 19.0 years. (inter-quartile range 18.1-25.3) and median BMI 24.9 kg/m^2 (inter-quartile range 22.1-27.1).

Primer sequences and PCR conditions. Exon 1 is comprised solely of 5'untranslated sequence and was not screened for mutations. Genomic DNA from subjects was randomly pre-amplified in a 50µl PEP (primer extension preamplification) reaction (*6*). PEP amplified DNA was diluted 8-fold in water and 4µl of this dilution was subjected to further amplification of the *AKT2* gene using gene specific primers (Table S2). Twenty one primer pairs were required to span the entire coding region of *AKT2* in PCR products of sizes up to 350bp. PCR was performed using standard protocols with AmpliTaq Gold (Roche, Indianapolis, IN). All primers were optimised to anneal at 60-65^{0}C. After PCR amplification, PCR products were subjected to SSCP and products showing abnormal patterns were subsequently sequenced using ABI big-dye terminator (Perkin Elmer, Wellesley, MA). Second confirmatory sequencing reactions from the proband and relatives was performed using genomic DNA not pre-amplified by PEP.

Supplementary Text:

During the first step of the clamp the glucose infusion rate (GIR) was 0.99 mg/kg/min, significantly lower than control data (Fig. S1). During the second step the GIR remained remarkably low at 3.6 mg/kg/min despite an insulin concentration of 7346pmol/l. In order to selectively examine the effects of insulin on hepatic glucose production (Ra) and peripheral glucose utilization (Rd), we considered the change in Ra and Rd per unit change in insulin concentration (UΔIC) during the first step of the clamp. In control subjects Ra was suppressed by 0.075μmol/kg/min/UΔIC (SD 0.034), versus 0.004 μmol/kg/min/UΔIC in the proband. Rd was increased by 0.156 μmol/kg/min/UΔIC (SD 0.09) in the controls versus 0.002μmol/kg/min/UΔIC in the proband. Even during the second step of the clamp Ra was not fully suppressed at 1.97 μmol/kg/min and Rd remained submaximal at 20.3 μmol/kg/min.

Fig. S1

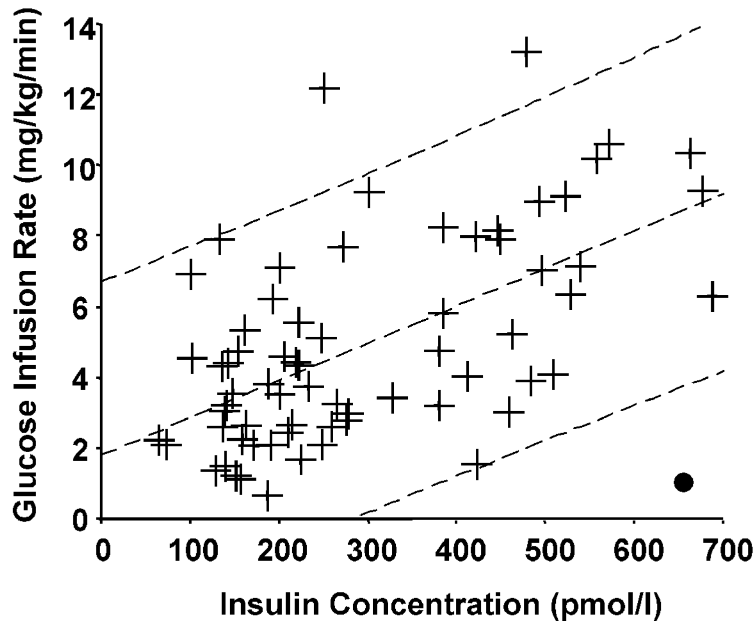

Fig. S1. Assessment of insulin sensitivity in the proband. Glucose infusion rate (mg/Kg/min) plotted against plasma insulin concentration (pmol/l) in 47 control subjects (black crosses) and the AKT2H274 proband (black circle) during the steady state of a hyperinsulinaemic clamp. The broken lines represent mean and 95% confidence intervals for the control population.

Table S1. Clinical and biochemical characteristics of subjects.

Subject	AKT2H274 Heterozygotes				Wild Type Homozygotes		
	(iii) 1 Proband	**(ii) 2**	**(ii) 3**	**(i) 2**	**(iii) 2**	**(ii) 1**	**(i) 1**
Gender	F	F	M	F	M	M	M
Age	35*	45	55	66	22	47	76
BMI (kg/m^2)	23.5	23.9	28.3	15.3	23.7	24.9	32.2
Diabetes	YES	YES	NO	YES	NO	NO	NO
Age of onset	30	38	N/A	37	N/A	N/A	N/A
Insulin Treatment	YES	YES	N/A	YES	N/A	N/A	N/A
Acanthosis nigricans	YES	YES	YES	YES	NO	NO	NO
Fasting insulin (pmol/l) (normal < 100 pmol/l)	180*	112	148	N/D	61	22	49
Post Prandial insulin (pmol/l) (normal<500 pmol/l)	5300*	N/D	2190	580	N/D	N/D	N/D
Hypertension	YES	YES	YES	N/K	NO	NO	YES

Reference numbers for subjects are as indicated in figure 1D. N/A Not applicable, N/K Not known, N/D Not determined. * Measurements made 12 years prior to diabetes onset.

Table S2 – Sequence of primers used for SSCP analysis and sequencing

Exon	Forward primer 5'-> 3'	Reverse primer 5' ->3'
2	ccctgaccagctcctctctt	tcaggctggtaagaccctcc
3	tcctctgggagaggcattct	gtacccaatgaaggagccgt
	ttcctgcctcatttcaggtg	agtcccacaagcccctaaga
4	gggcttctctccttccacac	cctctcgatgactgtggtcc
	ctgtctgttccctctgcctg	ccagggaaaatctctccagc
5	gaggtggcactcagctgttc	catctcctcagtcgtggagg
	actacaagtgtggctccccc	gccccctgaactgtgttatg
6	ccgtttggcaacagtgtctt	tcttcatggcgtagtagcgg
	tttggcaaagtcatcctggt	ataagcccacagcagcagaa
7	tttctgcatttatggctggg	ctcacactgtctgggaaggg
8	gtgtgcagaataagcagggc	catctcacccacagctcctc
9	tgccttgattggtccctcta	agccgagacaatctctgcac
	gtgtcttcacagaggagcgg	tgtgagtcccatgtggtgtg
10	atctggcctctctctgagcc	ttgcagaggccaaagtcagt
	ggaaaacctcatgctggaca	ttggcctcacacgttcctac
11	taggaacgtgtgaggccaag	ccatgaggatgagctcgaag
	gcccttctacaaccaggacc	acgacacactgcgaccctac
12	atcattaagggccatacaggg	caaccaaggtcaccacgagt
13	cactcgtggtgaccttggtt	atggactgggcggtaaattc
	tcaaacctcaggtcacgtcc	tgagagcagacttggggaaa
14	cctctcctgactggtcctcc	aagaactggaaaggggtga

References.

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