Protein	Yield (mg)	$S_{0.5}$ (mmol/L)	Hill (nH)	^{ATP}Km (mmol/L)	K_{cat} a (s^{-1})	K_{cat} b (s^{-1})	RAI	Predicted GSIS threshold for heterozygous carriers (mg/dl (mmol/L))
WT	31.25	7.98±0.16	1.58±0.02	0.51±0.01	61.47±0.32	49.50±0.56	1.00	90 (5.0)
R43P	3.41	222.38±2.43	1.59±0.01	0.35±0.01	7.09±0.05	7.23±0.08	0.000005	129.6 (7.2)
T206M**	5.07	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Supplemental Table 1. Kinetic characterization of mutant GCK enzymes. Results are based on the kinetic analysis of one preparation of human wild type and mutant GST-GCK proteins. Glucose affinity **($S_{0.5}$),** Hill number (**nH**), and turnover number (**K_{cat}**) values were determined in the presence of 0-100 mmol/L glucose (WT-GCK) or 0-1000mmol/Lglucose (R43P- and T206M-GCK). Affinity for ATP (**^{ATP}Km**) was determined in the presence 0-5mmol/Lof this substrate. **K_{cat} a** values were determined from glucose $S_{0.5}$ assays, and **K_{cat} b** values were determined from ^{ATP}Km assays. Relative activity indices (**RAI**) were calculated for each enzyme using the equation first described by Christesen et al which normalizes to a blood glucose of 90 mg/dl (5mmol/L) (K_{cat} values were taken from the glucose $S_{0.5}$ assay) (1). Data are presented as mean ±SEM. Glucose stimulated insulin secretion (GSIS) thresholds were calculated using the equation described by Gloyn et al (2). **As data were not quantifiable for this mutant protein a GSIS threshold could not be predicted. N/A = data unobtainable as mutation resulted in a protein with such poor affinity for glucose that its functional parameters could not be calculated.

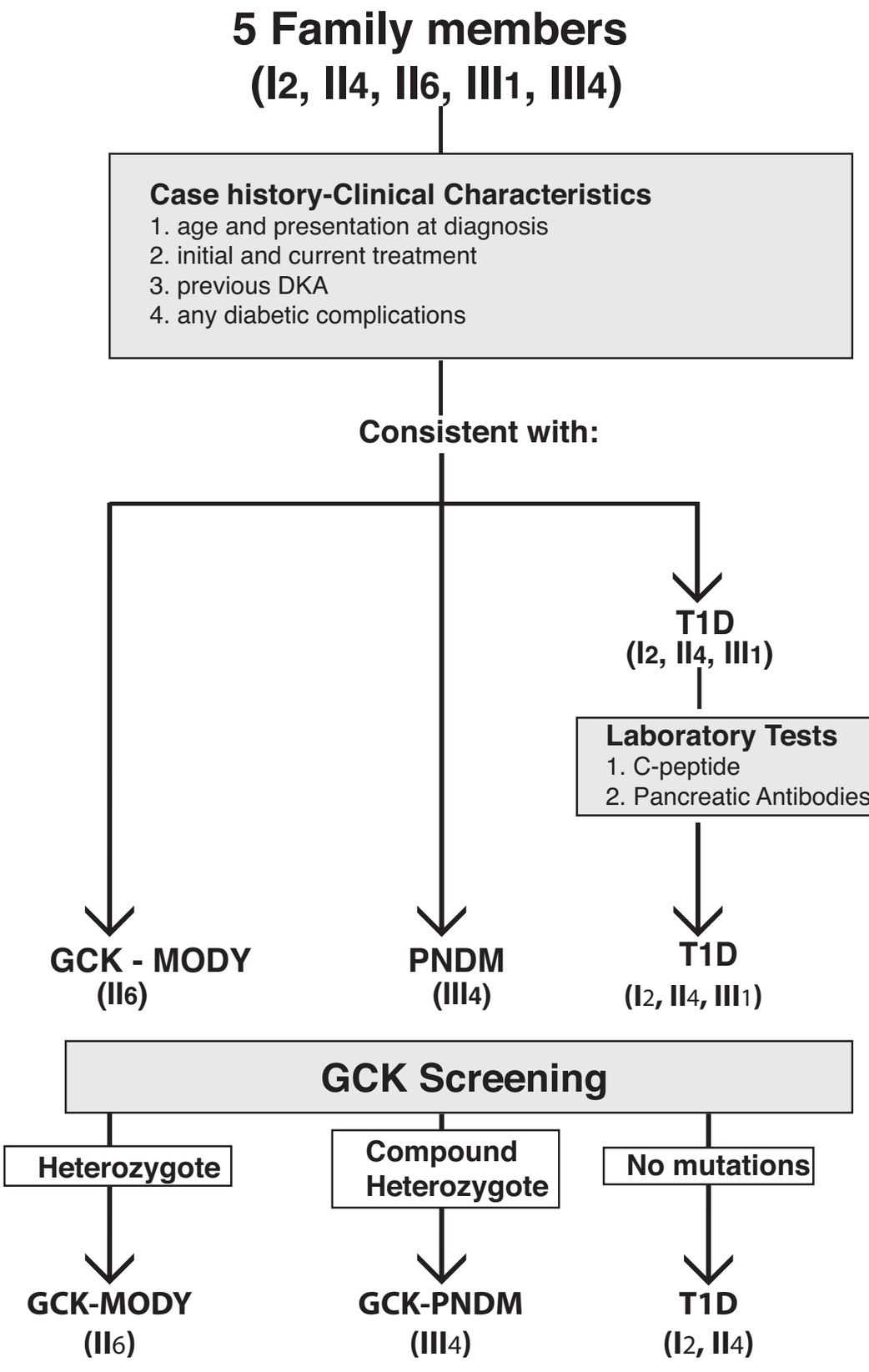
Supplemental Figure 1: Algorithm illustrating the systematic approach followed to investigate other family members with diabetes following identification of the p.T206M mutation in *GCK* and diagnosis of GCK-MODY in II-2.

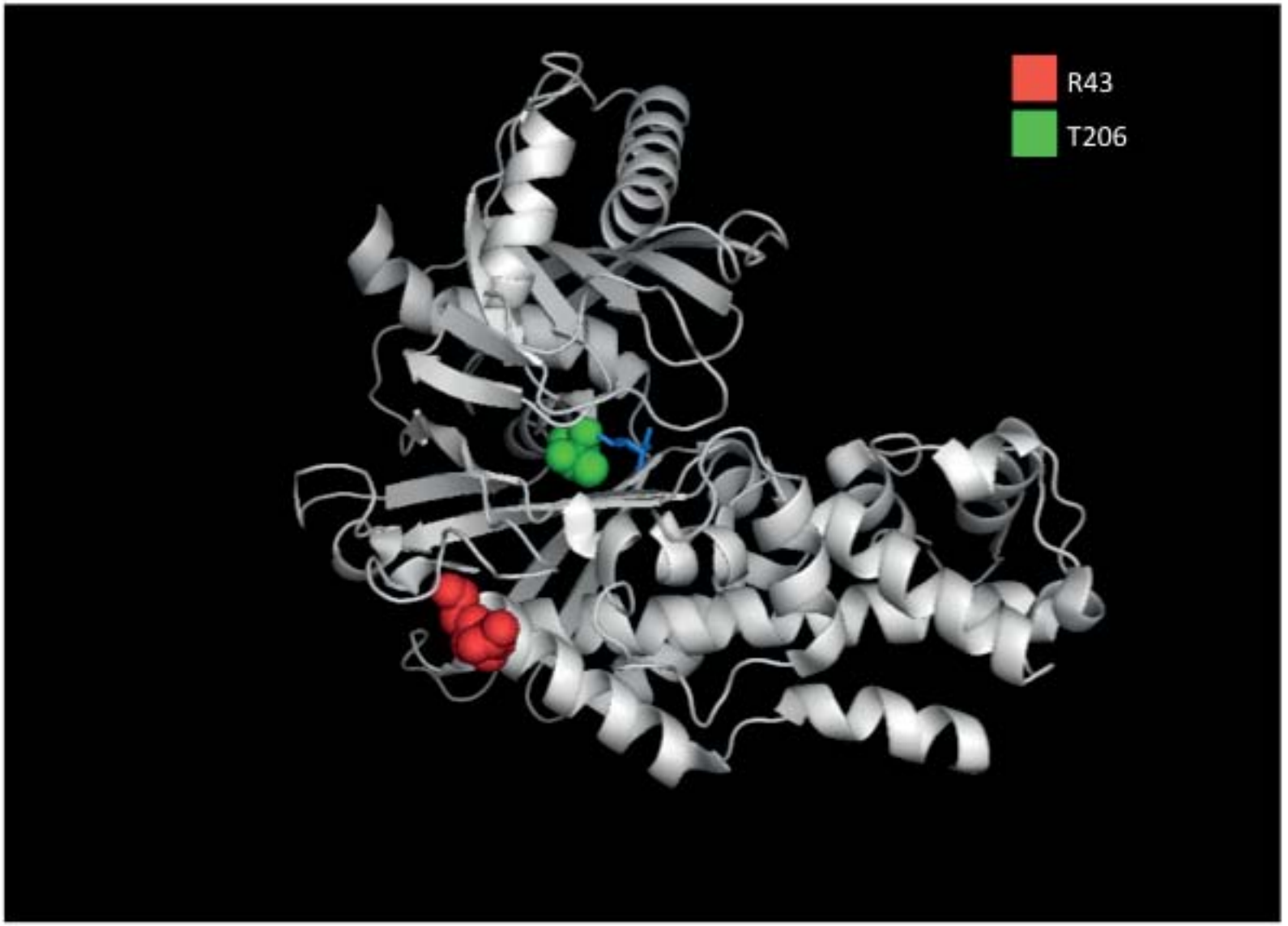
Supplemental Figure 2. Ribbon model of the closed (glucose-bound) form of human GCK illustrating each of the 2 missense mutations. Glucose is indicated in stick form in the centre of the active site.

References:

1. **Christesen, H.B., Jacobsen, B.B., Odili, S., Buettger, C., Cuesta-Munoz, A., Hansen, T., Brusgaard, K., Massa, O., Magnuson, M.A., Shiota, C. et al**. 2002 The second activating glucokinase mutation (A456V): implications for glucose homeostasis and diabetes therapy. Diabetes, 51, 1240-1246.

2. **Gloyn AL, Odili S, Buettger C, Njolstad PR, Shiota C, Magnuson MA, Matschinsky FM** 2004 Glucokinase and the Regulation of Blood Sugar. In: Matschinsky FM, Magnuson MA eds. Glucokinase and Glycaemic Disease: From Basics to Novel Therapeutics. Basel: Karger; 92-109


5 Family members
(I2, II4, II6, III1, III4)
Case history-Clinical Characteristics
1. age and presentation at diagnosis
2. initial and current treatment
3. previous DKA
4. any diabetic complications
Consistent with:
T1D
(I2, II4, III1)
Laboratory Tests
1. C-peptide
2. Pancreatic Antibodies
GCK - MODY
(II6)
PNDM
(III4)
T1D
(I2, II4, III1)
GCK Screening
Heterozygote
Compound Heterozygote
No mutations
GCK-MODY
(II6)
GCK-PNDM
(III4)
T1D
(I2, II4)


R43
T206