The previously reported T342P *GCK* missense variant is not a pathogenic mutation causing MODY

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Abbreviations

GCK Glucokinase
SNP Single nucleotide polymorphism

To the Editor: There is renewed interest in the difficulties of interpreting the role of missense variants identified in large scale medical resequencing projects made possible by next

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generation sequencing technologies. To assist in ascribing a role in disease causation for these variants, a combination of statistics, bioinformatic tools and, ultimately, functional studies, will be needed. Missense variants identified in highly penetrant Mendelian disorders where co-segregation studies can be performed provide a model to explore the utility of these tools.

The glycolytic enzyme glucokinase plays a key role in glucose stimulated insulin secretion and has been termed the pancreatic beta cell glucose sensor [1]. Heterozygous inactivating mutations in the *GCK* gene encoding glucokinase cause a subtype of MODY characterised by mild fasting hyperglycaemia (fasting plasma glucose 5.5–8 mmol/l, HbA_{1c} ≤7.5% [58 mmol/mol]) [2]. Over 600 different missense, nonsense, frameshift and splice site *GCK* mutations have been reported in patients with glucokinase (GCK)-MODY (dominant inactivating mutations), GCK-PNDM (permanent neonatal diabetes; recessive inactivating mutations) or GCK-HH (hyperinsulinaemic hypoglycaemia; dominant activating mutations) and are reviewed by Osbak and colleagues [3]. Of the mutations in this recent review, the majority are missense (402/620 [65%]) and most of these (392/402 [97%]) are inactivating mutations causing hyperglycaemia. A preponderance of missense mutations (224/402 [56%]) have only been reported in a single family [3]. Surprisingly, of the 29 single nucleotide polymorphisms (SNPs) described within the coding region of the pancreatic isoform, only one (D4N) results in a missense substitution [3]. T342P (c.1024A>C; p.Thr342Pro) has previously been reported as a pathogenic mutation following its identification in a single family (family SQ) [4]. We now report a second, unrelated family (family GB) with this variant and our clinical and functional studies suggest that T342P should be re-classified as a rare, non-pathogenic variant.

Capillary sequencing of *GCK* (NM_000162.3) in the probands (SQ and GB) from both families identified the heterozygous T342P variant. Eleven additional relatives underwent genetic testing and had fasting blood glucose measured. In both families we observed non co-segregation of the T342P substitution; four participants with the variant had a fasting glucose less than 5.5 mmol/l and three participants who did not inherit the variant had diabetes or a raised fasting glucose level (Fig. 1). At referral for genetic testing, SQ's most recent OGTT was consistent with a *GCK* mutation (0 h=6 mmol/l, 2 h=8.5 mmol/l). Ten years later she has a BMI of 40 kg/m², takes triple therapy for hyperglycaemia (metformin, glimepramide and pioglitazone) but remains hyperglycaemic with an HbA_{1c} level of 8.3% (67 mmol/mol). Abnormalities (mutations and gene

deletions) in *HNF1A* and *HNF4A* were excluded by direct sequencing and multiplex ligation-dependent probe amplification (MLPA) analysis, and a diagnosis of type 2 diabetes is most likely. Proband GB was referred for testing aged 9 years with an HbA_{1c} level of 11% (97 mmol/mol). On insulin she had good control but cessation of insulin resulted in thirst, lethargy and abdominal discomfort. Home blood glucose testing showed a rise to over 20 mmol/l over a 3 day period. This presentation is consistent with type 1 diabetes, although GAD65 and IA2 pancreatic auto-antibody tests were negative. Sequence and dosage analysis of *HNF1A/4A* failed to detect a mutation.

Functional characterisation of T342P-GCK was performed as previously described to investigate the effect of this amino acid substitution on enzyme kinetics [5]. Mutant

Fig. 1 Partial pedigrees showing status of (a) family SQ and (b) family GB. Affected (fully shaded), mild hyperglycaemia present (grey shading), unaffected (no shading). N/N indicates no mutation found. Arrow indicates proband. Current age for normoglycaemic participants provided and age at hyperglycaemia diagnosis provided for others; current BMI and current glycaemic status provided. BG, blood glucose; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus

T342P-GCK demonstrated near normal kinetics with a slight increase in enzyme activity (K_{cat}). Mathematical modelling based on the kinetic characteristics of wild type and mutant glucokinase predicted a threshold for glucose stimulated insulin release of 4.9 mmol/l comparable with wild type (5.0 mmol/l) (electronic supplementary material [ESM] Table 1). Our in vitro studies were in line with in silico predictions using SIFT, PolyPhen and Mutation Taster, which reported the T342P substitution as likely to be non-pathogenic. SIFT reported that proline is tolerated at residue 342, PolyPhen predicted the variant as benign (position-specific independent count [PSIC] score=0.092; scores of ≤ 0.5 are predicted to be benign) and Mutation Taster predicted T342P as a polymorphism, with a high probability value for the prediction ($p=0.9599$). Three-dimensional modelling in PyMol indicated the T342P substitution was unlikely to cause perturbation to the protein structure.

A previous study reported the GCK T342P mutation as a pathogenic loss-of-function MODY mutation [4]. At that time, bioinformatic prediction software was not widely available and it was believed that all missense variants in GCK were likely to be pathogenic. In the current study, of 13 participants from two families, it is clear that GCK T342P does not co-segregate with diabetes or fasting hyperglycaemia. Furthermore, kinetic analysis of T342P-glucokinase function shows that the catalytic activity of the enzyme is not impaired.

In addition to this direct evidence that T342P is not pathogenic, the sequence data now available for conservation analysis show that threonine is not strongly conserved at position 342, and three-dimensional protein modelling using PyMol suggests no impact on protein function. To date there are no other reports of GCK variants at this position (dbSNP, build 131; 1000 Genomes Project release 6, December 2010). Whilst in silico analyses should be interpreted with caution in the absence of other data, tools for predicting pathogenicity and effect upon protein structure have improved in recent years. A recent study showed >80% correct predictions by SIFT and PolyPhen for 67 loss-of-function mutations in ABCC8, KCNJ11 and GCK [6]. SIFT, PolyPhen and Mutation Taster all predicted GCK T342P to be a benign variant rather than a loss-of-function mutation. Thus, taken together with structural modelling and functional characterisation, the results of in silico analyses are consistent with the clinical data, which shows that the GCK T342P substitution is benign and not the cause of diabetes in the individuals reported. Therefore, the variant described in the original family was coincidental and was not causative of their diabetes [4].

There are previous reports of GCK mutations in which the enzyme produced has near normal or paradoxical kinetics [5, 7]. In some instances these mutations have

been shown to alter enzyme regulation by small molecular activators or glucokinase regulatory protein. In these cases, however, there has been clear support for pathogenicity from co-segregation studies [5, 8]. For the remaining variants where co-segregation has not been provided it is possible that these variants, like T342P, are rare polymorphisms.

Identification of a second family with the T342P variant and clinical phenotyping in both families has provided sufficient evidence that T342P is a rare polymorphism of no clinical significance. Unlike many genetic disorders, affection status in GCK-MODY is definitive since fasting hyperglycaemia is present from birth and easily measured. Our functional studies and in silico analysis were consistent with this finding. This study has important implications for diagnostic molecular genetics laboratories and clinicians alike. It highlights the importance of investigating all novel GCK missense substitutions by testing other family members for co-segregation of the variant with fasting hyperglycaemia. Such recommendations were included in the best practice guidelines for molecular genetic testing of MODY [2].

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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