Title	Prevalence of vascular complications among patients with glucokinase mutations and prolonged mild hyperglycemia
Date of revision	November 18, 2013
Word Count	3,133 (main text)
Authors	*Anna M Steele[1] (PhD), *Beverley M Shields[1] (PhD), Kirsty J Wensley[1] (AdDip Nursing), Kevin Colclough[2] (BSc), Sian Ellard[1,2] (PhD), Andrew T Hattersley[1] (DM) * These authors contributed equally to this work
Affiliations	1. NIHR Exeter Clinical Research Facility, Exeter Medical School, University of Exeter, UK 2. Department of Molecular Genetics, Royal Devon and Exeter NHS Foundation Trust, Exeter UK

Corresponding author:

Professor Andrew Hattersley, NIHR Exeter Clinical Research Facility, Exeter Medical School, Barrack Road, Exeter, Devon, EX2 5DW, UK

Email: A.T.Hattersley@exeter.ac.uk

Tel: +44 (0)1392 406806

Fax: +44 (0)1392 406767

Abstract

Context
Glycemic targets in diabetes have been developed to minimize complication risk. Patients with heterozygous inactivating glucokinase (*GCK)* mutations have mild fasting hyperglycemia from birth, resulting in an elevated HbA1c that mimics recommended levels for type 1 and type 2 diabetes.

Objective
To assess the association between chronic mild hyperglycemia and complication prevalence and severity in patients with *GCK* mutations

Design, Setting and participants
Cross sectional study in the UK between August 2008 and December 2010. Assessment of microvascular and macrovascular complications in participants ≥ 35 years was conducted in 99 *GCK* mutation carriers (median age 48.6 years), 89 non-diabetic, familial non-mutation carriers (controls) (52.2 years), and 83 individuals with type 2 diabetes diagnosed at <45 years (YT2D)(54.7 years).

Main outcome measures
Prevalence and severity of retinopathy, nephropathy, peripheral neuropathy, peripheral vascular disease, and cardiovascular disease

Results
Median HbA1c was 6.9% in *GCK* patients 5.8% in controls, and 7.8% in YT2D patients. *GCK* patients had a low prevalence of clinically significant microvascular complications (1% [95% CI 0-6%]) that was not significantly different from controls (2% [0.2-8%], p=0.52) and lower than in YT2D patients (36% [25-47%], p<0.001). Thirty percent of *GCK* patients had retinopathy (21-41%) compared to 14% of controls (7-23%, p=0.007) and 63% of YT2D patients (51-73%, p<0.001). Zero *GCK* patients or controls required laser therapy for retinopathy compared to 28% (18-39%) of the YT2D patients (p<0.001). Zero *GCK* patients or controls had proteinuria, and microalbuminuria was rare (*GCK* 1% [0.2-6%], controls 2% [0.2-8%]), whereas 10% (4-19%) of YT2D patients had proteinuria (p<0.001 vs. *GCK*) and 21% (13-32%) had microalbuminuria (p<0.001). Neuropathy was rare in *GCK* patients (2% [0.3-8%]) and controls (0% [0-4%]) but present in 29% (20-50%) of YT2D patients (p<0.001). *GCK* patients had a low prevalence of clinically significant macrovascular complications (4% [1-10%]) that was not significantly different from controls (11% [6-20%]; p=0.09), and lower in prevalence than YT2D patients (30% [21-41%], p<0.001).

Conclusion
Despite a median duration of 48.6 years of hyperglycemia, patients with a *GCK* mutation had low prevalence of microvascular and macrovascular complications. These findings may provide insights into the risks associated with isolated mild hyperglycemia.

Introduction

In both type 1 (T1D)[1] and type 2 diabetes (T2D)[2] hyperglycemia over time is associated with microvascular complications. Intensive treatment to lower blood glucose levels reduces the development of microvascular complications.[3,4] In T1D, lowering the blood glucose has been shown to have long-term beneficial effects on reducing macrovascular disease[5]. In T2D, the follow-up of the United Kingdom Prospective Diabetes Study (UKPDS) showed that lowering the level of hyperglycemia reduces the risk of macrovascular endpoints[6]. Additionally, associations have been seen between measures of glycemia and coronary heart disease throughout the non-diabetic range[7].

Except for during pregnancy, a target hemoglobin A1c (HbA1c) <7% has been recommended for people with diabetes,[8,9] yet longitudinal studies have few patients with sustained glycemia within this recommended target[1,2]. It is therefore of clinical importance to know the complication prevalence and severity in individuals with a long and sustained duration of glycemia at a level above that of the non-diabetic population but that mimics the current target range of 7%. Individuals with a heterozygous inactivating mutation in the *GCK* gene encoding the enzyme glucokinase have mild hyperglycemia that is present from birth. Their HbA1c is typically between 5.6% and 7.6%[10,11] and their fasting plasma glucose between 5.5 and 8.7 mmol/l [11,12]. These patients rarely require pharmacological treatment[13] and lipids and blood pressure are similar to the general population[14,15]. The hyperglycemia in patients with a *GCK* mutation is therefore an isolated risk factor for complications [14,15].

We assessed the prevalence and severity of microvascular and macrovascular complications in patients with *GCK* mutations to give further information about the relationship between current glycemic targets and diabetes-related complications. We also assessed these outcomes in non-diabetic, non-mutation carrying controls and patients with young-onset type 2 diabetes (YT2D).

METHODS

Study population

The study was approved by the Devon and Torbay Research Ethics Committee, United Kingdom (UK) and the NHS Scotland Research Coordinating Centre, UK. Recruitment was undertaken between August 2008 and December 2010 and each patient provided written consent. Individuals known to have a *GCK* mutation through genetic testing in Exeter (the UK testing center) were invited to participate. The majority of patients with a GCK mutation were from the South West of England and Scotland, the two areas with the largest number of GCK cases in the UK. All family members were invited to participate, both *GCK* mutation carriers and non-mutation carriers, if aged ≥18 years. Where genetic status was unknown in family members, diagnostic molecular genetic testing was performed to ascertain their mutation status, usually after the assessment of glycaemia and micro- and macrovascular complications. To identify potential survival bias within families, we examined pedigrees of recruited *GCK* patients and assessed vital status of siblings and parents not recruited. We attempted to determine their mutation status, and when DNA of the deceased was not available, used the high penetrance of GCK-MODY mutations and autosomal dominant form of transmission to make obligate

assignments of mutation carriage to the diabetic parent of a known mutation carrier when the other parent was known not to have diabetes. Deaths were balanced between mutation and non-mutation carrier siblings and parents (see supplementary data).

YT2D patients were diagnosed with Type 2 diabetes before the age of 45 years, did not use insulin therapy within one year of diagnosis, and were over 35 years at the time of the study. These patients were recruited from an existing research cohort from South West England, prioritized by proximity to Exeter. Patients unable to travel to Exeter were assessed at home or at a local hospital.

Assessment of glycemia

HbA1c was measured on all patients and analyzed in the biochemistry laboratory at the Royal Devon and Exeter NHS Foundation Trust (UK) on a Tosoh G8 anion exchange HPLC.

Assessment of complications

Detailed methods of assessments are described in the online supplementary data. Data were collected by one of two researchers. The assessment of retinal photographs, biochemical measurements and ECG assessment were blinded to assignment. However, researchers were not always blinded to participant group during clinical assessments (see supplementary data). Inter-rater and intra-rater reliability analysis showed acceptable levels of measurement agreement (see supplementary data. First void, mid-stream urine samples were analyzed to assess nephropathy. Persistent microalbuminuria was

diagnosed when two albumin creatinine ratios (ACRs) were between 2.5 mg/mmol and 30 mg/mmol (males) and 3.5 mg/mmol and 30 mg/mmol (females)[16]. Proteinuria was diagnosed when two ACRs were >30mg/mmol (male or female) and reported as a protein/creatinine ratio. Retinopathy was assessed using bilateral digital images. Images were graded by two readers, who were blinded to each other's results, using the English Retinopathy Minimum Grading Classification[17] (eTable 2). Peripheral neuropathy was assessed using Vibration Perception Threshold. Cutaneous perception was assessed using a 10g Semmes-Weinstein monofilament. To assess peripheral vascular disease (PVD), intermittent claudication was defined by clinical diagnosis or by a positive San Diego Claudication Questionnaire [18] and the ankle-brachial pressure index was measured. To assess cardiovascular disease, patients completed the World Health Organization (Rose) chest pain questionnaire[19]. A resting 12 lead electrocardiogram (ECG) was performed on those aged ≥40 years and assessed using Minnesota coding[20]. Participant-reported episodes of angina, myocardial infarction (MI) and stroke were documented and confirmed by reviewing participants' medical records. When adjudication of an endpoint was required this was provided by a senior clinician.

Statistical Analysis

The majority of data were not normally distributed so data are presented as median and inter-quartile range. For the linear association between HbA1c and age, a bivariable linear regression model was used to determine the increase in HbA1c per year of age, with HbA1c as the dependent and age as the independent variable. Model assumptions of linearity between predictor and outcome, normality of

residuals, and homoscedasticity were checked and met. Comparisons of complications between groups were assessed using Mann Whitney U and Kruskal-Wallis tests for continuous variables and Chi-squared and Fisher's Exact tests for discrete variables.

All significance tests were two sided and $p<0.05$ was considered statistically significant. Analysis was carried out using IBM SPSS Statistics v19.

RESULTS

We recruited 126 individuals aged >=18 years with an inactivating heterozygous *GCK* mutation (mutation details in eTable 1), 107 unaffected family members/spouses (controls), and 83 individuals with type 2 diabetes diagnosed ≤45 years (young-onset type 2 diabetes (YT2D)) from across the UK. All patients were white as reported by the patents' referring clinicians. Nine patients with a *GCK* mutation were excluded due to their potential for coincidental T1D or T2D. We identified these individuals using a robust outlier detection method as described by Horn to identify those above the normal range (HbA1c >7.6%).[21] Two controls who met American Diabetes Association criteria for diabetes (HbA1c > 6.5%) were also removed from analysis.

Figure 1 shows that patients of all ages with a *GCK* mutation have mild hyperglycemia as measured by HbA1c. HbA1c was consistently higher in *GCK* patients (n=117) compared with controls (n=105) (median (IQR) 6.8%, 6.5-7.1 vs. 5.7%, 5.5-5.9 ($p<0.001$). Glycemic levels were higher in older *GCK* carriers and controls, with a slope of 0.17%

per year for *GCK* carriers (95% CI 0.12-0.22, p<0.001) and a slope of 0.12% per year (95% CI 0.08-0.16, p<0.001) in controls, p=0.1 for difference between slopes).

To examine those most likely to have developed complications, assessments were performed in a subgroup of patients aged ≥35 years (99 *GCK* and 91 controls from 44 families, and 83 YT2D). We compared enrolled *GCK* patients with all UK *GCK* patients using data from the Molecular Genetics Diagnostic Laboratory, Exeter (UK). The 99 *GCK* patients were similar in age to the 360 UK patients known to have a *GCK* mutation (median (IQR) 48 (40, 62) vs. 49 (42, 61) years; p=0.90) but had a higher BMI (26.1 (22.3, 29.6) vs. 24.5 (22.0, 28.0) kg/m^2; p=0.05) and were different in gender distribution (20% vs. 32% male, p=0.02). The 83 YT2D participants were similar to the cohort of 397 YT2D they were recruited from the Diabetes Alliance for Research in England, in age (54 (49, 62) vs. 52 (46, 63) years; p=0.26), BMI (32.2 (28.3, 37.0) vs. 32.4 (28.0, 37.7) kg/m^2; p=0.98), and gender (63% vs. 60% male, p=0.62).

In the subgroup of patients aged ≥35 years, the level of hyperglycemia (HbA1c and FPG) was higher in GCK patients than in controls but milder than in patients with YT2D (Table 1). Duration of hyperglycemia was longer in patients with a *GCK* mutation (48 years (median), duration equal to current age) compared with YT2D patients (known to have diabetes for 17 years (median), p<0.001). 22% of patients with a *GCK* mutation were taking glucose lowering agents (0% insulin) compared with 90% of YT2D patients (60% insulin), p<0.001 (Table 1).

Microvascular Complications

Overall, the prevalence of clinically significant microvascular complications (>background retinopathy or persistent microalbuminuria or proteinuria) was low in patients with a *GCK* mutation (1% [95% CI 0-6%]) and not significantly different from controls (2% [0.2-8%], p=0.52). In contrast, 36% (95% CI: 25, 47%) of the YT2D patients had evidence of clinically significant microvascular disease (p<0.001 vs *GCK* mutation patients) (Table 3).

Persistent microalbuminuria was rare in patients with a *GCK* mutation (1/97 (1% (95% CI: 0.2, 6%)) and in controls (2/89 (2% (95% CI: 0.2, 8%)) (Table 3). This rate was lower than that seen in YT2D where 17/80 (21% (95% CI: 13, 32%)) were identified (p<0.001 vs. *GCK* mutation patients). Zero patients had persistent proteinuria in the *GCK* and control groups whereas 8/80 (10% (95% CI: 4, 19%)) of YT2D patients had this condition (p<0.001 vs. *GCK* mutation patients).

A higher prevalence of any level of retinopathy was seen *GCK* mutation carriers compared with controls (27/90, 30% (95% CI: 21, 41%) vs. 12/87, 14% (7, 23%), p=0.007). However, this was exclusively due to background retinopathy, and 22/27 (81%) of those with background retinopathy had minimal disease with fewer than five microaneurysms. A larger percentage of those with YT2D had any retinopathy (52/83(63% (95% CI: 51, 73%), p<0.001 vs. *GCK* mutation patients). Additionally, the degree of retinopathy in the YT2D group was more severe, with maculopathy in 17/83 (20% (95% CI: 12, 31%)) patients compared to 0/90 *GCK* mutation patients (p<0.001)

and laser therapy in 23/83 (28% (95% CI: 18, 39%)) patients compared to 0/90 *GCK* mutation patients (p<0.001).

Peripheral neuropathy was rare in patients with *GCK* mutations (2/93 (2% (95% CI: 0.3, 8%))) and zero cases were found in controls (0% (95% CI: 0, 4%)). In contrast, 24/83 (29% (95% CI: 20, 50%)) of the YT2D group had peripheral neuropathy (p<0.001 vs. *GCK* mutation patients).

Macrovascular Complications

The prevalence of known clinically diagnosed macrovascular complications was low in patients with a *GCK* mutation and controls (4/99, 4% (95% CI: 1, 10%) and 10/91, 11% (95% CI: 5, 19%) respectively). This contrasts with the YT2D patients where 30% (95% CI: 21, 41) had clinically diagnosed macrovascular disease (p<0.001, Table 2).

There were zero cases of intermittent claudication in patients with a *GCK* mutation or controls and 5/83 (6% (95% CI: 0, 13%)) in the YT2D group (Table 3). The prevalence of PVD (ABPI <0.5 or ABPI ≥ 1.40 or amputation or intermittent claudication) was low in patients with a *GCK* mutation and controls (1% and 3% respectively). The prevalence was significantly higher in the YT2D group (13/83 (16% (95% CI:8, 25%)), p<0.001 vs *GCK* mutation patients).

The presence of ischemic heart disease (IHD) was low in patients with a *GCK* mutation (2/99, 2% (95% CI: 0.2-7%)) and controls (5/91 ,5% (95% CI: 2-13%)). The prevalence

of IHD was higher in YT2D patients (13/83 (16% (95% CI: 10-29%)), p=0.001 vs. *GCK* mutation patients). Minnesota coding identified probable coronary disease in 9/69 (13%) of patients with a *GCK* mutation and 20/81 (25%) of controls, and in 19/75 (25%) of YT2D patients. Zero patients in either the *GCK* or control groups had suffered from a stroke compared with 4/83 patients (5%) in the YT2D group.

DISCUSSION

Patients with a *GCK* mutation have a low prevalence of clinically significant microvascular and macrovascular complications despite their hyperglycemia since birth [10,11]. In these patients, an average of nearly fifty years of isolated hyperglycemia within current target ranges for diabetes control had a negligible association with complication development. This work is, to our knowledge, the first systematic assessment of complication development in patients with a *GCK* mutation.

Previous studies have suggested that complications are rare in patients with *GCK* mutations, [10,14,15] but, unlike our study, these studies mainly used data from clinical notes[10,15], reviewed a small number of patients[10,14], did not include a control group[10,15,22] or had a mean age of study of <37 years[10,14,15]. We have used clinically recognized and standardized techniques for each participant, with high levels of intra and inter-observer reproducibility and reliability. The prevalence and severity of complications in patients with *GCK* mutations was similar to that of controls. The presence of complications in our control group was not surprising, as hyperglycemia related complications have previously been reported in the general population[23-27]. In contrast to other studies, we

have addressed the potential issues of selection bias and survival bias. The *GCK* and YT2D patients were generally representative of the large cohorts from which they were selected. There is no evidence to suggest that *GCK* patients were excluded from the study because of premature death.

Retinopathy (evidence of at least one microaneurysm) in people without diabetes occurs in 5–9% of the non-diabetic general population with an age range of 43-84 years[25,28]. The prevalence of background retinopathy in our control group was similar. We did identify a higher prevalence rate for background retinopathy in our patients with a *GCK* mutation, but the vast majority of these patients had mild background retinopathy with <5 microaneurysms. None of our patients with a *GCK* mutation had sight-threatening retinal disease. Previous studies in *GCK* mutation carriers found prevalence rates of proliferative retinopathy of 0-4%[14,15,22]. These studies used direct fundoscopy, whereas we used digital retinal imaging with primary, secondary and where required, arbitration grading and did not exclude concomitant Type 1 or Type 2 diabetes.

All other microvascular complications had comparably low prevalence rates in patients with a *GCK* mutation and in controls. Microalbuminuria has been reported in 6.6-9.4% of the non-diabetic population[23,24] and in 0-6% of patients with a *GCK* mutation[10,14,15,29]. Rather than relying on urinalysis sticks as in previous GCK studies[15,22] we excluded UTI and biochemically confirmed the diagnosis of microalbuminuria with 3 consecutive early morning urine samples. Our prevalence rates were similar to those previously reported in patients with a *GCK* mutation, but were lower than previously reported in the general

population. We did not identify any *GCK* patients with proteinuria. The prevalence of neuropathy was also low in *GCK* patients and controls in our study. Previous studies have reported a prevalence of 4-5% in *GCK* patients[10,14,15,29].

An increase in HbA1c of 1% results in a 10-20% increase in CVD risk and predicts cardiovascular risk both in people with diabetes and in the general population [30]. Our data suggest that macrovascular complications are not increased in individuals with *GCK* mutations and are in keeping with earlier studies reporting a prevalence of cardiovascular disease between 0.7-12%[10,14,15]. Our finding of a low prevalence of CVD in the *GCK* mutation carrier group, even with their slightly elevated HbA1c, provides evidence that isolated hyperglycemia is rarely associated with macrovascular complications. Although treatment is not advocated in patients with a *GCK* mutation outside pregnancy, 22% of our G*CK* cohort were treated with glucose lowering agents which is consistent with previous studies[13,22]. Recent work has shown that pharmacological treatment does not alter HbA1c in patients with a *GCK* mutation, so this is unlikely to confound our findings[13].

We have shown that patients with *GCK* mutations, who have mild hyperglycemia from birth, have significantly lower diabetes related complication prevalence and severity when compared to individuals with a shorter duration of more severe hyperglycemia (YT2D). Retinopathy, nephropathy, peripheral neuropathy and angina were all significantly more common in the YT2D group. However, several considerations warrant mentioning prior to extrapolating findings in patients with glucokinase mutations to other

subtypes of diabetes. Patients with *GCK* mutations have been shown to have a similar degree of insulin resistance and obesity as the general population[14,15] whereas the YT2D cohort has been exposed to many metabolic risk factors for complication development. Obesity, hypertension and dyslipidaemia found in T2D may alter the impact of glycaemia on the risk of complications. T1D and T2D patients have a more variable blood glucose profile compared with patients with a *GCK* mutation; this variability may impact on the complication prevalence and severity rates. Patients with *GCK* mutations are born with hyperglycemia so there may be early compensation for this, resulting in protection from vascular complications. It also means that *GCK* mutation patients are younger than patients diagnosed with diabetes later in life with the same duration of hyperglycemia. Age as well as duration is likely to be important in complication development. Despite these caveats, our ability to study individuals with isolated hyperglycemia has provided a useful natural experiment for understanding the development of complications.

Our study is limited by the relatively small number of patients known to have *GCK* mutations, which necessitates a cross sectional study rather than a longitudinal study and limits power. Although the prevalence of complications was low both in patients with a *GCK* mutation and controls, we are unable to prove equivalence with our sample size. We would need a control group over 10 times larger to detect statistical significance between patients with *GCK* mutations and controls at the prevalences we observed. However, with the exception of mild background retinopathy, the prevalence of all complications was very low in *GCK* mutation patients, with similar confidence intervals to controls, and significantly lower than those seen in the YT2D group. Even if proved to

be statistically different, it is unlikely that differences between the low prevalence rates seen would be of clinical significance. Our definition of the YT2D cohort's duration of hyperglycemia is not precise: individuals may be unaware they have T2D and complications can occur in up to 50% of people before a medical diagnosis for symptoms is sought [31]. Even with this likely underestimation of duration of hyperglycemia in the YT2D cohort, their duration will not be as long as the patients with a *GCK* mutation, whose hyperglycemia is present from birth. Because the researchers who conducted clinical assessments on macrovascular disease were aware of the clinical category of many of the patients, not all clinical assessments were collected in a blinded fashion. Finally, there were more females recruited in our *GCK* mutation group. This is unlikely to impact microvascular disease rates but could affect macrovascular disease rates. With so few cases in the *GCK* group, it is not possible to adjust for this difference statistically. However, the prevalence of coronary heart disease in the *GCK* mutation group for both men and women is similar to that reported in England for a similar age range (45-54 years)[32]: 5% v 3.6% in men and 1.2% vs. 1.3% in women. Although these results are similar to those of the general population, our confidence intervals were wide and larger numbers would be required to investigate this further.

Conclusions

Patients with *GCK* mutations over a median of 48.6 years had a low prevalence of vascular complications. These findings provide insights into the risks associated with isolated mild hyperglycemia.

Acknowledgements

Anna M Steele had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

No authors have any potential conflicts of interest, including financial interests, activities and/or relationships.

AMS was funded by the Diabetes UK (grant number BDA:RD07/0003473) and the National Institute for Health Research (NIHR). AMS, BS, KJW, ATH and SE are employed as core members of staff within the NIHR Exeter Clinical Research Facility where this research was undertaken. ATH is an NIHR Senior Investigator.

ATH and SE are Wellcome Trust Senior Investigators.

The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

The research leading to these results has also received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement nº223211, CEED3.

Role of the sponsors: The funding organizations had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Preliminary results pertaining to this paper have been presented at the Diabetes UK Annual Professional Conference. Steele AM et al 2013. Low prevalence of diabetes complications after 48 years of mild hyperglycaemia in patients with glucokinase mutations supports current glycaemic targets for diabetes management. Diabetic Medicine 30 (suppl.1) A70.

We gratefully acknowledge the Exeter Retinal Screening Team for their donation of equipment, training and grading of images, Mrs AH Keen for the Minnesota Coding and Mr Steve Aldington (DMS and FBIPP) for his advice with regard to retinal imaging techniques and grading.

List of supplemental material:

Methods: Anthropometry, brachial blood pressure, nephropathy, peripheral neuropathy (neurothesiometer), peripheral vascular disease, cardiovascular disease, intra-rater reliability for height and ABPI measurements, inter-rater reliability for height and ABPI measurements, information regarding family members not recruited

eTable 1: Number of patients (n=126) and families (n=49) studied according to *GCK* mutation. *GCK* gene mutations are numbered with respect to GenBank cDNA sequence NM_000162.3. Numbering is based on +1 as the A of the major start codon of exon 1A

eTable 2: Retinal images grading based on the English Retinopathy Minimum Grading Classification (UKNSC)

References

1. DCCT. The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial. *Diabetes.* Aug 1995;44(8):968-983.

2. Stratton IM, Adler AI, Neil HA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *Bmj.* Aug 12 2000;321(7258):405-412.

3. Reichard P, Nilsson BY, Rosenqvist U. The effect of long-term intensified insulin treatment on the development of microvascular complications of diabetes mellitus. *N Engl J Med.* Jul 29 1993;329(5):304-309.

4. UKPDS. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* Sep 12 1998;352(9131):837-853.

5. Nathan DM, Cleary PA, Backlund JY, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med.* Dec 22 2005;353(25):2643-2653.

6. DCCT. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med.* 1993 1993;329(14):977-986.

7. Khaw KT, Wareham N, Luben R, et al. Glycated haemoglobin, diabetes, and mortality in men in Norfolk cohort of european prospective investigation of cancer and nutrition (EPIC-Norfolk). *Bmj.* Jan 6 2001;322(7277):15-18.

8. ADA. Standards of Medical Care in Diabetes - 2011. *Diabetes Care.* 2011 2011;34(Supplement 1):S11-S61.

9. Nathan DM, Buse JB, Davidson MB, et al. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care.* Jan 2009;32(1):193-203.

10. Sagen JV, Bjorkhaug L, Molnes J, et al. Diagnostic screening of MODY2/GCK mutations in the Norwegian MODY Registry. *Pediatr Diabetes.* Apr 9 2008;9:442-449.

11. Steele AM, Wensley KJ, Ellard S, et al. Use of HbA1c in the Identification of Patients with Hyperglycaemia Caused by a Glucokinase Mutation: Observational Case Control Studies. *PLoS One.* 2013;8(6):e65326.

12. Stride A, Vaxillaire M, Tuomi T, et al. The genetic abnormality in the beta cell determines the response to an oral glucose load. *Diabetologia.* Mar 2002;45(3):427-435.

13. Stride A, Shields B, Gill-Carey O, et al. Cross-sectional and longitudinal studies suggest pharmacological treatment used in patients with glucokinase mutations does not alter glycaemia. *Diabetologia.* Oct 4 2013.

14. Page RC, Hattersley AT, Levy JC, et al. Clinical characteristics of subjects with a missense mutation in glucokinase. *Diabet Med.* Mar 1995;12(3):209-217.
15. Velho G, Blanche H, Vaxillaire M, et al. Identification of 14 new glucokinase mutations and description of the clinical profile of 42 MODY-2 families. *Diabetologia.* Feb 1997;40(2):217-224.
16. Physicians RCo. Joint Specialty Committee on Renal Medicine of the Royal College of Physicians and the Renal Association, and the Royal College of General Practitioners. *Chronic kidney disease in adults: UK guidelines for identification, management and referral.* London. 2006.
17. UKNSC. Essential Elements in Developing a Diabetic Retinopathy Screening Programme: Workbook 4. 2007:1-75.
18. Criqui MH, Denenberg JO, Bird CE, Fronek A, Klauber MR, Langer RD. The correlation between symptoms and non-invasive test results in patients referred for peripheral arterial disease testing. *Vasc Med.* 1996;1(1):65-71.
19. Rose GA. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. *Bull World Health Organ.* 1962;27:645-658.
20. Prineas RJ CR, Blackburn H. *The Minnesota Code manual of electrocardiographic findings*. Bristol: John Wright; 1982.
21. Horn PS, Feng L, Li Y, Pesce AJ. Effect of outliers and nonhealthy individuals on reference interval estimation. *Clin Chem.* Dec 2001;47(12):2137-2145.
22. Velho G, Vaxillaire M, Boccio V, Charpentier G, Froguel P. Diabetes complications in NIDDM kindreds linked to the MODY3 locus on chromosome 12q. *Diabetes Care.* Sep 1996;19(9):915-919.
23. Hillege HL, Janssen WM, Bak AA, et al. Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. *J Intern Med.* Jun 2001;249(6):519-526.
24. Yudkin JS, Forrest RD, Jackson CA. Microalbuminuria as predictor of vascular disease in non-diabetic subjects. Islington Diabetes Survey. *Lancet.* Sep 3 1988;2(8610):530-533.
25. Klein R, Klein BE, Moss SE, Wong TY. The relationship of retinopathy in persons without diabetes to the 15-year incidence of diabetes and hypertension: Beaver Dam Eye Study. *Trans Am Ophthalmol Soc.* 2006;104:98-107.
26. Gregg EW, Gu Q, Williams D, et al. Prevalence of lower extremity diseases associated with normal glucose levels, impaired fasting glucose, and diabetes among U.S. adults aged 40 or older. *Diabetes Res Clin Pract.* Sep 2007;77(3):485-488.
27. Gregg EW, Sorlie P, Paulose-Ram R, et al. Prevalence of lower-extremity disease in the US adult population >=40 years of age with and without diabetes: 1999-2000 national health and nutrition examination survey. *Diabetes Care.* Jul 2004;27(7):1591-1597.
28. van Leiden HA, Dekker JM, Moll AC, et al. Blood pressure, lipids, and obesity are associated with retinopathy: the hoorn study. *Diabetes Care.* Aug 2002;25(8):1320-1325.
29. Byrne MM, Sturis J, Menzel S, et al. Altered insulin secretory responses to glucose in diabetic and nondiabetic subjects with mutations in the diabetes

susceptibility gene MODY3 on chromosome 12. *Diabetes.* Nov 1996;45(11):1503-1510.

30. Khaw KT, Wareham N. Glycated hemoglobin as a marker of cardiovascular risk. *Curr Opin Lipidol.* Dec 2006;17(6):637-643.

31. UKPDS SG. UK Prospective Diabetes Study 6. Complications in newly diagnosed type 2 diabetic patients and their association with different clinical and biochemical risk factors. *Diabetes Res.* Jan 1990;13(1):1-11.

32. Townsend N WK, Bhatnagar P, Smolina K, Nichols M, Leal J, Luengo-Fernandez R, Rayner M. *Coronary heart disease statistics 2012 edition.* London: British Heart Foundation;2012.

Figure Legends

Figure 1. Scatterplot of HbA1c by age in *GCK* patients (n=117, black circles) compared with controls (unaffected family members, n=105, white circles). Regression lines show increasing HbA1c with age (dashed = controls, solid = *GCK*), p=0.1 for the difference between the lines.

Table 1: Characteristics of the three groups studied. Data presented as median (IQR) or %

Characteristics	**GCK (n=99)**	**Controls (n=91*)**	**YT2D (n=83)**	**GCK v Controls *P* Value**	**GCK v YT2D *P* Value**
Gender: n (%) male	20/99 (20%)	41/91 (45%)	52/83 (63%)	<0.001	<0.001
Current Age (years)	48.6 (40.1-62.7)	52.2 (42.3-64.8)	54.7 (49.2-62.0)	0.49	0.06
Body Mass Index (kg/m^2)	26.1 (22.3-29.3)	28.0 (25.3-31.2)	32.2 (28.3-37.1)	0.004	<0.001
Age hyperglycemia/diabetes diagnosis (years)	33 (24-44)	N/A	40 (35-42)	N/A	0.001
Duration hyperglycemia (years)	48 (40-62)	N/A	17 (9-23)	N/A	<0.001
Fasting Plasma Glucose (mmol/l)	7.0 (6.6-7.5)	5.2 (4.8-5.6)	8.0 (6.2-10.4)	<0.001	<0.001
HbA1c (%)	6.9 (6.5-7.1)	5.8 (5.5-5.9)	7.8 (7.2-8.7)	<0.001	<0.001
On glucose lowering agents: n (%)	22/99 (22%)	N/A	75/83 (90%)	N/A	<0.001
Systolic Blood Pressure (mm/Hg)	125 (116-141)	128 (119-140)	135 (123-149)	0.65	0.004
Diastolic Blood Pressure (mm/Hg)	78 (72-84)	79 (74-86)	79 (71-89)	0.31	0.38
% on antihypertensive treatment	25/99 (25%)	19/91 (21%)	56/83 (68%)	0.48	<0.001
Total cholesterol (mmol/L)	4.8 (4.2-5.4)	5.1 (4.5-6.0)	4.2 (3.7-4.8)	0.03	<0.001
Triglycerides (mmol/L)	0.97 (0.78-1.23)	1.15 (0.81-1.56)	1.49 (1.14-2.59)	0.03	<0.001
High Density Lipoproteins (mmol/L)	1.57 (1.27-2.00)	1.47 (1.26-1.76)	1.09 (0.95-1.39)	0.07	<0.001
Low Density Lipoproteins (mmol/L)	2.66 (2.14-3.27)	3.04 (2.44-3.92)	2.07 (1.66-2.50)	0.01	<0.001
Cholesterol/HDL ratio	2.9 (2.4-3.7)	3.5 (2.8-4.2)	3.6 (2.9-4.5)	0.002	<0.001
On lipid lowering treatment: n (%)	25/99 (26%)	11/91 (12%)	68/83 (82%)	0.02	<0.001
estimated Glomerular Filtration Rate[a] ($ml/min/1.73m^2$)	82 (68-92)	84 (73-93)	81 (62-100)	0.20	0.80
Smoking status: n (%) Never Ever	 59/99 (60%) 40/99 (40%)	 45/91 (49%) 46/91 (51%)	 28/83 (34%) 55/83 (66%)	0.16	0.001

[a]Measured using the 4-variable modification of diet in renal disease equation (MDRD) estimation of glomerular filtration rate (eGFR) [16].
*First degree relatives n=32, second degree relatives n=9, spouses n=38, distant relative n=3, non-blood relative n=9
Abbreviations: N/A: not applicable

Table 2: Prevalence of clinically significant microvascular and clinically significant macrovascular disease alone in patients with GCK mutations (GCK), controls and young type2 diabetes (YT2D). Clinically significant microvascular disease is greater than background retinopathy OR persistent microalbuminuria OR persistent proteinuria. Clinically significant macrovascular disease is intermittent claudication OR amputation OR angina OR myocardial infarction OR stroke

	GCK	**Controls**	**YT2D**	**GCK v controls *P* Value**	**GCK v YT2D *P* Value**
Clinically significant Microvascular disease alone	1/99 (1% (0-5))	0/91 (0% (0-4))	19/83 (23% (14-33))	0.5	<0.001
Clinically significant Macrovascular disease alone	4/99 (4% (1-10))	8/91 (9% (4-17))	14/83 (17% (10-27))	0.19	0.004
Clinically significant Microvascular and Macrovascular disease	0/99 (0% (0-4))	2/91 (2% (0.3-8%))	11/83 (13% (7-22))	0.23	<0.001
Total affected with clinically significant Microvascular and Macrovascular disease	5/99 (5% (2-11))	10/91 (11% (5-19))	44/83 (53% (42-64))	0.14	<0.001

Table 3: Prevalence and severity of complications in the three groups. Data presented as n (% (95%CI))

		GCK (n=99)	Controls (n=91)	YT2D (n=83)	GCK v Controls *P* value	GCK v YT2D *P* value
Microvascular Complications						
Renal complications	**Persistent microalbuminuria**	1/97 (1% (0.2-6))	2/89 (2% (0.2-8))	17/80 (21% (13-32))	0.60	<0.001
	Proteinuria	0/97 (0% (0-4))	0/91 (0% (0-4))	8/80 (10% (4-19))	>0.99	<0.001
Retinal complications	**Any degree of retinopathy**	27/90 (30% (21-41))	12/87 (14% (7-23))	52/83 (63% (51-73))	0.007	<0.001
	Background retinopathy alone <5 microaneurysms >5 microaneurysms	27/90 (30% (21-41)) 22/27 (81% (62-94)) 5/27 (19% (6-38))	12/87 (14% (7-23)) 12/12 (100% (74-100)) 0/12 (0% (0-26))	28/83 (34% (24-45)) 13/28 (46% (28-66)) 15/28 (54% (34-72))	0.02	0.6
	Pre-proliferative retinopathy	0/90 (0% (0-4))	0/87 (0% (0-4))	7/83 (8% (3-17))	>0.99	0.005
	Proliferative retinopathy	0/90 (0% (0-4))	0/87 (0% (0-4))	8/83 (10% (4-18))	>0.99	0.002
	Advanced eye disease	0/90 (0% (0-4))	0/87 (0% (0-4))	3/83 (4% (1-10))	>0.99	0.005
	Maculopathy	0/90 (0% (0-4))	0/87 (0% (0-4))	17/83 (20% (12-31))	>0.99	<0.001
	Laser therapy for retinopathy	0/90 (0% (0-4))	0/87 (0% (0-4))	23/83 (28% (18-39))	>0.99	<0.001
Peripheral neuropathy	**VPT >25v mean or monofilament ≤3sites**	2/93 (2% (0.3-8))	0/89 (0% (0-4))	24/83 (29% (20-50))	0.16	<0.001
Clinically significant microvascular disease	**>background retinopathy OR persistent microalbuminuria OR proteinuria**	1/99 (1% (0-5))	2/91 (2% (0.3-8))	30/83 (36% (25-47))	0.52	<0.0001
Macrovascular Complications						
Vascular complications	**Intermittent claudication** Clinically diagnosed OR positive San Diego questionnaire	0/97 (0% (0-4))	0/91 (0% (0-4))	5/83 (6% (0-13))	>0.99	0.14
	Significantly reduced ABPABPI <5.0	0/97 (0% 0-3))	0/91 (0% (0-3))	0/83 (0% 0-4))	>0.99	>0.99
	Significantly increased ABPI ABPI ≥1.40	1/97 (1% (0.2-6))	3/91 (3% (0.7-9))	9/83 (11% (5-20))	0.30	0.006
	Amputation	0/97 (0% (0-4))	0/91 (0% (0-4))	4/83 (5% (1-12))	0.10	0.04
	Peripheral Vascular Disease Clinically diagnosed OR ABPI <0.5 OR ABPI ≥1.40 OR amputation OR positive San Diego questionnaire	1/93 (1% (0-5))	3/89 (3% (0-9))	13/83 (16% (8-25))	0.30	0.0004
	Angina Clinically diagnosed OR positive Rose angina questionnaire	4/93 (4% (1-10))	10/89 (11% (5-19))	18/83 (22% (13-32))	0.07	<0.001
	Myocardial Infarction	2/89 (2% (0.2-7))	2/97 (2% (0.2-8))	5/83 (6% (2-14))	0.99	0.16
	Ischemic Heart disease (Myocardial Infarction OR Angina)	2/99 (2% (0.2-7))	5/91 (5% (2-13))	13/83 (16% (10-29))	0.32	0.001
	Minnesota coding: Coronary disease probable	9/69 (13% (6-23))	20/81 (25% (16-36))	19/75 (25% (16-37))	0.07	0.06
Cerebral vascular events	**Stroke**	0/99 (0% (0-4))	0/91 (0% (0-4))	4/83 (5% (1-12))	>0.99	0.04
Clinically diagnosed macrovascular disease	**Intermittent claudication OR Amputation OR Angina OR Myocardial Infarction OR Stroke**	4/99 (4% (1-10))	10/91 (11% (5-19))	25/83 (30% (21-41))	0.09	<0.001