# Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

## Methods

The patients were assessed clinically on a single occasion by one of two clinical researchers who had performed successfully on inter and intra-reliability studies (see below).
The assessments of retinal photographs, biochemical measurements (e.g. microalbuminuria,) and ECG assessment were blinded to assignment. The investigators who performed the clinical outcome assessments were not blinded to the cohort assignment. However, the mutation status of 65 subjects in glucokinase families (ie either GCK cases or non-mutation controls) was unknown at the time of assessment. Blood was taken for DNA analysis during the assessment process and the result of the genetic analysis was available only after all other data were collected.

The frequency of clinical assessments (all were performed on a single occasion) and types of assessments to evaluate patients for complications were identical between the groups and are therefore directly comparable.

***Anthropometry***
Weight was measured to the nearest 0.1kg using Tanita electric scales. Height was measured to the nearest 0.1cm using a Harpenden (UK) pocket Stadiometer.

***Brachial blood pressure***
4 blood pressure measurements with a 2 minute pause were taken on the dominant arm using an Omron M5-1 Intelli™Sense and a mean was calculated.
Each subject was asked to empty their bladder prior to measurement and where possible, the blood pressure was measured prior to taking blood samples. Each subject was asked to rest for 5 minutes in a quiet room at ambient temperature in a supine or seated position[1,2]. Each subject was in the same position (lying at a $30^0$ angle (supine)) when taking the blood pressure and tight or restrictive clothing was removed from the arm [1]. The blood pressure cuff covered 80% of the circumference of the upper arm and not more than 100%. The arm was supported either by a pillow or table at the level of the right atrium of the heart (mid sternum) with the hand relaxed and the palm upwards[1-3]. The volunteer and researcher did not converse while the cuff was inflating and deflating or during the 2 minute pauses [2]. Subjects were also asked to uncross their legs while the blood pressure was being measured [2].

***Nephropathy***
First void, mid-stream urine samples were tested using a urinalysis stick (Combur[7] Test®, Roche) and sent to the laboratory for analysis. If the sample showed signs of a urinary tract infection (UTI) using the urinalysis stick or microalbuminuria on laboratory analysis, subjects provided another 2 samples over the next 1-3 months. Persistent microalbuminuria was diagnosed when there were no signs of a UTI and when 2 albumin creatinine ratios (ACRs) were between 2.5 mg/mmol and 30 mg/mmol (males), 3.5 mg/mmol and 30 mg/mmol (females) [4]. Proteinuria was diagnosed when UTI was excluded and 2 ACRs were >30mg/mmol (male or female) and a protein/creatinine ratio provided.

***Retinopathy***
Following mydriasis (Tropicamide, 0.5%), 45º non-stereoscopic bilateral images of the macular and nasal fields were taken using a Canon CR6-45/ DGi, digital non-mydriatic camera. Images were taken by either of the 2 researchers or the local retinal screening team. All images were primary graded by the Principal Investigator and were based on the English Retinopathy Minimum Grading Classification [5] (eTable 2). All images were second graded by a senior retinal screener. Where first and second grading results differed, third grading/arbitration was performed by a third, separate senior screener, which occurred on 5 occasions. Second and third graders were blinded to the category status of the subjects. In each subject the microaneurysms were counted and classified into <5 or ≥5. The result of the worst eye in each volunteer was used for analysis.

***Peripheral Neuropathy***
**Neurothesiometer**
Vibration Perception Threshold (VPT) was assessed bilaterally using a neurothesiometer (Horwell NEU 1500) applied to the dorsum of the big toes. Initially, the vibratory head was placed on subjects' dorsum of a thumb and turned up to its highest frequency in order for the subject to understand what sensation to expect. The neurothesiometer was then turned down to its lowest frequency (0 volts) and the subject was informed that the device was contacting the big toe and they were to say when they first felt the sensation of vibration.
The vibratory head was held as still as possible and the voltage was increased at 1 volt per second until the subject indicated that they had felt the vibration. Three consecutive readings were taken at 10 second intervals

and the mean calculated. When the volunteer was unable to feel the vibration at <25volts (mean), they were identified as having large fiber dysfunction and significant neuropathy.
Cutaneous perception was assessed bilaterally on the feet using a 10g Semmes-Weinstein monofilament (Owen Mumford). The monofilament was applied perpendicular to the skin at 6 sites on the plantar aspect of each foot, these being the 5 metatarsal- phalangeal joints and the dorsum of the big toe. The participant was asked to say 'yes' when they felt the monofilament contact their skin. The monofilament had previously been applied to the subjects hand or forearm to ensure that they knew what sensation to expect. The subjects were asked to look away or close their eyes so that they could not pre-empt or see when the monofilament was being applied. Significant neuropathy was diagnosed if subjects were only able to feel the monofilament in 3 or less sites. The worst foot (remaining foot for amputees) was used for analysis.

***Peripheral Vascular Disease***
All subjects completed the San Diego Claudication Questionnaire[6] to assess symptoms of peripheral vascular disease (PVD).
Ankle-brachial pressure index (ABPI) measurement was obtained using bilateral dorsalis pedis and posterior tibial pulses. A manual sphygmanometer and Doppler (Huntleigh Healthcare, Mini Dopplex) were used. The sphygmanometer cuff was placed around the ankle, just above, but not covering, the malleolus. Pulses were located and the Doppler probe adjusted on the skin to achieve the best signal. The cuff was inflated to least 20 mm/Hg above the pressure at which the Doppler arterial signal was heard in order to be sure of cessation of flow. The cuff was slowly deflated and when the Doppler signal returned the result was documented.
The ABPI was calculated using the formula: highest pressure obtained from the ankle vessels for the leg/mean of the 4 brachial blood pressures (BPs). An abnormal result was defined as an ABPI of <0.9 (indicative of generalized atherosclerosis)[7], presence of clinically significant PVD defined using an ABPI of <0.5 (indicative of critical ischemia)[8]and an non-compressible calcified arteries were defined as an ABPI of $\geq 1.40$ [9]. The worst foot (remaining foot for amputees) was used for all analysis.

***Cardiovascular Disease***
Subjects completed the World Health Organisation (Rose) chest pain questionnaire [10]. A resting 12 lead electrocardiogram (ECG) was taken using a MAC® 1200 ST digital. The ECG was assessed using an external consultant (blinded to category and all clinical features) using Minnesota criteria [11]. Detailed Minnesota codes were grouped and summary coded into groups: 3 (CHD probable: mainly Q-wave items and complete left bundle branch block), 2 (CHD possible: mainly ST/T wave items) and 1(CHD unlikely: all other Minnesota codes and normal records).
Subject-reported episodes of angina, myocardial infarction (MI) and stroke were documented and confirmed by medical notes by the clinical investigators who made the primary decision about the endpoint. The outcome measure of yes/no from medical records was used as minimum data and the Action to Control Cardiovascular Risk in Diabetes (ACCORD) definitions were used as reference [12]. When there was uncertainty in the initial assessment there was adjudication by a senior consultant physician who was given all the available clinical material. This occurred on 3 occasions.

**Intra-rater reliability for height and ABPI measurements**
Intra-rater reliability (within observer) was assessed by repeat measurements by the two researchers on 9 individuals. Coefficient of Variance (CV) was calculated using the formula: (Standard deviation/mean) x100. Intra-rater reliability CVs were low (researcher 1: mean CV height 0.14, mean ABPI 4.8%, researcher 2: mean CV height 0.15, mean ABPI 6.1%) showing that each researcher had a high level of repeatability in their own measurements.

**Inter-rater reliability for height and ABPI measurements**
Inter-rater reliability for height and measurement of ABPI was determined by looking at the deviation from the mean between individuals using Bland and Altman plots[13].
The differences in measurement between the two researchers were plotted against their mean difference. This enabled the identification or random or systematic errors. A combined CV% was calculated in order to check that the variation between researchers was similar.
Repeat measurements were taken by each researcher on the same individual. For each individual researcher, a mean measure was calculated. The mean values were then combined and a mean and standard deviation obtained. These were then used to calculate the inter-rater CV.

When plotting the differences in the researchers' mean measurements, the mean of the differences for height was low at 0.15% and for ABPI it was 8.3% which is within previously reported acceptable limits[14].

***Information regarding family members who were not recruited***

There was no evidence of survival bias in recruitment. In the 44 families, there were 102 siblings and 152 parents of those in the study that were not recruited. Only 13 siblings were deceased, of which, 6 had mutations (2 confirmed, 4 imputed from pedigree analysis) and 7 did not (1 confirmed, 6 imputed from pedigree analysis). 77/152 parents had died and mutations status could be ascertained in 56: 28 were mutation carriers (8 confirmed, 20 imputed from pedigree), and 28 did not have the GCK mutation (7 confirmed, 21 imputed from pedigree).

**eTable 1**: Results of prevalence of neuropathy, clinically significant microvascular disease and clinically significant macrovascular complications

| | | |
|---|---|---|
| **Level 0** | **None** | |
| **Level 1** | **Background** | Microaneurysm(s) retinal hemorrhage(s) ± any exudate not within the definition of maculopathy |
| **Level 2** | **Pre-proliferative** | Venous beading, venous loop or reduplication<br>Intraretinal microvascular abnormality (IRMA)<br>Multiple deep, round or blot hemorrhages<br>Cotton Wool Spots (CWS) |
| **Level 3** | **Proliferative** | New vessels on disc (NVD)<br>New vessels elsewhere (NVE)<br>Pre-retinal or vitreous hemorrhage<br>Pre-retinal fibrosis ± tractional retinal detachment |
| **Level 4** | **Advanced eye disease** | Rubeosis<br>Stable treated proliferative retinopathy<br>Previous vitrectromy |
| **Maculopathy** | | Exudate within 1 disc diameter (DD) of the centre of the fovea<br>Circinate or group of exudates within the macula<br>Retinal thickening within 1DD of the centre of the fovea (if stereo available)<br>Any microaneurysm or hemorrhage within 1DD of the centre of the fovea only if associated with a best VA of ≤ 6/12 (if no stereo) |

Retinal images grading based on the English Retinopathy Minimum Grading Classification (UKNSC).

**eTable 2:** Number of participants (n=126) and families (n=49) studied according to glucokinase mutation

| Nucleotide Change | Protein Change | Subjects with mutation | Families with mutation |
|---|---|---|---|
| c.106C>T | p.R36W | 3 | 1 |
| c.110T>G | p.M37R | 2 | 1 |
| c.128G>A | p.R43H | 3 | 1 |
| c.128G>C | p.R43P | 4 | 1 |
| c.130G>A | p.G44S | 4 | 2 |
| c.183C>A | p.Y61X | 2 | 1 |
| c.184G>A | p.V62M | 5 | 1 |
| c.386G>A | p.C129Y | 2 | 1 |
| c.389T>C | p.I130T | 4 | 1 |
| c.391T>C | p.S131P | 2 | 1 |
| c.469G>A | p.E157K | 3 | 1 |
| c.478G>A | p.D160N | 1 | 1 |
| c.483+1G>A | IVS4+1G>A | 4 | 1 |
| c.483+2_483+16del | IVS4+2_+16del15 | 4 | 2 |
| c.544G>A | p.V182M | 1 | 1 |
| c.556C>T | p.R186X | 6 | 2 |
| c.571C>T | p.R191W | 12 | 3 |
| c.580-13_580-1del | IVS5-1_-13del13 | 2 | 1 |
| c.623C>T | p.A208V | 1 | 1 |
| c.626C>T | p.T209M | 1 | 1 |
| c.683C>A | p.T228K | 3 | 1 |
| c.683C>T | p.T228M | 2 | 1 |
| c.760A>C | p.N254H | 1 | 1 |
| c.772G>A | p.G258S | 2 | 1 |
| c.781G>A | p.G261R | 5 | 2 |
| c.864-1G>A | IVS7-1G>A | 1 | 1 |
| c.895G>C | p.G299R | 6 | 1 |
| c.930_931del | p.D311fs | 2 | 1 |
| c.995C>A | p.T332K | 1 | 1 |
| c.1000_1018del | p.F334fs | 3 | 2 |
| c.1019G>T | p.S340I | 6 | 2 |
| c.1130G>A | p.R377H | 2 | 1 |
| c.1159G>A | p.A387T | 7 | 2 |
| c.1166T>A | p.V389D | 2 | 1 |
| c.1169_1171del | p.I390del | 4 | 1 |
| c.1174C>T | p.R392C | 5 | 1 |
| c.1190G>T | p.R397L | 1 | 1 |
| c.1209delC | p.I404fs | 1 | 1 |
| c.1340G>A | p.R447Q | 1 | 1 |
| c.1358C>G | p.S453W | 5 | 1 |
| Totals: | | 126 | 49 |

*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 1.

**eReferences**


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