Intima-media thickness and endothelial dysfunction in GCK and HNF1A-MODY patients

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Abstract

Objective: Mutations in the glucokinase (*GCK*) gene, along with hepatocyte nuclear factor 1A (*HNF1A*) gene mutations, are the most frequent cause of maturity-onset diabetes of the young (MODY). GCK-MODY patients are typically characterized by a moderate fasting hyperglycemia; however, little is known about atherosclerosis and intermediate-related phenotypes in these subjects.

Design: To examine carotid artery intima-media thickness (IMT) and endothelial function assessed by brachial artery flow-mediated dilatation (FMD) in *GCK* gene mutations carriers and HNF1A-MODY.

Methods: A total of 64 subjects with *GCK* gene mutations, and 52 *HNF1A* gene mutation carriers as well as 53 nondiabetic controls were examined. IMT and FMD were assessed by ultrasonography. Appropriate statistical tests were performed to assess differences between the groups, and multivariate linear regression was done for the association with IMT and FMD.

Results: The clinical characteristics of all groups were similar with the mean age at examination of 35.1, 41.1, and 39.5 years for GCK, HNF1A and the control group respectively. The highest mean IMT value was in the HNF1A-MODY group: 7.0 ± 1.4 mm, whereas it reached 6.3 ± 1.4 mm in GCK mutation carriers and 6.3 ± 1.3 mm in controls ($P=0.008$). After adjustment for possible clinical and biochemical cofounders, IMT remained higher in HNF1A-MODY patients as compared with GCK-MODY patients ($P=0.02$) and controls ($P=0.0003$). FMD was significantly lower in HNF1A ($9.9 \pm 4.6\%$) and GCK-MODY ($11.1 \pm 4.6\%$) patients in comparison with controls ($13.9 \pm 4.7\%$; $P=0.0001$). After adjustment, FMD remained lower in HNF1A-MODY ($P=0.0005$) and GCK-MODY patients ($P=0.01$) as compared with controls.

Conclusions: Both examined MODY groups demonstrated evidence of endothelial dysfunction. In addition, HNF1-MODY patients seem to be more prone to an early atherosclerotic phenotype.

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Introduction

Monogenic forms account for a few percent of all diabetes cases, which corresponds to several million patients in Europe alone. Maturity-onset diabetes of the young (MODY) constitutes a heterogeneous group of relatively frequent autosomal single-gene disorders. Mutations in the glucokinase (*GCK*) gene, along with hepatocyte

nuclear factor 1A (*HNF1A*) gene sequence differences, are the most frequent causes of MODY (1). The *GCK* gene encodes glucokinase, a key regulatory enzyme of the β cell, catalyzing the first phosphorylation step of glucose metabolism. Thus, this enzyme is crucial for insulin secretion. *GCK* is also expressed in the liver where it

indirectly influences the processes of glycogen synthesis, gluconeogenesis, lipid synthesis, and urea production. Heterozygous loss-of-function *GCK* mutations result in mildly elevated fasting blood glucose levels with a moderate postprandial rise, usually not larger than 3 mmol/l, that correspond to prediabetes or fulfill the criteria of diabetes (2, 3). This fasting hyperglycemia is present from birth and patients are typically asymptomatic and diagnosed randomly, usually when glucose is measured for the first time (4). Pharmacotherapy is not effective in GCK-MODY patients (5). Therefore, *GCK* mutation carriers are exposed to higher fasting glucose for their whole lives, which in some patients, especially when overweight occurs, may overlap with clinically overt hyperglycemia due to type 2 diabetes.

HNF1 α is a transcription factor that controls pancreatic development and the function of genes involved in insulin synthesis (6). It is essential for the glucose-stimulated insulin secretory response (7). Individuals with HNF1A-MODY typically present with non-ketotic hyperglycemia, particularly post-prandially, which usually begins in the second or third decade of life and is progressive in nature. Patients can be initially treated with diet, but the majority will eventually need glucose-lowering agents (8). Oral sulfonylureas have been shown to be particularly effective in diabetic patients with HNF1A-MODY (9). Microvascular complications, particularly retinopathy, seem to be common in HNF1A-MODY patients. Despite a favorable lipid profile and decreased C-reactive protein (CRP) level described in these patients, they seem to be characterized by an increased risk of cardiovascular complications as compared with nondiabetic family members (10). Therefore, these complications seem to be mainly related to overall hyperglycemic exposure, rather than other nonglycemic factors. On the contrary, *GCK* gene mutation carriers are usually free from microvascular complications (11). However, very little is known about atherosclerosis and intermediate related phenotypes in both of the most prevalent subtypes of MODY.

In this study, we aimed to examine carotid artery intima-media thickness (IMT) and endothelial function, assessed by brachial artery flow-mediated dilatation (FMD) in *GCK* gene mutation carriers and HNF1A-MODY subjects as well as healthy controls.

Subjects and methods

Subjects

We examined 64 subjects with *GCK* gene mutations, either with diabetes ($n=45$) or with prediabetes ($n=19$), and 52

HNF1A-MODY patients (45 with diabetes and seven with prediabetes) as well as 53 nondiabetic control individuals. The individuals were considered as diagnosed with diabetes if at the study entry they were on hypoglycemic treatment or met the diagnostic criteria based on fasting glucose measurements. The subjects were white Caucasians, the residents of southeastern Poland. All the patients had a molecular diagnosis of MODY established during research activities performed at the Department of Metabolic Diseases, Jagiellonian University Medical College, Krakow, Poland. The pathogenicity of mutations was determined based on earlier reports, DNA sequence difference molecular character, and segregation within the families. The subjects received a standard questionnaire and underwent a basic physical examination. The individual was classified as having arterial hypertension if the following criteria were met: i) the diagnosis of hypertension in previous medical history; ii) anti-hypertensive treatment before the entry of the study; and iii) systolic or diastolic blood pressure > 140 mmHg or > 90 mmHg respectively. The diagnosis of coronary artery disease was based on their medical documentation. The presence of retinopathy was determined by a trained ophthalmologist based on color photographic documentation made using a fundus camera model CR-2 (Canon, Inc., Tokyo, Japan). We excluded all individuals with active infections, neoplasms, severe chronic diseases of the respiratory tract, kidney, or liver as well as pregnant women. This study was carried out according to the Helsinki Declaration and it was approved by the Bioethical Committee of Jagiellonian University.

Vascular examination

All the study individuals underwent two vascular examinations. To assess IMT of the carotid arteries, we used a Toshiba ultrasonograph (SSA-340A, type BF, Tokyo, Japan) equipped with an 8-MHz linear array transducer. IMT measurements were performed on the right and left common carotid arteries 1.0 cm proximal to the carotid bulb. Digital documentation was made and the mean IMT values were calculated with integrated software (12).

In addition, endothelial vasodilatory function was determined using FMD. By inflation of a blood pressure cuff for 5 min at a pressure of ca. 60 mmHg above the systolic blood pressure, ischemia was induced in the forearm distal to the location of the transducer. Ultrasonography continued for 4 min after cuff release with measurements at 30 s intervals. The widest lumen diameter was taken as a measure for maximal diameter

(FMD). Next, sublingual nitroglycerine spray in a dose of 0.4 mg was administered to assess an endothelium-independent nitroglycerin-mediated vasodilation (NMD). NMD measurements were obtained at 2.5 and 5 min. Computer-assisted analysis was performed. FMD and nitroglycerin (NTG) were expressed as percentage change relative to baseline diameter (13). IMT > 9 mm and FMD < 10% were considered abnormal (13, 14).

Laboratory examination

We performed basic clinical laboratory analyses, such as HbA1c level, fasting glucose, lipids, CRP, C-peptide, and creatinine. HbA1c was measured by HPLC (Bio-Rad). Fasting glucose level was determined by oxidase method. The concentrations of cholesterol and triglycerides were measured by enzymatic methods, with HDL-cholesterol (HDL-C) measured after precipitation of very LDL (VLDL). The concentration of LDL-cholesterol (LDL-C) was calculated using the Friedewald formula. Serum creatinine concentration was measured by the Jaffe method. Creatinine-based eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (15). CRP was determined by particle-enhanced immunoturbidimetric assay and C-peptide by enzyme immunoassay test.

Statistical analyses

Categorical variables are presented as counts (%). Continuous variables are presented as mean $\pm$ s.d. Differences between two (*t*-test or Mann–Whitney *U* test) or three groups (one-way ANOVA or the Kruskal–Wallis test with *post hoc* test (Tuckey test)) were analyzed. The χ^2 -test or Fisher's exact test were used for categorical variables. Multivariate linear regression for the association with IMT and FMD was performed. First, crude comparisons of IMT and FMD between the study groups were performed. Subsequently, they were adjusted in multiple regression to potentially confounding variables. To examine whether fasting glucose levels from the individual groups (HNF1A-MODY, GCK-MODY, and controls) were associated with examined traits, we performed multiple regression analysis. *P* values < 0.05 were considered to be statistically significant. All analyses were performed by using Statistica PL ver. 10.0 and R ver. 3.1.

Results

The basic clinical characteristics of all groups were similar, as given in Table 1, with the mean age of examination at

35.1, 41.1, and 39.5 years for the GCK, HNF1A, and control groups, respectively, and normal body weight (Table 1). There were differences in fasting glucose level between three groups; however, GCK-MODY compared with HNF1A-MODY patients revealed no statistically significant difference (*P*=0.06). The mean HbA1c in the GCK-MODY patients was $6.3 \pm 0.6\%$, while in HNF1A-MODY patients it reached $6.9 \pm 1.4\%$ (*P*=0.69). The majority of HNF1A-MODY patients required pharmacological therapy (34 individuals), and most of them (33 subjects) were on treatment with sulfonylurea. As expected, the majority of GCK mutation carriers were on diet only (49 patients), two out of 15 on hypoglycemic treatment used sulfonylurea. There was also no difference between the groups in total cholesterol and HDL-C level as well as in CRP concentration. The percentage of patients with a diagnosis of arterial hypertension was higher in the HNF1A-MODY (36%) and GCK-MODY (31%) groups as compared with controls (7%). In general, the prevalence of retinopathy and coronary artery disease was low in both groups with diabetes. None of the included subjects had an estimated GFR below 60 ml/min per 1.73 m².

There were very few patients with an abnormal mean IMT (three, four, and two in GCK-MODY, HNF1A-MODY and controls respectively). As given in Table 2, the highest mean IMT value was present in the HNF1A-MODY group: 7.0 ± 1.4 mm, whereas it reached 6.3 ± 1.4 mm in GCK-MODY patients and 6.3 ± 1.3 mm in controls (*P*=0.01). After adjustment for sex, age, BMI, CRP, total cholesterol, HDL-C, a diagnosis of arterial hypertension, and smoking status, the mean IMT remained higher in HNF1A-MODY patients as compared with controls (*P*=0.0003) and GCK-MODY patients (*P*=0.02) (given in Table 3).

In multiple regression analysis, there was no independent impact of fasting glucose on IMT values in our whole study (*P*=0.66) after adjustment for sex, age, BMI, CRP, total cholesterol, HDL-C, a diagnosis of arterial hypertension, and smoking status. Among the examined variables, four – age, CRP level, hypertension status, and BMI – had an independent impact on IMT (Table 4).

There were 31 individuals (48%) in the GCK-MODY group, 32 (61%) in the HNF1A-MODY cohort, and 14 (26%) from the controls with an abnormal FMD. FMD was significantly lower in HNF1A ($9.9 \pm 4.6\%$) and GCK-MODY ($11.1 \pm 4.6\%$) patients as compared with controls ($13.9 \pm 4.7\%$), as given in Table 2. After adjustment for sex, age, BMI, CRP, total cholesterol, HDL-C, a diagnosis of arterial hypertension, and smoking status, the maximum FMD remained lower in HNF1A-MODY

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Table 1 Clinical characteristics of MODY patients and controls without diabetes.

Clinical characteristic	Controls	HNF1A	GCK	P
Gender F/M (n)	27/26	31/21	35/29	0.67
Age at the examination (years)	39.5 ± 13.3	41.1 ± 15.0	35.1 ± 15.3	0.05
Age at diagnosis (years)	–	26.0 ± 11.4	24.6 ± 13.8	0.43
Time from diabetes diagnosis	–	16.2 ± 11.8	10.5 ± 8.2	0.003
BMI (kg/m ²)	24.1 ± 3.6	24.7 ± 3.9	23.7 ± 4.3	0.43
Fasting glucose (mmol/l)	5.6 ± 0.5	7.0 ± 2.7	6.8 ± 1.2	0.0001
HbA1c (%)	–	6.9 ± 1.4	6.3 ± 0.62	0.14
C-peptide (ng/ml)	1.9 ± 0.9	1.3 ± 0.7	1.4 ± 1.0	0.001
Total cholesterol (mmol/l)	5.0 ± 0.9	4.8 ± 1.0	4.9 ± 1.1	0.32
LDL-C (mmol/l)	3.1 ± 0.8	2.7 ± 0.9	2.9 ± 0.9	0.03
HDL-C (mmol/l)	1.3 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	0.004
Triglycerides (mmol/l)	1.2 ± 0.5	1.3 ± 1.0	1.0 ± 0.4	0.11
Creatinine (μmol/l)	79.4 ± 13.5	71.9 ± 14.5	71.1 ± 14.5	0.01
CRP (mg/l)	1.0 ± 0.8	0.8 ± 1.3	1.5 ± 2.8	0.0001
Smoking history (n)	15	16	14	0.57
Hypertension diagnosis (n)	4	19	20	0.002
Pharmacological treatment of diabetes (oral (n)/insulin (n)/none (n))	–	34/9/9	10/5/49	0.0001
Coronary artery disease (n)	0	5	1	0.02
eGFR (ml/min)	96.4 ± 16.7	101.0 ± 19.2	105.0 ± 18.6	0.05
Retinopathy (n)	–	10	0	0.0003

P value refers to the difference among three groups (one way ANOVA or the Kruskal–Wallis test, as appropriate) or, if data for the controls not available, between two MODY groups (t-test or Mann–Whitney U test).

($P=0.0005$) and GCK-MODY patients ($P=0.01$) as compared with controls (Table 3).

Unlike for IMT, we found that a rise in fasting glucose was associated with decreased mean maximum FMD when adjusted for sex, age, BMI, CRP, total cholesterol, HDL-C, a diagnosis of arterial hypertension and smoking status ($P=0.05$) (Table 4). FMD was also associated with sex ($P=0.04$) and a hypertension diagnosis ($P=0.01$).

Discussion

In this study, for the first time, we examined GCK and HNF1A gene mutations carriers with diabetes or prediabetes and nondiabetic controls for two intermediate atherosclerosis and endothelial dysfunction-related traits – IMT and FMD respectively. Our results showed that carrying a mutation (that causes diabetes or prediabetes) in the GCK gene or the HNF1A gene predisposes to arterial wall dysfunction as assessed by FMD. We also found that HNF1A-MODY was associated with significantly higher IMT values than the other two groups examined; however, the majority of subjects in all the study groups had no abnormalities in IMT value, with only single individuals showing an increase in this parameter.

Macrovascular complications, such as coronary artery disease or stroke, are the leading causes of increased mortality in type 1 and type 2 diabetes. Extensive research

has been carried out on their epidemiology, risk factors as well as preventive and therapeutic measurements. However, research data on atherosclerosis and related traits in monogenic diabetes, such as MODY, are so far very limited. This is probably related to the fact that although the true prevalence of MODY may reach hundreds of thousands or even several million subjects in Europe, most of them are not properly diagnosed, making such cardiovascular research difficult because of a limited number of identified individuals for potential inclusion. In addition, due to its clinical picture and the way the families with MODY have been ascertained around the world, most patients diagnosed so far on the molecular level are too young to develop clinically overt manifestations of atherosclerosis. Thus, the research aimed at the evaluation of surrogates, such as endothelial

Table 2 Crude comparison of IMT, FMD, and NMD in the examined groups.

Clinical characteristics	Controls	HNF1A	GCK	P
Mean IMT (mm)	0.63 ± 0.13	0.70 ± 0.14	0.63 ± 0.14	0.002
FMD max (%)	13.9 ± 4.7	9.9 ± 4.6	11.1 ± 4.6	<0.001
NMD max (%)	20.2 ± 5.8	21.4 ± 12.6	20.6 ± 6.4	0.89

P value refers to the difference among three groups (one way ANOVA or the Kruskal–Wallis test, as appropriate).

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Table 3 Estimates of differences in IMT or FMD between HNF1A-MODY, GCK-MODY and non-diabetic controls and effect estimates of clinical covariates on IMT and FMD from multiple linear regression models.

	IMT (mm)		FMD (%)	
	Estimate (95% CI)	P value	Estimate (95% CI)	P value
HNF1A-MODY vs control	0.06 (0.02, 0.1)	0.003	−3.9 (−5.9, −1.9)	<0.001
GCK-MODY vs control	0.01 (−0.02, 0.05)	0.5	−2.8 (−4.7, −0.9)	0.005
HNF1A-MODY vs GCK-MODY	0.05 (0.01, 0.08)	0.01	−1.1 (−3.0, 0.7)	0.22
Male vs female	0.02 (−0.02, 0.05)	0.35	−2.0 (−3.6, −0.4)	0.02
Age per 10 years increase	0.05 (0.04, 0.06)	<0.001	−0.3 (−0.9, 0.4)	0.38
BMI per 1 kg/m ² increase	0.01 (0.002, 0.01)	0.003	0.0 (−0.2, 0.3)	0.73
CRP per 1 mg/l increase	0.01 (0.004, 0.02)	0.002	−0.2 (−0.5, 0.2)	0.46
Total cholesterol per 1 mmol/l increase	−0.01 (−0.02, 0.01)	0.44	−0.3 (−1.1, 0.5)	0.44
HDL-C per 1 mmol/l increase	−0.03 (−0.07, 0.02)	0.23	−0.4 (−2.6, 1.8)	0.71
Positive vs negative smoking history	0.01 (−0.02, 0.04)	0.49	0.4 (−1.2, 2.0)	0.63
Hypertension diagnosis present vs absent	0.04 (0.002, 0.08)	0.04	−1.5 (−3.5, 0.5)	0.14

dysfunction or early subclinical atherosclerotic disease, may be useful to overcome, at least partially, this problem. Several noninvasive methods of such assessment have been proposed. FMD and IMT measurements are probably among those that are most commonly used in scientific reports (16).

So far, there was just one study published that examined one of these traits – IMT – in GCK-MODY (but not HNF1A-MODY) patients (17). This study, like the current report, showed no risk of IMT increase in GCK mutation carriers. Interestingly, the mean IMT in our HNF1A-MODY group was larger than that in GCK mutation carriers and the controls. This occurred in spite of a more favorable lipid profile and lower CRP levels in these patients. This seemingly antiatherogenic lipid profile and low CRP level in *HNF1A* mutation carriers have been described earlier (18, 19). The antiatherogenic profile of HNF1-MODY seems to be also associated with low sCD36, the scavenger protein that was shown to play a substantial role in the pathogenesis of atherosclerosis (20). Thus, the larger IMT may be a result of a more severe diabetic phenotype in the HNF1A-

MODY cohort or any other not yet identified atherosclerosis risk factor. It should be also noted that overall the number of individuals with an abnormal IMT was relatively low. This may be a consequence of the fact that our MODY patients were characterized by a relatively young age at the examination and their lack of some other risk factors, such as hyperlipidemia or obesity. While no earlier study had approached FMD measurements in monogenic diabetes, one previous study reported of microvascular function in HNF1A-MODY, which showed impaired maximum microvascular hyperemia in these patients (21). The relatively high prevalence of hypertension in both our diabetic cohorts should be treated with caution, as one criterion for diagnosis was the introduction of antihypertensive treatment. Thus, its proportion may reflect the common clinical practice of using angiotensin-converting enzyme (ACE) inhibitor in the prevention of diabetic kidney disease rather than the occurrence of truly elevated blood pressure.

Unlike in cases of IMT, we saw a high prevalence of FMD abnormalities in GCK mutation carriers and the HNF1A-MODY group as compared with the controls.

Table 4 Multiple linear regression models of association between IMT or FMD and clinical characteristics in all study participants.

	IMT (mm)		FMD (%)	
	Estimate (95% CI)	P value	Estimate (95% CI)	P value
Glucose per 1 mmol/l increase	−0.003 (−0.01, 0.00)	0.52	−0.4 (−0.9, 0.0)	0.06
Male vs female	0.01 (−0.02, 0.04)	0.55	−1.8 (−3.5, −0.1)	0.04
Age per 10 years, increase	0.05 (0.04, 0.06)	<0.001	−0.1 (−0.7, 0.5)	0.77
BMI per 1 kg/m ² increase	0.01 (0.00, 0.01)	<0.001	−0.0 (−0.3, 0.2)	0.81
CRP per 1 mg/l increase	0.01 (0.003, 0.02)	0.007	−0.1 (−0.5, 0.3)	0.56
Total cholesterol per 1 mmol/l increase	−0.01 (−0.03, 0.01)	0.16	0.1 (−0.8, 0.9)	0.83
HDL-C per 1 mmol/l increase	−0.01 (−0.05, 0.03)	0.71	−2.0 (−4.2, 0.2)	0.08
Positive vs negative smoking history	0.01 (−0.02, 0.05)	0.40	0.5 (−1.2, 2.2)	0.54
Hypertension diagnosis present vs absent	0.05 (0.01, 0.09)	0.01	−2.6 (−4.5, −0.6)	0.01

One of the possible explanations is that functional endothelial abnormalities precede structural changes related to the artery wall thickness; thus, their presence was easier to document in this relatively young study group. Interestingly, it was shown earlier that in young adults the status of systemic endothelial function assessed by FMD modified the association between cardiovascular risk factors and IMT (22). Multiple logistic regression results of this study suggest that FMD abnormalities may be, at least in part, related directly to the elevated glucose level at the time of examination. Unlike FMD, increased IMT, representing early structural changes, in HNF1A-MODY seems to be related to its more severe diabetic phenotype. Our results should be discussed in the light of the recent paper on GCK-MODY (11). This study showed low prevalence of self-reported macrovascular complications in the group of GCK mutation carriers. While our observations on IMT match the previous finding, as no difference was observed between the GCK group and the controls, data on FMD, a functional parameter predicting future cardiovascular events, suggest that hyperglycemia in GCK-MODY may cause abnormalities in endothelial function. Finally, some shortcomings of our research should be acknowledged. They are related to the relatively small size of the groups, young age of the study individuals, and the cross-sectional rather than prospective nature of the research. Moreover, examined outcomes were intermediate phenotypes, not clinically important end-points such as cardiovascular events. Our study should be perhaps criticized for including some individuals with prediabetes (diagnosed based on fasting glucose level). However, this is a quite common phenotype in GCK gene mutation carriers. On the other hand, HNF1A-MODY is characterized by substantial post-prandial hyperglycemia; thus, in this group, individuals with IFG are very likely to have diabetes by oral glucose tolerance test. Finally, we cannot entirely rule out the impact of sulfonylurea on FMD measurements, as one earlier small study suggested an advantageous effect of gliclazide on this parameter, possibly through antioxidant mechanism (23). Nevertheless, in spite of this unlikely hypothetical beneficial effect, the results in both MODY groups were worse than in the controls.

In summary, both the GCK-MODY and HNF1A-MODY groups demonstrated an evidence of abnormalities in endothelial function or/and presence of an early atherosclerotic phenotype. This seems to suggest that they are at increased risk of macroangiopathy and require systematic cardiovascular assessment as well as preventive measures.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

M Szopa, M T Malecki: study design, protocol development; J Skupien, B Matejko: data analysis; M Szopa, G Osmenda, G Wilk, B Zapala, T Guzik, M T Malecki, W Mlynarski: research data; M Szopa, B Matejko, J Skupien, M T Malecki: data interpretation; M Szopa, J Skupien, M T Malecki: writing the manuscript; T Guzik, B Matejko: critical review of the manuscript; M T Malecki: project coordination, final approval of the manuscript.

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