Functional Variants of Glucokinase Regulatory Protein and Apolipoprotein A5 Genes in Ischemic Stroke

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Abstract Both the natural variants of the apolipoprotein A5 (*APOA5*) and the glucokinase regulatory protein gene (*GCKR*) have been shown to associate with increased fasting triglyceride levels. Here, we investigated the possible association of the functional variants of these two genes with non-fasting triglyceride levels and their susceptibility nature in ischemic stroke. A total of 513 stroke patients and 172 healthy controls were genotyped. All the *APOA5* variants (T-1131C, IVS3+G476A, C56G, and T1259C) were associated with increased triglyceride levels in all stroke patients and controls; except for T1259C, they all conferred risk for the disease. No such association was found for the examined *GCKR* rs1260326 (C1337T) variant. Furthermore, we examined the effects of specific combinations of the *GCKR* rs1260326 and *APOA5* poly-morphisms. Our findings confirmed the previous results regarding the association of *APOA5* variants with triglyceride-level increase and stroke susceptibility of these alleles. By contrast, we could not detect any association of the studied *GCKR* allele with triglyceride levels or with the susceptibility of stroke in the same cohort of patients. In addition, the effect of *APOA5* did not change significantly when specific combinations of the two genes were present.

Keywords *GCKR* · *APOA5* · Stroke · Triglyceride

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Introduction

In the development of ischemic stroke, a common multi-factorial disease which is the third leading cause of death, the role of increased triglyceride level as a risk factor is still under debate (Rothwell et al. 2005). Recently, with the help of robust genome-wide association studies, several genetic factors associated with elevated triglycerides have been identified, opening new perspectives for the research of the fine details of the triglyceride-stroke relationship (Bansal et al. 2007; Nordestgaard et al. 2007; Szolnoki et al. 2003).

The apolipoprotein A5 (*APOA5*) gene is located near the apolipoprotein A (*APOA*) cluster containing the *APOAI-APOCIII-APOAIV* genes at 11q23 chromosome (Havasi et al. 2006; Martinelli et al. 2007a; Pennacchio and Rubin 2003; Szalai et al. 2004), encoding 366 amino acids. The mature *APOA5* protein following synthesis in the liver is secreted into the plasma and plays a central regulatory role in triglyceride metabolism (Groenendijk et al. 2001). Since the different proteins transcribed from the natural variants of the gene may have slightly different functions, the most common natural variants, the T-1131C (rs662799), T1259C (rs2266788), C56G (rs3135506), and IVS3+G476A

(rs2072560) alleles have been repeatedly reported to associate with elevated triglyceride levels; moreover, some of them were also found to be independent risk factors for cardio- and cerebrovascular diseases and metabolic syndrome (Maasz et al. 2008a, b; Willer et al. 2008).

The glucokinase regulatory protein (GCKR) gene, located on chromosome 2p23.3-p23.2, consists of 19 exons and encodes a protein of 625 amino acids (Hayward et al. 1998; Vaxillaire et al. 1994; Warner et al. 1995). The gene product functions as a regulatory protein that inhibits glucokinase in liver and pancreatic cells by binding the enzyme non-covalently to form an inactive complex (Farrelly et al. 1999; Grimsby et al. 2000; Hayward et al. 1996). Functional variants were discovered in a genome-wide association study in association with hypertriglyceridemia; later, an inverse relationship of the triglyceride and glucose levels has also been reported in relation with GCKR functional alleles. Moreover, demonstrating a central role of the gene, GCKR 446Leu (rs1260326) carriers are protected against type II diabetes (Vaxillaire et al. 2008). Recently, genome-wide association studies suggest that by combining genotype information for multiple loci, significant predictive value from genetic markers might be gained (Kathiresan et al. 2008a; Morrison et al. 2007; Perez-Martinez et al. 2009; Trichopoulou et al. 2008; Yiannakouris et al. 2006).

The aim of our work was to investigate the relationship of functional variants of APOA5 and GCKR genes with triglyceride levels and to study the allele distributions in stratified ischemic stroke patients.

Materials and Methods

Patients

The DNA samples were from the Central Biobank governed by the University of Pécs, as part of the National Biobank Network of Hungary (www.biobank.hu), which belongs also to the pan-European Biobanking and Biomolecular Resources Research Infrastructure (BBMRI) preparatory phase project (http://bbmri.eu/bbmri/). The governance, maintenance, and management principles of the Biobank had been approved by the national Scientific Research Ethics Committee, Budapest (ETT TUKEB). During the collection and use of DNA samples and the accompanying clinical and personal data, the guidelines and regulations of the Helsinki Declaration in 1975 and the currently operative National laws and regulations were also followed.

A total of 513 Caucasian patients were involved in the study (207 males, 306 females; mean age, 65.1±0.62 years) with acutely developing ischemic stroke, who had never suffered a previous stroke event. All the 513 subjects underwent a detailed clinical scrutiny, including medical and familial histories, estimation of vascular risk factors, general physical and neurological explorations, urine analysis, extended laboratory examinations, electrocardiography, extracranial and transcranial Doppler sonography of the brain-supplying arteries, transthoracic and/or transesophageal echocardiography where apposite, and magnetic resonance imaging (MRI) examinations within 2 days after the outbreak of symptoms. After collecting the data of the 513 patients, we classified them into one of three stroke categories; the first two groups essentially matched with the corresponding type defined by the Trial of Org 10172 in Acute Stroke Treatment classification. The first subgroup called "small-vessel occlusion type" consists of 232 patients (91 males, 141 females; mean age, 65.1±0.96 years) with small-vessel infarcts (one or more subcortical hemispheric or brainstem infarcts with a diameter <1.5 cm on MRI, with one of the features of the traditional clinical lacunar syndrome and without cerebral cortical dysfunction); the "large-vessel group" is composed of 139 patients (57 males, 82 females; mean age, 66.5±1.21 years) with large-vessel infarcts (cortical or cerebellar lesions and/or brainstem infarcts or subcortical hemispheric infarcts greater than 1.5 cm in diameter on the MRI, with a cerebral cortical impairment or a brainstem or cerebellar dysfunction). The "mixed group" contains 142 patients (59 males, 83 females; mean age, 63.8±1.06 years) with mixed vascular pathology (one or more lacunar and large-vessel infarcts on the MRI, and other nonspecific etiology). A total of 172 Caucasian subjects (49 males, 123 females; mean age, 56.5±1.20 years) served as controls. This cohort was a neuroimaging alteration-free group, with negative brain MRI findings.

Molecular Biology Methods

Genomic DNA was extracted from peripheral EDTA-anticoagulated blood by a standard desalting method (Miller et al. 1988).For polymerase chain reaction (PCR), the following forward and reverse primers were used: for GCKR rs1260326 forward: 5′-TGC AGA CTA TAG TGG AGC CG-3′ and reverse: 5′-CAT CAC ATG GCC ACT GCT TT-3′; APOA5 gene: for the T-1131C variant forward: 5′-CCC CAG GAA CTG GAG CGA AATT-3′ and reverse: 5′-TTC AAG CAG AGG GAA GCC TGT A-3′; for the T1259C single nucleotide polymorphism (SNP) 5′-TCA GTC CTT GAA AGT GGC CT-3′ and 5′-ATG TAG TGG CAC AGG CTT CC-3′; for the IVS3+G476A forward: 5′-CTC AAG GCT GTC TTC AG-3′ and the reverse 5′-CCT TTG ATT CTG GGG ACTG G-3′; for the C56G forward: 5′-AGA GCT AGC ACC GCT CCT TT-3′ and reverse: 5′-TAG TCC CTC TCC ACA GCG TT-3′ primers. The reactions were performed using an MJ Research PTC-200 thermal cycler (Bio-Rad, Hercules, CA, USA). The PCR conditions were the following: predenaturation at 96°C for

2 min; followed by 35 cycles of denaturation at 96°C for 20 s for the *GCKR* rs1260326 and all the *APOA5* SNPs; annealing at 60°C for 20 s; primer extension for 30 s at 72°C; and final extension at 72°C for 5 min. For the T-1131C, PCR conditions were as follows: predenaturation at 96°C for 2 min followed by 32 cycles of 30 s at 95°C; 30 s at 55°C; 30 s at 72°C, and a final extension at 72°C. The amplicons were digested by allele-specific restriction endonucleases, *Hpa*II for the *GCKR* rs1260326; *Tru*I for the *APOA5* T-1131C; *Bse*GI for T1259C; *Mnl*I for IVS3+G476A; and *Cfr*13I for C56G. All methods were designed to include an obligate cleavage site on the amplicon thus enabling us to monitor the efficacy of the digestion.

Statistical Analysis

All clinical data are represented as mean ± standard error of the mean (SEM). The Mann–Whitney test was used to assess the differences between the clinical parameters in patients and the controls. For discreet variables, χ^2 test was applied. Odds ratios (ORs) derived from multiple logistic regression were also used to determine whether the presence of the significant genotypes of the *APOA5* or *GCKR* gene were associated with clinical risk factors in the development of ischemic stroke. The ORs for the specific genotype combinations of the two genes (*GCKR* rs1260326 and *APOA5* gene variants) were calculated by χ^2 test. All statistical analysis were carried out using SPSS 15.0 package for Windows (SPSS Inc, Chicago, IL, USA).

Results

The main clinical features and general laboratory data of the patients and controls are shown in Table 1. Patients were divided into groups according to our previously established stroke classification. The gender ratio, age range and body mass index (BMI) of the subgroups were as indicated in Table 1. The major risk factors for ischemic stroke, serum total cholesterol, and triglyceride levels

showed significant differences between stroke patients and controls. The triglyceride levels of 1.67 ± 0.04, 1.78 ± 0.06, 1.76 ± 0.06, and 1.73 ± 0.03 mmol/l of the small vessel, large vessel, mixed, and overall groups, respectively, were significantly higher than the 1.53 ± 0.04 mmol/l triglyceride value of the controls ($p<0.05$). The serum total cholesterol levels were 5.85 ± 0.08, 5.96 ± 0.11, 5.90 ± 1.12, and 5.89 ± 0.06 mmol/l of the small vessel, large vessel, mixed, and overall cohorts, respectively, and showed a significant difference between the stroke subgroups and the controls ($p<0.05$).

Genotype profiles are summarized in Table 2. We analyzed the allele distribution of the different subgroups. All allele distributions were in Hardy–Weinberg equilibrium both in stroke groups and in controls. We detected a significant increase of allele frequencies of T-1131C, IVS3+G476A, and C56G variants of the *APOA5* gene in the following groups of stroke patients: −1131C (8.62%*, 8.27%*, 9.51%*, 8.77%*, respectively, in small vessel, large vessel, mixed, overall subgroups), IVS3+G476A (6.90%*, 5.04%*, 7.75%*, 6.63%*) in all stroke groups, and the 56G (7.55%* in large-vessel group; 7.75%* in mixed group; and 7.02%* in the overall cohort) in all groups except small-vessel (6.25%) associated stroke. The T1259C polymorphism did not accumulate in any stroke groups (9.48% in small-vessel group; 9.71% in large-vessel group; 11.27% in mixed group; and 10.0% in the overall group). The rs1260326 (C1337T) variant of *GCKR* gene showed similar allele frequencies both in the stroke and control groups (49.4% in small vessel; 47.8% in large vessel; 48.9% in mixed group; and 48.8% in overall cohort vs. controls).

Triglyceride levels are shown in Table 2. Both in stroke patients and in controls the level of triglycerides was elevated in all *APOA5* variant −1131C, 1259C, and IVS+G476A minor allele carriers compared with the non-carriers. By contrast, the *GCKR* gene's minor allele 1337 T was not associated with triglyceride level changes either in stroke patients or in controls.

After adjustment for age, gender, body mass index, ischemic heart disease, hypertension, diabetes mellitus and

Table 1 Major clinical and laboratory data of the patients

	Stroke patients				Controls ($n=172$)
	Small vessel ($n=232$)	Large vessel ($n=139$)	Mixed ($n=142$)	Overall ($n=513$)	
Males/females	91/141	57/82	59/83	207/306	49/123
Age (years)	65.1 ± 0.96	66.5 ± 1.21	63.8 ± 1.06	65.1 ± 0.62	56.5 ± 1.20
BMI (kg/m^2)	25.2 ± 0.13	24.8 ± 0.24	24.8 ± 0.14	24.9 ± 0.95	23.9 ± 0.16
Triglycerides (mmol/l)	1.67 ± 0.04*	1.78 ± 0.06*	1.76 ± 0.06*	1.73 ± 0.03*	1.53 ± 0.04
Total cholesterol (mmol/l)	5.85 ± 0.08*	5.96 ± 0.11*	5.90 ± 1.12*	5.89 ± 0.06*	5.37 ± 0.07

*$p<0.05$ vs. controls

Table 2 Individual effects of GCKR rs1260326 and APOA5 gene variants on ischemic stroke patients

		GCKR C1337T		MAF	APOA5 T-1131C		MAF	APOA5 T1259C		MAF	APOA5 IVS+G476A		MAF	APOA5 C56G		MAF
		CC	CT+TT	T allele	TT	TC+CC	C allele	TT	TC+CC	C allele	GG	GA+AA	A allele	CC	CG+GG	G allele
Stroke patients (n=513)																
Small (n=232)		(n=55)	(n=125+52)	49.4%	(n=197)	(n=30+5)	8.62%**	(n=190)	(n=40+2)	9.48%	(n=201)	(n=30+1)	6.90%**	(n=205)	(n=25+2)	6.25%
	TG	1.63±0.06	1.68±0.04		1.68±0.04	1.97±0.15*		1.67±0.04	1.97±0.12*		1.67±0.04	2.05±0.16*		1.64±0.03	1.95±0.13*	
	OR[a]	1.585 (0.867–2.899)			1.937* (1.026–4.541)			1.211 (0.616–2.381)			2.439* (1.272–6.120)			2.156 (0.836–5.561)		
Large (n=139)		(n=34)	(n=77+28)	47.8%	(n=118)	(n=19+2)	8.27%**	(n=113)	(n=25+1)	9.71%	(n=125)	(n=14+0)	5.04%**	(n=118)	(n=21+0)	7.55%**
	TG	1.79±0.12	1.78±0.07		1.73±0.06	2.11±0.20*		1.73±0.07	2.00±0.14*		1.73±0.06	2.28±0.21*		1.74±0.06	2.02±0.14*	
	OR[a]	1.231 (0.638–2.374)			2.813* (1.114–7.104)			1.204 (0.560–2.588)			1.888* (1.252–5.464)			2.873* (1.086–7.601)		
Mixed (n=142)		(n=34)	(n=77+31)	48.9%	(n=119)	(n=19+4)	9.51%**	(n=112)	(n=28+2)	11.27%	(n=121)	(n=20+1)	7.75%**	(n=121)	(n=20+1)	7.75%**
	TG	1.94±0.12	1.70±0.06		1.72±0.06	1.99±0.17*		1.71±0.06	1.94±0.14*		1.70±0.06	2.09±0.17*		1.73±0.06	1.91±0.12*	
	OR[a]	0.976 (0.498–1.914)			3.533* (1.421–8.781)			1.952 (0.932–4.091)			3.893* (1.445–10.489)			2.939* (1.079–8.010)		
Overall (n=513)		(n=123)	(n=279+111)	48.8%	(n=434)	(n=68+11)	8.77%**	(n=415)	(n=93+5)	10.0%	(n=447)	(n=64+2)	6.63%**	(n=444)	(n=66+3)	7.02%**
	TG	1.76±0.06	1.72±0.03		1.70±0.03	1.92±0.09*		1.69±0.03	1.89±0.07*		1.69±0.03	2.00±0.09*		1.69±0.03	1.96±0.08*	
	OR[a]	1.289 (0.790–2.104)			2.929* (1.418–6.051)			1.466 (0.838–2.566)			3.173* (1.408–7.150)			2.316* (1.059–5.067)		
Controls (n=172)		(n=48)	(n=80+44)	48.8%	(n=159)	(n=12+1)	4.07%	(n=145)	(n=27+0)	7.85%	(n=163)	(n=9+0)	2.62%	(n=160)	(n=12+0)	3.49%
	TG	1.58±0.09	1.51±0.04		1.51±0.04	1.84±0.12*		1.50±0.04	1.69±0.07*		1.52±0.04	1.80±0.11*		1.52±0.04	1.66±0.05*	

Values are means ± SEM. Triglycerides (TG) levels are mmol/l

MAF minor allele frequency

[a] Adjusted OR (95% CI) for differences in age, gender, BMI, total serum cholesterol, hypertension, diabetes mellitus, ischemic heart diseases, and smoking and drinking habits

*$p<0.05$ vs. non-carriers, **$p<0.05$ vs. controls

smoking and drinking habits, the adjusted ORs for carrying −1131C, 56G, and IVS3+476A demonstrated an independent risk for the development of stroke (Table 2). After inclusion of triglyceride levels into the adjustment parameters, the same positive correlations were found (results are not shown). In contrast, we could not detect any association of the 1259C allele with stroke; in addition, the *GCKR* 1337 T allele did not exhibit a risk or protective nature for the disease.

Besides single locus genetic analysis, we also tested the effect of the specific combinations of *GCKR* C1337T and *APOA5* polymorphisms. Individual *APOA5* genotypes were studied in combination with *GCKR* C1337T genotypes. We generated four groups for all *APOA5* variants as follows: NC_{C1337T}–$NC_{T-1131C}$; NC_{C1337T}–$C_{T-1131C}$; C_{C1337T}–$NC_{T-1131C}$ and C_{C1337T}–$C_{T-1131C}$ where "NC" refers to the wild genotype; "C" means carrying the minor allele of the variant indicated. Compared to individual ORs of the variants, the relative risk for ischemic stroke conferred by the presence of their combinations was significantly greater in certain groups. The relative risk for stroke was increased by C_{C1337T}–$C_{T-1131C}$ in the small-vessel group, in the large-vessel group, in the mixed group, and in the overall cohort; by C_{C1337T}–$C_{IVS+G476A}$ in the small and mixed groups as well as and in the whole stroke cohort; and by C_{C1337T}–C_{C56G} in the large-vessel group, in the mixed group, and in the whole group of stroke patients (results are shown in Table 3). For C_{C1337T}–$C_{T-1131C}$, lower ORs were seen in overall as well as in large and mixed-vessel groups. For the other combinations, we could not detect any changes affecting the risk of the disease. Although, for all the individual *APOA5* variants significant association with elevated non-fasting triglyceride level was found in all stroke subgroups, for the specific genotype combinations only C_{C1337T}–C_{T1259C}, C_{C1337T}–$C_{IVS+G476A}$ (small, overall), and C_{C1337T}–C_{C56G} (overall) showed the same effect (Table 3).

The C1337T variant of GCKR gene has an effect on triglyceride levels, impaired fasting glycemia, and have possible a risk of type II diabetes mellitus, as mentioned by Veiga-da Cunha (Veiga-da-Cunha et al. 2003). Carrying the 1337 T allele showed elevated triglyceride levels and higher risk of dyslipidemia but a lower fasting plasma glucose rates and a lower risk for the susceptibility of hyperglycemia (Vaxillaire et al. 2008). In our studied population, the presence of diabetes mellitus conferred increased risk for stroke in all stroke subgroups (adjusted OR in small vessel, 3.69*; in large vessel, 4.537*; in mixed, 6.157*, in overall, 4.395*; results are shown in Table 4). Subsequently, we examined the effect of *GCKR* C1337T variant on stroke patients with diabetes mellitus, the minor allele of *GCKR* (1337 T) in homozygous form showed an increased risk for stroke in two subgroups (in small-vessel group, 5.278*; in overall, 4.855*). Because of the low number of sample with

diabetes mellitus, we are unable to draw a far-reaching conclusion.

Discussion

The role of triglycerides in different occlusive vascular diseases, including ischemic stroke, has been under investigation for a long while (Freiberg et al. 2008). Results are still controversial, and as the delineation of the mechanism of triglyceride elevation has already begun, there might be a chance for verification of the possible roles of *APOA5* and *GCKR* genes (Hachinski et al. 1996; Haheim et al. 1993; Salonen et al. 1982; Simons et al. 1998).

In the postgenomic era, several new genes affecting the triglyceride metabolism had already been verified, like the *APOA5* variants. These polymorphisms can influence the function of the protein transcript, which can modify secondarily the interaction of *APOA5* with the lipoprotein lipase and eventually lead to increased circulating triglyceride levels (Pennacchio and Rubin 2003; Wright et al. 2006; Yang et al. 2004). Among the most frequently occurring variants of *APOA5*, the T-1131C, T1259C, C56G, and IVS3+G476A have already been reported to associate with elevated triglyceride levels in several different populations, and some of them were also found to confer risk for cardio- and cerebrovascular diseases (Havasi et al. 2006; Maasz et al. 2008a; b; Martinelli et al. 2007b; Pennacchio and Rubin 2003; Vaessen et al. 2006). In the present study, we could confirm the previous findings regarding associations with triglyceride levels and stroke susceptibility.

More recently, in a genome-wide association study, variants of different genes associated with elevated triglyceride levels were identified (Saxena et al. 2007). Glucokinase regulatory protein has an inhibitory effect on glucokinase, which depends on the presence of fructose-6-phosphate, antagonized by fructose-1-phosphate (Warner et al. 1995). The mutations in *GCKR* gene resulting in the synthesis of proteins with increased inhibitory activity might be diabetogenic, likely reflecting elevated sensitivity to fructose-6-phosphate or reduced susceptibility to antagonism by fructose-1-phosphate. Probably, glucokinase has a role in the development of maturity-onset diabetes of the young type II. The Diabetes Genetics Initiative genome-wide association study (Saxena et al. 2007) for type II diabetes and quantitative metabolic traits described one intronic polymorphism of the *GCKR* gene (rs780094). This SNP showed a trend toward association with lower fasting glycemia, less insulin resistance, and lower chance for the development of type II diabetes had been verified. Previously, it was proven that the rs780094 is in strong

Table 3 Analysis of disease risk and triglyceride levels in specific genotype combinations of GCKR rs1260326 and *APOA5* variants in stroke patients

		GCKR C1337T-*APOA5* T-1131C		GCKR C1337T-*APOA5* T1259C		GCKR C1337T-*APOA5* IVS+G476A		GCKR C1337T-*APOA5* C56G	
		NC_{C1337T}–$NC_{T-1131C}$	C_{C1337T}–$C_{T-1131C}$	NC_{C1337T}–NC_{T1259C}	C_{C1337T}–C_{T1259C}	NC_{C1337T}–$NC_{IVS+G476A}$	C_{C1337T}–$C_{IVS+G476A}$	NC_{C1337T}–NC_{C56G}	C_{C1337T}–C_{C56G}
Small (n=232)		(n=47)	(n=27)	(n=44)	(n=31)	(n=48)	(n=24)	(n=50)	(n=22)
	TG	1.65±0.05	1.88±0.10	1.67±0.06	1.88±0.10*	1.67±0.06	1.92±0.12*	1.60±0.06	1.96±0.16
	OR[a]	1	2.809** (1.189–6.631)	1	1.483 (0.727–3.028)	1	4.400** (1.545–12.533)	1	2.200 (0.918–5.272)
Large (n=139)		(n=28)	(n=15)	(n=27)	(n=19)	(n=30)	(n=10)	(n=31)	(n=18)
	TG	1.73±0.13	1.96±0.18	1.70±0.14	1.96±0.18	1.71±0.12	2.22±0.29	1.77±0.12	2.02±0.16
	OR[a]	1	2.619** (1.010–6.790)	1	1.481 (0.665–3.303)	1	2.993 (0.911–9.447)	1	2.903** (1.155–7.297)
Mixed (n=142)		(n=27)	(n=16)	(n=25)	(n=21)	(n=27)	(n=14)	(n=30)	(n=17)
	TG	1.84±0.13	1.80±0.16	1.83±0.13	1.80±0.16	1.84±0.13	1.98±0.22	1.93±0.13	1.89±0.14
	OR[a]	1	2.897** (1.124–7.467)	1	1.768 (0.797–3.923)	1	4.563** (1.477–14.096)	1	2.833** (1.117–7.186)
Overall (n=513)		(n=102)	(n=58)	(n=96)	(n=71)	(n=105)	(n=48)	(n=111)	(n=57)
	TG	1.72±0.05	1.88±0.08	1.72±0.06	1.88±0.08*	1.73±0.05	2.00±0.10*	1.74±0.06	1.95±0.09*
	OR[a]	1	2.780** (1.267–6.102)	1	1.557 (0.832–2.913)	1	4.023** (1.501–10.783)	1	2.568** (1.173–5.622)
Controls (n=172)		(n=44)	(n=9)	(n=40)	(n=19)	(n=44)	(n=5)	(n=45)	(n=9)
	TG	1.53±0.09	1.63±0.08	1.54±0.10	1.63±0.08	1.55±0.09	1.70±0.18	1.57±0.09	1.61±0.06

Values are means ± SEM. Triglycerides levels (TG) are mmol/l

NC carrying of the wild genotype; *C* carrying of at least one variant allele of the SNP marked in the subscript

[a] OR relative to "NC–NC" genotype (95% CI)

*$p<0.05$ vs. non-carriers, **$p<0.05$ vs. controls

Table 4 The effect of GCKR C1337T on stroke patients with diabetes mellitus

	Stroke patients with diabetes mellitus			
	Small-vessel (n=47; 20.3%)	Large-vessel (n=35; 25.2%)	Mixed (n=40; 28.2%)	Overall (n=122; 23.8%)
Adjusted OR[a]	3.69* (1.805–7.546)	4.537* (2.145–9.59)	6.157* (2.916–13.005)	4.395* (2.284–8.459)
Adjusted OR[a] for GCKR C1337T homozygous genotype	5.278* (1.091–25.54)	4.229 (0.796–22.468)	3.591 (0.693–18.613)	4.855* (1.16–21.315)

*$p<0.05$ vs. controls

[a] Adjusted for differences in gender, total serum cholesterol, triglyceride

linkage disequilibrium with the other nonsynonymous *GCKR* variant, the rs1260326 (Vaxillaire et al. 2008). The rs1260326 (Leu446Pro) is in connection with triglyceride levels and impaired fasting glycemia and might have a risk of type II diabetes mellitus (Veiga-da-Cunha et al. 2003). Vaxillaire et al. established that the minor allele (T) of rs1260326 variant in the *GCKR* gene could protect against diabetes mellitus type II (Vaxillaire et al. 2008). Although, the minor T-allele of rs1260326 was associated with increased triglyceride levels and higher risk of dyslipidemia, it showed lower fasting plasma glucose rates and decreased risk of hyperglycemia. S. Kathiresan et al. and C. J. Willer et al. investigated the effect of *GCKR* gene on triglyceride level elevation and have found a positive association with both the rs1260326 and the rs780094 SNPs (Kathiresan et al. 2008b; Willer et al. 2008).

In Hungarian population, in stroke patients, the *GCKR* gene has not been reported so far; accordingly, our goal was to scrutinize the possible gene variance and the correlation between gene effect and changes of triglyceride rates. We concluded that, except for T1259C, all *APOA5* variants had a significantly increased allele frequency and were associated with significantly elevated triglyceride levels in stroke patients collated with controls, suggesting an association with the development of stroke disease. By contrast, in the case of *GCKR* gene (rs1260326), we could not detect any difference in allele frequencies compared with controls and also found a nonsignificant triglyceride level rise. As a result, the variant of *GCKR* gene (rs1260326) is not in correlation with the susceptibility for the disease in our study group. Additionally, we observed that in all stroke subgroups diabetes mellitus was significantly associated with the development of stroke disease, despite the small number of cases. Perez-Martinez et al. (2009) studied the additive and/or synergistic effects of *GCKR* gene rs780094 variant with *APOA5* gene polymorphisms (T-1131C, C56G). We also examined the genotype combinations of the *GCKR* (rs1260326) and *APOA5* gene variants (T-1131C, T1259C, IVS+G476A, and C56G) but could not detect relevant changes—neither in triglyceride levels nor in odds ratios for the ischemic stroke disease—compared to

individual effects of these genes. In summary, in agreement with our previous results, the frequencies of *APOA5* minor alleles are higher in stroke patients than in controls, and we can conclude that there is a relationship between the triglyceride increase and the variants studied. We could detect a possible risk for the development of stroke in connection with the *APOA5* polymorphisms. However, we did not find any association between *GCKR* variant and either the triglyceride levels or the susceptibility for the disease; thus, in the Hungarian population, the *GCKR* gene polymorphism did not prove to be an independent risk factor. Examining the combinations of four *APOA5* polymorphisms with the *GCKR* variant, we found that in certain subgroups, the gene combinations increased the risk for the development of stroke. In conclusion, further well-powered studies investigating larger populations are required to understand the role of *GCKR* mutations in ischemic stroke susceptibility and to help in developing a more effective clinical prevention.

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