

Evaluation of the Obesity Genes *FTO* and *MC4R* and the Type 2 Diabetes Mellitus Gene *TCF7L2* for Contribution to Stroke Risk: The Mannheim-Heidelberg Stroke Study

Yaroslav Winter^a Tobias Back^b André Scherag^c Jakob Linseisen^{d,e} Sabine Rohrmann^d
Oliver Lanczik^f Anke Hinney^g Susann Scherag^g Michael Neumaier^h Peter A. Ringlebⁱ
Richard Dodel^a Johannes Hebebrand^g

^a Department of Neurology, Philipps University Marburg,

^b Department of Neurology, Saxon Hospital Arnsdorf, Arnsdorf/Dresden,

^c Institute for Medical Informatics, Biometry and Epidemiology, University of Duisburg-Essen, Essen,

^d Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg,

^e Institute of Epidemiology, Helmholtz Center Munich, Neuherberg,

^f Department of Neurology, Klinikum Mannheim, University of Heidelberg,

^g Department of Child and Adolescent Psychiatry, University of Duisburg-Essen, Essen,

^h Institute for Clinical Chemistry, Klinikum Mannheim,

ⁱ Department of Neurology, Klinikum Heidelberg, University of Heidelberg, Germany

Keywords

Genetics · Obesity genes · *FTO* · *MC4R* · *TCF7L2* · Stroke

Summary

Objective: Studies evaluating genetic markers for vascular risk and risk of stroke are limited, and none of them evaluated obesity genes. The objective was to investigate the genetic markers related to obesity genes *FTO* and *MC4R* and the gene of type 2 diabetes mellitus *TCF7L2* for their contribution to risk of stroke and transient ischemic attacks (TIA). **Methods:** We recruited 379 consecutive patients with stroke/TIA and 379 healthy population-based controls. The single-nucleotide polymorphisms (SNPs) rs9937053 (*FTO*), rs2229616 (*MC4R* V103I), rs17782313 (188kb downstream of *MC4R*), and rs7903146 (*TCF7L2*) were evaluated for association with stroke using logistic regression analyses. **Results:** The odds ratios for stroke/TIA were 1.14 (95%CI 0.91–1.42) for rs9937053/*FTO*, 1.11 (95%CI 0.49–2.51) for rs2229616/*MC4R*, 1.05 (95%CI 0.82–1.3) for rs17782313/*MC4R*, and 0.99 (95%CI 0.78–1.25) for rs7903146/*TCF7L2*. Further exploration revealed that male patients with the T allele of rs7903146/*TCF7L2* had a worse clinical outcome compared with male patients carrying the C allele. **Conclusion:** The observed trends of obesity risk alleles for risk of stroke/TIA as well as the possible sex-specific

differences in clinical outcomes found for the *TCF7L2* (rs7903146) require replication in future studies. Our study demonstrates that candidate gene studies for common stroke may benefit from focusing on polymorphisms that predispose to vascular risk.

Introduction

Stroke is the third leading cause of death in adults in the Western world. It imposes a significant economic burden on the society and individual patients [1–5]. The two principal types of stroke are ischemic and hemorrhagic stroke. Both are associated with vascular risk factors such as chronic arterial hypertension, type 2 diabetes mellitus (T2DM), hyperlipidemia, and obesity [6–8]. Studies in twins, families, and animal models support the role of genetic factors in the etiology of stroke [9]. However, the extent of genetic contribution to the risk of stroke is uncertain.

Mendelian disorders associated with stroke include metabolic disorders of inborn origin, hemoglobinopathies, dyslipoproteinemias, and cardioembolic disorders. Examples of such are sickle cell disease, cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy (CA-

DASIL), mitochondrial disorders such as mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS), familial atrial myxoma, and Fabry's disease [9]. However, all these well recognized and phenotypically distinct causes for stroke include only a small minority of stroke cases.

Defining the genetic basis of common stroke, which is thought to have a polygenic basis, has proven more challenging [10]. Most genetic studies which evaluated candidate genes in common stroke focused on genes involved in thrombosis and coagulation [10]. The majority of these candidate genes were evaluated initially in ischemic heart disease. Genes involved in the biology of well-known risk factors for stroke (e.g. arterial hypertension, hyperlipidemia, T2DM, and obesity) have received limited attention. To the best of our knowledge, studies evaluating the role of candidate genes of obesity in the formation of stroke are lacking to date. Data on the relation of T2DM genes with stroke are scarce and inconsistent [11, 12].

The objective of the Mannheim-Heidelberg Stroke Study was to evaluate confirmed genetic markers of the obesity genes 'fat mass and obesity associated' (*FTO*) and the 'melanocortin 4 receptor' (*MC4R*) as well as the T2DM susceptibility gene 'transcription factor 7-like 2' (*TCF7L2*) for their contribution to the risk of stroke and transient ischemic attacks (TIAs). The phenotype analysis of the study population had shown a positive association between anthropometric measures of obesity and increased risk of stroke/TIA [8].

Patients and Methods

Study Design

The Mannheim-Heidelberg Stroke Study is a case-control genetic study of the disease-oriented Genome Network 'Obesity and Related Disorders', which is a part of the German National Genome Research Network plus (NGFN^{plus}). For a detailed description of the study design, we refer to our previous publication [8]. In brief, the study sample consisted 379 consecutive cases of stroke and TIAs and 379 healthy controls. Cases were recruited in the Departments of Neurology of the University Hospitals of Mannheim and Heidelberg between February 1, 2005 and January 31, 2006. Those patients with recurrent cerebrovascular events or a history of cerebrovascular events prior to initiation of the study were not excluded. Clinical records were incomplete for 22 out of 401 initially recruited cases so that these cases were excluded from the analysis. Regional controls without a history of cerebrovascular disease were frequency-matched for age and sex using the database of 25,540 participants of the population-based cohort EPIC-Heidelberg [13]. For control selection, we draw individuals from the following age groups: <50, 50–55, 56–60, 61–65, 66–70, and >70 years. Note that the age range for the cases was between 25 and 90 years, whereas the age range of controls was between 45 and 75 years only. The study was approved by the local ethics committee, and all participants gave informed consent. Thus, patients with impaired consciousness or severe aphasia had to be excluded because no informed consent could be obtained in those instances.

Clinical Evaluation

Each patient received a complete medical and neurological examination, CT and/or MRI of the head. The diagnosis of stroke was defined according to the World Health Organization (WHO) [14]. The following markers of

obesity were evaluated in less than 48 h after admission: BMI, waist-to-hip ratio (WHR), waist circumference, and waist-to-stature ratio (WSR). BMI was calculated as weight (kilograms) divided by height (meters squared). WHR was calculated as waist divided by hip circumference. Waist circumference (cm) was measured at the level of the umbilicus. Hip circumference (cm) was measured at the level of the bilateral greater trochanters. WSR was defined as waist circumference divided by body height. The vascular risk factors included T2DM (fastening blood glucose \geq 126 mg/dl or use of insulin or oral hypoglycemic agents), hypertension (systolic blood pressure \geq 140 mm Hg and/or diastolic blood pressure \geq 90 mm Hg or treatment with antihypertensive agents), hyperlipidemia (total serum cholesterol level \geq 240 mg/dl or use of antihyperlipidemic agents), and physical inactivity (sport activities of less than 20 min 2 times per week).

Clinical outcomes were measured using the National Institute of Health Stroke Scale (NIHSS) and modified Rankin scale (mRS), which are valid and reliable measures of stroke outcome [15, 16]. The NIHSS is a standardized measure of stroke severity and level of stroke impairment. It is one of the most frequently used scales in stroke clinical trials. The NIHSS scores range from 0 to 42, with increasing scores indicating increasing stroke severity [16]. The mRS is a measure of the functional status and disability. Its scores range from 0 (no impairment) to 5 (severe impairment) [15].

Genotyping

The following single nucleotide polymorphisms (SNPs) were used in the analysis: rs17782313 (*MC4R*, obesity risk allele C) [17], rs2229616 (*MC4R* *V103I*, obesity risk allele A) [18], rs9937053 (*FTO*, obesity risk allele A) [19], rs7903146 (*TCF7L2*, T2DM risk allele T) [20].

The rationale for use of these SNPs in the analysis was based on following: The association of the common genetic variants of *FTO* and *MC4R* with BMI was shown in large-scale genetic studies [17, 19, 21]. The *MC4R* SNP rs17782313 is in high linkage disequilibrium with a SNP that is associated with waist circumference and insulin resistance [21]. The *TCF7L2* polymorphism rs7903146 is associated with T2DM; interestingly, this major T2DM risk allele is also weakly negatively associated with obesity [22]. Recently, the rs7903146 T allele has been shown to be associated with severity and mortality of coronary artery disease [23].

Genomic DNA was isolated from EDTA-anticoagulated blood using standard procedures. The *FTO* SNP rs9939609 and the *TCF7L2* SNP rs79031746 were genotyped using tetra-ARMS-PCR as described previously [24, 25]. For *MC4R* rs2229616 (*V103I*) DNA samples were genotyped as described previously [26], and rs17782313 was genotyped using TaqMan[®] SNP genotyping assay C_32667060_10 (Applied Biosystems, Darmstadt, Germany) with standard conditions; call rates were \geq 99%, with 100% concordance of duplicates.

Statistical Analysis

All genotype distributions (in cases, controls and in the sex-stratified subsamples) were tested for deviations from Hardy-Weinberg equilibrium using the PLINK software (Version 1.04), and no evidence for such deviations was detected (all $p \gg 0.12$). All phenotypes were analyzed by either linear or logistic regression analyses (crude (model 1 in table 3) and (simultaneously; model 2 in table 3) adjusted for the covariates age (years), sex (female/male), history of hypertension (yes/no), history of diabetes (yes/no), physical inactivity (yes/no), and smoking (yes/no)). Except for *MC4R* (rs2229616), where a dominant mode of inheritance was assumed, each SNP was analyzed under an (log)-additive genetic model. Power calculations were done with the software QUANTO (Version 1.2.3, <http://hydra.usc.edu/gxe>). 379 case-control pairs were estimated to yield a power of 83% to detect log-additive odds ratios (ORs) of 1.4 for a minor allele frequency of 25% (based on HapMap CEU data for rs7903146) and $\alpha = 0.05$ (two-sided). Confidence intervals were calculated with coverage of 95% (abbreviated 95% CI), and accordingly the level α for each test was 0.05 (two-sided). Unless otherwise stated, all reported p values are nominal, two-sided, and not adjusted for multiple testing.

Table 1. Demographics, clinical features of the study samples stratified by sex

	Men				p value	Women				
	controls (n = 238)		cases (n = 238)			controls (n = 141)		cases (n = 141)		p value
	mean ± SD	% yes	mean ± SD	% yes		mean ± SD	% yes	mean ± SD	% yes	
Age, years	65.0 ± 9.0		66.9 ± 10.9		0.018	65.0 ± 9.0		68.0 ± 14.1		0.021
Weight, kg	84.4 ± 12.0		84.7 ± 12.7		0.934	69.9 ± 11.1		74.8 ± 18.4		0.071
Height, cm	175.3 ± 6.6		173.9 ± 7.1		0.016	161.5 ± 6.2		161.7 ± 6.4		0.580
WC, cm	98.5 ± 10.3		103.6 ± 11.1		<0.001	85.0 ± 11.2		98.2 ± 15.5		<0.001
HC, cm	102.5 ± 6.4		103.5 ± 10.6		0.336	102.7 ± 8.3		105.3 ± 12.1		0.104
WHR	1.0 ± 0.7		1.00 ± 0.08		<0.001	0.8 ± 0.8		0.93 ± 0.1		<0.001
BMI	27.5 ± 3.6		28.0 ± 3.7		0.121	26.8 ± 4.3		28.6 ± 6.5		0.087
WSR	0.52 ± 0.08		0.60 ± 0.07		<0.001	0.52 ± 0.08		0.61 ± 0.10		<0.001
PIN		44.0		82.5	<0.001		41.8		87.2	<0.001
Smoking		16.3		30.2	<0.001		12.1		24.8	<0.001
AHTN		49.0		84.3	<0.001		49.6		80.4	<0.001
T2DM		11.9		30.9	<0.001		9.5		30.7	<0.001
HLP		55.6		50.4	0.087		56.0		53.9	0.322

AHTN = Arterial hypertension; HC = hip circumference; HLP = hyperlipidemia; PIN = physical inactivity; WC = waist circumference.

*Descriptive two-sided p value of the Wilcoxon-Mann-Whitney test.

Table 2. Genotype distributions in cases and controls

SNP (gene)	Genotypes	Men			p value*	Women		
		number of cases (%)	number of controls (%)			number of cases (%)	number of controls (%)	
rs9937053 (<i>FTO</i>)	AA	33 (16.4)	40 (17.3)	0.26	20 (16.9)	19 (14.0)	0.73	
	AT	104 (51.7)	102 (44.2)		64 (54.2)	73 (53.7)		
	TT	64 (31.8)	89 (38.5)		34 (28.8)	44 (32.4)		
rs17782313 (<i>MC4R</i>)	CC	12 (6.0)	15 (6.6)	0.87	10 (8.5)	4 (3.0)	0.18	
	CT	78 (38.9)	92 (40.7)		46 (40.0)	53 (39.9)		
	TT	111 (55.1)	119 (52.7)		62 (52.5)	76 (57.1)		
rs2229616 (<i>MC4R</i>)	GG	205 (96.7)	226 (96.6)	1.00	121 (96.0)	136 (97.1)	0.74	
	GA	7 (3.3)	8 (3.4)		5 (4.0)	4 (2.9)		
rs7903146 (<i>TCF7L2</i>)	CC	108 (50.9)	114 (49.1)	0.92	60 (47.6)	69 (50.7)	0.71	
	CT	87 (41.0)	100 (43.1)		58 (46.0)	56 (41.2)		
	TT	17 (8.0)	18 (7.8)		8 (6.3)	11 (8.1)		

*Descriptive two-sided p value of Fishers exact test.

Results

We included 379 cases with stroke (ischemic stroke n = 301, intracerebral hemorrhage (ICH) n = 37, or TIA n = 41) of whom 141 were female (37.2%). Demographics and distribution of known vascular risk factors are shown in table 1. Due to the fact that among the controls no individual was older than 75 years, the mean age of cases was slightly higher than the mean age of controls (67.3 ± 12.2 vs. 65.0 ± 8.3 years; p = 0.02). Cases had higher prevalences of obesity (29.8% of cases (n = 113) vs. 20.8% of controls (n = 79); p < 0.01) and

other vascular risk factors than controls. The mean NIHSS score at admission was 4.5 ± 3.9 (median 3) in male patients and 5.1 ± 4.5 (median 4) in female patients.

The genotype frequencies in cases and controls are shown in table 2. The T allele of *TCF7L2* showed a positive association with T2DM (OR 1.35, 95% CI 1.02–1.80), which remained significant after adjustment for age, sex, and vascular risk factors (OR 1.36, 95% CI 1.01–1.84). For all other markers, though not statistically detectable, we observed similar directions of allelic effects for BMI and WHR as reported previously (e.g. increased BMI (regression coefficient 0.15,

Table 3. ORs for the associations between genotype and risk of stroke/TIA

SNP (gene)	Effect allele	Genetic model	Model 1*			Model 2**		
			OR	95% CI	p value	OR	95% CI	p value
<i>All participants</i>								
rs9937053 (<i>FTO</i>)	A	ADD	1.14	0.91–1.42	0.25	1.11	0.85–1.44	0.46
rs17782313 (<i>MC4R</i>)	C	ADD	1.05	0.82–1.34	0.72	1.06	0.79–1.42	0.68
rs2229616 (<i>MC4R</i>)	A	DOM	1.11	0.49–2.51	0.80	0.80	0.31–2.08	0.64
rs7903146 (<i>TCF7L2</i>)	T	ADD	0.99	0.78–1.25	0.92	0.85	0.64–1.13	0.26
<i>Males</i>								
rs9937053 (<i>FTO</i>)	A	ADD	1.13	0.86–1.48	0.39	1.1	0.79–1.52	0.58
rs17782313 (<i>MC4R</i>)	C	ADD	0.92	0.67–1.25	0.59	0.90	0.63–1.31	0.61
rs2229616 (<i>MC4R</i>)	A	DOM	0.96	0.34–2.71	0.95	0.53	0.16–1.74	0.29
rs7903146 (<i>TCF7L2</i>)	T	ADD	0.96	0.72–1.29	0.80	0.82	0.58–1.17	0.28
<i>Females</i>								
rs9937053 (<i>FTO</i>)	A	ADD	1.16	0.80–1.69	0.43	1.16	0.73–1.84	0.54
rs17782313 (<i>MC4R</i>)	C	ADD	1.32	0.87–2.00	0.19	1.38	0.83–2.28	0.21
rs2229616 (<i>MC4R</i>)	A	DOM	1.41	0.37–5.35	0.62	1.74	0.32–9.60	0.52
rs7903146 (<i>TCF7L2</i>)	T	ADD	1.04	0.70–1.53	0.86	0.9	0.56–1.46	0.68

ADD = Additive mode of inheritance; DOM = dominant mode of inheritance.

*Model 1: not adjust; crude estimates.

**Model 2: simultaneously adjusted for age (years) and sex (female/male), history of hypertension (yes/no), history of diabetes (yes/no), physical inactivity (yes/no) and smoking (yes/no).

Table 4. Associations between genotype and risk of different cerebrovascular events

SNP (gene)	Effect allele	Genetic model	Model 1*			Model 2**		
			OR	95% CI	p value	OR	95% CI	p value
<i>TIA</i>								
rs9937053 (<i>FTO</i>)	A	ADD	1.57	0.94–2.59	0.08	1.47	0.87–2.49	0.15
rs2229616 (<i>MC4R</i>)	A	DOM	NA	NA	NA	NA	NA	NA
rs7903146 (<i>TCF7L2</i>)	T	ADD	1.11	0.65–1.89	0.72	1.07	0.62–1.86	0.81
<i>Ischemic stroke</i>								
rs9937053 (<i>FTO</i>)	A	ADD	1.03	0.82–1.29	0.83	0.97	0.74–1.27	0.84
rs2229616 (<i>MC4R</i>)	A	DOM	1.19	0.52–2.72	0.68	0.94	0.36–2.45	0.90
rs7903146 (<i>TCF7L2</i>)	T	ADD	0.95	0.75–1.21	0.69	0.82	0.61–1.08	0.15
<i>ICH</i>								
rs9937053 (<i>FTO</i>)	A	ADD	1.12	0.68–1.86	0.65	1.10	0.64–1.9	0.74
rs2229616 (<i>MC4R</i>)	A	DOM	1.75	0.39–7.74	0.46	1.47	0.32–6.74	0.62
rs7903146 (<i>TCF7L2</i>)	T	ADD	1.09	0.64–1.84	0.76	1.07	0.63–1.82	0.80

ADD = Additive mode of inheritance; DOM = dominant mode of inheritance; NA = not available.

*Model 1: not adjust; crude estimates.

**Model 2: adjusted for age (years) and sex (female/male), history of hypertension (yes/no), history of diabetes (yes/no), physical inactivity (yes/no) and smoking (yes/no).

p = 0.52) and larger WHR (regression coefficient 0.003, p = 0.54) for the obesity risk allele of the *FTO* polymorphism rs9937053).

The associations between variants of the three candidate genes and risk of stroke were investigated using logistic regression analysis (table 3). The ORs for stroke or TIA were 1.14 (95% CI 0.91–1.42; p = 0.25) for *FTO* rs9937053 A-allele,

1.11 (95% CI 0.49–2.51; p = 0.80) for *MC4R* V103I rs2229616 A-allele, 1.05 (95% CI 0.82–1.34; p = 0.72) for *MC4R* variant rs17782313 C-allele, and 0.99 (95% CI 0.78–1.25; p = 0.92) for rs7903146 (*TCF7L2*). After adjustment (model 2) the observation of the absence of an effect was underlined. In addition, explorative stratification by sex did not show any significant sex-specific associations (table 3).

Table 5. Sex-specific clinical outcome scores 7 days after stroke/TIA stratified by genotype

SNP (gene)	mRS mean (median) ± SD				NIHSS mean (median) ± SD			
	men	p value	women	p-value	men	p value	women	p value
rs9937053 (<i>FTO</i>)								
TT	1.4 (2) ± 1.5	0.22	2.7 (2) ± 2.6	0.79	1.9 (2) ± 2.6	0.24	5.3 (3) ± 5.7	0.85
AT	1.7 (2) ± 1.6		1.9 (2) ± 1.8		2.3 (2) ± 2.8		3.0 (3) ± 3.9	
AA	2.0 (2) ± 1.6		2.0 (2) ± 1.8		2.9 (2) ± 3.3		3.5 (3) ± 4.8	
rs17782313 (<i>MC4R</i>)								
CC	1.9 (2) ± 1.8	0.88	2.3 (2) ± 2.1	0.67	3.0 (3) ± 3.3	0.78	3.7 (3) ± 4.0	0.80
CT	1.8 (2) ± 1.7		1.8 (2) ± 1.7		2.7 (3) ± 3.4		2.8 (3) ± 3.5	
TT	1.7 (2) ± 1.5		2.1 (2) ± 1.8		2.2 (3) ± 2.7		3.6 (3) ± 4.6	
rs2229616 (<i>MC4R</i>)								
GG	1.8 (1) ± 1.6	0.58	2.1 (2) ± 1.8	0.58	2.5 (3) ± 3.0	0.50	3.6 (2) ± 4.5	0.66
GA	2.0 (1) ± 1.4		1.6 (2) ± 1.7		3.1 (3) ± 3.1		2.2 (2) ± 2.5	
rs7903146 (<i>TCF7L2</i>)								
CC	1.5 (1) ± 1.4	0.02	2.1 (2) ± 1.8	0.61	2.0 (2) ± 2.5	0.04	3.4 (3) ± 4.1	0.34
CT	2.1 (2) ± 1.7		2.1 (2) ± 1.9		3.1 (3) ± 3.2		3.4 (3) ± 4.9	
TT	1.9 (2) ± 1.7		1.4 (2) ± 1.6		3.1 (3) ± 4.0		2.3 (3) ± 1.7	

The analysis of different types of cerebrovascular disease showed a weak association of *FTO* rs9937053 A-allele with increased risk of TIA (OR 1.57, 95% CI 0.94–2.59; $p = 0.08$) (table 4). The OR decreased after adjustment (1.47, 95% CI 0.87–2.49; $p = 0.15$). For ischemic stroke and ICH no evidence for genetic associations was found.

Different genotypes were compared for association with stroke severity measured by NIHSS and mRS at admission and at follow-up on day 7 after admission. At admission, no differences in the stroke severity have been detected for the studied genotypes. The clinical outcome on day 7 was worse in male patients with allele T of rs7903146 (*TCF7L2*) compared to male patients with the C allele at this marker (mRS $p = 0.02$; NIHSS $p = 0.04$). There were no statistically significant differences in clinical outcome of female patients. The comparison of clinical outcomes by different genotypes related to *FTO* or *MC4R* did not provide evidence for statistically significant differences (table 5). We also did not find any associations between studied genotypes and the age at which the cerebrovascular event occurred (data not shown).

Discussion

We evaluated the confirmed variants in obesity and T2DM genes *FTO*, *MC4R* and *TCF7L2* with regard to their potential role in the risk of stroke/TIA. Obesity is an important vascular risk factor and its role in stroke has been intensely investigated in the last years [27]. For example, we have reported an association between abdominal obesity and increased risk of stroke/TIA [8] which has been replicated in two population-based studies [6, 7].

Large-scale studies searched for candidate genes for obesity and found associations with common genetic variants of the genes *FTO* and *MC4R* [17, 19, 21]. The minor (A) allele of the most common intragenic variant of *MC4R* (rs2229616) was shown to be associated with decreased BMI [28]. The role of *MC4R* as genetic risk factor contributing to the development of other common risk factors of cerebrovascular disease (e.g. insulin resistance and metabolic syndrome) was discussed in the recent years [21, 29]. However, genetic studies have so far not evaluated the association between *MC4R* and the risk of cerebrovascular disease outcomes directly. Despite the evidence from previous studies on the association of *MC4R* with cerebrovascular risk factors, the variant rs2229616 was not associated with risk of stroke/TIA in the present study.

FTO has also been shown to be associated with obesity and other cerebrovascular risk factors such as the metabolic syndrome [30], and a possible role in atherosclerosis is under discussion. Kivimaki et al. [31] showed that individuals with the A allele of the *FTO* polymorphism rs9939609 had a trend towards increased intima-media thickness of the carotid artery, which is a clinical marker of atherosclerosis. To the best of our knowledge, none of previous studies evaluated the *FTO* variants with a focus on cerebrovascular disease. Our study did not observe any evidence for an association between *FTO* and risk of stroke. Interestingly, we found a weak association between *FTO* rs9939609 and risk of TIA. Therefore, further studies should focus on evaluation of rs9939609 in risk of TIA.

T2DM is one of the most important risk factors for cerebrovascular disease [20, 32]. The *TCF7L2* polymorphism rs7903146 has been consistently associated with T2DM in dif-

ferent populations [33]. In addition, the same variant has been reported to be associated with obesity and the severity and mortality of cardiovascular disease [23]. However, data on its role in vascular diseases are inconsistent [34]. We observed that allele T of *TCF7L2* showed a positive association with T2DM which is consistent with the results of previous studies [33]. However, we did not observe any evidence for an association between *TCF7L2* and risk of stroke.

Little is known about the genetic determinants of outcome after stroke. The associations with survival and disability following stroke were found for genotypes of interleukin-1 receptor antagonist, interleukin-6, and insulin-like growth factor I [35–37]. Currently, genetic determinants of stroke outcome are being investigated in the Genes Affecting Stroke Risk and Outcome Study (GASROS) [38]. Our study found a sex-specific influence of the T allele of *TCF7L2* on the functional outcome after stroke measured by both mRS and NIHSS. This corresponds with data from other stroke studies showing that hyperglycemia is associated with a poor clinical outcome in stroke patients [39].

Strengths of our study are that we used a case-control design with controls ascertained from a population-based study. Cases were recruited consecutively in two large neurological clinics with a well-defined catchment area of approximately 800,000 inhabitants. The moderate sample size of our study is comparable to the samples sizes of other genetic studies in stroke (549 cases in the ARIC study [34], 540 cases in the Rotterdam Study [40], 297 cases in the Stroke Prevention in Young Women Study [41]). However, this study did not have sufficient statistic power to identify ORs for stroke that are in the order of 1.2 or lower and should be considered as a pilot study on the role of obesity genes (*FTO*, *MC4R*, and *TCF7L2*) in the risk of common stroke. In order to substantiate the possible association between *FTO* and the risk of stroke/TIA, a sample size of at least about 3,000 case-control pairs as based on the observed effect (OR ~ 1.1) and allele frequency (~40%) would be necessary for a power of 80%. As case-control studies focusing on stroke with such a sample size are rare, future meta-analyses will be necessary to address the question of potential smaller effects of the three investigated markers of the three genes *FTO*, *MC4R* and *TCF7L2* on the risk of stroke or

TIA. Additionally, very severely affected patients or patients with global aphasia were not included in this study as their informed consent could not be obtained. This may have limited the number of severe strokes so that these are under-represented. There were only individuals aged 45–75 years among the controls; so exact age matching was not possible. In order to avoid a systematic error of data, the plausibility proof was conducted by using only the exact age match for cases aged 45–75 years. This analysis confirmed all main results as robust [8]. Finally, we evaluated only three candidate genes of obesity and T2DM for their impact on stroke/TIA. *MC4R* and *FTO* are the most important obesity genes at the time of the study conduct [17, 19, 21], whereas *TCF7L2* is the most important genetic factor for T2DM. In or near the above mentioned candidate genes, we evaluated alleles of SNPs, which are known to be robustly associated with obesity or T2DM susceptibility despite the fact that these variants may not be the variants with a functional effect. Currently, a number of new candidate genes are suggested that may come under investigation soon [42, 43].

In conclusion, we evaluated variants of three candidate genes for obesity and T2DM, *MC4R*, *FTO* and *TCF7L2*, for a potential contribution to stroke susceptibility and observed no significant associations. In an explorative, sex-stratified analysis, we observed a weak association of the T allele of *TCF7L2* to clinical outcomes which requires replication. Future studies on genetics of common stroke should concentrate on evaluation of genes predisposing to cerebrovascular risk factors, such as obesity and T2DM, and consider the assessment of genetic determinants of clinical outcome.

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Disclosure Statement

The authors declare that they have no competing interests.

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