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Association of SGK1 Gene Polymorphisms with Type 2 Diabetes

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Key Words
SGK1 • Type 2 diabetes • Blood pressure • Obesity • Hypertension

Abstract
The serum and glucocorticoid inducible kinase SGK1 is genomically upregulated by glucocorticoids and in turn stimulates a variety of carriers and channels including the renal epithelial Na^+ channel ENaC and the intestinal Na^+ glucose transporter SGLT1. Twin studies disclosed an association of a specific SGK1 haplotype with moderately enhanced blood pressure in individuals who are carrying simultaneously a homozygous genotype for a variant in intron 6 [I6CC] and a homozygous or heterozygous genotype for the C allele of a polymorphism in exon 8 [E8CC/CT] of the SGK1 gene. A subsequent study confirmed the impact of this risk haplotype on blood pressure. SGK1 knockout mice are resistant to the insulin and high salt induced increase of blood pressure, glucocorticoid induced increase of electrogenic glucose transport, and glucocorticoid induced suppression of insulin release. The present study explored whether the I6CC/E8CC/CT haplotype impacts on the prevalence of type 2 diabetes. The prevalence of the I6CC genotype was 3.1% in a healthy German, 2.4 % in a healthy Romanian and 11.6 % in a healthy African population from Ghana (p=0.0006 versus prevalence in Caucasians). Comparison of genotype frequencies between type 2 diabetic patients and the respective control groups revealed significant differences for the intron 6 T>C variant. Carriers of at least one T allele were protected against type 2 diabetes (Romanians: p=0.023; OR 0.29; 95% CI 0.09-0.89; Germans: p=0.01; OR 0.37; 95% CI 0.17-0.81). The SGK1 risk haplotype (I6CC/E8CC/CT) was significantly (p=0.032; OR 4.31, 95% CI 1.19-15.58) more frequent in diabetic patients (7.2 %) than in healthy volunteers from Romania (1.8 %). The observations support the view that SGK-1 may participate in the pathogenesis of metabolic syndrome.

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Introduction

The serum- and glucocorticoid-inducible kinase 1 (SGK1) was originally cloned from rat mammary tumor cells [1-3]. The human isoform has been discovered as cell volume regulated gene [4]. A wide variety of further hormones upregulate SGK1 transcription [5] including mineralocorticoids [6-15]. Expressed SGK1 is activated by several stimuli including insulin [16, 17], IGF1 [16, 18, 19], and further growth factors [20] through a signaling cascade involving phosphatidylinositol-3-kinase (PI3-kinase) and the 3-phosphoinositide (PIP3)-dependent kinase PDK1 and PDK2 [16, 21-28]. Moreover, SGK1 may be activated by cAMP [17].

The gene encoding human SGK1 has been localised to chromosome 6q23. SGK1 transcripts have been found in virtually all tissues tested [4].

In the *Xenopus* oocyte expression system, SGK1 has been shown to be a potent regulator of a wide variety of ion channels and transporters [5]. Specifically, SGK1 has been shown to stimulate the epithelial Na^+ channel ENaC [8, 14, 29-38], the K^+ channels ROMK [39-41] and KCNE1/KCNQ1 [42, 43], the epithelial Ca^{2+} channel TRPV5 [44, 45], the Na^+/H^+ exchanger NHE3 [46, 47], the Na^+, K^+, $2Cl^-$ cotransporter NKCC2 [35], the Na^+/K^+ATPase [48-50], the glucose transporters SGLT1 [51], GLUT1 [52], and GLUT4 [53], the amino acid transporters ASCT2 (SLC 1A5) [54], SN1 [55], and EAAT1 [56], EAAT2 [57], EAAT3 [58], EAAT4 [59] and EAAT5 [60], the Na^+-coupled dicarboxylate transporter NaDC [61] and the Creatine transporter CreaT [62]. Clearly, those observations cannot be translated without reservations in the *in vivo* activity of the channels. However, the activity of several channels and carriers has indeed been shown to be decreased in gene targeted mice lacking functional SGK1.

Specifically, the ability of SGK1 to regulate renal Na^+ reabsorption is illustrated by the impaired renal sodium retention of gene targeted mice lacking functional SGK1 [63]. According to evidence in those mice, SGK1 is particularly important for the stimulation of renal Na^+ reabsorption by insulin [64]. Induction of hyperinsulinemia in mice by pretreatment with a high-fructose diet [64] sensitizes arterial blood pressure to high-salt intake in wild-type but not SGK1-deficient animals. Thus, SGK1 mediates the salt-sensitizing effect of hyperinsulinism on blood pressure.

SGK1 further mediates the stimulating effect of mineralocorticoids on salt appetite [65]. Thus, SGK1 influences salt balance by affecting both, NaCl intake and renal NaCl reabsorption [5].

Studies in SGK1 knockout mice further disclosed the pivotal role of SGK1 in the stimulation of intestinal electrogenic glucose transport by glucocorticoids [66]. Notably, SGK1 deficient mice show reduced glucocorticoid-induced intestinal glucose uptake [66]. At least partially due to its stimulating effect on glucose transporter GLUT1 [52], SGK1 also favours cellular glucose uptake from the circulation into several tissues including brain, fat and skeletal muscle [67]. Accordingly, following an i.p. glucose load, the plasma glucose concentration increases to higher peak values and declines slower in SGK1 knockout mice [67]. Likewise, the plasma glucose lowering effect of an i.p. insulin application is attenuated in SGK1 knockout mice [67].

The observation of SGK1 dependent effects on salt balance and blood pressure regulation in mice prompted the hypothesis that SGK1 may influence blood pressure in man. Genetic studies in twins indeed disclosed a specific SGK1 risk haplotype associated with moderately enhanced blood pressure [68, 69] in individuals who are carrying simultaneously a homozygous genotype for a variant in intron 6 [I6CC] and a homozygous or heterozygous genotype for the C allele of a polymorphism in exon 8 [E8CC/CT]). Of note this SGK1 risk haplotype was recently confirmed to be associated with hypertension in a large-scale population of more than 4800 Swedish subjects [70]. The correlation is presumably due to enhanced stimulation of ENaC by SGK1 in individuals carrying this SGK1 gene variant.

The SGK1 gene variants may further accelerate intestinal glucose absorption by stimulation of SGLT1 and glucose deposition in peripheral tissues including fat. Enhanced SGLT1 activity, and subsequently accelerated intestinal glucose absorption, may lead to excessive insulin release, fat deposition, a subsequent decrease of plasma glucose concentration and triggering of repeated glucose uptake and thus obesity [71]. Conversely, inhibitors of SGLT1 counteract obesity [72]. Thus, SGK1 may influence body weight. As a matter of fact, the same variant of the SGK1 gene associated with enhanced blood pressure proved to be similarly associated with increased body mass index [51].

Those observations are suggestive for a role of SGK1 in the pathogenesis of metabolic syndrome or syndrome X, a condition characterized by the coincidence of essential hypertension, procoagulant state, obesity and hyperinsulinemia [73]. The condition is associated with enhanced morbidity and mortality from cardiovascular disease [74-76]. Metabolic syndrome and Cushing´s

Parameter	Total population	intron 6 CC / exon 8 CC/CT carriers	non-carriers	p values (carriers vs. non-carriers)
Number (♀, ♂)	322 (♀ 157, ♂ 165)	6 (♀ 3, ♂ 3)	290 (♀ 139, ♂ 151)	1.00
Age	36.0 ± 0.9 (322)	36.2 ± 4.2 (6)	37.2 ± 0.9 (290)	0.74
Body weight (kg)	69.8 ± 0.7 (320)	71.5 ± 2.5 (6)	70.0 ± 0.7 (288)	0.62
Body mass index	23.0 ± 0.2 (320)	24.6 ± 1.4 (6)	23.1 ± 0.2 (288)	0.29
Systolic blood pressure (mmHg)	128.9 ± 0.9 (310)	132.3 ± 8.6 (6)	128.8 ± 1.0 (278)	0.79
Diastolic blood pressure (mmHg)	80.1 ± 0.6 (310)	83.5 ± 6.6 (6)	80.3 ± 0.7 (278)	0.72
Creatinine clearance (ml/min)	85.4 ± 1.6 (178)	63.3 ± 3.6 (4)	85.3 ± 1.8 (150)	0.01
Urinary output / 24 h	2145 ± 65 (184)	1976 ± 222 (4)	2136 ± 69 (156)	0.84
Plasma Na^+ (mM)	141.7 ± 0.2 (197)	143.5 ± 1.2 (6)	141.8 ± 0.2 (167)	0.11
Plasma K^+ (mM)	4.2 ± 0.1 (195)	4.0 ± 0.1 (6)	4.3 ± 0.1 (165)	0.86
Plasma Ca^{2+} (mM)	2.3 ± 0.0 (197)	2.5 ± 0.1 (6)	2.3 ± 0.0 (167)	0.01
Plasma Pi (mg/100 ml)	1.2 ± 0.1 (191)	1.6 ± 0.5 (6)	1.2 ± 0.1 (161)	0.13
Fractional excretion Na^+ %	0.9 ± 0.0 (179)	0.9 ± 0.2 (4)	1.0 ± 0.0 (151)	0.64
Fractional excretion K^+ %	14.5 ± 0.5 (177)	16.4 ± 2.1 (4)	14.2 ± 0.5 (149)	0.27
Fractional excretion Ca^{2+} %	1.6 ± 0.1 (176)	1.7 ± 0.3 (3)	1.6 ± 0.1 (149)	0.72
Fractional excretion Pi %	24.2 ± 0.8 (175)	23.6 ± 6.2 (4)	24.8 ± 0.9 (147)	0.98

Table 1. Parameters of Caucasian healthy controls from Tuebingen. Numbers in parenthesis indicate number of individuals.

Parameter	Total population	intron 6 CC / exon 8 CC/CT carriers	non-carriers	p values (carriers vs. non-carriers)
Number (♀, ♂)	169 (♀137, ♂32)	3 (♀ 2, ♂ 1)	166 (♀ 135, ♂ 31)	0.47
Age	39.7 ± 1.1 (167)	44.0 ± 15.5 (3)	39.6 ± 1.1 (164)	0.91
Body weight (kg)	67.7 ± 1.0 (155)	82.0 ± 3.0 (2)	67.5 ± 1.0 (153)	0.08
Body mass index	24.5 ± 0.3 (155)	29.5 ± 4.6 (2)	24.5 ± 0.3 (153)	0.14
Systolic blood pressure (mmHg)	130.2 ± 6.5 (155)	140.0 ± 15.0 (2)	123.6 ± 1.5 (153)	0.18
Diastolic blood pressure (mmHg)	70.7 ± 1.0 (155)	82.5 ± 17.5 (2)	70.5 ± 1.0 (153)	0.45

Table 2. Parameters of Caucasian healthy controls from Romania. Numbers in parenthesis indicate number of individuals.

syndrome share common attributes [77], but plasma cortisol levels are not usually elevated in metabolic syndrome [77]. Instead, the disorder may be caused by inappropriate activity of a downstream signaling element. SGK1 is a signaling molecule downstream of glucocorticoid receptors. Thus, a SGK1 gene variant leading to increased SGK1 activity would trigger glucocorticoid actions without the need for stimulation by enhanced plasma glucocorticoid concentrations.

The obesity of metabolic syndrome is expected to predispose for the development of type 2 diabetes [78, 79]. The present study thus explored whether diabetes is more common in families of I6CC/E8CC/CT carriers and whether this haplotype is more common in type 2 diabetic patients.

Materials and Methods

Volunteers and patients with type 2 diabetes

A total of 332 healthy unrelated students and employees of the University of Tuebingen were recruited by advertisement and volunteered for blood pressure measurements, systematic questionnaire and genetic analyses (table 1). No dietary recommendations were given and no subject did receive any antihypertensive treatment. All subjects were of Caucasian (German) origin. A second Caucasian control population of healthy volunteers from Romania was collected (n = 169, table 2). As an independent ethnic control population we included 112 healthy West Africans (Ghanians) which were randomly selected and recruited from hospital staff and medical students at Ghana Medical School, Accra, Ghana and belonged mainly to the Ga tribe [80]. The mean age of the Ghana population was 27±1 years, 42% of subjects were male.

The cases were a random sample of German and Romanian men and women with type 2 diabetes collected at the University Hospital Aachen, Germany and the Diabetes Clinic Craiova, Romania. The presence of type 2 diabetes in these subjects was defined according to 1999 World Health Organization criteria and/or based on medical record review, i.e. fasting hyperglycemia, insulin resistance, at least initially no absolute requirement for insulin treatment for survival [81, 82]. Determination of GADA antibodies was performed when latent autoimmune diabetes with onset in adults was suspected due to the course of disease and antibody-positive subjects were excluded from the study.

The study was approved by the respective local ethics committees in Germany, Ghana and Romania. Volunteers and patients gave their written informed consent. The age of the control populations was substantially lower than the age of the patients. Accordingly, the control population may include individuals, who may experience diabetes at later age. Thus, the observed differences may represent an underestimate of the differences between diabetic patients and individuals, who never develop diabetes.

Genetic Analyses

Genomic DNA was isolated using the QIAmp DNA Blood Mini Kit System (Qiagen, Hilden, Germany). Genotyping for both SGK1 variants rs1743966 intron 6 (T/C) and rs1057293 exon 8 (C/T), respectively, was performed by 5' nuclease assays using TaqMan technology. Primers and probes were designed using the Applied Biosystems (Foster City, Calif) primer express program (Primer Express version 1.5) in conjunction with manual adjustment (rs1057293:5'- AAC TAC TTT TCT ATT CAC TTT TTT ACA GAG ACT T-3', 5'- TTG TGT TCA ATG TTC TCC TTG CA-3', VIC- TCC TTA CTG ATT TCG GAC, FAM- TCC TTA CTG ACT TCG GA; rs1743966 : 5'- CCT TCA ACC TGT CAG GTT TAT AGT TAA TAG-3', 5'- GCA GGA GAC AGA ACA AAG TCA TTC-3', VIC- ATT CAT TTG CAA CCC AG, FAM- ATT CAT TCG CAA CCC A). TaqMan MGB probes were customized by Applied Biosystems. Details concerning primers and probes are available upon request (matthias.schwab@ikp-stuttgart.de). PCR was performed in a reaction volume of 25µl with 20ng genomic DNA, 200nM of each probe and 900nM of forward and reverse primers in 1x TaqMan Universal PCR Master Mix (Applied Biosystems). Amplification was performed using the following conditions: 1cycle of 50°C for 2min, 1 cycle of 95°C for 10min and 40 cycles each of 92°C for 15sec and 60°C for 1min. Detection of fluorescence signals VIC and FAM was performed using the ABI PRISM detection systems (ABI7500) and the results were analysed by allelic discrimination of the sequence detection software (Applied Biosystems). Each TaqMan run comprises four DNA samples homozygous for allele 1 (AL1), four samples homozygous for allele 2 (AL2) and four reactions in which no DNA template or allelic reference is included (no template controls, NTC). Approximately 10% of samples within each assay were re-typed as a quality control. Sequenced control samples for both alleles were used in each TaqMan run. Laboratory personnel were blinded to case status of the study participants during the entire genotyping process.

Clinical chemistry

The serum and urine concentrations of electrolytes and creatinine have been determined utilizing a BAYER ADVIA 1650 assay. The o-cresolphtalein-complexon method [83] was used to determine calcium concentrations. The phosphate concentrations have been determined utilizing photometric methods [84], sodium and potassium concentrations have been measured utilizing indirect potentiometric methods [85]. The creatinine concentrations have been determined by the picric acid reaction under alkaline conditions [86]. The appropriate test kits (B01-4145-01, B01-4144-01, B01-4171-51, B01-4173-51, B01-4126-01) have been obtained from BAYER (BAYER diagnostics, Fernwald, Germany).

Before starting the urine collecting period, the volunteers have been requested to empty their bladder completely and a blood specimen has been obtained for measurement of serum creatinine and electrolyte concentrations. Urine has been collected over 24 hours, and a urine specimen has been obtained from the whole amount of collected urine to determine the urine creatinine concentrations. Glomerular filtration rate (GFR) values have been calculated according to the formula:

$$GFR\ (ml/\min) = \frac{[creatinine]_{urine} \cdot \ urine\ volume}{[creatinine]_{serum}}$$

and corrected to a body surface area of 1.73 m^2.

Statistical evaluation

Sample size calculation was performed using the program nQuery Advisor Release 4.0 derived from the following assumptions. On the basis of our preliminary data, the probability of Caucasian individuals homozygous for the SGK1 intron 6 polymorphism T>C was estimated to be 2-3%. Consequently, a sample size of at least 165 patients would be sufficient to detect a significant difference in frequency distribution between patients with type 2 diabetes and controls based on a power of 70% and a 5% alpha level, respectively.

All data are given as means ± SEM. Data distribution was tested by the method of Shapiro-Wilk. Unpaired t-test, Mann-Whitney test, Wilcoxon test, Fisher exact test and chi-square analysis were applied as appropriate to assess differences or proportions of clinical and genetic data between different groups. Odds ratios (OR) are given with 95% confidence interval (CI) and two-sided p values. When the p-value (p) was smaller than the type I error rate of 0.05, differences were considered statistically significant. The packages SPSS (version 12.0) was used for statistical analyses.

Observed and expected allele and genotype frequencies within populations were compared by means of Hardy-Weinberg equilibrium calculations (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

Parameter	Total population	intron 6 CC / exon 8 CC/CT carriers	non-carriers	p values (carriers vs. non-carriers)
Romanian patients				
Number (♀,♂)	166 (♀ 95, ♂ 71)	12 (♀ 9, ♂ 3)	154 (♀ 86, ♂ 68)	0.24
Age	61.7 ± 0.8 (162)	57.8 ± 3.7 (12)	62.1 ± 0.8 (150)	0.21
Body weight (kg)	77.5 ± 1.0 (158)	80.1 ± 4.1 (12)	77.3 ± 1.1 (146)	0.47
Body mass index	28.9 ± 0.4 (158)	30.5 ± 1.3 (12)	28.8 ± 0.4 (146)	0.23
Systolic blood pressure (mmHg)	145.4 ± 2.0 (158)	149.2 ± 4.7 (12)	145.1 ± 2.1 (146)	0.24
Diastolic blood pressure (mmHg)	79.9 ± 0.9 (158)	79.6 ± 2.6 (12)	80.0 ± 1.0 (146)	0.89
German patients				
Number (♀,♂)	237 (♀104, ♂133)	12 (♀ 5, ♂ 7)	225 (♀ 99, ♂ 126)	1.00
Age	63.0 ± 0.7 (237)	62.0 ± 2.3 (12)	63.1 ± 0.7 (225)	0.52
Body weight (kg)	89.8 ± 1.3 (237)	99.3 ± 6.3 (12)	89.3 ± 1.3 (225)	0.08
Body mass index	30.7 ± 0.4 (237)	33.8 ± 1.9 (12)	30.6 ± 0.4 (225)	0.07
Hypertension	yes: 70.5% (167) no: 29.5% (70)	yes: 83.3% (10) no: 16.7% (2)	yes: 69.8% (157) no: 30.2% (68)	0.52
Plasma creatinine (mg/100ml)	1.03 ± 0.02 (237)	1.07 ± 0.09 (12)	1.03 ± 0.02 (225)	0.67
Creatinine clearance (ml/min)	95.0 ± 2.7 (237)	101.2 ± 9.8 (12)	94.6 ± 2.7 (225)	0.27
HbA1c (%)	7.5 ± 0.1 (235)	7.6 ± 0.2 (12)	7.5 ± 0.1 (223)	0.33

Table 3. Descriptive data of Romanian and German patients with type 2 diabetes. Numbers in parenthesis indicate number of individuals.

Results

As listed in table 1, in the German control population only the 24 h creatinine clearance was significantly lower (p=0.01) and plasma calcium concentration significantly higher (p=0.01) in carriers of the SGK1 risk gene (I6CC/E8CC/CT) than in non-carriers. No differences were observed in blood pressure values, plasma electrolyte concentrations other than Ca, urinary flow rate and renal electrolyte excretion (table 1). Moreover, descriptive parameters in Romanian controls were not different when comparing carriers of the SGK1 risk gene vs non-carriers (table 2).

Descriptive data of the Romanian and the German type 2 diabetes cohorts are given in table 3. Genotypes in all groups of subjects studied were in Hardy–Weinberg proportions assuming $p<0.05$ as the level of significance except a moderate deviation for the intron 6 polymorphism in the German and the Romanian type 2 diabetes cohort. This observation may indicate a role of SGK1 in the pathogenesis of type 2 diabetes.

Genetic analyses confirmed the strong linkage disequilibrium between intron 6 and exon 8 of the SGK1 gene. Exon 8TT was observed only in individuals with intron 6CC. As shown previously, average blood pressure is not altered in individuals carrying I6CC/E8TT, but is increased in individuals carrying either I6CC/E8CC or I6CC/E8CT gene variants [68, 69]. The prevalence of the I6CC genotype was 3.1% in the German control population, 2.4 % in the Romanian control population and significantly higher in the African population from Ghana (11.6 %; table 4) compared to Caucasians. The frequency of the C-allele was 31.7 % in Ghanaians and only 19.2 % in Caucasians (p=0.0001; OR, 2.0, 95% CI 1.4-2.8).

When genotype frequencies between type 2 diabetes patients and the respective control group were compared (table 4), significant differences were observed for the intron 6 T>C variant and carriers of at least one T allele were less likely to develop type 2 diabetes (Romanians: p=0.023; OR, 0.29; 95% CI, 0.09-0.89; Germans:

gene locus	genotype / allele	genotype / allele frequencies								statistics		
		Romanian				German			Ghanians	Romanian diabetics vs controls	German diabetics vs controls	Ghanians vs German controls
		diabetics (n=166)		controls (n=169)		diabetics (n=237)		controls (n=322)	controls (n=112)			
rs1743966 intron 6 (T/C)	TT	104	62.65%	112	66.27%	150	63.29%	207	64.69%	54	48.21%	TT+TC vs CC OR 0.29 (95%CI 0.09-0.89) p=0.023	TT+TC vs CC OR 0.37 (95%CI 0.17-0.81) p=0.010	TT+TC vs CC OR 4.07 (95%CI 1.73-9.57) p=0.0006
	TC	49	29.52%	53	31.36%	68	28.69%	103	32.19%	45	40.18%			
	CC	13	7.83%	4	2.37%	19	8.02%	10	3.13%	13	11.61%			
	T	257	77.41%	277	81.95%	368	77.64%	517	80.78%	153	68.30%	OR 0.76 (95%CI 0.52-1.10) p=0.14	OR 0.83 (95%CI 0.62-1.11) p=0.20	OR 1.95 (95%CI 1.38-2.75) p=0.0001
	C	75	22.59%	61	18.05%	106	22.36%	123	19.22%	71	31.70%			
rs1057293 exon 8 (C/T)	CC	142	85.54%	143	84.62%	189	79.75%	252	84.56%	102	91.07%	CC+CT vsTT OR 0.98 (95%CI 0.06-15.83) p=0.99	CC+CT vsTT OR 0.33 (95%CI 0.09-1.31) p=0.099	CC+CT vsTT OR 0.38 (95%CI 0.02-7.35) p=0.29
	CT	23	13.86 %	25	14.79%	41	17.30%	43	14.43%	10	8.93%			
	TT	1	0.60%	1	0.59%	7	2.95%	3	1.01%	0	0			
	C	307	92.47%	311	92.01%	419	88.40%	547	91.78%	214	95.54%	OR 1.07 (95%CI 0.61-1.88) p=0.82	OR 0.68 (95%CI 0.46-1.02) p=0.064	OR 0.52 (95%CI 0.26-1.05) p=0.065
	T	25	7.53%	27	7.99%	55	11.60%	49	8.22%	10	4.46%			
intron 6 CC/ exon 8 CC/CT (SGK1 risk)	carriers	12	7.23%	3	1.77%	12	5.1%	6	2.03%	13	11.61%	OR 4.31 (95% CI 1.19-15.58) p=0.032	OR 2.58 (95% CI 0.95-6.98) p=0.092	OR 6.35 (95% CI 2.35-17.15) p=0.0001
	non-carriers	154	92.77%	166	98.23%	225	94.9%	290	97.97%	99	88.39%			

Table 4. Allele and genotype frequencies of SGK-1 variants in patients with type 2 diabetes and controls.

p=0.010; OR, 0.37; 95% CI, 0.17-0.81). Of note, the SGK1 risk gene (I6CC/E8CC/CT) was significantly more frequent in Romanian patients with type 2 diabetes than in healthy Romanian volunteers. Accordingly, carriers of the I6CC/E8CC/CT gene variant are more prone to develop type 2 diabetes (p=0.032; OR, 4.31; 95% CI, 1.19-15.58). In Germans a trend of significance was found (p=0.09; OR, 2.58; 95% CI, 0.95-6.98).

Discussion

The present study confirms the previously published [68, 69] prevalence of the I6CC/E8CC/CT gene variant, which was similar in the German and Romanian control populations. Interestingly, the prevalence was significantly higher in the healthy population from Ghana. It is tempting to speculate that enhanced SGK1 activity augments the salt retaining capacity of the kidney and may thus be favorable in an arid environment [87]. Accordingly, African individuals may be particularly prone to develop hypertension and metabolic syndrome [87].

In the group of volunteers in Tübingen, the creatinine clearance has been significantly lower in the carriers of the I6CC/E8CC/CT gene variant. It is seducing to speculate that excessive SGK1 expression enhances the vulnerability to renal disease. Along those lines, SGK1 knockout mice have been shown to be protected against the proteinuria and fibrosis following mineralocorticoid and salt excess [88]. However, in the diabetic population from Aachen/Germany, the glomerular filtration rate was not significantly different between carriers of the I6CC/E8CC/CT gene variant and noncarriers. The other significantly different parameter in the healthy population from Tübingen was the plasma calcium concentration, which was significantly higher in the carriers of the I6CC/E8CC/CT gene variant than in the non carriers. SGK1 is known to stimulate the epithelial Ca^{2+} channel TRPV5 [44, 45] and thus, excessive SGK1 activity could indeed favor renal retention of calcium and thus hypercalcemia. The plasma calcium concentration in the SGK1 knockout mouse, is, however, not significantly different from that of wild type mice [89], as the decreased TRPV5 expression in those mice is presumbly more than compensated by enhanced proximal calcium reabsorption due to the volume depletion of those mice.

Most importantly, the present observations disclose an enhanced prevalence of the I6CC/E8CC/CT haplotype in patients with type 2 diabetes. This effect was statistically significant in the Romanian population

(p=0.032; OR, 4.31) and showed a tendency in our second independent case-control group deriving from Germany (p=0.092; OR, 2.58). The present observations do not allow any safe conclusions as to the mechanisms underlying the association of the I6CC/E8CC/CT gene variant with the development of type 2 diabetes. SGK1 has been shown to mediate the inhibition of insulin release by glucocorticoids [90]. Moreover, the association may result from the impact of SGK1 on body weight. Notably, carriers of the I6CC/E8CC/CT gene variant in the German diabetes group tended to have higher body weight, a difference, however, not reaching statistical significance (p=0.08). A significant association of the gene variant with body weight has been shown previously [51]. SGK1 may influence body weight via its influence on glucose transport [51-53]. Moreover, SGK1 may modify carbohydrate metabolism by phosphorylation of GSK3 and enhancement of glycogen synthase activity in hepatocytes [91].

SGK1 may not only be important in the pathogenesis of diabetes but may also participate in the development of diabetic complications. Excessive glucose concentrations increase SGK1 expression and excessive SGK1 transcript levels were observed in diabetic nephropathy [35, 92]. Intriguing evidence points to an active role of SGK1 in fibrosing disease during hyperglycemia [93], and DOCA induced cardiac [94] and renal [88] fibrosis.

The I6CC/E8CC/CT risk gene has initially been published as a gene variant associated with increased blood pressure [68, 69]. A subsequent study failed to detect a correlation of the gene variant with blood pressure in patients with renal failure [95]. The association between the gene variant and blood pressure was, however, later confirmed by a study in more than four thousand individuals [70]. This latter study further revealed a relatively strong correlation between insulinemia and blood pressure in individuals carrying the SGK1 gene variant, suggesting a particular role of SGK1 in the hypertension paralleling hyperinsulinemia [70]. The present cohorts may have been too small to yield a significant association of the gene variant with enhanced blood pressure.

Beyond its influence on blood pressure and glucose metabolism, SGK1 participates in the regulation of coagulation. SGK1 transcription is upregulated by thrombin [96] and the kinase contributes to the thrombin induced upregulation of NFκB, tissue factor expression and coagulation [96]. Thus, SGK1 is expected to enhance the risk of cardiovascular disease.

In conclusion, the haplotype I6CC/E8CC/CT of the serum- and glucocorticoid-inducible kinase gene is not only associated with increased blood pressure and body mass index, but appears to be more common in type 2 diabetic patients. Thus, the SGK1 variants may predispose to the development of type 2 diabetes. Accordingly, SGK1 presumably contributes to the development of metabolic syndrome, a condition characterized by the coincidence of essential hypertension, procoagulant state, obesity, insulin resistance and hyperinsulinemia and precipitating diabetes and cardiovascular disease.

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