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Genetic variance of *SGK-1* is associated with blood pressure, blood pressure change over time and strength of the insulin-diastolic blood pressure relationship

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Genetic variance of *SGK-1* is associated with blood pressure, blood pressure change over time and strength of the insulin-diastolic blood pressure relationship.

Background. Insulin stimulation of the serum- and glucocorticoid-regulated kinase 1 (SGK-1) prolongs the half-life of the epithelial sodium channel, a protein which is essential for blood pressure regulation. The aim of this study was to investigate if variation in the SGK-1 gene is associated with increased blood pressure and strength of the insulin-blood pressure relationship.

Methods. A promoter C/T, an intron 6 C/T and an exon 8 C/T polymorphism in the SGK-1 gene were genotyped in 4830 subjects from the Malmö Diet and Cancer (MDC) material of whom 4001 were free from antihypertensive medication. Of these, 2171 subjects had also been investigated 11.2 ± 4.4 years earlier in the Malmö Preventive Project (MPP).

Results. In untreated MDC subjects, intron 6 CC genotype carriers had higher diastolic blood pressure than carriers of the T allele ($P = 0.02$) and exon 8 C allele carriers had higher systolic blood pressure than TT genotype carriers ($P = 0.05$). Subjects simultaneously carrying the intron 6 CC genotype and the exon 8 CC or CT genotype (SGK-1 risk) had higher systolic blood pressure ($P = 0.03$) and higher diastolic blood pressure ($P = 0.009$) than noncarriers. From MPP to MDC, the percent change in blood pressure per year was higher for systolic blood pressure ($P = 0.002$) and diastolic blood pressure ($P = 0.001$) in SGK-1 risk carriers than noncarriers. The correlation between fasting plasma insulin concentration and diastolic blood pressure was stronger in SGK-1 risk carriers than in non-carriers ($P = 0.04$).

Conclusion. Our data suggest that SGK-1 risk carriers are at increased risk of hypertension and are more sensitive to the blood pressure elevating effects associated with hyperinsulinemia.

Key words: SGK-1, genetics, hypertension, blood pressure, insulin.

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Blood pressure is a complex phenotype as indicated by its normal distribution in the population, implicating that blood pressure is influenced by several environmental and genetic factors. It has been suggested that between 30% and 60% of blood pressure variation in the population is determined by genetic factors [1]. Both the environmental and genetic mechanisms that lead to blood pressure elevation and subsequently to hypertension are to large extents unknown. One strategy to unravel the complex genetic background of population blood pressure variation is to explore genes in which mutations are known to cause monogenic forms of hypertension and genes coding for key regulators of the proteins altered in these forms of hypertension. A number of mutations causing rare monogenic forms of hypertension have been described, all of which impair the kidneys' ability to excrete sodium [2]. One monogenic form of hypertension is Liddle's syndrome [3]. This disease is caused by mutations that delete or change a proline-rich segment of either the β or γ subunit of the amiloride-sensitive epithelial sodium channel (ENaC), rendering it refractory to down-regulation by the ubiquitin ligase "neural precursor cell expressed, developmentally down-regulated 4 like" (NEDD4L) in the distal nephron [4, 5]. This results in constitutive renal hyperreabsorption of sodium through the overexpressed ENaC, thereby leading to volume overload and hypertension. Although the ENaC locus has been linked to blood pressure variation, no specific genetic ENaC variants have been consistently associated with population blood pressure variation or primary hypertension [6–8]. Even though ENaC mutations that affect blood pressure seem to be rare in the population [6, 9], Liddle's syndrome has taught us that changes in ENaC expression or activity can strongly influence human blood pressure. In recent years the pathways through which ENaC and NEDD4L expression and activity are regulated have begun to unravel. The

Table 1. Clinical characteristics of blood pressure and hypertensive groups

	MPP without AHT ($N = 2171$)	MDC without AHT ($N = 4001$)	MPP to MDC ($N = 2171$)	MDC with AHT ($N = 829$)
Age *years*	47.2 ± 5.7	57.4 ± 6.0		59.5 ± 5.5
Body mass index kg/m^2	24.2 ± 3.2	25.7 ± 3.7		27.7 ± 4.3
Gender % *male*	54	41		45
Systolic blood pressure *mm Hg*	123 ± 13.2	140 ± 18.1		152 ± 19.2
Diastolic blood pressure *mm Hg*	81.9 ± 8.4	86.1 ± 9.0		92.1 ± 9.6
P-insulin *mIU/L*[a]	8.8 (5.0–9.0)	7.0 (5.0–9.0)		9.0 (6.0–12.0)
Diabetes mellitus % *yes*	0.5	6.3		19.1
Follow-up time *years*[a]			12.5 (7.79–14.51)	
ΔSystolic blood presure *mm Hg/year*[a]			1.56 (0.69–2.63)	
ΔDiastolic blood pressure *mm Hg/year*[a]			0.37 (0.00–0.95)	
ΔSystolic blood pressure%/*year*[a]			1.27 (0.54–2.24)	
ΔDiastolic blood pressure%/*year*[a]			0.78 (0.00–1.21)	

Abbreviations are: MPP, Malmö Preventive Project; MDC, Malmö Diet and Cancer; AHT, antihypertensive treatment. All variables except frequency variables are shown as mean ± SD.

[a]Median and interquartile range (IQR).

serum- and glucocorticoid-regulated kinase 1 (SGK-1) has been shown to be one of the key regulators of ENaC [10–13]. The *SGK-1* gene is located on chromosome 6q23 and is composed of 12 exons spanning 5.5 kb [14]. It is expressed in virtually all tissues [15], although predominantly in those of importance for water and electrolyte reabsorption such as renal collecting ducts and the colonic mucosa [16]. It has been shown that increased SGK-1 phosphorylating activity inhibits the capability of NEDD4L to down-regulate ENaC [5], thereby potentially promoting blood pressure elevation. It can therefore be hypothesized that subjects carrying genetic *SGK-1* variants that makes the protein more sensitive to stimulation or increases its expression or activity are prone to get elevated blood pressure. This hypothesis was recently supported by a study in German twins showing both linkage and association between the *SGK-1* gene and blood pressure variation [17].

Aldosterone and insulin have been shown to stimulate SGK-1, primarily by increasing its expression and activity, respectively [18–22]. Hypertension, in turn, is associated with peripheral insulin resistance [23] and fasting levels of circulating insulin correlate, weakly but consistently, with blood pressure at the population level [24]. As insulin has been shown to be an important stimulator of SGK-1 activity, we hypothesized that the strength of the direct relationship between fasting insulin concentration and blood pressure at the population level could be affected by genetically determined differences in expression, activity, intracellular localization, or sensitivity of SGK-1 so that genetic *SGK-1* variants associated with higher blood pressure would confer an increased strength of the insulin-blood pressure relationship.

In the present study, we studied three single nucleotide polymorphisms (SNPs) in the *SGK-1* gene, two of which have previously been shown to be associated with blood pressure phenotypes [17] and one located in the promoter thus potentially affecting *SGK-1* expression. The aims of this study were to test if these three SNPs and a genotype combination of two of the SNPs, which has previously been shown to be associated with blood pressure [17] are associated with blood pressure, blood pressure change over time and hypertension in a Swedish population. Additionally, we aimed at investigating whether these genetic variants also were associated with an increased strength of the insulin-blood pressure relationship.

METHODS

Subjects

All participants gave written informed consent and the study was approved by the local ethical committee.

The study population in the present study is a sample of a cohort study on diet and cancer in Malmö, Sweden [25]. A random 50% ($N = 11456$) of those who entered the study between November 1991 and February 1994 were invited to take part in a study of the epidemiology of carotid artery disease [26]. Of the 5540 subjects who agreed to participate (participation rate 48.4%) full phenotypic data, required for inclusion in the present study (Table 1), and successfully extracted DNA-samples, was obtained from 4830 subjects [Malmö Diet and Cancer (MDC) study]. The study of blood pressure as a continuous variable in MDC was only performed in subjects free from antihypertensive medication ($N = 4001$), whereas the patients on antihypertensive medication ($N = 829$) were included when the dichotomized phenotype of "hypertension" and "normotension" was studied in MDC. Of the subjects not on antihypertensive medication in MDC we were able to study the blood pressure change over time in 2171 subjects who previously also had been investigated in the Malmö Preventive Project (MPP) [27] with a mean follow-up time from MPP to MDC of 11.2 ± 4.4 years (range 1.0 to 19.3 years). Clinical characteristics of all subjects free from antihypertensive medication at

MPP and MDC and those of patients on antihypertensive medication at MDC are shown in Table 1. Of the 829 patients on antihypertensive medication, 37% were treated with diuretics, 57% with beta blockers, 25% with calcium antagonists, 16% with drugs blocking the renin-angiotensin system, and 2% were treated with other types of antihypertensive medications. Thirty-one percent were treated with two or more antihypertensive drugs.

Phenotyping

Blood pressure was measured at one occasion, by specially trained nurses, in the right brachial artery in the supine position after a 10-minute rest using a mercury sphygmomanometer. Korotkoff sounds corresponding to "phase I" was used to define the systolic blood pressure and "phase V" the diastolic blood pressure.

Blood pressure change over time from MPP to MDC was expressed as mm Hg increase of blood pressure per year [(blood pressure at MDC − blood pressure at MPP)/follow-up time in years]. In order to adjust for variations in baseline blood pressure at MPP, the blood pressure change over time was also expressed as percent increase in blood pressure per year [(blood pressure at MDC − blood pressure at MPP)/follow-up time in years]/blood pressure at MPP × 100.

Hypertension was defined as being on antihypertensive treatment or having systolic blood pressure or diastolic blood pressure ≥140/90 mm Hg according to modern diagnostic criteria [28] and normotension as having systolic blood pressure and diastolic blood pressure <140/90 mm Hg. Uncontrolled hypertension and poorly controlled hypertension was defined as being on antihypertensive treatment as well as having systolic blood pressure and diastolic blood pressure >140/90 mm Hg and >160/100 mm Hg, respectively. Body mass index (BMI) was calculated as the ratio of the weight in kilograms to the square of the height in meters (kg/m^2) measured bare foot in light clothing. Insulin concentration was measured in plasma, sampled after overnight fast, at the Department of Clinical Chemistry, Malmö University Hospital, by a non-specific radioimmunoassay [26]. Diabetes mellitus was defined as having a fasting blood glucose concentration of ≥6.1 mmol/L or being on antidiabetic medication.

SNP selection and genotyping

DNA was extracted from frozen granulocyte or buffy coat samples using QIAamp-96 spin blood kits (Qiagen, VWR, West Sussex, UK). Three SNPs in the SGK-1 gene [dbSNP accession number rs1743964 promotor (C/T), rs1743966″ intron 6 (C/T), and rs1057293 exon 8 (C/T)] were genotyped. SNPs rs1743966 and rs1057293 were selected based upon results from a previous study on blood pressure variation in German twins [17]. When analyzing the 4500 bp upstream of the first codon of the *SGK-1* gene using the MatInspector software (Genomatix Software GmbH, Munich, Germany) the rs1743964 was shown to be localized in a region harboring a multitude of transcription factor binding sites as well as being the only SNP within the putative promotor of *SGK-1*. According to the MatInspector software (Genomatix Software GmbH) the promotor stretches between 85 to796 bp upstream of the first codon. The rs1743964 was thus selected for genotyping. Genotyping was performed using the Sequenom Mass Array™ system (Sequenom Inc., San Diego, CA, USA) or by ABI 7900 (Applied Biosystems, Foster City, CA, USA). An extended genotyping section can be found at *www.endo.mas.lu.se/html/pub/SGK-1_primers&manuals.txt*. All primers were synthesized with mass spectrophotometric (MALDI) quality control (Metabion GmbH, Martinsried, Germany).

Statistics

Data was analyzed with SPSS Statistical Software (version 11.5) (SPSS Inc., Chicago, IL, USA). Frequency differences were analyzed by χ^2 test or Fisher's exact test where appropriate. Continuous variables are presented as mean ± standard deviation (SD) if normally distributed and as median and interquartile range (IQR) if not. Significance of differences in continuous variables were tested by *t* test and analysis of variance (ANOVA) or Mann-Whitney and Kruskal-Wallis test, depending on whether the variable was normally distributed or not. Multiple regression and multiple logistic regression analysis, respectively, were used to test if genotypic effects on blood pressure and hypertension were independent of covariates. After logarithmic transformation of the change in blood pressure from MPP to MDC, the general linear model was used to assess whether the effect of a *SGK-1* intron 6 and exon 8 genotype combination on blood pressure change was independent of covariates. Spearman's test for correlations was used to calculate correlations. Fisher's R to Z transformation was used to test if the correlation between fasting plasma insulin concentration and blood pressure was significantly stronger in carriers of a genotype combination in the *SGK-1* gene than in those who did not carry this combination, using a one-sided test. All other tests were two-sided and throughout. $P < 0.05$ was considered statistically significant.

RESULTS

Genotyping success rate and Hardy-Weinberg equilibrium

In the total material ($N = 4830$), the genotyping success rate was 97.3% ($N = 4700$) for the promotor SNP, 95.9% ($N = 4630$) for the intron 6 SNP, and 97.6% ($N = 4714$) for the exon 8 SNP. All SNPs were successfully genotyped in 95.4% ($N = 4608$) of all subjects. The numbers of subjects in the results section refer to the exact number of

Table 2. Blood pressure at Malmö Diet and Cancer (MDC) study according to promotor, intron 6 and exon 8 genotypes and a genotype combination [serum- and glucocorticoid-regulated kinase 1 (SGK-1) risk]

	CC ($N = 366$)	CT ($N = 1552$)	TT ($N = 1981$)		*P* value CC vs. TT
Promotor C/T polymorphism ($N = 3899$)					
Systolic blood pressure *mm Hg*	139 ± 17	140 ± 19	139 ± 18		NS
Diastolic blood pressure *mm Hg*	86 ± 8.7	86 ± 9.3	86 ± 8.8		NS
	CC ($N = 165$)	CT ($N = 1180$)	TT ($N = 2491$)	CT/TT ($N = 3671$)	*P* value CC vs. CT/TT
Intron C/T polymorphism ($N = 3836$)					
Systolic blood pressure *mm Hg*	141 ± 18	140 ± 18	139 ± 18	139 ± 18	NS
Diastolic blood pressure *mm Hg*	87.8 ± 8.5	86.1 ± 9.2	86.0 ± 8.9	86.0 ± 9.0	0.02
	CC ($N = 3122$)	CT ($N = 742$)	TT ($N = 40$)	CC/CT ($N = 3864$)	*P* value CC/CT vs. TT
Exon 8 C/T polymorphism ($N = 3904$)					
Systolic blood pressure *mm Hg*	139 ± 18	140 ± 19	134 ± 14	140 ± 18	0.04
Diastolic blood pressure *mm Hg*	86.1 ± 9.0	86.4 ± 9.1	86.5 ± 8.6	86.1 ± 9.0	NS
	SGK-1 risk ($N = 128$)		Other genotype carriers ($N = 3688$)		*P* value
Intron 6 CC + exon 8 CC/CT (SGK-1 risk) vs. all other subjects ($N = 3816$)					
Systolic blood pressure *mm Hg*	143 ± 18		139 ± 18		0.03
Diastolic blood pressure *mm Hg*	88.2 ± 8.3		86.0 ± 9.0		0.009

Analyses were made on subjects free from antihypertensive medication.

successfully genotyped subjects. Genotype distributions, in all groups of subjects studied, were in accordance with Hardy-Weinberg equilibrium. The genotype frequencies in the total material ($N = 4830$) were for rs1743964 CC 9.2%, CT 39.6%, and TT 49.6%; rs1743966 CC 4.0%, CT 30.8%, and TT 65.2%; and rs1057293 CC 80.4%, CT 18.7%, and TT 0.9%.

Variations in the *SGK-1* gene and blood pressure at MDC in subjects without antihypertensive medication

Blood pressure distributions in carriers of various *SGK-1* genotypes without antihypertensive medication at MDC are depicted in Table 2. Subjects homozygous for the C allele of the intron 6 polymorphism had significantly higher diastolic blood pressure than carriers of at least one T allele. Carriers of at least one C allele of the exon 8 polymorphism had significantly higher systolic blood pressure than subjects homozygous for the T allele. Subjects simultaneously carrying the intron 6 CC genotype and the exon 8 CC or CT genotype (SGK-1 risk) had significantly higher systolic blood pressure and diastolic blood pressure compared to subjects with any other genotype combination (noncarriers) (Table 2). In a multiple regression analysis, the effect of the SGK-1 risk on blood pressure was independent of the effects of age, gender, and BMI for both systolic blood pressure ($P = 0.02$) and diastolic blood pressure ($P = 0.01$). The promoter variant was not individually nor in any combination associated with any blood pressure phenotype. The three SNPs studied here were not a associated with blood pressure variation at MPP (data not shown).

Variations in the *SGK-1* gene and blood pressure change over time from MPP to MDC in subjects without antihypertensive medication

The change in systolic blood pressure and diastolic blood pressure from MPP to MDC in subjects without antihypertensive medication, expressed as mm Hg increase of blood pressure per year and as percent increase in blood pressure per year, was significantly higher in subjects homozygous for the C allele of the intron 6 polymorphism compared to carriers of at least one T allele, whereas these variables did not differ significantly when subjects were grouped by genotypes of the promoter or the exon 8 polymorphisms (Table 3). Both systolic blood pressure and diastolic blood pressure increased at a significantly faster rate from MPP to MDC in carriers of the SGK-1 risk compared to those who did not carry this combination, whether expressed as mm Hg increase of blood pressure per year or as percent increase in blood pressure per year (Table 3). Furthermore, when applying the general linear model, the effect of the SGK-1 risk on change in systolic ($P = 0.01$) and diastolic ($P = 0.006$) blood pressure from MPP to MDC was independent of age, gender, BMI at MPP, BMI at MDC, follow-up time, and blood pressure at MPP.

SGK-1 risk and strength of the insulin-blood pressure correlation at MDC in subjects without antihypertensive medication

At MDC, carriers of the SGK-1 risk ($N = 128$) as compared to noncarriers ($N = 3688$) had a significantly

Table 3. Blood pressure change from Malmö Preventive Project (MPP) to Malmö Diet and Cancer (MDC) study according to promotor, intron 6, and exon 8 genotypes and a genotype combination [serum- and glucocorticoid-regulated kinase 1 (SGK-1) risk]

	CC ($N = 213$)	CT ($N = 831$)	TT ($N = 1074$)		P value CC vs. TT
Promotor C/T polymorphism ($N = 2118$)					
ΔSystolic blood pressure *mm Hg/year*	1.5 (0.6–2.6)	1.6 (0.7–2.7)	1.5 (0.7–2.6)		NS
ΔDiastolic blood pressure *mm Hg/year*	0.3 (0.0–0.9)	0.4 (0.0–1.0)	0.4 (0.0–1.0)		NS
ΔSystolic blood pressure *%/year*	1.3 (0.5–2.5)	1.3 (0.6–2.3)	1.2 (0.5–2.2)		NS
ΔDiastolic blood pressure *%/year*	0.4 (0.0–1.1)	0.5 (0.0–1.2)	0.5 (0.0–1.2)		NS
	CC ($N = 81$)	CT ($N = 628$)	TT ($N = 1376$)	CT/TT ($N = 2004$)	P value CC vs. CT/TT
Intron C/T polymorphism ($N = 2085$)					
ΔSystolic blood pressure *mm Hg/year*	1.9 (1.2–3.3)	1.6 (0.6–2.7)	1.5 (0.7–2.6)	1.5 (0.7–2.6)	0.007
ΔDiastolic blood pressure *mm Hg/year*	0.7 (0.2–1.2)	0.4 (0.0–0.9)	0.4 (0.0–0.9)	0.4 (0.0–0.9)	0.001
ΔSystolic blood pressure *%/year*	1.6 (1.0–2.7)	1.3 (0.5–2.3)	1.3 (0.5–2.2)	1.3 (0.5–2.2)	0.007
ΔDiastolic blood pressure *%/year*	0.8 (0.2–1.6)	0.5 (0.0–1.2)	0.4 (0.0–1.2)	0.4 (0.0–1.2)	0.001
	CC ($N = 1699$)	CT ($N = 398$)	TT ($N = 18$)	CC/CT ($N = 2097$)	P value CC/CT vs. TT
Exon 8 C/T polymorphism ($N = 2115$)					
ΔSystolic blood pressure *mm Hg/year*	1.6 (0.7–2.6)	1.5 (0.7–2.9)	1.5 (0.8–2.1)	1.6 (0.7–2.6)	NS
ΔDiastolic blood pressure *mm Hg/year*	0.3 (0.0–0.9)	0.4 (0.0–1.1)	0.6 (0.1–0.9)	0.4 (0.0–1.0)	NS
ΔSystolic blood pressure *%/year*	1.3 (0.5–2.2)	1.3 (0.6–2.4)	1.3 (0.6–1.9)	1.3 (0.5–2.2)	NS
ΔDiastolic blood pressure *%/year*	0.4 (0.0–1.2)	0.5 (0.0–1.4)	0.7 (0.1–1.1)	0.4 (0.0–1.2)	NS
	SGK-1 risk ($N = 65$)		Other genotype carriers ($N = 2005$)		P value
Intron 6 CC + exon 8 CC/CT (SGK-1 risk) vs. all other subjects ($N = 2070$)					
ΔSystolic blood pressure *mm Hg/year*	2.1 (1.2–3.4)		1.6 (0.7–2.6)		0.002
ΔDiastolic blood pressure *mm Hg/year*	0.8 (0.2–1.3)		0.4 (0.0–0.9)		0.001
ΔSystolic blood pressure *%/year*	1.6 (1.1–2.7)		1.3 (0.5–2.2)		0.002
ΔDiastolic blood pressure *%/year*	0.9 (0.2–1.7)		0.4 (0.0–1.2)		0.001

Data are given as median (interquartile range) (IQR). Analyses were made on subjects being free from antihypertensive medication.

stronger correlation between fasting insulin concentration in plasma and diastolic blood pressure ($r = 0.32$ versus $r = 0.17$) ($P = 0.03$). The correlation coefficient between fasting insulin concentration in plasma and systolic blood pressure was not significantly higher in carriers of the SGK-1 risk than in noncarriers ($r = 0.24$ versus $r = 0.17$) (NS). In patients treated with anti hypertensive medication, the difference in the strength of the correlation coefficients between carriers and noncarriers of the SGK-1 risk was even more pronounced ($r = 0.34$ versus $r = 0.06$) ($P = 0.13$) for systolic blood pressure and $r = 0.41$ versus $r = 0.12$ ($P = 0.11$) for diastolic blood pressure. However, this difference did not reach significance, probably due to fact that the number of SGK-1 risk carriers on antihypertensive medication was very low ($N = 18$).

Effect of SGK-1 risk on hypertension and blood pressure control at MDC

We also analyzed the blood pressure phenotype as a dichotomous variable (hypertension and normotension) at MDC in all subjects successfully genotyped for the SGK-1 risk ($N = 4608$), thereby allowing inclusion also of subjects on antihypertensive medication who were excluded in the analyses of blood pressure. The SGK-1 risk was significantly more common among patients with hypertension than in subjects with normotension (Table 4). In a multiple logistic regression analysis with hypertension status (hypertension versus normotension) as dependent variable and SGK-1 risk status (carriers versus noncarriers), age, gender, and BMI as independent variables, the odds ratio with 95% confidence intervals (95% CI) for hypertension in carriers of the SGK-1 risk was 2.0 (95% CI = 1.3–3.1).

Of the hypertensive patients who were on antihypertensive treatment and had full genotypic information

Table 4. Proportion of hypertensive patients among carriers and non-carriers of serum- and glucocorticoid-regulated kinase 1 (SGK-1) risk ($N = 4608$)

	SGK-1 risk carriers ($N = 146$)	Non-carriers ($N = 4462$)
Number of hypertensive patients (%)	113 (77.4)	2883 (64.6)
Number of normotensive subjects (%)	33 (22.6)	1579 (35.4)

$P = 0.0001$

SGK-1 risk, carriers of the CC genotype of the intron 6 polymorphism and the CT or CC genotype of the exon 8 polymorphism; non carriers, carriers of any other genotype combination.

($N = 792$) 18 patients carried the SGK-1 risk. The proportion SGK-1 risk carriers with uncontrolled hypertension was 61.1% versus 43.9% in noncarriers (NS) and the proportion of patients with poorly controlled hypertension was significantly higher in carriers as compared to non-carriers of the SGK-1 risk (27.8% versus 8.9%) ($P = 0.02$). The on-treatment diastolic blood pressure (median, IQR) was higher in carriers as compared to noncarriers of the SGK-1 risk (99.0, 89.5–102.5 versus 90.0, 85.098.5) ($P = 0.05$), whereas the on-treatment systolic blood pressure did not differ between the two groups (150, 137–170 versus 150, 140–164) (NS).

DISCUSSION

In the present study we found that a combination of two SNPs in the *SGK-1* gene, referred to as the SGK-1 risk, was associated with elevated blood pressure, increased progression rate of blood pressure over time, and strength of the insulin-blood pressure correlation among subjects free from antihypertensive medication. When including subjects on antihypertensive medication, we found an association between the SGK-1 risk and hypertension and, finally, among patients on antihypertensive treatment the SGK-1 risk was associated with poorly controlled hypertension and higher on-treatment diastolic blood pressure.

SGK-1 variation and blood pressure

The finding of association between the SGK-1 risk and increased systolic blood pressure and diastolic blood pressure in the cross-sectional analysis of untreated subjects is consistent with a previous report where the SGK-1 risk construct was found to mediate a stronger association with blood pressure elevation than any of the SNPs alone [17]. Considering that the previous study was performed in a relatively small set of dizygotic twins and their parents ($N = 232$) as well as that false positive associations are common in genetic studies of complex traits, we found it worthwhile to replicate it in a larger material of unrelated subjects. In this study we extend and strengthen the evidence for a role of the SGK-1 risk in the development of hypertension by showing in a longitudinal analysis that the SGK-1 risk is associated with increased blood pressure progression rate over time during the clinically highly relevant age span of 47 to 57 years (Table 1), during which the incidence of hypertension increases dramatically in most populations [29]. The fact that the effect of the SGK-1 risk on blood pressure progression over time was independent of the initial blood pressure (Table 3) suggests that the genetic alteration acts continuously to increase blood pressure, at least between the ages of 47 and 57 years.

Selective increases of diastolic blood pressure in intron 6 CC genotype carriers and systolic blood pressure in carriers of at least one exon 8 C allele are difficult to explain in terms biologic mechanisms. However, simultaneous increases in both systolic blood pressure and diastolic blood pressure in carriers of SGK-1 risk are in accordance with the previous study in German twins [17]. Although statistical artefacts cannot be completely ruled out, the concordance between these two studies provides strong evidence that the SGK-1 risk truly contributes significantly to elevated cross-sectional blood pressure as well as blood pressure change over time.

In genetic studies of the complex phenotype of blood pressure, where the genetic effect is expected to be relatively small, adjusting blood pressure for the effects of antihypertensive treatment is highly error prone. In light of this, we excluded patients on antihypertensive treatment in the analyses of cross-sectional and longitudinal blood pressure (Table 1). To enable us to extract genetic information also from these subjects we performed an analysis of the dichotomized phenotype of "hypertension" and "normotension," using modern guidelines in the classification [28]. The prevalence of the SGK-1 risk was significantly higher in the hypertensive than in the normotensive group (Table 4). The genetic effect was independent of gender, BMI, and age. Some caution is warranted when interpreting these results as only the patients on antihypertensive medication had been clinically diagnosed with hypertension (Table 1), whereas the majority fell into the hypertensive category by exceeding the European Society of Hypertension/European Society of Cardiology (ESH/ECC) diagnostic blood pressure limits [28] based on recordings taken at one occasion. When applying these criteria, the prevalence of hypertension in our study population was 65%. Similar prevalence of hypertension (65% to 70%) was found in Europeans in the corresponding age span in a recent population survey using the same criteria to define hypertension [29]. However, in both studies a slight overestimation of the hypertension prevalence is to be expected due to the fact that the majority of included subjects were not clinically diagnosed with hypertension but merely exceeded the upper limit of the ESH/ECC criteria [28].

The discrepancy between invited and attending subjects of the MDC investigation was quite high with a participation rate 48.4%. This implies that the material is not fully representative of the inhabitants of the city of Malmö. It is probable, on the background of the low participation rate, that the individuals that participated in the investigation were healthier, came from higher social standards, and had a lower load of hazardous environmental factors than the average citizen [30]. However, the diminution of environmental factors could give genetic anomalies relatively greater impact on blood pressure elevation, thus increasing the power of this study to detect

an effect of genetic *SGK-1* variance. Among patients on antihypertensive treatment, the carriers of the SGK-1 risk had higher on-treatment diastolic blood pressure and the proportion of patients with poorly controlled hypertension was significantly higher than among noncarriers. This is interesting as it could imply that SGK-1 risk carriers respond less well to conventional antihypertensive treatment than noncarriers. Importantly, this study was not designed to explore the effect of genetic *SGK-1* variation on blood pressure treatment control and, additionally, the prevalence of SGK-1 risk carriers was very low among subjects on antihypertensive treatment. Therefore, these data have to be interpreted cautiously and warrant replication in studies specifically designed to address this issue.

Studies of monogenic forms of hypertension have taught us that ENaC plays an important role in the regulation of renal sodium reabsorption and blood pressure. Furthermore, it has been shown that SGK-1 and NEDD4L are important regulators of ENaC [31, 32]. Studies on *SGK-1* knockout mice [33], the Dahl salt-sensitive rat [34], and in vitro studies [35] have clearly indicated that *SGK-1* expression is important for renal sodium handling and blood pressure regulation. In *SGK-1* knockout mice, dietary NaCl restriction, as opposed to standard NaCl intake, revealed an impaired ability of the *SGK-1* knockout mice to adequately decrease sodium excretion despite increases in plasma aldosterone levels as well as decreases in blood pressure and glomerular filtration rate [33]. In the Dahl salt-sensitive rat the inability to down-regulate SGK-1 on a high salt diet indicated that the abundance of SGK-1 in the kidney play a role for the impaired salt adaptation and pathogenesis of hypertension in the Dahl salt-sensitive rat [34]. These animal studies support our hypothesis that genetically mediated increases in expression or activity of SGK-1 could be an important cause of elevated blood pressure also in humans. However, the mechanisms by which the SGK-1 risk increases human blood pressure remains to be elucidated. Since none of the two SNPs forming the SGK-1 risk alter the amino acid sequence of the protein, the most likely explanation is either that the polymorphisms alters *SGK-1* expression or that another nearby variant or variants, which are in linkage disequilibrium with the SGK-1 risk genotype combination, alters expression, activity, localization, or sensitivity to stimulation of SGK-1. It should, however, be mentioned that in the absence of functional in vitro or clinical studies of these and other polymorphisms in the *SGK-1* gene this remains highly speculative.

Impact of *SGK-1* variations on the insulin-blood pressure relationship

At the population level, insulin and blood pressure are weakly but consistently correlated with each other [24]; however, the cause of this relationship is unknown. One potential explanation is that hyperinsulinemia, as a consequence of selective insulin resistance in muscle, fat, and liver, results in increased insulin-induced sodium retention in the kidneys [36]. Although expression of *SGK-1* in the distal parts of the nephron is strongly induced by mineralocorticoids [10–13], aldosterone alone, the main mineralocorticoid in vivo, seems to have only a weak ability to activate SGK-1. On the other hand, insulin seems to be an important activator of SGK-1 via its classic signaling pathway through phosphatidylinositol-3 (PI3) kinase, PDK1 and PDK2 [18–20], suggesting that insulin stimulation of SGK-1 could be an important link between hyperinsulinemia and elevated blood pressure working through enhanced ENaC-mediated renal sodium retention. We therefore hypothesized that the strength of the direct relationship between circulating fasting insulin and blood pressure would be greater in carriers of *SGK-1* variants associated with higher blood pressure. We found that carriers of the SGK-1 risk had a stronger correlation between fasting circulating insulin levels and diastolic blood pressure than noncarriers, suggesting that insulin stimulation of SGK-1 results in relatively greater ENaC-mediated renal sodium reabsorption and greater blood pressure elevation in SGK-1 risk carriers than in noncarriers. One mechanism through which increased SGK-1 activity may prolong the half-life of ENaC in the luminal membrane in the renal tubules is that activated SGK-1 phosphorylates NEDD4L rendering it incapable to ubiquitinate and subsequently unable to remove ENaC from the cell membrane [4, 5]. It can thus be speculated that the stronger insulin-diastolic blood pressure correlation in SGK-1 risk carriers results from these subjects either having enhanced ability of SGK-1 to phosphorylate NEDD4L, increased SGK-1 sensitivity to insulin stimulation, prolonged half-life of the SGK-1 protein or a more favorable intracellular position of SGK-1 for phosphorylating NEDD4L. However, it should be emphasized that the statistical significance of the difference in correlation coefficient between carriers and noncarriers of SGK-1 risk is quite weak. Therefore, the finding of increased insulin-diastolic blood pressure correlation in SGK-1 risk carriers needs verification in other populations and further studies are warranted to explain its molecular mechanism.

CONCLUSION

This study provides confirmation of the previously reported association between the SGK-1 risk and increased cross-sectional blood pressure, in a large material of unrelated Caucasians. Additionally, we show that the SGK-1 risk is associated with increased blood pressure progression rate, higher prevalence of hypertension, and a

stronger correlation between fasting plasma insulin concentration and diastolic blood pressure. Our data suggest that SGK-1 risk carriers are at increased risk of hypertension and that they are more sensitive to the blood pressure elevating effects associated with hyperinsulinemia implicating that the previously described molecular interaction between insulin, SGK-1, NEDD4L, and ENaC is of clinical importance. Further support for this hypothesis was obtained from an exploratory analysis showing that hypertensive patients on pharmacologic treatment, who carried the SGK-1 risk genotype combination, had poorer blood pressure control than noncarriers, suggesting that these patients are resistant to conventional antihypertensive treatment. Our results encourage further studies exploring if SGK-1 risk carriers benefit more from interventions targeted more specifically at the "insulin, SGK-1, NEDD4L, and ENaC system" in the prevention and treatment of hypertension. Such interventions may include amiloride, dietary salt restriction, drugs, and lifestyle regimens lowering insulin concentration through improved insulin sensitivity and, in the future, perhaps selective SGK-1 antagonists and NEDD4L agonists. Finally, this study highlights the need for a more comprehensive genetic analysis of the *SGK-1* gene to unravel the possible functional variants hidden within the gene.

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