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Variant rs6749447 (T > G) in the serine threonine kinase gene is associated with cardiovascular complications, the Tampere adult population cardiovascular risk study

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Abstract

We have previously shown an association of *STK39* (serine threonine kinase) rs6749447 (T > G) with hypertension in the Tampere adult population cardiovascular risk study in 50-year-old subjects. These 1196 subjects were followed up to the age of 65 years to determine whether rs6749447 is also associated with coronary artery disease (CAD), transient ischemic attack (TIA), or early cardiovascular death.

DNA samples were collected by buccal swabs and genotypes were determined by PCR. Hypertension, TIA, and CAD were determined by questionnaire and the National Hospital Discharge Registry. Outcomes for death were collected from the National Statistics Centre. Linkage disequilibrium analysis and gene expression correlations for rs6749447 were done in silico.

After following the subjects up to the age of 60 years the rs6749447 G-allele still associated with hypertension ($P = .009$). The variation did not associate with CAD ($P = .959$). The risk for TIA was 5.2-fold among G-allele carriers compared to TT genotype even after adjusting for body mass index ($P = .036$, 95% CI 1.11-24.59). After follow-up of the subjects to the age of 65 years, adjusting for body mass index, the G-allele was associated with 3.2-fold risk of premature cardiovascular death ($P = .049$, 95% CI 1.00-10.01).

In conclusion, the *STK39* genetic variant rs6749447 was significantly associated with TIA and premature cardiovascular death in a Finnish cohort. The in silico results of linkage disequilibrium and gene expression analyses also showed associations that were distinct from the retention of salt effect on kidneys proposed earlier for this intronic variation.

Abbreviations: BMI = body mass index, CAD = coronary artery disease, LD = linkage disequilibrium, SPAK = proline-alanine-rich kinase, STK = serine threonine kinase, TAMRISK = Tampere adult population cardiovascular risk study, TIA = transient ischemic attack.

Keywords: death, genetic variation, STK39, vascular diseases

1. Introduction

The rs6749447 is an intron variant (T > G) in the serine threonine kinase 39 (*STK39*) gene that has been identified to be significantly associated with hypertension in several populations.[1–4] The minor G allele is associated with both higher systolic and diastolic blood pressure.[5] We have previously shown that there was a significant association between STK39 rs6749447 and hypertension in the Tampere adult population cardiovascular risk study (TAMRISK).[6] G-allele carriers had a 1.4-fold risk for hypertension compared to TT genotype by the age of 50 years. For the present study, we have followed up these subjects to the age of 65 years to determine whether this variation influences coronary artery disease (CAD), transient ischemic attack (TIA), or early cardiovascular death.

Editor: Yutang Wang.

Funding for the study was from grants from Competitive research funding of the Pirkanmaa Hospital District. The Genotype-Tissue Expression (GTEx) Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS.

The data that support the findings of this study are available from the corresponding author, Seppo T. Nikkari, upon reasonable request. However, the data obtained from the National Hospital Discharge Registry (HILMO) are confidential under the Act on National Personal Data Registers Kept under the Finnish Health Care System.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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How to cite this article: Kunnas T, Määttä K, Nikkari ST. Variant rs6749447 (T > G) in the serine threonine kinase gene is associated with cardiovascular complications, the Tampere adult population cardiovascular risk study. Medicine 2021;100:42(e27566).

Received: 18 June 2021 / Received in final form: 29 September 2021 / Accepted: 1 October 2021

http://dx.doi.org/10.1097/MD.0000000000027566

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2. Materials and methods

2.1. Subjects

TAMRISK study data was collected from periodic health examinations in 2003 to 2006 for 50-year-old women and men in Tampere, a city in southern Finland with 220,000 inhabitants. Health and disease related data were assessed using a structured questionnaire. Body mass index (BMI) was calculated from height (cm) and weight (kg) at the age of 50 years. Buccal swabs for DNA extraction and signed informed consent forms to use periodic health examinations and national registry data were obtained from the study subjects in 2006 to 2010. Cases in TAMRISK were subjects with self-reported physician-diagnosed hypertension by the age of 50 years (n = 450, 41% women). Controls were normotensive subjects of same gender, with similar smoking habits (n = 746, 43% women). Ethics Committee of Tampere University Hospital approved the study (R07007, R07075).

2.2. Outcomes

With follow-up, the original cases and controls were combined. Hospitalization data by ICD-10 codes for discharge diagnoses were obtained from the National Hospital Discharge Registry (HILMO) maintained by the National Institute of Health and Welfare. Diagnoses of CAD (I20-I25) and TIA (G45) were followed up to the age of 60 years (2013). In follow-up of the genotyped subjects, there were 87 with CAD (19 women, 68 men), and 11 with TIA (4 women, 7 men). Vital status from the age of 50 years up to the age of 65 years (2018) was determined by social security number, and death certificates were obtained from the National Statistics Centre (Statistics Finland). ICD10 codes were used to identify mortality from cardiovascular disease (0 women, 15 men) (ICD10: I21, I25, I71).

2.3. Genotyping

DNA from buccal swabs was extracted with a commercial kit (Qiagen Inc., Valencia, CA). Allele-specific primers for PCR were: 5′-CCTGTATTTACAAGCCCC ACA-3′ as a reverse primer and either 5′-AGTCTGCTAGTACTAGATTAGGAG-3′ or 5′-GAGTCTGCTAGTACT AGATTAGGAT-3′ as a forward primer, as described previously.[6]

2.4. In silico linkage disequilibrium (LD) analysis and gene expression correlations

The rs6749447 was uploaded into Variant Effect Predictor (VEP) (http://www.ensembl.org/Tools/VEP)[7] and the Linkage Disequilibrium Calculator was used to determine coefficient of linkage disequilibrium (D) of associated regulatory *STK39* single nucleotide polymorphisms in the population FIN (Finnish in Finland). Gene expression correlations were obtained from the Genotype-Tissue Expression (GTEx) project data (https://www.gtexportal.org/home/).

2.5. Statistical analysis

Chi-square test, *T* test or logistic regression were applied for the comparison of genotype groups. Kaplan-Meier survival analysis for cardiovascular deaths was also performed. Analyses were carried out using SPSS 23.0 for Windows (SPSS Inc., Chicago, IL). *P* values of $<.05$ were considered as statistically significant.

3. Results

Clinical characteristics of the TAMRISK participants at the age of 50 years have been previously described.[6] The 18 subjects who had rs6749447 genotype GG were combined with the GT group for statistical analyses.

When the 50-year-old subjects were followed up to the age of 60 years the *STK39* rs6749447 G-allele still associated with hypertension ($P = .009$) (Table 1). The variation did not associate with CAD ($P = .959$). Subjects with G-allele had higher incidence of TIA (1.6%), compared to those with the TT genotype (0.3%) ($P = .020$). Even after adjusting for BMI, the risk for TIA was 5.1-fold among G-allele carriers compared to TT genotype ($P = .037$, 95% CI 1.10-23.97).

After follow-up of the subjects to the age of 65, those with G-allele had higher incidence of cardiovascular death (2.0%), compared to those with the TT genotype (0.6%) ($P = .040$) (Table 1). After adjusting for BMI, the G-allele was associated with 3.2-fold risk of premature cardiovascular death ($P = .049$, 95% CI 1.01-10.05). The Kaplan-Meier survival curve illustrates the better survival of subjects with the TT genotype (upper curve) compared to those with the G-allele (lower curve) (Fig. 1; $P = .037$).

In the population FIN, LDs of intron variant *STK39* rs6749447 with regulatory region variants were only found with *STK39* rs1955337.[8] (D = .66), and rs1474055.[9] (D = .66), both of which are associated with Parkinson disease. In addition, LD with an uncharacterized regulatory region variant rs12987123 was found (D = .66). All other LD variants were intronic or intergenic in nature. For *STK39* rs6749447 gene expression correlations (The Genotype-Tissue Expression (GTEx) project), the highest effect sizes (effect of the alternative allele relative to the reference allele) were obtained for monocyte (–.44), pancreatic islet (–.41), sensory neuron (.37), macrophage

Table 1

Clinical characteristics of the study population stratified according to *STK39* rs6749447 genotypes.

Genotype (n)	TT (635)	(GT + GG) (561)	*P* value* TT vs (GT + GG)	*P* value† TT vs (GT + GG)
Body mass index at the age of 50 yrs (kg/m^2) (SD)	26.5 (4.4)	26.6 (4.5)	.791	
Hypertension by the age of 60 yrs % (n)	34.5 (219)	41.2 (231)	**.017**	**.009**
CAD by the age of 60 yrs % (n)	7.2 (46)	7.3 (41)	.966	.959
TIA by the age of 60 yrs % (n)	0.3 (2)	1.6 (9)	**.020**	**.037**
Cardiovascular death by the age of 65 yrs % (n)	0.6 (4)	2.0 (11)	**.040**	**.049**

CAD = coronary artery disease, SD = standard deviation, TIA = transient ischemic attack.

* *T* test or Chi-square test.

† Logistic regression adjusted by body mass index. *P* values $<.05$ are in bold.

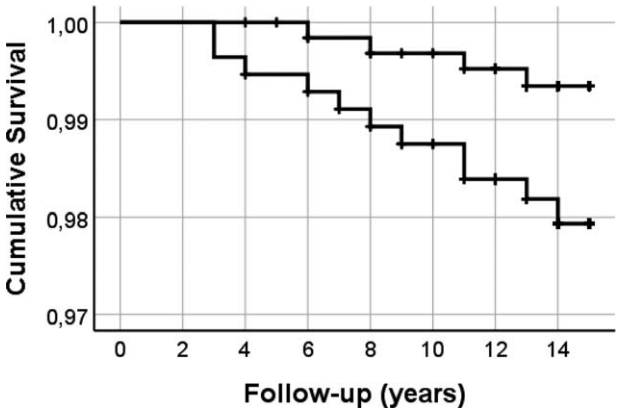

Figure 1. Kaplan-Meier survival analysis from cardiovascular deaths of subjects with the STK39 rs6749447 wild-type TT genotype (upper curve) and those with the G-allele (lower curve) ($P = .037$): The subjects were examined at baseline at 50 yrs of age and followed up for 15 yrs.

interferon-γ (.34), kidney cortex (–.33), regulatory T cell memory (.33), brain cerebellar hemisphere (.32), and naïve macrophage (–.26).

4. Discussion

STK39 codes for Ste20-related proline-alanine-rich kinase (SPAK), that participates in a kinase network that regulates Na^+ and K^+ excretion in kidneys.[10,11] If this kinase network is affected by single nucleotide polymorphisms in *STK39*, the response of kidneys to mineralocorticoid hormones may change. Consequently, Na^+ and K^+ excretion may be altered possibly leading to higher blood pressure due to retention of salt.[10,11]

We report that the *STK39* polymorphism rs6749447 G-allele still associated with hypertension when subjects were followed up to the age of 60 years, despite that some controls became hypertensive. Although the variation associated with hypertension, it did not associate with CAD. This is consistent with the finding that the present study has a relatively small number of subjects, and it has been estimated that at least 60 genetic loci are associated with CAD risk.[12]

Subjects with G-allele had higher incidence of TIA compared to those with the TT genotype. Risk factors for TIA are the same as for stroke, including genetics, age greater than 55 years, sex, hypertension, and hyperlipidemia.[13] Higher TIA risk for G allele carriers remained significant even after adjusting for BMI, indicating a role for *STK39* polymorphism rs6749447, possibly through hypertension.

Finally, when the subjects were followed up to the age of 65, those with G-allele had higher incidence of cardiovascular death, compared to those with the TT genotype. Even after adjusting for BMI, the G-allele was associated with 3.2-fold risk of premature cardiovascular death. Hypertension is strongly associated with cardiovascular endpoints, such as cardiovascular mortality, on average estimated to confer a threefold increase in risk.[14] Hypertension might thus explain our finding of the association of *STK39* rs6749447 with early cardiovascular death.

STK39 rs6749447 was in LD with *STK39* regulatory region variants rs1955337 and rs1474055, both of which are associated with Parkinson disease. Moreover, *STK39* rs6749447 gene expression correlations showed association to kidney cortex, but there were also several associations that were distinct from an effect on electrolytes, such as for leukocytes, pancreatic islet, sensory neuron, and brain cerebellar hemisphere. SPAK, coded for by *STK39*, has been indicated to be a kinase that has potential beyond regulation of ion co-transporters. A diverse function of SPAK is to bind to membrane receptors such as the β_2-adrenergic receptor.[15] This potential might also explain the associations of *STK39* to degenerative neurological disease,[8] hypertension,[1] and the present associations of rs6749447 with TIA and early cardiovascular death.

A limitation of the present study is that the BMI used in the adjustments were the BMIs calculated at 50 years of age. BMI will change over time, and new BMI data was not collected.

5. Conclusions

In conclusion, the *STK39* genetic variant rs6749447 was significantly associated with TIA and premature cardiovascular death in a Finnish cohort. The in silico results of LD and gene expression analyses for this intronic variation also showed associations that were distinct from the electrolyte effect on kidneys proposed earlier.[10,11]

Acknowledgments

We gratefully acknowledge the expert technical assistance by Mirka Pietiläinen and Nina Peltonen.

Author contributions

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