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Original Article

Determinants of concentrations of Nε-carboxymethyl-lysine and soluble receptor for advanced glycation end products and their associations with risk of pancreatic cancer

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Abstract: The soluble receptor for advanced glycation end-products (sRAGE) is shown to mitigate pro-inflammatory effects triggered by ligation of RAGE with Nε-carboxymethyl-lysine (CML)-AGE or other ligands. We examined the associations among host, lifestyle, and genetic determinants of CML-AGE or sRAGE and risk of pancreatic cancer in the prospective ATBC Study. We obtained baseline exposure information, data on serological and genetic biomarkers from 141 patients with pancreatic cancer and 141 subcohort controls. Stepwise linear and logistic regression models were used for data analysis. Multiple linear regression analyses showed that CML-AGE concentrations were independently inversely correlated with the minor allele of rs640742 of *DDOST*, physical activity, alcohol consumption, diastolic blood pressure (BP), and positively correlated with heart rate, serum sRAGE and HDL concentrations ($P < 0.05$). sRAGE concentrations were independently inversely correlated with the 82Ser allele of rs2070600 of *RAGE*, age, body mass index, heart rate, and serum HDL; and positively correlated with serum CML-AGE, sucrose consumption, and diastolic BP ($P < 0.05$). The minor allele of rs1035786 of *RAGE* was associated with reduced risk of pancreatic cancer (any T compared with CC: multivariate OR = 0.61, 95% CI: 0.38-0.98). We identified host metabolic profile, lifestyle and genetic factors that explained approximately 50% of variability of CML-AGE or sRAGE in Finnish men smokers. The association between *RAGE* SNPs and pancreatic cancer risk warrants further investigation.

Keywords: Advanced glycation end-products, metabolic syndrome, inflammation, sRAGE, single nucleotide polymorphism, pancreatic cancer

Introduction

The receptor for advanced glycation end-products (RAGE) is a multi-ligand receptor of the immunoglobulin superfamily and has been implicated in various chronic inflammatory diseases. Interaction of full-length RAGE with its ligands, including advanced glycation end-products (AGEs), S100/calgranulin protein family, and high-mobility group box 1 protein, triggers rapid generation of intracellular reactive oxygen species and activates an array of key cell signaling pathways culminating in activation of the pro-inflammatory NF-κB pathway [1, 2]. Nε-(carboxymethyl)-lysine (CML) is the predominant type of AGEs and a biomarker for long-time oxidative stress as a result of carbohydrate, protein, and lipid oxidation reactions [3, 4]. Elevated levels of circulating CML-AGE have been associated with increased risk of

diabetic retinopathy [5], chronic kidney disease [6], and heart disease [7, 8] in patients with diabetes. In humans, soluble RAGE (sRAGE) competitively binds with RAGE ligands, such as CML-AGE, but does not induce signaling cascade. sRAGE is therefore considered an anti-inflammatory factor in the course of various diseases by acting as a decoy receptor for RAGE ligands [9].

Host and lifestyle factors [10, 11] and genetic variations [12, 13] have been shown to influence CML-AGE and sRAGE concentrations. Although the associations between single nucleotide polymorphisms (SNPs) of *RAGE* and sRAGE concentrations have been well examined [12, 14], the genetic determinants of CML-AGE concentrations are less known. To fill this gap, we examined SNPs in glyoxalase (*GLO1*) and dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit (*DDOST*, aka *AGER-1*), two genes encoding two proteins involved in detoxification and clearance of AGEs compounds respectively, in relation to circulating CML-AGE levels. Moreover, a comprehensive evaluation of multiple factors in determining CML-AGE or sRAGE within a non-diseased population is lacking. We therefore examined the relationship between multiple host, lifestyle, and genetic factors and CML-AGE or sRAGE concentrations in the large well-phenotyped Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study. We previously found an inverse association between pre-diagnostic sRAGE and pancreatic cancer risk [15]. In the present study, we performed a novel exploratory evaluation of SNPs in *GLO1*, *DDOST*, and *RAGE* and risk of pancreatic cancer in this same study population.

Materials and methods

Study population

We performed the analysis in a previously defined case-cohort study nested in the ATBC Study [15, 16]. The ATBC Study included 29, 133 eligible Finnish men in the age range of 50 to 69 years recruited between 1985 and 1988 and who smoked at least five cigarettes per day. These men smokers were randomized to receive an active supplement or a placebo to examine their efficacy in reducing the incidence of lung cancer and other major cancers. Participants did not have any malignancy (other than nonmelanoma skin cancer) or carcinoma *in situ*, severe angina on exertion, chronic renal insufficiency, or cirrhosis of liver; and were not chronic alcoholics and had not received anticoagulant therapy. Although the trial ended in April 1993, participants continued to be followed for vital status and cancer through national registries [17]. For the current analysis, the follow-up was through 2004. The ATBC Study was approved by the institutional review groups of the U.S. National Cancer Institute and the National Institute for Health and Welfare, Helsinki, Finland. All participants provided written informed consent before randomization. The present study protocol was approved by NCI special studies Institutional Review Board, Institutional Review Board of Baylor College of Medicine and Michael E. DeBakey VA Medical Center.

Exposure assessment and measurement of biomarkers

At baseline before the ATBC Study intervention, all participants completed a self-administered questionnaire to provide comprehensive information on general demographics, medical, smoking and other lifestyle factors. Diet was assessed with a validated self-administered food frequency questionnaire that included 276 food items and mixed dishes and was accompanied by a picture booklet containing 122 pictures of foods and information on portion sizes [18]. Trained nurses obtained measurements on anthropometry, blood pressure, and heart rate according to a standardized protocol. Body mass index (BMI, weight (kg)/height $(m)^2$) was calculated. Study participants provided a venous blood sample after an overnight fast with serum isolated and stored at -70°C. Additional blood used for genomic DNA was collected toward the end of the trial.

Baseline serum concentrations of CML-AGE and sRAGE were measured in duplicate at the Microcoat Biotechnologie Company using an AGE-CML-ELISA kit (Microcoat Biotechnologie Company, Bernried, Germany) and a human sRAGE Quantikine ELISA kit (R&D system Inc, Minneapolis, MN), respectively [15]. Serum cholesterol concentrations were determined enzymatically (CHOD-PAP method, Boehringer Mannheim). High-density lipoprotein (HDL) cholesterol was measured after precipitation of very-low-density lipoprotein and low-density

Table 1. Baseline characteristics of cases and subcohort controls in the ATBC Study, 1985-1988

Characteristics (mean (SD))	Cases (n = 141)	Controls (n = 141)	*P* value
Age (year)	57.6 (4.6)	56.3 (4.8)	0.02
BMI (kg/m^2)	26.1 (3.4)	26.5 (3.8)	0.39
Duration of smoking (years)	35.8 (8.0)	35.2 (8.4)	0.34
Cigarette smoked per day	20.6 (0.7)	20.7 (0.7)	0.88
Systolic blood pressure (mmHg)	136.9 (16.3)	139.7 (16.8)	0.15
Diastolic blood pressure (mmHg)	85.1 (10.2)	86.5 (10.7)	0.27
Heart rate (per minute)	71.6 (11.0)	73.6 (12.6)	0.21
Frequency of physical activity at leisure time/week (N (%), heavy)	17 (12.4)	19 (13.5)	0.93
Alcohol intake (g/day)	18.3 (22.6)	16.1 (19.3)	0.68
Dietary variables (1000Kcal/day)			
Available carbohydrate (g)	99.1 (13.9)	101.1 (14.3)	0.25
Total Sugar (g)	41.2 (12.1)	41.8 (13.0)	0.79
Total sucrose (g)	20.7 (10.2)	20.0 (8.9)	0.74
Total fiber (g)	7.03 (3.4)	7.22 (3.1)	0.58
Total fat (g)	46.1 (5.8)	45.3 (6.8)	0.49
Protein (g)	34.8 (4.0)	35.7 (4.4)	0.04
Red meat (g)	27.4 (11.8)	26.5 (10.7)	0.60
Calcium (mg)	508 (158)	545 (182)	0.09
Vitamin E (μg)	4.37 (1.8)	4.22 (1.5)	0.88
Serological biomarkers			
CML-AGE (ng/ml)	522.1 (182.1)	557.3 (171.3)	0.10
sRAGE (pg/ml)	532.1 (274.1)	627.4 (313.7)	0.004
Glucose (mg/dl)	90.5 (17.8)	98.9 (17.6)	0.0002
Insulin (μIU/ml)	4.49 (3.10)	4.58 (2.95)	0.82
HDL (mmol/l)	1.17 (0.28)	1.21 (0.30)	0.33

lipoprotein cholesterol (LDL) with dextran sulfate and magnesium chloride. Serum levels of insulin and glucose were measured as previously described [15, 19].

Genotype data were abstracted from an earlier genome-wide association study for pancreatic cancer [20]. Genotype was determined using the human Hap500 Infinium Assay (Illumina, San Diego, CA) which covers 561, 466 SNPs. We identified nine *RAGE* SNPs from the array's marker map (genome build 37). We excluded five SNPs with minor allele frequency (MAF) < 0.01 (rs3176931, rs13209119, rs17846810, rs2856442, and rs17846803) among the controls, leaving four RAGE SNPs (rs3134940, rs1035798, rs2070600, and rs1800624) for subsequent analyses. As rs1800624 (C341T) was uniform in our population, it was not included in pancreatic cancer risk association analysis. Two *DDOST* SNPs (rs1091684 and rs640742) and six *GLO* SNPs (rs1937780, rs10484854, rs2736655, rs3799703, rs6458065, and rs6932648) were included in the analysis.

We previously performed a case-cohort study in the ATBC Study that included 255 incident cases of primary pancreatic cancer and 483 subcohort participants who were randomly selected from participants who had remained cancer-free through 2004 [15]. After excluding four outliers for sRAGE (< 100 or > 2000 pg/ml) and one outlier for CML-AGE (> 1400 ng/ml) identified by scatter plots, diet data were available to 248 cases and 461 controls. Because we found that sRAGE concentrations were significantly lower among those with self-reported common diseases, including pancreatitis, type 2 diabetes and hypertension, we excluded individuals with any of these conditions from our further analyses, which yielded 196 cases and 364 controls. Among them, genotype data were available to 141 cases and 141 controls [20].

Statistical analysis

We compared the distributions of demographic, clinical characteristics, lifestyle factors, concentrations of serological biomarkers between the cases and the subcohort controls using t-test and ANOVA for normally distributed continuous variables, the Wilcoxon rank-sum for non-normal variables, and chi-square test for categorical variables. All diet variables were energy adjusted using the density method. To meet assumptions necessary for performing linear regression, we evaluated continuous variables (arithmetic mean and standard deviation listed in **Table 1**) for normality using Skewness/Kurtosis test. The following variables followed a normal distribution: BMI, systolic and diastolic BP, intake of available carbohydrates, total starch, and CML-AGE concentrations. The following variables followed a normal distribution after a natural log transformation: intake of total sucrose, concentrations of sRAGE, insulin, and HDL. The normality of the following variables was not achieved after transformation and we categorized these variables into tertiles using the distribution in controls to establish cut-points: age, duration of smoking (years), heart rate, alcohol consumption, intakes of protein, red meats, total sugar, calcium, vitamin E, total fiber and glucose concentrations. The normalized or categorized variables were used in linear regression analyses.

Using univariate linear regression analysis, we examined the association between baseline concentrations of CML-AGE or sRAGE (dependent variables) and each explanatory variable. The variables with *P* values less than 0.25 (listed in **Table 1** for either CML-AGE or sRAGE model) were included in the multiple linear regression model. Other variables evaluated but not listed included: education, energy intake, total starch and vitamin D consumption, and serum total cholesterol concentrations. Effect sizes for the CML-AGE and sRAGE models were reported as β-coefficients with associated 95% confidence intervals (CI) and *P* values.

Genotyping data were analyzed using SNP & VARIATION SUITE 7 (SVS 7.0) (Golden Helix, Bozeman, MT). SNPs with MAF > 0.01, call rates > 90%, and *P* value for Hardy-Weinberg Equilibrium (HWE) test > 0.05 among controls were kept for further analyses. We used additive (comparison across three genotypes) or dominant (using major homozygous genotype as the reference) models in analyzing the association between SNPs and levels of biomarkers or risk of pancreatic cancer. We examined the association between SNPs in *GLO1*, *DDOST*, and *RAGE*, and risk of pancreatic cancer using logistic regression models. Our multivariate model adjusted for age, BMI, duration of smoking, HDL concentrations, and diastolic BP. Effect sizes were presented as odds ratios (ORs) with associated 95% CIs. The associations between sRAGE, factors were shown to be associated with sRAGE, and risk of pancreatic cancer were also examined using logistic regression models. Because use of Cox regression analysis accounting for the case-cohort design generated similar results, only the results based on logistic regression analysis are presented. All analyses were performed using STATA 12.0 (STATA Corporation, College station, TX) or SAS 9.2 (SAS institute, Cary, NC) with a *P* value < 0.05 to indicate statistical significance for two-sided tests.

Results

Cases were significantly slightly older and had significantly lower concentrations of sRAGE and glucose than controls. Controls had higher consumption of proteins than cases (**Table 1**). Among 141 subcohort controls, CML-AGE concentrations were lower among individuals who carried the variant G allele of rs640742 of *DDOST* (n = 90) than the individuals who carried the TT major genotype (n = 51) (539 versus 590 ng/ml, *P* = 0.07). Multiple linear regression analysis identified the variant G allele of rs640742, physical activity, alcohol consumption, and diastolic BP were inversely correlated, while heart rate, serum levels of HDL and sRAGE were positively correlated with CML-AGE (**Table 2**). Other SNPs of *GLO1*, *DDOST* and *RAGE* were not associated with CML-AGE concentrations (data not shown). Our multiple linear regression model explained 49% of the variation in observed CML-AGE concentrations (**Table 2**). The model without SNPs explained 47% of the variation. Among 141 cases, the same set of the explanatory variables explained 34% of variability for CML-AGE, with serum sRAGE and age the positive determinants for CML-AGE concentrations (*P* < 0.05).

Table 2. Multiple linear regression analysis on determinants of CML-AGE concentration among subcohort controls in the ATBC Study (N = 141)

Factors	β (95% CI)	*P*
Age (years)		
55-59 vs. < 55	-22.58 (-88.95 to 43.79)	0.50
≥ 60 vs. < 55	5.28 (-78.63 to 89.18)	0.90
BMI (kg/m^2)		
25-29.9 vs < 25	44.31 (-12.58 to 101.19)	0.13
≥ 30 vs < 25	63.96 (-20.10 to 148.02)	0.14
Heart rate per minute		
68-76 vs. < 68	17.22 (-42.01 to 76.45)	0.57
≥ 78 vs. < 68	61.93 (4.61 to 119.25)	0.03
Frequency of physical activity at leisure time/week		
Moderate vs. low	-80.97 (-159.39 to -2.55)	0.04
High vs. low	-73.22 (-147.00 to 0.56)	0.05
Duration of smoking (years)		
32-38 vs. < 31	-10.34 (-68.58 to 47.89)	0.73
≥ 39 vs. < 31	37.62 (-38.84 to 114.09)	0.33
Alcohol intake (gram/per day)		
4.8-23.4 vs. < 4.8	-30.16 (-86.76 to 26.45)	0.29
≥ 23.5 vs. < 4.8	-68.32 (-132.24 to -4.40)	0.04
Intake of vitamin E (mg/day)		
3.4-4.3 vs. < 3.4	13.02 (-43.03 to 69.07)	0.65
≥ 4.4 vs. < 3.4	-51.84 (-108.63 to 4.95)	0.07
Diastolic blood pressure (mm Hg)	-3.54 (-5.79 to -1.28)	0.002
Serum HDL*	244.15 (136.48 to 351.82)	< 0.0001
Serum insulin*	-32.67 (-78.80 to 13.46)	0.16
Serum sRAGE*	199.87 (148.97 to 250.77)	< 0.0001
RAGE rs2070600 (Gly82Ser vs. Ser82Ser)	55.99 (-30.91 to 142.89)	0.20
DDOST rs640742 (Any G allele vs. TT)	-55.81 (-103.28 to -8.33)	0.02
R^2	0.49	

*Logarithmically transformed (natural base) to meet assumption of linearity.

Among 141 subcohort controls, sRAGE concentrations were lower among the individuals who carried the variant Gly82Ser genotype (CT) of *RAGE* rs2070600 than those who carried the Gly82Gly genotype (CC) (533 versus 634 pg/ml, $P = 0.19$). Multiple linear regression analysis showed that the Gly82Ser genotype, age, BMI, heart rate, and serum HDL were independently inversely correlated with sRAGE concentrations, while diastolic BP, alcohol and sucrose consumption, and CML-AGE were positively correlated with sRAGE concentrations (**Table 3**). Other SNPs of *RAGE* were not associated with sRAGE concentrations (data not shown). Our multiple regression model explained 52% of the variation in observed sRAGE concentrations. The model without SNPs explained 50% of the variation. Among 141 cases, the same set of the explanatory variables explained 34% of the variation in sRAGE, with serum CML-AGE the positive and HDL the inverse determinant for sRAGE respectively ($P < 0.05$).

Out of 11 SNPs examined, the minor T allele of *RAGE* rs1035798 was associated with lower risk of pancreatic cancer (multivariate OR = 0.61, 95% CI 0.38-0.98, any T versus CC). None of other SNPs in *GLO1*, *DDOST*, and *RAGE* was strongly or significantly associated with risk of pancreatic cancer (**Table 4**). sRAGE concentrations were inversely associated with pancreatic cancer risk (multivariate OR = 0.40; 95% CI: 0.21-0.75 for the 3rd compared with the 1st tertle). The factors that significantly contribute to

Table 3. Multiple linear regression analysis on determinants of sRAGE concentrations (loge-transformed) among subcohort controls in the ATBC Study (N = 141)

Factors	β coefficient (95% CI)	*P*
Age (years)		
55-59 vs. < 55	-0.074 (-0.261 to 0.114)	0.44
≥ 60 vs. < 55	-0.252 (-0.484 to -0.021)	0.03
BMI (kg/m^2)		
25-29.9 vs. < 25	-0.101 (-0.257 to 0.054)	0.20
≥ 30 vs. < 25	-0.227 (-0.439 to -0.016)	0.04
Heart rate per minute		
68-76 vs. < 68	-0.204 (-0.371 to -0.038)	0.02
≥ 78 vs. < 68	-0.127 (-0.292 to 0.037)	0.13
Duration of smoking (years)		
32-38 vs. < 31	-0.134 (-0.297 to 0.029)	0.11
≥ 39 vs. < 31	-0.058 (-0.276~0.160)	0.60
Alcohol intake (gram/day)		
4.8-23.4 vs. < 4.8	0.168 (0.011 to 0.324)	0.04
≥ 23.5 vs. < 4.8	0.156 (-0.026 to 0.338)	0.09
Intake of vitamin E (mg/day)		
3.4-4.3 vs. < 3.4	0.0096 (-0.150 to 0.169)	0.90
≥ 4.4 vs. < 3.4	0.071 (-0.095 to 0.238)	0.40
Serum glucose (mg/dl)		
91-99 vs. ≤ 90	-0.038 (-0.214 to 0.138)	0.67
> 99 vs. ≤ 99	-0.096 (-0.268 to 0.077)	0.28
Diastolic blood pressure (mm Hg)	0.011 (0.005 to 0.018)	0.001
Serum HDL*	-0.787 (-1.084 to -0.489)	< 0.0001
Intake of sucrose*	0.172 (0.029 to 0.315)	0.02
Serum CML-AGE (ng/ml)	0.0015 (0.0011 to 0.0019)	< 0.0001
RAGE rs2070600 (Gly82Ser vs. Ser82Ser)	-0.249 (-0.490 to -0.008)	0.04
R^2	0.52	

*Logarithmically transformed (natural base) to meet assumption of linearity.

sRAGE concentrations, except for age, had no significant association with pancreatic cancer risk in multivariate logistic regression models (data not shown).

Discussion

In the present analysis in the ATBC Study, we made several novel observations. The *DDOST* (*AGER1*) SNP rs640742 and the *RAGE* (*AGER*) SNP rs2060700 were independent determinants for CML-AGE and sRAGE concentrations, respectively. The *RAGE* rs1035798 SNP was associated with risk of pancreatic cancer. In agreement with other studies, we found that CML-AGE and sRAGE were significantly correlated with each other and also with BMI, heart rate, diastolic BP, and HDL concentrations. Lifestyle factors, including alcohol consumption, intake of sucrose and physical activity, may also contribute to the inter-individual variation of CML-AGE or sRAGE.

Like several emerging studies [21-24], our study indicated that sRAGE, and to a lesser extent CML-AGE, was correlated with factors associated with metabolic profile, including obesity, dyslipidemia, and hypertension. Among generally lean study subjects, excluding men with self-reported pancreatitis, type 2 diabetes, and hypertension, we still observed an inverse correlation between sRAGE and BMI as reported previously [24-26]. An inverse correlation between circulating CML-AGE and obesity has been reported in old [27] or young individuals [28]. However, we did not observe a significant association between CML-AGE and BMI. We found both CML-AGE and sRAGE were associated with heart rate, diastolic BP and serum HDL. Similarly, among 44 non-diabetic individuals in Australia, plasma CML-AGE concentrations were inversely correlated with diastolic BP [29]. In a Bangladesh study of 98 younger non-diabetics [30], sRAGE was inversely correlated with diastolic BP [30]. These studies collectively suggest a role of CML-AGE/sRAGE in endothelial function. Some [31-33], but not all studies [34], also indicated the role of CML-AGE and sRAGE in insulin resistance. We found no significant association between CML-AGE/sRAGE and serum glucose or insulin. It is unknown whether difference in inclusion of diseased ver-

Table 4. Association between SNPs in *GLO1*, *DDOST* and *RAGE* and risk of pancreatic cancer

Gene/SNP	Cases (n = 141)	Controls (n = 141)	OR (95% CI)*
DDOST/rs640742 (T to G)			
TT	63 (44.7)	51 (36.2)	1.00
TG	62 (44.0)	66 (46.8)	0.76 (0.45-1.27)
GG	16 (11.4)	24 (17.0)	0.53 (0.25-1.12)
TG+GG	78 (55.3)	90 (63.8)	0.70 (0.43-1.13)
DDOST/rs10916846 (T to C)			
TT	118 (83.7)	118 (83.7)	1.00
TC	23 (16.3)	21 (14.9)	1.04 (0.54-2.00)
CC	0 (0)	2 (1.4)	
TC+CC	23 (16.3)	23 (16.3)	0.96 (0.50-1.81)
GLO1/rs10484854 (G to A)			
GG	80 (56.7)	80 (56.7)	1.00
GA	49 (34.8)	55 (39.0)	0.83 (0.50-1.37)
AA	12 (8.5)	6 (4.3)	1.76 (0.61-5.05)
GA+AA	61 (43.3)	61 (43.3)	0.92 (0.57-1.49)
GLO1/rs1937780 (C to T)			
CC	51 (36.2)	42 (29.8)	1.00
CT	66 (46.8)	76 (53.9)	0.67 (0.39-1.14)
TT	24 (17.0)	23 (16.3)	0.80 (0.39-1.65)
CT + TT	90 (63.8)	99 (70.2)	0.70 (0.42-1.16)
GLO1/rs2736655 (C to T)			
CC	79 (56.0)	79 (56.0)	1.00
CT	49 (34.8)	54 (38.3)	0.92 (0.56-1.53)
TT	13 (9.2)	8 (5.7)	1.70 (0.65-4.44)
CT + TT	62 (44.0)	62 (44.0)	1.02 (0.63-1.64)
GLO1/rs3799703 (A to G)			
AA	52 (36.9)	45 (31.9)	1.00
AG	58 (41.1)	71 (50.4)	0.65 (0.38-1.11)
GG	31 (22.0)	25 (17.7)	0.91 (0.46-1.81)
AG+ GG	89 (63.1)	96 (68.1)	0.71 (0.43-1.18)
GLO1/rs6458065 (C to T)			
CC	49 (34.8)	41 (29.1)	1.00
CT	61 (43.3)	73 (51.8)	0.64 (0.37-1.11)
TT	31 (22.0)	27 (19.2)	0.82 (0.41-1.62)
CT + TT	92 (65.3)	100 (70.9)	0.69 (0.41-1.15)
GLO1/rs6932648 (C to T)			
CC	119 (84.4)	112 (79.4)	1.00
CT	21 (14.9)	27 (19.2)	0.74 (0.39-1.40)
TT	1 (0.7)	2 (1.4)	0.31 (0.03-3.59)
CT + TT	22 (15.6)	29 (20.6)	0.71 (0.38-1.31)
RAGE/rs2070600 (C to T)			
82Gly/Gly (CC)	126 (89.4)	130 (92.2)	1.00
82Gly/Ser (CT)	15 (10.6)	11 (7.8)	1.45 (0.64-3.32)
RAGE/rs3134940 (A to G)			
AA	103 (73.0)	112 (79.4)	1.00

sus non-diseased study populations explains this inconsistency in findings.

In the present analysis, lifestyle factors were shown to determine CML-AGE and sRAGE concentrations, including alcohol consumption inversely for CML-AGE and positively sRAGE, physical activity inversely for CML-AGE, and sucrose consumption positively for sRAGE. One study administrated alcohol to diabetic mice and found that the interaction of acetaldehyde, the derivative of ethanol oxidation, with early glycation products in vitro produces a stable complex that prevents the formation of AGEs [35]. There has been no investigation on CML-AGE and physical activity. However, two studies have examined the effect of intervention of increased physical activity on sRAGE. One showed in 30 old healthy Japanese that sRAGE concentrations were decreased after 6-month intervention [10]; but another found in 75 diabetics that aerobic exercise increased sRAGE concentrations after 12-weeks of training [36]. These findings suggest that certain lifestyle factors modulate CML-AGE and sRAGE concentrations.

While most studies support anti-inflammatory role of sRAGE, some suggest that sRAGE is a pro-inflammatory molecule [37, 38]. Likewise, some studies have shown circulating CML-AGE was positively associated with risk of chronic diseases [6, 39] and some showed no association [40, 41]. Understanding the determinants of CML-AGE and sRAGE in disease-free individuals may aid in the interpretation of the study findings. We showed that CML-AGE and sRAGE explained

AG	33 (23.4)	27 (19.2)	1.33 (0.75-2.36)
GG	5 (3.6)	2 (1.4)	2.72 (0.52-14.3)
AG + GG	38 (27.0)	29 (20.6)	1.42 (0.82-2.48)
RAGE/rs1035798 (C to T)			
CC	69 (49.6)	53 (37.6)	1.00
CT	59 (42.5)	71 (50.4)	0.64 (0.39-1.05)
TT	11 (7.9)	17 (12.1)	0.50 (0.21-1.15)
CT + TT	70 (50.4)	88 (62.4)	0.61 (0.38-0.98)

*OR was adjusted for age, body mass index, duration of smoking, and serum HDL levels.

their intra-individual variability mutually as showed by several other studies [42-44]. We were able to further identify factors that explain approximately 50% of total inter-individual variability of CML-AGE and sRAGE among healthy Finnish men smokers. Other genetic and lifestyle factors that explain the remaining variability of CML-AGE and sRAGE need to be determined. For example, medications, such as statin and angiotensin-converting enzyme inhibitors, have been shown to modulate production of sRAGE in clinical studies or in animal models [45-47]. This source of variability can only be addressed in a cohort with such data collected.

We found genetic variations explain only a small proportion of variation of CML-AGE and sRAGE levels. Data on genetic and non-genetic determinants of CML-AGE are sparse. Interestingly, we found that the minor allele of rs640742 of *DDOST* that encodes the protein that degrades CML-AGE was associated with decreased levels of CML-AGE in multiple linear regression models. It suggests that CML-AGE metabolism is likely a determinant of circulating levels of AGEs. This novel finding warrants further confirmation.

We previously found that higher sRAGE was associated with lower risk of pancreatic cancer. This finding held true for this smaller subset of study participants. In the current analysis, higher sRAGE concentrations were correlated with younger age, lower BMI, higher diastolic BP, lower HDL, higher intake of sucrose, and higher CML-AGE concentrations. However, only age was shown to be positively associated with risk of pancreatic cancer in our study with moderate sample size. The *RAGE* gene (HUGO gene nomenclature *AGER*) is highly polymorphic. Although the variant 82 Ser allele of rs2070600 has been associated with reduced levels of sRAGE [25, 48] and it also affects the binding affinity with RAGE ligands [49-52], we only observed a moderately elevated risk of pancreatic cancer associated with the 82Ser containing genotype. Interestingly, we found that the T minor allele of rs1035798 of sRAGE was associated with reduced risk of pancreatic cancer. However, a Swedish study found that the same allele was associated with increased risk of a subtype of small-vessel diseases (n = 165) [53]. The functional significance of rs1035798 is unknown. Further large studies are needed to understand the associations between *RAGE* SNP and pancreatic cancer risk including in women and men from diverse racial backgrounds.

Our study has several strengths including that it used a case-cohort design utilizing a large and well-phenotyped cohort of healthy male smokers. However, our study also has several limitations. First, our secondary analysis was limited by modest sample size. The low MAFs of several SNPs further compromised our study power. Second, our study was performed in Finnish male smokers and may not be generalizable to other study populations. Finally, we did not find the same set of the variables explained the variability of CML-AGE or sRAGE in both cases and controls to the same extent although the blood was collected five years before cancer diagnosis in this prospective study. It is unknown whether pre-cancerous conditions or undiagnosed cancer had changed the metabolic profiles related to CML-AGE/sRAGE of the study subjects.

In generally healthy Finnish male smokers, CML-AGE and sRAGE were determined by each other and by host factors including medical conditions, BMI, diastolic BP, HDL, and genetic polymorphisms independently. Our results also suggested that the association between the *RAGE* polymorphisms and pancreatic cancer deserves further investigation. Additional research is needed to clarify the potential causal and modifying roles of CML-AGE and sRAGE as well as polymorphisms in *RAGE* in the pathogenesis of various diseases, including pancre-

atic cancer, and their potential value in clinical risk stratification.

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Disclosure of conflict of interest

None.

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