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ARTICLE

Soluble receptor for AGE (RAGE) is a novel independent predictor of all-cause and cardiovascular mortality in type 1 diabetes

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Abstract
Aims/hypothesis Activation of the receptor for AGE (RAGE) is implicated in the development and progression of vascular complications of diabetes. In this study, we explore factors and mortality outcomes associated with soluble RAGE (sRAGE) in a multicentre nationwide cohort of Finnish adults with type 1 diabetes.
Methods Baseline sRAGE concentrations were estimated in 3,100 adults with type 1 diabetes. Clinical and biological variables independently associated with sRAGE were identified using multivariate regression analysis. Independent predictors of mortality were determined using Cox and Fine–Gray proportional-hazards models.
Results The main independent determinants of sRAGE concentrations were estimated glomerular filtration rate, albuminuria, body mass index, age, duration of diabetes, HbA_{1c} and insulin dose (all $p<0.05$). During a median of 9.1 years of follow-up there were 202 deaths (7.4 per 1,000 patient years). sRAGE was independently associated with all-cause (Cox model: HR 1.03) and cardiovascular mortality (Fine–Gray competing risks model: HR 1.06) such that patients with the highest sRAGE concentrations had the greatest risk of mortality, after adjusting for age, sex, macrovascular disease, HDL-cholesterol, HbA_{1c}, triacylglycerol, high-sensitivity C-reactive protein (hsCRP) and the presence and severity of chronic kidney disease. Although polymorphisms in the gene coding for RAGE were significantly associated with sRAGE concentrations, none were associated with mortality outcomes.
Conclusions/interpretation Increased concentrations of sRAGE are associated with increased all-cause and cardiovascular mortality in type 1 diabetes, potentially reflecting the activation and production of RAGE in the context of accelerated vascular disease. These novel findings highlight the importance of the RAGE activation in the prevention and management of diabetic complications.

Keywords Advanced glycation end-products · AGE · Mortality · RAGE · Type 1 diabetes

Abbreviations

AIC	Akaike's information criteria
BIC	Bayesian information criteria
CKD	Chronic kidney disease

M. C. Thomas and J. Söderlund contributed equally to this study.

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CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CVD	Cardiovascular disease
CIF	Cumulative incidence function
eGFR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
FinnDiane	Finnish Diabetic Nephropathy
hsCRP	High-sensitivity C-reactive protein
RAGE	Receptor for AGE
SNP	Single nucleotide polymorphism
sRAGE	Soluble receptor for AGE

Introduction

Activation of the receptor for AGE (RAGE) is implicated in the development and progression of diabetic complications [1], including accelerated atherosclerosis [2, 3] and chronic kidney disease (CKD) [4]. RAGE-deficient mice and mice in which activation of the RAGE receptor is antagonised by recombinant soluble RAGE (sRAGE) are protected from vascular damage associated with diabetes [2, 3]. Endogenous sRAGE is largely produced by proteolytic cleavage of the membrane-bound form via the action of metalloprotein sheddases [5], with a smaller additional amount derived from the alternative splicing of RAGE (so called esRAGE) [6]. Circulating concentrations of sRAGE are increased in individuals with diabetes [7]. sRAGE concentrations are positively correlated with circulating AGEs in patients with diabetes. In addition, sRAGE concentrations are influenced by body mass [8], drug treatment [9] and kidney function [10, 11]. sRAGE production is also partly under genetic control, with a number of small studies showing an association between sRAGE and polymorphisms of *AGER* that codes for RAGE [12–15]. Recent studies have suggested that circulating concentrations of sRAGE are inversely associated with the presence and/or extent of vascular disease in non-diabetic [16] and general population samples [17]. In the present study, we explore the association between circulating sRAGE concentrations and mortality outcomes in a large nationwide multicentre cohort of Finnish adults with type 1 diabetes.

Methods

Study sample The Finnish Diabetic Nephropathy (FinnDiane) Study has been described in detail in previous publications [18–20]. In brief, the study was established in 1997 at the Helsinki University Central Hospital to study clinical, biochemical, environmental and genetic risk factors for type 1 diabetes and its complications. The patients were recruited from 21 university and central hospitals, 27 district hospitals and 29 primary healthcare centres across Finland (see Acknowledgements). In the participating centres, all adults with type 1 diabetes attending diabetic and/or renal outpatient clinics were asked to participate in the study. For this analysis, patients receiving renal replacement therapy (dialysis or transplantation) were excluded. The ethical committees of all participating centres approved the study protocol. Written informed consent was obtained from each patient, and the study was performed in accordance with the Declaration of Helsinki, as revised in the year 2000.

Characteristics of the participants Details on clinical status, including age at diagnosis, presence and severity of diabetic complications, insulin therapy and other regular medications were obtained from the attending physician using a standardised questionnaire. Data on smoking habits, alcohol intake, educational level and social class were obtained using a patient questionnaire. Fasting blood samples were obtained at baseline for the measurement of HbA_{1c}, lipids, high-sensitivity C-reactive protein (hsCRP) and isotope dilution mass spectrometry (IDMS)-adjusted serum creatinine. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [21], which is more accurate than other formulae, especially within the normal range of kidney function. Urinary AER in each patient was stratified according to International Diabetes Federation guidelines [22], on the basis of three consecutive timed urine collections and measured using standardised immunoturbidimetric methods. In addition, baseline blood samples (stored frozen at baseline) were used to determine serum concentrations of sRAGE by a commercial solid phase ELISA (Quantikine; R&D Biosystems, Minneapolis, MN, USA). Intra-assay coefficients of variability were <7%, and between-assay CVs were <9%, as previously described [7].

Genotyping of the AGER gene DNA was isolated from whole blood from a subgroup of 2,347 unrelated patients with either PUREGENE DNA Purification Kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's protocol or with a phenol–chloroform protocol modified from Vandenplas et al. [23]. TagSNPs for genotyping were selected in an unbiased manner from the HapMap Project's NCBI build 34 and 35 for the CEU population as defined by Gabriel et al. [24], to comprehensively capture the genetic variation in the *AGER* gene. Minor allele frequencies were required to be ≥5%. Genotyping was performed with TaqMan SNP Genotyping Assays (Foster City, CA, USA) with ABI PRISM 7900HT Sequence Detection System (Foster City, CA, USA) and the SEQUENOM (Hamburg, Germany) multiplex platform.

Hardy–Weinberg equilibrium for SNPs were tested in 703 control individuals using Haploview 4.0 (data not shown).

Follow-up of patients All patients were followed and their outcomes obtained through to 25 May 2009 (median follow-up 9 years). None were lost to follow-up. For the purposes of this paper, all-cause mortality was identified via a search of the Finnish National Death Registry and centre databases. All deaths were confirmed with death certificate data.

Statistical analysis To identify variables independently associated with sRAGE, we used multivariate linear regression analysis. To evaluate the independent predictors of all-cause mortality in individuals with type 1 diabetes, we used the Cox proportional-hazards model. The predictors of the cumulative incidence of cardiovascular mortality, accounting for the competing event of non-cardiovascular death, were ascertained using a Fine and Gray model [25], which extends the Cox proportional hazards model to competing risks data by considering the subdistribution hazard [26]. The strength of the association between each predictor variable and the outcome was assessed using the subhazard ratio [27], which is the ratio of hazards associated with the cumulative incidence function (CIF) in the presence of and in the absence of a prognostic factor. Model selection from candidate variables was accomplished by minimisation of the Akaike's (AIC) and Bayesian information criteria (BIC). In both models, covariate functional form (including assessment of non-linear effect) was adjudged by residual-by-time analysis and (cubic) regression splines, where appropriate. The potential for multiple colinearity was tested using the variance inflation factor and condition number, where a variance inflation factor <10 and condition number <30 are desirable. Overall, the Cox model fit was assessed by: (1) approximation of cumulative Cox–Snell residuals to (−log) Kaplan–Meier estimates and residual plots; and (2) Harrell's *C* statistic and 'added-variable' goodness-of-fit tests [28]. Cox model performance was adjudged by the explained variation (R^2) using 1000 bootstrap repetitions of the whole data set; adjusting for covariates, via the 'str2d' Stata module [29]. The Fine–Gray model was implemented in Stata statistical software (V11.1, 2010; College Station, TX, USA) using the 'stcrreg' module.

Results

Cohort characteristics The cohort in whom sRAGE was estimated comprised 3,100 adult patients with type 1 diabetes. Their baseline characteristics have been previously described in detail [18–20] and are summarised in Table 1. Briefly, approximately half of the FinnDiane participants were male (52%). The mean age of participants was 38 years with a median duration of diabetes of 20 years. At baseline, 8% of the cohort had pre-existing cardiovascular disease (CVD). One-third of the cohort had evidence of kidney disease, including 14% with a urinary AER in the microalbuminuric range and 15% with a urinary AER in the macroalbuminuric range. Twelve per cent of participants had an eGFR <60 ml min^{-1} 1.73 m^{-2}, denoting the presence of moderate to severe renal impairment.

Clinical determinants of sRAGE concentrations in adults with type 1 diabetes The mean concentration (±SD) of sRAGE was 1,255±558 pg/ml. Twenty-five per cent of patients had an sRAGE concentration ≥1,500 pg/ml, defining the upper quartile. These individuals had the highest burden of microvascular complications, including CKD and retinopathy (Table 1). On multivariate analysis, sRAGE concentrations (as a continuous variable) were independently associated with eGFR, BMI, albuminuria, HbA_{1c}, age, duration of diabetes and insulin dosing requirements (Table 2, all $p<0.05$). Glomerular filtration rate as estimated by the CKD-EPI equation was the strongest predictor of sRAGE concentrations, explaining 25% of the variability in sRAGE concentrations. Individuals with macroalbuminuria also had higher concentrations of sRAGE, when compared with those with urinary albumin excretion in the micro- or normoalbuminuric range ($p<0.01$), after adjusting for other predictive variables (Table 2). Individuals with macrovascular disease or retinopathy at baseline had higher concentrations of sRAGE than those without vascular complications, but these baseline associations were eliminated after adjusting for the presence and severity of co-morbid kidney disease.

AGER polymorphisms and sRAGE concentrations in adults with type 1 diabetes Previous studies have suggested that sRAGE concentrations are partly under genetic control [12–15]. To test this hypothesis, we undertook a comprehensive analysis of genetic variation in the *AGER* gene in 2,200 diabetic individuals, which was then correlated with circulating sRAGE concentrations. This subgroup of patients in whom genetic testing was undertaken was not significantly different from the total cohort, with respect to age, sex, duration of diabetes, metabolic control or co-morbidity (data not shown).

The coding polymorphism, rs2070600 (*G82S*), and the upstream polymorphism, rs204993, were associated with sRAGE concentrations (Fig. 1), independent of other predictors of sRAGE concentrations, including eGFR, albumin excretion rate, BMI, duration of diabetes and HDL cholesterol. However, in the total cohort, these

Table 1 Baseline characteristics of FinnDiane Study participants, stratified according to quartiles of circulating sRAGE concentrations

Characteristic	Q1	Q2	Q3	Q4
sRAGE (pg/ml)	<887	887–1,257	1,258–1,499	≥1,500
Age (years)	40±1*	39±1	37±1	38±1
Male sex (%)	51	52	54	51
Duration of diabetes (years)	22±1*	21±1	20±1	21±1
Insulin dose (U/kg)	0.7±0.1	0.7±0.1	0.7±0.1	0.7±0.1
HbA_{1c} (%)	8.4±0.1	8.5±0.1	8.5±0.1	8.5±0.1
BMI (kg/m^2)	26.1±0.1*	25.2±0.1	24.9±0.1	24.4±0.1*
Systolic blood pressure (mmHg)	134±1*	132±1	131±1	134±1*
Diastolic blood pressure (mmHg)	80±1*	79±1	79±1	80±1*
Medication use (%)				
ACE inhibition	30*	23	22	31*
Angiotensin receptor blocker	5	5	4	5
Calcium channel blocker	7	8	9	12*
Beta blocker	8	9	7	16*
Diuretic	7	8	9	16*
Lipid-lowering therapy	11	9	9	12*
Total cholesterol (mmol/l)	5.0±0.1	5.0±0.1	4.9±0.1	5.0±0.1
LDL-cholesterol (mmol/l)	3.1±0.1	3.1±0.1	3.1±0.1	3.1±0.1
HDL-cholesterol (mmol/l)	1.3±0.1	1.3±0.1	1.3±0.1	1.3±0.1
Triacylglycerol (mmol/l)	1.3±0.1	1.3±0.1	1.3±0.1	1.3±0.1
Retinopathy requiring laser therapy (%)	31	26	27	35
Current smoker (%)	23	23	24	28
Established macrovascular disease (%)	8	6	8	8
eGFR <60 ml min^{-1} 1.73 m^{-2} (%)	7	8	9	25*
Microalbuminuria (%)	18	16	14	12
Macroalbuminuria (%)	11	12	14	28*
hsCRP (geometric mean) (mg/l)	2.5	2.3	2.2	2.1*

Data shows means ± SEM unless otherwise stated

*p value vs Q2/3<0.05

genetic factors determined less than 5% of the total variability in sRAGE concentrations.

All-cause mortality and sRAGE concentrations The median follow-up was 9.1 years, during which time there were 202 deaths (7.4 per 1,000 patient years). Circulating concentrations of sRAGE were significantly associated with all-cause mortality in a Cox regression analysis (Table 3). This association occurred regardless of the presence and severity of kidney disease, and independent of other predictive variables including age, glycaemic control (HbA_{1c}), systemic inflammation (hsCRP), triacylglycerol and HDL-cholesterol concentrations, smoking status and the presence of macrovascular disease on Cox regression analysis (Table 3). The functional form of the relationship between sRAGE and all-cause mortality was linear across the range of concentrations, with no evidence of non-linear effects in fully adjusted Cox models, as adjudged by restricted splines [30]. Overall, the Cox model was well specified (Harrell's C statistic=0.85, May–Hosmer goodness-of-fit test, p=0.77) with a good predictive utility (R^2=0.79; 95% CI 0.73–0.87) and no interactions were detected. In this model, the explained variation in all-cause mortality due to sRAGE (R^2=0.15; 95% CI 0.05–0.28) was stronger than traditional risk factors such as hsCRP and HbA_{1c} (R^2=0.07 (0.01–0.16) and 0.06 (0.01–0.15) respectively). Despite their strong association with circulating sRAGE levels, no *AGER* polymorphisms were associated with mortality outcomes.

Table 2 Independent predictors of sRAGE concentrations in adults with type 1 diabetes from the FinnDiane cohort

Predictor variable	β coefficient	95% CI	$p>\|t\|$
Age	−2.83	−4.85, −0.81	0.006
Estimated GFR	571.8	490.0–653.5	0.0001
BMI	2,109.4	1,755.3–2,463.6	0.0001
Duration of diabetes	−3.82	−5.83, −1.81	0.0001
HbA_{1c}	16.20	2.97–29.47	0.016
Insulin dose	−169.8	−261.3, −78.2	0.0001
Macroalbuminuria	204.9	143.5–266.3	0.0001

BMI is modelled as a −1 fractional polynomial; estimated GFR modelled as a −0.5 fractional polynomial. Albuminuria is modelled as a categorical variable, denoting the presence or absence of macroalbuminuria. All other variables are modelled as a linear relationship

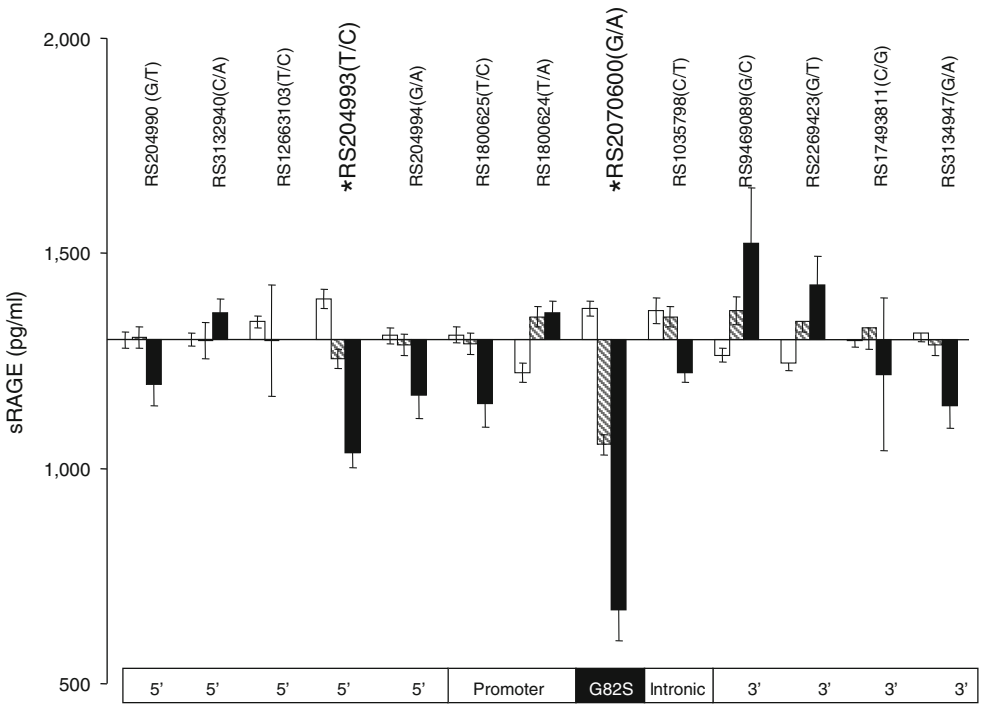

Fig. 1 The mean level of circulating sRAGE in patients with type 1 diabetes stratified according to polymorphisms in the *AGER* gene. White columns denote the major allele, solid bars denote the minor allele, cross-hatched columns denote heterozygotes. Association between the polymorphism and sRAGE concentrations adjusted for age, duration of diabetes, BMI, sex, HbA_{1c}, albuminuria, eGFR, smoking, blood pressure and lipid concentrations and treatment modalities: $*p<0.05$

Table 3 Cox regression model for predictors of all-cause mortality in adults with type 1 diabetes from the FinnDiane cohort

Predictor variable	Hazard ratio	95% CI	*p* value
Male sex	1.43	0.996–2.055	0.05
Age (years)	1.06	1.042–1.077	<0.001
Current smoking (yes/no)	1.73	1.211–2.479	0.01
Macrovascular disease (yes/no)	2.53	1.726–3.700	<0.001
Triacylglycerol (mmol/l)	1.21	1.005–1.454	0.044
HDL-cholesterol (mmol/l)[a]	1.09	0.910–1.301	<0.001
HDL-cholesterol (mmol/l)	0.91	0.799–1.027	0.355
HDL-cholesterol (mmol/l)	1.15	1.003–1.326	0.123
HbA_{1c} (%)[b]	1.17	0.987–1.374	0.045
HbA_{1c} (%)[b]	0.80	0.719–0.897	<0.001
hsCRP (mg/l)	1.03	1.017–1.039	<0.001
Estimated GFR (ml min^{-1} 1.73 m^{-2})	0.99	0.981–0.997	0.006
Albumin excretion rate (log)	1.18	1.063–1.306	0.002
sRAGE (per 100 pg/ml)	1.03	1.010–1.056	0.005

The represented coefficient effects for HDL and HbA_{1c} are not subject to literal effect interpretation (compared with other coefficients) as their underlying scale was transformed in the process of spline generation (interpretation of their functional form is demonstrated in electronic supplementary material [ESM] Fig. 1)

[a] HDL first coefficient is the basis-function, second and third coefficients are the knots at 1.54 and 1.08

[b] HbA_{1c} first coefficient is the basis-function, second coefficient is the knot at 7.4

Cardiovascular mortality and sRAGE concentrations Our study specifically examined cardiovascular deaths while taking into consideration (in an estimation sense) the competing risk of non-cardiovascular death (Fine and Gray model) [25]. This strategy may be especially important in patients with diabetes, as diabetes is also associated with increased non-cardiovascular mortality [31], which may potentially confound cause-specific analysis. In our competing risk model, circulating levels of sRAGE were also significantly associated with the cumulative incidence of cardiovascular mortality (Table 4). Again, this association was linear and independent of other predictive variables including age, duration of diabetes, the presence and severity of CKD and the presence of macrovascular disease.

Table 4 Competing risk model of variables associated with the cumulative incidence of cardiovascular mortality in patients with type 1 diabetes from the FinnDiane cohort, taking into account the competing risk of non-cardiovascular death

Predictor variable	Subhazard ratio	95% CI	*p* value
Age (years)	1.05	1.02–1.09	0.002
Macrovascular disease (yes/no)	2.66	1.39–5.07	<0.001
Albumin excretion rate (log)	1.42	1.23–1.65	0.012
sRAGE (per 100 pg/ml)	1.06	1.03–1.09	<0.001
Duration of diabetes (years)	1.07	1.04–1.10	<0.001

For graphical interpretation, see Fig. 2

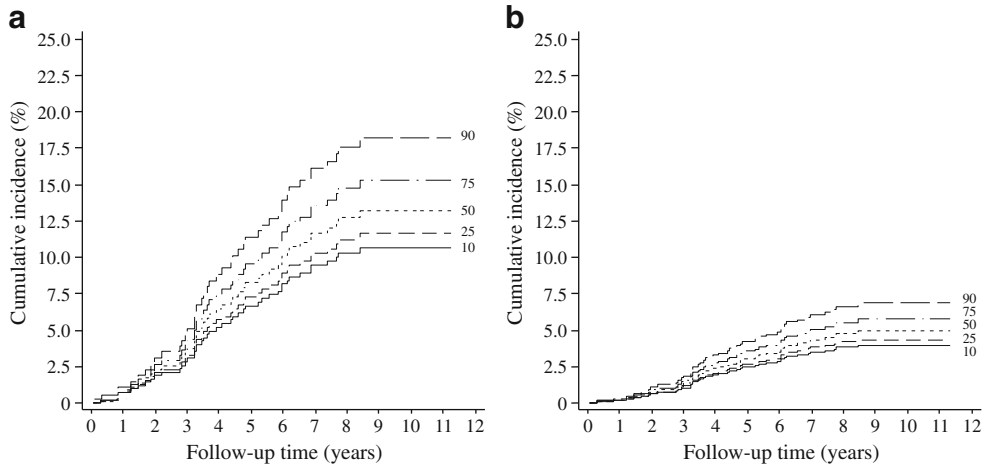

Fig. 2 The relationship between circulating level of sRAGE and the cumulative incidence of cardiovascular mortality in a competing risks model, taking into account non-cardiovascular deaths, stratified for the presence (**a**) or absence (**b**) of established cardiovascular disease at baseline and adjusted for other predictive variables (Table 4). sRAGE percentiles (concentration) are set at 10th (n=789), 25th (n=1,011), 50th (n=1,386), 75th (n=1,921) and 90th (n=2,536). The age was arbitrarily set at mean (39.1), log albumin excretion rate at 75% (3.8) and duration at median (20.5)

Discussion

Activation of RAGE is a key mediator of vascular complications in type 1 diabetes [1–3]. sRAGE is widely viewed as an endogenous antagonist of RAGE activation, as recombinant sRAGE is able to prevent diabetic complications in experimental models [2, 3]. An inverse association between circulating concentrations of sRAGE and surrogate measures of atherosclerotic burden has also been described in cross-sectional studies in non-diabetic [16] and population samples [17]. This has led to the suggestion that sRAGE may be a protective factor. Indeed, cardioprotective effects have been observed in experimental models of diabetes [2, 3]. However, in this study, we demonstrate that sRAGE concentrations are positively associated with all-cause and cardiovascular mortality in patients with type 1 diabetes, with the greatest risk observed in individuals with the highest sRAGE concentrations, independent of glycaemic control, the presence and severity of CKD and other conventional risk factors.

Although the relationship between sRAGE and mortality in our patients is strong and significant, it does not appear to be (directly) causal, as we also examined the genetic determinants of sRAGE and their association with mortality. This 'Mendelian randomisation' provides a relatively unbiased system to assess the consequences of lifelong exposure, independently of other risk factors [32]. In our study, two *AGER* polymorphisms were strongly associated with sRAGE concentrations in diabetic patients. However, neither of these polymorphisms was associated with mortality. A similar phenomenon has been demonstrated for hsCRP, leading to the suggestion that increased hsCRP concentrations are a marker of atherogenic processes [33]. In the same way, it is possible to hypothesise that the positive association of sRAGE with all-cause mortality observed in our study may indirectly reflect the overactivity of the AGE–RAGE axis in diabetic patients [11, 34], which may not only accelerate the development and progression of vascular complications but also lead to the auto-induction of the production of RAGE and subsequent increased shedding of sRAGE into the circulation.

Our findings are clearly different from previous studies in other settings, in which an inverse association between circulating concentrations of sRAGE and atherosclerotic burden has been demonstrated [16, 17]. However, none of these studies were performed in type 1 diabetes. By contrast, small cross-sectional analyses of selected patients with type 1 diabetes from the EURODIAB Prospective Complications Study have shown that individuals with CVD (n=116) had higher levels of sRAGE than those without CVD or any microvascular complications (n=178) [35]. In addition, an increased incidence of fatal and non-fatal CVD and all-cause mortality with higher baseline levels of sRAGE has recently been described in a prospective follow-up study of 169 individuals with diabetic nephropathy and 170 individuals with persistent normoalbuminuria attending the Steno clinic [36]. Our data, now obtained in a large cohort of 3,100 diabetic individuals, support and extend these findings, as well as demonstrate the independent nature of this association, which cannot be accurately assessed in small studies.

Reduced concentrations of the splice variant esRAGE have been associated with increased mortality in patients with end-stage renal disease (ESRD) [37]. However, it has

been suggested that esRAGE and cleaved forms may have distinct relationships with clinical outcomes [38]. It is possible that reduced production of esRAGE partly reflects increased production of full-length RAGE due to the suppression of alternative splicing required for full-length RAGE production, and hence this negative association. Another possibility could be the confounding effects of ESRD and renal replacement. Indeed, paradoxical associations are often observed in ESRD patients, possibly due to poor nutrition.

It is not clear why the association between sRAGE and mortality in people with type 1 diabetes should be different from that demonstrated in other clinical settings [16, 17]. However, it is not uncommon for risk factors to be paradoxical in type 1 diabetes. For example, we have previously shown that increased plasma adiponectin concentrations are associated with mortality in type 1 diabetes [20], whereas in patients with type 2 diabetes and the general population the relationship is the opposite [17, 39]. In addition, none of these reports adjusted for the potentially confounding effects of renal function, which is a major determinant of sRAGE concentrations in individuals with type 2 diabetes [10, 15, 40, 41] and in the general community [11]. The association between sRAGE and renal impairment partly represents the impaired renal clearance of AGEs and other RAGE ligands and may lead to increased tissue RAGE levels and/or sRAGE shedding. In addition, sRAGE may accumulate in the plasma of individuals with renal impairment, like a number of low molecular mass proteins including β2-microglobulin, cystatin C, β-trace protein and adiponectin [42]. Although the presence and severity of nephropathy is certainly the major determinant of mortality in patients with type 1 diabetes, both in the FinnDiane [19] and other diabetic cohorts [43], the association between sRAGE and mortality was statistically independent to the presence and severity of CKD, with no evidence of significant interactions, suggesting that sRAGE is more than simply a marker of impaired kidney function, and that its association with adverse outcomes was not confounded by CKD.

In our cohort, circulating concentrations of sRAGE were also inversely associated with body size, as estimated by the BMI (Table 2), as previously described in patients with type 2 diabetes [8] and in the general population [8]. Any physiological basis for this association remains to be established. Certainly, in our study the association between BMI and sRAGE was not influenced by systemic inflammation, smoking, poor metabolic control [44] or the confounding interaction of renal function and body size (i.e. smaller people generally have lower renal function). In addition, sRAGE concentrations were negatively associated with the required insulin dose (adjusted for body mass), a marker of insulin resistance in type 1 diabetes. This is consistent with a recent report showing that insulin sensitisers are able to increase sRAGE concentrations in patients with type 2 diabetes.

Notably, after adjusting for renal function, the circulating concentration of sRAGE was not associated with blood pressure. This finding is different from that observed in patients with essential hypertension where sRAGE is inversely associated with pulse pressure [45]. In addition, the number or type of antihypertensive therapies, including the use of agents that inhibit the renin angiotensin system (ACE inhibitors or angiotensin receptor blockers), was not correlated with sRAGE concentrations after adjusting for renal function. This finding is potentially confounded by indication, as high-risk individuals were more likely to receive renin angiotensin system blockade in clinical practice. However, in previous studies we have demonstrated that normotensive individuals with type 1 diabetes and microalbuminuria treated with ACE inhibitors achieve higher concentrations of sRAGE compared with placebo or equivalent blood pressure reduction with amlodipine [9].

Strengths of our study include its very large cohort of individuals with type 1 diabetes, high participation rate, access to subsidised care (75–100% of costs) and contemporary treatment regimens, including a range of insulin regimens, statins, blockers of the renin angiotensin system and self-monitoring technologies. We used validated methods to identify deaths and all deaths in our cohort were confirmed through death records. Few changes in diabetes treatment and healthcare over the short study period will have affected mortality results. In our statistical analysis we specifically incorporated the non-linear effects of predictive variables, modelled for interactions and in the case of cardiovascular mortality, modelled within the paradigm of a formal competing risks (Fine and Gray) model, which looked at the cumulative incidence of cardiovascular deaths while taking into consideration (in an estimation sense) the competing risk of other causes of death [26].

It should be noted that our data are essentially observational. Although observational studies have a number of potential advantages [46], it is also possible that associations demonstrated in this study may be due to confounding by unmeasured factors or ones that are difficult to quantify. For example, sRAGE concentrations may be associated with a range of occult differences in inflammatory or metabolic pathways that may themselves impact on mortality outcomes in diabetic individuals. This hypothesis is supported by the absence of any demonstrable association between *AGER* polymorphisms and mortality outcomes in our patients.

In summary, this large multicentre study demonstrates that sRAGE is positively associated with mortality in adults with type 1 diabetes. These data support the accumulating body of experimental data demonstrating that RAGE activation is a key mediator of premature mortality in patients with type 1 diabetes.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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