

RAGE Gly82Ser polymorphism in patients with type 2 diabetes with comorbid depression

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Abstract

Objective: To examine receptors for advanced glycation end-products Gly82Ser polymorphism in patients of type 2 diabetes with comorbid depression.

Methods: The case-control study was conducted at Fujian Provincial Hospital, Fuzhou, China, between December 2011 and December 2012, and comprised unrelated Chinese Han patients of type 2 diabetes, and diabetics with diagnosed clinical depression. Gly82Ser polymorphism was determined using polymerase chain reaction amplification-high resolution melting curve protocol. Serum levels of endogenous secretory receptor for advanced glycation end products were measured using enzyme-linked immunosorbent assay. SPSS 16 was used for data analysis.

Results: Of the 114 subjects, 72(63.15%) were clinically depressed. Lower levels of endogenous secretory receptor were found in the depression group compared with the other group ($p=0.049$). No difference in genotypes or allele frequencies existed between the two groups ($p>0.05$). Gly82Ser carriers had significantly higher Hamilton Rating Scale scores ($p<0.001$) and lower serum endogenous secretory receptor ($p=0.012$) among the depressed diabetics. There were also significant differences in body mass index ($p=0.005$), abdominal circumference ($p=0.038$), carotid intima-media thickness ($p=0.037$) and high-sensitivity C-reactive protein ($p=0.005$) concentration between the different genotypes.

Conclusion: Receptors for advanced glycation end-products-ligands system may be involved in type 2 diabetes with comorbid depression at the genetic level.

Keywords: Type 2 diabetes, Depression, RAGE, Polymorphism, esRAGE. (JPMA 69: 828; 2019)

Introduction

Extensive epidemiological data indicates that diabetes greatly advances the risk of depression.^{1,2} Besides the psychological stress associated with chronic disease, it is now being questioned if there is a common biological mechanism between diabetes and depression.

In recent years, Cytokine hypothesis is one of the research focuses in the pathogenesis of depression, which postulated that cytokines may cause depressive illness³ and depression is a psycho-neuro-immunological disorder.⁴ A study clearly stated that depression is an inflammatory disease, but cell-mediated immune activation is the key component of depression.⁵ Besides, in patients with type 2 diabetes (T2D), the interaction between receptors for advanced glycation end products (RAGE) with its ligands results in the activation of multiple signalling pathways, which, in turn, increase the secretion of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF- α). These considerations led to the hypothesis that the increasing

cytokines derived from activation of the interaction between RAGE with its ligands contributed to the pathology of diabetic depression. However, soluble RAGE (sRAGE) as an isoform of RAGE plays a protective role by neutralising the harmful effects due to its special structures that carry all the extracellular domains, but lack the transmembrane and cytoplasmic domains. A previous study⁶ showed that, compared with the common T2Ds, diabetics with comorbid depression had lower endogenous secretory RAGE (esRAGE) level and higher serum high-sensitivity C-reactive protein (hsCRP) level, confirming the association between T2D with comorbid depression and RAGE ligands. Recent studies have shown that advanced glycation end-product (AGE) inhibitors prevent or limit diabetes-accelerated medial calcification, thus inhibiting the association of AGE-RAGE or the downstream signalling-reduced medial calcification in diabetes.⁷ RAGE-mediated regulation of adiposity and inflammation may attribute to T2D and diabetic vascular complications.⁸ However, the mechanism is still unclear.

Growing evidence⁹⁻¹¹ indicates that circulating levels of sRAGE are associated with RAGE Gly82Ser (G82S) polymorphism, and Ser82 allele carriers had lower plasma levels of sRAGE. RAGE G82S polymorphism. (Gly82Ser,

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rs2070600) is a non-synonymous single nucleotide polymorphism (SNP) which is identified within the V-type immunoglobulin (IG) domain in the extracellular region of the receptor, and leads to a change from glycine to serine at codon 82 in exon 3.⁹ Additionally, the Ser82 allele has been shown in in-vitro studies to increase ligand affinity in transfection cells, which, upon further analysis, demonstrated upregulation of key pro-inflammatory signal transduction pathways, and the prompted Ser82 alleles may play an important role in inflammatory disease.¹⁰

However, information regarding the relation between these polymorphisms and serum esRAGE in T2D patients with comorbid depression remains limited. The current study was planned to explore whether these polymorphisms and levels of serum esRAGE were related in T2D patients with depression as a comorbidity.

Patients and Methods

The case-control study was conducted at Fujian Provincial Hospital, Fuzhou, China, between December 2011 and December 2012, and comprised unrelated Chinese Han T2D patients, and diabetics with diagnosed clinical depression. T2D was diagnosed by the 1999 World Health Organisation (WHO) criteria.¹²

After approval was obtained from the institutional ethics committee, all patients hospitalized in the Department of Endocrinology were included. Complete medical records were available for all patients, and all subjects provided written informed consent.

Clinical assessment was based on the criteria established by the Chinese Classification and Diagnosis of Mental Diseases-3rd edition¹³ and the Hamilton Rating Scale for Depression (HAM-D).¹⁴ The HAM-D scale was used to evaluate the severity of depression symptoms.¹⁵ A score of <7 indicated no depression, 7-17 indicated possible depression, and a score ≥ 17 confirmed depression. Patients with scores ≥ 17 were selected as patients, while those with score <7 were selected as controls. Current and past medical histories, personal background, and medications were recorded for all subjects. Patients with age <18 or >75 years were excluded, and so were those with presence of a central nervous system (CNS) disease that could cause depression, with the presence of a severe primary physical illness, and those with presence of familial hereditary disease.

Serum samples were isolated from fasting subjects and stored at -80°C prior to analysis. The serum levels of esRAGE were measured using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems,

Minneapolis, MN). The intra-assay coefficient of variation (CV) was <4% and the inter-assay CV was <10%. Blood lipids, C-reactive protein (CRP) and glycated haemoglobin (HbA1C) levels were quantified using an automatic biochemical analyser (Bio-Rad, Benicia, CA). Colour Doppler ultrasound was used to locate the thickest site of the carotid artery intima-media and to measure the intima-media thickness (IMT) at the site, two other upstream sites, and one site that was 1cm downstream, each of which were measured six times to obtain average values. Abdominal circumference, height and body weight were measured, and body mass index (BMI; kg/m²) was calculated.

Human genomic deoxyribonucleic acid (DNA) was extracted from blood samples using the TIANamp Blood DNA Kit (TIANGEN, China). High-resolution melting (HRM) was used to analyse RAGE G82S polymorphism.⁹ The sequences of HRM primers (design and synthesis by Shanghai Huirui Biotechnology Co., Ltd) were 5'-CTGGGACAGTGTGGCTCGT-3' (forward) and 5'-CGGAAAATCCCCTCATCCT-3' (reverse). Polymerase chain reaction (PCR) was performed in a final volume of 20 μ l containing 10ng DNA, 250nM 0.5 μ l of each primer, and 1X10 μ L genotype quantitative PCR (qPCR) Master Mix (Shanghai Huirui Biotechnology Co., Ltd). The amplification was carried out according to the following protocol: initial denaturation at 5°C for 5min, followed by 40 cycles of 95°C for 10s, annealing at 58°C for 30s, and elongation at 72°C for 30s, one cycle of 72°C for 5 min, 95°C for 1 min, 60°C for 2 min. HRM curves were generated by monitoring the fluorescence of the sample during a temperature ramp from 60°C to 95°C at 0.03°C/s. All PCR amplifications were carried out on a Light Cycler 480 instrument (Roche) according to the manufacturer's instructions. All samples were tested in duplicate. Two random samples from each genotype group were sequenced to confirm the accuracy of the genotyping.

Data analysis was done using SPSS 16. Categorical variables were compared using chi-square test. Normally and homoscedastic distributed continuous variables were tested with student's t test, while non-normally distributed variables were log-transformed or assessed with Mann-Whitney U test. Hardy-Weinberg equilibrium of RAGE genotypes was evaluated by chi-square test. The factors influencing HAM-D score or esRAGE were analysed using Pearson correlation analysis for normally distributed variables, and Kendall's tau-b correlation for non-normally distributed. Multiple backward stepwise linear regression analysis was used to evaluate significant predictors of HAM-D score in T2D patients with complicating depression. Descriptive data was expressed

as means \pm standard error (SE) for normally distributed variables and as median (interquartile range) for variables with a skewed distribution, unless otherwise stated. Categorical variables were presented as frequencies and percentages.

Results

Of the 114 subjects, 72(63%) were clinically depressed patients, and 42(37%) were non-depressed controls. There were no significant differences between the groups in clinical parameters ($p>0.05$). Compared with the control group, the patients had elevated BMI ($p<0.001$), abdominal circumference ($p<0.001$), carotid (IMT) ($p=0.008$), HbA1c ($p=0.034$), hsCRP ($p=0.002$), morning urine micro-albumin ($p=0.049$), and lower age ($p=0.019$). Serum esRAGE level was significantly lower in the

depression group ($p=0.049$). Also, there were significant differences between the different genotypes in T2D patients with depression (Table-1).

There was no difference in genotypes or allele frequencies between the two groups (Table-2).

Next, esRAGE was found to be negatively correlated with hsCRP and BMI and there was no significant correlation of esRAGE with other variables (Table-3).

HAMD score was positively correlated with hsCRP, BMI, and abdominal circumference, but it was negatively correlated with esRAGE, and was not significantly correlated with other variables (Table-4).

Finally, esRAGE, RAGE G82S polymorphism and hsCRP

Table-1: Demographic and clinical characteristics.

	All subjects(n=114)		T2D with depression(n=72)	
	T2D with depression	T2D without depression	82G/82G	82S/82G+82S/82S
Number of patients (n)	72	42	45	27
male/female (n)	26/46	26/16**	17/28	9/18
Age(years)	58.99 \pm 10.57	63.43 \pm 7.77*	59.07 \pm 10.57	58.85 \pm 11.44
Duration of type 2 diabetes (years)	10	9.5	10	9.5
BMI(Kg/m ²)	23.78 \pm 3.06	21.43 \pm 3.02**	23.01 \pm 3.38	25.06 \pm 0.69**
Abdominal circumference(cm)	87.60 \pm 9.82	78.85 \pm 7.48**	85.75 \pm 9.80	90.69 \pm 9.24*
HbA1C (% , mmol/mol)	9.78 \pm 2.70	8.78 \pm 2.35*	9.86 \pm 2.70,	9.64 \pm 2.75,
	83.0 \pm 29.0	72.0 \pm 25.7*	84.0 \pm 29.0	82.0 \pm 30.1
TG (mg/dl)	124	124	176 \pm 239	134 \pm 61
CHOL (mg/dl)	193 \pm 55	187 \pm 44	188 \pm 58	200 \pm 52
LDL (mg/dl)	113 \pm 53	112 \pm 36	109 \pm 48	120 \pm 46
HDL (mg/dl)	43	43	43	46
IMT (mm)	1.20	1.05**	1.17 \pm 0.28	1.35 \pm 0.45*
hsCRP (mg/L)	1.46	0.69**	1.31	2.76**
Morning urine microalbumin (mg/L)	16.6	13.95*	21.6	14.6
esRAGE (ug/L)#	2.13	4.49*	2.78	1.54*
HAMD scores			23.31 \pm 3.96	28.48 \pm 4.72**

T2D: Type 2 diabetes; esRAGE: Endogenous secretory receptor for advanced glycation end products; hsCRP: High-sensitivity C-reactive protein; BMI: Body mass index; HAMD: Hamilton Rating Scale for Depression; HbA1c: Glycated haemoglobin; TG: Triglycerides; CHOL: Cholesterol; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; IMT: Intima-media thickness.

The Student's t test or Mann-Whitney U test for continuous variables and χ^2 test for categorical variables for significant differences. * $P<0.05$, ** $P<0.01$

#Log transformed before test.

Table-2: Genotype and allelic frequencies.

RAGE G82S (rs2070600)		Type 2 diabetes with depression N(%)	Type 2 diabetes without depression N(%)	P Value
Genotypes	GG(82G/82G)	45 (62.50%)	29 (69.05%)	0.48
	GA(82S/82G)	25 (34.72%)	10 (23.81%)	
	AA(82S/82S)	2 (2.78%)	3 (7.14%)	
Alleles	G(82G)	115 (79.86%)	68 (80.95%)	0.84
	A(82S)	29 (20.14%)	16 (19.05%)	

χ^2 test was used.

RAGE: Receptors for advanced glycation end-products.

Table-3: Relationship of esRAGE with other clinical indicators in type 2 diabetic patients with and without depression (n=114).

	esRAGE	
	Kendall's tau_b correlation	P
CRP	-0.367	<0.001
BMI	-0.130	0.041

esRAGE: Endogenous secretory receptor for advanced glycation end products
CRP: C-reactive protein
BMI: Body mass index.

Table-4: Relationship of HAMD score with other clinical parameters in type 2 diabetic patients with depression (n=72).

	HAMD score	
	r	P
esRAGE	-0.442 a	<0.001
CRP	0.357 a	<0.001
BMI	0.342 b	0.003
Waist circumference	0.287 b	0.014

^a Kendall's tau_b correlation

^b Pearson correlation

esRAGE: Endogenous secretory receptor for advanced glycation end products
CRP: C-reactive protein
BMI: Body mass index
HAMD: Hamilton Rating Scale for Depression.

Table-5: Multiple backward stepwise linear regression analysis for significant predictors of HAMD score in type 2 diabetic patients complication depression (R²a= 0.45,n=72).

	Unstandardized Coefficients		Standardized Coefficients	t	Sig	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
esRAGE (ug/L)	-0.367	0.095	-0.372	-3.849	<0.001	-0.557	-0.177
Genotype (82S/82G+82S/82S VS. 82G/82G)	3.419	0.960	0.339	3.562	0.001	1.504	5.335
hsCRP (mg/L)	0.460	0.222	0.208	2.072	0.042	0.017	0.903

Variables included in the model: sex, age, Duration of type 2 diabetes, BMI, HbA1C, esRAGE, hsCRP and the RAGE G82S polymorphism.

esRAGE: Endogenous secretory receptor for advanced glycation end products
hsCRP: High-sensitivity C-reactive protein.

were found to be significant predictors for HAMD score (Table-5).

Discussion

In T2D patients, elevated levels of blood glucose and accelerated Maillard reaction lead to generation of AGEs. By receptor-dependent means, AGEs exert cell-mediated effects via engagement of cellular binding receptors. RAGE was first identified as a signal transduction receptor for AGEs. On the one hand, the combination of AGEs with RAGE activates downstream signalling pathway, eventually leading to pathological damage. On the other hand, the combination increases the expression of RAGE through a positive feedback via activation of nuclear

factor kappa B (NF- κ B). RAGE belongs to the IG superfamily of cell surface receptors and is capable of interacting with multiple ligands, including AGEs, advanced oxidation protein products (AOPPs), S100/calgranulins, high mobility group box-1 (HMGB1), amyloid- β peptide and β -sheet fibrils.¹⁶

It is well known that the interaction between RAGE with its ligands results in activation of multiple signalling pathways which amplifies immuno-inflammatory responses. Numerous studies indicated that RAGE interaction with pro-inflammatory ligands, such as S100/calgranulins and HMGB1, is responsible for chronic inflammation via modulating properties of inflammatory cells (monocytes/macrophages, T lymphocytes and dendritic cells).^{17,18}

Recent studies have focussed on the role of inflammation in depression. A study indicated that the patients of depression had significant higher level of pro-inflammatory cytokines along with increased acute phase proteins (e.g. CRP), chemokines, cell adhesion molecules, and other inflammatory mediators such as prostaglandins.¹⁹ The underlying molecular mechanisms of this response is innate immune activation, including IL-1a, IL-1b, IL-6, and TNF- α .²⁰ A proof for it is that persons

who were being treated with interferon alpha (IFN- α) for malignant melanoma showed more psychomotor retardation and weight loss than the medically-healthy depressed sample.²¹

The current study observed lower levels of sRAGE in the depression group and an inverse correlation between the esRAGE level and hsCRP concentration as well as the esRAGE level and HAMD score. This supports the concept that RAGE-ligands systems and immune inflammatory response may be the link between diabetes, depression and esRAGE. It could be beneficial to those who have depression along with T2D by neutralising the harmful effects of RAGE-ligands systems and immune

inflammatory response. Future studies need to be performed to look into this aspect.

Based on literature cited above, we speculate that the binding of RAGE/ligands causes activation of pro-inflammatory signalling pathways accompanied by release of various pro-inflammatory molecules. Some pro-inflammatory cytokines, particularly IL-1b, can access and ultimately influence the brain by several special pathways before stimulating the release of corticotropin-releasing hormone (CRH) or arginine vasopressin (AVP) from paraventricular nucleus of the hypothalamus, which activates the release of adrenocorticotrophic hormone (ACTH) from the pituitary, which, in turn, travels to the adrenals, stimulating the release of cortisol.²² It is known that corticosteroid secreting rhythm disturbance plays a critical role in the mechanism of depression.²³ Thus, T2S with comorbid depression occurs possibly through such a mechanism.

The current study also showed that G82S carriers had significant higher HAMD scores and lower serum esRAGE in T2D group with depression. There were also significant differences between G82S polymorphism with BMI, abdominal circumference, IMT and hsCRP concentration. Furthermore, multiple backward stepwise linear regression analysis for significant predictors of HAMD score in the patients group showed that the HAMD score was associated with RAGE G82S polymorphism, esRAGE and hsCRP concentration. To the best of our knowledge, this investigation for the first time found that the functional RAGE Gly82Ser polymorphism increased depression symptoms in T2D patients, and confirmed the association between RAGE-ligands system with T2D with comorbid depression. However, mechanisms whereby SNP rs2070600 (Gly82Ser) affects the sRAGE plasma concentrations remain unknown. The traditional view²⁴ is that the generation of soluble receptors either derive from an alternatively spliced messenger ribonucleic acid (mRNA) or are cleavage products of the membrane-bound form. On the one hand, genetic variations in the gene coding for RAGE, also known as advanced glycosylation end-product-specific receptor (AGER), affect gene expression and ligand binding affinity.²⁵ The higher binding affinity of RAGE to ligands, such as AGEs, leads to a diminished ability of esRAGE to capture ligands. On the other hand, esRAGE are also cleavage products of the membrane-bound form. A study¹¹ hypothesised that the alteration of the amino-terminal glycosylation (N-glycosylation) state of the protein, caused by the Gly82Ser polymorphism, induces structural changes in the protein that makes RAGE more vulnerable for the action of proteinases, such as a disintegrin and metalloproteinases

10 and matrix metalloproteinase-9, and affects the binding of antibody (in ELISA kits obtained from R&D Systems) to esRAGE ultimately affecting our immunoassay. Thus SNP rs2070600 (Gly82Ser) is associated with depression via affecting the concentration of esRAGE. In addition, according to a study,¹⁰ RAGE Ser82 isoform may display the enhanced ligand-binding affinity and up-regulate the inflammatory response on engagement of S100/calgranulins, thereby contributing to the increased generation of pro-inflammatory mediators. In our study, hsCRP as the downstream cytokines of AGEs-RAGE axis was higher in diabetes with depression, and was a proof for the pro-inflammatory mechanism.

The current study also observed a positive correlation between HAMD score with BMI and abdominal circumference, but a negative correlation between esRAGE level with BMI. In this background, we speculate that there may be some common underlying mechanisms in diabetes, depression, RAGE systems, inflammation and obesity. Increasingly, epidemiological data is revealing that obesity increases risk for depression.²⁶ Consistent with our finding, a study²⁷ observed that concentrations of sRAGE were significantly lower in both obese groups compared to lean volunteers. A study²⁸ observed that serum CRP was significantly associated with the G82S variant. This result implies that obesity and depression may have biological correlation via RAGE-ligands pathway. As is known, obesity is closely associated with low-grade inflammation. Thus, chronic inflammation may be an important mechanism.

However, no difference in genotypes or allele frequencies was observed between the two groups. But we can't deny the association of the Gly82Ser polymorphism with the onset of depression in T2D. Maybe the sample size was not large enough and we should consider other influences such as race, environmental factors and linkage disequilibrium with other genes.

There were some limitations of the current study. First, the patients enrolled were all hospitalised patients who were relatively severe T2D cases and might not be representative of the standard T2D patients. Second, the sample size was not large enough to make definitive conclusions. Finally, we did not investigate depression in patients without diabetes as controls. Given that this is only a clinical correlation study, it would be of interest in the future to establish functional studies of Gly82Ser polymorphism with T2D in conjunction with comorbid depression.

Conclusion

RAGE G82S polymorphism was associated with esRAGE

levels and the severity of depression symptoms. Thus esRAGE may be a potential protective factor for T2D with comorbid depression.

Disclaimer: The Abstract was presented at the 21st Scientific Meeting of the Chinese Diabetes Society, Nov 22-25, 2017, held in Chongqing, China.. It was also published in the Abstract Book of the event (The name of the Abstract Book is DIABETES-METABOLISM RESEARCH AND REVIEWS, Abstract number 698696, Page1) .

Conflicts of Interest: None.

Source of Funding: None.

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