

## **G82S RAGE polymorphism is associated with Alzheimer's disease**

**Rani Cathrine Chellappa<sup>1</sup>, Palaniswamy Rani<sup>1</sup>**

*<sup>1</sup>Department of Biotechnology, PSG College of Technology, Peelamedu, Coimbatore, Tamil Nadu, 641004, India*

### **TABLE OF CONTENTS**

1. Abstract
2. Introduction
3. Materials and methods
  - 3.1. Study subjects
  - 3.2. Quantification of RAGE and TNF- $\alpha$  expression by qPCR
  - 3.3. Quantification of A $\beta$ 42 and sRAGE in plasma
  - 3.4. G82S RAGE polymorphism
  - 3.5. Statistical analysis
4. Results
  - 4.1. Analysis of expression of RAGE variants in PBMC
  - 4.2. Quantification of sRAGE in plasma samples
  - 4.3. Analysis of G82S RAGE polymorphism
5. Discussion
6. Acknowledgments
7. References

### **1. ABSTRACT**

Receptor for advanced glycation end products (RAGE) has been implicated in the pathophysiology of Alzheimer's disease (AD) due to its ability to interact with amyloid beta and to elicit an inflammatory response. sRAGE, one of the splice variants of RAGE, has been reported to be a decoy receptor for amyloid beta peptides. The present study addresses the occurrence of G82S RAGE polymorphism in AD, and its association with the expression of sRAGE and amyloid beta load (A $\beta$  peptide). The results indicated that the heterozygous genotype (GS) was distributed more than the wild genotype (GG) in patients with AD. Moreover, in patients with AD, there was decreased expression of sRAGE and increased expression of tRAGE and TNF- $\alpha$ . The data show that G82S RAGE polymorphism is highly associated with the development of AD, with decreased expression of sRAGE and increased expression of tRAGE and TNF- $\alpha$ .

### **2. INTRODUCTION**

Alzheimer's disease (AD) is the sixth leading cause of death worldwide. In India, about 3.7 million people have AD and it is estimated that by 2030, the incidence will rise by two-fold (1). AD is a complex multistep and multifactorial disease involving genetic and environmental factors. Major pathological features of AD include the presence of senile plaques, neurofibrillary tangles (NFTs), and neuronal loss (2). In addition to the established pathology of senile plaques and NFT, there is a significant oxidative damage in the brains that are involved with AD (3). The pathology in the brain with AD is associated with the overproduction, deposition and failure in clearance of A $\beta$  peptide (4).

Receptor for advanced glycation end products (RAGE) is a transmembrane receptor which binds to multiple ligands namely amyloid (A $\beta$ ), S100, amphoterin, and mac-1 and mediates inflammatory

## Expression of RAGE variants in AD

response. It also acts as a cargo transporter for circulating plasma A $\beta$  across blood brain barrier (BBB) into the brain (5). Activation of full-length RAGE (fRAGE) by A $\beta$  binding, stimulates the expression of  $\beta$  site APP cleaving enzyme through T1 activation leading to enhanced A $\beta$  production (6). RAGE also enhances the production of pro-inflammatory cytokines via the activation of the NF- $\kappa$ B pathway (7).

Within the brain, A $\beta$  is cleared by phagocytosis, interstitial fluid drainage and also by crossing through the BBB to circulation. This is mediated by RAGE and the low-density lipoprotein receptor-related protein (LRP1) (8, 9). LRP1 and p-glycoprotein regulate the efflux of A $\beta$  to circulation from the blood. A $\beta$  is cleared out from systemic circulation by the liver/kidney. Soluble LPR (sLRP) in the blood prevents re-entry of A $\beta$  back into the brain. However, the A $\beta$  peptides also escape the sLRP-mediated mechanism and re-enter the brain through RAGE, further augmenting oxidative stress and neuronal damage (10).

There are about thirty single nucleotide polymorphisms (SNPs) reported to be present in the RAGE gene. The polymorphism in ligand-binding regions of the RAGE could affect the expression and function of RAGE (11). Several epidemiological studies have investigated the association between the G82S polymorphism in the RAGE gene and increased risk of AD development (12, 13, 14, and 15). A splice variant of RAGE termed soluble RAGE (sRAGE) acts as a decoy receptor for A $\beta$  due to the absence of membrane - spanning domain. The present study focuses on analyzing the association of G82S RAGE with AD in south Indian population and to study the expression of RAGE variants and its correlation with A $\beta$  in AD pathology.

## 3. MATERIALS AND METHODS

### 3.1. Study subjects

Individuals with AD (n=40) were recruited from PSG Institute of Medical Sciences and Research (PSG IMS&R), Coimbatore and Alzheimer's and Related Disorders Society of India (ARDSI), Thrissur, India. The present study

was approved by the Institutional Ethics Committee of PSG IMS&R and all procedures involving human subjects were in accordance with the Declaration of Helsinki. Informed consents were obtained from control subjects and from the relatives/legal guardians of the AD patients for their inclusion in the study. The cognitive status of each subject was assessed using Mini-Mental State Examination (MMSE) (16) and the patients were categorized according to the criteria of NINCDS-ADRDA and DSM-IV for dementia (17). AD patients showing co-morbidity with cardiovascular disease and diabetes were excluded from the study. The control subjects (n=40) included in the study were in the age group ranging between 65-85 years with a negative history of cardiovascular disorders, diabetes and neurological disorders. Subjects with habits of smoking or alcohol consumption or having certain advanced malignancies such as AIDS and other major illnesses were excluded from the study. The blood samples (5 ml) from AD subjects and age matched healthy controls were collected individually in EDTA tubes and 4 ml was utilized for separation of plasma, PBMC and RNA. The remaining 1 ml was utilized for DNA isolation.

### 3.2. Quantification of RAGE and TNF- $\alpha$ expression by qPCR

Peripheral blood mononuclear cells (PBMC) were isolated by HiSep LSM density gradient separation (Himedia, Mumbai, India). Total RNA from PBMC was isolated using RNA-XPress™ reagent (Himedia, Mumbai, India). Quality of RNA was analyzed using a nanospectrometer, (Imple, USA). 250 ng of total RNA was then reverse-transcribed using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) and stored at -20° C until further use.

Expression analysis was performed for total RAGE (tRAGE), sRAGE and TNF- $\alpha$  using Quantitative real-time PCR (CFX96 Touch™ Real-Time PCR - Bio Rad) with 2X SYBR Green qPCR master mix (Applied Biosystems, USA). Information regarding the primer sequences used

## Expression of RAGE variants in AD

**Table 1.** Primers sequence used for analysis of RAGE variants by qPCR

Gene	Forward (5'-3')	Primer (5'-3')	Product size (base pair)
Trage	GGGCAGTAGTAGGTGCTCAA	TCCGGCCTGTGTTTCAGTTTC	120
Srage	AGCATCATCGAACCAGGCGA	TTTTCTGGGGCCTCCATTC	134
TNF- $\alpha$	CCCCAGGGACCTCTCTAATC	GGTTTGCTACAACATGGGCTACA	98
GAPDH	TGCACCACCAACTGCTTAG	GGATGCAGGGATGATGTTC	177

for real-time PCR analysis can be found in Table 1. The primers for tRAGE were designed with reference to the fRAGE gene (GenBank ID – NM001136) using Primer-Blast tool ensuring that it was comprised the exon-exon junction to avoid amplification of contaminating genomic DNA. On the other hand, the primers for soluble RAGE (containing intron 9) were designed to have an exon-intron junction and an exon-exon junction, ensuring it amplified all the prominent soluble isoforms (Figure 1). Though primers used for tRAGE can amplify other variants excluding sRAGE, the canonical variant fRAGE has been reported to be the major variant (70% to 80%) expressed in human tissues (18).

The PCR conditions were used as follows: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 10s, and 60°C for 1 min. Relative quantification of gene transcripts was calculated using  $2^{-\Delta\Delta Ct}$  method and normalized to Glyceraldehyde-3-phosphate dehydrogenase (GADPH) transcripts.

### 3.3. Quantification of A $\beta$ 42 and sRAGE in plasma

Samples of blood collected in EDTA tubes were centrifuged at 400 g for 15 minutes to separate plasma, which was stored at -20 °C in aliquots till use. A $\beta$ 42 and sRAGE were quantified in plasma using a commercially available ELISA kit from CUSABIO (CSB-E10684h) and Quantikine R&D systems (DRG00, USA), respectively, according to the manufacturer's instructions.

### 3.4. G82S RAGE polymorphism

The Polymerase chain reaction – restriction fragment length polymorphism (PCR- RFLP) was designed to identify G82S RAGE polymorphism. The genomic DNA was isolated using Xpress DNA kit

(Magenome, India), according to the manufacturer's instruction. The PCR was carried out in EmeraldAmp® PCR Master Mix (Takara, Japan) with genomic DNA (200 ng) and 10  $\mu$ M of forward (GTCCCTCACCCACCCCTGTC) and reverse primers (GCCACACACCCACCCAC) for amplification of exon 3 region of RAGE gene containing G82S polymorphism. The amplified product (817 bp) was subjected to restriction digestion by *Alu I* (5 units) for 2h at 37 °C followed by electrophoresis on 2% agarose gel.

### 3.5. Statistical analysis

Statistical analysis was performed using the statistical package SPSS Version.16, Chicago, USA. An independent sample t-test was used to compare cases between AD and control. Receiver operating characteristic (ROC) curve analysis was performed to study the reliability of sRAGE in plasma as marker for AD diagnosis. Pearson's correlation was done wherever applicable. For genotype and allelic frequency, the level of significance was analyzed using chi-squared test. The disease relationship with genotype and allelic frequencies is presented as an odds ratio with 95% confidence interval.

## 4. RESULTS

The baseline clinical characteristics of the study groups (AD and control) are shown in Table 2. The MMSE score in AD group indicated severe cognitive dysfunction. The plasma A $\beta$  42 level was found to be significantly higher in the AD group compared to controls ( $p < 0.001$ ).

### 4.1. Analysis of expression of RAGE variants and TNF- $\alpha$ in PBMC

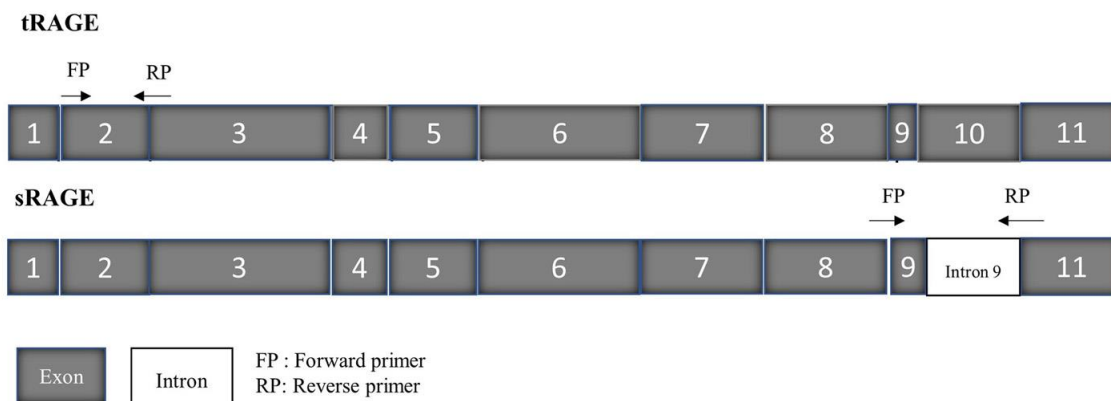
Expression of tRAGE, sRAGE and TNF- $\alpha$  were quantified using qPCR with gene specific primers. To analyze mRNA expression of RAGE

## Expression of RAGE variants in AD

**Table 2.** Clinical characteristics of study groups

Parameters	Alzheimer's patients	Control subjects
Number of subjects	40	40
Sex (W/M)	23/17	15/25
Age (Years)	74.45 ± 0.95 <sup>b</sup>	70.4 ± 1.4 <sup>b</sup>
MMSE <sup>a</sup>	2.0 ± 0.28 <sup>b</sup>	25.9 ± 0.32 <sup>b</sup>
Aβ42 (pg/ml)	191.57 ± 9.5 <sup>b</sup>	88.48 ± 6.7 <sup>b</sup>

Abbreviations: W = Women, M = Men; <sup>a</sup> Mini Mental State Examination (MMSE) score = Profound 0-4, Severe 4-13, Moderate 13-16, Mild 17-23, Normal 24-30; <sup>b</sup> Mean ± SEM.



**Figure 1.** Schematic representation of qPCR primer designed to amplify tRAGE and sRAGE variants. RAGE gene exons, intron; Forward primer: FP, Reverse primer: RP.

variants, the comparative threshold cycle (Ct) method was applied with GAPDH as the endogenous reference gene. The first, synthesized strand cDNA was verified by the amplification of GAPDH. Melt curve analysis of sRAGE, tRAGE and TNF- $\alpha$  revealed a single peak which represents a single amplicon and specific amplification (Figure 2A). The results revealed that while the expression sRAGE was down regulated by 3.4-fold, tRAGE expression was upregulated by 1.9-fold in AD samples compared to controls. There was also an upregulation in the expression of TNF- $\alpha$  (2.2 fold) in the AD group associated with lower expression of sRAGE in AD (Figure 2B, Table 3).

### 4.2. Quantification of sRAGE and A $\beta$ 42 in plasma samples

The levels of sRAGE in plasma was significantly lower in patients AD (151.91±15.9

pg/mL,  $p < .001$ ) as compared to the levels found in cognitively healthy age matched control (753.79±58.12 pg/mL) (Figure 3A). The plasma A $\beta$ 42 level was found to be higher in AD (191.57 ± 9.5 pg/ml,  $p < .0001$ ) compared to controls (88.48 ± 6.7 pg/ml) Table 4. The correlation analysis between plasma sRAGE and A $\beta$  levels has shown no significant relation (AD R = 0.122; age matched healthy control subjects R = -0.080) (Figure 3B). ROC curve analysis was conducted to assess the cutoff points of sRAGE to differentiate the AD group from the controls. The area under the curve was found to be 0.981, indicating good diagnostic accuracy for sRAGE with a cutoff value of  $\leq 278.09$  pg/ml (Sensitivity 100 %, Specificity 85%) suggesting that plasma sRAGE could be used to distinguish AD from controls ( $p = 0.001$ ; 95% CI, 0.960 – 1.00, Figure 3C).

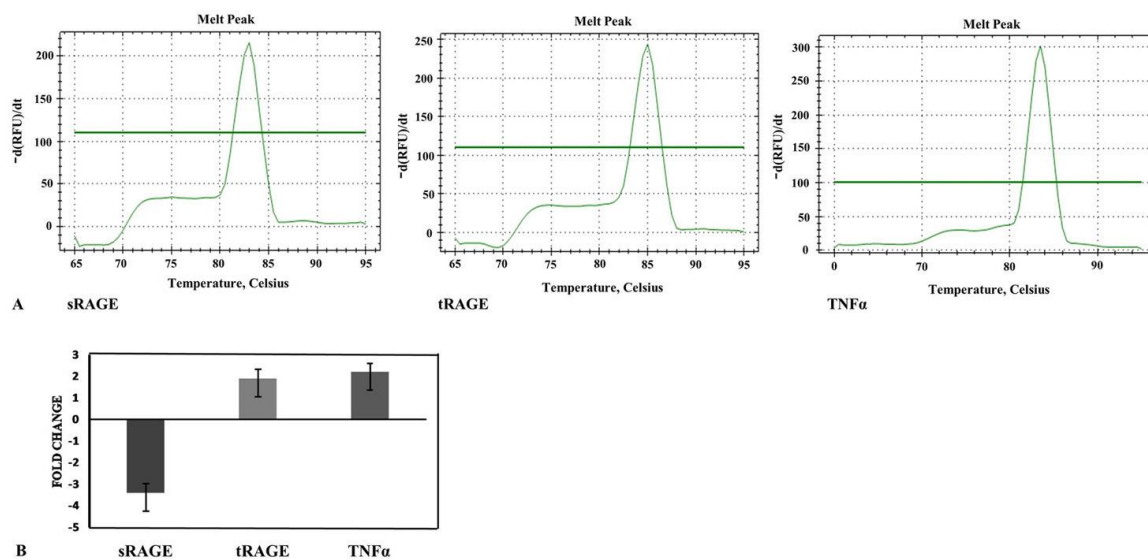
### 4.3. Analysis of RAGE G82S polymorphism

In the present study PCR-RFLP was adopted to analyze the G82S polymorphism.

## Expression of RAGE variants in AD

**Table 3.** The expression levels of tRAGE, sRAGE and TNF- $\alpha$  mRNA

Gene	Values in AD compared to age matched healthy controls
Trage	1.9-fold
Srage	3.4-fold
TNF- $\alpha$	2.2-fold



**Figure 2.** Expression profile of RAGE variants and TNF- $\alpha$ . (A) Melt curves from qPCR for sRAGE, tRAGE and TNF- $\alpha$ . (B) Analysis of RAGE variants and TNF- $\alpha$  mRNA expression in PBMC. Bar graph depicts mean gene expression levels and error bars represents the standard error mean obtained from the average of 40 nos. Expression values are obtained by  $2^{-\Delta\Delta CT}$ .

Information on the primers used, nucleotide sequence of PCR products and *AluI* restriction site in the PCR product are all represented in Figure 4A. The representative gel image of six samples after digestion of the PCR-amplified products (Figure 4B) and schematic pattern of RFLP after digestion with *AluI* enzyme can be found in Figure 4C. The distribution of genotype was in accordance with the Hardy–Weinberg equilibrium. The genotypic and allelic frequency of G82S RAGE polymorphism in AD and control group along with the resulting odds ratio and significance levels is represented in Table 5.

The S82S genotype was not observed in both groups. Allelic frequency of S allele in the AD and control group was 0.225 and 0.037 respectively, whereas for G allele, it was 0.775 and 0.963 respectively. The observed difference in genotype distribution and allelic frequency between controls and the AD group was

statistically significant ( $\chi^2$  test,  $p < 0.05$ ) (Table 5). Reduced sRAGE in subjects with GS genotype was also observed compared to GG genotype and the results were statistically significant (Figure 5).

## 5. DISCUSSION

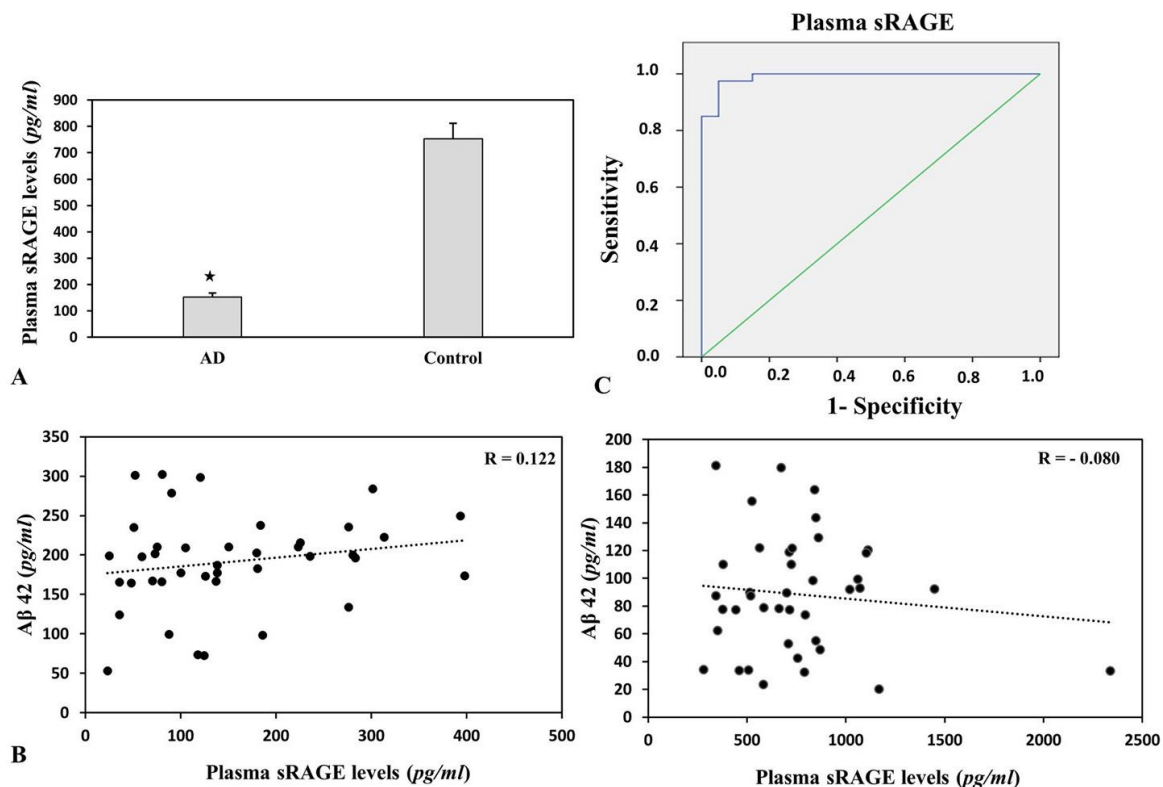
In the present study, the differential expression of tRAGE and sRAGE was analyzed in PBMC of AD and age matched control. The upregulation of tRAGE and down regulation of sRAGE in AD patient highlights the role of RAGE variants in AD pathogenesis. sRAGE being the circulatory form, was analyzed in plasma, also indicated down regulation of its expression and this was correlated with increased A $\beta$  load in AD. The graphical representation of these results represented in Figure 6.

The risk association of G82S carrier with

## Expression of RAGE variants in AD

**Table 4.** Plasma levels of A $\beta$ 42 and sRAGE

Marker	Plasma levels	
	Age matched healthy controls (pg/mL)	Individuals with AD (pg/mL)
A $\beta$ 42	88.48 $\pm$ 6.7	191.57 $\pm$ 9.5
Srage	753.79 $\pm$ 58.12	151.91 $\pm$ 15.9



**Figure 3.** Plasma sRAGE levels in AD. (A) Plasma sRAGE in AD and control. The values are shown as mean  $\pm$  SEM and were found to be statistically significant compared to control, ( $p < 0.001$ ). (B) The correlation between concentration of sRAGE and A $\beta$ 42 (i) AD ( $R = 0.122$ ) (ii) controls ( $R = -0.080$ ). (C) Receiver operating characteristic curve analysis of plasma sRAGE: AUC: 0.981 ( $p < 0.001$ ), cutoff value of  $\leq 278.09$  (Sensitivity 100 %, Specificity 85%). The quality of diagnosis test is determined by AUC: 0.9-1 excellent, 0.8-0.9 good, 0.7-0.8 fair, 0.6-0.7 poor, 0.5-0.6 fail.

AD was analyzed in South Indian population. The frequency of occurrence of 82S risk allele (heterozygous form) is higher in AD compared to age matched control indicating that 82S RAGE allele is probably the risk factor for AD. Association of G82S polymorphism with AD is also reported in Chinese (12, 13), Korean (14) and Turkish population (15). sRAGE by binding to circulating A $\beta$  in the plasma regulates A $\beta$  equilibrium between brain and peripheral circulation thus enhanced A $\beta$  clearance (19). The ELISA and qPCR analysis of RAGE

variants showed significant decrease in sRAGE in plasma along with an increase in tRAGE in PBMC in AD compared to controls. Both sRAGE and sLRP was found to be decreased in Chinese population (20,21). Reduced sRAGE might interfere with the clearance of A $\beta$ . The decreased level of sRAGE could be responsible for increased load of A $\beta$  observed in plasma of AD patients and ROC analysis supports the use of plasma sRAGE as a diagnostic marker for identifying the cases of AD with sensitivity and specificity of 100 % and 85% respectively. The

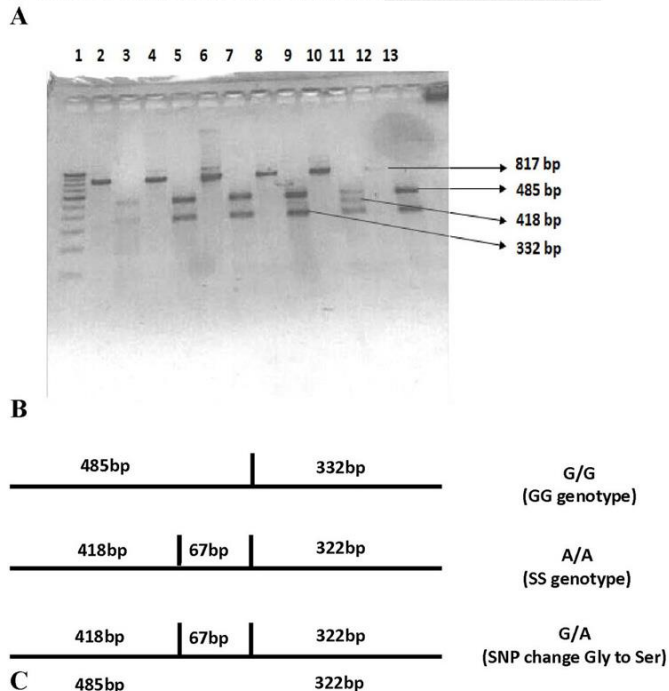


## Expression of RAGE variants in AD

```

GTCCCTCACCCACCCTGTCCAAATTTTGTAGCCCTCATTCCCTTCTACCCCTCTACCATGGTGCTATCTCCAGG
GGCAGTAGTAGGTGCTCAAAACATCACAGCCCGGATGGCGAGCCACTGGTGCTGAAGTGTAAAGGGGCCCAAGA
AACCACCCAGCGGCTGGAATGGAACCTGGTAAGCGGGGCTCCTGTTGCAGCCTCCCACTTCCAGGGAGACCAGCA
ATGATTTGGATCCCCGCTCACTCTGCCTCACAGTCCTTCCCAAAGGCCCTGCACTGTTTAGGCCCTGCTTCTCTGCT
TCTAGAACACAGGCCGGACAGAGCTGGGAAGGTCCCTGCTTCCCAAGGGA
GGAGGCCCTGGGACAGTGTGGCTCGTGTCTTCCCAACAGCTCCCTCTT
CCTTCCGGCTGTCGGGATCCAGGATGAGGGGATTTCCGGTGCCAGGCAAT
GAACAGGAATGAAAGGAGACCAAGTCCAACTACCGAGTCCGTGTCTACCG
TAAGAATTCCAGGGTCTTCTCCAAGGCCCTCCCTCTTACCTAAGAAAAAGCC
TTCACCCAGCCTTGGCCCATGAGGGCCTCTGACTTCCACTGGCCTCATT
TCCACACACAGAGTTTGAGAACCTTCACAATTACAGCCTCTGACTGGATTT
TTCCTCCTCAGAGATTCCTGGGAAGCCAGAAATTGTAGATTCTGCCTCTG
AGCTCAGGCTGGTGTTCCTCAATAAGGTAGTGGAAGAAAGCAGGAGAAGTA
GAAAACGGCCCTGTGAACAGGAGCGAGTGTGTGGGTGTGGGTGTGTGGC
  
```

GGC → Glycine (G)  
 AGC → Serine (S)  
 AluI site AGCT  
 G82S

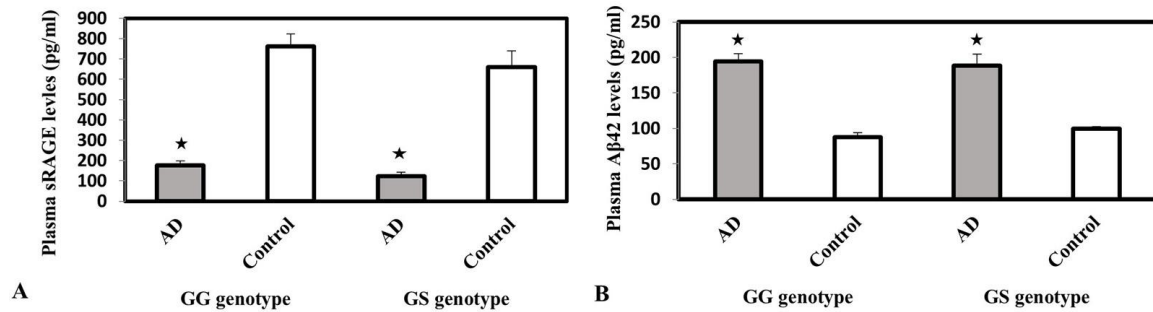


**Figure 4.** G82S RAGE Polymorphism. (A) Nucleotide sequence of the PCR-amplified RAGE gene showing the restriction site of *AluI* that are highlighted in grey and yellow color and indicated by arrow. The yellow color represents the restriction site that occurred due to single nucleotide change from G to A that shifted Gly 82 to Ser. Primer sequences are highlighted in gray. (B) PCR RFLP results of G82S RAGE gene polymorphism. PCR product: Lane - 2,4,6,8,10,12; Restricted samples (*AluI* digest): Lane 3,5,7,9,13- Genotype GG, Lane 11 - Genotype GS, Lane 1- 100 bp Ladder. (C) Restriction pattern of RAGE for homozygous GG and SS and heterozygous GS. After *AluI* digestion, the G82G genotype of RAGE gives two fragments and S82S genotype gives three fragments. Due to single nucleotide polymorphism, the heterozygous GS allele gives four fragments.

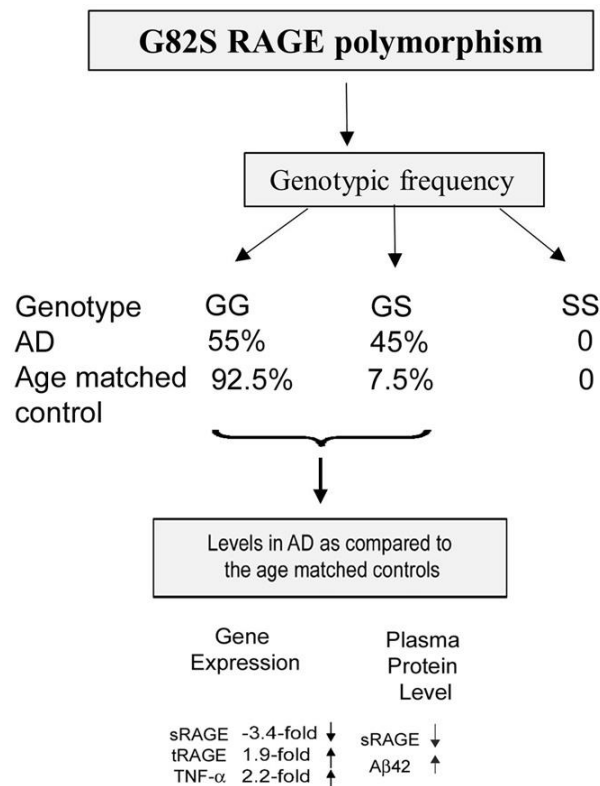
observed increase in fRAGE expression may be due to the consequences of A $\beta$  overload, since it has been reported that A $\beta$  toxicity induces fRAGE expression leading to further oxidative damage. While plasma TNF- $\alpha$  was shown to be enhanced during aging (22) the observed increase in TNF- $\alpha$  (2.2-fold) levels in AD compared to age matched healthy controls indicate the association of TNF- $\alpha$  in AD probably due to its effect as an early mediator of acute phase response (2,12, 23, 24).

sRAGE was decreased in G82S RAGE genotype in both AD and control subjects compared to the G82G genotype. The decreased sRAGE observed in plasma could be due to alteration in N81 glycosylation of RAGE caused by G82S polymorphism which makes it more susceptible for metalloproteases (25) and also due to alteration in transcription and expression of RAGE gene. Park *et al.* had confirmed that the Asn<sup>81</sup> site is fully glycosylated in G82S RAGE with significant amounts

## Expression of RAGE variants in AD



**Figure 5.** Association of G82S RAGE genotype on sRAGE and Aβ42 expression in AD. (A) sRAGE in different RAGE genotypes. It is found that the expression of sRAGE was reduced in AD, which is further reduced in G82S RAGE genotype compared to G82G RAGE in AD and control. (B) Aβ42 levels in different RAGE genotypes. The expression of Aβ42 was observed to be high in AD in both genotypes compared to control. The values are shown as mean ± SEM. The effects of G82S polymorphism on the levels of sRAGE and Aβ42 expression in AD were found to be statistically significant with the p value,  $p < 0.0001$



**Figure 6.** The G82S RAGE polymorphism showing genotypic frequency, and levels of sRAGE, tRAGE, TNF-α. Arrows point to up or downregulation.

of *N*-linked glycan, proved through the combination of MS analysis and PNGase-F digestion (26). Asn<sup>81</sup> is located in close proximity to a hydrophobic cavity in the RAGE V-domain. Hydrophobic interactions involving residues within this cavity are critical for

RAGE- S100B protein ligand binding (27) and are those most likely to be influenced by Asn<sup>81</sup> glycosylation. The effect of *N*-linked glycosylation on the outcome of the RAGE-S100B ligand interaction was confirmed through measures of NF-κB



## Expression of RAGE variants in AD

activation. Based on these studies, the change in the structure and conformation of RAGE due to alteration in glycosylation in G82S RAGE polymorphism has been hypothesized for RAGE- A $\beta$  interaction as well. Higher level of 82S risk allele with lower levels of sRAGE would further favor the formation of A $\beta$  fibrils significantly based on the concept that Chaney *et al.* (2005) (28) established the abolishment of A $\beta$  filamentous aggregation, when A $\beta$  peptide is incubated with sRAGE for longer time. A longitudinal study with the incorporation of MCI to AD is needed to use the circulating sRAGE as a preliminary marker for AD diagnosis.

## 6. ACKNOWLEDGMENTS

The authors thank Dr. Pranesh, PSG IMSR, Coimbatore, India and Mr. R. Narendhar, ARDSI, Cochin, India, for their kind support and valuable input while conducting this study. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. This study was carried out in accordance with the recommendation from the Institutional Ethical Committee, PSG Institute of Medical Science and Research (PSG IMS&R), Coimbatore. All subjects provided written informed consent in accordance with the Declaration of Helsinki. The study was supported by the Department of Science and Technology (DST), Ministry of Science and Technology, New Delhi, India. SR/WOS-A/LS-1312/2014 (G). We acknowledge the Editing Refinery, USA, for their editing service of this manuscript.

## 7. REFERENCES

1. Dementia India report, <http://www.ardsi.org/downloads/ExecutiveSummary.pdf> (2010)
2. D Allan Butterfield, Jennifer Drake, Chava Pocernich and Alessandra Castegna: Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid  $\beta$ -peptide. *Trends Mol Med* 7(12) 548-54 (2001)

DOI: 10.1016/S1471-4914(01)02173-6

3. Lawrence M Sayre, George Perry, Peggy L R Harris, Yahua Liu, Kathryn A Schubert and Mark A Smith: In situ oxidative catalysis by neurofibrillary tangles and senile plaques in Alzheimer's disease: a central role for bound transition metals. *J Neurochem* 74(1), 270-79 (2000)  
DOI: 10.1046/j.1471-4159.2000.074-0270.x
4. Rani Palanisway, Sreeram Krishnan and Rani Cathrine Chellappa: Study on Analysis of Peripheral Biomarkers for Alzheimer's Disease Diagnosis. *Front Neurol* 8, (2017)  
DOI: 10.3389/fneur.2017.00328
5. Rashid Deane, Shi Du Yan, Ram Kumar Submamaryan, Barbara LaRue, Suzana Jovanovic, Elizabeth Hogg, Deborah Welch, Lawrence Manness, Chang Lin, Jin Yu, Hong Zhu, Jorge Ghiso, Blas Frangione, Alan Stern, Ann Marie Schmidt, Don L Armstrong, Bernd Arnold, Birgit Liliensiek, Peter Nawroth, Florence Hofman, Mark Kindy, David Stern and Berislav Zlokovic: RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. *Nat Med* 9, 907-13 (2003)  
DOI: 10.1038/nm890
6. Cho HJ, SM Son, SM Jin, H. S. Hong, D. H. Shin, S. J. Kim, K. Huh, and I. Mook-Jung: RAGE regulates BACE1 and A $\beta$  generation via NFAT1 activation in Alzheimer's disease animal model. *FASEB J* 23, 2639-49 (2009)  
DOI: 10.1096/fj.08-126383
7. Granic Ivica, Amalia M Dolga, Ingrid M Nijholt, Gertjan van Dijk, and Ulrich LM Eisel: Inflammation and NF- $\kappa$ B in

- Alzheimer's Disease and Diabetes. *J Alzheimers Dis* 16(4), 809-821(2009)  
DOI: 10.3233/JAD-2009-0976
8. Shibata, Masayoshi, Shinya Yamada, S. Ram Kumar, Miguel Calero, James Bading, Blas Frangione, David M. Holtzman, Carol A. Miller, Dudley K. Strickland, Jorge Ghiso, and Berislav V. Zlokovic: Clearance of Alzheimer's amyloid- $\beta$ 1-40 peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J Clin Investig* 106, 1489-99 (2000)  
DOI: 10.1172/JC110498
  9. Deane Rashid, and Berislav V. Zlokovic: Role of blood brain barrier in the pathogenesis of Alzheimer's disease. *Curr Alzheimer Res* 4, 191-7 (2007)  
DOI: 10.2174/156720507780362245
  10. Sagare, Abhay, Rashid Deane, Robert D. Bell, Bradley Johnson, Katie Hamm, Ronan Pendu, Andrew Marky, Peter J Lenting, Zhenhua Wu, Troy Zarcone, Alison Goate, Kevin Mayo, David Perlmutter, Mireia Coma, Zhihui Zhong and Berislav V Zlokovic: Clearance of Alzheimer's amyloid- $\beta$  with circulating lipoprotein receptors. *Nat Med* 13, 1029-31(2007)  
DOI: 10.1038/nm1635
  11. M A Hofmann, S Drury, B I Hudson, M R Gleason, W Qu, Y Lu, E Lalla, S Chitnis, J Monteiro, M H Stickland, L G Bucciarelli, B Moser, G Moxley, S Itescu, P J Grant, P K Gregersen, D M Stern and A M Schmidt: RAGE and arthritis: the G82S polymorphism amplifies the inflammatory response. *Genes Immun* 3(3), 123-35 (2002)  
DOI: 10.1038/sj.gene.6363861
  12. Li Keshen, Dawei Dai, Bin Zhao, Lifan Yao, Songpo Yao, Binyou Wang, and Ze Yang: Association between the RAGE G82S polymorphism and Alzheimer's disease. *J Neural Transm* 117, 97-104 (2010)  
DOI: 10.1007/s00702-009-0334-6
  13. Daborg Jonny, Malin von Otter, Annica Sjolander, Lennart Minthon, Deborah R. Gustafson, Ingmar Skoog, Eric Hanse, Kaj Blennow and Henrik Zetterberg: Association of the RAGE G82S polymorphism with Alzheimer's disease. *J Neural Transm* 117(7), 861-67 (2010)  
DOI: 10.1007/s00702-010-0437-0
  14. Jang Yangsoo, Ji Young Kim, Seok-Min Kang, Jung-Sun Kim, Jey Sook Chae, Oh Yoen Kim, Soo Jeong Koh, Hyun Chul Lee, Chul WooAhn, Young DukSong, and Jong HoLee: Association of the Gly82Ser polymorphism in the receptor for advanced glycation end products (RAGE) gene with circulating levels of soluble RAGE and inflammatory markers in nondiabetic and nonobese Koreans. *Metabolism* 56(2), 199-205 (2007)  
DOI: 10.1016/j.metabol.2006.09.013
  15. Atac Zehra Simin, Merve Alaylıoğlu, Erdinç Dursun, Duygu Gezen-Ak, Selma Yılmaz, and Hakan Gürvit: G82S polymorphism of receptor for advanced glycation end products gene and serum soluble RAGE levels in mild cognitive impairment and dementia of Alzheimer's type patients in Turkish population. *J Clin Neurosci* 59, 197-201 (2019)  
DOI: 10.1016/j.jocn.2018.10.072
  16. Marshal F. Folstein, Susan E. Folstein, and Paul R. McHugh: Mini-mental state: a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 12, 189-98 (1975)

- DOI: 10.1016/0022-3956(75)90026-6
17. McKhann Guy, David Drachman, Marshall Folstein, Robert Katzman, Donald Price, and Emanuel M. Stadlan: Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 34, 939-44 (1984)  
DOI: 10.1212/WNL.34.7.939
  18. Srikanth Velandai, Annette Maczurek, Thanh Phan, Megan Steele, Bernadette Westcott, Damian Juskiw, and Gerald Münch: Advanced glycation endproducts and their receptor RAGE in Alzheimers disease. *Neurobiol Aging* 32(5), 763-77 (2011)  
DOI: 10.1016/j.neurobiolaging.2009-.04.016
  19. Yan, Shirley ShiDu, Doris Chen, Shiqian Yan, Lan Guo, and John Xi Chen: RAGE is a key cellular target for A $\beta$ -induced perturbation in Alzheimer's disease. *Front Biosci (Schol Ed)* 4, 240 (2012)  
DOI: 10.2741/s265
  20. Xiao-Yan Xu, Chang-Qing Deng, Jian Wang, Xiao-Juan Deng, Qian Xiao, Yuan Li, Qi He, Wen-Hui Fan, Feng-Ying Quan, Yao-Ping Zhu, Ping Cheng and Guo-Jun Chen: Plasma levels of soluble receptor for advanced glycation end products in Alzheimers disease. *Int J Neurosci* 127, 454-58 (2016)  
DOI: 10.1080/00207454.2016.1193861
  21. Liang, Furu, Jianping Jia, Shuying Wang, Wei Qin, and Guorong Liu: Decreased plasma levels of soluble low densitylipoprotein receptor-related protein-1 (sLRP) and the soluble form of the receptor for advanced glycation end products (sRAGE) in the clinical diagnosis of Alzheimer's disease. *J Clin Neurosci* 20, 357-61 (2013)  
DOI: 10.1016/j.jocn.2012.06.005
  22. Bruunsgaard H, Skinhøj P, Pedersen AN, Schroll M, and Pedersen BK: Ageing, tumour necrosis factor- alpha (TNF-  $\alpha$ ) and atherosclerosis. *Clin Exp Immunol* 121(2), 255-60 (2000)  
DOI: 10.1046/j.1365-2249.2000.01281.x
  23. Cacquevel M, Lebeurrier N, Chéenne S, and Vivien D: Cytokines in neuro-inflammation and Alzheimer's disease. *Curr Drug Targets* 5(6), 529-34 (2004)  
DOI: 10.2174/1389450043345308
  24. Tanaka N, Yonekura H, Yamagishi SI, Fujimori H, Yamamoto Y, and Yamamoto H: The receptor for advanced glycation end products is induced by the glycation products themselves and tumor necrosis factor- $\alpha$  through nuclear factor- $\kappa$ B, and by 17 $\beta$ -estradiol through Sp-1 in human vascular endothelial cells. *J BIOL CHEM* 275(33), 25781-90 (2000).  
DOI: 10.1074/jbc.M001235200
  25. Raucci, Angela, Simona Cugusi, Antonella Antonelli, Silvia M. Barabino, Lucilla Monti, Angelika Bierhaus, Karina Reiss, Paul Saftig, and Marco E. Bianchi: A soluble form of the receptor for advanced glycation endproducts (RAGE) is produced by proteolytic cleavage of the membrane-bound form by the sheddase a disintegrin and metalloprotease 10 (ADAM10). *FASEB J* 22, 3716-27 (2008)  
DOI: 10.1096/fj.08-109033
  26. Park, Sun Jin, Torsten Kleffmann, and Paul A Hessian: The G82S polymorphism promotes glycosylation of the receptor for

## Expression of RAGE variants in AD

advanced glycation end products (RAGE) at asparagine 81 comparison of wild-type rage with the g82s polymorphic variant. *J Biol Chem* 286 (24), 21384-92 (2011)  
DOI: 10.1074/jbc.M111.241281

27. Park H, Boyington JC: The 1.5 Å crystal structure of human receptor for advanced glycation endproducts (RAGE) ectodomains reveals unique features determining ligand binding. *J Biol Chem* 285(52), 40762-70 (2010)  
DOI: 10.1074/jbc.M110.169276
28. Chaney MO, Stine WB, Kokjohn TA, Kuo YM, Esh C, Rahman A, Luehrs DC, Schmidt AM, Stern D, Du Yan S and Roher AE: RAGE and amyloid beta interactions: atomic force microscopy and molecular modeling. *Biochim Biophys Acta Mol Basis Dis* 1741(1-2), 199-205 (2005)  
DOI: 10.1016/j.bbadis.2005.03.014

**Abbreviations:** AD: Alzheimer's disease; RAGE: Receptor for advanced glycation end products; A $\beta$  42: Amyloid peptide 1-42; CSF: Cerebral spinal fluid; BBB: Blood brain barrier; LRP: low-density lipoprotein receptor-related protein, sRAGE: soluble receptor for advanced glycation end products, tRAGE: total receptor for advanced glycation end product, TNF- $\alpha$ : tumor necrosis factor alpha, A $\beta$ 42: amyloid peptide

**Key Words:** Amyloid toxicity, oxidative stress, RAGE, sRAGE, polymorphism, AD

**Send correspondence to:** Palaniswamy Rani, Department of Biotechnology, PSG College of Technology, Peelamedu, Coimbatore, Tamil Nadu, India, Tel: 91 9443161653, Fax:0422-2573833, E-mail: rani.bio@psgtech.ac.in