Brief Genetics Report

Identification of Polymorphisms in the Receptor for Advanced Glycation End Products (RAGE) Gene

Prevalence in Type 2 Diabetes and Ethnic Groups

B.I. Hudson, M.H. Stickland, and P.J. Grant

The continued exposure to hyperglycemia is now established to be the major cause of diabetic vascular complications (1,2). Evidence indicates that nonenzymatic glycation of proteins or lipids leading to the formation of advanced glycation end products (AGEs) may be the underlying mechanism (1,2). The AGEs are a heterogeneous group of compounds that cause a plethora of adverse effects, including reduction of enzymatic activity, damage to nucleic acids, impaired degradation of proteins, cross-linking of proteins, and induction of cytotoxic pathways. Evidence to indicate the role of AGEs in diabetic complications is supported by the inhibitory effects of aminoguanidine, which prevents the formation and cross-linking of AGEs and reduces the severity of diabetic complications in animal models (2).

The effects of AGEs have been shown to be partially mediated via cellular receptors, the most defined to date being the receptor for AGEs (RAGE). RAGE is a 35-kDa polypeptide of the immunoglobulin superfamily of receptors located on chromosome 6 in the HLA region (3) and is complexed with a lactoferrin-like polypeptide, which together bind, internalize, and trancytose AGEs to the subendothelium (4). Binding of AGEs to RAGE has been shown to induce multiple effects, resulting in oxidative stress and cellular dysfunction and bringing about the generation of oxygen free radicals and the cellular activation of NF-κB (5), an oxidative stress marker. NF-κB, in turn, induces expression of procoagulant tissue factor (6), the vasoconstrictive endothelin-1 (7), and increased levels of vascular cellular adhesion molecule-1, an early marker of atherosclerosis (8).

From the Unit of Molecular Vascular Medicine, Leeds General Infirmary, University of Leeds, Leeds, U.K.

Address correspondence and reprint requests to Barry I. Hudson, Unit of Molecular Vascular Medicine, Research School of Medicine, G Floor, Martin Wing, Leeds General Infirmary, Leeds, LS1 3EX, U.K. E-mail: medbh@medphysics.leeds.ac.uk.

Received for publication 22 October 1997 and accepted in revised form 2 March 1998.

Additional information can be found in an on-line appendix at www.diabetes.org/diabetes/appendix.htm.

AGE, advanced glycation end product; PCR, polymerase chain reaction; RAGE, receptor for advanced glycation end products; SSCP, single-strand conformation polymorphism.

Studies indicate that susceptibility to diabetic complications may be influenced by a genetic factor because environmental influences do not appear to explain all the variation in presentation of vascular disorders (9). Sequence variants within the RAGE gene may influence development of complications by altering the AGE-RAGE interaction. To identify candidates for this process, polymorphisms in the coding region of RAGE were screened by polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP).

PCRs were carried out with overlapping primers, designed to span each exon of RAGE. DNA from 40 random blood donors and 40 random type 2 diabetes patients was amplified by PCR, and SSCP was then conducted under different running conditions to maximize mutation detection. All subject information and methods are contained in the on-line appendix at www.diabetes.org/diabetes/appendix.htm. SSCP analysis revealed different allelic fragments in five regions, with the addition of glycerol being required to detect two polymorphisms in exon 10, further demonstrating the requirement of multiple running conditions for effective mutation detection. All allelic fragments were confirmed as genuine nucleotide substitutions by automated nucleotide cycle sequencing, of which seven were located in exons and two occurred in introns (Fig. 1*A*). Four functional amino acid changes were detected in total: Gly82Ser (exon 3), Thr187Pro (exon 6), Gly329Arg (exon 8), and Arg389GIn (exon 10). With the exception of the glycine–to–serine polymorphism at codon 82, all other substitutions resulting in functional amino acid changes were only detected once, and therefore only Gly82Ser was considered further because of its relatively high prevalence. Gly82Ser polymorphism was found to result in the formation of an *Alu*I restriction site (AG↑CT) and thus facilitated screening by PCR–restriction fragment length polymorphism (Fig. 1*B* and *C*). However, the observed digestion pattern did not match the predicted one when using the genomic sequence published by Sugaya et al. (3) and further sequencing revealed that nucleotide number 7128 (according to the nucleotide sequence of RAGE, accession number D28769, Genbank) was in fact a T, not an A, resulting in the loss of an *Alu*I restriction site (Fig. 1*B*).

To assess any possible significance of the Gly82Ser polymorphism, we examined the genotype frequency between different ethnic groups and in relation to macrovascular disease of diabetic patients. There were 196 Caucasian (mean age 58.5

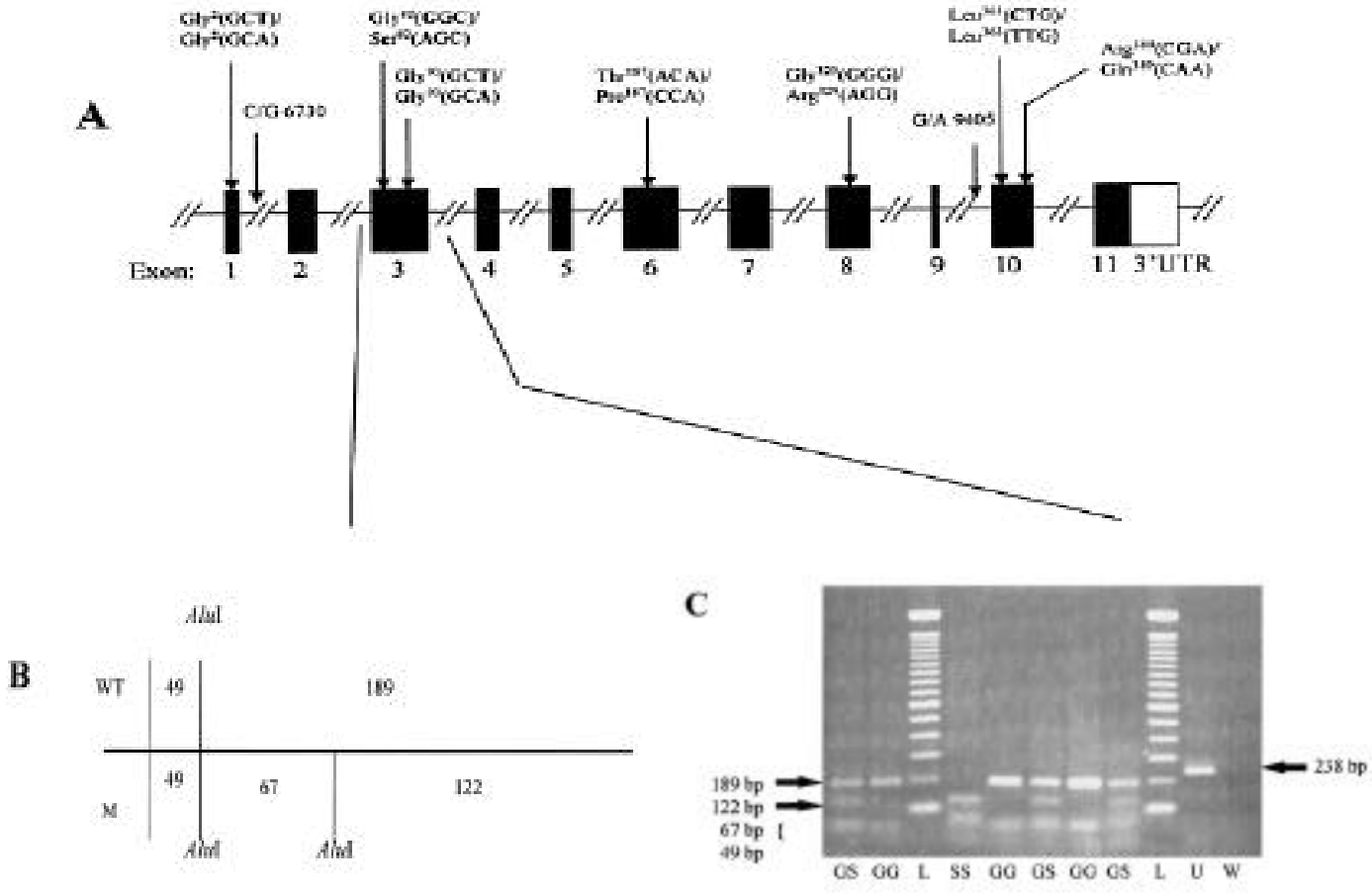

FIG. 1. *A:* **Diagrammatic representation of the polymorphisms identified within the coding region of RAGE. Sequence changes in exons (boxes) are highlighted in bold, with codon number and amino acid change. Intron (lines) sequence changes are shown with the nucleotide number.** *B:* **Restriction map of the PCR product of exon 3. The dotted line represents an *Alu*I restriction site that exists in the published sequence of RAGE by Sugaya et al. (3) but was found to be absent by sequencing. M, mutant digestion pattern; WT, wild-type digestion pattern.** *C:* **Restriction digest of the exon 3 PCR product with *Alu*I, separated on an agarose gel. *Lane GS* represents a heterozygote for the Gly82 and Ser82 allele, *lane GG* represents a wild type for the Gly82 allele, *lane L* is a 100–base pair (bp) ladder, *lane SS* represents a homozygote for the Ser82 allele, *lane U* is the undigested PCR product, and *lane W* is a water blank.**

years) and 156 Asian (mean age 44.3 years) control subjects who were recruited and defined as healthy individuals with no history of vascular disease; 258 type 2 diabetic subjects (mean age 62.6 years) were also recruited and diagnosed according to World Health Organization criteria.

Genotype and allele frequencies were not significantly different between the Asian (90% GG, 10% GS, 0% SS) and Caucasian (87% GG, 12% GS, 1% SS) nondiabetic control subjects ($P > 0.05$), with allele frequencies of 94 and 92% for the Gly allele, respectively. In addition, there was no difference in genotype (92% GG, 7% GS, 0% SS) or allele (96% Gly, 4% Ser) frequencies between the type 2 diabetic patients and the Caucasian control subjects. Genotype and allele frequencies did not differ in the type 2 diabetic subjects with (93% GG, 7% GS, 0% SS) or without (92% GG, 8% GS, 0% SS) macrovascular disease ($P > 0.05$), with allele frequencies of 97 and 95% for the Gly allele, respectively. None of the genotype frequencies differed significantly from Hardy-Weinberg equilibrium ($P > 0.05$).

Additionally, the effect of the Gly82Ser polymorphism on receptor function is of interest. This polymorphism occurs at a predicted N-linked glycosylation site and in the same immunoglobulin variable domain as the AGE binding site, but because the tertiary structure of RAGE is unknown, it is impossible to predict any structural effects of this polymorphism.

It also remains possible that polymorphisms in the promoter region could be implicated in contributing to the vascular complications of diabetes, as evidenced by the increased expression of RAGE observed in these pathological settings (10). We are currently investigating these possibilities in further studies of AGE-RAGE interactions and RAGE promoter functions. Because of its location in the HLA region, it remains possible that any association found with RAGE and diabetic complications may be due to linkage disequilibrium with other sequence variation in the DQ or DR loci. However, although an association exists between the etiology of diabetes and variation in the HLA loci, little or no evidence exists for an association with the development of diabetic complications.

In summary, we have identified four polymorphisms resulting in functional amino acid changes in the RAGE gene. The frequency of one of these, the Gly82Ser polymorphism, did not differ between ethnic groups, type 2 diabetic patients, or in relation to macrovascular disease. Further investigation is required, however, to establish the

significance of such polymorphisms in the pathogenesis of the microvascular complications of diabetes.

REFERENCES

1. Vlassara H, Bucala R, Striker L: Pathogenic effects of advanced glycosylation: biochemical, biologic, and clinical implications for diabetes and aging. *Lab Invest* 70:138–151, 1994
2. Brownlee M: Lilly Lecture 1993: glycation and diabetic complications. *Diabetes* 43:836–841, 1994
3. Sugaya K, Fukagawa T, Matsumoto KI, Mita K, Takahashi EI, Ando A, Inoko H, Ikemura T: Three genes in the human MHC class II region near the junction with the class II: gene for receptor of advanced glycosylation end products, PBX2 homeobox gene and a notch homolog, human counterpart of mouse mammary tumor gene *int*-3. *Genomics* 23:408–419, 1994
4. Schmidt AM, Hasu M, Popov D, Zhang JH, Chen J, Yan SD, Brett J, Cao R, Kuwabara K, Costache G, Simionescu M, Stern D: Receptor for advanced glycation end products (AGEs) has a central role in vessel wall interactions and gene activation in response to circulating AGE proteins. *Proc Natl Acad Sci U S A* 91:8807–8811, 1994
5. Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D: Cellular receptors for advanced glycation end products: implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. *Arterioscler Thromb* 14:1521–1528, 1994
6. Bierhaus A, Luther T, Sernau T, Haase M, Kasper M, Quehenberger P, Wahl P, Schmidt AM, Stern DM, Muller M, Nawroth PP: Induction of tissue factor by advanced glycation endproducts (AGEs) is suppressed in the presence of antisense oligonucleotides directed against the receptor for AGEs (RAGE) (Abstract). *Thromb Haemostasis* 73:1092, 1995
7. Quehenberger P, Greten J, Riedesel JV, Ziegler R, Wahl P, Speiser W, Bierhaus A, Nawroth PP: Induction of endothelin-1 by advanced glycation endproducts in cultured bovine cells (Abstract). *Thromb Haemostasis* 73:917, 1995
8. Schmidt AM, Hori O, Chen JX, Li JF, Crandall J, Zhang J, Cao R, Yan SD, Brett J, Stern D: Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice: a potential mechanism for the accelerated vasculopathy of diabetes. *J Clin Invest* 96:1395–1403, 1995
9. Newfield RS, Polak M, Marchase R, Czernichow P. Epidemiology and genetics of diabetic complications. *Diabetologia* 40 (Suppl. 3):B62–B64, 1997
10. Bierhaus A, Ritz E, Nawroth RP: Expression of receptors for advanced glycation end-products in occlusive vascular and renal disease. *Nephrol Dial Transplant* 11:87–90, 1997