



2007

Identification and Characterization of *OGG1* Mutations in Patients with Alzheimer's Disease

Guogen Mao

University of Kentucky, gmao2@uky.edu

Xiaoyu Pan

University of Kentucky

Beibei Zhu

University of Kentucky, bzhu2@uky.edu

Yanbin Zhang

University of Kentucky

Fenghua Yuan

University of Kentucky

See next page for additional authors

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Follow this and additional works at: https://uknowledge.uky.edu/toxicology_facpub

 Part of the [Medical Toxicology Commons](#)

Repository Citation

Mao, Guogen; Pan, Xiaoyu; Zhu, Beibei; Zhang, Yanbin; Yuan, Fenghua; Huang, Jian; Lovell, Mark A.; Lee, Maxwell P.; Markesbery, William R.; Li, Guo-Min; and Gu, Liya, "Identification and Characterization of *OGG1* Mutations in Patients with Alzheimer's Disease" (2007). *Toxicology and Cancer Biology Faculty Publications*. 32.

https://uknowledge.uky.edu/toxicology_facpub/32

This Article is brought to you for free and open access by the Toxicology and Cancer Biology at UKnowledge. It has been accepted for inclusion in Toxicology and Cancer Biology Faculty Publications by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

Authors

Guogen Mao, Xiaoyu Pan, Beibei Zhu, Yanbin Zhang, Fenghua Yuan, Jian Huang, Mark A. Lovell, Maxwell P. Lee, William R. Markesbery, Guo-Min Li, and Liya Gu

Identification and Characterization of *OGG1* Mutations in Patients with Alzheimer's Disease**Notes/Citation Information**

Published in *Nucleic Acids Research*, v. 35, no. 8, p. 2759-2766.

© 2007 The Author(s)

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/2.0/uk/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Digital Object Identifier (DOI)

<http://dx.doi.org/10.1093/nar/gkm189>

Identification and characterization of OGG1 mutations in patients with Alzheimer's disease

Guogen Mao¹, Xiaoyu Pan¹, Bei-Bei Zhu², Yanbin Zhang¹, Fenghua Yuan¹, Jian Huang⁵, Mark A. Lovell^{3,4}, Maxwell P. Lee⁶, William R. Markesbery^{2,3}, Guo-Min Li^{1,2} and Liya Gu^{1,2,*}

¹Graduate Center for Toxicology, ²Department of Pathology & Laboratory Medicine, ³Sanders-Brown Center on Aging, ⁴Department of Chemistry, University of Kentucky, Lexington, KY, USA, ⁵College of Life Sciences, Wuhan University, Wuhan, China and ⁶Laboratory of Population Genetics, National Cancer Institute, Bethesda, MD, USA

Received January 31, 2007; Revised and Accepted March 15, 2007

ABSTRACT

Patients with Alzheimer's disease (AD) exhibit higher levels of 8-oxo-guanine (8-oxoG) DNA lesions in their brain, suggesting a reduced or defective 8-oxoG repair. To test this hypothesis, this study investigated 14 AD patients and 10 age-matched controls for mutations of the major 8-oxoG removal gene *OGG1*. Whereas no alterations were detected in any control samples, four AD patients exhibited mutations in *OGG1*, two carried a common single base (C₇₉₆) deletion that alters the carboxyl terminal sequence of *OGG1*, and the other two had nucleotide alterations leading to single amino acid substitutions. *In vitro* biochemical assays revealed that the protein encoded by the C₇₉₆-deleted *OGG1* completely lost its 8-oxoG glycosylase activity, and that the two single residue-substituted *OGG1* proteins showed a significant reduction in the glycosylase activity. These results were consistent with the fact that nuclear extracts derived from a limited number of AD patients with *OGG1* mutations exhibited greatly reduced 8-oxoG glycosylase activity compared with age-matched controls and AD patients without *OGG1* alterations. Our findings suggest that defects in *OGG1* may be important in the pathogenesis of AD in a significant fraction of AD patients and provide new insight into the molecular basis for the disease.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of adult dementia and is characterized by progressive cognitive deterioration, impairment of activities of daily

living, and a variety of neuropsychiatric symptoms and behavioral disturbances. It is generally accepted that the production and accumulation of beta-amyloid peptide in the brain in AD are important in the pathogenesis of the disease (1). Increasing evidence supports that mutations in the beta-amyloid precursor protein and the presenilins lead to some early-onset AD cases (1). Although mutations can be induced by numerous endogenous and exogenous agents, oxidative stress has been thought to be the main source for DNA damage in AD (2). Indeed, previous studies have demonstrated that increased nuclear and mitochondrial DNA oxidation is present in the brain in AD (3–6), with 8-hydroxyguanine or its isomeric form 7,8-dihydro-8-oxoguanine (8-oxoG) being the major DNA lesion (7).

The 8-oxoG is highly mutagenic because adenine is preferentially incorporated opposite the oxidized base upon replication by DNA polymerases, yielding GC to TA transversions. To prevent 8-oxoG-induced mutagenesis, cells possess a mechanism called the GO system, which consists of MutY homolog (MYH), *OGG1* (8-oxoG-DNA glycosylase I, the eukaryotic homolog of MutM), and MutT (8–10). The MutT protein is a phosphatase that specifically converts 8-oxo-dGTP in the nucleotide pool into 8-oxo-dGMP to keep the oxidized nucleotides from being incorporated into DNA during replication. Both MYH and *OGG1* are 8-oxoG-specific DNA glycosylases. Unlike MutT, *OGG1* and MYH are components of base excision repair and involved in processing 8-oxoG (9,10). Whereas, *OGG1* actually removes 8-oxoG from damaged DNA, MYH excises the adenine residues incorporated by DNA polymerases opposite 8-oxoG. Mutations of *OGG1* and MYH have been implicated in the development of certain human diseases including cancer (11–13). Gene knockout mice defective in *OGG1* accumulate higher levels of 8-oxoG lesions compared with wild-type controls

*To whom correspondence should be addressed. Tel: +1 859 323 0285; Fax: +1 859 323 1059; Email: lgu0@uky.edu

(14,15), and exhibit elevated spontaneous mutations, especially when exposed to higher levels of oxidative stress (16). These observations suggest that OGG1 acts as a major pathway responsible for 8-oxoG removal (17).

To determine the impact of OGG1 in AD development, DNA and/or protein samples derived from the brain of AD patients and age-matched controls were analyzed for defects in *OGG1* and OGG1 glycosylase activity. Mutations of the *OGG1* gene were indeed identified in AD patients. Biochemical studies revealed that these mutations either completely inactivate or severely reduce the OGG1 DNA-glycosylase activity, suggesting that defects in OGG1 are associated with the development of AD.

MATERIALS AND METHODS

AD sample collection

Brain specimens were from AD patients and normal control subjects who were longitudinally followed with annual detailed assessments in the University of Kentucky Alzheimer's Disease Center. The specimens were evaluated in a blinded manner in our study. These samples were collected as described previously (3). Briefly, 10–15 g specimens of frontal, temporal and parietal lobes and cerebellum were dissected en bloc at the time of autopsy from patients with AD and age-matched normal control subjects. The tissue was snap-frozen and stored at -70°C until used for analysis. All AD patients met standard clinical diagnostic criteria for probable AD (18) and accepted histopathological criteria for the diagnosis of AD (19). The AD patients were in the late stage of AD. Control subjects were individuals without a history of dementia, other neurological disorders or systemic disease affecting the brain. Neuropathological studies revealed no significant neuropathological alterations in the control subjects. The mean age for the control cases was 92.3 and 72.0 years for the AD cases. The average postmortem interval was 2.90 ± 0.31 h for AD and 3.36 ± 0.47 h for control subjects.

OGG1 activity assay

Preparation of nuclear extracts from brain specimens was performed as described previously (20). Because of potential variations in water content in tissues collected from various individuals (both AD and control subjects), data were normalized to protein content for each isolated nuclear fraction, rather than using the same amount of starting materials for extract preparations. The OGG1 glycosylase activity was measured essentially as described (21) using an oligonucleotide duplex consisting of the ^{32}P -labeled 5'-ATTGCCATTCTCGATA[8-oxoG]GATCCGGTCAAACCTAGAC-3' and the cold complementary strand 5' GTCTAGGTTTGACCGGATCCTATCGAG AATGGCAAT-3'. Briefly, incision reactions (20 μl) contained 50 mM sodium phosphate buffer (pH 6.8), 2 mM EDTA, 2 mM DTT, 80 mM KCl, 0.1 mg/ml of BSA, 10% glycerol, 50 fmol of ^{32}P -labeled duplex and 40 μg of nuclear extracts or indicated amount of purified wild-type or mutant OGG1 protein. After incubation at 37°C for

1 h, the reactions were terminated by adding an equal volume of a stop solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol). The reaction products were resolved on a 15% denaturing gel and visualized by a phosphorimager.

Site-directed mutagenesis, overexpression and purification of OGG1

The His-tagged human *OGG1* expression vector (pET28d) was kindly provided by Drs Sankar Mitra and Tapas K. Hazra, University of Texas Medical Branch at Galveston. Construction of the *OGG1* mutants associated with AD was performed using an overlap-extension PCR technology as described (22), which is depicted in Figure 1, showing the strategy for the generation of the C_{796} -deletion of *OGG1*. Two sets of PCR primers were used: forward primer 1 (RP1), 5'-GGGGAATTGTGAGCGGATAAC-3', and reverse primer 1 (RP1), 5'-ATATGGACATCCACGGCACAGCCTGGGGC TT-3'; FP2, 5'-AGGCTGTGCCGTGGATGTCCATAT GT-3' and RP2, 5'-GGTGGTGGTGGTGGTCTCGAG-3', where the bold types represent the mutation site. PCR products from the two sets of reactions were mixed, denatured and reannealed. Because these PCR products overlapped at their 3' ends, a full-length mutant *OGG1* was generated via 3' end extension (Figure 1). The full-length mutant gene was amplified using primers FP1 and RP2, digested with XhoI and XbaI, and cloned into the XhoI/XbaI-digested pET28d expression vector. The C_{796} deletion results in a frame-shift mutation that allows the mutated gene to encode a protein 59 amino acids longer than the wild-type OGG1 (see below for details). Thus, a pair of primers (5'-ACTGTCAGTCTCACCAG-3' and 5'-ATACTCGAGTCAATATCCCCACCCCATCTTGTTG-3') were used to amplify part of the exon 7 sequence of *OGG1* and its 3' untranslated region (3'-UTR) from genomic DNA derived from patient AD2. After digestion with BspMI and XhoI, the PCR product

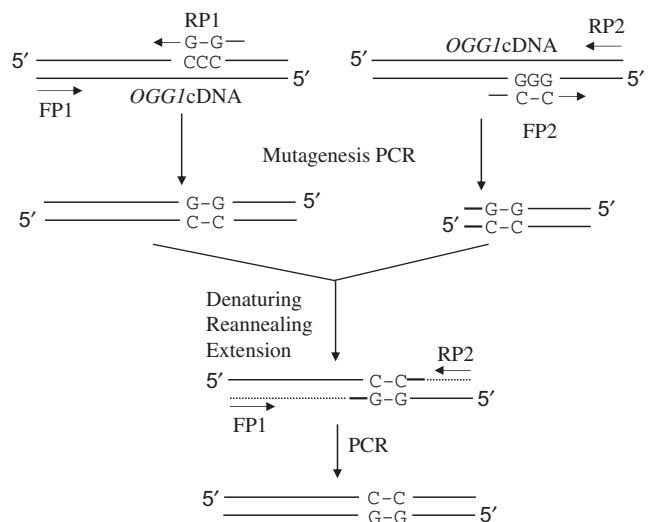


Figure 1. Diagram of PCR-based site-directed mutagenesis. Deletion of a C from C_{796} – C_{797} – C_{798} in *OGG1* is shown as an example.

was cloned into pET28d plasmid containing the C₇₉₆-deleted *OGG1*. The presence of the C₇₉₆ deletion and the extended 3'UTR was confirmed by DNA sequencing. Other *OGG1* mutants identified in AD were similarly constructed.

The wild-type and mutant *OGG1* plasmids were transformed into *Escherichia coli* strain BL21 DE3 for expression and the recombinant proteins were purified as described by Hill *et al.* (23). The purified proteins (>98% purity, as judged by Coomassie Brilliant Blue R-250 staining on an SDS-PAGE gel) were aliquoted and stored at -80°C for use.

Single-strand conformational polymorphism and DNA sequencing analyses

Nuclear DNA was isolated from brain regions of AD patients and controls as described by Gabbita *et al.* (3). PCR-based single-strand conformation polymorphism (SSCP) was used to determine mutations in *OGG1*. PCR primers (Supplementary Data, Table S1) were used to amplify all seven exons and flanking splice sites of the *OGG1* gene as described by Murata *et al.* (24). Variant and wild-type PCR products were excised and eluted from SSCP gels, and then reamplified. The resulting PCR products were sequenced using T7 Sequenase, version 2.0 DNA sequencing kit (USB Corp., Cleveland, OH, USA).

Statistical analysis

Statistical analyses were performed using *t*-test function in *R* package. Specifically, one-sided *t*-test was used to compare 8-oxoG glycosylase activity shown in Figure 2 and 8-oxoG lesion level in Table 1.

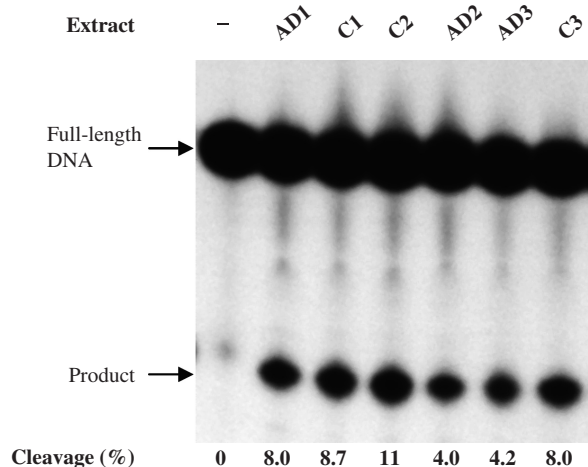


Figure 2. The 8-oxoG glycosylase activity of nuclear extracts derived from brain of AD patients. Nuclear extracts (40 µg) from AD patients (AD1, AD2 and AD3) or age-matched controls (C1, C2 and C3) were incubated with 50 fmol of ³²P-labeled 36-mer duplex containing a centrally located 8-oxoG residue. 8-oxoG cleavage activity in AD patients, except AD1, was lower than that in age-matched controls.

RESULTS

Reduced 8-oxoG DNA glycosylase activity in AD patients

The abundance of 8-oxoG in the brain of AD patients prompted us to determine 8-oxoG glycosylase activity in AD patients. Nuclear extracts obtained from the brain specimens of AD patients were examined for their ability to cleave a duplex DNA containing a single 8-oxoG lesion. As shown in Figure 2, the nuclear extracts derived from all three age-matched controls (C1, C2 and C3) exhibited high levels (8% or more) of 8-oxoG glycosylase activity, whereas extracts from two (AD2 and AD3) of the three AD patients tested had only about one-half of the glycosylase activity observed in the control samples. These results were repeatable. Statistical analysis revealed that the observed difference in glycosylase activity between extracts derived from AD patients with lower 8-oxoG glycosylase activity (AD2 and AD3) and those isolated from control subjects (C1-3) or an AD patient with normal glycosylase activity (i.e. AD1) is highly significant ($P=0.00298$). These observations suggest that reduced repair of 8-oxoG is associated with AD patients.

OGG1 mutations in AD patients

Because the major DNA glycosylase for removal of 8-oxoG is *OGG1* (17), we reasoned that the reduced 8-oxoG glycosylase activity in AD patients could be due to a reduction in *OGG1* activity. PCR primers (Supplementary Data, Table 1) were therefore designed to amplify all seven individual exons of the *OGG1* gene from the brain specimens of AD patients and age-matched controls. The resulting PCR products were analyzed by SSCP as described (24). Whereas, no abnormal SSCP products were detected in exons 1-4, 6 and 7 of *OGG1* in all samples tested (data not shown), two AD patients appeared to display aberrant SSCP bands in exon 5. As shown in Figure 3A, two specific products (bands 1 and 4), presumably the normal alleles of the *OGG1* exon 5, were seen in the age-matched controls (data for C1 and C2 not shown) and patient AD1 but both normal and additional alleles (bands 2 and 3) were detected in AD2 and AD3, the two AD patients exhibiting reduced 8-oxoG glycosylase activity (Figure 2). These results seem to suggest a heterozygous alteration of *OGG1* in these patients.

To determine if the abnormal SSCP products of exon 5 in AD2 and AD3 were associated with mutation(s), SSCP bands 1-4 shown in Figure 3A were eluted from the gel, reamplified and sequenced. As expected, bands 1 and 4 from all samples indeed showed the wild-type *OGG1* sequence, and examples of these sequences are shown in Figure 3B (see two gels on the left side for AD1 and C3). However, bands 2 and 3 exhibited a mutated *OGG1* sequence as a C (C₇₉₆, C₇₉₇ or C₇₉₈) was deleted from nucleotide positions 796-798. For simplicity, the mutation was referred to as C₇₉₆-deleted. Interestingly, both AD2 and AD3 carried the same deletion (Figure 3B, two gels on the right side). The status of the heterozygous mutation in AD2 and AD3 was further confirmed by cloning the PCR products of exon 5 into a T-A cloning vector (Invitrogen, Carlsbad, CA, USA), followed by DNA sequencing.

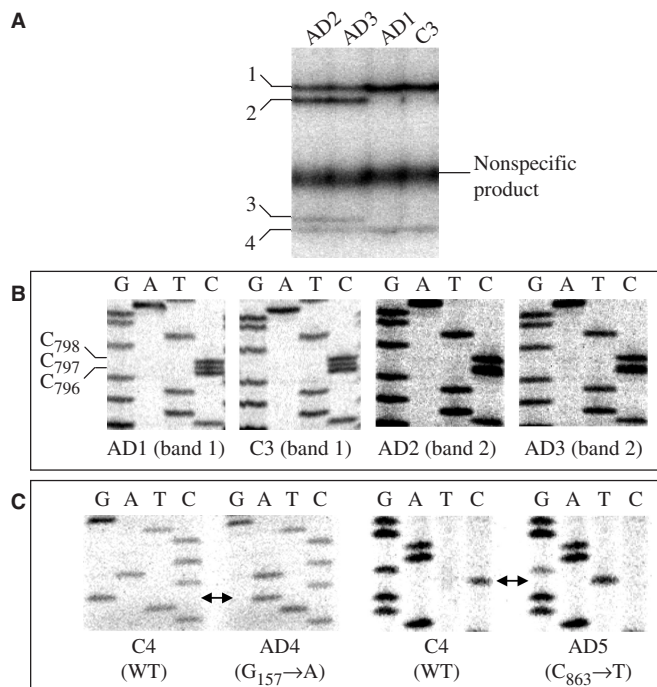


Figure 3. Mutations of *OGG1* identified in AD patients. (A) SSCP analysis of AD patients and age-matched controls. Whereas, samples with higher levels of 8-oxoG cleavage activity (AD1 and C3) exhibited two specific SSCP products (bands 1 and 4), samples with lower levels of 8-oxoG cleavage activity showed two additional products (bands 2 and 3). (B) *C*₇₉₆ deletion in AD2 and AD3. The indicated SSCP bands from patients or controls were eluted, PCR-amplified and sequenced. (C) Base-base substitutions in AD4 and AD5. Arrows point to the altered nucleotides.

The results revealed an approximate 1:1 ratio of wild-type and mutant clones (data not shown).

The association of a defective *OGG1* in AD2 and AD3 prompted us to investigate 11 additional AD samples for mutations in *OGG1*. Abnormal SSCP products were identified in two patients (data not shown). DNA sequencing analysis revealed that one patient (AD4) had a G₁₅₇→A transition and another patient (AD5) carried a C₈₆₃→T transition in *OGG1* (Figure 3C). The G→A substitution changes amino acid residue alanine₅₃ (A₅₃) to threonine and the C→T substitution changes alanine₂₈₈ (A₂₈₈) to valine. However, no nucleotide alterations were identified in any of the seven additional age-matched controls (C4 in Figure 3C, data for C5–C10 not shown). Taken together, our results suggest that a substantial fraction (28.6% or 4 out of 14) of AD patients are associated with mutations in the *OGG1* gene.

The *C*₇₉₆-deleted *OGG1* gene encodes a proteins lacking 8-oxoG glycosylase activity

Given a single nucleotide deletion in the *OGG1* gene, AD2 and AD3 were expected to express an altered OGG1 protein that possesses a carboxyl terminal different from that of the wild-type OGG1. The wild-type *OGG1* gene encodes 345 amino acid residues (Figure 4A). However, the *C*₇₉₆ deletion altered the coding frame of *OGG1* at the

A Wild type *OGG1* (partial sequence)

```

261 K P Q A V P V D V H M W H I A Q R D Y S
781 AAGCCCCAGGCTGTGCCC GTGGATGTCCATATGTGGCACATGCCCAACGTGACTACAGC

281 W H P T T S Q A K G P S P Q T N K E L G
841 TGGCACCCCTACCACGTCCCAGGCGAAGGGACCGAGCCCCAGACCAACAAGAACTGGGA

301 N F F R S L W G P Y A G W A Q A V L F S
901 AACTTTTCCGGAGCCTGTGGGGACCTTATGCTGGCTGGGCCAAGC GGTGCTGTTCAGT
Exon 7

321 A D L R Q S R H A Q E P P A K R R K G S
961 GCCGACCTGCCCAATCCC GCCATGCTCAGGAGCCACCAGCAAAGCGCAGAAAGGGTTCC

341 K G P E G -
1021 AAAGGCCGGAAGGC TAG

```

B *C*₇₉₆-deleted *OGG1* (partial sequence)

```

261 K P Q A V P W M S I C G T L P N V T T A
781 AAGCCCCAGGCTGTGCCC GTGGATGTCCATATGTGGCACATGCCCAACGTGACTACAGCT

281 G T L P R P R R R D R A P R P T R N W E
841 GGCACCCCTACCACGTCCCAGGCGAAGGGACCGACCCCCAGACCAACAAGAACTGGGA

301 T F S G A C G D L M L A G P K R C C S V
901 AACTTTTCCGGAGCCTGTGGGGACCTTATGCTGGCTGGGCCAAGC GGTGCTGTTCAGT
Exon 7

321 P T C A N P A M L R S H Q Q S A E R V P
961 CCGACCTGCGCCCAATCCGCCATGCTCAGGAGCCACCAGCAAAGCGCAGAAAGGGTTCCA
BspMI

341 K G R K A R W G T L D K E I P Q A P S P
1021 AAAGGCCGGAAGGC TAGATGGGGCACCTGGCAAGAAGAAATCCCCAAGCACCTTCCCCT

361 P F P T S L S P S P P S L M L L G R G L P
1081 CCATTTCCCACTTCTCTCTCCCATCCCCACCCAGTCTCATGTGGGGAGGGGCCCTCCCT

381 V T T S K A R H P Q I K Q S V C T T R W
1141 GTGACTACCTCAAAGGCCAGGCACCCCAATCAAGCAGTCAGTTTGCACAACAAG ATGG

401 G G G Y -
1201 GGTGGGGATAT TGA

```

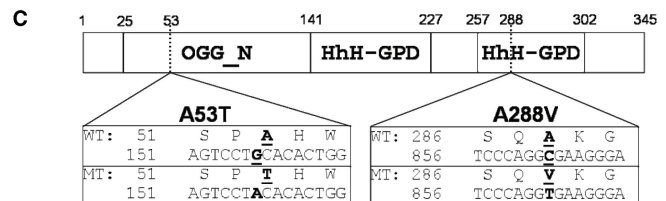


Figure 4. Locations of the identified mutations in OGG1. (A) Partial protein and DNA sequences of wild-type OGG1. (B) Partial protein and DNA sequences of OGG1-59aa. Mutation site and original stop codon are highlighted. Recognition sequence of the restriction endonuclease BspMI is also highlighted, which was used to clone part of the exon 7 and 3'-UTR sequences from patient AD2 into pET28d plasmid. (C) Partial DNA and peptide sequences of mutants A53T and A288V. Important functional domains (modified from www.sanger.ac.uk/cgi-bin/Pfam/swisspfamget.pl?name=O15527) of OGG1 are also shown.

mutation site. As a result, the translation does not terminate at codon 346, instead, it terminates 59 amino acid residues downstream of the previous termination site (Figure 4B). Thus, not only does the mutated protein contain different amino acid residues from the wild-type OGG1 following the mutation site, but also is 59-residue bigger than OGG1 (Figure 4B). The mutant protein was therefore referred to as OGG1-59aa.

To determine if the mutated OGG1 retains 8-oxoG glycosylase activity, site-directed mutagenesis was performed to generate the OGG1-59aa as described in Materials and Methods section. Both the wild type and

OGG1-59aa proteins were purified (Figure 5A) and assayed for the 8-oxoG glycosylase activity. Whereas, the wild-type OGG1 (W) was very active in cleaving the 8-oxoG-containing duplex, little such activity was detected in reactions with OGG1-59aa (M), regardless of the reaction incubation time and the amount of protein used (Figure 5B and C). Mixing experiments using various ratios of wild-type OGG1 versus OGG1-59aa indicated that the mutant protein had no negative inference on the wild-type OGG1 activity (data not shown), ruling out a dominant negative role for OGG1-59aa. These results indicate that a defective OGG1 is present in patients AD2 and AD3, which may account for the reduced 8-oxoG glycosylase activity in these patients.

OGG1 with A53T or A288V substitution possesses a reduced 8-oxoG glycosylase activity

To determine the impact of A53T or A288V substitution on OGG1 glycosylase activity, genes encoding OGG1(A53T) and OGG1(A288V) were created using a similar strategy as described in Figure 1, and the mutant proteins were purified (see Figure 5A) and examined for 8-oxoG glycosylase activity. Unlike OGG1-59aa, which is completely defective in 8-oxoG glycosylase activity, both OGG1(A53T) and OGG1(A288V) proteins were capable of cleaving the 8-oxoG-containing oligonucleotide duplex (Figure 6A and B). However, the glycosylase activity of both altered proteins was only 50–60% of the wild-type OGG1 activity (Figure 6C). Further analysis revealed that both mutant proteins possess a K_m higher than that of the wild-type OGG1 (Figure 6D), indicating that the reduction in glycosylase activity for both mutant proteins is due to the reduction in enzyme's affinity to the DNA substrate. Although protein samples of patients AD4 and AD5 were not available for biochemical assays, our results strongly suggest that reduced 8-oxoG glycosylase activity was likely present in the brain of these AD patients.

DISCUSSION

Patients with AD have been shown to exhibit a much higher level of 8-oxoG DNA lesions in brain than age-matched normal control subjects (7). The increased level of 8-oxoG lesions in AD is likely due to reduced DNA repair in these patients. We provide here both the genetic and biochemical evidence that a significant fraction (28.6%) of AD patients examined in this study exhibited mutations in the 8-oxoG removing gene *OGG1* and that OGG1 proteins with the mutations identified in AD patients have either no or greatly reduced 8-oxoG glycosylase activity.

Interestingly, of four unrelated patients with *OGG1* mutations, two (AD2 and AD3) had a C_{796} deletion mutation, which changes the *OGG1* reading frame and leads to a different carboxyl terminal sequence, including an extra fragment of 59 amino acid residues (Figure 4). This mutation completely abolishes the OGG1 glycosylase activity (Figure 5). This result is consistent with previous crystal structure studies of the human OGG1 protein. Bruner *et al.* (25) demonstrated that the 8-oxoG lesion is

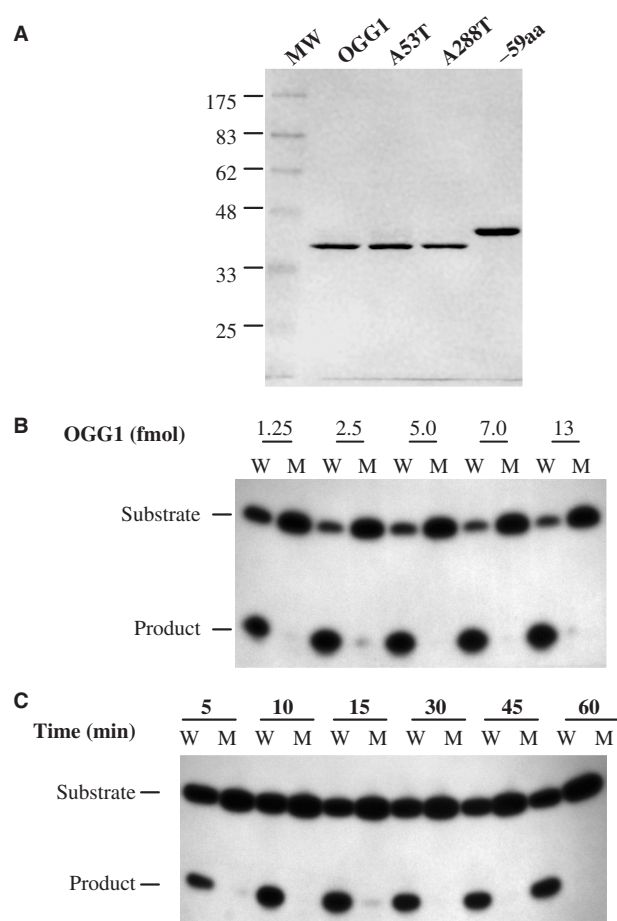


Figure 5. OGG1-59aa has no glycosylase activity. 8-OxoG glycosylase activity was determined as described in the Figure 2 legends, but using purified proteins. (A) SDS-PAGE of purified recombinant wild-type and mutant OGG1 proteins, as indicated. (B) 8-oxoG glycosylase activity of wild-type OGG1 (W) and OGG1-59aa (M) using the indicated amount of proteins. Reactions were incubated at 37°C for 30 min. (C) Time course of 8-oxoG glycosylase activity assays of OGG1 (W) and OGG1-59aa (M). Each reaction contained 1.25 fmol of the indicated proteins.

extruded from the DNA helix and inserted into an extrahelical pocket composed of amino acid residues Phe₃₁₉, Gln₃₁₅, Gly₄₂ and Cys₂₅₃ of OGG1. In this active-site pocket, Phe₃₁₉ and Cys₂₅₃ sandwich the 8-oxoG lesion via interactions with the lesion; the Gln₃₁₅ amide-NH₂ and a water molecule tightly bound to the residue cooperate to recognize O⁶ of the oxoG; and the side-chain carbonyl of Gln₃₁₅ and a second tightly bound water form hydrogen bonds with N1, NH₂ and O⁶ of the oxoG (25). In addition, residue Asp₂₆₈ is required to deprotonate Lys₂₄₉ and His₂₇₀ is needed to protonate Lys₂₄₉, which acts to displace the oxoG base and to promote conjugate elimination of the 3' phosphodiester through Schiff base chemistry (25). However, the C_{796} deletion alters the *OGG1* reading frame beginning at amino acid residue 267, leading to a protein no longer containing these important residues (i.e. Asp₂₆₈, His₂₇₀, Gln₃₁₅ and Phe₃₁₉) at the corresponding positions (see Figure 4A and B). Therefore,

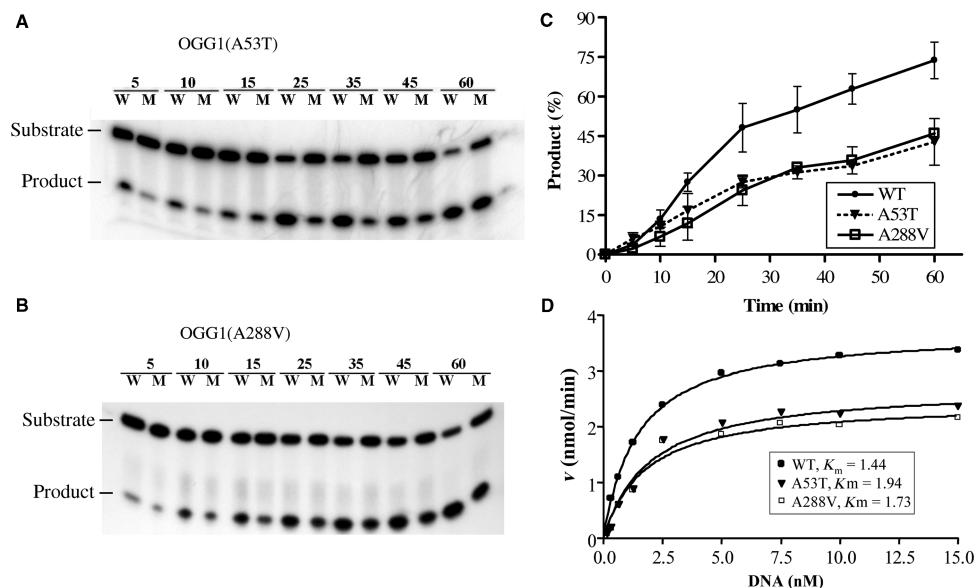


Figure 6. Reduced glycosylase activity in OGG1(A53T) and OGG1(A288V). Unless otherwise mentioned, 8-oxoG glycosylase activity was assayed in a 20- μ l reaction containing 25 fmol of wild-type (W) or mutant (M) OGG1 and 50 fmol of oligonucleotide duplexes containing a single 8-oxoG. Reactions were incubated at 37°C for 1 h. (A) Time course of glycosylase activity of OGG1(A53T). (B) Time course of glycosylase activity of OGG1(A288V). (C) Comparison of glycosylase activities among various OGG1 proteins. Each point represents the average value of three independent experiments, and error bars show SDs. (D) OGG1(A53T) and OGG1(A288V) possess a K_m that is higher than that of OGG1. Reactions were assembled similarly as described in panels A and B, but incubated at 37°C for 30 min and in the presence of increasing DNA concentrations as indicated.

it is understandable that the OGG1-59aa has no 8-oxoG glycosylase activity.

Two additional OGG1 mutations, A53T and A288V, were also identified in AD patients and both were found to reduce 8-oxoG glycosylase activity (Figure 6). The observed activities for these mutants appear to be justified by the roles of A₅₃ and A₂₈₈ in the OGG1 structure. OGG1 has two important domains (see Figure 4C): the OGG-N domain partially contributes to the 8-oxoG-binding pocket and the HhH-GPD domain (a helix-hairpin-helix structural element followed by a Gly/Pro-rich loop and a conserved aspartic acid) provides both the catalytic and DNA-binding functions of the DNA glycosylase (26–28). A₅₃ is located in the region linking two β sheets (β B and β C) in the OGG-N domain, and A₂₈₈ is part of the polypeptide connecting two α -helices (α M and α N) involved in the catalytic activity of the glycosylase (25). Thus, both alanine residues are not in the active center of their corresponding domains. However, substitutions of these alanines with other residues may allow OGG1 to adapt a conformation that is slightly different from the wild-type conformation, leading to the observed reduced glycosylase activity. Whether or not these alterations represent polymorphism in the population remains to be defined. It is also important to determine if individuals with these types of OGG1 alterations are at higher risk to develop AD.

It is interesting that, although a defective OGG1 was detected in the brain of patients AD2 and AD3, extracts derived from these tissues still contained partial 8-oxoG glycosylase activity (Figure 2). This phenomenon could be attributed to two possibilities. First, the alteration is a

heterozygous mutation, thus, the patients possessed both wild-type and mutant OGG1. This possibility is supported by the fact that both wild-type and mutant alleles of the OGG1 exon 5 were detected in patients AD2 and AD3 (Figure 3A) and that the mutant OGG1 protein does not interfere with the wild-type protein on its glycosylase activity (data not shown). A second explanation is that there are multiple 8-oxoG glycosylase activities in human cells. Evidence for this assumption came from gene knockout mice defective in OGG1. Although *Ogg1*^{-/-} mice manifest a steady-state level of 8-oxoG that is 3- to 10-fold higher than wild-type controls (14,15), there is slow but significant removal of 8-oxoG from proliferating *Ogg1*-null cells (29), indicating that alternative 8-oxoG repair pathways are present in mammalian cells. Indeed, such activities have been recently identified (17,30).

The presence of multiple 8-oxoG glycosylase activities in mammalian cells may also explain why some AD patients who were previously shown to exhibit higher levels of 8-oxoG lesions in brain (3–6) seemed to be normal in OGG1 glycosylase activity, implying that deficiency in other 8-oxoG removal pathway(s) may also contribute to the high level of 8-oxoG in AD. Previous studies have shown significant differences in 8-oxoG levels between AD patients and age-matched controls (3–6). Interestingly, when 8-oxoG levels reported previously (3–6) were compared between AD patients with and without OGG1 mutations, it was found that the 8-oxoG level in AD with OGG1 alterations was higher than that in those without OGG1 alterations (Table 1). And *t*-test analysis revealed that the difference in 8-oxoG levels

Table 1. 8-OxoG lesion levels in AD patients with and without OGG1 alterations^a

	8-OxoG (lesions/10 ⁶ bases)	OGG1 mutation
AD patient		
AD2	241.2	Frameshift
AD4	237.5	A53T
AD6	171.9	None
AD7	133.3	None
AD8	192.5	None
Control		
C4	61.4	None
C5	70.0	None

^aData were adapted from References (3) and (6) with permission.

between these two groups is highly significant as judged by $P=0.025$. These data indicate that whereas OGG1 is the major 8-oxoG removal enzyme, abnormality in other 8-oxoG removal pathway(s), and 8-oxoG tolerance (MYH) and cleansing (MutT homologs) pathways can also result in accumulation of the oxidized lesion. The identification of only 28.6% AD patients with OGG1 alterations also support the possibility that the remaining 71.4% of the AD patients could have mutations in MYH, MutT homologs and/or other 8-oxoG removal pathways. Although this hypothesis remains to be investigated, our results shown here indicate that defects in DNA repair, including the OGG1 pathway, are present in AD patients, providing a possible molecular basis for the development of AD.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR online.

ACKNOWLEDGEMENTS

We thank Drs Sankar Mitra and Tapas Hazra for the human *OGG1* expression vector. This work was supported by a program project grant (P01 AG05119) and an Alzheimer's Disease Center Grant (1P30 AG0 28383) from the National Institute on Aging. X.P. was supported by a pre-doctoral fellowship from China Scholarship Council. G.M.L. is the James-Gardner Endowed Chair in Cancer Research, and W.R.M. is the Commonwealth Endowed Chair in Aging. Funding to pay the Open Access publication charges for this article was provided by the James F. Madeline and Edith D. Gardner Endowment to G.M.L.

Conflict of interest statement. None declared.

REFERENCES

- Hardy, J. and Selkoe, D.J. (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*, **297**, 353–356.
- Markesbery, W.R. (1997) Oxidative stress hypothesis in Alzheimer's disease. *Free Radic. Biol. Med.*, **23**, 134–147.
- Gabbita, S.P., Lovell, M.A. and Markesbery, W.R. (1998) Increased nuclear DNA oxidation in the brain in Alzheimer's disease. *J. Neurochem.*, **71**, 2034–2040.
- Lyras, L., Cairns, N.J., Jenner, A., Jenner, P. and Halliwell, B. (1997) An assessment of oxidative damage to proteins, lipids, and DNA in brain from patients with Alzheimer's disease. *J. Neurochem.*, **68**, 2061–2069.
- Wang, J., Markesbery, W.R. and Lovell, M.A. (2006) Increased oxidative damage in nuclear and mitochondrial DNA in mild cognitive impairment. *J. Neurochem.*, **96**, 825–832.
- Wang, J., Xiong, S., Xie, C., Markesbery, W.R. and Lovell, M.A. (2005) Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. *J. Neurochem.*, **93**, 953–962.
- Lovell, M.A. and Markesbery, W.R. (2001) Ratio of 8-hydroxyguanine in intact DNA to free 8-hydroxyguanine is increased in Alzheimer disease ventricular cerebrospinal fluid. *Arch. Neurol.*, **58**, 392–396.
- Michaels, M.L. and Miller, J.H. (1992) The GO system protects organisms from the mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxoguanine). *J. Bacteriol.*, **174**, 6321–6325.
- Hazra, T.K., Hill, J.W., Izumi, T. and Mitra, S. (2001) Multiple DNA glycosylases for repair of 8-oxoguanine and their potential in vivo functions. *Prog. Nucleic Acid Res. Mol. Biol.*, **68**, 193–205.
- Lu, A.L. and Wright, P.M. (2003) Characterization of an *Escherichia coli* mutant MutY with a cysteine to alanine mutation at the iron-sulfur cluster domain. *Biochemistry*, **42**, 3742–3750.
- Boiteux, S. and Radicella, J.P. (2000) The human OGG1 gene: structure, functions, and its implication in the process of carcinogenesis. *Arch. Biochem. Biophys.*, **377**, 1–8.
- Al-Tassan, N., Chmiel, N.H., Maynard, J., Fleming, N., Livingston, A.L., Williams, G.T., Hodges, A.K., Davies, D.R., David, S.S., Sampson, J.R. *et al.* (2002) Inherited variants of MYH associated with somatic G:C→T : A mutations in colorectal tumors. *Nat. Genet.*, **30**, 227–232.
- Parker, A.R., O'Meally, R.N., Sahin, F., Su, G.H., Racke, F.K., Nelson, W.G., DeWeese, T.L. and Eshleman, J.R. (2003) Defective human MutY phosphorylation exists in colorectal cancer cell lines with wild-type MutY alleles. *J. Biol. Chem.*, **278**, 47937–47945.
- Klungland, A., Rosewell, I., Hollenbach, S., Larsen, E., Daly, G., Epe, B., Seeberg, E., Lindahl, T. and Barnes, D.E. (1999) Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proc. Natl Acad. Sci. USA*, **96**, 13300–13305.
- Minowa, O., Arai, T., Hirano, M., Monden, Y., Nakai, S., Fukuda, M., Itoh, M., Takano, H., Hippou, Y., Aburatani, H. *et al.* (2000) Mmh/Ogg1 gene inactivation results in accumulation of 8-hydroxyguanine in mice. *Proc. Natl Acad. Sci. USA*, **97**, 4156–4161.
- Arai, T., Kelly, V.P., Komoro, K., Minowa, O., Noda, T. and Nishimura, S. (2003) Cell proliferation in liver of Mmh/Ogg1-deficient mice enhances mutation frequency because of the presence of 8-hydroxyguanine in DNA. *Cancer Res.*, **63**, 4287–4292.
- Friedberg, E.C., Walker, G.C., Siede, W., Wood, R.D., Schultz, R.A. and Ellenberger, T. (2006) *DNA Repair and Mutagenesis*. ASM Press, Washington, DC.
- McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D. and Stadlan, E.M. (1984) 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**, 939–944.
- The National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. (1997) Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. *Neurobiol. Aging*, **18**, S1–S2.
- Lovell, M.A., Xie, C. and Markesbery, W.R. (2000) Decreased base excision repair and increased helicase activity in Alzheimer's disease brain. *Brain Res.*, **855**, 116–123.

21. Souza-Pinto, N.C., Croteau, D.L., Hudson, E.K., Hansford, R.G. and Bohr, V.A. (1999) Age-associated increase in 8-oxo-deoxyguanosine glycosylase/AP lyase activity in rat mitochondria. *Nucleic Acids Res.*, **27**, 1935–1942.
22. Aiyar, A., Xiang, Y. and Leis, J. (1996) Site-directed mutagenesis using overlap extension PCR. *Methods Mol. Biol.*, **57**, 177–191.
23. Hill, J.W., Hazra, T.K., Izumi, T. and Mitra, S. (2001) Stimulation of human 8-oxoguanine-DNA glycosylase by AP-endonuclease: potential coordination of the initial steps in base excision repair. *Nucleic Acids Res.*, **29**, 430–438.
24. Murata, H., Khattar, N.H., Kang, Y., Gu, L. and Li, G.M. (2002) Genetic and epigenetic modification of mismatch repair genes hMSH2 and hMLH1 in sporadic breast cancer with microsatellite instability. *Oncogene*, **21**, 5696–5703.
25. Bruner, S.D., Norman, D.P. and Verdine, G.L. (2000) Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA. *Nature*, **403**, 859–866.
26. Bruner, S.D., Norman, D.P., Fromme, J.C. and Verdine, G.L. (2000) Structural and mechanistic studies on repair of 8-oxoguanine in mammalian cells. *Cold Spring Harb. Symp. Quant. Biol.*, **65**, 103–111.
27. Labahn, J., Scharer, O.D., Long, A., Ezaz-Nikpay, K., Verdine, G.L. and Ellenberger, T.E. (1996) Structural basis for the excision repair of alkylation-damaged DNA. *Cell*, **86**, 321–329.
28. Nash, H.M., Bruner, S.D., Scharer, O.D., Kawate, T., Addona, T.A., Spooner, E., Lane, W.S. and Verdine, G.L. (1996) Cloning of a yeast 8-oxoguanine DNA glycosylase reveals the existence of a base-excision DNA-repair protein superfamily. *Curr. Biol.*, **6**, 968–980.
29. Osterod, M., Hollenbach, S., Hengstler, J.G., Barnes, D.E., Lindahl, T. and Epe, B. (2001) Age-related and tissue-specific accumulation of oxidative DNA base damage in 7,8-dihydro-8-oxoguanine-DNA glycosylase (Ogg1) deficient mice. *Carcinogenesis*, **22**, 1459–1463.
30. Hazra, T.K., Izumi, T., Kow, Y.W. and Mitra, S. (2003) The discovery of a new family of mammalian enzymes for repair of oxidatively damaged DNA, and its physiological implications. *Carcinogenesis*, **24**, 155–157.