Human Polymorphic Variants of the NEIL1 DNA Glycosylase*

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In mammalian cells, the repair of DNA bases that have been damaged by reactive oxygen species is primarily initiated by a series of DNA glycosylases that include OGG1, NTH1, NEIL1, and NEIL2. To explore the functional significance of NEIL1, we recently reported that neil1 knock-out and heterozygotic mice develop the majority of symptoms of metabolic syndrome (Vartanian, V., Lowell, B., Minko, I. G., Wood, T. G., George, S., Ballinger, S. W., Corless, C. L., McCullough, A. K., and Lloyd, R. S. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 1864–1869). To determine whether this phenotype could be causally related to human disease susceptibility, we have characterized four polymorphic variants of human NEIL1. Although three of the variants (S82C, G83D, and D252N) retained near wild type enzyme specificity and kinetics, whereas G83D was devoid of glycosylase activity on abasic (AP) site-containing DNA, G83D did not catalyze the wild type β,δ-elimination reaction but primarily yielded the β-elimination product. The AP nicking activity of the C136R variant was significantly reduced. Glycosylase nicking activities were measured on both thymine glycol-containing oligonucleotides and γ-irradiated genomic DNA using gas chromatography/mass spectrometry. Two of the polymorphic variants (S82C and D252N) showed near wild type enzyme specificity and kinetics, whereas G83D was devoid of glycosylase activity. Although insufficient quantities of C136R could be obtained to carry out gas chromatography/mass spectrometry analysis, this variant was also devoid of the ability to incise thymine glycol-containing oligonucleotide, suggesting that it may also be glycosylase-deficient. Extrapolation of these data suggests that individuals who are heterozygous for these inactive variant neil1 alleles may be at increased risk for metabolic syndrome.

A major source of DNA lesions in eukaryotic cells is the interaction of reactive oxygen species with DNA constituents.

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DNA bases are particularly susceptible to reactive oxygen species, with the major DNA damages being, among others, 8-hydroxyguanine (8-OH-Gua), 8-hydroxyadenine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), and 4,6-diamino-5-formamidopyrimidine (FapyAde) (1, 2). To reverse the potentially deleterious effects of oxidatively induced DNA base lesions, cells primarily utilize the base excision repair pathway to restore the DNA to its original state. This pathway is initiated by lesion-specific DNA glycosylases that hydrolyze the bond attaching the damaged base to the deoxyribose, and many of these enzymes also possess an activity that catalyzes a β- or β,δ-elimination reaction at the newly formed abasic (AP) site. These incision intermediates are further processed by an AP endonuclease to yield a free 3'-OH that serves as a primer for repair synthesis and ligation.

Human and mouse NEIL1 proteins have been shown to possess a strong substrate preference for FapyAde and FapyGua, with no specificity for 8-OH-Gua when genomic DNA containing multiple lesions was used as a substrate (3, 4). However, in experiments using oligonucleotides with a single lesion, NEIL1 has exhibited some specificity for methyl-FapyGua, urea, (5R)- and (5S)-thymine glycols (opposite Thy, Cyt, and Gua and to a much lesser extent Ade), 5,6-dihydrouracil (and tandem 5,6-dihydrouracil), 5-formyluracil, 5-(hydromethyl)uracil, 5-hydroxycytosine, and mismatches bases uracilcytosine and thymine, and 8-OH-Gua opposite Gua or Thy, and AP sites (reviewed in Ref. 2).

In analogy to its prokaryotic homolog, NEIL1 utilizes its N-terminal proline to catalyze sequential glycosylase, β- and

3 The abbreviations and trivial names used are: 8-OH-Gua, 8-hydroxyguanine; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; FapyAde, 4,6-diamino-5-formamidopyrimidine; AP, abasic; GC/MS, gas chromatography/mass spectrometry.
δ-eliminations (3, 7), and mutations at Pro-2 compromise enzyme activity. The structure of human NEIL1 was solved by x-ray crystallography (8). This structure not only confirmed the identity of residues involved in the catalytic mechanism, but also showed that, although NEIL1 does not contain a zinc-finger motif similar to its Escherichia coli counterparts, endonuclease VIII and formamidopyrimidine DNA glycosylase, it maintains a similar overall fold termed a “zinc-less finger” motif.

The potential biological importance of NEIL1 has recently been demonstrated in studies showing a correlation of inactivating mutations in neil1 with human gastric cancer (9). In addition, RNA interference knockdown experiments in which an ~80% reduction in the mRNA levels of neil1 was achieved significantly sensitized cells to the killing effects of ionizing radiation (10). These data may indicate a critical role for NEIL1 in long term maintenance of genetic integrity.

To determine what role NEIL1 might play in the overall repair of DNA containing oxidatively induced lesions, we have constructed mice in which the neil1 gene has been knocked out. In the absence of exogenous oxidative stress, neil1 knock-out (neil1−/−) and heterozygotic (neil1+/−) mice develop severe obesity, dyslipidemia, and fatty liver disease, and also have a tendency to develop hyperinsulinemia (11). In humans, this combination of clinical manifestations, including hypertension, is known as the metabolic syndrome and is estimated to affect more than 40 million people in the United States. These data suggest an important role for NEIL1 in the prevention of the diseases associated with the metabolic syndrome. In the present study, we report on the enzymatic characterization of four polymorphic variants of human NEIL1 and on their specificities and excision kinetics for removal of oxidatively induced base lesions from DNA.

**EXPERIMENTAL PROCEDURES**

**Expression Constructs**—Human neil1 was amplified by PCR from Image Clone corresponding to BC010876 using primers (WT forward and reverse, Table 1). The amplified DNA was restricted with NdeI and HindIII (New England Biolabs) and cloned into pET22b(+) vector (Novagen) that had been similarly digested. Single amino acid polymorphisms were identified using Primer Designer (Stratagene). Clones were confirmed by sequencing (Molecular Microbiology and Immunology Core Facility, Oregon Health and Science University).

**Expression of hNEIL1 in E. coli**—Plasmids were transformed into BL21-CodonPlus(DE3)-RP (Stratagene) and grown in LB supplemented with ampicillin (50 μg/ml) and chloramphenicol (50 μg/ml) to an optical density of 0.6 at 37 °C. Expression of hNEIL1 was induced with 0.5 mM isopropyl 1-thio-β-d-galactopyranoside at 25 °C for 3.5 h. Cells were harvested, and the frozen pellet was resuspended on ice in 1X equilibration/wash buffer (10 mM imidazole, 300 mM NaCl, 50 mM sodium phosphate, pH 8.0) containing a protease inhibitor mixture (Roche Applied Science), 2 units/μl DNase I solution, 100 μM phenylmethylsulfonyl fluoride, and 1 mg/ml lysozyme for 30 min on ice. Cells were subjected to quick freeze and thaw, followed by brief sonification. Lysates were centrifuged for 20 min at 14,000 revolutions/min and soluble proteins recovered and the recombinant proteins purified from the supernatant with Talon resin according to the manufacturer’s instructions for native batch/gravity flow column purification (Clontech). Protein concentrations were determined using the Bradford protein assay (Bio-Rad) and quality assessed on 10% SDS-polyacrylamide gels stained with Coomassie Blue.

**AP Lyase Assays**—DNA AP lyase assays were performed as previously described (12). Briefly, a 33-bp oligonucleotide containing a centrally placed uracil was 5’-end-labeled with [γ-32P]ATP and annealed to the complementary strand containing an Ade opposite the Ura. The duplex uracil-containing oligonucleotide was digested with uracil DNA glycosylase (New England Biolabs) for 1 h to create the site-specific AP site. Purified protein (0.5 μg; 556 nm) was incubated with 125 nM AP-containing oligonucleotide for 30 min at 37 °C in 5 mM EDTA, 0.1 mg/ml bovine serum albumin, 60 mM NaCl, and 50 mM sodium phosphate (pH 8.0). For kinetic analyses, 0.24 μg (178 nm) of purified protein was incubated with 83 nm AP-containing oligonucleotide for the specified reaction times (45 s or 1.5, 3, 6, 12, and 20 min). Reactions were terminated by the addition of 100 mM NaBH₄, a 5× volume of 95% formamide and heating at 95 °C for 5 min. Samples were analyzed by electrophoretic separation of the substrate and product DNAs through 20% PAGE 8 M urea sequencing gels and results visualized and quantified by phosphorimaging analyses (Storm 820, Amersham Biosciences).

**Thymine Glycol-containing DNA Nicking Assays**—To assay for glycosylase activity on DNAs containing a site-specifically defined lesion, nicking assays were carried out using an oligonucleotide containing a single thymine glycol (TG) lesion (5’-GATCCTCTAGAGTGGACACCTGAGGATC-3'), (generous gift of Dr. Richard Cunningham, State University of New York, Albany, NY). Complementary strands were synthesized so that, when annealed to the lesion-containing strand, they were either fully duplexed (5’-TGATCCTCTAGAGTGGACACCTGAGGATC-3’) or contained a centrally located 9-base mismatched bubble (underlined) encompassing the region containing the thymine glycol (5’-TGATCCTCTAGAGTGGACACCTGAGGATC-3’) or contained a centrally located 9-base mismatched bubble (underlined) encompassing the region containing the thymine glycol (5’-TGATCCTCTAGAGTGGACACCTGAGGATC-3’) or contained a centrally located 9-base mismatched bubble (underlined) encompassing the region containing the thymine glycol (5’-TGATCCTCTAGAGTGGACACCTGAGGATC-3’). The damaged strand (75 pmol) was 32P-labeled and annealed with equal molar concentrations of the two complementary oligonucleotides described above. The DNA 125 nm was incubated with 0.5 μg (556 nm) of wild type and polymorphic variants of NEIL1 for 30 min at 37 °C in 12.5 mM sodium phosphate, 6.25 mM EDTA, 12.5 μg/ml bovine serum albumin. Substrate and product DNAs

### Table 1

| Oligonucleotides used in PCR | WT      | Forward | 5’-ACCTACCTGCATACCTGAGGCTCCACAGCTCC-3’ |
|-----------------------------|---------|---------|----------------------------------------|
| G83D Forward                | 5’-CCCTCCTAGAGTGGACACCTGAGGATC-3’ |
| G83D Reverse                | 5’-ACCTACCTGCATACCTGAGGCTCCACAGCTCC-3’ |
| S82C Forward                | 5’-ACCTACCTGCATACCTGAGGCTCCACAGCTCC-3’ |
| S82C Reverse                | 5’-CCCTCCTAGAGTGGACACCTGAGGATC-3’ |
| C136R Forward               | 5’-CCCTCCTAGAGTGGACACCTGAGGATC-3’ |
| C136R Reverse               | 5’-ACCTACCTGCATACCTGAGGCTCCACAGCTCC-3’ |
| D252N Forward               | 5’-ACCTACCTGCATACCTGAGGCTCCACAGCTCC-3’ |
| D252N Reverse               | 5’-CCCTCCTAGAGTGGACACCTGAGGATC-3’ |
were separated by denaturing PAGE and analyzed by phosphor-imaging analyses.

Preparation of DNA Samples, Enzymic Assays, and GC/MS—The preparation of N₂O-saturated aqueous solutions of calf thymus DNA and their exposure to ionizing radiation in a ⁶⁰Co γ-source were performed as described previously (13). Enzymic assays were performed as described previously (14). For the measurement of excision kinetics, DNA solutions were γ-irradiated at 2.5, 5, 10, 20, 40, and 60 gray. Aliquots of stable isotope-labeled analogs of modified DNA bases (purchased from Cambridge Isotope Laboratories, Cambridge, MA) as internal standards were added to 50-μl aliquots of irradiated DNA. The samples were dried in a SpeedVac under vacuum. Two sets of these samples with three replicates were prepared. One set of the samples was dissolved in 50 μl of the incubation buffer and then incubated with 454 nM wild type NEIL1 (1 μg) at 37 °C for 30 min. The concentration ranges of the FapyAde and FapyGua were 0.32–4.29 μM and 0.92–8.69 μM, respectively. After incubation, 150 μl of cold ethanol (−20 °C) were added to the samples to stop the reaction and precipitate DNA. The samples were kept at −20 °C for 2 h. Subsequently, the samples were centrifuged at 10,000 × g for 30 min at 4 °C. DNA pellets and supernatant fractions were separated. Ethanol was removed from supernatant fractions under vacuum in a SpeedVac. Aqueous supernatant fractions were lyophilized to dryness for 18 h. The other set of irradiated samples was used to determine the levels of modified DNA bases in each sample. The dried samples were hydrolyzed with 0.5 ml of 60% formic acid in evacuated and sealed tubes for 30 min at 140 °C. The hydrolysates were frozen in liquid nitrogen and then lyophilized for 18 h.

Dried supernatant fractions of enzyme-treated irradiated samples or dried acid-hydrolysates of irradiated samples were trimethylsilylated and subsequently analyzed by GC/MS using a gas chromatograph (Model 6890 Series) mass-selective detector (Model 5973N) system (Agilent Technologies, Rockville, MD) according to the published procedures (15).

RESULTS
Experimental Rationale—Because it has been estimated that single nucleotide polymorphisms occur once every ~1250 bp in the human genome (16), it is anticipated that many DNA repair genes contain polymorphisms as low penetrance alleles affecting cancer risk (17–19). Of the many DNA repair gene polymorphisms that have been identified, some have been shown to affect protein function (19–22) and can be associated with an increased incidence of cancer (18, 23).

Interestingly, relatively few single nucleotide polymorphisms within neil1 have been reported. The National Institute of Environmental Health Sciences Environmental Genome Project at the University of Washington has sequenced the neil1 gene in a sample set of individuals who are representative of the United
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States population in the Polymorphism Discovery Resource. These sequencing efforts identified variants that predict changes in the amino acid sequence of NEIL1 (S82C, G83D, C136R, and D252N). In a separate investigation, an additional variant, I182M, was identified near the completion of these studies and has not yet been characterized for functionality. All five neil1 single nucleotide polymorphisms that result in an amino acid change are low frequency variants occurring at \(-1\%\) within this population sample.

The location of these predicted amino acid changes in the NEIL1 crystal structure (8) are shown in Fig. 1A. Within the eukaryotic family of NEIL1 proteins, the identity of the amino acid residues at the sites of these polymorphic variants is well conserved. Fig. 1B shows a sequence alignment and local context of the residues from human, mouse, rat, and Xenopus that are germane to this study. Human NEIL1 is 14.9 and 14.6% identical to the E. coli endonuclease VIII and formamidopyrimidine DNA glycosylase, respectively (3, 7). Alignment of human NEIL1 with these prokaryotic enzymes reveals that, among the variant sites, only G83 is conserved. Additionally, relevant to this study, it is of particular interest that two of the variants, S82C and G83D, reside in close proximity to the M81 residue that has been implicated in stabilization of the enzyme-DNA complex during catalysis (8). The C136R variant appears to reside in a linker region connecting the two major domains of the protein and could affect the overall stability of the enzyme structure.

Cloning, Expression, and Purification of the Wild Type and Polymorphic Variants of NEIL1—The human neil1 gene was PCR-amplified from the BC010876 image clone such that NdeI and HindIII restriction enzyme sites were introduced immediately 5’ and 3’ to the neil1 gene, respectively, and cloned into pET22b(+) using the same restriction sites. Following complete DNA sequence confirmation of the wild type gene, bidirectional site-directed mutagenesis was used to create the following mutations and resulting amino acid changes in the NEIL1 protein: S82C, G83D, C136R, and D252N. All complete genes encoding variants of NEIL1 were confirmed by DNA sequence analyses.

Plasmids carrying the wild type and variant genes of neil1 were transformed into E. coli BL21plus DE3 RP and proteins expressed following isopropyl 1-thio-β-D-galactopyranoside induction. The His₆-tagged enzymes were purified to apparent homogeneity with the exception of C136R. This enzyme could be expressed at levels equivalent to that of the wild type enzyme and was soluble within E. coli, as evidenced by no significant differences in the total amount of the C136R variant expressed in E. coli versus the concentration measured in the high speed supernatant. No evidence was found for inclusion body formation in cells expressing C136R, as no appreciable enzyme was in the cell pellet fraction and microscopic examination of cells revealed no opalescent bodies. Additionally, and in contrast to wild type NEIL1 and the other variants, only a very small fraction of the C136R protein bound to the Talon resin under native conditions. However, when the proteins in the high speed supernatant were denatured prior to loading onto the Talon beads, C136R was efficiently recovered. These data suggest that the C136R mutation may alter the folding of this variant, obscuring the His tag, except under denaturing conditions. Low concentrations of C136R that bound to the Talon resin could be assayed using site-specifically modified oligonucleotides containing either an AP site or a thymine glycol but could not be sufficiently concentrated for the more extensive GC/MS analyses.

Catalytic Activity on Abasic Site-containing DNA—To evaluate the effect that specific variant mutations might have on the complex sequential DNA glycosylase, β-elimination, and δ-elimination reactions, we chose to initially assay for the effects on the β,δ-elimination reaction using AP site-containing DNAs. This reaction relies on the incision of AP site-containing DNA using the secondary amine of the N-terminal Pro-2 as the nucleophile (3, 7). Mutation of either Pro-2 or Glu-3 results in the total loss of catalytic activity (3, 7). Fig. 2 shows data surveying the incision activity of wild type, S82C,
G83D, D252N, and C136R on duplex DNA containing an AP site in the labeled strand, in which the AP site was generated by the action of uracil DNA glycosylase on a centrally positioned uracil. AP-containing DNA (lane 1) was reacted with wild type (Fig. 2, lane 2), S82C (lane 3), G83D (lane 4), D252N (lane 5), and C136R (lane 6) followed by reaction with 100 mM NaBH₄ to reduce any unreacted AP sites.

Wild-type, S82C, and D252N displayed qualitatively similar reactions, generating the expected β,δ-cleavage. However, although G83D incised the abasic DNA, the product was predominantly that of the β-elimination reaction, a result similar to that observed for AP lyases that utilize an ε-amino group of lysine or a primary amino group of an N-terminal amino acid (reviewed in Ref. 24). Nicking activity of C136R was significantly reduced relative to any of the other enzymes, and similar to G83D, showed an uncoupling of the β,δ-elimination steps. However, as described above, this variant displayed reduced binding to Talon beads under native conditions, and thus it cannot be accurately ascertained whether the decrease in activity was due to improper folding or an intrinsic decrease in catalytic efficiency. Because the abasic DNA shown in lane 1 was incubated in buffer for an equivalent amount of time and then treated with NaBH₄, these data show that no appreciable amount of nicking occurred in buffer alone, and thus the incised products observed in lanes 2–6 are interpreted to be the result of the catalytic activities of the enzymes. When the AP-containing DNA was not treated with NaBH₄ prior to denaturing gel electrophoresis analyses, the heat denaturation resulted in a near quantitative conversion to the β-elimination product (data not shown).

Kinetic analyses of AP site incision were performed with the wild type, S82C, G83D, and D252N NEIL1 proteins (Fig. 3). Fig. 3A shows a representative nicking assay in which it is evident that the wild type, S82C, and D252N NEIL1 proteins all directly produced the δ-elimination product, whereas the G83D NEIL1 yielded almost exclusively the β-elimination product. Fig. 3B shows both data sets plotted individually and reveals only modest differences in the catalytic efficiencies of these variants relative to the wild type NEIL1.

Incision Activity of NEIL1 and Variants on Thymine Glycol-containing Oligodeoxynucleotides—The major biological role of NEIL1 is inferred to be recognition and initiation of repair at oxidatively damaged bases. Prior investigations have shown that, using oligonucleotides containing thymine glycol lesions, both mouse and human NEIL1 catalyze glycosylase/β- and δ-elimination reactions. To test the NEIL1 polymorphic variants for this activity, wild type and each variant were surveyed using a 30-mer-containing thymine glycol at position 13 from the 5′ end in which the lesion was located either within a 9-base bubble or fully duplexed DNA. As shown in Fig. 4, the wild type and each variant displayed consistent activity (or lack thereof) on both substrates. S82C and D252N (Fig. 4, lanes 4, 9 and 6, 11, respectively) catalyzed the nicking reactions similar to that of wild type NEIL1 (lanes 3 and 8). In contrast, and although G83D incised AP-containing DNA with high efficiency, it was completely devoid of glycosylase nicking (Fig. 4, lanes 5 and 10). Similar to the loss of AP lyase activity, the C136R variant was
unable to initiate incision (Fig. 4, lanes 7 and 12). To assure that these proteins were not carrying out the glycosylase reaction without the /H9252- and /H9254-elimination reaction, the DNA reaction products were treated with hot piperidine to cleave any AP sites. These data revealed no additional products, thus demonstrating both that the G83D and C136R variants were inactive for glycosyl bond cleavage and that the wild type, S82C, and D252N generally carry out the combined glycosylase/ /H9252- and /H9254-elimination reactions (data not shown).

Substrate Specificities of Human NEIL1 and Its Variants—Excision of modified bases from DNA by NEIL1 and its polymorphic variants was further investigated using DNA samples that had been previously exposed to ionizing radiation in N2O-saturated buffered aqueous solution and subsequent GC/MS analyses. DNA samples were irradiated at a dose of 60 gray and subsequently incubated with pure wild type or individual NEIL1 polymorphic variants, or heat-inactivated enzyme, or no enzyme either as a function of enzyme concentration or time. Supernatant and pellet fractions of DNA samples were separated and then analyzed by GC/MS with isotope dilution. An efficient excision of FapyAde and FapyGua from DNA by wild type NEIL1 of FapyAde and FapyGua on the enzyme concentration. Additionally, the time dependence of excisions was measured using 10-, 15-, 20-, 30-, and 45-min incubation times, with the excision increasing with time until it reached a plateau after 45 min (data not shown). Some excision of 5-hydroxy-5-methylhydantoin was also observed, but it was significantly less when compared with the excision of FapyAde and FapyGua (data not shown). Consistent with the lack of incision of thymine glycol-containing oligonucleotides, G83D exhibited no activity for any of these products. In all cases, other modified bases including 8-OH-Gua were not significantly excised. The heat-inactivated enzymes had no activity.

Excision Kinetics—To determine the full kinetic parameters for the wild type and glycosylase-active polymorphic variants, kinetic analyses were performed. DNA samples were irradiated at six doses, i.e. 2.5, 5, 10, 20, 40, and 60 gray, to obtain different levels of modified bases to measure the dependence of excision on substrate concentration. Levels of excised modified bases found in supernatant fractions were used for the determination of the kinetic parameters (Table 2). Concentration ranges of FapyAde and FapyGua in DNA samples incubated with the enzyme were 0.32–4.29 and 0.92–8.69 M, respectively. Excision followed Michaelis-Menten kinetics (25). As examples, Lineweaver-Burk plots for the excision of FapyAde and FapyGua by wild type NEIL1 are illustrated in Fig. 6. The calculations of the kinetic constants and S.D. (n = 6) were achieved using a program with the linear least squares analysis of the data. The kinetic constants of the excision of FapyAde and FapyGua by wild type NEIL1 are given in Table 2. The relatively low excision of 5-hydroxy-5-methylhydantoin from DNA did not permit an accurate determination of its excision kinetics. In the case of all three enzymes, the specificity constant (kcat/Km) for excision of FapyAde was significantly greater than that for excision of FapyGua, indicating a preference of these enzymes for the former over the latter.

DISCUSSION

Human exposure to both endogenous and exogenous sources of reactive oxygen species are hypothesized to be causative factors in
the etiology of a variety of disparate diseases (26, 27), including but not limited to cancer, hypertension, obesity, atherosclerosis, fatty liver disease, diabetes, and stroke. However, the molecular mechanisms by which these reactive compounds trigger cascades that ultimately result in disease manifestation are not well elucidated. The products resulting from reaction of reactive oxygen species with nucleic acids, proteins, and lipids are well characterized, with DNA base damage including at least 27 adducts (28). Consequently, repair of these lesions are critical to the stable maintenance of the genomes of these organisms. To accomplish the restoration of damaged bases to their original status, organisms possess a base excision repair mechanism. In humans, the repair of oxidatively induced lesions can be initiated by at least four glycosylases: NEIL1, NEIL2, OGG1, and NTH1.

We have previously described the consequences of inactivating or partially inactivating the gene encoding NEIL1 in mice, in which they developed symptoms consistent with metabolic syndrome (11). The observation that neil1 heterozygotes also develop disease prompted us to ask whether there are human polymorphic variants of NEIL1 that possess compromised catalytic efficiencies. Further encouraging us to initiate such a study was the observation that an 80% small interfering RNA-directed reduction in the neil1 message resulted in a 3–4-fold increase in cytotoxicity following ionizing radiation exposure (10). Hypothetical extrapolation of these data to humans may suggest that similar reductions in the effective intracellular concentrations of NEIL1 could genetically predispose individuals to a subset of diseases that are associated with the metabolic syndrome.

Toward this end, the present study shows that human wild type NEIL1 and its polymorphic variants S82C and D252N possess an efficient activity to release FapyAde and FapyGua from high molecular weight DNA containing multiple lesions. All three enzymes had a preference for FapyAde over FapyGua, with some activity toward 5-hydroxy-5-methylhydantoin. FapyAde and FapyGua are among the major hydroxyl radical-and UV radiation-induced products in DNA (reviewed in Ref. 1). Recently, these compounds have been shown to be premutagenic lesions, with FapyGua being even more mutagenic than 8-OH-Gua in mammalian cells (29, 30), pointing to their importance in the biological effects of oxidative DNA damage.

This investigation reveals that the G83D and potentially the C136R variants of human NEIL1 were devoid of DNA glycosylase activity. In the case of the G83D mutant, we conclude that access to flipping the AP site into the active site of catalyzing predominantly a β-elimination reaction. These data suggest that the G83D variant was not compromised but that following the formation of the Schiff base intermediate and β-elimination, the addition of water dissociated the complex prior to the δ-elimination reaction. This would suggest that the active site pocket of the G83D variant was more open than the wild type counterpart or that the introduction of an acidic residue at the opening of the active site pocket would change the hydration of the region of the mole-

| Substrate | Vmax (nM min⁻¹) | kcat (min⁻¹) | KM (nM) | kcat/KM × 10³ (min⁻¹ nM⁻¹) |
|-----------|-----------------|--------------|---------|----------------------------|
| FapyAde   | 5.17 ± 0.58*    | 0.0114 ± 0.0020 | 2420 ± 284 | 0.470 ± 0.104 |
| FapyGua   | 3.79 ± 0.45     | 0.0083 ± 0.0018 | 3572 ± 469 | 0.233 ± 0.052 |
| FapyAde   | 3.34 ± 0.51     | 0.0074 ± 0.0021 | 1677 ± 277 | 0.438 ± 0.130 |
| FapyGua   | 3.17 ± 0.38     | 0.0070 ± 0.0016 | 3493 ± 466 | 0.200 ± 0.045 |
| FapyAde   | 3.04 ± 0.65     | 0.0067 ± 0.0030 | 1378 ± 308 | 0.485 ± 0.020 |
| FapyGua   | 3.73 ± 1.01     | 0.0082 ± 0.0041 | 6841 ± 1817 | 0.127 ± 0.064 |

* Values represent the mean ± S.D. (n = 6). kcat = Vmax/[enzyme]. The enzyme concentration was 454 nM. Concentration ranges of FapyAde and FapyGua were 0.32–4.29 μM and 0.92–8.69 μM, respectively.
In this study, two previous reports have identified cancer-associated variants in the *neil1* gene (9, 32). Shimura et al. (9) identified five *neil1* variants associated with gastric cancer; two were from primary tumor samples, one from a gastric cancer cell line, and two from blood samples. The G245R and the R334G variants have WT catalytic activity, whereas the deletion of Glu-28 has activity similar to a deletion of Pro-2. The gastric cell line mutation results in a splice variant that affects nuclear localization of NEIL1, whereas the other blood sample mutation was an unspecified intrinsic variant. Interestingly, in a screen of 94 familial colorectal cancer cases for *nth1*, *neil1*, *neil2*, *mpg*, *tdg*, *ung*, and *smug1* variants, no missense *neil1* mutations were identified (32). This study found only three novel missense mutations (one each in *neil2*, *tdg3*, and *ung2*). The only variant found in *neil1* was within intron 1 at the second nucleotide of the splice site. It is unclear whether this variant has any functional significance.

Collectively, our data suggest that at least one, and possibly two, of the four studied polymorphic variants of *NEIL1* have severely diminished or abolished activities. If data obtained in the mouse model and *in vitro* cell culture systems can be extrapolated to the human condition, we speculate that individuals carrying this (these) allele(s) may be at a greater risk of susceptibility to developing metabolic syndrome, because we hypothesize that deficiencies in NEIL1 lower the threshold of oxidative stress that is required to transition from a disease-free state to disease onset.

REFERENCES

1. Evans, M. D., Dizdaroglu, M., and Cooke, M. S. (2004) Mutat. Res. **567**, 1–61.
2. Dizdaroglu, M. (2003) Mutat. Res. **531**, 109–126.
3. Hazra, T. K., Izumi, T., Boldogh, I., Imhoff, B., Kow, Y. W., Jaruga, P., Dizdaroglu, M., and Mitra, S. (2002) Proc. Natl. Acad. Sci. U. S. A. **99**, 3523–3528.
4. Jaruga, P., Birincioglu, M., Rosenquist, T. A., and Dizdaroglu, M. (2004) Biochemistry **43**, 15909–15914.
5. Dou, H., Mitra, S., and Hazra, T. K. (2003) *J. Biol. Chem.* **278**, 49679–49684.
6. Parsons, J. L., Zharkov, D. O., and Dianov, G. L. (2005) *Nucleic Acids Res.* **33**, 4849–4856.
7. Bandaru, V., Sunkara, S., Wallace, S. S., and Bond, J. P. (2002) DNA Repair (Amst.) **1**, 517–529.
8. Double, S., Bandaru, V., Bond, J. P., and Wallace, S. S. (2004) *Proc. Natl. Acad. Sci. U. S. A.* **101**, 10284–10289.
9. Shimura, K., Tao, H., Goto, M., Igarashi, H., Taniguchi, T., Maekawa, M., Takezaki, T., and Sugimura, H. (2004) *Carcinogenesis* **25**, 2311–2317.
10. Rosenquist, T. A., Zai, E., Fernandes, A. S., Zharkov, D. O., Miller, H., and Grollman, A. P. (2003) *DNA Repair (Amst)* **2**, 581–591.
11. Vartanian, V., Lowell, B., Minko, I. G., Wood, T. G., Ceci, J. D., George, S., Ballinger, S. W., Corless, C. L., McCullough, A. K., and Lloyd, R. S. (2006) *Proc. Natl. Acad. Sci. U. S. A.* **103**, 1864–1869.
12. McCullough, A. K., Sanchez, A., Dodson, M. L., Marapaka, P., Taylor, J. S., and Lloyd, R. S. (2001) *Biochemistry* **40**, 561–568.
13. Dizdaroglu, M., Bauche, C., Rodriguez, H., and Laval, J. (2000) *Biochemistry* **39**, 5586–5592.
14. Jaruga, P., Jabil, R., McCullough, A. K., Rodriguez, H., Dizdaroglu, M., and Lloyd, R. S. (2002) *Photochem. Photobiol. Biol.* **75**, 85–91.
15. Jaruga, P., Birincioglu, M., Rodriguez, H., and Dizdaroglu, M. (2002) *Biochemistry* **41**, 3703–3711.

16. Venter, J. C., Adams, M. D., Myers, E. W., Li, P. W., Mural, R. J., Sutton, G. G., Smith, H. O., Yandell, M., Evans, C. A., Holt, R. A., Gocayne, J. D., Amanatides, P., Ballew, R. M., Huson, D. H., Wortman, J. R., Zhang, Q., Kodira, C. D., Zheng, X. H., Chen, L., Skupski, M., Subramanian, G., Thomas, P. D., Zhang, J., Gabor Miklos, G. L., Nelson, C., Broder, S., Clark, A. G., Nadeau, J., McKusick, V. A., Zinder, N., Levine, A. J., Roberts, R. J., Simon, M., Slyman, C., Hunkapiller, M., Bolanos, R., Delcher, A., Dew, L., Fasulo, D., Flanigan, M., Florea, L., Halpern, A., Hannenhalli, S., Kravitz, S., Levy, S., Mobarry, C., Reinert, K., Remington, K., Abu-Threideh, I., Beasley, E., Biddick, K., Bonazzi, V., Brandon, R., Caggil, M., Chandramouliwaran, I., Charlal, K., Chaturvedi, K., Deng, Z., Di Francesco, V., Dunn, P., Eibbeck, K., Evangelista, C., Gabrielian, A. E., Gan, W., Ge, W., Gong, F., Gu, Z., Guan, P., Heiman, T. J., Higgins, M. E., Ji, R. R., Ke, Z., Ketchum, K. A., Lai, Z., Lei, Y., Li, Z., Li, Jiang, Y., Lin, X., Lu, F., Merkulov, G. V., Milshina, N., Moore, H. M., Naik, A. K., Narayanan, V. A., Neelam, B., Nusskern, D., Rusch, D. B., Salzberg, S., Shaw, W., Shue, B., Sun, J., Wang, Z., Wang, A., Wang, X., Wang, X., Wei, M., Wides, R., Xiao, C., Yan, C., Yao, A., Ye, J., Zhan, M., Zhang, W., Zhang, H., Zhao, Q., Zheng, L., Zhong,
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F., Zhong, W., Zha, S., Zhao, S., Gilbert, D., Baumhueter, S., Spier, G., Carter, C., Cravchik, A., Woodage, T., Ali, F., An, H., Awe, A., Baldwin, D., Baden, H., Barnstead, M., Barrow, I., Beeson, K., Busam, D., Carver, A., Center, A., Cheng, M., Curry, L., Danaher, S., Davenport, L., Desilets, R., Dietz, S., Dodson, K., Doup, L., Ferriera, S., Garg, N., Gluecksmann, A., Hart, B., Haynes, J., Haynes, C., Heiner, C., Hladun, S., Hostin, D., Houck, J., Howland, T., Ibegwam, C., Johnson, J., Kalush, F., Kline, L., Koduru, S., Love, A., Mann, F., May, D., McCawley, S., McMullen, L., Moy, M., Moy, L., Murphy, B., Nelson, K., Pfannkoch, C., Pratts, E., Puri, V., Qureshi, H., Reardon, M., Rodriguez, R., Rogers, Y. H., Romblad, D., Ruhfel, B., Scott, R., Sitter, C., Smallwood, M., Stewart, E., Strong, R., Suh, E., Thomas, R., Tint, N. N., Tse, S., Veich, C., Wang, G., Wetter, J., Williams, S., Williams, M., Windsor, S., Winn-Deen, E., Wolfe, K., Zaveri, J., Zaveri, K., Abril, J. F., Guigo, R., Kejariwal, A., Mi, H., Lazareva, B., Hatton, T., Narechania, A., Diemer, K., Muruganujan, A., Guo, N., Sato, S., Bafna, V., Istrail, S., Lippert, R., Schwartz, R., Walenz, B., Yooseph, S., Allen, D., Basu, A., Saxenla, J., Blick, L., Caminha, M., Carnes-Stine, J., Caulk, P., Chiang, Y. H., Coyne, M., Dahlke, C., Mays, A., Dombroski, M., Donnelly, M., Ely, D., Esparham, S., Fiset, S., Gire, H., Glanowski, S., Glasser, K., Glodek, A., Gorokhov, M., Graham, K., Groisman, E., Kann, R., Harris, M., Heil, J., Henderson, S., Hoover, J., Jennings, D., Jordan, C., Jordan, J., Kash, J., Kagan, L., Kraft, C., Levitsky, A., Lewis, M., Liu, X., Lopez, J., Ma, D., Majoros, W., McDaniel, J., Murphy, S., Newman, M., Nguyen, T., Nguyen, N., Nodell, M., Pan, S., Peck, J., Peterson, M., Rowe, W., Sanders, R., Scott, J., Simpson, M., Smith, T., Sprague, A., Stockwell, T., Turner, R., Venter, E., Wang, M., Wex, M., Wu, D., Wu, M., Xia, A., Zandieh, A., and Zhu, X. (2001) Science 291, 1304–1351

17. Mohrenweiser, H. W., and Jones, I. M. (1998) Mutat. Res. 400, 15–24
18. Mohrenweiser, H. W., Wilson, D. M., III, and Jones, I. M. (2003) Mutat. Res. 526, 93–125
19. Xi, T., Jones, I. M., and Mohrenweiser, H. W. (2004) Genomics 83, 970–979
20. Lunn, R. M., Helzlsouer, K. J., Parshad, R., Umbach, D. M., Harris, E. L., Sanford, K. K., and Bell, D. A. (2000) Carcinogenesis 21, 551–555
21. Kohno, T., Shimura, K., Tosaka, M., Tani, M., Kim, S. R., Sugimura, H., Nohmi, T., Kasai, H., and Yokota, J. (1998) Oncogene 16, 3219–3225
22. Hadi, M. Z., Coleman, M. A., Fidelis, K., Mohrenweiser, H. W., and Wilson, D. M., 3rd. (2000) Nucleic Acids Res. 28, 3871–3879
23. Goode, E. L., Ulrich, C. M., and Potter, J. D. (2002) Cancer Epidemiol. Biomarkers Prev. 11, 1513–1530
24. McCullough, A. K., Dodson, M. L., and Lloyd, R. S. (1999) Annu. Rev. Biochem. 68, 255–285
25. Copeland, R. A. (1996) Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis, pp. 93–119, VCH Publishers, Inc., New York
26. Valko, M., Leibfried, D., Moncol, J., Cronin, M. T., Mazur, M., and Telser, J. (2007) Int. J. Biochem. Cell Biol. 39, 44–84
27. Fishel, M. L., Vasko, M. R., and Kelley, M. R. (2007) Mutat. Res. 614, 24–36
28. Dizdaroglu, M. (2005) Mutat. Res. 591, 45–59
29. Kalam, M. A., Haraguchi, K., Chandani, S., Loechler, E. L., Moriya, M., Greenberg, M. M., and Basu, A. K. (2006) Nucleic Acids Res. 34, 2305–2315
30. Wiederholt, C. I., and Greenberg, M. M. (2002) J. Am. Chem. Soc. 124, 7278–7279
31. Hegde, V., Kelley, M. R., Xue, Y., Mian, I. S., and Deutsch, W. A. (2001) J. Biol. Chem. 276, 27591–27596
32. Broderick, P., Bagratuni, T., Vijayakrishnan, J., Lubbe, S., Chandler, I., and Houlston, R. S. (2006) BMC Cancer 6, 243