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Biochemical Characterization of Eight Genetic Variants of Human DNA Polymerase κ Involved in Error-free Bypass Across Bulky *N*^{2}-Guanyl DNA Adducts

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Abstract

DNA polymerase (pol) κ, one of the Y-family polymerases, is thought to function in the error-free translesion DNA synthesis (TLS) opposite the bulky *N*^{2}-guanyl DNA lesions induced by many carcinogens such as polycyclic aromatic hydrocarbons. We analyzed the biochemical properties of eight pol κ variants positioned in the polymerase core domain, using the recombinant pol κ (residues 1–526) protein and the DNA template containing an *N*^{2}-CH_2(9-anthracenyl)G (*N*^{2}-AnthG). The truncation R219X was devoid of polymerase activity, and the E419G and Y432S variants showed much lower polymerase activity than wild-type pol κ. In steady-state kinetic analyses, E419G and Y432S displayed 20- to 34-fold decreases in k_{cat}/K_m for dCTP insertion opposite G and *N*^{2}-AnthG compared to wild-type. The L21F, I39T, and D189G variants, as well as E419G and Y432S, displayed 6- to 22-fold decreases in k_{cat}/K_m for next-base extension from C paired with *N*^{2}-AnthG, compared to wild-type pol κ. The defective Y432S variant had a 4- to 5-fold lower DNA-binding affinity than wild-type, while a slightly more efficient S423R variant possessed a 2- to 3-fold higher DNA-binding affinity. These results suggest that R219X abolishes —and the E419G, Y432S, L21F, I39T, and D189G variations substantially impair—TLS ability of pol κ opposite bulky *N*^{2}-G lesions in the insertion step opposite the lesion and/or the subsequent extension step, raising the possibility that certain non-synonymous pol κ genetic variations translate into individual differences in susceptibility to genotoxic carcinogens.

Graphical Abstract

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Supporting Information. Analysis of human pol κ^{1-526} wild-type and variant proteins by SDS-polyacrylamide gel electrophoresis (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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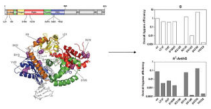

Keywords

DNA polymerase κ; translesion DNA synthesis; carcinogen; DNA adduct; genetic variation

(INTRODUCTION)

The cellular genome is continuously damaged by endogenous and exogenous agents. The various cellular transactions dealing with DNA damage determine the level of residual DNA lesions and their final biological outcome. The cellular capacity for efficient and faithful repair and replication against DNA damage can be important determinants for genome maintenance and cell survival.[1] Cells utilize robust DNA repair systems to remove the potentially harmful DNA lesions, but these are often imperfect and can leave unfixed lesions in DNA. Unrepaired DNA lesions can induce replication errors and/or blockage of DNA polymerases during DNA replication, which can lead to genetic mutation and cell death.[2] Cells are equipped with DNA damage tolerance system involving specialized DNA polymerases, mainly belonging to the Y-Family, to carry out translesion DNA synthesis (TLS) at DNA lesions that block replicative polymerases and thus avoid the permanent cell-cycle arrest and cell death in the face of potential mutagenic risks.[3] Each of the Y-Family polymerases can perform its unique TLS, differing in both the efficiency (e.g. high to low) and fidelity (e.g. error-free to error-prone) depending on the lesion type, and thus they can play potentially dual roles in both the lesion bypass and mutagenesis in cells.[4]

The exocyclic N^2-amino group of guanine in the DNA minor groove is often attacked by various potential carcinogens, to form guanyl N^2-adducts varying in size and shape.[5] Bulky N^2-G adducts are commonly formed from covalent interaction of activated bulky carcinogens, including the oxidation metabolites of estrogens, heterocyclic amines, polycyclic aromatic hydrocarbons, and tamoxifen catalyzed by cytochrome P450 enzymes.[6] These bulky N^2-G adducts can frequently induce DNA replication errors and mutations in bacterial and mammalian cells.[7] We have previously addressed the biochemical details of replicative bypass across various N^2-G adducts by various DNA polymerases. Y-Family DNA polymerases could bypass bulky N^2-G adducts that block replicative DNA polymerases, and the lesion bulk could be an important factor for determining the extent of replication blockage and errors (e.g. misincorporation) by each DNA polymerase.[8–16]

DNA polymerase (pol) κ is one of the four human Y-Family DNA polymerases and is specialized in bypassing bulky minor-groove N^2-G adducts very efficiently in an error-free manner.[8] Prokaryotic and archaeal orthologs of pol κ, *Escherichia coli* dinB (pol IV) and *Sulfolobus solfataricus* P2 DNA polymerase IV (Dpo4), are also able to copy DNA relatively efficiently and faithfully past some N^2-G adducts.[15,17] However, some subtle differences are apparent in that replicative bypass of benzo[*a*]pyrene-derived N^2-G adducts is error-free by pol κ but is mutagenic by Dpo4,[18–20] which might be related to their distinct structural features, for example, an additional N-terminal extension and an enlarged

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structural gap between the core and little finger domains of pol κ, compared to Dpo4.[20,21] Multiple lines of *in vitro* and *in vivo* evidence have suggested that a group of bulky N^2-G adducts such as N^2-benzo[*a*]pyrene diol epoxide (BPDE)-G, N^2-(1-carboxyethyl)-G, N^2-(estradiol-6-yl)-G, and α-(N^2-deoxyguanosyl) tamoxifen, as well as an acrolein-mediated N^2-G interstrand cross-link, might be among the cognate or most-favored lesion substrates of pol κ.[3, 22–28] Pol κ-deficient mouse embryonic fibroblast cells show a high sensitivity to both killing and mutagenesis to benzo[*a*]pyrene[23] and an impaired TLS (i.e. decrease in both bypass efficiency and fidelity) across an N^2-benzo[*a*]pyrene diol epoxide-G on a shuttle vector plasmid.[27] Pol κ is specifically required for recovery from S-phase checkpoint arrest in mouse cells following exposure to BPDE.[28] One haplotype involving a noncoding variation in the *POLK* gene was significantly associated with a reduced risk of lung cancer,[29] although its specific mechanism has not been elucidated. In these respects, it can be postulated that pol κ might play a role in preventing mutagenesis from bulky carcinogen-bound N^2-G adducts in cells, and thus the altered functional status of pol κ by genetic variations might modify an individual's risk of mutation and cancer from exposure to certain chemical carcinogens.

The human *POLK* gene encodes the pol κ protein consisting of 870 amino acids. Its N-terminal polymerase core region (amino acids 19–526) is known to be indispensable for catalytic activity of pol κ and its ternary complex crystal structure has been determined.[30,31] To the present time, a total of ~136 germline variations in *POLK* gene have been described for human individuals in dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP), but the functional effects of these genetic variations have not been biochemically evaluated yet. Biochemical investigations to assess the direct effects of germline genetic variations on the pol κ function are important for understanding and predicting their biochemical mechanisms and biological consequences prior to or after exploring their clinical association. In the present study, we paid particular attention to the non-synonymous coding *POLK* gene variations located in the polymerase core domains and have been predicted to have deleterious effects by *in silico* prediction tools such as SIFT and PolyPhen,[32–34] in that they would be likely to affect the catalytic property of pol κ and thus quantitatively and/or qualitatively modify its TLS function. In order to identify and characterize the functional genetic variations of human pol κ, we investigated the biochemical impact of eight selected missense or nonsense genetic variations on the enzymatic properties of human pol κ regarding normal incorporation and bulky N^2-G adduct bypass. We performed the experiments with full-length primer extensions, steady-state kinetics, and pol-DNA binding, using the recombinant catalytic core (1–526 amino acids) proteins of wild-type and eight variant human pol κ enzymes with oligonucleotide DNA templates containing an undamaged G or a bulky N^2-CH_2(9-anthracenyl)G (N^2-AnthG) adduct. Here we describe that seven germline genetic variations could alter enzyme function for DNA synthesis upon normal and/or N^2-AnthG-adducted DNA substrates, some of which also alter DNA-binding affinity. These findings are discussed in the context of understanding the likely mechanistic and functional consequences of altered TLS with pol κ variants.

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EXPERIMENTAL PROCEDURES

Materials.

T4 polynucleotide kinase and dNTPs were purchased from New England Biolabs (Ipswich, MA). [γ−^{32}P]ATP (specific activity 3 × 10^3 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA). Bio-spin columns were purchased from Bio-Rad (Hercules, CA). A protease inhibitor cocktail was obtained from Roche Applied Science (Indianapolis, IN). The pBAD-TOPO TA expression kit was from Invitrogen (Carlsbad, CA) and the QuickChange mutagenesis kit was from Stratagene (La Jolla, CA). FPLC columns were purchased from GE Healthcare (Uppsala, Sweden).

DNA Substrates.

24-mer (5′−GCC TCG AGC CAG CCG CAG ACG CAG−3′) and 25-C-mer (5′−GCC TCG AGC CAG CCG CAG ACG CAG C−3′) oligonucleotides were obtained from Midland Certified Reagent Co. (Midland, TX). Two 36-mer oligonucleotides (3′−CGG AGC TCG GTC GGC GTC TGC GTC XCT CCT GCG GCT−5′; X = G or N^2-CH_2(9-anthracenyl)G (N^2-AnthG)) containing a G or N^2-AnthG (Figure 1) were prepared as previously described.[9, 10] An 18-FAM-mer (5′−(FAM)-AGC CAG CCG CAG ACG CAG−3′; FAM = 6-carboxyfluorescein) was prepared from Bioneer (Daejeon, Korea). 24-mer and 25-mer were used as primers, and 36-mers were used as templates. Primers were 5′ end-labeled using T4 polynucleotide kinase with [γ−^{32}P]ATP and annealed with template to make duplex primer-template DNA substrates.

Selection of Human *POLK* Gene Variations Having Potentially Functional Impact.

We made an effort to search for human *POLK* gene variations that might be highly probable to alter enzyme function. We first looked for the naturally occurring genetic variations in the coding region of the *POLK* gene from the public database dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP). We then narrowed these to the likely dysfunctional variations based on three criteria: i) non-synonymous coding variations that result in amino acid change (missense) or premature termination of translation (nonsense), ii) variations located in the polymerase core domain (amino acid residues 19 to 526), and iii) missense variations expected to exert deleterious or damaging effects on protein function by SIFT and Polyphen prediction programs.[32–34] We thus selected seven missense and one nonsense genetic variants and performed detailed biochemical analyses using recombinant protein pol κ^{1-526} proteins purified from *Escherichia coli*. Current information for the eight *POLK* gene variations included in this study is summarized in Table 1, based on public databases such as dbSNP, HapMap (http://www.hapmap.org), and 1000 genomes (http://browser.1000genomes.org).

Construction of Expression Vectors for Catalytic Cores of Eight Pol κ Variants.

The gene fragment covering the catalytic core of wild-type pol κ was obtained by PCR amplification from the vector pAcHLT/HPOLK[8] as template using AccuTaq LA DNA polymerase (Sigma Aldrich, St. Louis, MO) with a pair of primers: 5′-GCC ATG GGA CAT CAC CAC CAT CAT CAC ATG GAT AGC ACA AAG GAG-3′ and 5′-CTA TTG TTG

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GTG TTT CCT GTC-3′. The resulting PCR products of 1.6 kb *POLK core* were cloned into the vector pBAD-TOPO. The N-terminal leader sequence encoding 12 extra amino acids in the vector was removed by *Nco*I digestion and the resulting vector was relegated to form the pBAD-wtPOLK$^{1-526}$ vector, which can generate the pol κ core protein (amino acids 1–526) with an *N*-terminal MGHHHHHH tag. Following sequence confirmation of the wild type pol κ core gene insert, each of the eight different substitutions in the *POLK* gene was created with a QuickChange mutagenesis kit with the pBAD-wtPOLK$^{1-526}$ vector as template. The oligonucleotide primers for introducing the point mutations in *POLK* were 5′-CTT CTG CTT AGG ATG GGA TTT AAT GAT AAT AAA GCA GGA ATG G-3′ for L21F, 5′-GAA AAT TAA CAA AAC TAT AAT GGA AGC CAC GAA GGG GTC C-3′ for I39T, 5′-GCT GAT TAT GGT CCC AAT TTT ATG GCC ATG AGT CTT-3′ for D189G, 5′-GGC CTG AGG ATA AAA TAA GGT ATT TCA TCA AAA TGG GAA GC-3′ for R219I, 5′-GCC TGA GGA TAA ATG AAG GTA TTT CAT CAA AAT GGG AAG C-3′ for R219X, 5′-GGA AAA GTA TGA GCG TTG GGA GGA CAT TCA GTG AG-3′ for E419G, 5′-CGT TGA GAG GAC ATT CCG TGA GAT AAA TAA AGC GG-3′ for S423R, 5′-CGG AAG AGC AAT CCA GCC TAT GTC AAG AAC TTT GC-3′ for Y432S (and its corresponding antiparallel primer for each mutation). All eight substitutions were confirmed by nucleotide sequence analyses of the constructed vectors.

Expression and Purification of Recombinant Proteins.

The wild type and variant forms of recombinant pol κ core proteins were expressed in *E. coli* strain TOP10 cells. *E. coli* TOP10 harboring each vector for the recombinant protein was grown in Luria-Bertani broth supplemented with ampicillin (100 μg ml^{-1}) at 37 °C, with aeration, to an OD_{600} of 0.6. L-Arabinose was added to 0.02% (w/v), and incubation was continued for 14 h at 16 °C. The cells were harvested by centrifugation and resuspended in lysis buffer (50 mM Tris-HCl, pH 7.4, containing 300 mM NaCl, 10% glycerol (v/v), 5 mM β-mercaptoethanol, 1 mg lysozyme mL^{-1}, and protease inhibitor cocktail (Roche Applied Sciences, Indianapolis, IN)), cooled on ice for 30 min, and then lysed by sonication (12 × 10 s duration with a Branson digital sonifier microtip, (VWR, West Chester, PA), 45% amplitude, with intervening cooling time). The cell lysate was clarified by centrifugation at 4 × 10^4 × *g* for 60 min at 4 °C. The resulting supernatant was loaded onto a 1-mL HisTrap HP column and the column was washed with 20 mL of Buffer A (50 mM Tris-HCl (pH 7.4), 300 mM NaCl, 10% glycerol (v/v), and 5 mM β-mercaptoethanol) containing 40 mM imidazole. His-tagged pol κ core fractions (eluted with 400 mM imidazole in buffer A) were collected and diluted 6-fold with buffer B (50 mM Tris-HCl (pH 7.4), 1 mM EDTA, 10% glycerol (v/v), and 5 mM β-mercaptoethanol). Pol κ core was further purified to near homogeneity with the use of a Mono S column and a 50 mM to 2 M NaCl gradient in buffer B. Pol κ core was eluted at ~400 mM NaCl. Protein concentrations were estimated using a Bradford protein assay, and the quality of purified proteins was assessed by SDS-polyacrylamide gel electrophoresis and Coomassie Brilliant Blue staining (Figure S1, Supporting Information).

DNA Polymerization Assays and Steady-State Kinetic Analysis.

Standard DNA polymerase reactions of 8 μL were performed in 50 mM Tris-HCl (pH 7.5) buffer containing 50 mM NaCl, 5 mM dithiothreitol, 100 μg mlL^{-1} bovine serum albumin

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(BSA) (w/v), and 10% glycerol (v/v) with 100 nM primer-template substrate at 37 °C. Reactions were initiated by the addition of dNTPs and $MgCl_2$ (5 mM final concentration) to preincubated enzyme/DNA mixtures and terminated with six volumes of a solution of 20 mM EDTA in 95% formamide (v/v). For steady-state kinetic analysis, the primer-template was extended in the presence of 0.1–10 nM pol κ with increasing concentrations of individual dNTPs for 5 or 10 min, when the maximum amount of extension products was ≤20% of total DNA substrates. Products were resolved using a 16% polyacrylamide (w/v) gel electrophoresis system containing 8 M urea and visualized using a Bio-Rad Personal Molecular Imager and Quantity One™ software (Bio-Rad). The product formation rates (as a function of dNTP concentration) were plotted to estimate the kinetic parameters K_m and k_{cat} by non-linear regression fitting to the Michaelis-Menten equation using Graph Pad Prism 5.0 software (GraphPad, San Diego, CA). Misinsertion frequency (f) opposite G or N^2-AnthG was calculated as $f = (k_{cat}/K_m)_{dNTP}/(k_{cat}/K_m)_{dCTP}$.[35]

Fluorescence Anisotropy Experiments.

The 5 nM 5'−6-carboxyfluorescein-labeled 18-FAM-mer primer annealed with 36-mer template DNA containing an unmodified G or N^2-AnthG was incubated with varying concentrations (0 – 500 nM) of pol κ for 20 min at 25 °C. The polymerase-DNA binding reaction was performed in the presence of 50 mM HEPES buffer (pH 7.5) containing 10 mM potassium acetate, 10 mM KCl, 0.1 mM $ZnCl_2$, 5 mM $MgCl_2$, 0.1 mM EDTA, 2 mM β-mercaptoethanol and 0.1 mg mL^{-1} BSA as modified from a previous study.[36] Fluorescence anisotropy was measured with a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, Santa Clara, CA) equipped with a manual polarizer, using excitation and emission wavelengths of 471 and 525 nm, respectively. The anisotropy data were plotted vs. enzyme concentration and fit to a quadratic equation to estimate $K_{d,DNA}$ using the equation: $A = A_0 + (A_{max} - A_0)((D_t + E_t + K_d) - ((D_t + E_t + K_d)^2 - (4D_tE_t))^{½})/(2D_t)$, where A is measured anisotropy, A_0 is initial anisotropy (DNA alone), A_{max} is maximum anisotropy, D_t is the concentration of fluorescein-labeled DNA, E_t is enzyme concentration, and K_d is the equilibrium dissociation constant for enzyme binding to DNA.

RESULTS

Overall Strategy.

The objective of this study was to characterize the functional germ-line genetic variations in human pol κ. For this, we first chose human *POLK* genetic variations likely to impair the enzyme function of pol κ, from the dbSNP database. We selected eight functional candidate variations by searching for non-synonymous coding variations that reside in the polymerase core domains and also are predicted to be dysfunctional by prediction programs or a premature translation termination site. Thereafter, we investigated the biochemical effects of eight variations on the catalytic properties of pol κ in both normal and translesion DNA replication across G and N^2-AnthG. A set of experiments, including full-length primer extension, steady-state kinetics of nucleotide insertion opposite the lesion and the subsequent extension, and pol-DNA binding assay, was performed sequentially using the recombinant catalytic core (1–526 amino acids) of these pol $κ^{1-526}$ enzymes and oligonucleotides containing a normal G or a cognate N^2-AnthG adduct at a defined site.

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Primer Extension Across G and N^2-AnthG with All Four dNTPs by Wild-type and Variant Pol κ Enzymes.

In order to assess the possible alterations in activities for normal and translesional DNA synthesis by wild-type and eight pol κ variants, we extended the 24-mer primers annealed to 36-mer templates containing unmodified G or N^2-AnthG (Figure 1) at the 25th position in the presence of all four dNTPs using the wild-type and variant pol κ proteins (Figure 2). N^2-AnthG was chosen as a model bulky minor-groove N^2-G DNA adduct, which was found to be bypassed efficiently and accurately by human pol κ.[8] Wild-type pol κ readily extended the primers past G and yielded products up to about 33-mers in proportion to enzyme concentration, and this pattern was also observed with the L21F, I39T, D189G, R219I, and S423R variants. However, the E419G and Y432S variants showed much less activity than wild type pol κ, indicating the severe impairment of polymerase activities due to those substitutions. For TLS polymerization across N^2-AnthG, wild-type pol κ readily extended primers past N^2-AnthG and yielded substantial products up to about 33-mers, which was similarly observed with the R219I and S423R variants (although the extent of bypass synthesis past N^2-AnthG was less than that past G). However, the L21F, I39T, and D189G variants generated substantially reduced amounts of extended products compared to wild-type pol κ, indicating considerable attenuation of their lesion-bypass activities. The E419G and Y432S variants generated only a trace of one-base or almost no extension at N^2-AnthG with 1 nM enzyme concentrations, indicating the severe diminution of their lesion-bypass activities. As expected, the R219X variant did not show any activity with either DNA substrate, indicating the total loss of polymerase activity due to its premature translation termination at the codon 219.

Primer Extension Beyond an N^2-AnthG:C Pair with All Four dNTPs by Wild-type and Variant Pol κ Enzymes.

Primer extension (“next-base”) experiments were done with a 25-C-mer primer containing a 3′-end C positioned opposite an N^2-AnthG of a 36-mer template in the presence of all four dNTPs by the wild-type and eight variant pol κ proteins to qualitatively evaluate the subsequent extension activities of pol κ variants from the N^2-AnthG:C pair in comparison with wild-type pol κ (Figure 3). The R219I and S423R variants extended the primers past N^2-AnthG to a similar extent as wild-type pol κ; however the L21F, I39T, and D189G variants generated extension products less efficiently than wild type pol κ, indicating considerable attenuation of their extension ability following C insertion opposite an N^2-AnthG due to amino acid substitution. The E419G and Y432S variants yielded only trace extension products past N^2-AnthG, while the truncated R219X showed no extension, as expected.

Steady-State Kinetics of Nucleotide Incorporation Opposite N^2-AnthG by the Wild-type and Variant Pol κ Enzymes.

Steady-state kinetic parameters were determined for incorporation of single nucleotides into 24-mer/36-mer duplexes opposite a G or N^2-AnthG by pol κ variants in comparison to wild-type (Tables 2 and 3). The specificity constant (k_{cat}/K_m) and misinsertion frequency, $f = (k_{cat}/K_m)_{\text{incorrect dNTP}}/(k_{cat}/K_m)_{\text{correct dNTP}}$, were utilized as semi-quantitative indicators for

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the catalytic efficiency and fidelity of a distributive enzyme, pol κ, as in previous work.[37] For correct dCTP insertion opposite undamaged G, the L21F, I39T, D189G, and R219I variants showed k_{cat}/K_m values similar to wild-type pol κ, with relative efficiencies of 0.5 to 1.0 (Table 2). Those variants also had relatively low misinsertion frequencies (for incorrect nucleotides), comparable to wild-type pol κ. However, the E419G and Y432S variants showed 22- and 34-fold reductions in k_{cat}/K_m values for correct dCTP insertion opposite G compared to wild-type pol κ, which was accompanied by about 4- to 20-fold increase in misinsertion frequencies for dGTP and dATP. Similar trends of results were observed with the N^2-AnthG template (Table 3). The E419G and Y432S variations caused about 20-fold reductions in the k_{cat}/K_m values for correct dCTP insertion opposite N^2-AnthG compared to the wild-type enzyme, as well as 4- to 17-fold increases in misinsertion frequencies for dGTP, dATP, and dTTP opposite N^2-AnthG. Noticeably, the L21F, I39T, and D189G variants showed 2- to 3-fold decreases in k_{cat}/K_m for correct dCTP insertion opposite N^2-AnthG compared to wild-type pol κ, which might in part explain the substantially diminished bypass at N^2-AnthG with those variants. On the other hand, the S423R variant displayed 2- to 3-fold increases in k_{cat}/K_m for correct dCTP insertion opposite G and N^2-AnthG compared to wild-type pol κ, while showing interesting alterations in nucleotide insertion fidelity, 3- to 4-fold decreases in dATP and dGTP misinsertion frequencies opposite N^2-AnthG but 3- to 4-fold increases in dTTP and dATP misinsertion frequencies opposite G.

Steady-State Kinetics of Next-Base Extension from an N^2-AnthG:C Pair by Wild-type and Variant Pol κ Enzymes.

Our finding that the primer extensions past N^2-AnthG were considerably retarded with many pol κ variants (Figures 2 and 3) led to further steady-state kinetic analysis for the subsequent extension step following dCTP insertion opposite N^2-AnthG by pol κ enzymes. Steady-state kinetic parameters were determined for incorporation of the next complementary nucleotide, dGTP, opposite template C following the adducted N^2-AnthG:C base pair into 36-N^2-AnthG-mer/25-C-mer template-primer DNA by wild-type pol κ and the variants, in comparison with next-base extension from the normal G:C pair of a 36-G-mer/25-C-mer substrate (Table 4). Concerning the k_{cat}/K_m values for next-base extension from the G (or N^2-AnthG):C pair, the pol κ variants showed similar trends as with correct nucleotide insertion opposite G (or N^2-AnthG) in Tables 2 and 3. For instance, the E419G and Y432S variations caused severe (~20-fold) reductions in k_{cat}/K_m values in next-base extension from the N^2-AnthG:C pair compared to wild-type pol κ , which was similarly observed in the corresponding cases for dCTP incorporation opposite N^2-AnthG with those variants. However, the L21F, I39T, and D189G variants showed large (6- to13-fold) decreases in k_{cat}/K_m for next-base extension only from N^2-AnthG:C pair (but not from G:C pair) compared to wild-type pol κ, indicating that those variations might significantly impair the subsequent extension step beyond N^2-AnthG (but not G), which might in large part explain the exceptionally reduced bypass only at N^2-AnthG but not at undamaged G with those variants in Figure 2B.

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Binding of the Wild-type and Variant Pol κ Enzymes to DNA Containing G or N^2-AnthG.

The effects of eight genetic variations on DNA binding affinity of pol κ were evaluated using fluorescence anisotropy experiments. The equilibrium dissociation constants ($K_{d,DNA}$) of the wild type and variant pols κ were estimated by fitting the anisotropy of fluorescein-labeled primer-template DNA (18-FAM-mer/36-mer) as a function of increasing concentration of pol κ to a quadratic equation (Table 5). The $K_{d,DNA}$ of the wild-type pol κ for unmodified DNA was similar to that for N^2-AnthG-adducted DNA, indicating that DNA-binding affinity of pol κ is not affected by an N^2-CH_2(9-anthracenyl) group at G, which was also similarly observed in most of variant pol κ enzymes. The $K_{d,DNA}$ values of Y432S were 4- to 5-fold higher for both N^2-AnthG-adducted and unmodified DNA substrates than for wild-type pol κ, indicating a relatively large decrease in DNA-binding affinity of pol κ by the Y432S substitution. In contrast, the S423R variant showed 2- to 3-fold increases in binding affinity for DNA substrates, which might partly be related to slight increases in relative efficiencies for correct nucleotide insertion opposite G and N^2-AnthG with this variant compared to wild-type pol κ in Tables 2 and 3. It was also observed that the L21F variation caused a slight (2-fold) reduction in the $K_{d,DNA}$ of pol κ only for unmodified DNA but not for N^2-AnthG-adducted DNA (compared to wild-type). However, this was not a large change, which might in part explain a slightly less diminution of relative efficiency in dCTP insertion opposite G compared to N^2-AnthG with this variant in Tables 2 and 3.

DISCUSSION

In this work, we have investigated the biochemical properties of eight non-synonymous coding variants of human pol κ, a key player in the error-free TLS of minor-groove DNA adducts. Seven missense and a nonsense variations were selected for study because they are presumed to possibly cause dysfunctional impacts on pol κ on the basis of their locations, types, and predicted effects. Our biochemical data reveal that the E419G and Y432S variations severely impair both the efficiency and fidelity of pol κ for both normal and translesion DNA synthesis across G and N^2-AnthG, while the L21F, I39T, and D189G variations considerably impair pol κ efficiency chiefly for translesion DNA synthesis (particularly in the subsequent extension step) past N^2-AnthG. As anticipated, the truncated R219X variant, which loses large parts of the palm, thumb, and PAD domains, lacked polymerase activity. Two variations positioned in the PAD domain were also found to substantially influence DNA binding of pol κ but in opposite ways, in that the Y432S variation reduces DNA-binding affinity by 4- to 5-fold, while S423R increases it by 2- to 3-fold.

This is the first study, to our knowledge, to analyze functional alterations in germline genetic variants of human pol κ using biochemical approaches. Seven among the eight studied *POLK* genetic variations were found to at least partially affect the enzymatic function of pol κ in translesion DNA synthesis and/or DNA binding. These functional genetic variations seem to be rare, in that their minor allele frequencies are < 1% or not available yet in human populations (beyond their reported occurrence in a human) (Table 1). Recent articles in the literature propose the possible relevance of rare genetic variants to human disease risks. The rare genetic variations that can be really abundant in human populations and more likely to

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be functional than common variations, have been proposed to be potential sources of unexplained heritability in complex human diseases, while only a small fraction of the known disease heritability have been explained by common genetic variants in genome-wide association studies.[38–41] Even spontaneous new (*de novo*) germline mutations, i.e. the most extreme form of rare genetic variations, have been implicated in rare and common forms of neurodevelopmental diseases.[42] Three somatic *POLK* mutations, which might result in a missense change at codon 205 or abnormal splicing, were reported in prostate tumors, although their functional impacts are not revealed yet.[43] Rare variants are being increasingly identified with technological progress and application of the high-throughput next-generation sequencing. The number of non-synonymous *POLK* gene variations reported in dbSNP increased from 21 in 2008 into 58 in 2011 and 91 in 2013, all of which are likely to be rare because their minor allele frequencies are < 1% or unavailable. Our biochemical approach is a good initial step to identify the functional pol κ variants and reveal their mechanistic insights, although we have focused on only eight selected "probably functional" coding variants in this study. Nine additional non-synonymous coding *POLK* variations, which are putatively deleterious, have been added in dbSNP since our study began and are worth exploring further.

Seven functional pol κ variants identified in this study can be categorized into four types according to the changes of polymerase efficiencies represented by overall bypass efficiencies[44] across G and N^2-AnthG (Fig. 4), which are the variant devoid of activity (R219X), variants severely impaired of both normal and TLS efficiency (E419G and Y432S), variants impaired mainly in TLS efficiency (L21F, I39T, and D189G), and the variant showing slight enhancement of polymerase efficiency (S423R). We also note that the values for overall bypass efficiencies with each pol κ variants are in good agreement with the qualitative results of full-length primer extension experiments in Fig 2. The fidelity of nucleotide insertion opposite G and N^2-AnthG did not seems to be affected in most variants but was severely impaired with the E419G and Y432S variants (Tables 2 and 3), due to a greater reduction of insertion efficiency for correct dCTP than for the other nucleotides with those defective variants.

Structural views of pol κ may be helpful in understanding the mechanistic aspects of genetic variations (Fig. 5). Pol κ possesses the classic polymerase domains (finger, palm, and thumb), the Y-family specific "polymerase associated domain (PAD)" (also known as little finger) which makes the majority of DNA contacts, and the unique N-clasp domain which fully encircles DNA to allow the efficient extension of mismatched or distorted primer termini.[30] In the crystal structures with DNA containing bulky N^2-G adducts at the post-insertion position, Dpo4, which is an archaeal ortholog of pol κ but lacks the N-clasp domain, accommodates an N^2-CH_2(2-naphthyl) group of G in the *syn* (or *anti* in a disordered nonproductive conformation) orientation between the template DNA and the little finger domain in a productive conformation,[15] while flipping/looping-out bulkier N^2-BPDE-G extrahelically into the gap between the little finger and core domains in productive conformation (or intercalating the benzo[*a*]pyrene ring into the DNA helix in a nonproductive conformation).[20] Computational modeling of human pol κ postulates a good accommodation of an N^2-BPDE-G in the minor-groove side and a structural role of the N-clasp domain in near error-free TLS of N^2-BPDE-G.[45] In these circumstances, it can be

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postulated that pol κ might accommodate an N^2-AnthG, which is smaller than N^2-BPDE-G but bulkier than N^2-CH_2(2-naphthyl)G, well in the active site by means of N-clasp and PAD domains. We can also assume that non-synonymous *POLK* genetic variations in the N-clasp and PAD domains as well as in classic polymerase domains seem to be likely to affect the lesion bypass capability of pol κ from the available structural information.[30,31,46]

It is noteworthy that L21F and I39T variations, located in the N-clasp domain, caused considerable catalytic impairment of N^2-AnthG bypass, particularly in the subsequent extension step from C inserted opposite N^2-AnthG, which might be due to local perturbation at the N-clasp domain (proposed to tolerate distortions at the primer terminus).[30,45] Interestingly the D189G variation at a loop between αE helix and β5 strand of the catalytic palm domain also seemed to impair the subsequent extension step of lesion bypass, which might come from the local structural alteration of that loop positioned near the 3′ terminal nucleotide of the primer at the post-insertion position. It is also remarkable that two E419G and Y432S variations (though not S423R) positioned at the PAD domain caused severe reductions in general polymerase activity of pol κ, indicating the necessity of an intact PAD domain to hold DNA near the active site for full catalytic efficiency of pol κ. A strong catalytic defect of the E419G variant, in spite of its normal DNA binding affinity, seems to be related to the loss of a long side chain with negative charge in a Glu residue lying in the template DNA side across the active site residues, which might perhaps induce a conformational deformation in the active site cleft. Differing DNA binding affinities between the Y432S and S423R variants might in part explain the opposite functional changes in these two variants. A severe catalytic defect of the Y432S variant, which loses an aromatic benzene ring in a Tyr residue at the solvent-facing αQ helix supporting the four-stranded β sheet of the PAD domain, is likely to be attributable to a moderate reduction in DNA binding affinity, as well as possible structural destabilization of the PAD domain. In contrast, a slight increase in polymerase efficiency with the S423R variant might be related to its higher DNA binding affinity, possibly due to gain of a positively charged Arg residue near the phosphosugar backbone at 5′ unpaired region of template DNA.

How would these functional *POLK* genetic variations influence TLS process across bulky N^2-G DNA adducts relating to mutagenesis in cells? Eukaryotic TLS processing of DNA lesions is supposed to involve polymerase switching between replicative and TLS polymerases, mediated by the protein-protein interactions including PCNA and the additional post-translational modifications such as ubiquitination at a site of DNA damage, where a set of Y-family polymerases are recruited and utilized.[47, 48] Accordingly, the mutation rate and mutational spectrum of a specific DNA lesion in cells might be influenced by overall kinetic behavior of various DNA polymerases, mainly TLS polymerases, utilized for the lesion. When encountering a bulky N^2-G adduct such as N^2-BPDE-G during DNA replication, normal cells might best utilize pol κ, which is most kinetically favored (i.e. most efficient) and, moreover, most accurate at bulky N^2-G lesion bypass among Y-family pols,[8] and thus enable largely error-free TLS. If a cell possesses only kinetically defective pol κ variants, e.g., E419G and Y432S, we can speculate that other less accurate TLS pols (e.g., pols η and ι) can be more frequently employed at bulky N^2-G lesions, which would increase error-prone TLS events and thus result in more mutations in cells. Several observations support this view, especially a possible alternative role of pol η in pol κ-deficient cells: pol

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κ-deficient mouse embryonic fibroblasts show markedly elevated G to T transversions after benzo[*a*]pyrene exposure[23] and preferential dATP misinsertion opposite a site-specific N^2-BPDE-G in a shuttle vector,[27] which is in good agreement with the proclivity of pol η to preferentially misincorporate dATP opposite N^2-BPDE-G *in vitro*.[19,49] Conversely, if the cell has inherently more efficient pol κ variants with similar or higher accuracy, e.g. S423R, and utilize it correctly at bulky N^2-G damage sites, more error-free TLS events at bulky N^2-G lesions would result, with and fewer mutations in cells. It is also worth examining other non-synonymous coding variations located in protein interaction domains of pol κ to bind other proteins such as PCNA, ubiquitin, and REV1, in that they might alter cellular TLS events such as polymerase switching and coordination. It is also plausible that noncoding functional variations in regulatory regions for pol κ might be able to alter pol κ expression levels in cells and thus influence TLS events, although this aspect was not examined here. Pol κ has been also suggested to be involved in nucleotide excision repair,[50,51] homologous recombination repair,[52] and replication checkpoint response,[53] all of which have a role in preventing DNA damage-induced mutagenesis, and assuring the maintenance of genome stability. Collectively, it is likely that functional genetic variations (either coding or noncoding) of pol κ might alter the multiple cellular pathways of TLS, DNA repair, and checkpoint control of genotoxic stress in cells, which might affect in turn overall genomic stability and thus modify an individual's risk to cancer. In these aspects, it is desirable to perform further studies to verify the *in vivo* impact of those functional pol κ variants in both cellular and organismal contexts.

In conclusion, our results suggest that seven germline genetic variations in human *POLK* gene may either hamper or facilitate the error-free TLS capability of pol κ across bulky N^2-G DNA lesions, generating different mutation phenotypes in the affected individuals, i.e. predisposing or protecting from mutagenesis following exposure to certain genotoxic carcinogens, e.g. polycyclic aromatic hydrocarbons. The identification of dysfunctional genetic variations for human pol κ in this work may provide insight into our understanding of individual differences in mutation phenotypes to specific mutagens and related disease risks in human populations and in future investigations for deciphering genetic markers on various TLS DNA polymerases for individual mutation risks related to cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations:

A	adenine

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BPDE	benzo[*a*]pyrene diol epoxide
BSA	bovine serum albumin
C	cytosine
dNTP	deoxynucleoside triphosphate
EDTA	ethylenediaminetetraacetic acid
G	guanine
***N*^{2}-Anth**	N^2-CH_2(9-anthracenyl)
PCR	polymerase chain reaction
pol	DNA polymerase
SDS	sodium dodecyl sulfate
T	thymine
TLS	translesion DNA synthesis

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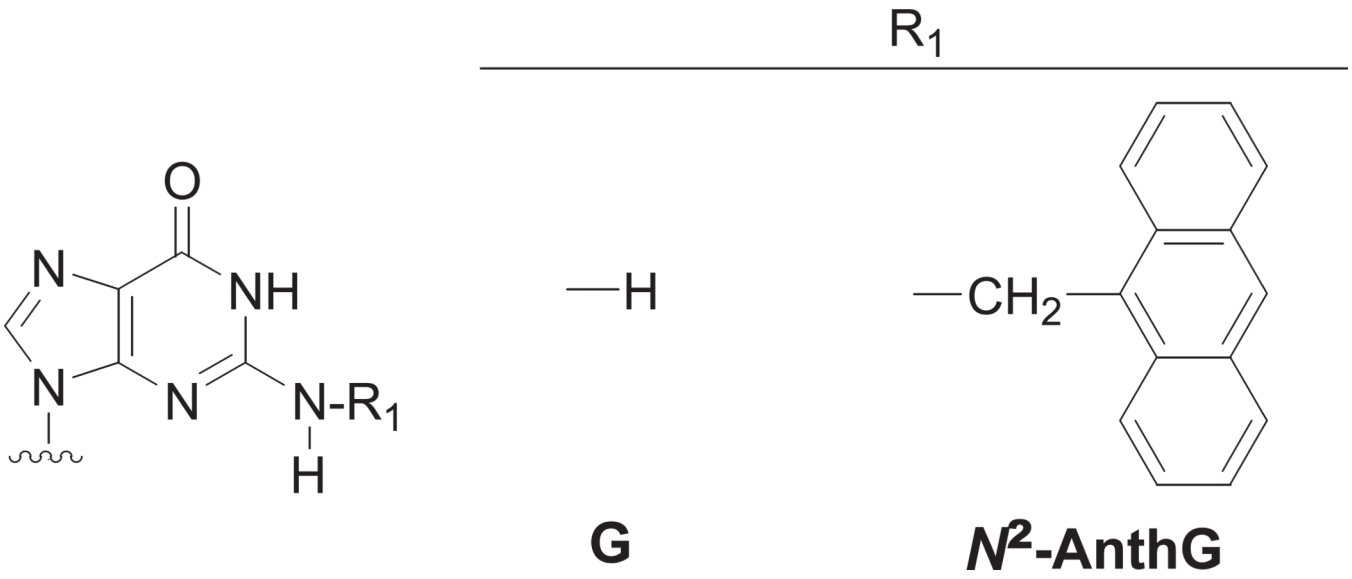

Figure 1. ***N*^{2}-CH$_2$(9-anthracenyl)G (*N*^{2}-AnthG).**
Structures of *N*^{2}-AnthG and normal G are shown.

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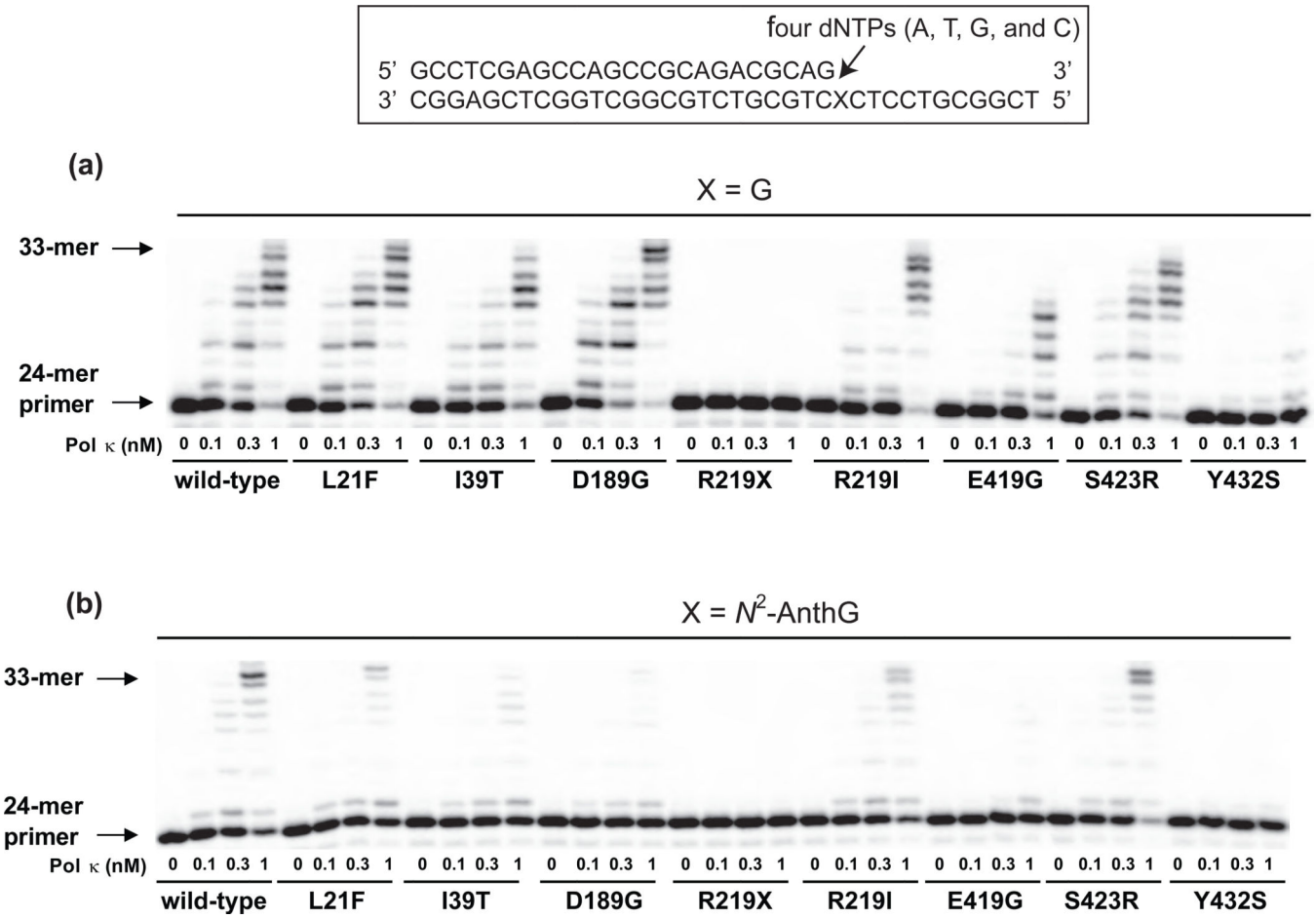

Figure 2. Extension of a ^{32}P-labeled 24-mer primer across G or N^2-AnthG at position 25 by human wild-type and variant pol κ^{1-526} enzymes.

Primer (24-mer) was annealed with each of the two different 36-mer templates, containing an unmodified G or N^2-AnthG placed at the 25th position from the 3′-end. Reactions were done for 15 min with increasing concentrations of pol κ^{1-526} (0 − 1 nM) and DNA substrate (100 nM primer/template) as indicated. ^{32}P-labeled 24-mer primer was extended in the presence of all four dNTPs (50 μM each). The reaction products were analyzed by denaturing gel electrophoresis and phosphorimaging. (A) Extension across G. (B) Extension across N^2-AnthG.

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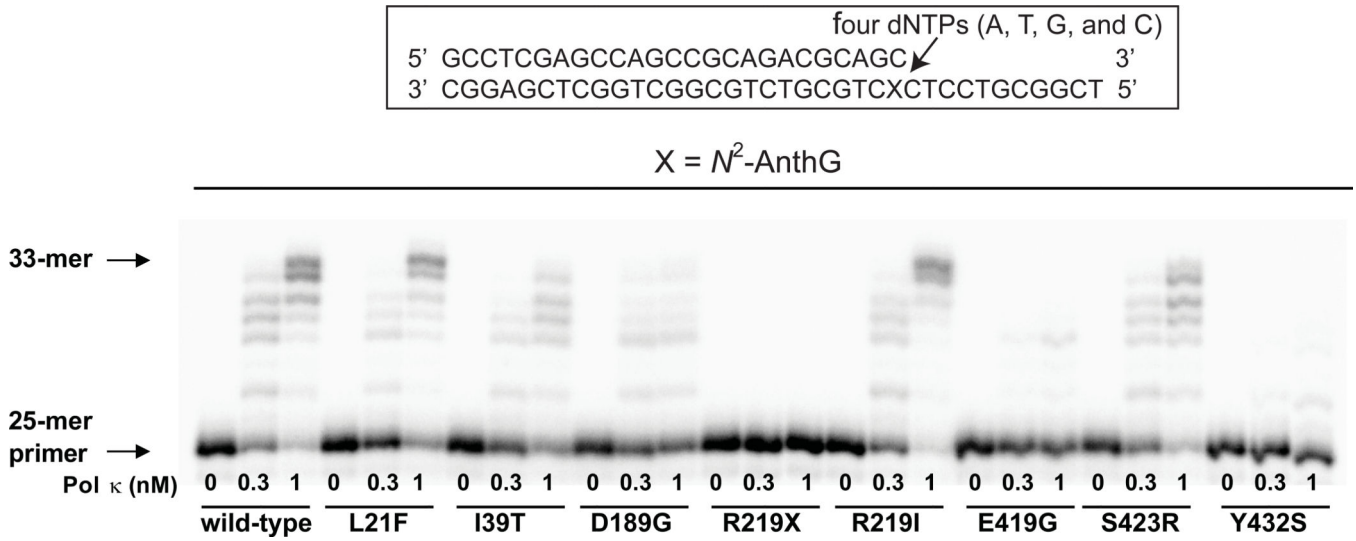

Figure 3. Extension of a ^{32}P-labeled 25-mer primer beyond an N^2-AnthG:C pair by human wild-type and variants pol κ^{1-526} enzymes.

Primer (25-C-mer) containing a C at the 3'end was annealed with the 36-mer template (36-N^2-AnthG-mer) containing an N^2-AnthG placed at the 25th position from the 3′-end. Reactions were done for 15 min with increasing concentrations of pol κ^{1-526} (0 − 1 nM) and DNA substrate (100 nM primer/template) containing an N^2-AnthG:C pair as indicated. ^{32}P-labeled 25-mer primer was extended in the presence of all four dNTPs (50 μM each). The reaction products were analyzed by denaturing gel electrophoresis and phosphorimaging.

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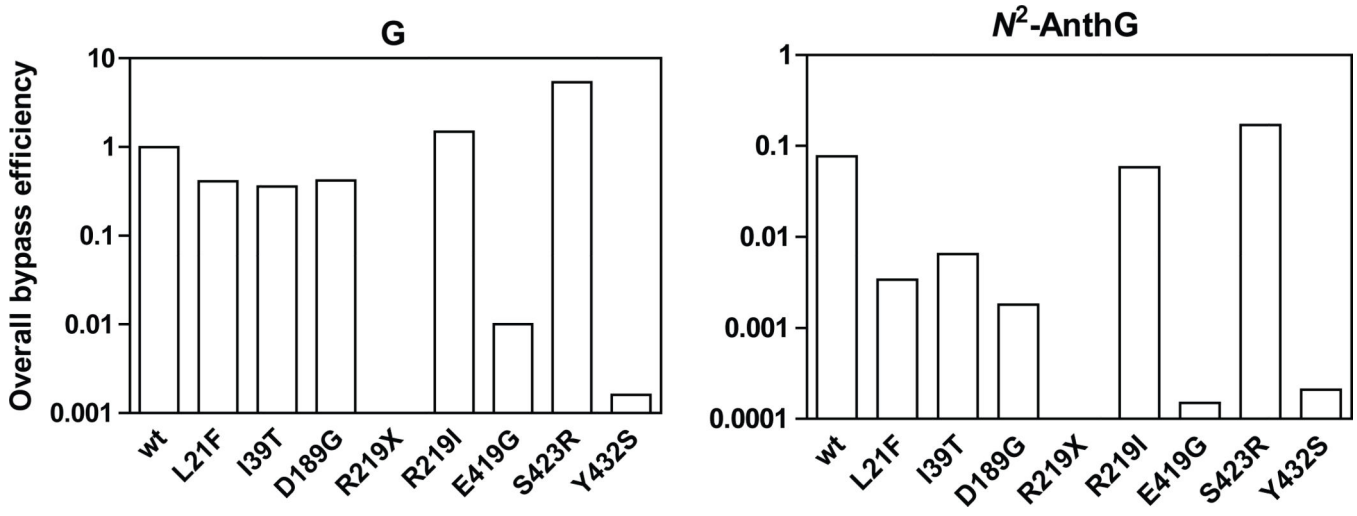

Figure 4. Overall bypass efficiencies of human variant pol κ^{1-526} enzymes for G and N^2-AnthG compared to the wild-type.

The overall bypass efficiency was calculated by multiplying the relative efficiency for dCTP insertion opposite G (or N^2-AnthG) and the relative efficiency for the subsequent next-base extension by each pol κ^{1-526}, based on the insertion and extension kinetic parameters in Tables 2–4 as described previously.[44]

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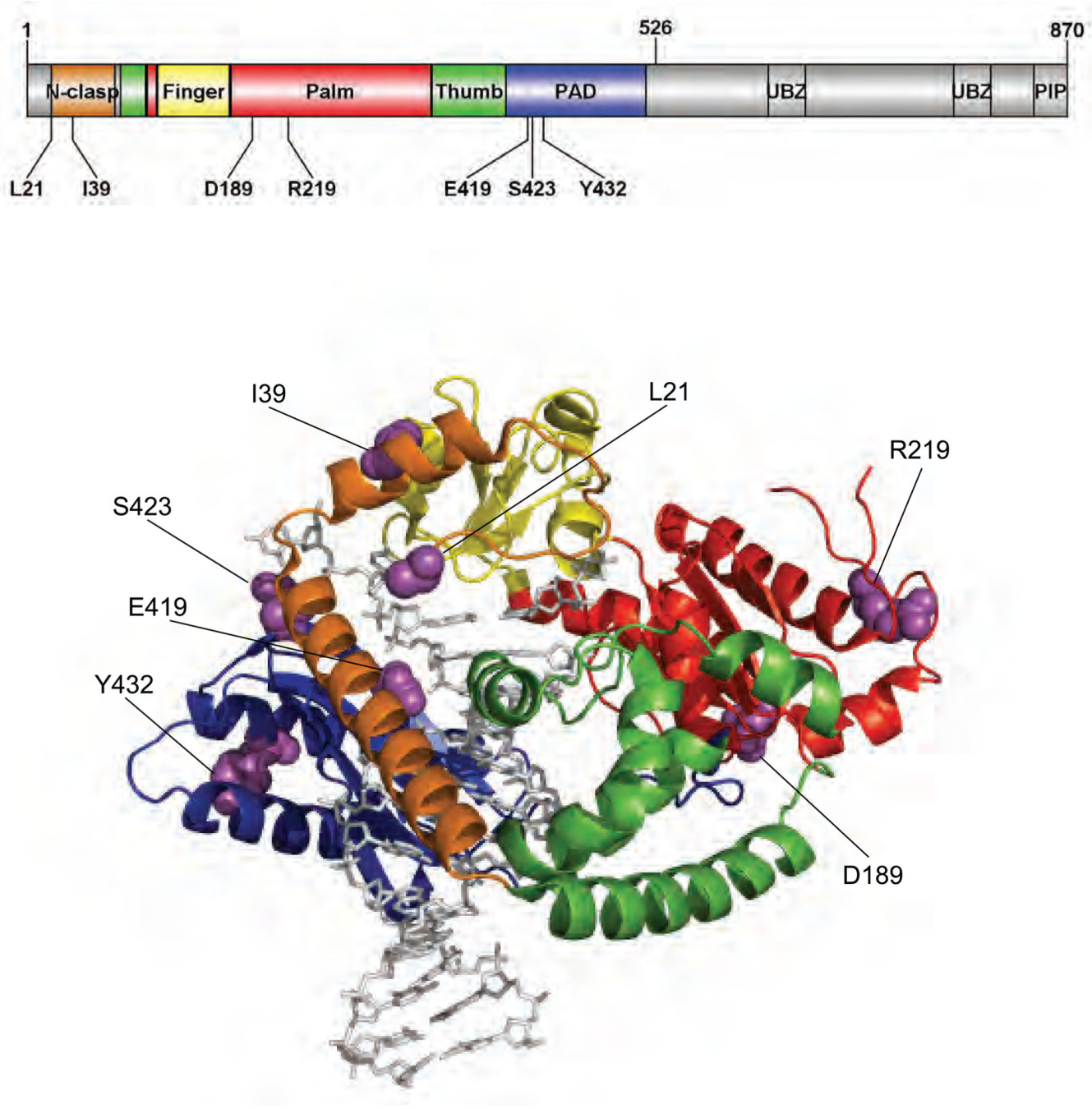

Figure 5. Locations of genetic pol κ variations.

Structure of human pol κ^{21-517} (PDB code, 2OH2) bound to primer/template DNA and incoming nucleotide is shown using Pymol. Pol κ^{21-517} is shown in cartoon ribbons, and the primer/template DNA and dNTP are shown in gray sticks. The N-clasp, finger, palm, thumb, and PAD domains are colored orange, yellow, red, green, and blue, respectively. The amino acid residues (in purple spheres) of genetic pol κ variants are indicated. The structural

domains of pol κ are shown in the upper schematic diagram using DOG (version 2.0),[54] where locations of amino acids related to eight studied variations are indicated.

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Table 1.

POLK gene variations studied.

rs ID[a]	nucleotide change	amino acid change	protein domain	minor allele frequency	prediction[b]	
					SIFT	PolyPhen-2
rs3104729	c.61C>T	L21F	N-clasp	0.006[c]	damaging	probably damaging
rs3094258	c.116T>C	I39T	N-clasp	N/A[d]	damaging	probably damaging
rs111689950	c.566A>G	D189G	Palm	N/A	damaging	probably damaging
rs3094265	c.655A>T	R219X[e]	Palm	N/A	N/A	N/A
rs3104717	c.656G>T	R219I	Palm	0.008[c]	damaging	probably damaging
rs111584802	c.1256A>G	E419G	PAD	N/A	damaging	probably damaging
rs35257416	c.1267A>C	S423R	PAD	0.004[f]	damaging	possibly damaging
rs77612491	c.1295A>C	Y432S	PAD	N/A	damaging	probably damaging

[a] A reference SNP identification number provided by dbSNP.

[b] Possible functional effects of genetic variations are predicted *in silico* using SIFT and PolyPhen-2.[32–34]

[c] From International HapMap Project.

[d] Not available.

[e] X denotes a termination codon.

[f] From 1000 Genomes project.

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Table 2.

Steady-state kinetic parameters for dNTP incorporation opposite G by human wild-type and variant pol κ^{1-526} enzymes

pol κ^{1-526}	dNTP	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($s^{-1}M^{-1}$)	f_{ins} [a]	relative efficiency [b]
wild-type	A	470 ± 70	0.0074 ± 0.0003	0.016	0.00055	
	T	1600 ± 100	0.19 ± 0.01	0.12	0.0041	
	G	150 ± 9	0.029 ± 0.0004	0.19	0.0066	
	C	21 ± 3	0.61 ± 0.026	29	1	1
L21F	A	520 ± 90	0.0025 ± 0.0002	0.0048	0.00024	
	T	630 ± 160	0.023 ± 0.002	0.037	0.0019	
	G	200 ± 40	0.013 ± 0.001	0.065	0.0033	
	C	55 ± 9.6	1.1 ± 0.06	20	1	0.69
I39T	A	820 ± 60	0.0078 ± 0.0002	0.0095	0.00043	
	T	980 ± 400	0.059 ± 0.01	0.060	0.0027	
	G	250 ± 40	0.014 ± 0.001	0.056	0.0025	
	C	27 ± 4	1.1 ± 0.06	22	1	0.76
D189G	A	1000 ± 300	0.0067 ± 0.001	0.0067	0.00048	
	T	1900 ± 310	0.033 ± 0.003	0.017	0.0012	
	G	740 ± 110	0.010 ± 0.001	0.014	0.0010	
	C	81 ± 11	1.1 ± 0.05	14	1	0.48
R219I	A	530 ± 80	0.018 ± 0.001	0.034	0.0012	
	T	840 ± 60	0.18 ± 0.01	0.21	0.0072	
	G	170 ± 30	0.013 ± 0.001	0.076	0.0026	
	C	38 ± 3	1.1 ± 0.03	29	1	1.0
E419G	A	900 ± 140	0.0084 ± 0.001	0.0093	0.0072	
	T	2200 ± 380	0.017 ± 0.002	0.0077	0.0059	
	G	600 ± 110	0.020 ± 0.002	0.033	0.025	
	C	150 ± 34	0.19 ± 0.018	1.3	1	0.045
S423R	A	140 ± 30	0.016 ± 0.001	0.11	0.0023	
	T	530 ± 60	0.28 ± 0.01	0.53	0.011	
	G	93 ± 20	0.016 ± 0.001	0.17	0.0036	
	C	17 ± 1.7	0.80 ± 0.02	47	1	1.6
Y432S	A	1500 ± 200	0.014 ± 0.001	0.0093	0.011	
	T	1200 ± 300	0.057 ± 0.003	0.0048	0.0056	
	G	500 ± 60	0.020 ± 0.001	0.040	0.047	
	C	130 ± 19	0.11 ± 0.0066	0.85	1	0.029

[a] Misinsertion frequency, calculated by dividing k_{cat}/K_m for each dNTP incorporation by the k_{cat}/K_m for dCTP incorporation opposite G.

[b]Relative efficiency, calculated by dividing k_{cat}/K_m of each pol κ^{1-526} for dCTP incorporation opposite G by k_{cat}/K_m of wild type pol κ^{1-526} for dCTP incorporation opposite undamaged G.

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Table 3.

Steady-state kinetic parameters for dNTP incorporation opposite N^2-AnthG by human wild-type and variant pol κ^{1-526} enzymes

pol κ^{1-526}	dNTP	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($s^{-1}M^{-1}$)	f_{ins} [a]	relative efficiency [b]
wild-type	A	160 ± 30	0.00074 ± 0.00003	0.0046	0.0014	
	T	360 ± 70	0.0015 ± 0.0001	0.0042	0.0013	
	G	32 ± 3	0.00029 ± 0.000003	0.0091	0.0028	
	C	380 ± 30	1.2 ± 0.03	3.2	1	0.11
L21F	A	400 ± 90	0.00020 ± 0.00001	0.00050	0.00055	
	T	510 ± 90	0.0013 ± 0.0001	0.0025	0.0027	
	G	73 ± 10	0.00023 ± 0.00001	0.0032	0.0035	
	C	1100 ± 68	1.0 ± 0.03	0.91	1	0.031
I39T	A	390 ± 90	0.00039 ± 0.00002	0.0010	0.00059	
	T	330 ± 10	0.0012 ± 0.00002	0.0036	0.0021	
	G	69 ± 10	0.00020 ± 0.00001	0.0029	0.0017	
	C	820 ± 230	1.4 ± 0.17	1.7	1	0.059
D189G	A	450 ± 80	0.000067 ± 0.000003	0.00015	0.00015	
	T	520 ± 120	0.0010 ± 0.0001	0.0019	0.0019	
	G	510 ± 40	0.00067 ± 0.00002	0.0013	0.0013	
	C	1000 ± 200	1.0 ± 0.06	1.0	1	0.034
R219I	A	230 ± 40	0.00053 ± 0.00003	0.0023	0.00068	
	T	330 ± 30	0.0024 ± 0.0001	0.0073	0.0021	
	G	85 ± 10	0.00053 ± 0.00001	0.0062	0.0018	
	C	590 ± 180	2.0 ± 0.27	3.4	1	0.12
E419G	A	760 ± 36	0.0025 ± 0.0001	0.0033	0.024	
	T	1100 ± 220	0.0015 ± 0.0002	0.0014	0.010	
	G	77 ± 14	0.00011 ± 0.00001	0.0014	0.010	
	C	1600 ± 290	0.23 ± 0.01	0.14	1	0.0048
S423R	A	120 ± 10	0.00053 ± 0.00002	0.0044	0.00050	
	T	230 ± 40	0.0035 ± 0.0001	0.015	0.0017	
	G	27 ± 3	0.00018 ± 0.00001	0.0067	0.00076	
	C	170 ± 11	1.5± 0.03	8.8	1	0.30
Y432S	A	600 ± 100	0.00057 ± 0.00003	0.00095	0.0059	
	T	520 ± 50	0.0011 ± 0.00003	0.0021	0.013	
	G	81 ± 9	0.00029 ± 0.00001	0.0036	0.023	
	C	320 ± 110	0.050 ± 0.004	0.16	1	0.0055

[a] Misinsertion frequency, calculated by dividing k_{cat}/K_m for each dNTP incorporation by the k_{cat}/K_m for dCTP incorporation opposite N^2-AnthG.

[b] Relative efficiency, calculated by dividing k_{cat}/K_m of each pol κ^{1-526} for dCTP incorporation opposite N^2-AnthG by k_{cat}/K_m of wild type pol κ^{1-526} for dCTP incorporation opposite undamaged G in Table 2.

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Table 4.

Steady-state kinetic parameters for next base extension from G (or N^2-AnthG): C pair template:primer termini by human wild-type and variant pol κ^{1-526} enzymes

base-pair at 3′ primer termini (template:primer)	pol κ^{1-526}	extension with dGTP (the correct nucleotide opposite next template C)			
		K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m (mM^{-1} s^{-1})	relative extension efficiency[a]
G:C	wild-type	5.0 ± 0.9	0.28 ± 0.02	56	1
	L21F	9.0 ± 1.3	0.30 ± 0.02	33	0.59
	I39T	6.0 ± 1.3	0.16 ± 0.01	27	0.48
	D189G	8.2 ± 0.7	0.40 ± 0.01	49	0.88
	R219I	6.0 ± 1.0	0.50 ± 0.03	83	1.5
	E419G	23 ± 7	0.30 ± 0.03	13	0.23
	S423R	1.4 ± 0.2	0.27 ± 0.01	190	3.4
	Y432S	25 ± 4	0.080 ± 0.004	3.2	0.057
N^2-AnthG:C	wild-type	14 ± 2	0.54 ± 0.02	39	0.70
	L21F	65 ± 18	0.41 ± 0.06	6.3	0.11
	I39T	97 ± 12	0.60 ± 0.03	6.2	0.11
	D189G	200 ± 10	0.60 ± 0.02	3.0	0.054
	R219I	7.3 ± 3.0	0.20 ± 0.02	27	0.48
	E419G	27 ± 6	0.048 ± 0.004	1.8	0.032
	S423R	3.7 ± 0.4	0.12 ± 0.03	32	0.57
	Y432S	24 ± 3	0.051 ± 0.003	2.1	0.038

[a]Relative extension efficiency, calculated by dividing k_{cat}/K_m of each pol κ^{1-526} for each dGTP incorporation opposite the next template C from the G (or N^2-AnthG):C pair by k_{cat}/K_m of wild type pol κ^{1-526} for dGTP incorporation opposite the next template C from the undamaged G:C pair.

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Table 5.

Binding affinities of wild-type and variant pol κ enzymes for DNA substrates

pol κ^{1-526}	K_d (nM)	
	18-FAM-mer/36-G-mer	18-FAM-mer/36-N^2-AnthG-mer
wild-type	50 ± 12	52 ± 16
L21F	27 ± 5	60 ± 9
I39T	42 ± 8	45 ± 15
D189G	58 ± 15	54 ± 11
R219I	50 ± 12	50 ± 8
E419G	40 ± 9	44 ± 9
S423R	21 ± 5	19 ± 3
Y432S	270 ± 60	220 ± 30