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# Increased dietary cholesterol promotes enhanced mutagenesis in DNA polymerase kappa-deficient mice

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ABSTRACT

DNA polymerase kappa (Polκ) bypasses planar polycyclic $N^2$-guanine adducts in an error-free manner. Cholesterol derivatives may interact with DNA to form similarly bulky lesions. In accordance, these studies examined whether increased mutagenesis of DNA accompanies hypercholesterolemia in *Polk*$^{-/-}$ mice. These mice also carried a*poE* gene knockouts to ensure increased levels of plasma cholesterol following exposure to a high cholesterol diet. The mice carried a reporter transgene (the λ-phage *cII* gene) for subsequent quantitative analysis of mutagenesis in various tissues. We observed significantly increased mutation frequencies in several organs of *apoE*$^{-/-}$*Polk*$^{-/-}$ mice following a high cholesterol diet, compared to those remaining on a standard diet. Regardless of dietary regime, the mutation frequency in many organs was significantly higher in *apoE*$^{-/-}$*Polk*$^{-/-}$ than in *apoE*$^{-/-}$*Polk*$^{+/+}$ mice. As expected for polycyclic guanine adducts, the mutations mainly consisted of G:C transversions. The life expectancy of *apoE*$^{-/-}$*Polk*$^{-/-}$ mice maintained on a high cholesterol diet was reduced compared to *apoE*$^{-/-}$*Polk*$^{+/+}$ mice. Overall, this study demonstrates a role for Polκ in bypass of cholesterol-induced guanine lesions.


## 1. Introduction

Many types of base damage can arrest semi-conservative DNA synthesis by high fidelity DNA polymerases [1]. In recent years a new class of highly error-prone DNA polymerases (so-called specialized DNA polymerases) has been discovered and extensively studied [2]. Nine such enzymes have been identified in mammalian cells, all of which share in common a high rate of misincorporation of nucleotides [3]. One of these enzymes, called DNA polymerase eta (Polη) encoded by the *Polh* gene, has been convincingly shown to *accurately* bypass thymine–thymine pyrimidine dimers generated by exposure of cells to ultraviolet (UV) radiation [4]. All human subjects examined to date who suffer from the variant form of the skin cancer-prone hereditary disease xeroderma pigmentosum (XP) carry inactivating mutations in the *Polh* gene [5]. These experiments suggest that most, if not all the remaining mammalian specialized DNA polymerases evolved to accurately replicate past other types of naturally occurring base damage by a process referred to as translesion DNA synthesis (TLS) [3].

The molecular mechanism of TLS remains to be established. However it has been hypothesized that the process involves a series of DNA polymerase switching events [6,7]. In one such switch a particular specialized DNA polymerase is thought to be brought into a primer-template position from which it can bypass the site of replication arrest, either accurately (thereby avoiding a mutation in the non-template strand) or inaccurately (in which case a mutation is generated). The mechanism by which a particular polymerase is selected for TLS past a particular form of base damage is also not understood. When the lesion in question is bypassed by the incorporation of one, or in the case of pyrimidine dimers two nucleotides, it is suggested that the replicative machinery replaces the specialized polymerase by a second switch and high fidelity DNA replication continues.

Our laboratory, as well as several others have provided evidence in support of the hypothesis that a specialized polymerase called DNA polymerase kappa (Polκ) encoded by the *Polk* gene, evolved to accurately bypass base damage associated with polycyclic compounds covalently bound to guanine in DNA. For example, accurate TLS past guanine residues carrying benzo[*a*]pyrenediolepoxide is well documented [8–13]. Accurate TLS by Polκ past other polycyclic $N^2$-guanine adducts has also been reported [14,15]. Notably too, the promoter region of the mouse and human *Polk* gene contains two xenobiotic response elements that facilitate the catabolism of polycyclic aromatic hydrocarbons [16].

Cholesterol and many of its metabolic derivatives are known to interact with DNA [17]. Correspondingly, various sex hormones and steroids, all of which share cholesterol as a synthetic precursor, have been reported to promote reduced survival in

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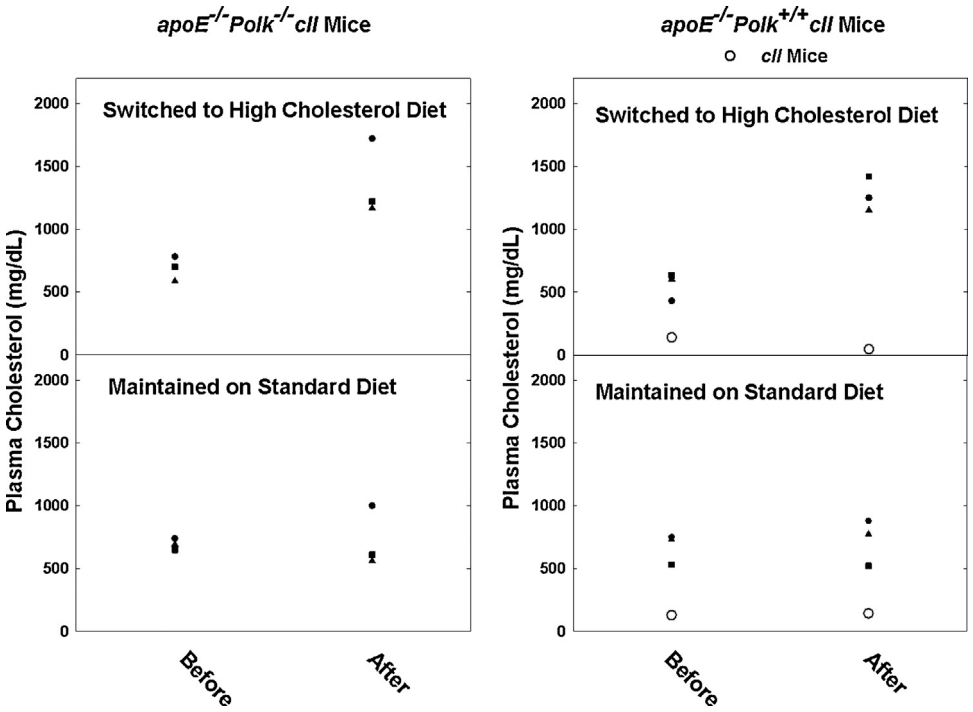


**Fig. 1.** Plasma cholesterol levels of mice used in the mutagenesis studies. For each mouse, cholesterol levels were determined immediately before (9 months of age) and after (12 weeks later) the diet. Symbols of before- and after-diet are identical for individual mice.

*Polk*$^{-/-}$ DT40 cells [18]. These results led us to hypothesize that hypercholesterolemia may stimulate the mutation frequency of DNA in *Polk*$^{-/-}$ mouse cells. Here we document the increased spontaneous mutation frequency in multiple tissues from *Polk*$^{-/-}$ mice genetically modified to facilitate raised levels of serum cholesterol when placed on a high cholesterol diet. We also report that the mutation spectra primarily involves G:C base pairs, consistent with the supposition that guanine is a major site of DNA damage.

## 2. Materials and methods

### 2.1. Production and maintenance of experimental mice

Mice were in a mixed 129 × C57BL/6 background. Mice carrying the *Polk*$^{tm1.1Esp}$ allele (*Polk*$^{-/-}$ mice) have been described previously [19]. *cII*, the reporter gene of transgenic mice for the λ Select-*cII* Mutation Detection System (Stratagene) will be used as a genotypic descriptor, rather than Big Blue [13]. *Polk*$^{+/-}$*cII* mice were cross-bred with *apoE*$^{-/-}$ mice obtained from Jackson Laboratories. Resultant a*poE*$^{-/-}$*Polk*$^{+/-}$*cII* mice served as breeders to generate the mice used in these studies. Mice were housed in a conventional mouse facility that was not specific-pathogen-free. Food (6% fat mouse chow) and water were provided *ad libitum*. High cholesterol mouse food contained 2% (w/v) added cholesterol (Harlan Teklad).

### 2.2. Genotyping

DNA was isolated from tails with a tissue DNA kit (Qiagen). Genotyping for *Polk* and *cII* were reported previously [13]. For *apoE* genotyping, primers apoE WT-F (5′-GCCTAGCCGAGGGAGAGCCG-3′), apoE WT-R (5′-TGTGACTTGGGAGCTCTGCAGC-3′), and apoE MUT-R (5′-GCCGCCCCGACTGCATCT-3′) (0.5 mM each) were used at a 68 °C annealing temperature to amplify either a 245 bp fragment (mutant), a 155 bp fragment (WT), or both (heterozygous).

### 2.3. Identification and characterization of λ-cII$^{-}$ mutants

This procedure has been described in more detail elsewhere [13]. Tissues harvested at the time of sacrifice (12 months) of various genotypic combinations of *cII* mice were flash frozen and stored at −80 °C. DNA was isolated from kidney, liver, spleen, lung and brain using the RecoverEase DNA isolation kit (Stratagene).

The *cII* genes from these DNA preparations were inserted into λ phage using Stratagene Transpack packaging extract. Packaging extracts were diluted in SM buffer, then transformed into G1250 *E. coli* culture in 10 mM $MgSO_4$. Cells were plated onto TB1 plates using molten TB1 top agar (55 °C). Two sets of triplicate transformation plates were used for phage titering and grown at 37 °C overnight. The remainder of the transformed G1250 was used to screen for mutants. These plates were grown at room temperature (24 °C) for 48 h.

Putative mutant plaques were cored and stored in SM buffer in 96-well plates at 4 °C. These plaques were individually subjected to low density transformation on TB1 media and grown at 24 °C for 48 h. Visible plaques were considered to be verified mutants. These were cored, transferred to SM buffer in 96-well plates, and stored at 4 °C.

Verified mutant plaques in SM buffer were used as PCR templates using CII-F and CII-R primers [13]. Aliquots of the PCR reactions were treated with ExoSap-It (USB). Samples were then sequenced with the CII-R primer using the ABI Big Dye Terminator Cycle Sequencing Kit on an automated ABI Prism 3100 Genetic Analyzer. Finally, *cII* mutations were analyzed with Sequencher 4.9 (Gene Codes Corp.) and mutation frequencies were determined as described previously [13].

Statistical significance of differences in mutation frequencies between high cholesterol and standard diet experimental groups was calculated for each organ using the Wilcoxon rank-sum test to obtain a two-tailed *p*-value (http://elegans.swmed.edu/~leon/stats/utest.cgi).