Research Article

Association of Base Excision Repair Gene Polymorphisms with ESRD Risk in a Chinese Population

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The base excision repair (BER) pathway, containing OGG1, MTH1 and MUTYH, is a major protector from oxidative DNA damage in humans, while 8-oxoguanine (8-OHdG), an index of DNA oxidation, is increased in maintenance hemodialysis (HD) patients. Four polymorphisms of BER genes, OGG1 c.977C > G (rs1052133), MTH1 c.247G > A (rs4866), MUTYH c.972G > C (rs3219489), and AluYb8MUTYH (rs10527342), were examined in 337 HD patients and 404 healthy controls. And the 8-OHdG levels in leukocyte DNA were examined in 116 HD patients. The distribution of MUTYH c.972 GG or AluYb8MUTYH differed between the two groups and was associated with a moderately increased risk for end-stage renal disease (ESRD) (P = 0.013 and 0.034, resp.). The average 8-OHdG/10 6 dG value was significantly higher in patients with the OGG1 c.977G, MUTYH c.972G or AluYb8MUTYH alleles (P < 0.001 via ANOVA). Further analysis showed that combination of MUTYH c.972GG with OGG1 c.977GG or AluYb8MUTYH increased both the risk for ESRD and leukocyte DNA 8-OHdG levels in HD patients. Our study showed that MUTYH c.972GG, AluYb8MUTYH, and combination of OGG1 c.977GG increased the risk for ESRD development in China and suggested that DNA oxidative damage might be involved in such process.

1. Introduction

Oxidative stress is characterized by an excess of reactive oxygen species (ROS) and leads to cellular injury via reactions with proteins, nucleic acids, and lipids [1, 2]. The DNA bases, especially guanine (G), are particularly susceptible to oxidation, for which ROS frequently lead to a plethora of oxidized guanine products [3]. 8-hydroxy-2′-deoxyguanosine (also known as 8-oxoguanine; 8-OHdG) is one of the most common mutagenic products and pairs with adenine in double-stranded DNA during DNA replication [3, 4]. If the mispairing is not repaired, it will lead to a G:C to T:A transversion mutation in cells [5].

Several repair pathways are involved with the DNA insults that result from either endogenous sources or exogenous sources, including the direct reversal pathway, the mismatch repair (MMR) pathway, the nucleotide excision repair (NER) pathway, and the base excision repair (BER) pathway [6]. Base excision repair (BER) is the primary DNA repair pathway that corrects base lesions that arise due to oxidative, alkylation, deamination, and depurination/depyrimidination damage, such as 8-OHdG [7]. Actually, the BER pathway specifically prevents those G: C-to-T : A mutations by the repair of 8-OHdG. It includes the MTH1, OGG1, and MUTYH genes that prevent, recognize and remove the misincorporated oxidized nucleotide, 8-OHdG, and the adenine paired with 8-OHdG, respectively, when initiated by the BER pathway.

Increasing evidence has shown that genetic polymorphisms in DNA repair genes may modulate DNA repair capacity, result in DNA damage accumulation, and then contribute to some complex diseases [8, 9]. Kasahara et al. have reported that MUTYH Gln324His (c.972G > C) is associated with increased risk of colorectal cancers [10]. Marchand et al. have described the effect of OGG1 Ser326Cys (c.977C > G) on the risk of lung cancer [11]. We have also shown that
the AluYb8 insertion in MUTYH (AluYb8MUTYH) might be a risk factor for age-related diseases and type 2 diabetes mellitus [12, 13].

The kidney is highly vulnerable to any of the results caused by ROS, and leukocyte 8-OHdG content is a surrogate biomarker for oxidation-induced DNA damage in patients with end-stage renal disease (ESRD), especially those on maintenance hemodialysis (HD). Oxidative injury is thought to alter the structure and function of glomeruli and is suggested to be related to renal diseases risk and eventual ESRD, as well as atherosclerosis, dialysis-related amyloidosis and anemia in incident dialysis patients [14, 15]. The primary role of DNA repair in ESRD may be complex. Fukushima et al. [16] demonstrated that the polymorphism of the hOGG1 (Ser326Cys) was associated with progression of IgA nephropathy. Most recently, Trabulus et al. [17] showed that XRCC1 Arg399Gln polymorphism may confer increased risk for the development of ESRD in Turkey, which is the first report showing an association between DNA repair gene polymorphisms and ESRD development. However, the genetic variations involved in antioxidant defense still need to be clarified in this disease, especially in China.

Based on the association of BER polymorphisms, oxidative DNA damages, and ESRD, we hypothesized that genetic variation in the BER genes might lead to repair impairment or disability, oxidative DNA damage accumulation, and pathogenesis of ESRD. Given the potential roles of OGG1 c.977C > G, MTH1 c.247G > A, MUTYH c.972G > C, and AluYb8MUTYH in the oxidative DNA repair pathway; we examined the association between these four polymorphisms in the BER pathway and ESRD in a Chinese cohort. We also assessed the leukocyte DNA 8-OHdG levels in HD patients to reveal the correlation between oxidative damage and end-stage renal disease arises.

### 2. Materials and Methods

#### 2.1. Subjects

The allelic frequency of OGG1 (NG_012106.1) c.977C > G, MTH1 (NC_000007.13) c.247G > A, MUTYH (NG_001819.1) c.972G > C, and AluYb8MUTYH (AluYb8 insertion at intron 15 of MUTYH [12]) was investigated in 337 HD patients, regardless of cause, in Nanjing, Jiangsu province, China, between October 2009 and February 2010. All patients had been maintained on hemodialysis protocols for >3 months and were reviewed for age, sex, and presentation of clinical and laboratory data. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg and/or use of antihypertensive medication [18]; anemia was defined as an Hgb <11 g/dL or use of recombinant human erythropoietin [19].

Healthy individuals with normal renal function were recruited from volunteers receiving health checkups in the same region. Detailed interview and various laboratory analyses were made upon every individual, including albumin excretion rate (AER) and serum creatinine. The subjects were excluded if their albumin excretion rate (AER) ≥ 30 mg/24 h, serum creatinine ≥ 1.2 mg/dL and ultrasound of the kidney and ureter was abnormal in size and appearance. They were ruled out if they suffering from certain diseases, such as acute inflammation, and diabetes, hypertension, autoimmune diseases or cancer according to past history and the clinical or laboratory characteristics. A total of 404 sex and age matched subjects were selected for inclusion in the control cohort. The Institutional Ethics Committee of Nanjing University School of Medicine approved this study, and written informed consents were obtained from all participants.

#### 2.2. High-Resolution Melting Analysis

In this study, OGG1 c.977C > G, MTH1 c.247G > A, and MUTYH c.972G > C were genotyped using the dsDNA dye LCGreen in combination with HRM analysis. DNA was extracted from peripheral blood samples, and PCR was performed to amplify the target sequences. The PCR primers were designed by LightScanner primer design software (Idaho Technology) (Table 1). Each PCR reaction was initially performed in a final reaction volume of 10 μL, using 25 ng of genomic DNA, 0.2 pmol of each primer, 0.8 μL 2.5 mM dNTPs, 1 μL 25 mM MgCl2, 1 μL 10 × Taq buffer with (NH4)2SO4, 0.4 U Taq DNA Polymerase (Fermentas), and 0.4 μL dimethyl sulfoxide (DMSO). The reaction mixture was incubated at 95°C for 5 min and then subjected to 40 cycles of 95°C for 30 sec, 55–58°C (Table 1) for 30 sec, and 72°C for 30 sec, followed by 72°C for 7 min using a PTC-200 thermal cycler (Bio-Rad).

The 9 μL reaction was supplemented with 1 μL 1 × LCGreen PLUS (Idaho Technology), and the 96-well plate (Bio-Rad) was transferred to the Light Scanner (Idaho Technology).
Figure 1: Demonstration of genotyping and sequence. (a) HRM directly discriminated the heterozygotes (OGG1 c.977 CG) and homozygotes (OGG1 c.977 CC or GG). (b) homozygous PCR products (CC or GG) were measured by LightScanner after being mixed with an equal amount of a known product (CC), which distinguished the wild homozygous samples (CC) from the variant ones (GG), as the mutational homozygotes (GG) were converted into heterozygotes (CG). (c) random samples from OGG1 c.977C > G testing were sequenced for confirmation. (d) The PCR products were separated using 1% agarose gels to assess the pattern of AluYb8 insertion into the MUTYH gene. Lanes 2, 4, 5, and 10: absence/absence (A/A); 3, 6, and 9: absence/presence (A/P); 1, 7, and 8: presence/presence (P/P); M: DNA Marker 2000.

Fluorescence data was collected over a temperature range of 70°C–97°C, as the samples were melted. Melting curve analysis was performed according to the manufacturer’s software. HRM could directly discriminate the heterozygote (CG) and homozygote (CC or GG) genotypes of OGG1 c.977C > G through melt scanning (Figure 1(a)). After mixing homozygous DNA with an equal amount of known PCR products (e.g., CC), it further distinguished between the CC and GG genotypes (Figure 1(b)). For further confirmation, 10% of samples from each group detected by HRM were randomly selected and subjected to DNA sequencing (Figure 1(c)). Similarly, the MTH1 c.247G > A and MUTYH c.972G > C polymorphisms were genotyped by HRM.

2.3. Agarose Gel Assay for AluYb8MUTYH Polymorphism. The PCR primers were listed in Table 1, and the PCR condition was carried out with an initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, extension at 72°C for 50 sec, and then a final extension at 72°C for 10 min. The PCR products were run out on 1% agarose gels (Invitrogen, Carlsbad, CA, USA). The AluYb8MUTYH genotypes were classified as homozygous absence of this variation (only 500 bp products, absence/absence, A/A), homozygous presence of this variation (only 826 bp products, presence/presence, P/P), and heterozygote (500 bp and 826 bp products, absence/presence, A/P), according to the variant fragment absence or presence (Figure 1(d)).

2.4. Measurement of 8-OHdG Levels in Genomic DNA of Blood Cells. For measuring the level of 8-OHdG, 116 patients were randomly rerecruited from the HD cohort and investigated by the method reported previously [12]. Briefly, DNA extraction from fasting venous whole blood (10 mL, with EDTA added to prevent coagulation) was performed within 1 h of collection, using the salting out method [20]. The purity of the DNA sample was checked by OD260 nm/OD280 nm and OD260 nm/OD230 nm using an Eppendorf BioPhotometer Plus (Eppendorf, North America). Acceptable DNA stored frozen at −80°C until all samples could be assayed at the same time.

The DNA (200 μg) of each sample was dissolved in 135 μL of water. Sodium acetate (15 μL, 200 mM) and Nuclease P1 (15 μL, 6 units, Sigma, USA) were added to the DNA solution and incubated at 37°C for 30 min. Tris-HCl buffer (15 μL, 1 M, pH 7.4) and alkaline phosphatase (7 μL, 2 units, TAKARA, Shiga, Japan) were added and incubated at 37°C
for another 30 min. The hydrolysate was filtered through Millipore Microcon columns at 14000 rpm for 10 min, and 50 µL of digested DNA was applied to one well of an ELISA kit (Highly Sensitive 8-OHdG Check, JaICA, Fukushima, Shizuoka, Japan). Results were measured in nanograms per milliliter, and then 1 ng/mL was converted to 4.8 8-OHdG/10^{6} dG based on Halliwell [21].

2.5. Statistical Analysis. All statistical analyses were carried out using the statistical program SPSS, version 15.0. Descriptive statistical values included mean ± SD values for continuous data and percentages for categorical data. Chi-squared tests were used to compare the genotype and allelic frequencies for patients and healthy controls. Odds ratios (OR) are shown with 95% confidence intervals (CIs). Separate comparisons of variables among subjects with different genotypes were conducted with ANOVA and followed by post hoc analysis. Since 8-OHdG levels in leukocyte DNA were positively skewed, a natural logarithmic transformation was used to normalize the distributions for analyses. In all cases, a P value of less than 0.05 was considered statistically significant.

3. Results

Of the 337 HD patients, 212 (62.9%) were men, and 125 (37.1%) were women. The average age was 53.1 ± 15.8 yrs (ranging from 22 to 85 yrs), and duration of hemodialysis was 4.0 ± 3.5 yr. Primary glomerulonephritis (GN) was the most prevalent kidney disease in the HD group: 207 (61.4%) developed ESRD as a result of GN, 51 (15.1%) as a result of hypertensive nephropathy (HN), 36 (10.7%) as a result of diabetic nephropathy (DN), 16 (4.7%) as a result of congenital or inherited causes, 6 (1.8%) as a result of systemic lupus erythematosus (SLE) and 21 (6.2%) as a result of other causes. Additionally, 267 (79.2%) of the patients had anemia, and 250 (74.2%) had hypertension. The 404 healthy individuals were age and sex matched, with a mean age of 53.1 ± 16.2 yrs, and 254 (62.9%) were males.

3.1. Genotyping of BER Polymorphisms in HD Patients. The frequencies of the OGG1 c.977C > G, MTH1 c.247G > A, MUTYH c.972G > C, and AluYb8MUTYH genotypes associated with HD were shown in Table 2. The distribution in the healthy controls of these polymorphisms was consistent with Hardy-Weinberg equilibrium (P > 0.05 for all).

Compared to healthy controls, the distribution of the genotypes in OGG1 c.977 C > G (namely, CC, CG, and GG) and the allele frequencies were not significantly different in the patients (P > 0.05, χ² test). For the c.247 G > A in MTH1, the frequency of heterozygous MTH1 c.247 G > A was only 6.2% and 7.7% in the patients and controls (P = 0.444), while the homozygote was not detected. Thus, the MTH1 polymorphism (c.247 G > A) was not included in further analysis.

Interestingly, both of the polymorphisms in the MUTYH gene showed an individual risk effect for ESRD (Table 2). For MUTYH c.972 G > C, the distribution of the three genotypes, namely CC, CG, and GG, and the allele frequencies were significantly different in HD patients (P = 0.046 and 0.026) compared with healthy controls. Furthermore, the frequency of the MUTYH c.972GG genotype was statistically higher in the HD cases (40.9%) than in the controls (32.2%), and the OR of GG adjusted by age and gender was 1.46 (95% CI: 1.08–1.98; P = 0.013). For AluYb8MUTYH, the distribution of the three genotypes and alleles in the HD patients was almost identical to that in the controls. Compared to the A/A genotype, the AluYb8MUTYH insertion carriers (A/P or P/P) were significantly higher in HD patients, and the OR was 1.40 (95% CI, 1.03–1.90; P = 0.034).

Regarding the effect of the MUTYH c.972GG genotype and the AluYb8MUTYH P allele on ESRD, a combined risk analysis was performed and shown in Table 3. Individuals carrying the MUTYH c.972GG genotype might have a higher risk for ESRD, and the OR of MUTYH c.972GG adjusted by age and gender was 2.23 (95% CI: 1.37–3.64; P = 0.001) among those with the OGG1 c.977GG genotype. Meanwhile, the presence of MUTYH c.972GG also added to the risk of AluYb8MUTYH A/P or P/P genotypes for ESRD development (OR, 1.46; 95% CI, 1.07–1.99; P = 0.017).

3.2. BER Polymorphisms in the Patients with Different Clinical Characteristics. The HD patients were stratified into six subgroups on the basis of the primary diagnoses (i.e., GN, HN, DN, congenital or inherited causes, SLE, or other causes). Similar to ESRD, the effects of BER polymorphisms on HD risk were confirmed in the 207 patients with primary diagnosis of glomerulonephritis when compared to the whole cohort (Table 4). The frequency of the MUTYH c.972 G > C GG genotype was significantly higher in cases than in controls, and the OR was 1.75 (95% CI: 1.24–2.47; P = 0.001). The frequency of the MUTYH AluYb8MUTYH A/P or P/P genotype was significantly higher in cases than in controls, and the OR was 1.73 (95% CI: 1.20–2.52; P = 0.003).

In addition, the association of BER polymorphisms with risk of HD complication status was further analyzed (Table 4). Among 267 patients with anemia, the frequency of MUTYH c.972G > C GG was markedly higher in patients than controls (42.7% versus 32.2%; OR (95% CI) = 1.57 (1.14–2.16); P = 0.006), whereas the AluYb8MUTYH insertion (A/P or P/P) significantly increased the risk for patients with anemia (73.4% versus 62.6%; OR (95% CI) = 1.78 (1.22–2.60); P = 0.003). A similar relationship was detected among 250 patients with hypertension. The frequency of MUTYH c.972GG carriers was higher in cases than controls (41.2% versus 32.2%; OR (95% CI) = 1.48 (1.07–2.05); P = 0.019). The frequency of AluYb8MUTYH insertion carriers (A/P or P/P) was higher in cases than controls (70.4% versus 62.6%; OR (95% CI) = 1.42 (1.01–1.99); P = 0.042).

3.3. Predictor Effect of BER Polymorphisms to 8-OHdG. The 8-OHdG levels in leukocyte DNA were evaluated in 116 HD patients divided into different subgroups according to the polymorphism genotypes and compared (Figure 2(a)). The genotypic frequencies of the three polymorphisms were
Table 2: Genotypes of OGG1, MTH1, and MUTYH and the risk for HD.

| Genotype                        | Patients (n = 337) | Controls (n = 404) | P valuea | OR (95%CI) |
|---------------------------------|--------------------|--------------------|----------|------------|
| OGG1 c.977 C > G               |                    |                    | 0.394    |            |
| CC                              | 56 (16.6%)         | 77 (19.1%)         |          |            |
| CG                              | 160 (47.5%)        | 200 (49.5%)        |          |            |
| GG                              | 121 (35.9%)        | 127 (31.4%)        | 0.199    | 1.22 (0.90–1.66) |
| CC or CGb                       | 216 (64.1%)        | 277 (68.6%)        |          | 1.00       |
| C allele                        | 0.404              | 0.438              |          | 1.00       |
| G allele                        | 0.596              | 0.562              | 0.180    | 1.15 (0.94–1.42) |
| MTH1 c.247 G > A               |                    |                    | 0.444    | 1.00       |
| GG                              | 316 (93.8%)        | 373 (92.3%)        |          |            |
| GA                              | 21 (6.2%)          | 31 (7.7%)          |          |            |
| AA                              | 0                  | 0                  |          |            |
| GA or AA b                      | 21 (6.2%)          | 31 (7.7%)          | 0.444    | 0.80 (0.45–1.42) |
| G allele                        | 0.969              | 0.962              |          | 1.00       |
| A allele                        | 0.031              | 0.038              | 0.453    | 0.81 (0.46–1.42) |
| MUTYH c.972 G > C              |                    |                    | 0.046    |            |
| CC                              | 44 (13.1%)         | 63 (15.6%)         |          |            |
| CG                              | 155 (46.0%)        | 211 (52.2%)        |          |            |
| GG                              | 138 (40.9%)        | 130 (32.2%)        | 0.013    | 1.46 (1.08–1.98) |
| CC or CGb                       | 199 (59.1%)        | 274 (67.8%)        |          | 1.00       |
| C allele                        | 0.361              | 0.417              |          | 1.00       |
| G allele                        | 0.639              | 0.583              | 0.026    | 1.27 (1.03–1.57) |
| AluYb8MUTYH                     |                    |                    | 0.099    |            |
| A/A                             | 101 (30.0%)        | 151 (37.4%)        |          | 1.00       |
| A/P                             | 164 (48.7%)        | 172 (42.6%)        |          |            |
| P/P                             | 72 (21.3%)         | 81 (20.0%)         |          |            |
| A/P or P/P b                    | 236 (70.0%)        | 253 (62.6%)        | 0.034    | 1.40 (1.03–1.90) |
| A allele                        | 0.543              | 0.587              |          | 1.00       |
| P allele                        | 0.457              | 0.413              | 0.092    | 1.19 (0.97–1.47) |

Note: CI: confidence interval; OR: odds ratio. aP value for comparison using χ² test to assess correlation between HD risk and predicted high-risk OGG1, MTH1, and MUTYH genotypes and alleles; bgenotypes were combined properly to assess their association with HD and the genotype 1.00 as the reference category.

similar between the 116 patients tested for leukocyte DNA 8-OHdG levels and all 337 patients investigated in the present study. In a parallel investigation of healthy controls in our laboratory, the HD patients exhibited increased 8-OHdG levels compared to the healthy individuals [12].

For the OGG1 c.977 C > G polymorphism, the genotypic frequencies (CC/CG/GG ratios of 15.5%/43.1%/41.4%) for the 116 patients whose leukocyte DNA 8-OHdG levels had been analyzed did not vary significantly from the whole study population of 337 patients (16.6%/47.5%/35.9%). The leukocyte 8-OHdG levels for patients carrying GG (26.7 ± 4.7/10⁶ dG) or CG (26.6 ± 5.5/10⁶ dG) were significantly higher than the patients carrying CC (18.4 ± 8.9/10⁶ dG) (P < 0.001 via ANOVA). For the AluYb8MUTYH polymorphism, out of the 116 patients, 37, 52, and 27 showed the A/A, A/P and P/P genotypes, which did not differ from the whole population. The patients carrying P/P (29.2 ± 3.9/10⁶ dG) or A/P (25.4 ± 5.6/10⁶ dG) had significantly higher 8-OHdG levels than the patients carrying A/A (22.6 ± 7.9/10⁶ dG) (P < 0.001 via ANOVA).

The combined impacts of these polymorphisms on 8-OHdG levels were further investigated (Figures 2(b) and 2(c)). Based on the risk for HD, 43 patients carrying the MUTYH c.972GG genotype were analyzed; 6, 21, and 16 showed the CC, CG, and GG genotypes of OGG1 c.977C > G, respectively (Figure 2(b)). The OGG1 c.977C > G GG or CG genotypes significantly increased the 8-OHdG level when compared with patients with the OGG1 c.977C > G CC genotype among patients with the MUTYH c.972GG genotype (29.2 ± 3.2/10⁶ dG, 28.1 ± 4.8/10⁶ dG versus 21.8 ± 9.0/10⁶ dG; P = 0.01 via ANOVA). This indicates that
Figure 2: Individual (a) and combined [(b) accompanied with MUTYH c.972GG genotype; (c) accompanied with AluYb8MUTYH A/P or P/P genotype] analysis of the effect of BER polymorphism on mean levels of leukocyte DNA 8-OHdG in 116 HD patients. Every group includes three different bars stratified according to the polymorphism genotypes. Statistical significance was calculated using one-way ANOVA testing followed by post hoc analysis. ∗P < 0.05 and ∗∗P < 0.01 versus subjects with the genotype in the blank bar.

Table 3: Combined analysis of genetic polymorphisms in OGG1 and MUTYH with HD risk.

| Genotypes | Patients (n = 337) | Controls (n = 404) | P valuec | OR (95% CI) |
|-----------|-------------------|-------------------|-----------|-------------|
| MUTYH c.972GG<sup>a</sup> | | | | |
| OGG1 | | | | |
| c.977CC | 23 (6.8%) | 27 (6.7%) | 0.939 | 1.02 (0.58–1.82) |
| c.977CG | 67 (19.9%) | 75 (18.6%) | 0.650 | 1.09 (0.75–1.57) |
| c.977GG | 48 (14.2%) | 28 (6.9%) | 0.001 | 2.23 (1.37–3.64) |
| AluYb8MUTYH | | | | |
| A/A | 18 (5.3%) | 19 (4.7%) | 0.691 | 1.14 (0.59–2.22) |
| A/P | 58 (17.2%) | 54 (13.4%) | 0.146 | 1.35 (0.90–2.02) |
| P/P | 62 (18.4%) | 57 (14.1%) | 0.113 | 1.37 (0.93–2.03) |
| AluYb8MUTYH A/P or P/P<sup>b</sup> | | | | |
| OGG1 | | | | |
| c.977CC | 38 (11.3%) | 42 (10.4%) | 0.701 | 1.10 (0.69–1.74) |
| c.977CG | 120 (35.6%) | 136 (33.7%) | 0.579 | 1.09 (0.80–1.48) |
| c.977GG | 78 (23.1%) | 75 (18.6%) | 0.125 | 1.32 (0.93–1.89) |
| MUTYH | | | | |
| c.972CC | 11 (3.3%) | 12 (3.0%) | 0.818 | 1.10 (0.48–2.53) |
| c.972CG | 105 (31.2%) | 130 (32.2%) | 0.766 | 0.95 (0.70–1.30) |
| c.972GG | 120 (35.6%) | 111 (27.5%) | 0.017 | 1.46 (1.07–1.99) |

Note: CI: confidence interval; OR: odds ratio. ∗Trend test assessing correlation between HD risk and predicted high-risk OGG1 and MUTYH genotypes combined with the MUTYH c.972GG genotype. bTrend test assessing correlation between HD risk and predicted high-risk OGG1 and MUTYH genotypes combined with the AluYb8MUTYH A/P or P/P genotype. c: P value for comparison using χ<sup>2</sup>-test between patients and controls.

MUTYH and OGG1 may have synergistic roles in the prevention of DNA oxidative damage. Similarly, out of 79 patients carrying the AluYb8MUTYH insertion (A/P or P/P), 6, 41, and 32 showed the MUTYH c.972G > C CC, CG, and GG genotypes, respectively (Figure 2(c)). The 8-OHdG levels of individuals carrying the GG or CG genotypes were higher than in individuals carrying the CC genotype among patients with the AluYb8MUTYH insertion (29.5 ± 3.4/10<sup>6</sup> dG, 25.6 ± 5.3/10<sup>6</sup> dG versus 19.4 ± 5.3/10<sup>6</sup> dG; P < 0.001 via ANOVA).

4. Discussion

End-stage renal disease (ESRD) is a troublesome health problem worldwide, and the mortality rate for ESRD patients
| Genotype | Controls ($n = 404$) | Primary glomerulonephritis ($n = 207$) | Anemia ($n = 267$) | Hypertension ($n = 250$) |
|----------|----------------------|----------------------------------------|-------------------|------------------------|
| $OGG1$ c.977 C > G | | | | |
| CC or CG | 277 (68.6%) | 132 (63.8%) | 178 (66.7%) | 158 (63.2%) |
| GG | 127 (31.4%) | 75 (36.2%) | 89 (33.3%) | 92 (36.8%) |
| $P$ value | 1.00 | 0.233 | 0.607 | 0.158 |
| OR (95% CI) | 1.00 | 1.24 (0.87–1.76) | 1.09 (0.78–1.52) | 1.27 (0.91–1.78) |
| $MUTYH$ c.972 G > C | | | | |
| CC or CG | 274 (67.8%) | 113 (54.6%) | 253 (57.3%) | 147 (58.8%) |
| GG | 130 (32.2%) | 94 (45.4%) | 114 (42.7%) | 103 (41.2%) |
| $P$ value | 0.001 | 0.006 | 0.019 | 0.019 |
| OR (95% CI) | 1.75 (1.24–2.47) | 1.57 (1.14–2.16) | 1.48 (1.07–2.05) | |
| $AluYb8MUTYH$ | | | | |
| $A/A$ | 151 (37.4%) | 53 (25.6%) | 71 (26.6%) | 74 (29.6%) |
| $A/P$ or $P/P$ | 253 (62.6%) | 154 (74.4%) | 196 (73.4%) | 176 (70.4%) |
| $P$ value | 0.003 | 0.004 | 0.042 | 0.042 |
| OR (95% CI) | 1.73 (1.20–2.52) | 1.65 (1.18–2.31) | 1.42 (1.01–1.99) | |

Note: CI: confidence interval; OR: odds ratio. $P$ value for comparison using $\chi^2$-test and trend test assessing correlation with HD risk among subgroup of primary glomerulonephritis$^a$, anemia$^b$, and hypertension$^c$, compared with the healthy controls.
is 10 to 20 times higher than similarly aged individuals from the general population [22]. Maintenance hemodialysis (HD) is an efficient way to treat ESRD, and its use is increasing due to the epidemic of ESRD. New epidemiological studies show that China is also anticipating an increasing burden from ESRD and HD in the near future, although it used to be severely underestimated. It was reported that the number of patients with chronic kidney disease was 119.5 million in China [23], and the annual incidence of HD was estimated to be as high as 36.1 per million population (pmp) [24].

In this study, we investigated polymorphisms of base excision repair (BER) genes in a case-control Chinese population and demonstrated that individual and combined BER variations, mainly MUTYH polymorphisms, might increase the risk for ESRD. The underlying mechanical linkage might be an increase in 8-OHdG levels in leukocyte DNA, which was confirmed to be genetically determined. Oxidative DNA damage is unavoidable and is continuously generated by oxidative byproducts of normal cellular metabolism. The BER pathway is a critical process for genomic maintenance, as highlighted by the severe phenotypes seen in cells and animals deficient in BER function. MUTYH and OGG1 double knockout cells are more sensitive to oxidants, and the double knockout resulted in a reduction of S phase and an increase in G2/M phase than wildtype cells, suggesting multiple roles of MUTYH and OGG1 in the maintenance of genome stability [25].

The effectiveness of DNA repair is subject to modulation by gene polymorphism. In the hemodiagnosis population, OGG1 c.977C>G, MUTYH c.972G>C, and AluYb8MUTYH showed significant effects on 8-OHdG levels in peripheral leukocytes, both individually and in combination. Our previous study illustrated that leukocyte 8-OHdG levels are variable among the Chinese population, regardless of AluYb8MUTYH variations [12, 13]. In this study, we confirmed a relationship among ESRD patients. Patients carrying the OGG1 c.977 GC or CG genotypes had significantly higher 8-OHdG levels than those with the OGG1 c.977CC genotype. Similarly, Kohno et al. previously reported that 326Ser-containing (c.977CC) OGG1 has a seven-fold higher activity for repairing 8-oxoguanine than 326Cys-containing (c.977GG) OGG1 [26]. In a background of the MUTYH c.972GG genotype, OGG1 c.977C>G still showed a significant increase in 8-OHdG levels in peripheral leukocytes. The same results were detected for the MUTYH c.972G>C combined with the AluYb8MUTYH P allele. However, the patients carrying MUTYH c.972GG had significantly higher 8-OHdG levels than the patients carrying MUTYH c.972CC. In contrast, Ali et al. reported that the glycosylase and DNA-binding activity was partially impaired in the MUTYH c.972CC genotype [27], whereas Shimamura and Yokota showed the same activity levels despite variation in the MUTYH c.972G>C polymorphism [28]. Therefore, the genetic variations in the BER pathway may be enough to maintain 8-OHdG levels in nuclear DNA, although the underlying mechanisms are not extensively studied or understood.

Intriguingly, the genotype frequencies of MUTYH c.972GG or AluYb8MUTYH carriers (A/P or P/P) in the HD patients were markedly higher than those in the controls. The combined analysis showed that the risk of HD was further increased among the individuals carrying both the MUTYH c.972GG and OGG1 c.977GG genotypes as well as those with both the AluYb8MUTYH (A/P or P/P) and MUTYH c.972GG genotypes. The findings from Trabulus et al. [17] confirmed the association between DNA repair gene polymorphisms and ESRD development in Turkish population. And the combined effect of DNA repair variants added to such association, which was similarly illustrated in the present study.

It has been noted that the disease profile of ESRD is different in China from Western countries. Zuo and Wang showed that the glomerulonephritis remained the leading cause and accounted for nearly 50% of cases [24]. In this study, 61.4% of HD cases were the result of primary glomerulonephritis (GN). Additionally, the MUTYH GG genotype also significantly increased the risk for ESRD from GN (OR = 1.75). This association remained in persons with the AluYb8MUTYH P allele, which increased the risk for ESRD from GN by a factor of 1.73. Thus, MUTYH c.972GG or AluYb8MUTYH could be the novel genetic risk factor for ESRD, and screening for these genetic variants or combined analysis may have predictive value in assessing potential risk in China.

Based on the correlation between BER polymorphisms and 8-OHdG levels, patients with different BER genetic polymorphisms were found to be at increased risk of cumulative oxidative DNA damage. Thus, we proposed that the relationship between genetic factors and ESRD and the effect of increased 8-OHdG levels underly this process. Increasing evidence has shown that the accumulation of 8-OHdG in DNA could increase the risk of DNA mutations and cancer development [29, 30]. We have also demonstrated that increased DNA oxidation might contribute to age-related diseases [12]. 8-OHdG levels in leukocyte DNA of HD patients are significantly higher than healthy controls, which has been confirmed by other groups [31, 32]. However, to date, there has been no direct evidence demonstrating a cause-and-effect relationship between oxidative DNA damage and the development of GN and ESRD. Our study using genetic analysis supports such a relationship, but further studies are needed to elucidate the pathologic significance of oxidative DNA damage among people with respect to ESRD development.

Anemia and hypertension are the most frequent complications of ESRD and related to the increased mortality rates [33, 34]. Increased DNA damage is responsible for depressed production of erythropoietin (EPO), hypertension formation, and cardiovascular disease (CVD) [35, 36]. Observational studies have revealed a strong association between the severity of anemia and the risk of morbidity and mortality from cardiovascular disease and other causes in HD patients [19, 37]; hypertension is also likely to be a major contributing factor to these events [38]. In this study, we illustrated that mutations in BER genes were tightly linked with the complications of anemia or hypertension among HD patients. As cardiovascular events are the primary cause of death in HD patients, these data suggest that oxidative
DNA damage might be involved in the risk for complications and long-term outcomes.

However, not all relationships regarding BER polymorphisms in the present study can be explained thoroughly. It was demonstrated that the BER polymorphisms were related to the disability to repair oxidative DNA and then accumulation of the levels of 8-OHdG in leukocyte DNA. The high levels of 8-OHdG, therefore, contribute to the related to the disability to repair oxidative DNA and then disease condition were not consistent. Take OGG1 c.977C > G for instance, the GG and/or CG variations significantly increased the 8-OHdG levels, which predicts a high risk for oxidative DNA damage. But the OGG1 c.977C > G polymorphism did not appear to be related to ESRD among the investigated Chinese population. Tarng et al. also showed similar results among patients undergoing HD, but did not provide a detailed interpretation [31].

In summary, our study showed that the polymorphisms in BER system, including MUTYH c.972GG and AluYb8MUTYH, increased the risk for ESRD development in China, especially their combined effect with OGG1 c.977GG. It suggests that oxidative DNA damage might be one common risk factor for related renal diseases, and the genes in BER pathway may be involved in the progress of renal function deterioration and complications. Those homozygous or heterozygous for BER polymorphisms might be candidate genetic factors for ESRD development. Screening those polymorphisms would be helpful for preventing progression of chronic kidney disease and improving the patients' long-term outcomes of hemodialysis.

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