

# Genetic polymorphism of hOGG1 and risk of pterygium in Chinese

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## Abstract

**Purpose** Ultraviolet irradiation is known to cause oxidative DNA damage and is thought to be a major factor implicated in the pathogenesis of pterygium. The highly mutagenic 8-hydroxy-2'-deoxyguanosine, a marker for the evaluation of photo-oxidative DNA damage, can be repaired by human 8-oxoguanine glycosylase I (hOGG1). A transition of C to G at nucleotide position 1245 in exon 7 of the hOGG1 gene is associated with the substitution of cysteine for serine at codon 326. In this study, we investigated the association of the hOGG1 Ser326Cys polymorphism with pterygium in a Chinese population.

**Methods** In all, 70 patients and 86 controls were enrolled in this study. The Ser326Cys polymorphism was determined by the polymerase chain reaction-restriction fragment-length polymorphism analysis. The association between this genetic polymorphism and risk of pterygium was examined by  $\chi^2$ -test and logistic regression.

**Results** The allelic frequencies for the Ser and Cys variants of hOGG1 gene were not significantly different between the two groups. However, when compared with Ser/Ser and Ser/Cys genotypes combined, we found that the homozygous Cys/Cys genotype was more prevalent in pterygium patients than controls ( $P=0.024$ ) with the odds ratio being 2.2 (95% CI: 1.1–4.5). In the pterygium group, the mean age of patients with the Cys/Cys genotype was younger than those with the other two genotypes ( $P=0.025$ ).

**Conclusions** Our findings suggest that the 1245C→G transition in exon 7 of the hOGG1 gene, which results in Ser326Cys substitution of the enzyme, might play a role in the susceptibility of humans to pterygium.

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## Introduction

Pterygia are common ocular surface lesions, which are especially prevalent in the population of tropical and subtropical areas. The prevalence of pterygia in adults over 40 years old in Southeast Asia has been estimated to be 7%.<sup>1</sup> Pterygia may invade adjacent cornea and cause astigmatism and visual impairment. Surgical excision is the current treatment strategy if the visual axis is threatened or in cases of extreme irritation. However, the recurrent rate is still significant.

Pterygium was regarded as an ocular degenerative disease with pathological evidence of degeneration in the conjunctival collagen fibre. However, pterygia display some tumour-like properties in their clinical appearance of local invasion and epithelial cell metaplasia.<sup>2</sup> Furthermore, the presence of oncogenic viruses, such as the human papilloma virus and herpes simplex virus,<sup>3,4</sup> loss of heterozygosity,<sup>5</sup> and abnormal p53 expression in pterygium lesions,<sup>6,7</sup> all suggest the possible neoplastic nature of pterygium. A model of pterygium formation is then proposed, in which genetic predisposition, environmental factors, and viral infections may participate in the multistep process.<sup>8</sup> However, there is still a lack of strong evidence regarding the genetics of the disease.

Although the pathogenesis of pterygium is still uncertain, some risk factors including ultraviolet (UV) exposure,<sup>9–12</sup> age,<sup>1,11</sup> race,<sup>13</sup> and family history<sup>14</sup> have been proposed. Among these risk factors, UV exposure has been documented to have strong correlation with

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pterygium, but the mechanism by which UV light induces pterygium remains elusive. Nolan *et al*<sup>15</sup> demonstrated that heparin-binding epidermal growth factor-like growth factor, a potent mitogen, was localized in pterygium tissue, and was significantly induced by UVB in pterygium-derived epithelial cells. Lu *et al*<sup>16</sup> has proposed photo-damage in pterygium from the point of view of lipid peroxidation. UV irradiation may damage various cellular biomolecules, especially the DNA. Once DNA is modified without proper repair, altered genetic expression and even mutation may occur. The photo-oxidative damage to DNA results in pyrimidine dimers or various DNA modifications such as 8-hydroxy-2'-deoxyguanosine (8-OHdG), strand breaks, sites of base loss, and DNA-protein crosslinks.<sup>17</sup> Among all the photo-oxidative DNA products, the 8-OHdG is regarded a sensitive and stable biomarker for evaluating the degree of DNA damage.<sup>18</sup> The 8-OHdG is highly mutagenic, because it frequently mispairs with adenine during DNA replication, ultimately giving rise to the G-C to T-A transversion mutations.<sup>19</sup> The increase in 8-OHdG content in DNA has been shown to increase cancer risks.<sup>20</sup>

In human genome, there is a base excision repair system to correct 8-OHdG. The human 8-oxoguanine glycosylase I (hOGG1) is the key component responsible for the removal of 8-OHdG in DNA.<sup>21</sup> Previous genetic studies have revealed the presence of several polymorphisms within the hOGG1 locus.<sup>22,23</sup> Among them, a common polymorphism has been shown to have a functional difference. A transition of C to G at nucleotide position 1245 in exon 7 of the hOGG1 gene is associated with the substitution of cysteine for serine at codon 326. It has been proved in an *Escherichia coli* complementation assay<sup>23</sup> that the DNA repair activity of hOGG1-Cys<sup>326</sup> protein is lower than that of hOGG1-Ser<sup>326</sup>. The hOGG1 Cys/Cys homozygosity has been reported to increase the risks of lung cancer,<sup>24</sup> oesophageal cancer,<sup>25</sup> orolaryngeal cancer,<sup>26</sup> and stomach cancer.<sup>27</sup> Additionally, some studies revealed the impact of this genetic polymorphism on patients suffering from chronic oxidative stress.<sup>28,29</sup> In this study, we tried to investigate whether the hOGG1 genetic polymorphism is associated with the susceptibility of humans to pterygium.

## Materials and methods

We recruited pterygium patients from the Department of Ophthalmology at the Taipei Veterans General Hospital from August 2002 to February 2003. The diagnosis of pterygium was established according to the clinical finding of a wing-shaped fold of conjunctiva and fibrovascular tissue that has invaded the adjacent cornea

for which there was no alternative explanation (for example, trauma). Patients with ocular diseases other than pterygium were excluded from this study. The volunteers in the control group were healthy subjects recruited when they attended their routine health examination. All the subjects were nonrelated. Clinical examination and diagnosis were all performed by the same ophthalmologist. To eliminate the factors that might affect the systemic oxidative stress, the exclusion criteria for the patients and control subjects were a history of smoking, taking vitamins or nutrition supplements, as well as those suffering from benign or malignant tumours, inflammatory or infectious diseases. Each eligible subject was interviewed to obtain data on age, sex, and occupational history. For analysis, the occupations were combined into two groups: outdoor (soldier, farmer, fisherman, and postman) or indoor (professional, office worker, teacher, and housekeeper). Our Institutional Review Board approved this study, and informed consent was obtained from all subjects after explanation of the nature and possible consequences of the study.

Each subject donated 5 ml of blood that was collected in a heparinized tube. The genomic DNA was prepared by serial phenol/chloroform extraction and then ethanol precipitation. The genotype of hOGG1 was determined by the polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) analysis. An aliquot of 100 ng of genomic DNA was added to a 50  $\mu$ l PCR mixture containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 0.1% Triton X-100, 2 mM MgCl<sub>2</sub>, 200  $\mu$ M of each dNTP, 100 pmol of each primer, and 1 U of Taq DNA polymerase (Biotools; B & M Labs, Madrid, Spain). PCR was performed using the primers described by Kohno *et al*,<sup>11</sup> that is, 5'-AGGGGAAGGTGCTTGGGGGAA-3' as the forward primer and 5'-ACTGTCACTA GTCTACCAG-3' as the reverse primer. The thermal profile consisted of 35 cycles of denaturation at 94°C for 15 s, annealing at 58°C for 15 s, and extension at 72°C for 40 s, preceded by an initial denaturation step at 94°C for 2 min and followed by a terminal extension at 72°C for 5 min. Following PCR, 10  $\mu$ l of the product was subjected to *Fnu*4HI digestion (2.5 U of enzyme in a 15  $\mu$ l digest). The presence of C to G transversion at nucleotide position 1245 created an *Fnu*4HI recognition site, which led to digestion of the 200 bp PCR product into two DNA fragments of 100 bp. *Fnu*4HI digests of PCR products were separated by electrophoresis on a 3% agarose gel, followed by staining with ethidium bromide.

The distributions of categorical variables (eg, gender, occupation type, and genotype) in patients and control groups were compared with  $\chi^2$ -test. The continuous variable (eg, age) was examined using the Student's *t*-test. A *P*-value less than 0.05 was considered to be

statistically significant. The odds ratio (OR) and 95% confidence interval (CI) were calculated and adjusted for age, gender, and occupation type using logistic regression.

## Results

In total, 70 patients with pterygium and 86 healthy subjects were enrolled in this study. The mean age in the pterygium patients and control groups were  $71.7 \pm 10.1$  and  $68.3 \pm 13.2$  years, respectively. The proportions of the male gender in two groups were 85.7 and 74.4%, respectively. The proportions of outdoor occupation were 77.1 and 64.0%, respectively. The differences between pterygium and control groups for age, sex, and occupation type were not statistically significant.

The allelic frequencies for the C1245 (Ser) and G1245 (Cys) genotype of the hOGG1 gene were found to be 0.414 and 0.586 among the pterygium patient group, compared with 0.465 and 0.535 among the control group ( $P = 0.369$ ) (Table 1). The distributions of hOGG1 genotypes among pterygium patients (Ser/Ser, 22.9%; Ser/Cys, 37.1%; Cys/Cys, 40.0%) were different from those of the control subjects (Ser/Ser, 16.3%; Ser/Cys, 60.5%; Cys/Cys, 23.3%) ( $P = 0.014$ ). The frequency of the Cys/Cys genotype in pterygium patients was higher than that of normal controls. When compared with Ser/Ser and Ser/Cys genotypes combined, homozygous Cys/Cys genotype significantly increased the risk of developing pterygium ( $P = 0.024$ ), with the OR adjusted for age, sex, and occupation type being 2.2 (95% CI: 1.1–4.5) (Table 1).

Among the pterygium patients, the mean age of patients with Cys/Cys genotype ( $67.8 \pm 14.1$  years old) was significantly lower than those with Ser/Ser and

Ser/Cys genotypes combined ( $74.3 \pm 4.8$  years old) ( $P = 0.025$ ).

## Discussion

Our findings showed that the hOGG1 Ser326Cys polymorphism was associated with the susceptibility of humans to pterygium. Individuals who carried the homozygous Cys/Cys genotype were at a 2.2-fold higher risk to develop pterygium. Besides, the mean age of pterygium patients with Cys/Cys genotype was younger than those with the other two genotypes combined.

Since the genetic polymorphism of hOGG1 has been associated with many kinds of neoplasia, it is reasonable to expect it to play a role in human cancer susceptibility.<sup>24–27</sup> In order not to interfere with our analysis of hOGG1 polymorphism on the susceptibility to the pterygium, the subjects suffering from benign or malignant tumours were excluded. On the other hand, owing to the fact that the veterans accounted for approximately 50% of our patients, there were higher proportions of male sex and outdoor occupation type in our patients and controls. However, the association between hOGG1 Ser326Cys polymorphism and pterygium was still statistically significant after OR was adjusted for age, sex, and occupation type.

The hOGG1 is a specific DNA glycosylase/apurinic lyase that is responsible for the removal of 8-OHdG in DNA. The hOGG1 molecule can recognize 8-OHdG, and then catalyse both the release of 8-OHdG and the cleavage of DNA at the resulting apurinic site.<sup>21</sup> The enzyme itself is very sensitive to UVB irradiation, which causes photolysis of tryptophan residues at the active site and leads to hOGG1 inactivation.<sup>30</sup> It has been demonstrated that a CG polymorphism at nucleotide position 1245 in exon 7 of the hOGG1 gene results in an amino-acid substitution from serine to cysteine at residue 326. According to the functional study by Kohno *et al*,<sup>23</sup> the Cys326 allele is about seven-fold less capable of complementing a repair-deficient *E. coli* strain than the Ser326 allele in an in vitro complementation assay. Besides, the inactivation of hOGG1 increases the frequency of G–C to T–A transversion mutations in *Saccharomyces cerevisiae*.<sup>31</sup> It could be possible that the Cys326 allele has reduced enzymatic activity toward 8-OHdG or increased sensitivity to UVB inactivation. As a result, individuals bearing the homozygous Cys/Cys genotype might allow the persistent presence of 8-OHdG in genomic DNA and accelerate the accumulation of G–C to T–A mutations, which may lead to an increased susceptibility of an individual to develop pterygium. Further studies on the effect of the genetic polymorphism on the enzyme function of hOGG1 in vivo are warranted.

**Table 1** Allelic frequency, genotype frequency, and OR of hOGG1 Ser326Cys among pterygium patients and control groups

	Control group (n = 86)	Pterygium group (n = 70)	P-value
Allelic frequency			
Cys	0.535	0.586	0.369
Ser	0.465	0.414	
Genotype distribution			
Cys/Cys	20 (23.3%)	28 (40.0%)	0.024
Ser/Cys and Ser/Ser	66 (76.7%)	42 (60.0%)	
OR (95% CI) <sup>a</sup>		2.2 (1.1–4.5)	0.369

<sup>a</sup>OR and 95% CI were calculated by logistic regression, with the Ser/Ser and Ser/Cys genotypes combined as the reference group and adjusted for age, gender, and occupation type.

In conclusion, our study demonstrated for the first time that Ser326Cys polymorphism in hOGG1 is associated with the risk of pterygium in Chinese. In view of the complexity in the aetiology of pterygium, no single genetic marker is sufficient for prediction of the risk of this disease. Therefore, a panel of susceptibility biomarkers including genetic polymorphisms in other DNA repair pathways or photo-oxidative reactions is warranted to define individuals at a high risk of developing pterygium.

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