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Contribution of Radiation Sensitive Protein 51 Genotypes to Pterygium Risk in a Taiwanese Population

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Abstract. *Background/Aim: In current literature, there is a notable lack of studies investigating the role of radiation-sensitive protein 51 (RAD-51) in pterygium diagnosis. Nevertheless, reports indicate elevated expression levels of RAD-51 among recurrent pterygium cases compared to those with primary pterygium. However, the genomic involvement of RAD-51 has yet to be explored in any population. This study aimed to assess the contribution of RAD-51 genotypes to pterygium risk in a representative Taiwanese population. Materials and Methods: RAD-51 rs1801320 genotyping was successfully conducted in a Taiwanese cohort comprising 140 pterygium cases and 280 non-pterygium controls using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technology. Results: The distribution of RAD-51 rs1801320 genotypes (GG, CG, and CC) in the pterygium group (70.0%, 25.7%, and 4.3%, respectively) did not significantly differ from that in the non-pterygium group (73.6%, 23.6%, and 2.8% for GG, CG, and CC genotypes, respectively; p for trend=0.6337). Carriers of the variant CG and CC RAD-51 rs1801320 genotypes exhibited 1.15- and 1.58-fold increased pterygium risk, respectively (95%CI=0.72-1.84 and 0.53-4.67, p=0.6552 and p=0.5914, respectively). In the dominant model, there appeared to be a slight association between variant genotypes CG and CC and pterygium risk (OR=1.19, 95%CI=0.76-1.87, p=0.0223). Allelic analysis revealed that the RAD-51 rs1801320 variant C allele was not significantly linked to pterygium risk (17.1% versus 14.6%, OR=1.20, 95%CI=0.82-1.78, p=0.3991). Conclusion: Variant genotypes at RAD-51 rs1801320 were firstly identified to associate with susceptibility to pterygium among Taiwanese individuals. Nonetheless, these findings warrant validation in larger and more diverse populations.*

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Pterygium presents as a common ocular surface ailment characterized by irregular epithelial and fibrovascular proliferation, infiltration, and restructuring of the extracellular matrix (ECM) (1, 2). This fibrovascular proliferation, leading to an abnormal wing-shaped growth, bears resemblance to the excessive growth observed in neoplastic formations (3). The migration of these abnormal, wedge-shaped tissues from the bulbar conjunctiva to the cornea also shares certain characteristics of tumorigenesis seen in solid cancers (4). The

Table I. *Demographics of pterygium and non-pterygium patients.*

Characteristic	Controls (n=280)			Cases (n=140)			*p*-Value
	n	%	Mean±SD	n	%	Mean±SD	
Age (years)			62.3±7.4			62.2±7.9	0.2980[a]
<60	99	35.4%		56	40.0%		0.4109[b]
≥60	181	64.6%		84	60.0%		
Sex							
Male	162	57.9%		81	57.9%		1.0000
Female	118	42.1%		59	42.1%		

SD, Standard deviation; [a]based on unpaired Student's *t*-test; [b]based on Chi-square test with Yates' correction.

multifaceted nature of pterygium, influenced by various factors including heat, dust, atmospheric particles, immunological cytokines, rearrangement of the extracellular matrix, UV radiation, and growth factors, contributes to its complex etiology (5-13). Additionally, several studies have indicated the significant role of genetic variations in predisposing individuals to pterygium (14-17). However, a practical and readily accessible marker for pterygium remains notably elusive.

From a molecular standpoint, cellular DNA repair pathways become activated to uphold genetic stability and integrity upon exposure to various endogenous or exogenous DNA-damaging agents. Failure to rectify these lesions may culminate in genomic instability and the gradual accrual of mutations, which constitutes a hallmark of cancer (18, 19). Among the diverse types of DNA damage, DNA double-strand breaks (DSBs) pose a particular menace to cells. Two primary repair pathways, namely non-homologous end joining (NHEJ) and homologous recombination (HR), undertake the task of mending DSBs (20, 21). Generally, NHEJ represents an error-prone repair mechanism that juxtaposes the broken ends, while HR executes a precise and error-free repair process (22, 23).

From a molecular perspective, the DSB repair protein RAD-51 homolog 1, encoded by RAD-51 and situated at chromosome locus 15q15.1, assumes a critical role in preserving genetic stability and integrity under the assault of various DNA-damaging agents (24). This genomic region, notable for its propensity for loss of heterozygosity in malignancies, such as breast, colorectal, and lung cancers, holds significance in elucidating cancer progression. RAD-51, comprising 339 amino acids in humans, is indispensable for HR during the repair of DSBs (25-27). Overexpression of RAD-51 has been documented across a spectrum of cancers, encompassing (28-33), pancreatic (34, 35), head and neck (36), prostate (37), soft tissue sarcoma (38) and esophageal cancer (39). Remarkably, RAD-51 overexpression has been detected in non-small cell lung cancer, with renal cell carcinoma representing the sole exception, demonstrating under-expression (40, 41). In genomic investigations, a widely scrutinized polymorphism is the G to C polymorphism in *RAD-51*'s promoter region, denoted as rs1801320 (G-135C) (42). Literature underscores the association of *RAD-51* rs1801320 genotypes with susceptibility to diverse cancers, including breast (43-49), laryngeal (50), colorectal (51, 52), prostate (42), ovarian (53-55), cervical (56), endometrial (57, 58), and glioblastoma malignancies (59).

Regarding pterygium, there is a lack of literature exploring genetic variations of *RAD-51* in relation to this condition. Building upon this gap, the present study endeavors to examine the potential association between *RAD-51* rs1801320 genotypes, a single-nucleotide polymorphic (SNP) site, and the susceptibility to pterygium within a representative Taiwanese cohort comprising 140 pterygium cases and 280 non-pterygium controls.

Materials and Methods

Recruitment of pterygium and non-pterygium population. The research concepts, association hypotheses, and experimental protocols employed in this study have been sanctioned by the Institutional Review Board of Changhua Christian Hospital (number: 151225). Furthermore, written informed consent has been procured from either one or both parents of each participant. A cohort comprising 140 individuals diagnosed with pterygium, alongside a double size of non-pterygium control subjects, was enrolled for the study. Each participant willingly completed a questionnaire and furnished peripheral blood samples for genotyping. Non-pterygium control subjects were chosen based on the absence of pterygium, endometriosis, myoma, or any malignancy. Demographic characteristics of all participants are delineated in Table I.

RAD-51 rs1801320 genotyping procedures. Genomic DNA isolated from peripheral blood leukocytes of both patient cohorts and controls was extracted utilizing the QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan, ROC) and processed following established methodologies (60-63). Polymerase chain reaction (PCR) cycling conditions for *RAD-51* rs1801320 genotyping were as follows: an initial denaturation step at 94˚C for 5 min, followed by 35 cycles of denaturation at 94˚C for 30 s, annealing at 55˚C for

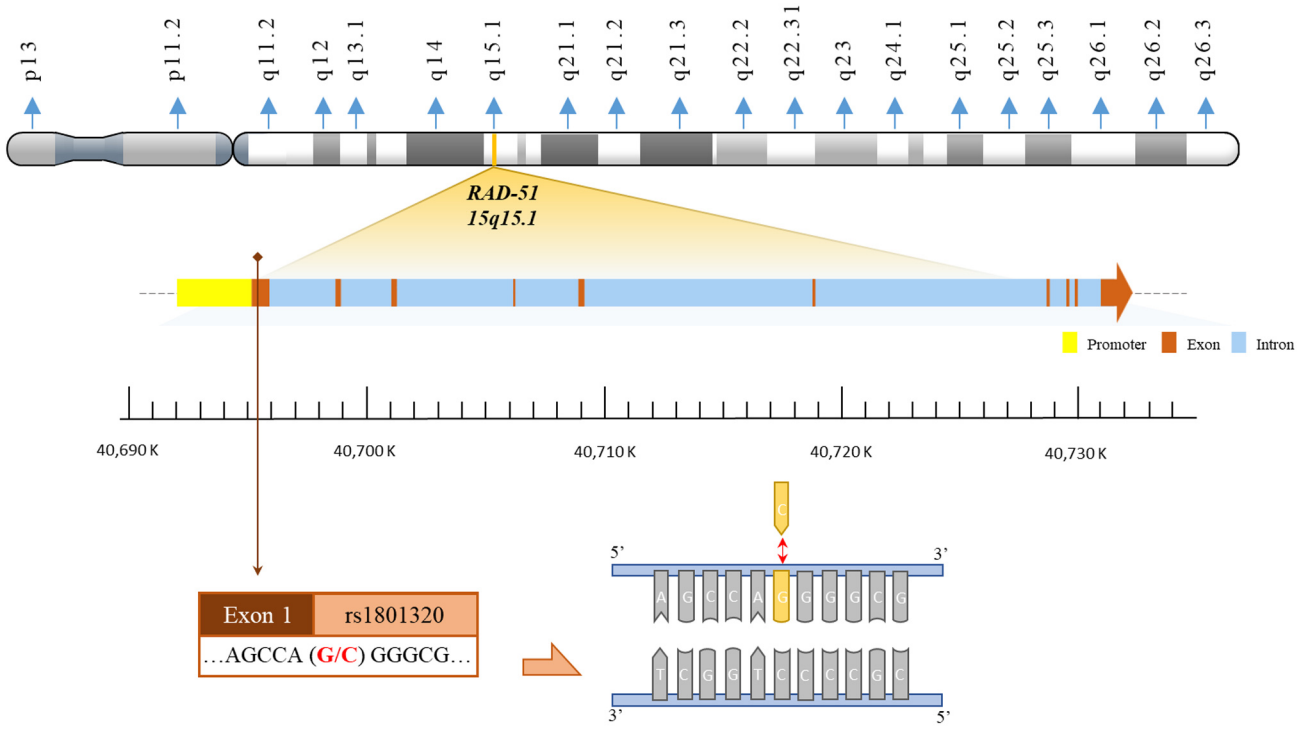

Figure 1. *The physical map of RAD-51 rs1801320 polymorphic site. The polymorphic site rs1801320 is located within exon 1 of the RAD-51 gene. Specifically, rs1801320 represents a G to C polymorphism.*

30 s, and extension at 72˚C for 30 s, with a final extension step at 72˚C for 10 min. The forward and reverse primers for *RAD-51* rs1801320 were 5'-CAGGATCAAGCTCTCGAGCT-3' and 5'-GGTGTTGCCTATAAAGGCTC-3', respectively. Subsequently, PCR products underwent digestion by the restriction enzyme *PspG* I (New England BioLabs, Ipswich, MA, USA). The G-allele contigs were cleaved into 333- and 281-base pair fragments, while the C-allele contigs remained unaltered, presenting as 614-base pair products. Genotypic analysis was conducted independently and blindly by laboratory personnel, with repeated data demonstrating 100% concordance. The physical map illustrating the locations of *RAD-51* rs1801320 is presented in Figure 1.

RAD-51 rs1801320 statistical analyzing methodologies. Age comparison between the pterygium patient and non-pterygium control groups was presented as the mean±standard deviation (SD), with unpaired Student's *t*-test employed for analysis. Evaluation of the impact of *RAD-51* rs1801320 polymorphisms on pterygium risk was conducted using Pearson's chi-square test. Associations were further assessed through odds ratios (ORs) accompanied by their 95% confidence intervals (CIs). Statistical significance was considered when the resulting *p*-value was below 0.05.

Results

Comparison of age and sex distributions between the pterygium patient and non-pterygium control groups. Firstly, it is imperative to scrutinize the age distributions across the pterygium and non-pterygium cohorts. The mean ages of both groups were evaluated, revealing no notable disparity between them (p=0.2980). This observation persisted even upon stratification of data by employing a threshold age of 60 years (p=0.4109). Secondly, it is noteworthy that, as a component of our recruitment strategy, we meticulously matched the pterygium and non-pterygium groups, thereby ensuring equitable distribution of sex across these cohorts (p=1.0000).

Association of RAD-51 rs1801320 genotypes and pterygium risk. Table II presents the distribution of *RAD-51* rs1801320 genotypes within the pterygium and non-pterygium cohorts. Notably, the *RAD-51* rs1801320 genotypes did not display divergent distributions between the pterygium and non-pterygium groups (p for trend=0.6337). Specifically, the heterozygous variant CG and homozygous variant CC of *RAD-51* rs1801320 did not exhibit a significant association with pterygium risk (OR=1.15 and 1.58, 95%CI=0.72-1.84 and 0.53-4.67, p=0.6552 and 0.5914, respectively). Confirmation of the negative association of *RAD-51* rs1801320 polymorphic variants with pterygium risk was obtained in the recessive model (OR=1.52, 95%CI=0.52-4.48, p=0.6308). Notably, a borderline association of *RAD-51* rs1801320 genotype with pterygium risk was observed in the dominant model (p=0.0223; lower section of Table II).

Table II. *Genotypic frequency distribution of RAD-51 rs1801320 among pterygium and non-pterygium patients.*

Genotypes	Controls, n (%)	Cases, n (%)	OR (95%CI)	*p*-Value[a]
rs1801320				
GG	206 (73.6)	98 (70.0)	1.00 (Reference)	
CG	66 (23.6)	36 (25.7)	1.15 (0.72-1.84)	0.6552
CC	8 (2.8)	6 (4.3)	1.58 (0.53-4.67)	0.5914
p_{trend}				0.6337
p_{HWE}				0.3400
Carrier analysis				
GG+CG	272 (97.2)	134 (95.7)	1.00 (Reference)	
CC	8 (2.8)	6 (4.3)	1.52 (0.52-4.48)	0.6308
GG	206 (73.6)	98 (70.0)	1.00 (Reference)	
CG+CC	74 (26.4)	42 (30.0)	1.19 (0.76-1.87)	0.0223*

OR: Odds ratio; CI: confidence interval; [a]data based on Chi-square test with Yates' correction; p_{trend}: *p*-Value based on trend analysis; p_{HWE}: *p*-Value based on Hardy-Weinberg Equilibrium; *statistically significant.

Table III. *Allelic frequencies for RAD-51 rs1801320 polymorphisms among the pterygium patients and the non-pterygium patients.*

Allelic type	Controls, n (%)	Cases, n (%)	Odds ratio (95% Confidence internal)	*p*-Value[a]
rs1801320				
Allele G	478 (85.4)	232 (82.9)	1.00 (Reference)	
Allele C	82 (14.6)	48 (17.1)	1.20 (0.82-1.78)	0.3991

[a]Data based on Chi-square test with Yates' correction.

Carriers of the CG and CC variants at *RAD-51* rs1801320 exhibited a 1.19-fold increased risk of pterygium development compared to those with the GG genotype (95%CI=0.76-1.87; lower section of Table II).

Association of RAD-51 rs1801320 allelic frequencies and pterygium risk. Allelic frequency analysis results suggested that the presence of the variant C allele at *RAD-51* rs1801320 does not exhibit an association with pterygium risk (17.1% *versus* 14.6%, OR=1.20, 95%CI=0.82-1.78, *p*=0.3991) (Table III).

Discussion

Among ophthalmologists, there remains a lack of consensus regarding the optimal comprehension of pterygium etiology and its management. This divergence is partly ascribed to the complexity of involved risk factors and the lack of a dependable marker for tailoring personalized therapeutic approaches. In 2007, Tsai and his colleagues have revealed the genotypes at *Ku70* promoter T-991C (rs5751129) can serve as novel predictor for pterygium risk (64). RAD-51 is known to play a central role in HR during the repair of DNA DSBs. Although little is known about the role of RAD-51 protein in pterygium, it has been reported that the expression level of RAD-51 was higher in the peripheral blood lymphocytes from patients with recurrent pterygium in comparison to those patients with primary pterygium (65). As mentioned in the introduction part, the genomic contribution of *RAD-51* to pterygium remains unrevealed and we are the first team to assess the impact of *RAD-51* rs1801320 genotype on pterygium risk. Among the non-pterygium healthy controls, the percentages of wild-type GG and variant CG and CC genotypes were 73.6%, 23.6%, and 2.8%, respectively, and fitted well with the Hardy-Weinberg equilibrium (*p*=0.6337, Table II). The percentages of wild-type G and variant C alleles at *RAD-51* rs1801320 in Taiwan population were 85.4% and 14.6%, respectively (Table III). In the global 1000 Genomes Project, the percentages of wild-type G and variant C alleles at *RAD-51* rs1801320 for East Asians were 85.0% and 15.0%, respectively, based on a sample size of 1170 subjects (66). The adherence to Hardy-Weinberg Equilibrium indicated that our collection of non-pterygium samples can be representative of the whole Taiwanese population without sampling bias.

Although our data show that the C allele of *RAD-51* rs1801320 does not appear to be a significant contributor to individual pterygium susceptibility, however, it's noteworthy that *RAD-51* rs1801320 variant CG and CC genotypes were more prevalent in the pterygium group compared to the non-

pterygium group (25.7% *versus* 23.6% and 4.3% *versus* 2.8%, Table II). More important, in the dominant model, CG or CC carriers at *RAD-51* rs1801320 exhibited a significantly higher risk of developing pterygium than GG carriers (Table II). These finding raises considerable interest, and larger pterygium populations could be beneficial to validate the diagnostic role for *RAD-51* rs1801320 genotypes. To the best of our knowledge, our current study is the first to demonstrate the potential contribution of *RAD-51* rs1801320 variant genotypes to pterygium susceptibility on a global scale.

There are several directions we may extend our study from the current findings. First, we did not extend our stratification analysis to investigate the impact of other factors (such as age and sex) combined with *RAD-51* rs1801320 genotype on pterygium risk since the sample size for variant CC genotype were only 8 and 6 for the control and case groups, respectively. It is very essential to enlarge the sample size. Second, it is reported that the X-ray repair cross complementary 1 (*XRCC-1*) codon 194 polymorphism was associated with a decreased risk of developing pterygium, but the codon 399 polymorphism was associated with an elevation of pterygium risk (67). Since pterygium is a UV-related disease, we may figure out the role of those proteins and genes involved in the nucleotide excision and single strand break repair pathways as some literatures suggested (68-70). In addition to RAD-51, a significant elevated expression of XRCC-2 and XRCC-3 was found among recurrent pterygium patients, compared to those patients with primary pterygium (65). Third, lower level of RAD-51 expression in pterygium patients compared to the non-pterygium control group, providing another piece of clue to involvement of DSB repair pathway in pterygium in addition to the current study (65). Up to now, the knowledge for the involvement of *Ku80*, *DNA-PKcs*, *ligase 4* and other DSB genes are still lacking.

In summary, this study initially investigated the genotypic impact of *RAD-51* rs1801320 within a Taiwanese pterygium cohort and identified potential associations between *RAD-51* rs1801320 variant genotypes and increased susceptibility to pterygium among individuals in Taiwan. It is pertinent to validate these findings in larger and more diverse population cohorts before clinical practices.

Conflicts of Interest

All the Authors declare no conflicts of interest regarding this study.

Authors' Contributions

Research design: Hsia NY, Hu PS, Bau DT, Chang WS; patient and questionnaire summaries: Hsia NY, Hu PS, Chuang CL, Chen HC; experimental work: Wang YC, Tsai CW, Chang WS; statistical analysis: Chuang CL, Mong MC, Chen JC, Wang YC; manuscript writing: Hu PS, Bau DT, Chang WS; manuscript checking and discussing: Hsia NY, Hu PS, Chuang CL, Mong MC, Chen HC, Tsai CW, Wang YC, Chen JC, Bau DT, Chang WS.

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