Evaluation of the ERCC2 (Lys751Gln), MSH2 (gIVS12-6TC), RAD54 (Ala730Ala), XPC (Lys939Gln), XPG (Asp1104Hist), XRCC1 (Arg399Gln) and XRCC3 (Thr241Met) gene polymorphisms in the Ecuadorian population with retinoblastoma

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Abstract

Background Retinoblastoma is a neoplasia that starts in the retina and may have inheritable or sporadic genetic predisposition. This affects children, mainly those who are under 5 years old. Approximately 9,000 new cases are diagnosed per year worldwide. In Ecuador this disease has an incidence of 1 per each 20,000 live births. The genetic predisposition to develop retinoblastoma is strongly influenced by RB1 gene, and may be influenced by the presence of genetic polymorphisms which intervene in the DNA repair system. **Methods** This study has analyzed the genotype frequency of ERCC2 (Lys751Gln), MSH2 (gIVS12-6TC), RAD54 (Ala730Ala), XPC (Lys939Gln), XPG (Asp1104Hist), XRCC1 (Arg399Gln), and XRCC3 (Thr241Met) polymorphisms of different repair genes, genotyping 90 individuals affected with retinoblastoma and 80 healthy individuals through polymerase chain reaction / restriction fragments length polymorphism and sequencing analysis. **Results** The presence of the (C/C) mutant homozygous genotype of XPC (Lys939Gln) polymorphism triggers a significant risk of developing retinoblastoma with an odds ratio (OR) of 3 (CI: 1.22-9.84; $p < 0.05$). Likewise, the A/G heterozygous genotype and the combination A/G+G/G of XRCC1 (Arg399Gln) polymorphism presented ORs of 9.7 (CI: 4.45-21.08; $p < 0.001$) and 7.55 (IC: 3.57-16; $p < 0.001$), respectively. **Conclusions** The genetic variants XPC (Lys939Gln) and XRCC1 (Arg399Gln) may be associated with the risk of developing retinoblastoma in the Ecuadorian population.

Background

Retinoblastoma (Rb) is a neoplasia characterized by the presence of malignant tumors in the retina. According to the Union for International Cancer Control, 95% of individuals are diagnosed before being 5 years old. Rb has an incidence of 1 per each 15,000–20,000 live births worldwide, and it accounts for 3% of all the cancer cases affecting children [1–3]. In Ecuador, according to the Cancer National Records by SOLCA, the incidence of this neoplasia is of 1 per 20,000 live births [4]. In developing countries, including Peru, Bolivia, Ecuador and India, the incidence of Rb increases since most of the cases are not detected on time due to the lack of access to health services [2, 3].

Most cancers find a way to impair *RB1* function, either through direct mutation of the *RB1* gene or, more commonly, through the altered expression of *RB1* regulators, which include cyclin D, CDK4 and CDK6, and their principal inhibitor, p16 [5]. RB1 is a multi-functional protein involved in a wide range of biological processes including transcriptional regulation by recruiting chromatin remodelling enzymes, DNA replication via interaction with DNA polymerase complex components, apoptosis and plays a central role in DNA repair [6]. In addition, proteins from the Rb family use their amino-terminal domains to interact with other proteins and recognize DNA damage [7].

RB1 is the tumor suppressor gene responsible for retinoblastoma. Rb usually occurs when both alleles of the *RB1* get inactivated in a precursor retinal cell, which is followed by mutations in some other specific genes. Both alleles may be lost from a retinal cell from which a tumor arises (nonheritable Rb or sporadic Rb) developing a somatic mutation, or it could be a germ line mutation (heritable Rb), in which case, there

is a predisposition for the development of multiple retinal tumors during childhood and even other cancers later in life [8]. Heritable RB encompasses 45% of all reported cases with bilateral (80%), unilateral (15%), or trilateral (5%) tumors [9].

On the other hand, DNA damage may be caused by endogen or hexogen factors. The endogen damages are mainly caused by free radicals, accelerating the rupture of DNA chains. Ionizing radiation and UV radiation cause hexogen damages responsible for the rupture of chain or pyrimidine dimers [10]. The DNA repair processes are important to eliminate different types of damage caused by those endogen or hexogen factors. Nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), and homologous recombination repair

(HR) are pathways to repair genetic material [11].

Some repair gene polymorphisms may affect the function of the protein that alters the capacity of repairing DNA, causing genetic instability and carcinogenesis. NER is responsible for repairing damage with voluminous adducts and thymine dimers. The *ERCC2*, *XPC*, and *XPG* genes are important members of this pathway [12, 13]. BER repairs damage caused by ionizing radiation or through alkylation replacing the mutated base with the correct nucleotide. The *XRCC1* gene is involved in this pathway, and codifies a protein that facilitates the repair carried out by hexogen factors. The Arg399Gln polymorphism of the *XRCC1* gene has a functional importance on the gene and could alter repair capacity and could influence cancer susceptibility [14, 15]. The *MSH2* gene is involved in DNA repair due to mismatch. *MSH2* is found in the short arm of the chromosome 2. In order to be able to perform its function of DNA repair, it must work together with other proteins, such as MSH6 and MSH3. It was previously found that the gIVS12–6TC polymorphism, which involves a replacement of T with C in an intron junction site, has been associated with the development of lymphomas. This polymorphism was found in 7.52% of the normal individuals (allele frequency of 0.05) and in 22.73% of the lymphomas (allele frequency of 0.11) [16, 17].

One of the main mechanisms to repair the rupture of DNA double chain is HR because it repairs the genetic material during cell replication [18]. The *XRCC3* gene is part of the repair by HR, its Thr241Met polymorphism could be associated with the damage in the repair function since replacing methionine with threonine may influence the function of the enzyme through the elimination of a phosphorylation site [19]. The *RAD54* gene also repairs DNA by HR and meets the function of modifying the topology of double-stranded DNA. *RAD54* encodes a protein of 747 amino acids; it is member of the SNF2 family and depends on DNA-ATPase. This gene was previously associated with the development of meningioma [20].

Single-nucleotide polymorphisms (SNPs) present in the previously explained genes may drive the development of Rb in Ecuador. Therefore, the objective of this research was to study the association of several SNPs of the DNA repair genes *ERCC2* (Lys751Gln), *MSH2* (gIVS12–6TC), *RAD54* (Ala730Ala), *XPC* (Lys939Gln), *XPG* (Asp1104Hist), *XRCC1* (Arg399Gln) and *XRCC3* (Thr241Met) with the risk of developing Rb in the Ecuadorian population.

Methods

Biological samples

The Bioethics Committee of the Universidad de las Americas approved this retrospective study (2015–0401) following the Declaration of Helsinki. A total of 170 individuals were included into the analysis. Concerning the 90 individuals with Rb, DNA from peripheral blood samples and paraffin-embedded tumor samples were collected per patient between the years 1999 and 2015 from the Solón Espinosa Ayala Oncological Hospital (SOLCA), Baca Ortiz Children's Hospital, Metropolitano Hospital, and Eugenio Espejo Hospital. All referred cases to these hospitals were captured including their respective medical history. With regards to control group, peripheral blood samples (n = 80) were obtained randomly from healthy children who did not suffer from Rb, did not present family antecedents of Rb, and went through a routine test at the Baca Ortiz Children's Hospital. Furthermore, all the participants signed their respective informed consent.

DNA Extraction

Histopathologists reviewed all tissue sections of paraffin-embedded tumors of the affected group. Subsequently, DNA was extracted from the area of interest (tumor tissue). Ten sections (10 µm thick) were cut and deparaffinized using a standard protocol. Genomic DNA was extracted using a protocol based on the High Pure PCR template preparation kit (Roche, USA) following the manufacturer's instructions. DNA extraction from peripheral blood (controls and cases) was carried out by means of the PureLink™ Genomic DNA kit (Invitrogen, USA). quantification of the extracted DNA was performed through the spectrophotometer Nanodrop™ 2,000 (Thermo Scientific, USA).

Genotyping

Regarding the affected individual cases, genotyping was performed analyzing DNA from paraffin-embedded tumor samples in order to correlate them with the genotypes from the blood samples. The genomic DNA from all individuals was amplified by the polymerase chain reaction (PCR), using specific primers for each polymorphism of the different repair genes (Table 1). Each reaction consists of 8.13 µl of water Mili-Q, 1.5 µl of genomic DNA (50–100 ng/µl), 10 mM of each deoxynucleoside triphosphate (dNTPs), 10 mM of each primer, 3 mM of MgCl₂, 1X Buffer of PCR and 5 U of Taq Polymerase (Invitrogen; Thermo Fisher Scientific, Inc.) in a total volume of 15 µl. The PCR program for all the polymorphisms was carried out in a thermocycler Sure Cyclor 8800 (Agilent, Santa Clara, CA), and consists of an initial denaturalization of 5 minutes at 95 °C, followed by 35 cycles of 1 minute at 94 °C, 45 seconds at different annealing temperatures, being 67 °C for the *ERCC2* gene, 51 °C for *MSH2*, 62 °C for *RAD54*, 64 °C for *XPC*, 61 °C for *XPG*, 60 °C for *XRCC1* and 62 °C for *XRCC3*, finishing with an elongation at 72 °C for 5 minutes.

The fragments amplified for the *ERCC2* (Lys751Gln), *XPC* (Lys939Gln), *XPG* (Asp1104Hist), *XRCC1* (Arg399Gln), and *XRCC3* (Thr241Met) polymorphisms were digested with 5 U of the *PstI*, *PvuII*, *Hsp92II*, *MspI* and *N1aIII* enzymes, respectively. The digested fragments were divided by conventional agarose gel electrophoresis in 4% agarose gel dyed with ethidium bromide and the genotypes were identified through amplicon observation under the UV light of the transilluminator. The products of the digestion of the different polymorphisms are detailed in Table 2. The *MSH2* (glVS12–6TC) and *RAD54* (Ala730Ala) polymorphisms were directly sequenced after amplification. The genotyping analysis of these last polymorphisms and the confirmation of the genotypes obtained by enzyme digestion were made by the genetic analyzer 3130 (Applied Biosystems, Austin, TX) using the BigDye Terminator v.3.1 kit (Applied Biosystems, USA).

Statistical Analysis

Each patient's clinical background was analyzed. Genotyping frequencies, allele frequencies, and Hardy-Weinberg equilibrium were calculated for the different polymorphisms. through the IBM SPSS version 11.5 program, the following tests were carried out: odds ratio (OR) (with a 2x2 contingency table and a 95% confidence interval) and p-values in order to determine the association between SNPs and the risk of developing Rb.

Results

The analysis included 90 cases of retina cancer and 80 controls from the Ecuadorian population. The relevant information of the studied population is shown in Table 3. The patients have a mean age of 3 years, being 51.11% males and 48.89% females. All patients underwent enucleation surgery for the removal of the eye at risk. As for the control group, blood samples were collected from 80 healthy individuals of 1–6 years old and a mean age of 3.40 years, being 62.50% males and 37.50% females. Regarding the age ranges, individuals between 2 and 5 years old had a non-significant risk (OR) of 1.35 (IC: 0.43–4.33; $p = 0.77$), and individuals under 2 years old had a non-significant OR of 1.32 (IC: 0.36–4.64; $p = 0.68$) in developing Rb, compared to the reference group (children older than 5 years). According to laterality, 81.11% of cases were unilateral, 17.78% were bilateral and 1.11% was trilateral.

Figure 1 shows the distribution of all 90 cases with Rb in 20 Ecuadorian provinces. The Sierra Region (the Highlands) presented 60% of cases followed by the Coast Region (25.60%) and the Amazon Basin (14.40%). The provinces with the most cases of Rb were Pichincha (28.90%), Guayas (11.10%), Imbabura (7.80%), Santo Domingo (6.70%), Sucumbíos (5.60%) and Chimborazo (5.60%). Regarding the type of Rb, 73 cases were unilateral, of which 58.90% were from Sierra, 26% from Coast and 15.10% from the Amazon Basin; 16 cases were bilateral, of which 68.80% were from Sierra, 25% from Coast and 6.20% from the Amazon Basin; finally, the only trilateral case was from the Amazon Basin.

Table 4 shows the genotype distribution and allele frequencies of the studied SNPs. The genotypes of the *ERCC2* (Lys751Gln), *XPC* (Lys939Gln), *XPG* (Asp1104Hist), *XRCC1* (Arg399Gln), and *XRCC3*

(Thar241Met) genetic polymorphisms were obtained according to the band pattern shown in agarose gel resulting from the enzyme digestion; the presence of the genotypes of each polymorphism was verified by means of electropherograms of sequences, excepting *MSH2* and *RAD54* which were directly analyzed by the electropherograms obtained in the sequencing process. The genotype frequencies of the study group, excepting those of the *XPG* gene, were in Hardy-Weinberg equilibrium. The frequency of the allele C of *ERCC2* was higher in cases (0.13) than controls (0.12). The frequency of the allele T of *MSH2* was higher in cases (0.06) than controls (0.04). The frequency of the C allele of *XPC* was higher in cases (0.48) in contrast with controls (0.36). The allele frequency of the G allele of *XRCC1* was higher in cases (0.46) than controls (0.29). Lastly, the allele frequency of the G allele of *XRCC3* was higher in cases (0.10) than controls (0.08).

Table 5 shows the association between different polymorphisms of repair genes and the risk of developing retina cancer. The statistical analysis has given rise to a higher risk of Rb in presence of certain genotypes of the *XPC* and *XRCC1* genes. The risk was 3.47 times higher for the group with the (C/C) mutant homozygous genotype of the *XPC* gene (OR = 3.47; CI: 1.22–9.84; p = 0.03). In addition to that, risk was observed for the heterozygous genotype (A/G) of the *XRCC1* gene with an OR of 9.74 (CI: 4.45–21.08; p < 0.01). On the other hand, the different genotypes of the *ERCC2*, *MSH2*, *RAD54*, *XPG* and *XRCC3* genes had no significant risk in developing Rb in the Ecuadorian population.

Discussion

Li *et al* (2013) performed an enrichment analysis of genes related to Rb through Gen Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database [21, 22]. They determined that 119 genes were related to Rb compared to normal retina. The enrichment analysis of the 119 genes generated 2 GO terms with significant false discovery rate (FDR) < 0.01 relevant for Rb: cell cycle (GO:0007049) and M phase of mitotic cell cycle (GO:0000087). For KEGG analysis, only the cell cycle pathway (hsa04110) was significantly enriched. However, the most influential gene in retinoblastoma is *RB1* [21, 22].

Rb is caused by mutations of the *RB1* gene altering functional proteins, triggering cell division in the retina, and leading to the development of tumors [23]. According to Leone *et al* (2003), a previous study in Ecuadorians with Rb has described new mutations and SNPs [24]. For instance, a mutation found in exon 15 with a replacement of G to A (g.76920 G>A), and a mutation found in exon 22 with a replacement of T to C (g.IVS22–14 T>C). Additionally, three SNPs were found, two were deletions in introns 15 (g.76983) and 16 (g.78064) and the third one was a transition C to T in intron 26.

On the other hand, several mechanisms are involved in the development of Rb such as the disruption of the DNA repair system [25]. The genomic alteration of this system has been associated with other types of cancer such as colorectal cancer (CRC) [26], glioma [27], lung cancer [28, 29], head and neck squamous cell carcinoma [30], melanoma [31] and breast cancer [32].

The disruption of the DNA repair system may be caused by the inter-individual variability in the capacity of repairing the genetic material, due to the presence of SNPs in several DNA repair genes found in coding or regulating regions [33]. The *XPC*, *XPB*, *XPV*, *XRCC1* and *XRCC3* repair genes may present polymorphisms leading to replacement of amino acids that may affect the function of the resulting proteins. These changes occur in a relatively high frequency, affecting this way a wide section of the world population [34]. The deficient result to DNA damage caused by hexogen and endogen agents may lead to genetic alterations in the DNA repair pathway, triggering serious biological effects [35].

NER pathway is involved in the elimination of short segments of nucleotides that have damaged bases by exposure to UV radiation. Important members of this pathway are *ERCC2*, *XPC*, and *XPB*. *ERCC2* synthesizes the *XPB* protein [36]. This protein is an essential subunit of a group of proteins called transcription factor IIH (TFIIH) complex. This complex is involved in gene transcription process and it helps to repair damaged DNA. According to Lanara *et al* (2013), a huge meta-analysis study has demonstrated that *ERCC2* Lys751Gln is positively correlated with breast and lung cancers [37], and this study contributes in understanding the association between this SNP and Rb in Ecuador.

A higher frequency of the A allele (0.58) was obtained for the Lys939Gln polymorphism of the *XPC* gene, whereas the C allele was represented by 0.42. In contrast with a meta-analysis that studied 679 cases of several types of cancer and 902 controls, the most prevalent allele was A with 0.62 [38], coinciding the allele frequency of the studied population. In a meta-analysis made in a Chinese population, the Lys939Gln polymorphism was significantly associated with the risk of CRC in the A/C heterozygous pattern (OR = 1.40, CI of 95% = 1.16–1.69; $p < 0.05$) [39]. Another research demonstrated that the patients with the C/C genotype have 2.09 times more risk of developing bladder cancer than normal A/A genotype [40]. In this study was found that the C/C genotype has 3.47 times more risk of developing retina cancer than a normal A/A genotype ($p < 0.05$). According to He *et al* (2013), the risk of developing any type of cancer may be assumed due to the fact that the polymorphisms of the *XPC* gene may influence the capacity of DNA repair and the function of the protein of an individual, affecting genetic instability and modifying predisposition to cancer, since this gene is involved in the recognition of DNA damage; thus, *XPC* is the main component to initiate NER pathway [41].

Several studies have shown that SNPs of the *XPB* gene are associated with several cancers. The Asp1104His polymorphism helped to increase the risk of CRC [25, 42]. He *et al* (2014) reported a significant decrease in the risk of melanoma for the C/C genotype (OR = 0.32; CI of 95% = 0.13–0.75; $p < 0.05$) [25]. In other studies a statistically significant risk of prostate cancer in individuals with C/C genotype (OR = 2.53, CI of 95% = 0.99–6.56; $p < 0.05$) was found. Concerning Rb, we had no significant results for G/C + C/C; thus, these are not associated with the disease (OR = 0.47, CI: 0.23–0.97, $p > 0.05$).

BER pathway repairs the oxidative DNA damage caused by alkylating agents. The *XRCC1* gene is an important component of this pathway. The Arg399Gln polymorphism of the *XRCC1* gene showed more frequency of G allele (0.63) in contrast with A allele (0.37). Vaezi, Feldman, & Niedernhofer (2011) suggest that the mutant G allele correlates with lower capacity of DNA repair, leading to increased

genomic instability and sensibility to agents that damage DNA [43]. In a meta-analysis of CRC, the frequency of G allele was significantly different among three populations, being highest in the black population (0.83), intermediate in Taiwanese origin (0.74), and lowest in whites (0.63). These differences suggest a possible ethnic variability in allele distribution of *XRCC1* [44]. Another study analyzed 1,635 patients with cervical cancer and 2,361 controls, showing that the Arg399Gln polymorphism decreases the susceptibility of such cancer (G vs. A: OR = 0.39, CI of 95% = 0.29–0.51; $p < 0.05$) [45]. In a meta-analysis in the Caucasian populations, it was found that the A/G and G/G genotypes were associated with the risk of developing lung cancer [46]. Likewise, a meta-analysis showed that the association between the Arg399Gln polymorphism and the risk of prostate cancer is affected by ethnicity; significantly marked associations were found among Asians, rather than African people [47]. In the current study it was also found an association between the Arg399Gln polymorphism with the risk of developing retina cancer for A/G genotype (OR = 9.74; CI: 4.45–21.08; $p < 0.05$), as well as for the combined genotype A/G + G/G (OR = 7.55; CI: 3.57–16.00; $p < 0.05$).

MMR pathway plays an important role in maintaining genomic stability and repairs damage mainly caused in base-base imbalance and the insertion/deletion errors generated during DNA replication and recombination [48]. *MSH2* belongs to this repair pathway. In a study on patients with lymphoma, it was found that 22.73% of these individuals had a replacement of T with C in intron junction site of the *MSH2*, whereas only 7.50% of the same variation was observed in normal individuals ($p < 0.01$); consequently, the allele frequency of the group was 0.95 for the T allele, 0.05 for the C mutant allele, and there were no homozygotes for the C allele in the group with lymphoma, suggesting this replacement probably influences susceptibility to cancer because it is twice more often in people who have developed lymphoma, rather than a non-carcinogenic population [17]. In this research no significant differences were obtained for the C/T heterozygote (OR = 1.43; CI: 0.46–3.92; $p > 0.05$), which suggests that such polymorphism has no association with the risk of Rb.

Several replication errors cause double-chain breaks that are generated by hexogen agents, such as ionizing radiation. The pathway that repairs such damages is homologous recombination to which the *RAD54* and *XRCC3* genes belong. As for the *RAD54* gene, according to Paz-y-Miño *et al* (2010), a statistically significant association was found between frequencies calculated for the subgroup with chronic myelogenous leukemia (0.14), in contrast with the control group [49]. Concerning meningioma, an association between the T allele and the development of meningioma tumor ($p < 0.05$) was found [20]. In this research an association of the *RAD54* with Rb was not found (OR = 0.63; CI: 0.25–1.52; $p > 0.05$). Regarding the *XRCC3* gene, in an analysis of melanoma a significant association of the T/T genotype and cancer (TT versus CC: OR = 1.06; CI of 95% = 0.87–1.29) was found. Another study showed a protective effect in the individuals with the C/C genotype (OR: 0.32; $p < 0.05$), whereas the T/T mutant genotype indicated that the risk of developing lung cancer is 4 times higher. In this study a statistically significant association with Rb was not found since the OR was of 1.31 for C/T + T/T with a $p > 0.05$.

Conclusions

The *RB1* gene has been associated with different pathways that are involved in the development of cancer such as DNA repair. Consequently, according to the evidence obtained in this research, it is suggested that Rb is directly related to the NER (*XPC*) and BER (*XRCC1*) repair pathways.

Abbreviations

Rb: Retinoblastoma; NER: Nucleotide excision repair; BER: base excision repair; MMR: mismatch repair; HR: homologous recombination repair; SNPs: Single-nucleotide polymorphisms; PCR: polymerase chain reaction; dNTPs: deoxynucleoside triphosphate; OR: odds ratio; KEGG: Kyoto Encyclopedia of Genes and Genomes; CRC: colorectal cancer; TFIIH: transcription factor IIH.

Declarations

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Availability of data and material

All data generated or analyzed during this study are included in this published article. All these data supports the conclusions of the study.

Authors' contributions

PEL designed the study and wrote the manuscript. PGR performed the experiments and wrote the manuscript. SQ performed the data collection of clinic history. SZ was the pathologist and histopathologically analyzing the paraffin-embedded tumor samples. IAC, JMGC, SG, ALC, AP, VY and AKZ analyzed the data. CPyM supervised the research project. All authors read and approved the final manuscript.

Ethical approval and consent to participate

This study was approved by the Bioethics Committee of the Universidad de las Americas (2015–0401) and met the tenets of the Declaration of Helsinki, and informed consent was obtained from all of the

subjects prior to the study.

Consent for publication

Not applicable

Competing interests

The authors have no conflict of interest to declare.

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Tables

Table 1. Primer sequences, annealing temperatures, and genetic variations.

Gene	Polymorphism	Primer sequence	Fragment (bp)	Annealing temperature (°C)
ERCC2	Lys751Gln	FW-5'-TCAAACATCCTGTCCCTACTGGCCAT-3'	343	67
	rs13181	RV-5'-CTGCGATTAAAGGCTGTGGACGTGAC-3'		
	A/C			
MSH2	glVS 12-6TC	FW-5'-CGCGATTAATCATCAGTG-3'	353	51
	rs2303428	RV-5'-GGACAGAGACATACATTTCTATC-3'		
	T/C			
RAD54	Ala730Ala	FW-5'-AGTGCCCTAACCATTATC- 3'	258	62
	rs1048771	RV-5' -TGGAAGACGAAGGTGATA- 3'		
	C/T			
XPC	Lys939Gln	FW-5'-ACCAGCTCTCAAGCAGAAGC-3'	365	64
	rs2228001	RV-5'-GAATCTGACAAGGGCTGGAG-3'		
	A/C			
XPG	Asp1104His	FW-5'-TGGATTTTTGGGGGAGACCT-3'	159	61
	rs17655	RV-5'-CGGGAGCTTCCTTCACTGAGT-3'		
	G/C			
XRCC1	Arg399Gln	FW-5'-GCCCCCTCAGATCACACCTAA-3'	344	60
	rs25487	RV-5'-AGGTCCTCCTTCCCTCATCT-3'		
	A/G			
XRCC3	Thr241Met	FW-5'-TTCAGACGGTCGAGTGACAG-3'	358	62
	rs861539	RV-5'-CCGCATCCTGGCTAAAAATA-3'		
	C/T			

bp, base pairs

Table 2. Enzyme digestion with RFLPs and fragment weights.

Gene	Polymorphism	Enzyme	Genotype	Fragment (bp)
ERCC2	Lys751Gln	<i>PstI</i>	A/A	234, 110
			A/C	234, 172, 110, 62
	rs13181		C/C	172, 110, 62
XPC	Lys939Gln	<i>PvuII</i>	A/A	365
			A/C	365, 250, 150
	rs2228001		C/C	250, 150
XPG	Asp1104His	<i>Hsp92II</i>	G/G	159
			G/C	159, 100, 59
	rs17655		C/C	100, 59
XRCC1	Arg399Gln	<i>MspI</i>	G/G	184, 161
			A/G	344, 184, 161
	rs25487		A/A	344
XRCC3	Thr241Met	<i>N1aIII</i>	C/C	341, 18
			C/T	341, 243, 100, 18
	rs861539		T/T	243, 100, 18

bp, base pairs

Table 3. Baseline characteristics (at diagnosis) of cases and controls.

Variable	Cases	Controls	OR	95% CI	p-value
	n=90 (%)	n=80 (%)			
Gender					
Male	46 (51.11)	50 (62.50)		-	
Female	44 (48.89)	30 (37.50)			
Age at diagnosis					
> 5	6 (6.67)	7 (8.74)		Reference (1.0)	
2-5	65 (72.22)	56 (70.01)	1.35	0.43 – 4.33	0.77
< 2	19 (21.11)	17 (21.25)	1.32	0.36 – 4.64	0.68
Type of Rb					
Unilateral	73 (81.11)	-		-	
Bilateral	16 (17.78)	-			
Trilateral	1 (1.11)	-			

n, number of individuals; Rb, retinoblastoma; OR, odds ratio.

Table 4. Genotype distribution and allele frequency of single nucleotide polymorphisms.

Gene	Genotype	Genotypic frequency			Allele frequency		
		Cases	Controls	All	Cases	Controls	All
<i>ERCC2</i>	A/A	0.74	0.76	0.75	0.87	0.88	0.88
	A/C	0.26	0.24	0.25	-	-	-
	C/C	0.00	0.00	0.00	0.13	0.12	0.12
<i>MSH2</i>	C/C	0.91	0.90	0.92	0.94	0.96	0.95
	C/T	0.07	0.10	0.07	-	-	-
	T/T	0.02	0.00	0.01	0.06	0.04	0.05
<i>RAD54</i>	C/C	0.90	0.85	0.88	0.95	0.93	0.94
	C/T	0.10	0.15	0.12	-	-	-
	T/T	0.00	0.00	0.00	0.05	0.08	0.06
<i>XPC</i>	A/A	0.23	0.38	0.30	0.52	0.64	0.58
	A/C	0.58	0.54	0.56	-	-	-
	C/C	0.19	0.09	0.14	0.48	0.36	0.42
<i>XPG</i>	G/G	0.31	0.18	0.24	0.65	0.58	0.62
	G/C	0.68	0.81	0.75	-	-	-
	C/C	0.01	0.01	0.01	0.35	0.42	0.38
<i>XRCC1</i>	A/A	0.13	0.54	0.34	0.54	0.71	0.63
	A/G	0.81	0.34	0.57	-	-	-
	G/G	0.05	0.13	0.09	0.46	0.29	0.37
<i>XRCC3</i>	C/C	0.81	0.85	0.83	0.90	0.92	0.91
	C/T	0.18	0.14	0.16	-	-	-
	T/T	0.01	0.01	0.01	0.10	0.08	0.09

Table 5. Association between DNA repair genes and retinoblastoma risk among cases and controls.

Gene /	Genotypes	Cases	Controls	OR	95% CI	p-value
Polymorphism		n=90 (%)	n=80 (%)			
<i>ERCC2</i> rs13181	A/A	67 (74.44)	61 (76.25)		Reference (1.0)	
	A/C	23 (25.56)	19 (23.75)	1.12	0.55 - 2.22	0.86
	C/C	0 (0.0)	0 (0.0)	-	-	-
	A/C + C/C	23 (25.56)	19 (23.75)	1.12	0.55 - 2.22	0.86
<i>MSH2</i> rs2303428	C/C	81 (90)	73 (91.25)		Reference (1.0)	
	C/T	9 (10)	6 (7.50)	1.43	0.46 - 3.92	0.60
	T/T	0 (0.0)	1 (1.25)	-	-	-
	C/T + T/T	9 (10)	7 (8.75)	1.16	0.41 - 3.24	0.82
<i>RAD54</i> rs1048771	C/C	81 (90)	68 (85)		Reference (1.0)	
	C/T	9 (10)	12 (15)	0.63	0.25 - 1.52	0.36
	T/T	0 (0.0)	0 (0.0)	-	-	-
	C/T + T/T	9 (10)	12 (15)	0.63	0.25 - 1.52	0.36
<i>XPC</i> rs2228001	A/A	21 (23.33)	30 (37.50)		Reference (1.0)	
	A/C	52 (57.77)	43 (53.75)	1.73	0.87 - 3.44	0.16
	C/C	17 (18.90)	7 (8.75)	3.47	1.22 - 9.84	0.03 ^a
	A/C + C/C	69 (76.66)	50 (62.50)	1.97	1.01 - 3.84	0.07
<i>XPG</i> rs17655	G/G	28 (31.11)	14 (17.50)		Reference (1.0)	
	G/C	61 (67.77)	65 (81.25)	0.47	0.23 - 0.97	0.06
	C/C	1 (1.12)	1 (1.25)	0.52	0.03 - 8.61	1
	G/C + C/C	62 (68.88)	66 (82.50)	0.47	0.23 - 0.97	0.06
<i>XRCC1</i> rs25487	A/A	12 (13.33)	43 (53.75)		Reference (1.0)	
	A/G	73 (81.11)	27 (33.75)	9.74	4.45 - 21.08	< 0.01 ^a
	G/G	5 (5.56)	10 (12.50)	1.82	0.51 - 6.25	0.49
	A/G + G/G	78 (86.66)	37 (46.25)	7.55	3.57 - 16	< 0.01 ^a
<i>XRCC3</i> rs861539	C/C	73 (81.11)	68 (85)		Reference (1.0)	
	C/T	16 (17.77)	11 (13.75)	1.36	0.59 - 3.13	0.53
	T/T	1 (1.12)	1 (1.25)	0.93	0.06 - 15.22	1
	C/T + T/T	17 (18.88)	12 (15)	1.31	0.59 - 2.92	0.55

OR, odds ratio; CI, confidence intervals; n, number of individuals.

^a Significant

Figures

Figure 1

Distribution and laterality of Rb cases in Ecuador.