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ORIGINAL ARTICLE

RB1 gene mutations in retinoblastoma and its clinical correlation

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KEYWORDS

Retinoblastoma;
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Multiplex-PCR

Abstract *Purpose:* To find correlation between the type of mutations observed and the severity of the disease using multiple techniques like polymerase chain reactions (PCR), quantitative multiplex PCR, sequencing and RNA analysis.
Methods: Prospective, observational study. Patients who had been screened for mutations in the *RB1* gene were included in the study. Patient details including demographic data; age and sex, laterality, international classification of intraocular retinoblastoma (ICIOR) staging, modality of management, histopathology high risk factors if the eyes were enucleated and metastasis rate were assessed.
Results: Seventy four patients were studied. Fifty three patients had bilateral and 21 unilateral disease. Complete genetic data was analyzed for 74 patients and complete clinical correlation was established for all the 49 patients with mutations. Of the total mutations identified, 11/49 (22.4%) of patients had large deletions, 12/49 (24.5%) had small deletions or insertions, 14/49 (28.6%) had nonsense mutations, 7/49 (14.3%) had splice mutations and 5/49 (10.2%) of patients

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had missense mutations. Four cases were familial. Group E ICIOR stage at presentation was noted in 40% of patients with large deletions, 33% with small deletions whereas 38.5% with splice mutations and 44.4% of patients with missense mutations presented with Group B ICIOR. Twenty five percentages of eyes with large deletions had high risk features on histopathology and one patient among these developed metastasis.

Conclusion: Current laboratory testing of *RB1* mutations may be feasible in determining the severity of the disease and patient counseling. The study provides a starting point for looking at correlations.

1. Introduction

Retinoblastoma (Rb) is the most common intraocular malignancy in children, with a reported incidence ranging from 1 in 15,000 to 1 in 18,000 live births (Bishop and Madsen, 1975). It is second only to uveal melanoma in the frequency of occurrence of malignant intraocular tumors. There is no racial or gender predisposition in the incidence of retinoblastoma. Retinoblastoma is bilateral in about 25–35% of cases (Shields and Shields, 1992). The average age at diagnosis is 18 months, unilateral cases being diagnosed at around 24 months and bilateral cases before 12 months (Shields and Shields, 1992).

Retinoblastoma (Rb) is brought about by biallelic inactivation of the human retinoblastoma susceptibility gene, *RB1* (GenBank accession number L11910), located on chromosome 13q14 that codes for the RB protein. The cytogenetic deletions examined in retinoblastomas have assigned the genetic locus of the disease to q14 of chromosome 13 linked with the polymorphic marker gene enzyme esterase D (Friend et al., 1986). A successful labeling of normal and tumor cells with homozygous *RB1* gene deletion in the human tumors, showed that most *RB1* deficient tumor cells resemble cone photoreceptors, suggesting that cone photoreceptors is the cell of origin (Xu et al., 2009).

Retinoblastoma arises due to two genetic events involving both the alleles of *RB1* and occurs in two forms, the hereditary and non-hereditary. Mutation of both the alleles of *RB1* is required for tumor initiation. In case of hereditary transmission of the disease, one allele is mutated in the germline and the other mutation occurs somatically in the developing retina. In non-hereditary disease, mutations of both the alleles somatic (Knudson, 1971; Mairal et al., 2000; Corson and Gallie, 2007). The *RB1* gene shows a high degree of mutational heterogeneity in retinoblastoma with over 900 mutations reported till date (Valverde et al., 2005). Approaches using multiple techniques including quantitative multiplex PCR and sequencing (Richter et al., 2003), or DHPLC (denaturing high-performance liquid chromatography) along with quantitative multiplex PCR for short fluorescent fragments (QMPSF) Houdayer et al., 2004 could achieve detection rate of 80–89% (Richter et al., 2003; Houdayer et al., 2004). Precise identification of the *RB1* gene mutation could help in enhancing the clinical management of the relatives at risk (Gallie et al., 1995). Genetic testing of retinoblastoma is being employed to screen for and detect carriers of *RB1* mutations among relatives of affected individuals and for prenatal testing (Gallie et al., 1999). This in turn can facilitate prompt management of the disease and better visual outcome in affected children (Gallie et al., 1999).

Our group first developed and published a combinatorial approach for detection of *RB1* mutations (Parsam et al., 2009) and in the present study the authors attempted to correlate clinical features of the disease with *RB1* mutations in 49 patients with known mutations.

2. Materials and methods

2.1. Study: prospective, observational

2.1.1. Technique

Mutational analysis of *RB1* was carried out to detect different types of mutations as previously described (Parsam et al., 2009). Once our group had the genetic data, authors then correlated the different types of mutations with patient details like demographic data; age and sex, laterality, international classification of intraocular retinoblastoma (ICIOR) staging, modality of management, high risk factors if the eyes were enucleated and metastasis rates. The following histopathological entities were taken as high risk features: (Honavar et al., 2002) anterior chamber seeding, iris infiltration, ciliary body infiltration, massive choroidal infiltration, invasion of optic nerve lamina cribrosa, retrolaminar optic nerve invasion, invasion of optic nerve transection, scleral infiltration and extrascleral extension.

3. Results

Seventy four patients were studied. Fifty three patients had bilateral and 21 unilateral retinoblastoma. By combining the three different approaches as elucidated in the techniques above, mutations were detected in 49 patients (44 bilateral and five unilateral) (Parsam et al., 2009). Complete genetic data was analyzed for 74 patients and complete clinical correlation was established for all the 49 patients with mutations. Since the study is about the clinical correlation among patients of retinoblastoma where mutations were identified, all the results would restrict to these 49 patients. There were 28 males (57%) and 21 females (43%) among the study subjects. The mean age was 17.2 months (range 3–38 months). Of the total mutations identified, 11/49 (22.4%) of patients had large deletions, 12/49 (24.5%) had small deletions or insertions, 14/49 (28.6%) had nonsense mutations, 7/49 (14.3%) had splice mutations and 5/49 (10.2%) of patients had missense mutations. Four cases were familial. 23/49 (46.94%) of the mutations identified were not reported earlier to the best of our knowledge and these were novel mutations identified in our study (Parsam et al., 2009). The clinical details among each class are as follows.