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ORIGINAL ARTICLE

RB1 gene mutations in retinoblastoma and its clinical correlation

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KEYWORDS

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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.

3.1. Large deletions

Eleven of the 49 patients had large deletions accounting for 22.4% of all the mutations. There were six male and five female patients. 9/11 (81.8%) patients had bilateral retinoblastoma whereas 2/11 (18.2%) had unilateral retinoblastoma at presentation. Of the 20 eyes that were studied in this group, eight (40%) presented with Group E ICIOR followed by groups B, D and C ICIOR. Two of the 20 (10%) presented with extra ocular extension and one eye had presented after enucleation elsewhere. Seven (35%) eyes were enucleated. The other modalities of management in this group included chemoreduction and focal therapy. Focal therapy here included cryotherapy and transpupillary thermotherapy. Plaque brachytherapy was evaluated separately. External beam radiotherapy (EBRT) was given as a part of multimodal approach for the extra ocular retinoblastomas. Five of the 20 eyes (25%) had high risk histopathological features and these patients underwent adjuvant chemotherapy. One patient with extra ocular extension presented with metastasis and was subjected for palliative treatment (Table 1).

3.2. Small deletions

Twelve of the 49 patients had small deletions accounting for 24.5% of all the mutations. There were eight male and four female patients. All these 12 patients had bilateral retinoblastoma at presentation. Of the 24 eyes that were studied in this group, eight (33.3%) presented with Group E ICIOR followed by groups B, D and C ICIOR. Two (8.3%) presented with extra ocular extension and one eye had presented after enucleation elsewhere. Eight (34%) eyes were enucleated. The other modalities of management in this group included chemoreduction, focal therapy and plaque brachytherapy. External beam radiotherapy (EBRT) was given as a part of multimodal approach for the extra ocular retinoblastoma. Two (8.3%) of the eyes had high risk histopathological features and these patients underwent adjuvant chemotherapy (Table 2).

3.3. Nonsense mutations

Fourteen of the 49 patients had nonsense mutations accounting for 28.6% of all the mutations. There were six male and eight female patients. Thirteen of the 14 patients (92.8%) had bilateral retinoblastoma whereas the remaining one (7.2%) patient had unilateral retinoblastoma at presentation. Of the 27 eyes that were studied in this group, nine (33.3%) presented with Group B ICIOR followed by groups C, E and D ICIOR. Six (22.2%) eyes were enucleated. The other modalities of management in this group included chemoreduction, focal therapy and plaque brachytherapy. Two (7.4%) of the eyes had high risk histopathological features and these patients underwent adjuvant chemotherapy (Table 3).

3.4. Splice mutation

Seven of the 49 patients had splice mutations accounting for 14.3% of all the mutations. There were five male and two female patients. Six out of seven (85.7%) patients had bilateral retinoblastoma whereas one (14.3%) had unilateral retinoblastoma at presentation. Of the 13 eyes that were studied in this group, five (38.5%) presented with Group B ICIOR followed by groups C, D and E ICIOR. Two (15.4%) eyes were enucleated. The other modalities of management in this group included chemoreduction, focal therapy and plaque brachytherapy. Two (15.4%) of the eyes had high risk histopathological features and these patients underwent adjuvant chemotherapy (Table 4).

3.5. Missense mutation

Five of the 49 patients had nonsense mutations accounting for 10.2% of all the mutations. There were three male and two

Table 1 Clinical profile of retinoblastoma patients with large *RB1* deletions.

No. of patients	Sex	No. of eyes	Laterality	Stage-eyes	High risk features (HRF)	Metastasis	Management
Large deletions							
11	6 M 5 F	20	Unilateral-2 patients Bilateral-9 patients	B-4 C-2 D-3 **E-8 (40%)** Extra ocular extension-2 Enucleation else-1	5	1	Enucleation-7 (35%) Chemoreduction-9 External beam radiotherapy-2 Focal therapy-5 Pallative therapy-1 Adjuvant chemotherapy-5

Table 2 Clinical profile of retinoblastoma patients with small *RB1* deletions.

No. of patients	Sex	No. of eyes	Laterality	Stage-eyes	High risk features (HRF)	Metastasis	Management
Small deletions							
12	8 M 4 F	24	All bilateral RB patients	B-5 C-2 D-5 **E-8 (33%)** Extra ocular extension-2 Enucleation else where-1	2	Nil	Enucleation-8 (34%) Chemoreduction-11 External beam radiotherapy-2 Focal therapy-11 Pallative therapy-0 Adjuvant chemotherapy-5

Table 3 Clinical profile of retinoblastoma patients with *RB1* nonsense mutations.

No. of patients	Sex	No. of eyes	Laterality	Stage-eyes	High risk features (HRF)	Metastasis	Management
Nonsense mutations							
14	6 M 8 F	27	Unilateral patients-1 Bilateral patients-13	**B-9 (33.3%)** C-6 D-4 E-6 Phthisis bulbi-1 Enucleation else where-1	2	Nil	Enucleation-6 (22%) Chemoreduction-14 External beam radiotherapy-0 Focal therapy-9 Plaque brachytherapy-1 Adjuvant chemotherapy-2

Table 4 Clinical profile of retinoblastoma patients with *RB1* splice mutations.

No. of patients	Sex	No. of eyes	Laterality	Stage-eyes	High risk features (HRF)	Metastasis	Management
Splice mutations							
7	5 M 2 F	13	Unilateral patients-1 Bilateral patients-6	**B-5 (38.5%)** C-3 D-3 E-2	2	Nil	Enucleation-2 (15.4%) Chemoreduction-6 External beam radiotherapy-0 Focal therapy-6 Plaque brachytherapy-1 Adjuvant chemotherapy-2

Table 5 Clinical profile of retinoblastoma patients with *RB1* missense mutations.

No. of patients	Sex	No. of eyes	Laterality	Stage-eyes	High risk features (HRF)	Metastasis	Management
Missense mutations							
5	3 M 2 F	9	Unilateral patients-1 Bilateral patients-4	**B-4 (44.5%)** C-1 D-2 E-2	1	Nil	Enucleation-2 (22%) Chemoreduction-3 External beam radiotherapy-0 Focal therapy-4 Adjuvant chemotherapy-2

female patients. Four of the five patients (80%) had bilateral retinoblastoma whereas the remaining one (20%) had unilateral retinoblastoma at presentation. Of the nine eyes that were studied in this group, four (44.4%) presented with Group B ICIOR followed by groups D, E and C ICIOR. Two (22%) eyes were enucleated. The other modalities of management in this group included chemoreduction and focal therapy. One (11.1%) of the eyes had high risk histopathological features and this patient underwent adjuvant chemotherapy (Table 5).

4. Discussion

In the present study a practical correlation between clinicopathological features of retinoblastoma with genetic mutational status was attempted. The caveat in such studies is that several different categories of mutation such as deletions or insertions of different sizes, and nonsense mutations have the same impact at the protein level. Essentially, the protein would be truncated in case of all frame-shifting and nonsense mutations, and so would be grossly defective or non-functional or would have undergone degradation either at the mRNA or protein level.

Alonso et al. (2001) studied the spectrum of germ line *RB1* mutations in a Spanish population and tried to bring out the phenotypic and molecular implications of these mutations. They found 29 mutations in 49 hereditary retinoblastoma patients, most of them (62%) not reported earlier. Sixty nine percentages of these were nonsense mutations and the remainder splice mutations. Splice mutations were associated with delayed onset at presentation (32 months on average) whereas nonsense mutations had early onset (8.7 months on average). In comparison our study showed that 23 (46.94%) of the mutations identified were not reported earlier to the best of our knowledge, however early onset retinoblastomas in our study were seen more with large deletions (mean age 13.64 months, range 3–22 months) and nonsense mutations (mean age 14.43 months, range 5–26 months).

Taylor et al. (2006) were the first to study genotype–phenotype correlations in hereditary familial retinoblastoma. This was the first large scale study of familial retinoblastoma with high level of homogeneity in the clinical and genetic analysis of patients and their relatives thereby allowing reliable intrafamilial genotype–phenotype correlations. They studied the mutations and correlated them with demographic features,

carrier and disease status and disease eye ratio. In comparison since most of the patients (90% or more) presenting in our clinic are sporadic cases, our series comprised sporadic and a few familial cases. We took into consideration clinical manifestations, stages at presentation and modalities of management.

Another factor in our study that limits interpretation is the relatively small numbers of patients within each of the subgroups of patients having deletions, nonsense, splice and missense mutations. Group E ICIOR stage at presentation was noted in 40% of patients with large deletions and 33% with small deletions. However, 38.5% of patients 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 them developed metastasis. The tumor stages could have also been influenced by delayed presentation, however. More accurate data on age at onset, and comparison of cases with early presentation would enhance correlations. The current study despite its limitations, may be helpful in promoting further larger studies and provides a starting point for looking at correlations. We also feel the need for a larger more uniform sample size with longer follow up to identify the genetic factors that modify the relationship between genotype and phenotype in retinoblastoma.

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