RESEARCH COMMUNICATION

Genotype-phenotype Correlation Analysis in Retinoblastoma Patients from India

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Abstract

Background: Genetic analysis has a beneficial impact on retinoblastoma management enabling definite risk assessment. However, information regarding genotype-phenotype correlation in retinoblastoma is limited. Aim: To analyze the retinoblastoma susceptibility gene for mutations in retinoblastoma patients and correlate the genotypes to the phenotypes. Methodology: Eleven retinoblastoma patients, who underwent molecular genetic studies were classified into high, moderate or low disease severity groups based on phenotype. Results: Seven patients had high disease severity and four moderate disease severity. Eleven truncating mutations were detected; six were in the N-terminus region of the retinoblastoma protein and two in the A/B pocket (p=0.03). Conclusions: No significant association between mutation type and disease severity could be established in the present study. However a positive correlation between location of the mutations in certain domains of the retinoblastoma protein and disease severity was observed. To the best of our knowledge this is the first genotype-phenotype correlation study in retinoblastoma patients from India.

Key Words: Genotype, phenotype, RB1 mutations, Retinoblastoma

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Introduction

Retinoblastoma (RB1; MIM# 180200) is a malignant tumor of the eye that arises from un-differentiated retinal precursor cells. It occurs in children below the age of five years (Brantley and Harbour, 2001). Retinoblastoma manifests in its earliest clinical stage as a small (less than 2 mm in basal diameter), slightly translucent lesion in the sensory retina. Larger tumors present with leukocoria, strabismus and/or neovascular glaucoma. The disease has been categorized as low, moderate or high severity according to disease stage, age at presentation and subsequent clinical course (Qi et al., 2005). Treatment depends on the tumor stage with smaller lesions treated by local modalities such as cryotherapy, photocoagulation, and brachytherapy and larger tumors necessitating external beam radiation therapy (EBRT), chemotherapy, and/or enucleation.

The RB1 gene is a large 180 kilobase, 928 – aminoacid protein at chromosome 13q14, that plays a role in cell growth and development. Mutations in both alleles of RB1 gene are required for development of the tumor (Cavenee et al., 1985). While a de novo or inherited germline mutation gives rise to bilateral retinoblastoma, somatic mutations in both the alleles account for 85% of unilateral tumors (Richter et al., 2003). Most RB1 mutations are point mutations (single-base substitutions, short length-alterations, and complex mutations). These can be broadly classified as truncating, non-truncating and promoter mutations (Qi et al., 2005). The RB1 gene codes for the retinoblastoma protein pRB, that has 4 domains (Figure 1); the N terminus (aminoacids 1-392), the A/B domain (aminoacids 393-772), the C pocket (aminoacids 773-869), and the C terminus (aminoacids 870-928).

Genotype-phenotype associations are of great significance since they might help prognosticate the effect of an individual mutation in a carrier (Lohmann and Gallie, 2004). To date conflicting reports exist about correlation between the genotype and phenotype in retinoblastoma. A retrospective analysis of 88 germline RB1 mutation carriers from Canada and the United States (Qi et al., 2005) revealed a significant association of high disease severity with truncating mutations. However, mean residual protein length and location of mutations in domains did not show any association to clinical severity (Qi et al., 2005). Albrecht et
al observed a positive correlation between presence of gross
deletion with one breakpoint in RB1 gene and occurrence
of bilateral retinoblastoma (Albrecht et al., 2005). Alonso
et al reported an association between mutation type and time
of onset of retinoblastoma (Alonso et al., 2001). Mutations
affecting splice junctions resulted in a delayed onset of
tumors while nonsense and frameshift mutations were
associated with an early age at diagnosis (Alonso et al.,
2001). However, studies on Chinese and Indian
retinoblastoma patients did not demonstrate any association
between the type of mutations and the phenotype (Ata-ur-
Rasheed et al., 2002; Choy et al., 2002). Further, families
with the same mutation have been shown to demonstrate
phenotypic differences viz. the development of lipomas in
only one of two retinoblastoma families having an identical
splice mutation (resulting in skipping of exon 13) (Lohmann
and Gallie, 2004).

We report the results of mutational analyses of the RB1
gene, and the correlation between the genotype and
phenotype in 11 retinoblastoma patients from our center.

Materials and Methods

The study was carried out at Vision Research Foundation,
Sankara Nethralaya, Chennai, India, a tertiary eye care center
for retinoblastoma in India. The study was approved by the
institutional ethics review board and adhered to the tenets
of the declaration of Helsinki.

The study population comprised of 11 retinoblastoma
patients who presented to our hospital during the period
1999-2000. All the cases were staged according to the Reese-
Ellsworth classification (Shields and Shields, 2004). Enucleation had to be performed in all the patients due to
advanced disease. Diagnosis of retinoblastoma was
confirmed by current histopathological criteria.

Histopathological examination included determination of
tumor differentiation, and presence or absence of choroidal
and/or optic nerve invasion, tumor cells at the cut end of the
optic nerve, and any extra ocular extension. Patients were
classified into 3 disease severity groups viz. high, moderate
and low, according to criteria adopted by Qi et al (2005)
with some modifications (Table 1).

RB1 mutation screening was performed on DNA from
fresh tumor and peripheral blood following methods
described in a previous study (Kumaramanickavel et al.,
2003). Mutations were classified based on the type and
aminoacid location in the retinoblastoma protein. They were
divided into 3 groups viz. (i) large deletions that included
mutations which resulted in loss of multiple exons, (ii)
truncating mutations that included nonsense, frameshift or
splice site mutations resulting in premature creation of stop
codon and (iii) non-truncating mutations, which resulted in
change of aminoacids. Predicted residual protein length and
domains disrupted by the mutations were calculated for
patients with truncating mutations.

Statistical Analysis- The correlation of disease severity
with the type of mutations was analyzed using ‘Z’ test for
proportions with SPSS version 13. The exact test based on
Montecarlo assumption was used to test for correlation
between mean residual protein length and disease severity.

Results

All 11 patients (3 unilateral retinoblastomas / 8 bilateral
retinoblastomas) were identified to have moderate to high
disease severity, with 7 patients categorized as having highly
severe disease and 4 patients identified as having moderately-
severe disease. No patient could be placed in the low severity
group (Table 2). A total of 13 mutations were detected in
the study of which eight were found in patients with a highly
severe phenotype and five were found in patients with moderately-severe disease. These mutations included nine nonsense mutations, one frameshift mutation (eight bp deletion), and one splice site mutation (all resulting in truncation) and two large deletions (Table 3). Both large deletions were present in the high disease severity group. Of 11 truncating mutations, six were found in the high disease severity group and five were found in the moderate disease severity group (Figure 1). The mean residual protein length was 289 amino acids for the moderate disease severity group and 588 amino acids for the high disease severity group (Table 3).

When considering the location of the mutations in the RB1 gene, six truncating mutations (five nonsense, one frameshift and one large deletion) were present in the N terminus region, in moderate and high severity groups; two nonsense mutations were seen in the A/B pocket and three nonsense mutations were in the C pockets (Figure 1). Of these, one patient with a mutation in A/B pocket (N12) and another patient with a mutation in C pocket (S161) had a second mutation in the N terminus. These patients had moderate and high severe retinoblastoma respectively. In the patient who had an intron 20 deletion, the intron 2 was intact; however the location of the deletion cannot be stated with absolute certainty as PCR was performed using microsatellites in RB1 introns 2 and 20. This deletion was therefore placed between the N terminus and the A/B pocket.

### Table 2. Correlation of RB1 Mutations versus Disease Severity

<table>
<thead>
<tr>
<th>Severity</th>
<th>RB1 Truncating mutations</th>
<th>Mean residual protein length</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>8</td>
<td>588</td>
</tr>
<tr>
<td>Moderate</td>
<td>5</td>
<td>289</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>-</td>
</tr>
</tbody>
</table>

No statistically significant association was found between disease severity (phenotype) and mutation type \( (p>0.05) \). The mean predicted protein length also did not show any statistically significant difference between moderate and high disease severity groups. However, the number of truncating mutations in the N terminus region were significantly more (6/10) when compared to the A/B pocket region (2/10; \( p=0.03 \)) and C pocket region (3/10; \( p=0.08 \)).

### Discussion

Genotype-phenotype information is important for genetic counseling of retinoblastoma families, enabling risk assessment and prognostication of the disease (Lohmann and Gallie, 2004). While no genotype-phenotype correlation could be drawn in Chinese (Choy et al., 2002) and Indian (Ata-ur-Rasheed et al., 2002) populations, gross deletions (with one breakpoint in RB1 gene) were significantly associated with laterality in another study (Albrecht et al., 2005). In the present study, no significant association was observed between truncating mutations and disease severity. This could be because of the small sample size. Additionally, we did not find any correlation between mean predicted residual protein length and disease status for the truncating mutations. While Qi et al (2005) could not detect a relationship between truncating mutations and their location, we found that majority of the truncating mutations (6/10) were in the N terminus of the protein \( (p=0.03) \). Nonsense or frameshift mutations occurring in exons 2-25 have been previously associated with bilateral retinoblastoma (Lohmann and Gallie, 2004). Seven patients (S161, N29, N15, N19, N34, N26 and N37) in this study showed a similar association. However, occasionally such mutations have also been found in isolated unilateral patients.
Table 3. Phenotype and Genotype Data

<table>
<thead>
<tr>
<th>S No.</th>
<th>ID</th>
<th>Clinical features</th>
<th>Severity</th>
<th>Mutation/pRB domain (exon)</th>
<th>Mutation type</th>
<th>Truncation Effect on Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S161</td>
<td>Optic nerve invasion</td>
<td>High</td>
<td>Intron 2 del./NA</td>
<td>Large deletion</td>
<td>Loss of expression</td>
</tr>
<tr>
<td>2</td>
<td>N15</td>
<td>Bilateral, age &gt;1 yr</td>
<td>Moderate</td>
<td>C&gt;T / C(E23)</td>
<td>Nonsense</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>N26</td>
<td>Bilateral, age &lt;1 yr</td>
<td>High</td>
<td>8 bpdel./N (E04)</td>
<td>Frameshift</td>
<td>136X</td>
</tr>
<tr>
<td>4</td>
<td>N29</td>
<td>Advanced disease</td>
<td>High</td>
<td>C&gt; G /N(E07)</td>
<td>Nonsense</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>N12</td>
<td>Unilateral, no family</td>
<td>High</td>
<td>C&gt;T /N(E08)</td>
<td>Nonsense</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>N19</td>
<td>Bilateral, age &gt;2 yrs</td>
<td>Moderate</td>
<td>R445X/N(E14)</td>
<td>Nonsense</td>
<td>+ R445X</td>
</tr>
<tr>
<td>7</td>
<td>N34</td>
<td>Bilateral, age &gt;2 yrs</td>
<td>Moderate</td>
<td>C&gt;T/N (E08)</td>
<td>Nonsense</td>
<td>+ R255X</td>
</tr>
<tr>
<td>8</td>
<td>N8</td>
<td>Bilateral, age &lt;1 yr</td>
<td>High</td>
<td>Intron 11 splice /NA</td>
<td>Splice site</td>
<td>aa 378-405 del; aa 378-379 mutation; 380+ truncation</td>
</tr>
<tr>
<td>9</td>
<td>N26</td>
<td>Bilateral, age &lt;1 yr</td>
<td>High</td>
<td>C&gt;T/Spacer (E18)</td>
<td>Nonsense</td>
<td>+ R579X</td>
</tr>
<tr>
<td>10</td>
<td>N3</td>
<td>Unilateral, age &lt;2 yrs, Choroidal infiltration</td>
<td>High</td>
<td>Intron 20 del./NA</td>
<td>Large deletion</td>
<td>- Loss of expression</td>
</tr>
<tr>
<td>11</td>
<td>Q56</td>
<td>Unilateral, age &lt;2 yrs</td>
<td>High</td>
<td>C &gt;A /C(E23)</td>
<td>Nonsense</td>
<td>+ S780X</td>
</tr>
<tr>
<td>12</td>
<td>N37</td>
<td>Bilateral, age &lt;1 yr</td>
<td>High</td>
<td>C&gt;T/C(E23)</td>
<td>Nonsense</td>
<td>+ R787X</td>
</tr>
</tbody>
</table>

(Ohmann et al., 1997) and in the current study patients N12 (two nonsense mutations) and Q56 (one nonsense mutation) had unilateral retinoblastoma. On the other hand, intron 11 splice mutation in patient N8 resulted in bilateral retinoblastoma. This could be caused by protein truncation and complete penetrance of mutation at invariable splice sites (Ohmann and Gallie, 2004).

Qi et al concluded that large deletions were associated with high disease severity (2005). In the present study, two large deletions, one at intron 2 and another at intron 20 were found in a bilateral (S161) and unilateral retinoblastoma patient (N3), respectively. Though both are large deletions, intron 2 deletion could be expected to result in definite loss of major domains of the retinoblastoma protein critical for most of its function. Moreover, this patient also had a nonsense mutation R787X in the C pocket, which could have disrupted the interaction of the A/B domain with E2F and resulted in the bilateral phenotype. The i20 deletion could have occurred anywhere between introns 2 and 20 resulting in some protein expression and function causing unilateral phenotype. Six out of 9 nonsense mutations detected here occurred in the N terminus region and were associated with moderate and high disease severity. This association is much favorable to the expected outcome of loss of protein beyond the N terminus and thereby loss of A/B pocket and its crucial function in E2F binding. However, patients in low severity group and more number of mutations are required to clearly understand these correlations.

In summary, though no significant association between type of mutations and disease severity could be established in the present study, we found a positive correlation between location of the mutations in certain domains of the retinoblastoma gene and disease severity.

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References


