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Unilateral retinoblastoma, lack of familial history and older age does not exclude germline RB1 gene mutation

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ABSTRACT

Conclusive identification of *RB1* mutations in retinoblastoma is predicted to improve the clinical management of affected children and relatives. However, despite clear clinical benefits, *RB1* screening remains difficult, most of the alterations being unique and randomly distributed throughout the entire coding sequence. In this report, we present the results of a constitutional *RB1* analysis undertaken in our institution over the last four years. The detection of *RB1* gene deletion or mutation was performed by Southern blot and sequence analyses in 73 patients (including three families with 2, 3 and 3 probands, respectively). Complementary constitutional chromosome and fluorescent in situ hybridization (FISH) analyses of *RB1* gene were applied in cases where hereditary retinoblastoma was suspected despite negative detection. Altogether, germline abnormalities were found in 11% (4/36 patients) of sporadic unilateral retinoblastoma (median age, 21.5 months) and 86% (32/37 patients) of sporadic bilateral or positive familial history retinoblastoma (median age, 5 months). The spectrum of germline alterations found in 31 distinct families included 12 nonsense mutations (39%); 10 point insertions or deletions with frameshift (32%); 4 mutations and 1 deletion affecting splice sites (16%); 2 missense mutations (6%); and 2 large deletions (6%). A total of 15 mutations have not been previously reported. In this small series, splicing mutations were associated with bilateral disease whilst most of the frameshift mutations were identified in patients with an early age at diagnosis, bilateral disease or hereditary forms of the disease.

This study confirms that screening for constitutional *RB1* mutation should become an integral part of current management of any patient affected by retinoblastoma irrespective of the tumour laterality and familial background.

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1. Introduction

Retinoblastoma, an embryonic neoplasm of retinal origin, is the most common intraocular malignant tumour in childhood, with an average incidence of one case every 15,000–20,000 live births. It occurs as a result of mutations in the retinoblastoma gene (*RB1*) located at 13q14. Approximately, 45% of retinoblastoma patients are hereditary cases (15% unilateral and as many as 30% bilateral cases) whilst the others are sporadic and present as unilateral tumours [1]. Approximately, 12–15% of patients with retinoblastoma already have a family history, where the tumour phenotype segregates as an autosomal dominant trait with high (90%) penetrance. Individuals harbouring a germline *RB1* gene mutation are predisposed to the development of multiple other cancers throughout life including bone and soft tissue sarcomas, melanoma, brain tumours and have a 50% risk of transmitting their germline *RB1* gene mutation to an offspring [1,2].

While most patients with hereditary retinoblastoma can be clinically identified (bilateral or multifocal tumours, positive family history), 5–15% of patients have single-eye tumours and no familial history, making them indistinguishable from patients with non-hereditary retinoblastoma. Identification of germline alterations in this subset of unilateral retinoblastoma probands is crucial for appropriate treatment and long-term follow-up. Furthemore, precise identification of the *RB1* mutation in a child has been predicted to enhance the quality of clinical management of the affected patient and relatives [3,4]. Children at risk for retinoblastoma undergo intensive clinical surveillance with regular ophthalmologic examinations under anaesthesia. If a germline *RB1* gene mutation is detected, only relatives with the *RB1* gene anomaly require this follow-up whereas those proven not to be carriers require no further examinations [5,6]. Moreover, pre-implantation genetic diagnosis for retinoblastoma can be planned in pregnancies at risk [7].

Although enucleation and external beam radiotherapy have been the most common treatments for retinoblastoma in the last decade, standard therapeutic approach has evolved to systemic chemotherapy combined with local therapies to preserve eye and vision. Identification of patients with germline *RB1* mutation is crucial for the therapeutic choice. External irradiation will indeed be excluded in patients with constitutional *RB1* mutation to avoid development of secondary osteosarcoma. Conservative management will be attempted particularly in patients with bilateral retinoblastoma or at risk of developing bilateral tumours.

If *RB1* screening has demonstrated clear clinical benefits, *RB1* testing remains extremely challenging, as the majority of the mutations are unique and randomly distributed over the entire coding sequence. To date, more than 368 mutations have been reported in *RB1* germline mutation database [8–12]. All these difficulties explain why extensive *RB1* mutation detection has not been widely implemented [10].

In the current study, we present the results of the constitutional *RB1* analysis that was carried out over the last four years to improve the management of retinoblastoma patients and their families. Detection of *RB1* alteration was performed in 73 patients (including three families with 2, 2 and 3 probands) by the means of Southern blot and sequence of analyses of the 27 exons as well as part of the promoter and intronic regions. Constitutional chromosome and fluorescent in situ hybridization (FISH) analyses of gene were restricted to patients with a suspicion of hereditary retinoblastoma despite negative Southern blot and sequence analyses. We present the spectrum of *RB1* constitutional alterations, and discuss the benefits of this screening strategy in daily clinical practice with reference to existing literature [10–17].

2. Patients and methods

2.1. Patients

A total of 73 probands from 68 families with retinoblastoma, mostly of European countries including 18% of families from Spanish origin (12/68), were assessed for the presence of a constitutional *RB1* alteration. All the patients were followed at the Cliniques Universitaires Saint Luc, Brussels, Belgium. We studied 37 patients with bilateral retinoblastoma with or without familial history (including three families with 2, 3 and 3 probands, respectively) and 36 patients with unilateral retinoblastoma. Only one of the unilateral patients had a positive familial history at diagnosis. The sex ratio (M/F) was 0.7 for unilateral and 1.4 for bilateral groups and the median age was 19 and 5 months, respectively. All participating families provided an informed consent for the genetic analysis. Ophthalmologic examination was performed in each parent of the affected children. Southern blot and sequence analyses of the *RB1* gene were performed in all patients. Complementary constitutional chromosome and FISH analysis of *RB1* gene were carried out on blood DNA from bilateral retinoblastoma patients and patients under 1 year of age when Southern blot and sequence analyses were negative. They were also carried out initially on probands with abnormal phenotype such as developmental delay or facial dysmorphism.

2.2. Southern blot analysis

Detection of specific DNA fragments by gel transfer hybridization was performed from peripheral blood lymphocytes. DNA was digested with *Hin*dIII enzyme (Roche 656313) and separated by an agarose electrophoresis gel. The DNA was transferred to Zeta-Probe GT Genomic membrane (Biorad, ref:162-0194). Labelling and hybridization were performed according to the manufacturer's instructions by sequentially using two radioactive probes derived from cDNA *RB1* 3.8 and 0.6 kb (Dryja). After each experiment, labelled DNA probes hybridized to complementary DNA bands were visualized by autoradiography.

2.3. Sequence analysis of the retinoblastoma gene

Genomic DNA was isolated from peripheral blood lymphocytes. Amplification of each of the 27 individual exons, the promoter (520 bp) and intronic regions (5–80 bp, according to the location of the exons) was performed with primers and according to conditions listed in Table 1. Amplification products were purified from free nucleotides and primers using the QIAquick PCR Purification kit (Qiagen). Sequence analysis