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Original article

Molecular diagnosis in patients with retinoblastoma: Report of a series of cases☆,☆☆

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ABSTRACT

Objectives: To report the benefits of genetic diagnosis in patients with retinoblastoma.
Method: Observational study. Patients with retinoblastoma and their families were included. Demographic and clinical data were recorded. Blood and tumor samples were obtained. Next generation sequencing was performed on the samples. When deletion 13 q syndrome was suspected, cytogenetics microarray was performed (Cytoscan® HD, Affymetrix, Santa Clara, CA, USA), with a high density chip of 1.9 million of non-polymorphic probes and 750 thousand SNP probes.
Results: Of the 7 cases were analyzed 4 were male. The mean age at diagnosis was 21 months (range 5–36). Three cases had bilateral retinoblastoma, and 4 unilateral. None had family history. In all patients, blood was analyzed, and a study was performed on the tissue from 2 unilateral enucleated tumors, in which 6 mutations were identified, all *de novo*. Just one was novel (c.164delC; case 1). One case of unilateral tumor revealed blood mosaicism, showing that his condition was inheritable, and that there is a high risk of developing retinoblastoma in the unaffected eye. The patient also has an increased risk of presenting with other primary tumors.
Conclusion: Molecular diagnosis of *RB1* in patients with retinoblastoma impacts on the decision process, costs, treatment, and prognosis of patients, as well as their families.

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☆☆ Impact Genetics offers genetic testing service as a clinical laboratory.

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Diagnóstico molecular en pacientes con retinoblastoma: reporte de una serie de casos

Palabras clave:
Retinoblastoma
Gen *RB1*
Estudio genético
Cariotipo molecular
Tumor ocular

RESUMEN

Objetivos: Reportar los beneficios del diagnóstico genético en una serie de pacientes con retinoblastoma.

Métodos: Estudio longitudinal observacional. Se incluyó a pacientes con retinoblastoma y sus familias. Se registraron datos demográficos y clínicos. Se obtuvieron muestras de sangre y del tumor, realizándose análisis mediante *next generation sequencing*. Con la sospecha de síndrome de deleción 13q, se realizó análisis citogenético con cariotipo molecular (Cytoscan® HD, Affymetrix, Santa Clara, CA, EE. UU.), utilizando un chip de alta densidad con 1,9 millones de sondas únicas no polimórficas y 750.000 sondas de SNP.

Resultados: Se analizan 7 casos. Cuatro eran hombres. La mediana de edad del diagnóstico fue de 21 meses (rango 5–36). Tres casos presentaron retinoblastoma bilateral y 4, unilateral. Ninguno tenía antecedentes familiares. En todos se estudió la sangre y en los 2 pacientes unilaterales enucleados se estudió el tejido tumoral. Se encontraron 6 mutaciones, todas fueron *de novo*. Solo una era nueva (c.164delC; caso 1). Un caso de tumor unilateral reveló un mosaicismo en sangre, por lo que su enfermedad es heredable, tiene riesgo de desarrollar retinoblastoma en el ojo contralateral sano y riesgo de presentar otros tumores primarios.

Conclusión: El diagnóstico molecular de *RB1* en pacientes con retinoblastoma influye sobre la toma de decisiones, los costos, el tratamiento y el pronóstico de los pacientes y sus familias.

Introduction

Retinoblastoma is the most frequent ocular cancer in the pediatric population. The incidence of retinoblastoma at the world level remains constant at a rate of 10–15 cases/10,000 live births. Between 40 and 50% of cases exhibited an inheritable germinal mutation.[1] Penetrance is incomplete. Only 10–15% of patients inherit the germinal mutation of their parents,[2] because some constitutional expressions are *de novo* mutations.

Germinal retinoblastoma patients, typically with bilateral or focal expressions, are at significant risk of new ocular tumors in the first years of life and higher risk of malign neoplasia in other organs (second primaries) in the long-term and with familial recurrence.[3] Molecular genetic diagnostic is crucial for diagnosing germinal retinoblastoma, identifying familial areas and avoiding an excessive amount of examinations under anesthesia during follow-up.[4] In addition, said diagnostic influences therapeutic decisions, as some authors recommend that all germinal retinoblastoma patients should receive systemic chemotherapy to prevent the expression of new tumors and pineoblastoma.[3]

Early diagnostic is critical for survival and for the preservation of the ocular globe, as retinoblastoma is treatable in its early stages.[1,3] In our country, survival is comparable to that of developed countries,[5,6] although mortality in Latin America remains close to 20%.[7]

Retinoblastoma is caused by mutations in the *RB1* (13q14) tumor suppressant gene.[2] The translated RB1 protein (retinoblastoma protein [pRB]) halts the cellular division cycle in its non-phosphorylated stage. Genetic studies have shown that mutations in the 2 RB1 alleles are required but that epigenetic factors, methylations or chromosome aberrations also predispose the appearance of retinoblastoma.[2]

Even though the molecular diagnostic of *RB1* in patients with retinoblastoma is not routine practice in developing countries, it enables a significant change in the management of patients and involved relatives. The objective of this publication is to demonstrate the benefits of genetic diagnostic in a series of patients.

Subjects, material and methods

The present study was approved by the Ethics Committee of the Alemana Clinic. Informed consents were obtained from the parents. Seven patients with confirmed retinoblastoma diagnostic and their families were referred by the San Juan de Dios hospital (PINDA network). All patients underwent examination under general anesthesia. Demographic and clinical data were obtained as well as blood and tumor samples, processed at the Genetics and Genomic Center of the Development University. DNA was drawn from whole blood through magnetic pearls (Prepito DNA 250 *blood kit* for the Chemagic Prepito-D equipment; PerkinElmer, Waltham, MA, USA). The tumors were frozen with liquid nitrogen and pulverized in a mortar to extract DNA through silica columns (PureLink Genomic DNA Mini Kit; Thermo Scientific, Wilmington, DE, USA). The DNA samples were assessed with the spectrophotometric method (NanoDrop 2000; Thermo Scientific, Wilmington, DE, USA) and sent at ambient temperature to Impact Genetics (Toronto, Canada) for study.