CONSTITUTIVE MOLECULAR ALTERATIONS IN THE 13q14 CHROMOSOME REGION IN PATIENTS WITH RETINOBLASTOMA

Vanessa dos Santos Mendonça Silva, Anna Claudia Evangelista dos Santos, Hector Nicolas Seuanez Abreu.
Genetics Program, CPQ, Instituto Nacional de Câncer

INTRODUCTION

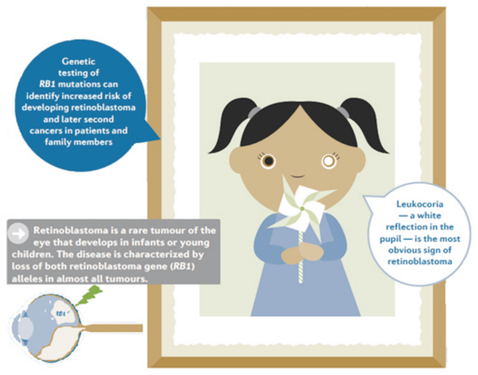


Figure 1: Characteristics of retinoblastoma. Adapt from Dimaras et al. (2015).

The majority of constitutive mutations in the *RB1* gene consist of substitutions of one or several nucleotides but, in some cases, large deletions and duplications may occur which may extend to the whole chromosome region 13q14 where this gene is located. As large rearrangements may not be identified by direct sequencing, the main method used for investigating *RB1* mutations, while MLPA (*Multiplex Ligation-dependent Probe Amplification*) is a method of choice.
Identification of the first mutational hit enables us to establish prevention strategies by genetic counseling of families carrying mutations. This study identified constitutive alterations in the 13q14 chromosome region by MLPA in patients that did not show pathogenic mutations by direct DNA sequencing. This study contributed to the Genetic Counseling Program of the National Cancer Institute of Brazil (INCA).

METHODS

This study is a part of the project entitled "Cytomolecular Studies in Retinoblastoma" (protocol 40/00), approved in 2001 by the Ethics Committee of the National Cancer Institute (INCA). Informed consents were signed by all the participants (parents or guardians). Peripheral blood samples were collected for identifying constitutional mutations. MLPA was carried out with the SALSA MLPA P047 RB1 kit according to the manufacturer's instructions with samples of 59 patients who did not show alterations by direct sequencing. This kit contains probes for 26 *RB1* exons, probes for two genes upstream of *RB1* (*ENOX1* and *ITM2B*), probes for three genes downstream of *RB1* (*RCBTB2, DLEU1, PCDH8*) and probes for 14 autosomal genes outside 13q for control of the reaction.

Figure 2 shows the steps of the MLPA reaction.

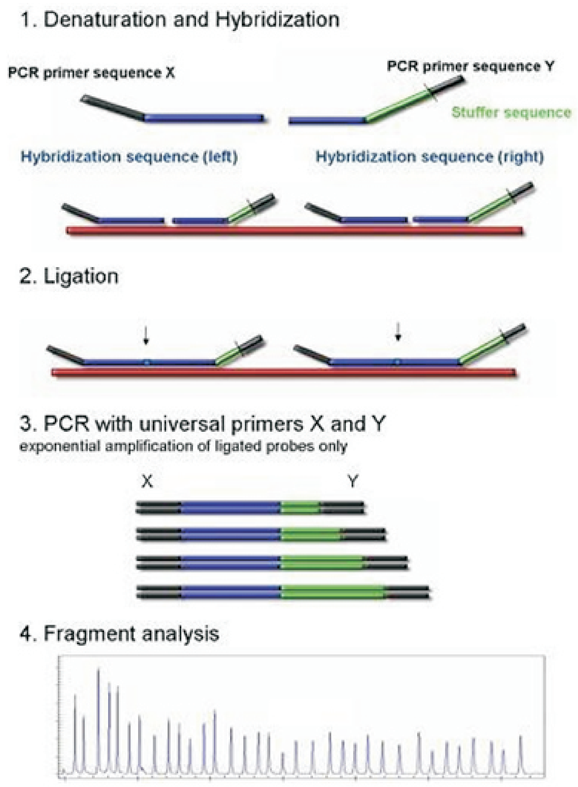


Figure 2: Steps of MLPA reaction. From Schouten et al. (2002).

RESULTS

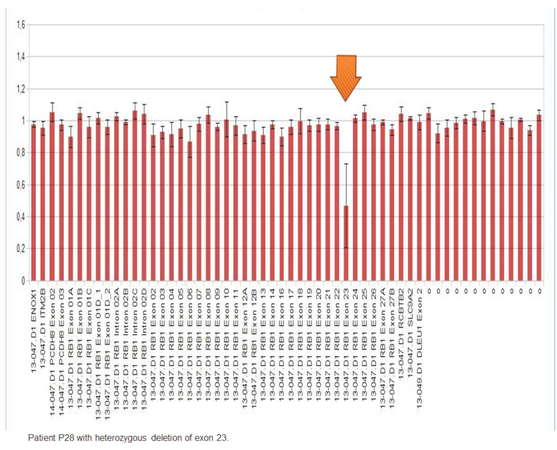
Fifty nine patients were studied and six of them showed alterations (Table 1). Available samples from parents were also analyzed for identifying hereditary mutations.

Table 1 – Patient information, alterations and MLPA findings in parents.

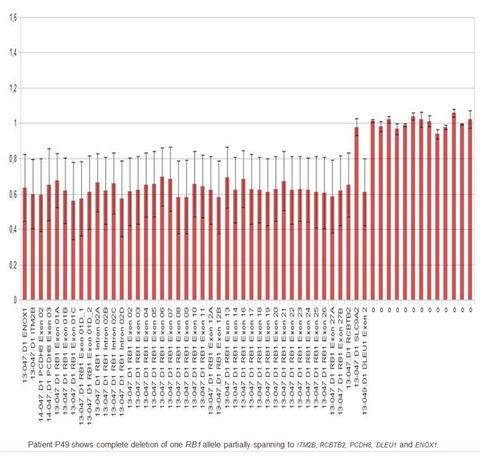
Patient	Sex	Presentation	Age at Diagnosis	Alterations	Parents
P48	Male	Bilateral	8 months	Amplification of the region between exon 12 and 17 of the *RB1*	**Father:** Sample not available
					Mother: Without alterations
P30	Female	Bilateral	24 months	Deletion of exon 3 of *RB1*	**Father:** Without alterations
					Mother: Without alterations
P28	Male	Bilateral	9 months	Deletion of exon 23 of *RB1*	**Father:** Sample not available
					Mother: Without alterations
P49	Female	Bilateral	17 months	Heterozygous deletion of *RB1* and partial deletion of *ITM2B, RCTB2, PCDH8, DLEU 1* (13q14.2) and *ENOX 1* (13q14.11).	**Father:** Sample not available
					Mother: Sample not available
P53	Female	Unilateral	20 months	Heterozygous deletion of *RB1* and partial deletion of *ITM2B* and RCTB2 (13q14.2).	**Father:** Sample not available
					Mother: Without alterations
P12	Male	Unilateral	17 months	Heterozygous deletion of RB1 gene and partial deletion of *ITM2B, RCTB2, PCDH8, DLEU1* (13q14.2).	**Father:** Without alterations
					Mother: Without alterations

Patient P48 shows amplification of five consecutive exons of *RB1*.

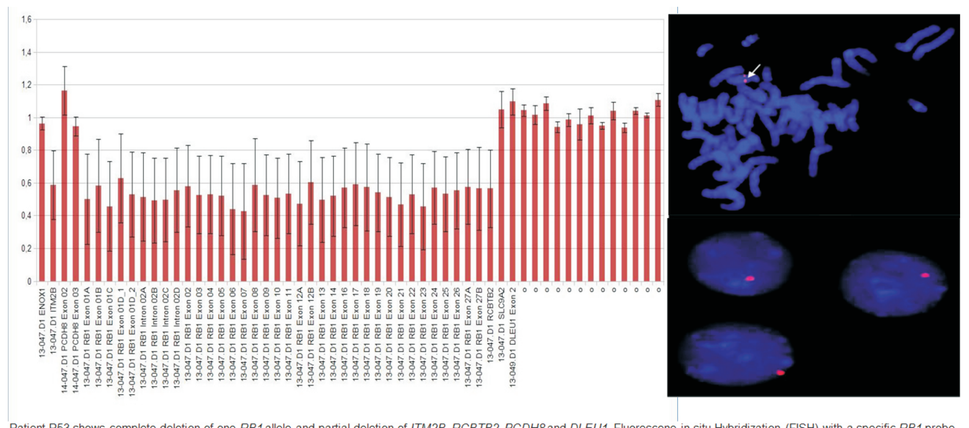
Patient P30 with heterozygous deletion of exon 03.



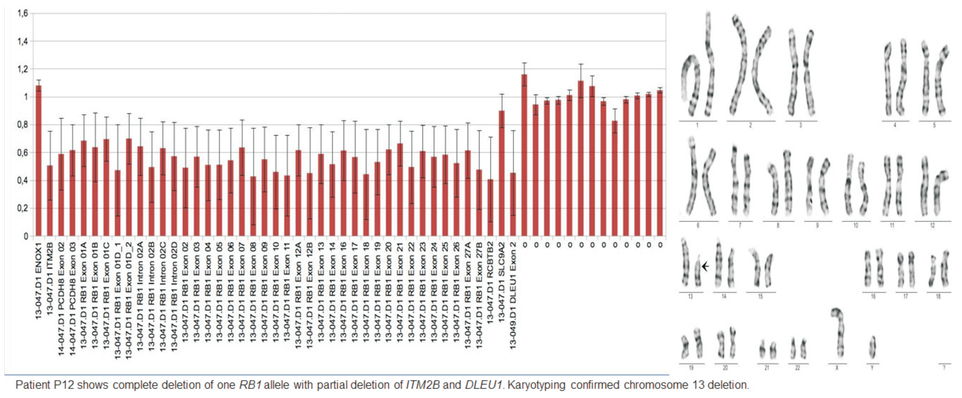
Patient P28 with heterozygous deletion of exon 23.



Patient P49 shows complete deletion of one *RB1* allele partially spanning to *ITM2B, RCBTB2, PCDH8, DLEU1* and *ENOX1*.



Patient P53 shows complete deletion of one *RB1* allele and partial deletion of *ITM2B, RCBTB2, PCDH8* and *DLEU1*. Fluorescene in situ Hybridization (FISH) with a specific *RB1* probe.



Patient P12 shows complete deletion of one *RB1* allele with partial deletion of *ITM2B* and *DLEU1*. Karyotyping confirmed chromosome 13 deletion.

MLPA was successful in showing alterations that had been undetected by direct sequencing, a reason why it has been adopted as a routine procedure for patients who do not show apparent RB1 mutations. This procedure is simple and easy to standardize for mutational screening for our Genetic Counseling Program.

PERSPECTIVES

The fact that 53 patients with retinoblastoma did not show any alteration with direct sequencing and MLPA should be further investigated. Despite the predominance of RB1 alterations as trigger for retinoblastoma, it has recently been reported that, in some in 1.4% of patients, this gene is not affected. In this cohort, increased levels of the NMYC oncogene (V-Myc Avian Myelocytomatosis Viral Oncogene Neuroblastoma Derived Homolog) have been identified in tumor samples. It is not known whether retinoblastoma might be caused by inactivation of both of RB1 alleles with increased levels of NMYC or only by NMYC amplification. And it is also unknown whether NMYC amplification might be responsible for keeping a once established retinoblastoma phenotype. Regardless of these questions, the characterization of this retinoblastoma subtype (RB1+/+NMYCa) is essential for genetic counseling because it strongly suggests a nonhereditary form of retinoblastoma, with a normal risk for developing retinoblastoma in the unaffected eye or other types of cancer, as well as excluding familial risk. NMYC amplification will be studied by quantitative PCR or Fluorescence in situ hybridization (FISH).
Furthermore, a study of a set of 40 tumor samples will be screened by Next-Generation Sequencing (NGS) to look for presence of somatic RB1 alterations.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA