CASE REPORT

Retinoblastoma and mosaic 13q deletion: a case report

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Abstract

**Background:** Patients with 13q-syndrome are at risk of retinoblastoma when the RB1 gene, located in the chromosomal band 13q14.2, is deleted. This syndrome is frequently associated with congenital malformations and developmental delay, although these signs could be mild. Mosaic 13q-deletion patients have been previously reported in the literature; their phenotype is variable, and they may not be recognized.

**Case presentation:** Retinoblastoma diagnosed in a child with 13q-mosaicism confirmed in blood, oral mucosa, healthy retina and retinoblastoma. A second RB1 hit is present exclusively in the retinoblastoma sample (RB1 c.958C>T p.Arg320Ter). Other detected molecular events in retinoblastoma are 6p12.3pter gain and 6q25.3qter loss. Clinical examination is unremarkable except for clinodactyly of the right fifth finger.

**Discussion and conclusions:** We describe a case of mosaic 13q deletion syndrome affected by retinoblastoma. Molecular data obtained from the tumor analysis are similar to previous data available about this malignancy. High clinical suspicion is essential for an adequate diagnosis of mosaic cases.

**Keywords:** Retinoblastoma, 13q-syndrome, Mosaicism, Cytogenetics, Molecular genetics

**Background**

Retinoblastoma is a rare tumor that occurs in young children’s retina. About 40% of patients diagnosed with retinoblastoma have a predisposing genetic condition [1]. Most of them carry heterozygous truncating RB1 mutations in the germline. Some patients present isolated deletions of one of the two RB1 alleles, and at-risk patients are exceptionally 13q-syndrome cases [2]. Because of the fact that 98% of retinoblastoma cases begin after a double RB1 hit, according to Knudson’s hypothesis [3], all these children are at a major risk of being affected.

13q deletion syndrome was first described by Alldredge et al. after studying two pediatric patients in 1969 [4]. The first patient affected by the syndrome including retinoblastoma was reported in 1983 [5]. Several cases have been communicated during the past 50 years and the syndromic phenotype has been characterized. Intellectual disability, facial anomalies, several malformations and retinoblastoma risk stand out as the most prominent signs amongst other previously described abnormalities. However, the tumor would not be able to progress easily in 13q-syndrome even if a second RB1 hit were present. It has been hypothesized that some genes deleted together with RB1 would be necessary for retinoblastoma development. Available data suggest that 13q deletions larger than 1 Mb—and particularly those including MED4 and SUCLA2—are associated with unilateral forms or without retinoblastoma development [6].

Improvements in cytogenetic analysis has enabled better molecular characterization of 13q-syndrome cases and more accurate genotype–phenotype correlations. Depending on the deleted chromosomal bands, three clinical groups may be established [7]:

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• Group 1: 13q12.2–13q32. Mild intellectual disability, growth delay, limb malformations, and retinoblastoma risk (when the \textit{RB1} gene is deleted [chromosomal position 13q14.2]).
• Group 2: 13q32. Severe brain malformations and developmental delay.
• Group 3: 13q33–13q34. Minor congenital malformations but severe intellectual impairment.

Some patients with 13q-syndrome are affected by a mosaic disease and a few cases have been described [8–11]. Bestetti et al. reported a patient with mosaic 13q

![Fig. 1](image)

**Fig. 1** \textit{RB1} deletion in the context of mosaic 13q deletion. **A** Multiplex Ligation-dependent Probe Amplification (MLPA) assay looking for \textit{RB1} gene deletions or duplications (SALSA P047-C1) in germline DNA from peripheral blood lymphocytes. The detected values were low but not consistent with a heterozygous deletion. The suspicion was a complete \textit{RB1} deletion in mosaicism. **B** Genomic SNP array (AffymetrixCytoScan 750 array) reports a 13q deletion in mosaicism. It is a deletion of 35.7 Mb from 13q12.13 to 13q21.2 (arr[hg19] 13q12.13q21.2(26,555,387–62,280,955) × 1–2) observed in about 40% of all determinations.
deletion syndrome including RB1 but no retinoblastoma [8].

**Case presentation**

A 6-month-old girl conceived by in vitro fertilization (IVF) (own oocytes and anonymous donor sperm) was admitted to the hospital because of leukocoria and strabismus. Past medical history and physical examination were unremarkable except for clinodactyly of the right fifth finger. Indirect ophthalmoscopic examination and examination under anesthesia was performed by ophthalmologists. Orbital ultrasound and magnetic resonance imaging (MRI) scans showed a 14 × 13 × 11 mm left intraocular mass located in the lower-external retinal side. Retinal detachment was also detected. Other tumoral lesions were ruled out by an ophthalmologist and MRI in both retina and brain. Diagnosis of Retinoblastoma was made and, based on *International Classification for Intraocular Retinoblastoma*, a grade E was established. The patient received intra-arterial melphalan but due to a local vasospasm in her left leg, the treatment was discontinued. Afterwards, four courses of conventional chemotherapy were administered (vincristine, carboplatin and etoposide). A partial response was achieved, but, despite chemotherapy, the disease progressed few weeks later and the affected eye was enucleated.

On the basis of global recommendations, the RB1 gene was studied in germline DNA from peripheral blood lymphocytes. Exon–intron boundaries of RB1 were amplified by conventional PCR and then sequenced by the Sanger method; no mutations were detected. A Multiplex Ligation-dependent Probe Amplification (MLPA) assay was used to test for RB1-gene deletions and duplications (SALSA P047-C1). The detected values were relatively low but within the normal range (Fig. 1A) and a complete RB1 deletion in mosaicism was suspected. A genomic SNP array (AffymetrixCytoScan 750 array) was performed and a 13q deletion of 35.7 Mb from 13q12.13 to 13q21.2 (arr[hg19] 13q12.13q21.2(26,555,387–62,280,955) × 1–2) detected in around 40% of cells (Fig. 1B) was confirmed. This result was further confirmed by cytogenetic karyotype analysis of cultivated lymphocytes previously stimulated with phytohemagglutinin. Fifty metaphases were analyzed and two cell clones were detected. A majority cell line (44 cells) presented 46 chromosomes whose identification with G bands (resolution level of 400–500 bands) did not show numerical or structural alterations (46, XX). A minor cell line (6 cells) with 46 chromosomes showed the presence of an interstitial deletion in the long arm of chromosome 13.

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**Fig. 2** Cytogenetic karyotype from cultivated lymphocytes previously stimulated with phytohemagglutinin. Karyotype 46,XX,del(13)(q12q21) [6]/46,XX[44]. A A majority cell line (44 cells): 46 chromosomes whose identification with G bands (resolution level of 400–500 bands) does not show numerical or structural alterations (46, XX). B A minor cell line (6 cells): 46 chromosomes but shows the presence of an interstitial deletion in the long arm of chromosome 13.
deletion in mosaicism but in about 50% of the studied cells. However, all retinoblastoma sample cells carried the deletion in heterozygosity (Fig. 3). Neither LOH (Loss of Heterozygosity) nor chromothripsis were detected in 13q bands. Furthermore, 6p12.3pter gain (3 total copies) and 6q25.3qter loss (1 total copy) were reported exclusively in the tumor sample.

Looking for second hit mutations in RB1, we applied a custom designed NGS panel (Onconano V2) that included the RB1, BCOR and CREBPP genes (among other 400 commonly mutated genes in pediatric cancer). The study detected only one pathogenic single-nucleotide variant, RB1 c.958C>T (p.Arg320Ter) (NM_000321.2 chromosomal position 13–48,941,648-C-T; allele frequency of 25%). Copy number variations in 6p, 6q and 13q were again observed.

After molecular diagnosis and completing the treatment, the patient was placed on surveillance. The right eye has been free of disease and the child is 42 months old now. She does not present growth retardation at the moment (weight and height in the 50th percentile; cranial perimeter in the 90th). Neither cardiac, eye nor other malformations have been detected and neurological development has been normal (Fig. 4).

Informed consent for genetic studies and for taking and sharing pictures was obtained from both parents.
Discussion and conclusions
We described the case of a child with 13q-mosaicism affected by retinoblastoma. The unilateral presentation agrees with previous data available for 13q deletions larger than 1 Mb including MED4 and SULCL2 [6]. As in this case, retinoblastoma with both genes deleted is associated with less tumor aggressiveness compared with tumors whose genes are conserved [6].

Retinoblastoma seems to be caused by a double hit in RB1 approaching 98% cases (by mutation, deletion, promoter methylation or intra-genic chromothripsis) [12, 13]. Few retinoblastoma cases would start because of MYCN amplification [13]. 13q deletion syndrome patients would not be an exception. In fact, we confirmed a second RB1 hit (RB1 p.Arg320Ter) in the tumor.

However, double RB1 hit only gives rise to retinoma; therefore, subsequent epigenetic or genetic changes would give an advantage for tumor progression. The sequence of events capable of causing a malignant phenotype is only partially known. Epigenetic deregulation secondary to homozygous RB1 loss drives an increase in KIF14 and E2F3 levels [14] and could lead to the expression of the SYK oncogene as well. Moreover, cellular control mediated by p53 is inactivated as a result of high expression of MDM2 and MDM4 in retinoblastoma [14].

In addition, cytogenetic analysis has shown recurrent CNVs (copy number variation) among retinoblastoma tumors, which are mainly chromosomal gains at 1q, 2p, 6p, 13q and 19q and losses at 13q, 16q and 17p [15]. These recurrent aberrations allow to establish as a possible hypothesis that genes located at these loci could be related to retinoblastoma progression [15], yet no conclusive data are available about this at the moment. We looked for CNVs in the tumor and discovered a chromosomal gain in 6p12.3pter, which is one of the most frequently reported CNVs in retinoblastoma [15]. However, we also detected a less common deletion of 6q25.3qter. The deletion of this region has already been described among non-13q-deletion syndrome patients, although rarely [16]. Sixty OMIM genes are located in this region, and several of them are associated with different cancers, but none with retinoblastoma. A terminal 6q deletion may be present in ovarian cancer and neuroblastoma [17] and seems to be related to bad prognosis in neuroblastoma [17]. The fact that this deletion could play a role in retinoblastoma development in the context of 13q-syndrome is unknown.

Furthermore, NGS approaches have detected a low rate of mutations in retinoblastoma. Several studies support retinoblastoma as one of the less mutated human tumors. Only BCOR (mutated in 13% of tumors) and CREBPP mutations occur frequently in retinoblastoma [18]. Therefore, retinoblastoma presents a stable genome with few genetic events described and epigenetic deregulation appears to have a notable role [19]. Studies based on RNA-sequencing could continue to shed light on the genes and signaling pathways involved in retinoblastoma development [20]. In regards to common mutated genes in retinoblastoma, we determined BCOR and CREBPP status without detecting pathogenic variants. We did not find other variants considered pathogenic or likely pathogenic in 400 genes commonly mutated in pediatric cancer beyond RB1.

The patient carries the deletion 13q12.13–13q21.2 and, therefore, fits in Group 1 of the clinical classification for 13q-syndrome [7]. Patients with band 13q14 deleted typically present with mild facial anomalies such as high forehead, short nose, small upper lip, curly hair and down-turned corners of the mouth [6]. Our patient does not show these facial features. Furthermore, deletion of NUFIP1, located in 13q14.12, and PCDH8, in 13q21.1, may be crucial for developmental delay [6]. Both of them are deleted in our patient, but the degree of mosaicism in her central nervous system is unknown. In fact, she is neurologically normal. Moreover, other common abnormalities in Group 1 are micrognathia and microcephaly but these are related to loss in the 13q21.33q31.1 and 13q21.32q21.33 regions, respectively [6]. Our patient’s deletion finishes at 13q21.2; therefore, she does not present either micrognathia or microcephaly, because those regions are not affected. About 75% of patients with large deletions present short height, but this is not the case of our patient (50th percentile). Genes involved in short height have not been clearly defined.

The BRCA2 gene, located in 13q13.1, may be lost in some 13q-patients. Heterozygous mutations in this
gene predispose to breast and ovarian cancer syndrome in adulthood [21] and a complete deletion of this gene might predispose to these tumors as well. However, the occurrence of these two tumors has not been reported in 13q-syndrome to date. Our patient loses BRCAl2; therefore, she may benefit from risk-adapted surveillance strategies for breast/ovarian cancer.

After confirming retinoblastoma diagnosis in a child, genetic study of RBl in the germline is mandatory. Any phenotypic manifestation, including minor peculiarities (clinodactyly of the fifth finger in our case) should raise suspicion of 13q-syndrome, and it should be studied, given the fact that mosaic forms exist.

Abbreviations
MRI: Magnetic resonance imaging; LOH: Loss of heterozygosity; CNV: Copy number variation; NGS: Next generation sequencing.

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Authors’ contributions
All authors have made substantial contributions to the conception. All authors have substantially revised the work. SO, YY; VS and IC contributed in the genetic studies. JB, HB, AJR, VC and AC were responsible for clinical evaluation, management, treatment and surveillance. MLL performed the histological examination of retinoblastoma and retina. All authors read and approved the final manuscript.

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Availability of data and materials
Data generated or analyzed during this study are included in this published article.

Declarations
Ethics approval and consent to participate:
The study was accepted by CEIm la Fe (Ethics Committee La Fe) on March 13, 2018. Project reference number: 2017/0546. Project title: genetic predisposition to childhood cancer. From NGS to clinical consultation.

Consent for publication
Parents consented to participate in the study and they allowed us to publish pictures of their child. Documents are available for consultation.

Competing interests
The authors declare that they have no competing interests.

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