Metachronous, non-pineal, trilateral retinoblastoma in a patient with a seemingly reduced-expressivity RB1 germline deletion

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Abstract
The clinical course of trilateral retinoblastoma can be unpredictable, and expressivity of germline RB1 variants may vary during development. We describe an unexpected fatal case of trilateral retinoblastoma with an intracranial tumor in an unusual location and discuss genetic copy number analyses as a useful diagnostic tool with therapeutic potential.

KEYWORDS
DNA copy number analysis, intracranial tumor, RB1, retinoblastoma, trilateral

1 | INTRODUCTION

Retinoblastoma is a rare childhood cancer of the retina (incidence 1 in 16,000 live births). In high income countries, survival rate is >95%.1

Retinoblastoma arises from a cell that nearly always harbors pathogenic variants in both copies of the RB1 gene, a first and second “hit” as described by Knudson in 1971 (Knudson’s two-hit hypothesis).2 In non-heritable retinoblastoma, both RB1 variants arise in the developing retina, whereas in heritable retinoblastoma, a germline RB1 variant is present. The majority of heritable retinoblastoma patients have a de novo RB1 variant, as only 10% inherit the variant from a parent (autosomal dominant transmission).2 Patients with heritable retinoblastoma have a significantly increased risk for other primary cancers later in life, including osteosarcoma, melanoma, and pinealoblastoma.1,3

Retinoblastoma can affect one eye (unilateral) or both eyes (bilateral). Children with bilateral disease have a pathogenic germline RB1 variant (hence heritable retinoblastoma), but
some children with heritable retinoblastoma only develop unilateral disease, due to variation in penetrance and expressivity. Trilateral retinoblastoma refers to an intracranial tumor associated with heritable retinoblastoma and occurs in 3.5% of patients with heritable retinoblastoma.4

In this case study of trilateral retinoblastoma, we describe a patient with an intracranial tumor in an unusual location and a challenging clinical investigation process involving extensive genetic testing.

2 | CASE HISTORY

A 15-month-old boy presented with deviation of the right eyeball that had been slowly progressing since onset one month earlier. There was no family history of retinoblastoma, and the patient had an unremarkable prior history with normal growth and developmental milestones. The diagnosis of retinoblastoma was made on the basis of ophthalmoscopy and ultrasound of the right eye that revealed large, calcified tumor masses. The anterior chamber was normal. A baseline brain MRI was performed and revealed no extraocular extensions or trilateral disease (Figure 1).

The eye was staged Group E according to International Intraocular Retinoblastoma Classification,5 cT3 TNMH.6 Pathology showed retinoblastoma with minimal invasion of the choroid, and no extrascleral tumor cells, invasion of the optic nerve, or tumor cells in the anterior chamber, pT2a (Figure 2).6 The patient was treated according to guidelines with surgical enucleation of the right eye, without adjuvant chemo- or radiation therapy.7 Because of the risk of contralateral retinoblastoma, the patient was monitored with retinal examination under anesthesia every 4 weeks.

A germline deletion encompassing exon 2 and at least part of exon 1 of RB1 was detected on DNA extracted from blood. The deletion was not detected in DNA from blood from either of the parents. However, mosaicism in either parent could not be ruled out, and the couple was offered prenatal testing in the future pregnancies.

Almost 2 years after the primary diagnosis, at age 37 months, the patient was admitted to a local pediatric center with a left side peripheral facial nerve palsy and vomiting. In the 8 weeks prior to admission, the patient had experienced hoarseness, fatigue, weight loss, and a dry cough that did not respond to antibiotic treatment. The parents had noticed a lump on the left side of the neck 3 weeks prior to admission, which had been growing.

The patient was transferred to a specialized children’s oncology unit. MRI of the neuroaxis revealed a large tumor (3.5 × 5.5 × 5.5 cm) in the cerebellopontine angle, subependymal tumor elements in the rim of the lateral ventricle anterior horns, several drop metastases in the thoracolumbar spinal canal, and enlarged lymph nodes on the left side of the neck (Figure 3). There were no signs of tumor masses in the left eye or the corpus pineale.

A surgical decompression with partial resection of the cerebral tumor was performed, and pathology review confirmed the diagnosis of a malignant neuroblastic tumor that could represent either a medulloblastoma or a retinoblastoma, the latter being the most likely diagnosis due to the patient history. The patient was treated with eight cycles of intravenous and intrathecal chemotherapy, followed by high-dose chemotherapy and stem cell reinfusion. Brain MRIs showed regression throughout the treatment, and a supplemental positron emission tomography (PET) scan performed at one-month follow-up showed no pathological activity in residual lesions in the left cerebellopontine angle and the spinal canal. The three-month follow-up brain and spine MRI showed no changes.

Five months after treatment was completed, the patient was admitted with sudden-onset vomiting, headache, fatigue, and loss of balance. MRI showed multiple infra- and supratentorial processes bilaterally, and several small nodules in the ventricles. Aspergillosis was suspected, but biopsy showed malignant cells that were confirmed to be retinoblastoma (Figure 2). The patient received palliative care and died 2 weeks later, at 50 months of age.

![Baseline brain MRI at age 15 months](image-url)
A heterozygous deletion of exon 2, and at least part of exon 1, of \textit{RB1} was detected in DNA from the patient’s blood. Quantitative multiplex PCR (QM-PCR) confirmed that only one copy of exon 2 was present. For exon 1, three sets of non-overlapping primers showed only one copy, the first primer being located at the position NM_000321.2(\textit{RB1}): c.101. Analysis of the \textit{RB1} promoter and exon 3 revealed 2 copies.

It was suggested that the deletion might be a reduced-expressivity variant, as the patient was only unilaterally affected at the time, and it is known that some exon 1 variants show reduced penetrance due to utilization of alternative transcription start sites \cite{8,9}. The germline deletion was also detected at a heterozygous allele frequency in the patient’s primary, intraocular tumor; a second \textit{RB1}

\section{MOLECULAR GENETIC INVESTIGATIONS}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Histopathology images of the retinal (A and B) and cerebral (c and d) tumors. A. The overview of the right eye shows an intraocular tumor of the retina (asterisks) with both endo- and exophytic growth and with minimal invasion of the choroid and no extrascleral extension (bar = 800 \textmu m). B. The retinal tumor cells are seen with round or oval hyperchromatic nuclei and surrounded by a scant amount of cytoplasm. Homer-Right rosettes are seen (white arrows): The tumor is consistent with a retinoblastoma (bar = 40 \textmu m). C. The cerebral tumor shows poorly differentiated round tumor cells with hyperchromatic nuclei. In general, the tumor shows a diffuse growth pattern (bar = 40 \textmu m). D. In small areas of the cerebral tumor, rosettes are also seen (bar = 40 \textmu m). The cerebral tumor is consistent with a retinoblastoma.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Trilateral retinoblastoma diagnosed at age 37 months. MRI shows (A) a large tumor (3.5 x 5.5 x 5.5 cm) in the cerebellopontine angle and subependymal tumor elements in the rim of the lateral ventricle anterior horns and (B) several drop metastases in the thoracolumbar spinal canal.}
\end{figure}
pathogenic variant was not identified after sequencing all
RBI coding sequence and flanking noncoding regions,
methylation-sensitive PCR analysis of the RBI promoter, and
and copy number analysis by multiplex ligation-dependent
probe amplification (MLPA, SALSA P047-D1 RBI MRC-Holland, Amsterdam, The Netherlands). To confirm
presence of tumor DNA in the intracranial tumor sample,
analysis for well-recognized retinoblastoma somatic copy
number changes in the genes KIF14, DEK, E2F3, CDH11,
and MYCN was performed using QM-PCR. The analysis
showed three copies of DEK and four copies of MYCN,
confirming the presence of tumor DNA.

When the patient was diagnosed with an intracranial
tumor, further genetic analyses were performed to help
determine whether the tumor was a primary tumor (tri-
lateral retinoblastoma) or a metastasis. MLPA analysis
of the intracranial tumor identified homozygous loss of RBI
exons 1–2 (Table 1) as well as a gain of RBI exons 3–27.
This result potentially suggests that the intracranial tumor
DNA may have developed by loss of the normal RBI allele
and two rounds of endoreduplication of the abnormal
copy (loss of heterozygosity, LOH). LOH is frequently the
second mutational event leading to the development of
a tumor in both retinoblastomas as well as in other can-
cers. Analysis for somatic copy number changes in the
KIF14, DEK, E2F3, CDH11, and MYCN genes showed
differences in comparison with the intraocular tumor
(Table 2), suggesting that the intraocular and the intracra-
nial tumors were independent in origin, predisposed by
the presence of the germline RBI variant.

4 | DISCUSSION

We describe a clinical case of trilateral retinoblastoma that
is atypical in several aspects. Non-pineal tumors in trilat-
eral retinoblastoma usually occur synchronously, and al-
most exclusively in the supra- or parasellar regions. Accordingly, we have identified only two previous cases
with a non-pineal tumor not located to the supra- or para-
ellar regions. As described in our presented case, the
tumors were instead located in relation to the fourth ven-
tricle and cerebellum. In both previous cases, however,
the patients had been treated for bilateral retinoblastoma
prior to the diagnosis of metachronous, trilateral retino-
blastoma. The first case, presented by Finelli et al. in 1995,
describes a girl with a family history of retinoblastoma,
who was diagnosed with bilateral retinoblastoma at age
7 weeks, at which point a brain CT was normal. At age five
months, she had developed bilateral recurrent ocular dis-
ease and an MRI of the brain showed a mass in the fourth
ventricle, extending from the inferior vermician region
along the right cerebellar hemisphere. Histopathology
showed a primitive neuroectodermal tumor with neuronal
differentiation. The second case, presented by Elias et al.
in 2001, describes a girl who was diagnosed with retino-
blastoma of the right eye at age 9 months, and a second
primary neoplasm of the left eye one month later. MRI of
the brain was normal. Cytogenetic investigations revealed
a germline deletion on chromosome 13 with breakpoints
at q12.3 and q21.3, thus encompassing the RBI gene that
originated from a balanced chromosomal rearrangement
in the mother. At age 4 years, the patient had no sign of
recurrent eye disease, but had developed a large, symp-
tomatic tumor located to the midline cerebellum. She
died months later from disseminated disease with spinal
involvement. Autopsy and thorough histopathological as-
essment were performed, and the cerebellar tumor was
found to be a medulloblastoma that originated separately
from the ocular tumors, based on histomorphological and
immunocytochemical features. In our presented case,
the same conclusion was reached by supplement of ge-
netic copy number analysis, to confirm the diagnosis of
trilateral retinoblastoma with a primary tumor located to
the cerebellopontine angle.

In addition to being a diagnostic tool, characterization
of somatic alterations is the basis of personalized oncol-
ogy therapies, developed to improve treatment outcomes
and minimize long-term sequelae by selectively targeting
cancer cells. For some cancer types, targeted treatments
are well established, such as the use of tyrosine kinase
inhibitors in Philadelphia chromosome-positive leu-
kemia and the use of PARP inhibitors in ovarian- and
breast cancers harboring somatic or germline pathogenic
variants in certain genes, including BRCA1 and BRCA2. Pediatric cancer tumors in general—and retinoblastomas
in particular—harbor less somatic genomic alterations
than cancerous tumors in adults, but the potential for
targeted treatments is promising. Among possible
targets being studied in retinoblastoma are E2F3 and
MYCN, which were amplified in the tumors of the pa-
tient described in this case report.

More than 50% of trilateral retinoblastomas can be di-
agnosed by a baseline brain MRI in relation to the primary
diagnosis of retinoblastoma, and this is now generally ac-
ccepted to be the standard approach. Whether further
screening for trilateral retinoblastoma should be imple-
mented remains unsolved. In a recent meta-analysis by De
Jong et al., it is estimated that if a brain MRI screening was

| Sample          | RBI allele 1 | RBI allele 2 |
|-----------------|--------------|--------------|
| Intracranial tumor | del1->2      | del1->2      |
| Eye tumor       | del1->2      | Normal       |
| Blood           | del1->2      | Normal       |
TABLE 2 Genetic copy number analysis of the patient’s tumors

| Tumor sample | Copy numbers |
|--------------|--------------|
|              | KIF14 | DEK | E2F3 | CDH11 | MYCN |
| Intracranial | 5     | 6   | 5    | 2     | 5    |
| Eye          | 2     | 3   | 2    | 2     | 4    |

implemented, with scans every six months from diagnosis to age 36 months, it would take at least 311 scans to detect one asymptomatic pineal trilateral retinoblastoma and 776 scans to save a single life. The authors also found that there is no association between age at diagnosis of intraocular- and pineal retinoblastoma, or between the laterality of intraocular retinoblastoma and the age at diagnosis of pineal trilateral retinoblastoma. They suggested that their findings could be due to an independent development of intraocular and pineal retinoblastoma, and that penetrance and expressivity of the germline RB1 variant may vary during development.

Similarly, in a review by Yamanaka et al., no difference in latency period between intraocular and tertiary retinoblastoma of any location was found, when comparing patients according to laterality of their intraocular retinoblastoma. In line with these findings, our report of a patient with non-pineal trilateral retinoblastoma demonstrates an apparent variation in expressivity. The patient presented with unilateral and localized intraocular disease, was found to carry a seemingly reduced-expressivity RB1 germline variant, and was expected to have a good prognosis. However, the patient developed aggressive tertiary disease that initially responded to treatment but recurred shortly after.

In conclusion, the clinical course of trilateral retinoblastoma can be unpredictable. Whether screening for trilateral retinoblastoma should be implemented is still a subject of debate, and no international consensus exists. Genetic analysis can be a useful diagnostic tool in challenging clinical investigations of trilateral retinoblastoma and has therapeutic potential.

ETHICAL APPROVAL
None.

CONSENT
Informed consent was obtained from both of the patient’s parents for publication of this case report and clinical images. Written documentation of consent in accordance with the institution’s patient consent policy is available upon request.

DATA AVAILABILITY STATEMENT
None.

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CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS
Saga Elise Eiset and Pernille Axél Gregersen drafted the manuscript. Mikkel Funding, Hilary Racher, Steffen Heegaard, Brenda Gallie, and Steen F. Urbak contributed to relevant parts of the manuscript. All authors reviewed and approved the final manuscript.
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