SECONDARY OSTEOSARCOMAS IN PATIENTS WITH RETINOBLASTOMA

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INTRODUCTION

- Bone sarcomas are among the most common second primary neoplasms occurring in retinoblastoma survivors, and their cumulative incidence following retinoblastoma has been estimated to be 7% at 20 years of age
- The most common type of second bone malignancy is osteosarcoma, and both chondrosarcoma and Ewing sarcoma have also been reported
- Osteosarcoma is the most common primary bone malignancy in children, adolescents and young adults

INTRODUCTION

- Retinoblastoma is the most frequently occurring primary ocular malignancy in pediatric ages
- Retinoblastoma can be hereditary (30–40%) or non-hereditary (60–70%)
- Recently early diagnosis and treatment have greatly improved the survival rates and quality of vision of affected patients
- Cure rates of >90% have been achieved in developed countries
- Second malignancies now represent the primary cause of death in retinoblastoma survivors

INCIDENCE

- We report the clinical and therapeutic features of second primary osteosarcoma after retinoblastoma in five patients diagnosed in Rasool Akram Hospital
- Among the 468 patients with retinoblastoma, 5 patients (1.06%) developed osteosarcoma as a second malignancy in historical cohort survey from 2001 to now

DEMOGRAPHIC

Case number	*Sex*	*Laterality*	*Family History*	*Interval for second malignancy*	*Age retinoblastoma*	*Age osteosarcoma*
1 (B.NA)	Female	Bilateral	-	10 years	3 months	11 years
2 (AH.K)	Male	Bilateral	-	5 years	8 months	7 years
3 (A.TF)	Male	Bilateral	-	8 years	4 months	9 years
4 (NZ.S)	Male	Bilateral	-	9 years	5 months	9 years
5 (H.NT)	Female	Bilateral	-	13 years	15 months	12 years

SECOND PRIMARY OSTEOSARCOMA

Case Number	Site	Radiation history	Genetic abnormality
1	Cheek	+	No
2	Cheek	-	Yes
3	Right distal of tibia	-	Yes
4	Right Distal Femur	-	Yes
5	Right Distal Femur	-	Yes

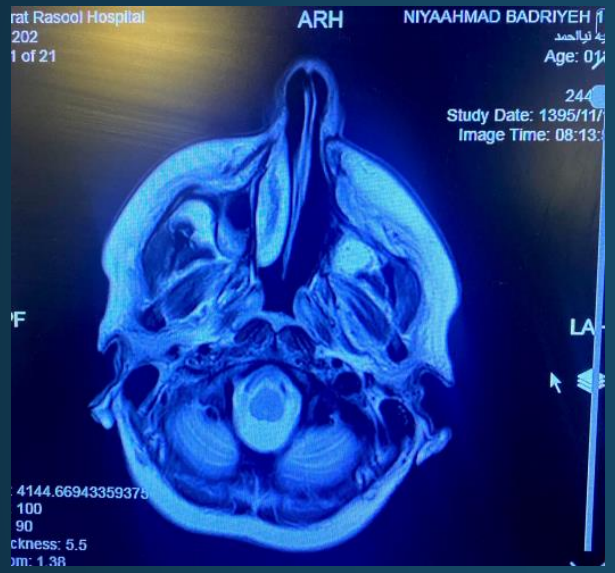
TREATMENT FOR RETINOBLASTOMA

Case Number	Enucleation	Systemic chemoreduction	Arterial infusion chemotherapy	Nuclear Plaque	Radiation therapy
1	Both eye	OPEC High dose VEC	No	-	EBRT
2	Left eye	High Dose VEC	Melphalan Carboplatin	+	-
3	No	High dose VEC	Melphalan Topotecan	_	-
4	Left eye	High Dose VEC	Melphalan Topotecan Carboplatin	+	-
5	No	High dose VEC	No	_	-

TREATMENT FOR SECOND PRIMARY OSTEOSARCOMA

Case Number	Metastasis	Surgery	Chemotherapy	Outcome
1	-	+	Inter group 0133	Alive Off treatment
2	Right Tibia Right Radius Lung	+	Inter group 0133	Death
3	Left Radius	+	Inter group 0133	On treatment
4	-	+	Inter group 0133	On treatment
5	Lung	+	Inter group 0133	On treatment

CASE 1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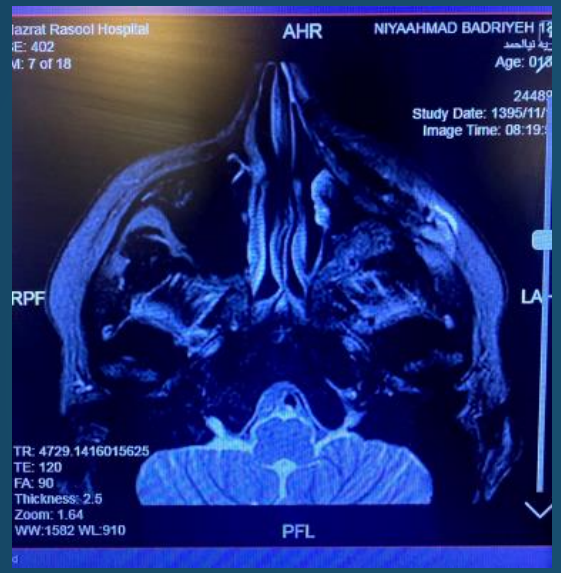
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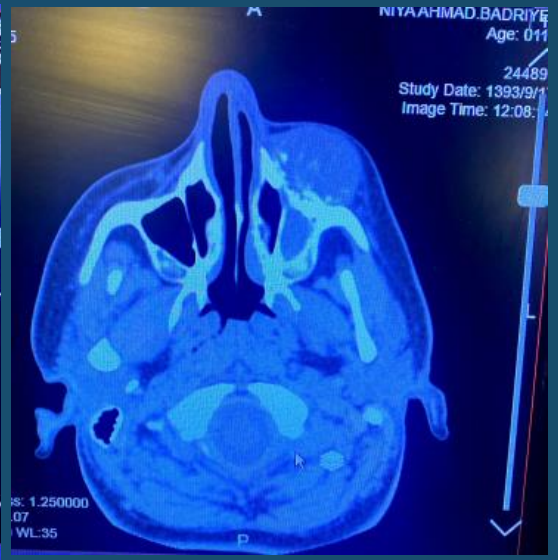
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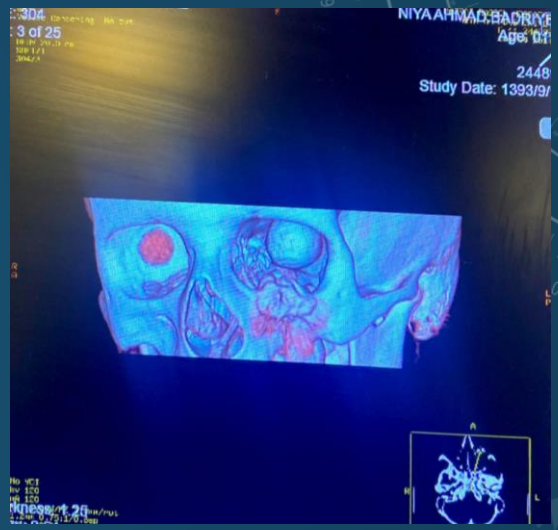
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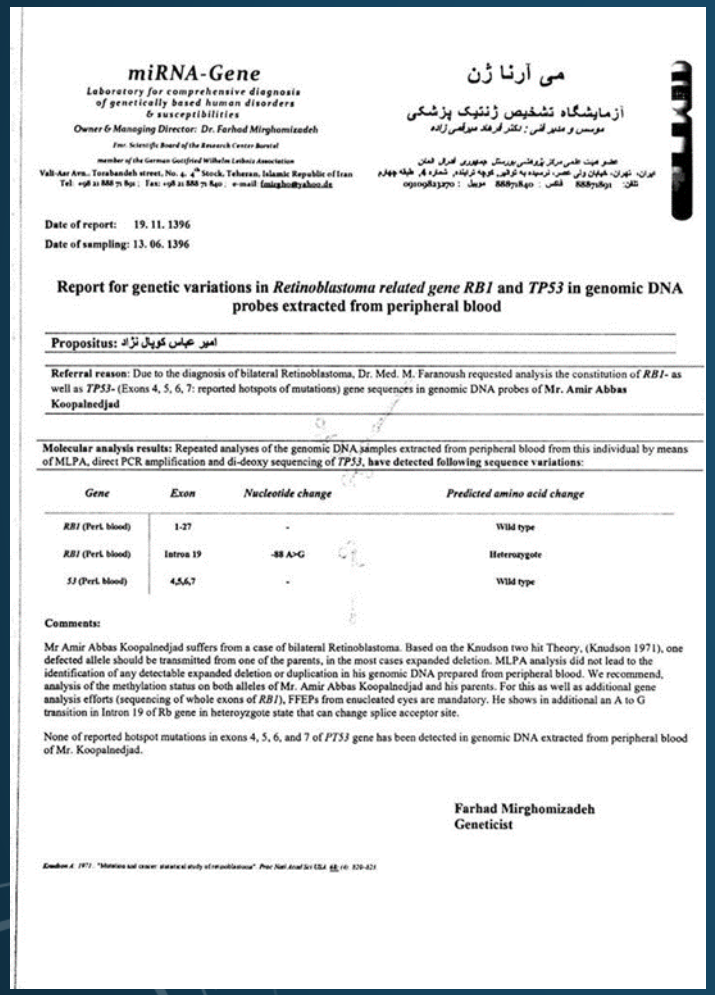

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Laboratory for comprehensive diagnosis of genetically based human disorders & susceptibilities

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می آرنا ژن

آزمایشگاه تشخیص ژنتیک پزشکی

موسس و مدیر فنی : دکتر فرهاد میرقمی زاده

عضو هیئت علمی مرکز پژوهشی بورسیتک جمهوری فدرال آلمان
تهران، خیابان ولی عصر، ترسیده به تقاطع، کوچه ترابنده، شماره ۴، طبقه چهارم
تلفن: 88871840 فکس : 88871840 موبیل : 09109183270

Date of report: 19. 11. 1396

Date of sampling: 13. 06. 1396

Report for genetic variations in *Retinoblastoma related gene RB1* and *TP53* in genomic DNA probes extracted from peripheral blood

Propositus: امیر عباس کوپال نژاد

Referral reason: Due to the diagnosis of bilateral Retinoblastoma, Dr. Med. M. Faranoush requested analysis the constitution of *RB1*- as well as *TP53*- (Exons 4, 5, 6, 7: reported hotspots of mutations) gene sequences in genomic DNA probes of **Mr. Amir Abbas Koopalnedjad**

Molecular analysis results: Repeated analyses of the genomic DNA samples extracted from peripheral blood from this individual by means of MLPA, direct PCR amplification and di-deoxy sequencing of *TP53*, **have detected following sequence variations:**

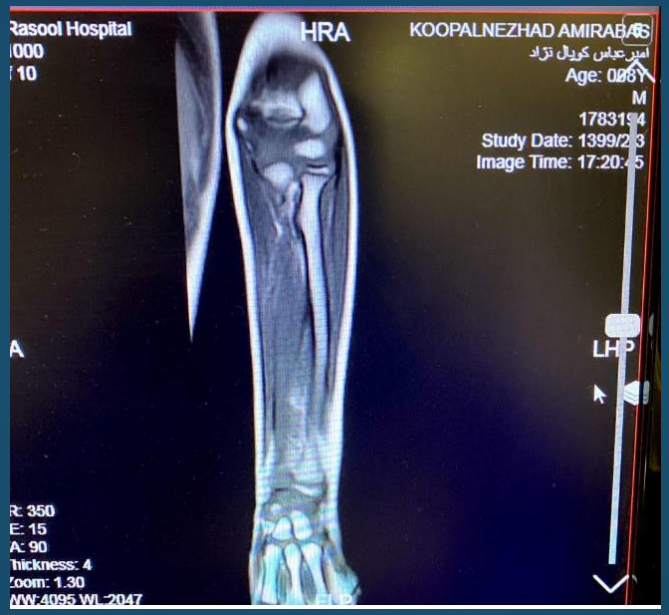
Gene	*Exon*	*Nucleotide change*	*Predicted amino acid change*
RB1 (Perf. blood)	1-27	-	Wild type
RB1 (Perf. blood)	Intron 19	-88 A>G	Heterozygote
53 (Perf. blood)	4,5,6,7	-	Wild type

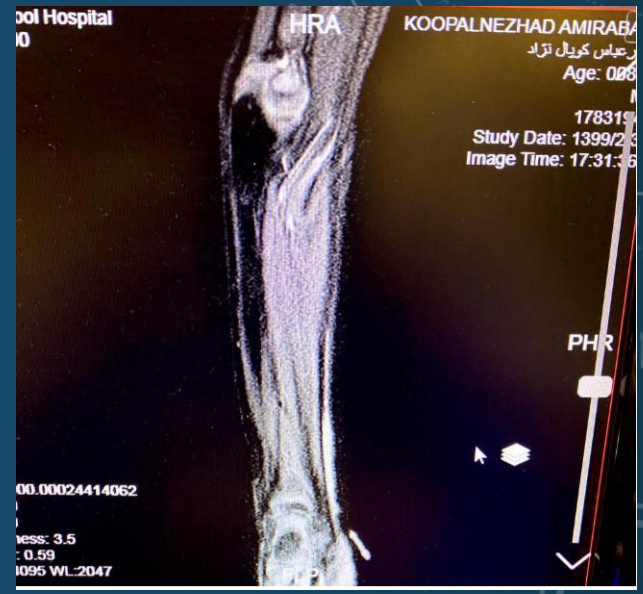
Comments:

Mr Amir Abbas Koopalnedjad suffers from a case of bilateral Retinoblastoma. Based on the Knudson two hit Theory, (Knudson 1971), one defected allele should be transmitted from one of the parents, in the most cases expanded deletion. MLPA analysis did not lead to the identification of any detectable expanded deletion or duplication in his genomic DNA prepared from peripheral blood. We recommend, analysis of the methylation status on both alleles of Mr. Amir Abbas Koopalnedjad and his parents. For this as well as additional gene analysis efforts (sequencing of whole exons of *RB1*), FFEPs from enucleated eyes are mandatory. He shows in additional an A to G transition in Intron 19 of Rb gene in heteroyzgote state that can change splice acceptor site.

None of reported hotspot mutations in exons 4, 5, 6, and 7 of *PT53* gene has been detected in genomic DNA extracted from peripheral blood of Mr. Koopalnedjad.

Farhad Mirghomizadeh
Geneticist





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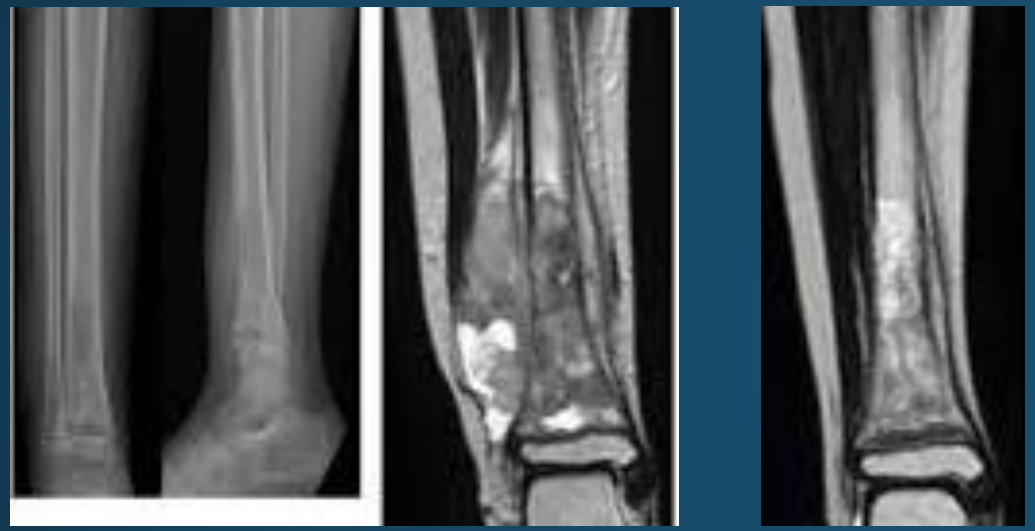


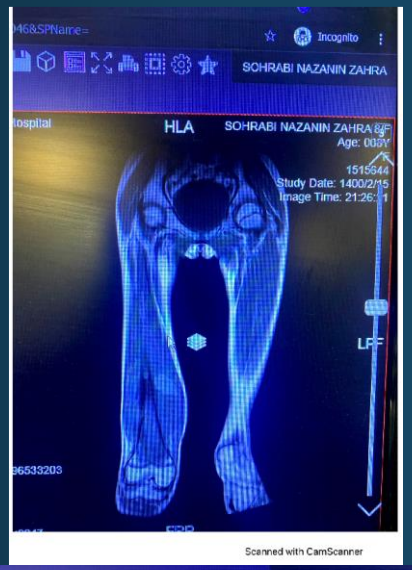
ابوبکر طاهری

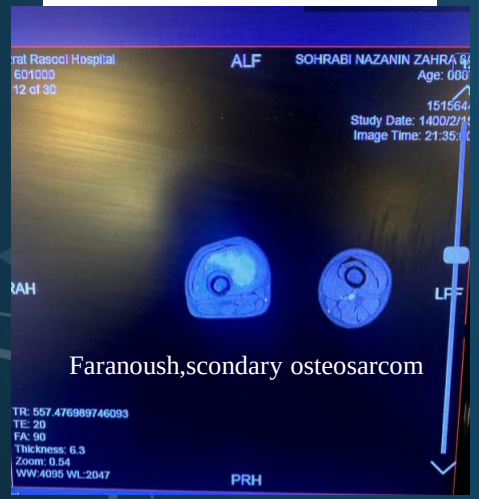
آنزوم‌های زیر انجام شده ول موتاسیونی

در این آنزوم‌ها یافت نشده است.

Exon = 1
Exon = 5
Exon = 6
Exon = 7
Exon = 9
Exon = 10
Exon = 11
Exon = 12
Exon = 14
Exon = 15
Exon = 16
Exon = 17
Exon = 18
Exon = 19
Exon = 20
Exon = 21
Ex = 22
Ex = 23
E = 24
E = 25
E = 26

CASE 4





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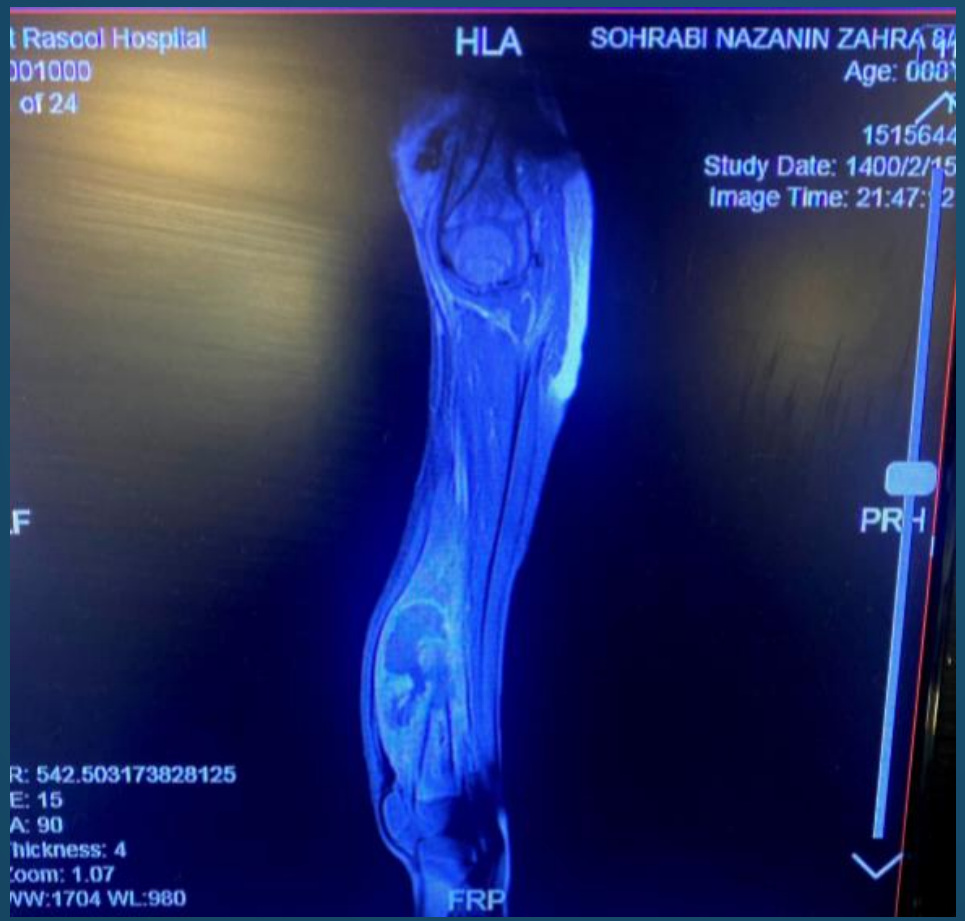

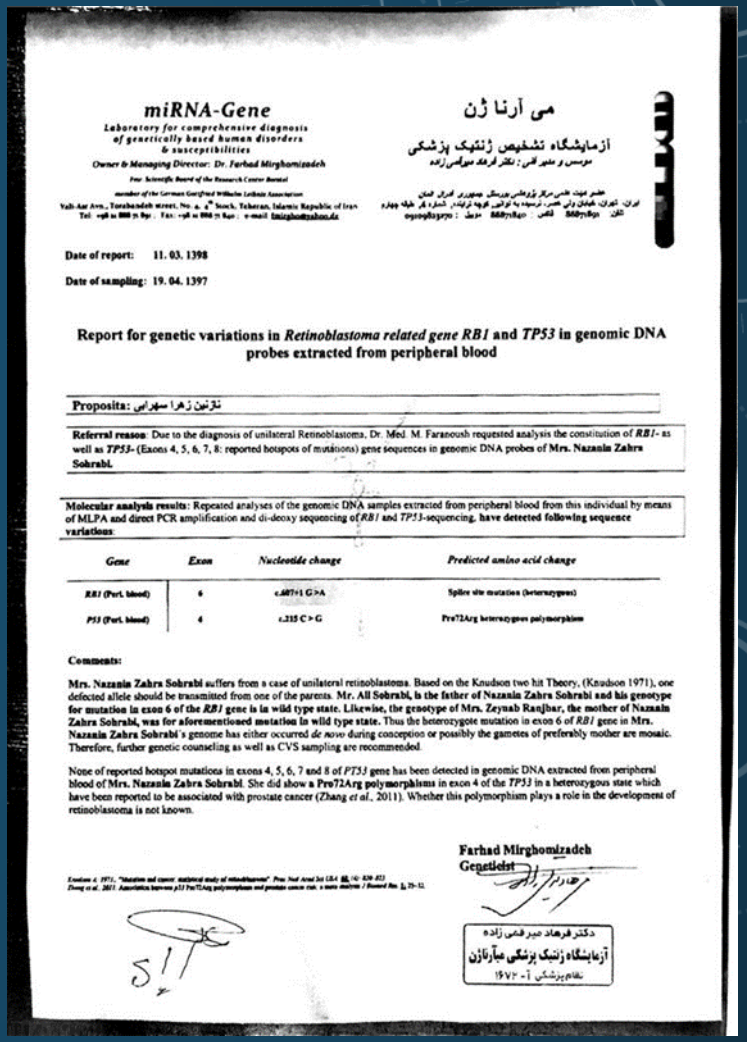

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می آرنا ژن

آزمایشگاه تشخیص ژنتیک پزشکی

مؤسس و مدیر فنی: دکتر فرهاد میرقمی‌زاده

Date of report: 11. 03. 1398

Date of sampling: 19. 04. 1397

Report for genetic variations in *Retinoblastoma related gene RB1* and *TP53* in genomic DNA probes extracted from peripheral blood

Proposita: نازنین زهرا سهرابی

Referral reason: Due to the diagnosis of unilateral Retinoblastoma, Dr. Med. M. Faranoush requested analysis the constitution of ***RB1***- as well as ***TP53***- (Exons 4, 5, 6, 7, 8: reported hotspots of mutations) gene sequences in genomic DNA probes of **Mrs. Nazanin Zahra Sohrabi.**

Molecular analysis results: Repeated analyses of the genomic DNA samples extracted from peripheral blood from this individual by means of MLPA and direct PCR amplification and di-deoxy sequencing of *RB1* and *TP53*-sequencing, **have detected following sequence variations**:

Gene	*Exon*	*Nucleotide change*	*Predicted amino acid change*
RB1 (Perl. blood)	6	c.607+1 G>A	Splice site mutation (heterozygous)
P53 (Perl. blood)	4	c.215 C>G	Pro72Arg heterozygous polymorphism

Comments:

Mrs. Nazanin Zahra Sohrabi suffers from a case of unilateral retinoblastoma. Based on the Knudson two hit Theory, (Knudson 1971), one defected allele should be transmitted from one of the parents. **Mr. Ali Sohrabi, is the father of Nazanin Zahra Sohrabi and his genotype for mutation in exon 6 of the *RB1* gene is in wild type state. Likewise, the genotype of Mrs. Zeynab Ranjbar, the mother of Nazanin Zahra Sohrabi, was for aforementioned mutation in wild type state.** Thus the heterozygote mutation in exon 6 of *RB1* gene in **Mrs. Nazanin Zahra Sohrabi**'s genome has either occurred *de novo* during conception or possibly the gametes of preferably mother are mosaic. Therefore, further genetic counseling as well as CVS sampling are recommended.

None of reported hotspot mutations in exons 4, 5, 6, 7 and 8 of *P53* gene has been detected in genomic DNA extracted from peripheral blood of **Mrs. Nazanin Zahra Sohrabi**. She did show a **Pro72Arg polymorphism** in exon 4 of the *TP53* in a heterozygous state which have been reported to be associated with prostate cancer (Zhang *et al.*, 2011). Whether this polymorphism plays a role in the development of retinoblastoma is not known.

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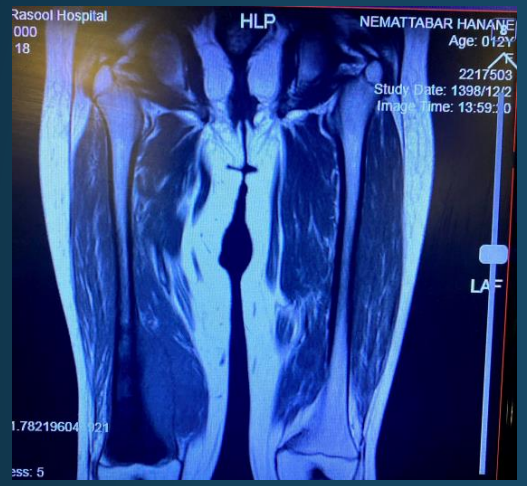
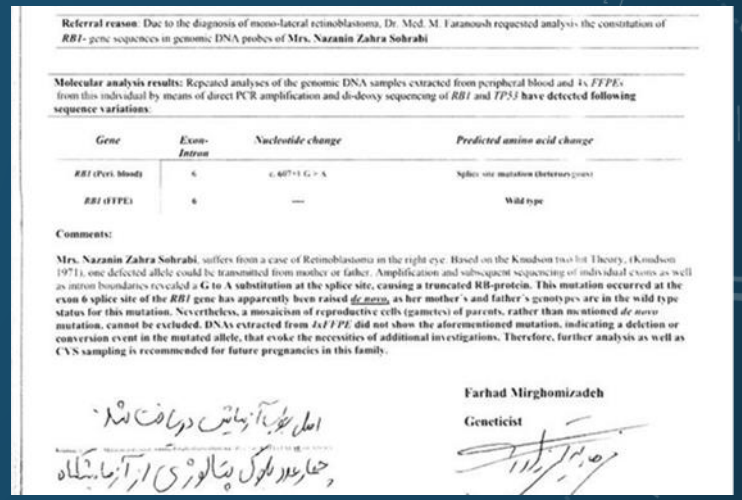
Geneticist

دکتر فرهاد میرقمی زاده
آزمایشگاه ژنتیک پزشکی می‌آرنا‌ژن
نظام‌پزشکی آ-۱۶۷۲

CASE 5





Referral reason. Due to the diagnosis of mono-lateral retinoblastoma, Dr. Med. M. Faranoush requested analysis the constitution of *RB1*- gene sequences in genomic DNA probes of **Mrs. Nazanin Zahra Sohrabi**

Molecular analysis results: Repeated analyses of the genomic DNA samples extracted from peripheral blood and 4x *FFPEs* from this individual by means of direct PCR amplification and di-deoxy sequencing of *RB1* and *TP53* **have detected following sequence variations**:

Gene	*Exon-Intron*	*Nucleotide change*	*Predicted amino acid change*
RB1 (Peri. Blood)	6	c.607+1 G > A	Splice site mutation (heterozygous)
RB1 (FFPE)	6	—	Wild type

Comments:

Mrs. Nazanin Zahra Sohrabi, suffers from a case of Retinoblastoma in the right eye. Based on the Knudson two hit Theory, (Knudson 1971), one defected allele could be transmitted from mother or father. Amplification and subsequent sequencing of individual exons as well as intron boundaries revealed a **G to A substitution at the splice site, causing a truncated RB-protein. This mutation occurred at the exon 6 splice site of the *RB1* gene has apparently been raised *de novo*, as her mother's and father's genotypes are in the wild type status for this mutation. Nevertheless, a mosaicism of reproductive cells (gametes) of parents, rather than mentioned *de novo* mutation, cannot be excluded. DNAs extracted from *4xFFPE* did not show the aforementioned mutation, indicating a deletion or conversion event in the mutated allele, that evoke the necessities of additional investigations. Therefore, further analysis as well as CVS sampling is recommended for future pregnancies in this family.**

Farhad Mirghomizadeh

Geneticist

LITERATURE REVIEW

- Improvements in diagnosis and treatment for retinoblastoma have allowed most patients to survive their ocular cancer
- Second malignant neoplasms have become the major cause of death in retinoblastoma survivors
- Osteosarcoma being the most common second malignancy
- Risk factors for second primary osteosarcoma have been well characterized
- HD-MTX-based multi-agent chemotherapy provided a good tumor response and, with wide tumor resection, it contributed to a good oncologic outcome.
- The clinical outcomes for second primary osteosarcoma of an extremity occurring in retinoblastoma survivors may be more favorable than those for conventional osteosarcoma.

REVIEW OF DATA

- In patients with hereditary retinoblastoma, a genetic predisposition is an important risk factor for the development of second malignancies
- Previous reports have revealed that the incidence of osteosarcoma after heritable retinoblastoma is 300 times greater than that in the general population
- In our series, 4/5 patients had genetically predisposed and possess a risk for second primary osteosarcoma
- All cases had bilateral retinoblastoma, and no family history of retinoblastoma
- The age at the onset considerably younger than in general (average, 7 months)
- Germline mutations of the RB1 gene were detected in 80% of patients

RISK FACTORS

- Radiation therapy for retinoblastoma is another risk factor for the development of second malignancies
- One patient who developed radiation-induced second primary osteosarcoma were bilateral retinoblastoma survivors, highly likely to be heterozygous carriers of an RB1 mutation
- The available data on bilateral patients who were initially treated, among 148 bilateral patients with EBRT, five patients (2.7%) developed osteosarcoma in an extremity and one (0.7%) developed osteosarcoma in the radiation field of a cranial lesion.
- Incidence rates of second primary osteosarcoma were certainly increased by the radiation therapy.

RISK FACTORS

- Chemotherapy containing alkylating agents alone or in combination with radiotherapy might be another risk factor
- Some study focusing on the risk factors for the development of second malignancies found that focal and systemic chemotherapy was not a significant risk factor

OSTEOSARCOMA

- Response of osteosarcoma to chemotherapy is one of the most important factors affecting prognosis , the chemo sensitivity of second primary osteosarcoma is a clinically important issue
- Tumor response to pre-operative chemotherapy (including MTX, ADM, IFM and etoposide) was good in five of patients with second primary osteosarcoma after retinoblastoma.

OUTCOME

- In the present study, all of three patients with a second primary osteosarcoma in an extremity survived with no evidence of disease,
- One of two patients with a second primary osteosarcoma in cheeck survived with no evidence of disease,
- Therefore, the results of the present study indicated that clinical outcomes for second primary osteosarcoma in retinoblastoma survivors may be more favorable than those for conventional osteosarcoma.
- Second primary osteosarcomas affecting sites other than the limbs have a poorer prognosis, largely because of the limitations to wide excision of these tumors.
- Therefore, an early diagnosis of second primary osteosarcoma in the craniofacial region is important to ensure complete resection.

SUMMARY

- The clinical outcomes of second primary osteosarcoma of an extremity occurring in retinoblastoma survivors may be more favorable than those for conventional osteosarcoma.
- Careful and long-term follow-up is necessary for patients with retinoblastoma, and it is important to educate parents of children who need to rapidly seek medical advice for any pain or abnormalities in the head, face, or extremities to ensure an early diagnosis of second primary malignancies.

Thank you
Any Question