Retinoblastoma discordance in families with twins

Aloysius Abraham, Kannan Thirumalairaj, Namrata Gaikwad[1], Veerappan Muthukkaruppan[2], Alla G Reddy[3], Kumarasamy Thangaraj[3], Usha Kim[1], Ayyasamy Vanniarajan

Retinoblastoma has an increased inheritance risk of germline *RB1* mutations in offspring and siblings, especially twins. Three families, each having one retinoblastoma-affected twin, were selected for genetic analysis and DNA profiling. Germline *RB1* mutations were found in all probands. DNA profiling carried on similar-looking twins of families I and II, proved them to be fraternal. This study demonstrates the importance of genetic analysis of *RB1* gene for risk prediction in retinoblastoma families. It also emphasizes that DNA profiling is a mandate for genetic screening of families with twins, thus adding a new dimension in counseling of retinoblastoma.

Key words: DNA profiling, genetic analysis, Retinoblastoma 1(*RB1*) gene, retinoblastoma, twins

Retinoblastoma (RB), an intraocular childhood malignancy, is the prototype genetic cancer caused by biallelic inactivation of *RB1*. A wide spectrum of *RB1* mutations, including point mutations, exonic, or whole *RB1* deletions were commonly reported.[1] The cause for acquisition of *RB1* mutations still remains elusive, which would be possible through study of twins from large representative samples and determine the status of disease among them. In 1929, Benedict first reported the occurrence of RB in homologous eyes of identical twins,[2] Duncan and Maynard further reported bilateral RB in identical twins, neither of whom survived.[3] Though twin studies have started in the early 19th century, about eight cases without discordance were reported based on physical appearance alone. In 1964, Kantar and Harris reported a case of RB discordance in identical twins, where hair color, blood types, amniotic sac number, placenta, and finger-ridge count with a mean difference of six were also considered to confirm twin zygosity.[4] With the evolution of DNA profiling, which is based on varying lengths of short tandem repeats (STRs), authentic results on zygosity were obtained. Among 200 RB families screened for *RB1* mutations, three families with twins were identified and DNA profiling was done in similar-looking twins, believed to be from the same zygote. As reviewed by Dimaras in 2015, various RB research groups in India have carried out genetic testing but this is the first report on usage of DNA profiling for RB patients in Indian population.

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Each family had only one twin affected by bilateral RB, confirmed by clinical and radiological investigations. Families I and II had similar-looking twins. Family III had a positive family history of RB. Mutational screening was done with blood DNA in sequential manner as described[5] based on the calculated frequency of mutations in *RB1* gene.

In family I, twin A was presented at 15 months with OS asymmetric nystagmus and intermittent leukocoria in both the eyes [Fig. 1]. Her twin sister (twin B), examined as a part of sibling screening, was found to have a small suspicious lesion in her right eye. Multiplex Ligation-dependent Probe Amplification (MLPA) revealed deletion of exons 4, 5, and 6 in twin A [Fig. 2]. Parents and sibling (twin B) blood samples did not show any deletion.

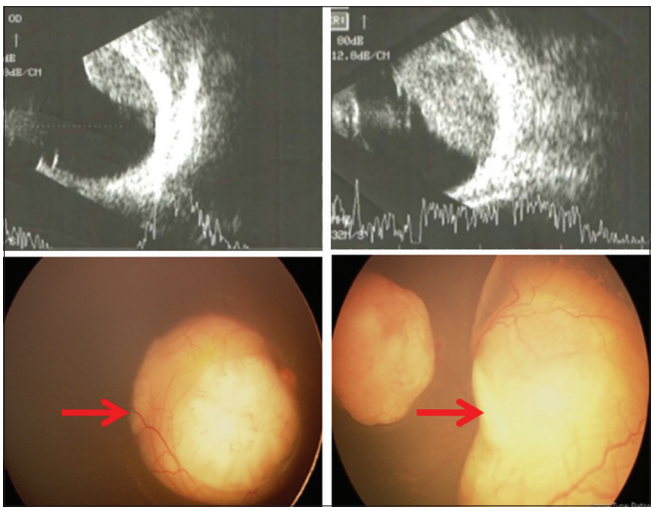

Figure 1: Ultrasonography B-scan and RetCam images of twin A in family I. The right eye (top and bottom left) had Group B retinoblastoma while the left eye (top and bottom right) had Group D. The red arrows in the RetCam image show the extent of tumor growth

Department of Molecular Genetics, Aravind Medical Research Foundation, [1]Department of Orbit, Oculoplasty and Oncology, Aravind Eye Hospital, [2]Department of Immunology and Stem Cell Biology, Aravind Medical Research Foundation, Madurai, [3]Council of Scientific and Industrial Research Centre for Cellular and Molecular Biology, Hyderabad, India

Correspondence to: Dr. Usha Kim, Department of Orbit, Oculoplasty and Oncology Services, Aravind Eye Hospital, 1, Anna Nagar, Madurai, Tamil Nadu - 625 020, India. E-mail: usha@aravind.org

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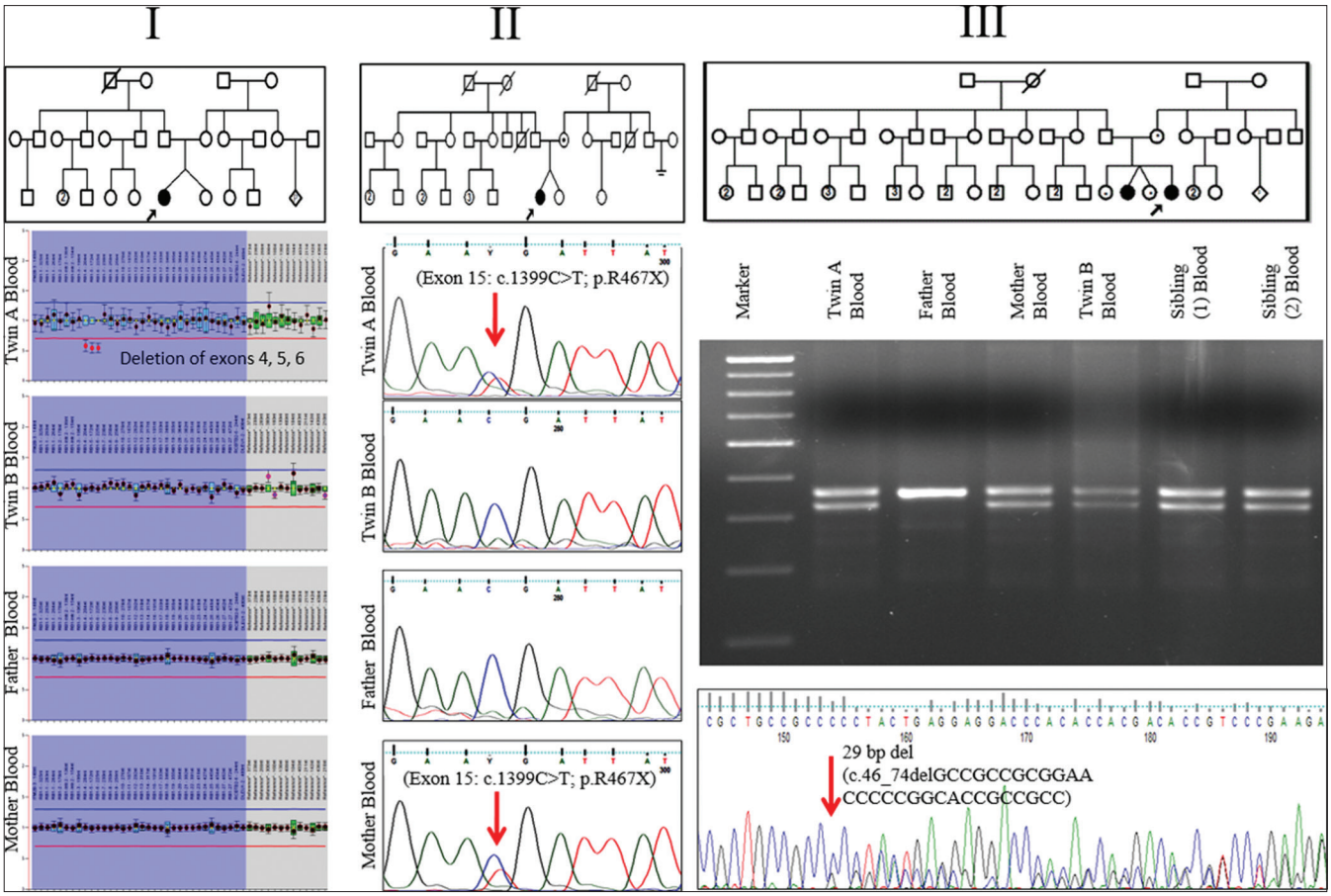

Figure 2: Pedigree and mutation profile of retinoblastoma (RB) families. Positive history of RB was found in family III. In family I, Multiplex Ligation-dependent Probe Amplification (MLPA) profile showed exonic deletions only in twin A. In family II, electropherogram showed nonsense mutation in twin A and mother. In family III, 29 bp deletion in exon-1 was found in all family members except the father

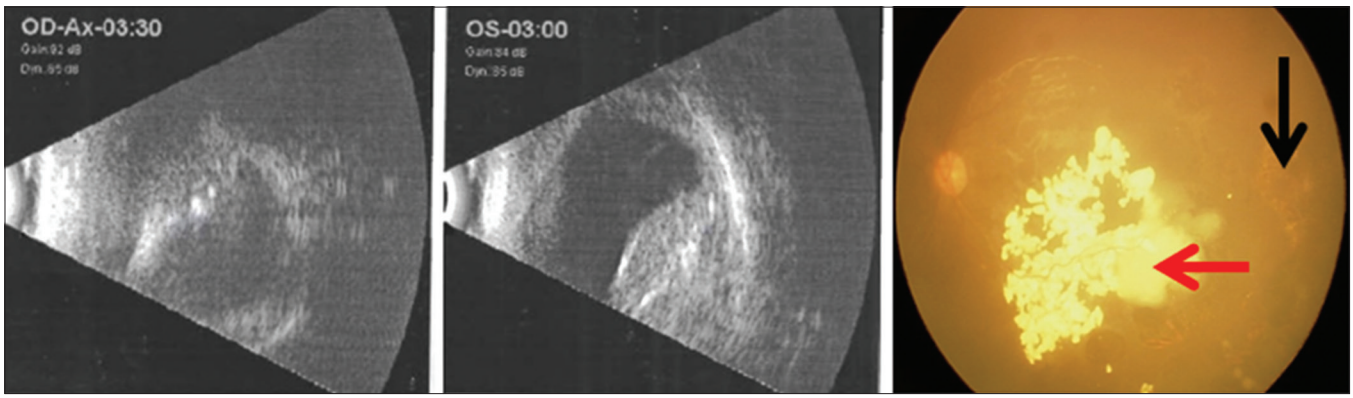

Figure 3: Ultrasonography B-scan and RetCam fundus image of twin A in family II. The right eye had Group E retinoblastoma for which the fundus picture was unavailable. Ultrasound B-scan shows a diffuse tumor (left). The left eye had Group D retinoblastoma. Ultrasound B-scan shows a compact tumor (middle). The red arrow in the RetCam image (right) shows the subretinal and vitreous seeding and black arrow points out the scars due to chemotherapy in left eye of the proband

In family II, twin A was presented at 30 months with bilateral RB having exophytic tumors with vitreous seedings [Fig. 3]. Sibling screening confirmed that twin B was normal. Sanger sequencing of twin A blood revealed a nonsense mutation in exon 15 of the *RB1* gene, leading to premature protein truncation. Twin B and father did not have this mutation, but mother was found to have this same mutation [Fig. 2].

DNA profiling was carried out in families I and II for zygosity mapping using the AmpFLSTR™ Identifiler® PCR Amplification Kit (Thermo Fisher Scientific, Massachusetts,

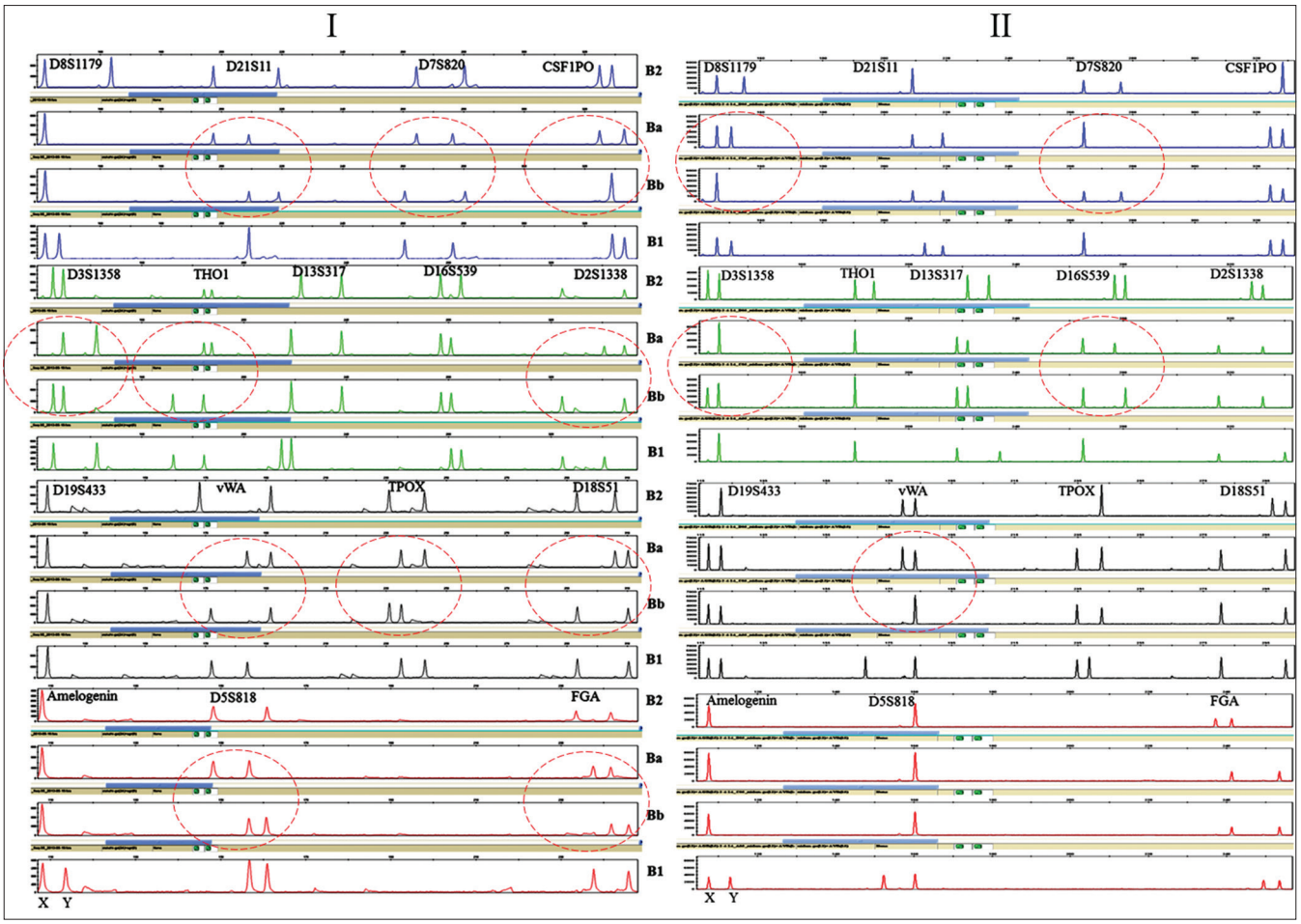

Figure 4: DNA profiling of family I and family II. Ba-Twin A, Bb-Twin B, B1-Father, B2-Mother. Discordant loci are shown in dotted red circles

USA) according to the manufacturer's instructions.[6] Profiles of families I and II clearly demonstrated the discordance among the twins for many loci such as D7S820, D3S1350, and vWA [Fig. 4].

In family III, apart from the affected twin, there was an affected sibling who was not her twin. The affected twin had Group C in right eye and Group E in left eye. Co-segregation analysis by the Sanger sequencing revealed 29 bp deletion in exon-1 of all family members, except the father [Fig. 2].

Discussion

Disease penetrance of RB is about 90% in bilateral cases with germline mutations. Monozygotic twins harboring such mutations would generally display concordant phenotypes.[7] Here, discordance of RB was observed in three families with twins. In family I, proband (twin A) had *de novo* germline deletion and twin B was diagnosed with regressed RB, which did not correlate with the genetic analysis. Therefore, the suspicious lesion was re-examined and confirmed as a gliotic tuft. In family II, the proband had a *RB1* null mutation inherited from the mother. Parents did not give consent for their eye examination and hence the possibility of regressed RB in the mother could not be ruled out. In family III, 29 bp deletion in exon-1 was detected in all family members expect the father, which could be speculated as a low penetrant mutation. Also, second *RB1* mutational event in retinal cells might not have occurred in those unaffected carriers.

Concordance and discordance of RB in monozygotic twins have been reported in various studies earlier but the techniques used for zygosity differentiation were of low resolution and archaic.[2,4,8–10] Owing to these hitches, carriers in the family could be missed. Combining high-resolution techniques like DNA profiling and genetic testing of *RB1*, risk prediction for all members of affected families was made. The quality of disease management was enhanced through close follow up of the affected child with appropriate treatment and the unaffected child was relieved from the burden of continuous monitoring. As a tool of establishing individual identity, DNA profiling revealed the twins to be fraternal and therefore discordance of the disease was due to individual zygote undergoing mutational events *de novo*. This high-resolution technique not only gives the zygosity but also the biological identity of families. The genotypes of the twins were clearly matching with their parents in both the families [Fig. 4]. DNA profiling also helps in avoiding sample switch-overs in diagnostic labs with huge patient sample load.

Conclusion

Our report demonstrates the importance of genetic evaluation in families with retinoblastoma. It also shows that DNA

profiling is useful for genetic screening of families with twins, thus adding a new dimension in counseling.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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