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A LINKAGE ANALYSIS IN TWO FAMILIES WITH BILATERAL RETINOBLASTOMA*

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SUMMARY: Emre S, Sungur A, Hazar V, Bilgiç S, Büyükpamukçu M, Günalp İ. (Departments of Medical Biology, Pathology and Ophthalmology, and Oncology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). A linkage analysis in two families with bilateral retinoblastoma. Turk J Pediatr 1996; 38: 413-417.

Using polymerase chain reaction (PCR) amplification from blood samples, XbaI and BamHI polymorphisms were analyzed in two families with bilateral retinoblastoma. In one of the families it was predicted using the BamHI polymorphism that the 200 bp allele co-segregates with the disease. This family was uninformative for XbaI polymorphism. The second family was uninformative for both XbaI and BamHI polymorphism. *Key words: retinoblastoma, polymorphism, polymerase chain reaction.*

Retinoblastoma (RB) is the most common intraocular tumor in children. It may affect one or both eyes and occurs in both familial and sporadic forms. In familial cases the inheritance follows an autosomal-dominant pattern[1]. Sporadic cases represent the majority of both unilateral and bilateral forms of the disease. Random inactivation or loss of both alleles at the RB locus are thought to cause unilateral unifocal cases[2,3]. Individuals with bilateral sporadic disease are known to have new germline mutations and frequently have affected offspring. In other words, they carry the mutation in their germline. Familial cases are more frequently bilateral and multifocal and are believed to be either the result of inheritance of one defective RB allele from an affected or carrier parent or the somatic inactivation or loss of the remaining functional allele[4,5]. The RB gene has been mapped to chromosome region 13q14. The RB gene contains 27 exons and spans 180 kb of genomic DNA[6]. The message (4.7 kb) contains a relatively short 5' untranslated sequence followed by a coding region of 2.7 kb which encodes 928 aminoacids. The sequence for the entire RB cDNA is now available, and

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the sequences of XbaI and BamHI polymorphic sites have also been determined; thus linkage analysis in RB families can be performed using the polymerase chain reaction (PCR).

In this study, XbaI and BamHI polymorphic sites were analyzed and the segregation of the alleles was detected by PCR in two families with bilateral retinoblastoma.

Material and Methods

Patients and DNA Preparation: Patients were identified through the Department of Ophthalmology at Hacettepe University and Ankara University. The diagnosis of retinoblastoma had been established by current Ophthalmologic criteria[9]. DNA from whole blood samples was prepared using standard phenol/chloroform extraction followed by ethanol precipitation[10].

PCR-Based Detection of Polymorphic Sites

BamHI Site: Two primers (Table I), one from exon 1 and the other from intron 1 of the human RB gene, were used to amplify a genomic DNA fragment containing the polymorphic BamHI site. For the BamHI polymorphism an approximately 200 bp genomic DNA fragment containing the intron 1 splice donor site was amplified. Fragments of 140 bp and 60 bp were generated after BamHI digestion of the PCR product. PCR was carried out in a total volume of 50 ml containing approximately 1 µg DNA and buffer consisting of 50 pmol of each dNTP (dATTP, dTTP, dCTP, dGTP), 50 mmol/l KCl, 10 mmol/l TrisHCl, (pH 9.0 at 25 °C) 1.5 mmol/l MgCl, 10 percent dimethyl sulphoxide (DMSO), and two units Taq DNA polymerase (Promega).

Table I: Sequence of PCR Primers Used to Amplify Polymorphic Restriction Enzyme Sites within the RBI Gene

	Primers	
Polymorphic Site	Sense	Antisense
BamHI	5'-CAGGACAGCGCCCGGAG-3	5'-CTGCAGACGCTCCGCCGT-3'
XbaI	5'-TTCCATGAAGAACAAATGG-3	5'-GCAATTGCACAATCCAAGTT-3'

Amplification was performed using a programmable thermal cycler (Perkin Elmer Cetus 480). Amplification consisted of three steps (after an initial 15 minute denaturation step at 96 °C): denaturation at 96 °C for 20 seconds, annealing at 60 ° for 20 seconds followed by an extension step at 72 °C for 60 seconds. After 30 cycles of amplification, the amplified product was digested overnight in restriction enzyme BamHI. DNA fragments were resolved by electrophoresis through two percent agarose gels.

XbaI Site: A genomic DNA fragment containing the polymorphic XbaI site (21.8 kb downstream from exon 17) was amplified using primers (Table I) from intron 17 of the human RB gene. XbaI digestion of this fragment identifies two alleles: an allele 945 bp and an allele consisting of two fragments 630 bp+315 bp long. The PCR conditions were the same as those used for the BamHI site except that the PCR mix did not contain DMSO and the annealing temperature was 50 °C. The amplification product was digested overnight with XbaI. DNA fragments were resolved by electrophoresis through one percent agarose gels.

Results

XbaI and BamHI polymorphisms were analyzed using PCR amplification, from blood samples in two families with bilateral retinoblastoma.

The first family (RB-1) pedigree is shown in Figure 1. The father developed tumors in both eyes, and one of the eyes was enucleated. There was no history of RB on the maternal side of the family. At the age of 14 months, the firstborn child, a boy, was diagnosed with bilateral RB. Blood samples were taken from all of the family members. Using BamHI polymorphism, the mother was found to be homozygous for the 140/60 bp allelle. The father and proband were heterozygous for the 200/140-60 bp allele (Fig. 1). Homozygous individuals are described as 140/60 bp. Heterozygous individuals are described as 200/140-60 bp. Using the other set of primers (Table I), an approximately 945 bp genomic DNA fragment containing the polymorphic XbaI site was amplified. At the XbaI locus, the mother, father and proband were all heterozygous for the 945/630-315 bp allele (Fig. 2). Heterozygous individuals are described as 945/630-315 bp.

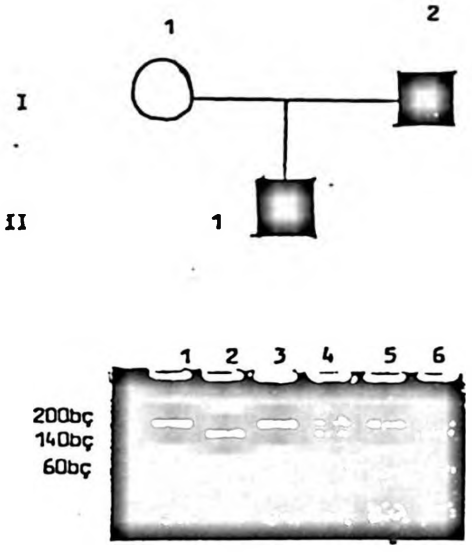

Fig. 1: Pedigree of family RB-1. PCR amplification of the polymorpic BamHI site from DNA samples from the members of family RB-1. In all cases a dominant band of the expected size, ± 200 bp long was observed. The amplified DNA from each patient was digested with BamHI. lane (1, 3, 5): PCR products (± 200 bp). The mother (lane 2) is homozygous for the 140/60 allele, but the father (lane 4) is heterozygous. The proband (lane 6) is also heterozygous. The RB gene was therefore segregating with the 200 bp allele from the father.

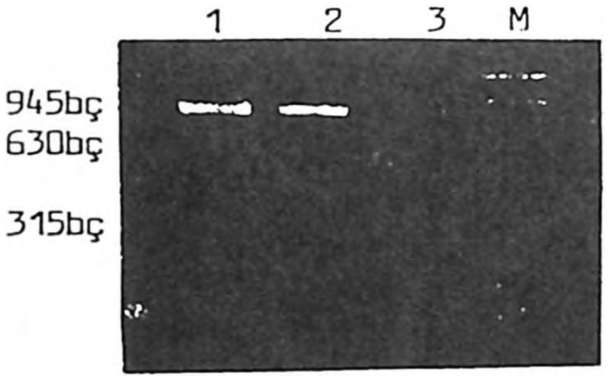

Fig. 2: Segregation of the alleles for the polymorphic Xbal site in family RB-1. The father (lane 1), mother (lane 2) and proband (lane 3) here heterozygous for the 945/630-315 allele. M: ØX174 HaeIII digested. (Marker lane).

In the second family (RB-2), three affected siblings were born to unaffected parents. This family pedigree is shown in Figure 3. There was no previous history of RB in this family.

All three children were boys, ages 8, 9 and 11, with bilateral RB. All the children had one enucleated eye. Blood samples were obtained from all the family members, including healthy individuals, and analyzed for the polymorphisms. All the affected and unaffected individuals were found to be heterozygous for BamHI and Xbal polymorhism (Fig. 3).

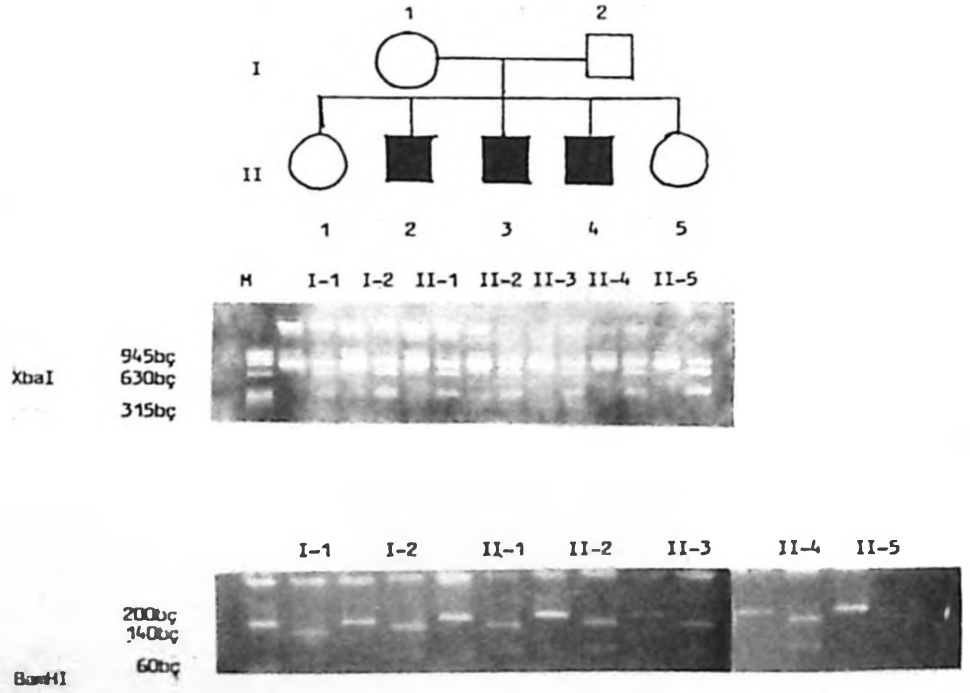

Fig. 3: Pedigree of family RB-2. Segregation of the alleles for the polymorphic Xbal and BamHI site in family RB-2. The father, (I-2), mother (I-1), all probands (II-2, II-3, II-4) and healthy children (II-1, II-5) are heterozygous for Xbal and BamHI sites. Thus the family was non-informative for mutant allele segregation. M: ØX174 HaeIII digested (marker lane).

Discussion

Predisposition to cancer can be assessed by identifying mutant alleles in affected pedigrees. By identifying carriers of the mutant gene, management of the disease and early intervention become possible. Now with the use of various DNA markers, linkage analysis allows prenatal diagnosis in RB families[11, 12].

In this study, two families identified as RB-1 and RB-2 were analysed. No tumor tissue had been saved from enucleated eyes; only blood samples were obtained and analyzed from all of the members of the two families.

Family RB-1 with hereditary retinoblastoma was investigated by linkage analysis using the BamHI polymorphism. Our prediction is that it is the upper allele (200 bp) which cosegregates with the disease. This test would allow counseling of this family in the future. This family was uninformative for the Xbal polymorphism. The family RB-2 was uninformative for both the Xbal and BamHI polymorphisms. In this family it was not possible to determine whether one of the parents was a true case of incomplete penetrance or whether one or the other was a germline mosaic for the mutation.

This segregation analysis with polymorphic DNA markers is often not feasible. Therefore, direct detection of the germline defect is the method of choice for identifying individuals at risk.

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