Constitutional retinoblastoma gene deletion in Egyptian patients

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Background: **Retinoblastoma is a neuroblastic tumor of childhood with an incidence of 1: 20 000. Retinoblastoma gene (Rb1) is a tumor suppressor gene that is located on the long arm of chromosome 13 at region 14. This study was to evaluate the constitutional monoallelic Rb1 deletion among retinoblastoma families.**

Methods: **Nine families with an affected Rb proband were evaluated. Clinical examination, pedigree analysis, and complete eye examination were given to patients, their sibs and parents. Standard cytogenetic and fluorescence *in situ* hybridization (FISH) analyses were carried out for all of them. Also, two sib fetuses were tested for Rb1 deletion.**

Results: **No dysmorphic features were detected in any patient. Developmental milestones were within normal limit except in one proband who had a mild delay. The age of onset ranged from one month to 4 years. Positive family history was found in two families. In one, the father and 3 sibs had retinoblastoma, and in the other, 2 sibs were affected, but the parents were free. Chromosomal study revealed no abnormalities in all parents and sibs. Two patients had mosaic chromosome 13 abnormalities, 46,XY/46,XY,del(13)(pter→q14:) and 46,XX/46,inv(13)(q14q22). FISH analysis detected mosaic Rb1 deletion in two patients and excluded Rb1 deletion in two fetuses.**

Conclusions: **The detection of genetic alterations affecting the Rb1 locus is important for the establishment of carriers, and prenatal and presymptomatic diagnosis. The search for deleted Rb1 mosaic cell lines is important for genetic counseling. Germline mutation may be considered as genetic transmission method of the Rb1 gene.**

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Introduction

Retinoblastoma (MIM 180200) is the most common primary intraocular malignancy in childhood, with no sex or racial predisposition.[1] It accounts for 5% of blindness in childhood, 3% of childhood cancer, and 1% of all deaths caused by childhood cancer.[2] Patients are usually accidentally discovered during regular pediatric check-ups, as they commonly present with leukocoria.[3,4]

Retinoblastoma (RB) is caused by mutations or deletions of the Rb1 gene, which is located on chromosome 13q14. The gene produces a tumor suppressor protein that normally regulates tightly the cell cycle and growth. Only one functioning Rb1 allele in a retinal cell is necessary to prevent the cell from cancerous changes. Disease occurs from inactivation of both normal Rb1 alleles.[1]

The frequency of constitutional interstitial deletion chromosome 13q14 affecting a locus of Rb1 is generally around 5%-8%.[5] This percentage indicates the limitations of conventional cytogenetics in detecting such minute interstitial deletion. The sensitivity and resolution of fluorescence *in situ* hybridization (FISH) technology have increased the availability of locus-specific Rb1 probes. Implementation of FISH to familial and non-familial RB to detect constitutional Rb1 deletion revealed changes in 12%-17% of the patients.[6]

Forty percent of patients with retinoblastoma have the inherited form, transmitted as pre-existing familial mutations in 10%, or as new-onset germline mutations in 30%. The type of inheritance is autosomal dominant.[5] Individuals with this inherited form are more likely to have multiple, multifocal and bilateral tumors. The rest of the patients (60%) with

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retinoblastoma have the sporadic form, which occurs as result of spontaneous somatic non-hereditary mutations. Such mutations lead to predominant formation of single, unifocal and unilateral tumors.

The present study aimed to investigate Rb1 gene deletion among retinoblastoma families using the FISH technique.

Consents were obtained from all the parents to do the test and in condition of publication to keep the privacy of personal data. This study was approved by the ethics committee of the University.

Methods

Subjects and clinical data

Nine families with a proband suffering from retinoblastoma were examined at Clinical Genetics Clinic, National Research Centre (NRC) and Ophthalmology Department, Kasr El-Aini Hospital, Cairo University. Cytogenetic studies were performed in Human Cytogenetics Department, NRC. Consent forms were obtained from the parents. Family history and pedigree data were documented from the families. Probands were examined clinically and essential body anthropometric measurements were taken. The psychomotor development of the patients was assessed using Vineland social maturity scale and Portage guide.[7,8] Similarly, affected family members were examined carefully and investigated. All patients, their parents and available sibs were given a full ophthalmological evaluation with meticulous fundus examination performed by an expert ophthalmologist.

Cytogenetics and FISH

Standard cytogenetic analysis following GTG banding technique was carried out for the nine patients, their parents and available sibs. Twenty-five metaphases derived from phytohemagglutinin (PHA) stimulated peripheral blood lymphocytes were karyotyped.[9] Nomenclature was according to International System for Human Cytogenetic Nomenclature (ISCN).[10]

Spectrum orange labeled Rb1 locus specific (13q14) probe (Vysis, Abbot Inc., Il) was used for analysis of Rb1 gene deletion. *In situ* hybridization was performed according to Pinkel et al[11] with some modification according to the manufacturer instructions. Scoring of Rb1 signal(s) was done in metaphases and interphases. A minimum of 200 cells were evaluated in each case. We set our cut-off value for FISH at ≥80%.

Results

Clinical data

Nine patients and their family members were studied. They were 6 males and 3 females, aged from one month to 4 years (Table). Parents were consanguineous in three families (33.3%). Positive family history was found in 2 families (22.2%). The patients consisted of 2 familial patients, 3 patients with sporadic (non-familial) bilateral RB and 4 patients with sporadic unilateral RB. The familial patients were patient 3 who had an affected father and 3 dead sibs with retinoblastoma, and patient 7 who had 2 affected sibs while the parents were clinically and ophthalmologically normal (Fig. 1). No dysmorphic features were detected in any patient. The developmental milestones were within normal limit in 8 probands. Patient 1, a male with mosaic deletion, showed a mild developmental delay. Leukocoria was the most presenting sign in 5 patients, followed by squint in 2 patients and nystagmus in 2 patients.

Table. Clinical and cytogenetic data of the patients

Pt. No.	Sex	Age of onset	Family history	Parental cons	Origin	Tumor	Pt. karyotype	Pt. FISH	Father's & mother's karyotype	Karyotype of sib(s)	First presenting sign of Rb
1	M	1 mon	- ve	+	Sohag	Bilateral	46,XY/46,XY, del(13)(pter→q14:)	+ ve del in 50%	Normal	Normal (2 fetuses)	Red eye & leukocoria
2	M	10 mon	- ve	+	Sohag	Bilateral	46,XY	Normal	Normal	-	Squint
3	M	13 mon	+ ve	-	Sohag	Unilateral (familial)	46,XY	Normal	Normal (clinically affected father)	Died (3 affected sibs according to pathological reports)	Leukocoria
4	M	25 mon	- ve	-	Giza	Unilateral	46,XY	Normal	Normal	-	Nystagmus
5	M	4 mon	- ve	-	Monofia	Bilateral	46,XY	Normal	Normal	-	Nystagmus
6	F	3 years	- ve	-	Fayoum	Unilateral	46,XX/46,XX, inv(13)(q14q22)	+ ve del in 40%	Normal	Normal	Leukcoria
7	F	6 mon	+ ve	-	Fayoum	Bilateral (familial)	46,XX	Normal	Normal	3 normal (2 clinically affected)	Leukocoria
8	F	20 mon	- ve	-	Cairo	Unilateral	46,XX	Normal	Normal	-	Squint
9	M	4 years	-ve	+	El Mansoura	Unilateral	46,XX	Normal	Normal	Normal	Leukocoria

cons: consanguinity; del: deletion; F: female; M: male; mon: months; Pt.: patient; Rb: retinoblastoma.

Cytogenetics and FISH

Chromosomal study revealed mosaic chromosome 13 abnormality in 2 patients. Patient 1 (male) had mosaic deletion 46,XY[50%]/46,XY,del(13)(pter→q14:)[50%], (Fig. 2A), and patient 6 (female) had mosaic inversion 46,XX[60%]/46,XX,inv(13)(q14q22)[40%]. Cytogenetic analysis of the parents and available sibs revealed normal karyotypes.

FISH analysis confirmed Rb1 deletion in 50% of cells in patient 1, who had mosaic chromosome 13 deletion [46,XY,ish(13)(q14)(Rb×2)/46,XY,ish del(13) (q14)(Rb-)] (Fig. 2B). Also in this family two subsequent sib fetuses were subjected to prenatal diagnosis and proved that they have no Rb1 deletion (Fig. 2C). FISH for patient 6 with mosaic chromosome 13 inversion showed 40% Rb1 deletion. FISH analysis of the available parents and sibs revealed no abnormality (Fig. 2D, Table).

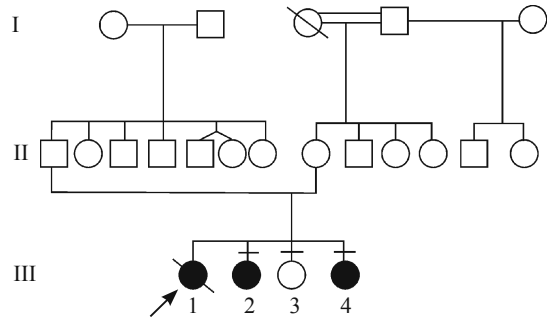

Fig. 1. Pedigree of retinoblastoma family (case 7).

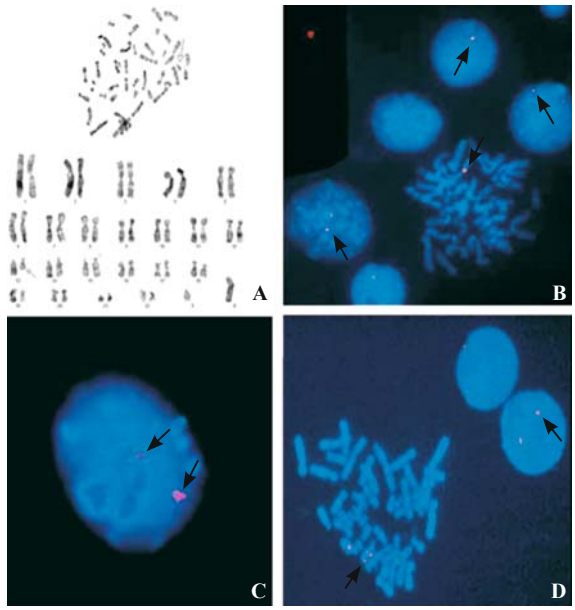

Fig. 2. A: Karyotype of a male patient showing 46,XY,del(13)(pter→q14:); **B**: FISH analysis showing mosaic deletion for Rb1 gene; **C**: FISH analysis of amniotic fluid cells showing normal copy number of Rb1 gene; **D**: FISH analysis showing normal copy number of Rb1 gene.

Discussion

Retinoblastoma is an embryonic malignant neoplasm of retinal origin. It almost always presents in early childhood and is often bilateral. The retinoblastoma gene Rb1 is the first tumor suppressor gene cloned.[12] Retinoblastoma is sporadic in approximately 60% of cases, but two groups of individuals are genetically predisposed to develop the tumor: (1) individuals with an autosomal dominant mutated gene and (2) individuals with partial deletion of the long arm of chromosome 13. Patients with RB and deletion 13q were reported to have systemic abnormalities, especially retardation of physical and mental development.[13,14] Our study revealed 5 probands had normal cytogenetics and FISH results, two probands had affected sibs and normal karyotype, and two probands had mosaic deletions (patient 1 and 6). In the two patients with mosaic cytogenetic abnormalities, physical growth measurements (height, weight, and head circumference) ranged from mean to -2SD. Evaluation of their developmental milestones using portage program revealed a mild delay in the patient 1. Parental consanguinity was present in three families (33%), which is within the population range of Egyptian consanguinity.[15] Leukocoria is an early sign of RB,[4] which was the presenting sign in 5 patients. Although RB cannot be prevented, appropriate screening should be applied to all at-risk individuals to ensure that tumor(s) are diagnosed at an early stage. The earlier the diagnosis, the more likely that an eye can be salvaged and vision maintained.

Standard chromosomal GTG-banding technique has shown that chromosomal deletion in patients with RB is specific to chromosome 13, and that the extent of the deleted 13q segment varied from one patient to the other but band 13q14 appears to be missing in all patients with deleted type RB.[16,17]

FISH analysis confirmed the cytogenetic findings and detected 50% and 40% cell mosaicism, respectively (Table 1). This finding agrees with the published specificity of the FISH technique to detect Rb1 constitutional deletion mosaicism.[6] Hereditary retinoblastoma transmitted by germline mosaicism has also been reported.[18] Therefore, constitutional mosaic cell line Rb1 deletion should be given a serious consideration during genetic counseling.

Family of patient 1 had mosaic deletion 13q14 although the deletion was a post zygotic event. According to the request of the parents, we performed prenatal diagnoses for two subsequent fetuses and they had no deleted Rb, and regular eye examination revealed no abnormality.

Genetic counseling is very important in patients with mosaic deletion as they have the risk of transmitting the disease to their offspring with a risk according to the percentage of mosaic cell line in their gonads. Also the

recurrence risk in future offsprings who have the deletion will be transmitted as autosomal dominant familial type.

Two families had more than one affected family member with normal cytogenetic and FISH results (patient 3 and 7). Patient 3 had three affected dead sibs and affected father. The patient and his affected father had unilateral RB, normal karyotype and normal FISH results. These data support the inheritance of one dominant mutated allele from the father. In the other family (patient 7, Fig. 1), the first affected girl had bilateral RB and normal karyotype. Her parents were clinically, ophthalmologically and cytogenetically free, and two of her three sisters had RB. The sister (III-2) had an early eye examination, and at the age of one month a unilateral RB was discovered and started early treatment for RB. Another sister (III-4) developed RB at the age of 1 year, while the third sister (III-3) is monitored by regular ophthalmo-clinical examination. This highlights the importance of regular ophthalmo-clinical examination in familial cases of RB. The occurrence of three sibs with an autosomal dominant trait who have normal parents can be explained on the basis of either low-penetrant mutation present in one of the parents,[19] or due to germline (gonadal) mosaicism. Sometimes, early in the development, an individual could have a mutation that is limited to the germline (egg or sperm) while sparing the somatic cells. These individuals are phenotypically normal, but have the risk of transmitting the mutated gene to their offspring.[1] Barbosa et al[18] found Rb1 mutation in four affected children descended from three different unaffected fathers and one unaffected mother, indicating that mosaicism is restricted to the maternal germline.

In conclusion, the detection of genetic alterations affecting the Rb1 locus is important for carrier detection, and prenatal and presymptomatic diagnosis. Our results emphasize the usefulness of regular ophthalmo-clinical evaluation combined with cytogenetic and molecular cytogenetic techniques in early diagnosis and detection of constitutional Rb1 deletions. We recommend using the FISH technique to screen a large number of cells to search for mosaic Rb1 deletion. Germline mutation may be considered as a genetic transmission of the Rb1 gene, especially in familial retinoblastoma cases.

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Contributors: Mohamed AM designed, analyzed, interpreted and approved the final version of the article. Kamel AK analyzed and interpreted molecular cytogenetics. Hammad SA analyzed cytogenetic data. Afifi HH performed clinical and psychological evaluation, interpreted and revised the article. El Sanabary Z and Ezz El Din M performed the ophthalmological evaluation.

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