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CLINICAL STUDY

Genetic screening in Iranian patients with retinoblastoma

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Abstract

Purpose **The most common intraocular tumor in childhood, retinoblastoma, is largely associated with mutations in the *RB1* gene. In the most comprehensive RB1 screening in Iran, we evaluated the *RB1* mutations in 106 patients with retinoblastoma, including 73 bilateral (heritable) and 33 unilateral (sporadic) cases.**

Patients and methods **Mutations were identified using amplification refractory mutation system (ARMS) PCR and direct sequencing of the 27 coding exons of *RB1* and multiplex ligation-dependent probe amplification (MLPA).**

Results and Conclusion **We found 33 (31%) and 64 (60%) patients with sporadic unilateral and bilateral retinoblastoma, respectively as well as 9 (8.5%) cases with hereditary bilateral retinoblastoma. In total, we identified 52 causative *RB1* mutations in 106 patients (global mutation rate of 49%). Of the 52 patients, 48 (92%) had sporadic and familial bilateral and 4 (8%) had sporadic unilateral RB. Therefore, the detection rate of *RB1* mutations was 66% (48/73) and 12% (4/33) in bilateral and unilateral cases, respectively. Mutations were classified as nonsense in 31 (60%), missense in 1 (2%), large deletion in 11 (21%), small deletion in the 7 novel (15%) and splice site mutation in 2 (4%) patients with RB. Of 31 nonsense mutations, 23 (74%) occurred in the 11 Arginine codons of the *RB1*. Seven mutations (13%) were novel, and 45 (87%) had been previously reported. Thirty-three mutations were single-base substitutions leading to 31 nonsense amino acid changes and 2 splice site mutations in introns 12 and 16 of *RB1*. The altered 3D model structures of the RB1 novel mutant proteins are also predicted in this study.**

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Introduction

Retinoblastoma (RB, OMIM 180200) is the most common pediatrics intraocular tumor, derived from neural retinal germ cells,[1] and largely caused by *RB1* mutations. The annual incidence and occurrence of retinoblastoma varies from 10–14 per million under 5 years of age[2] to 1 : 15 000–1 : 20 000 live births with no sex predominance. Worldwide, 40% of RB cases are heritable, mostly associated with bilateral disease; the mean age at diagnosis is 18 months.[3] Although mutations in both alleles are necessary for RB, the predisposition to develop the disease in familial cases is inherited in an autosomal dominant pattern.[4] A germline *RB1* mutation predisposes patients (with penetrance $\geq 90\%$) to 'multifocal bilateral heritable RB' within the first two years of life as well as secondary tumors like 'bone and connective tissue tumors' later in adulthood.[5,6] The germline mutation is either inherited *de novo* (acquired via gametogenesis during gestation) or classically inherited from an involved parent.[4,7] Somatic RB accounts for ~60% of cases and does not increase a patient's risk of second cancers. In unilateral cases, biallelic *RB1* mutations in a single retinal cell lead to RB formation.[1,8,9]

Depending on screening techniques and inclusion criteria for patient selection, a wide range of detection rates (5.5–94.8%) has been reported for highly heterogenous mutations in the 27 coding exons and the promoter of *RB1*.[10–17] Though loss of RB1 function is the initiating event, the most common *RB1* alterations leading to RB are nonsense and single-base mutations. Some genetic RB modifiers, (eg, *MDM2*), have been recently found to be regulators of disease severity.[18–20]

In this study, we analyzed the *RB1* open reading frame (27 exons) in an Iranian retinoblastoma cohort to identify the pattern of scattered mutations and to provide efficient genetic counseling. Since alterations in protein structure lead to altered function, we also predicted the 3D structures of novel RB1 mutant proteins. In this study, most known mutations and all the novel ones produced a truncated RB1 protein that could have reduced or absent function. Since the RB1 protein resembles several

proteins with known crystallography structures in the protein database (pdb), we used fold recognition to predict the structure of certain parts of RB1. Although a limited number of retinoblastoma patients and their mutations has been previously reported by this group,[21–23] the current study represents the most comprehensive report of *RB1* mutation in Iranian patients with retinoblastoma.

Materials and methods

Patients

Of 106 unrelated RB cases recruited between November 2009 and August 2013, 74 had bilateral disease. Of these, 72 had no family history and two were familial cases. In addition, 34 patients had unilateral RB without a positive family history. For the 49 girls and 57 boys, the age at diagnosis ranged from 2.5 to 120 months. All patients were examined and treated at the ophthalmology department of the Rassoul Akram University Hospital, IUMS, Tehran. Detailed demographic data including sex, age, laterality, age at diagnosis, presenting signs and familial history of Rb, were collected. Comprehensive fundus examination and fundus photography by Ret Cam (Massie industries, Dublin, CA, USA) was performed under general anesthesia for each patient. Blood samples (5 ml) were obtained in standard EDTA collection tubes from patients and available relatives. The samples were coded and stored at − 20 °C until nucleic acid extraction was performed. In accordance with the Declaration of Helsinki, the study was approved by Ethical Committee of Eye Research Center (IUMS), and a written consent for genetic analysis was obtained from parents or legal guardians.

Molecular analysis

DNA was extracted from peripheral blood using the salting out method.[24] Screening of *RB1* mutations was performed by initial ARMS, multiplex ligation-dependent probe amplification (MLPA) (MRC-Holland, Netherland), followed by PCR-directed sequencing of the 27 coding exons and their flanking intronic regions, using the primers indicated in Table 1.[21,22,25] PCR reactions were performed in a thermal cycler (Eppendorf, Hauppauge, NY, USA), in a total volume of 25 µl containing 100–200 ng of genomic DNA and 5 picomoles of each primer, using Maxime PCR Premix Kit (iNtRON BIOTECHNOLOGY, South Korea). Reactions were performed for 35 cycles of 94 °C for 1 min, annealing at the specific temperature for 1 min, extension time of 72 °C for 1 min, and a final extension step at 68 °C for 7 min. After the unincorporated dNTPs and primers were removed using Exonuclease I, the PCR products were directly sequenced using the ABI BigDye Terminator and run on an ABI 3130 Genetic Analyzer. The sequence data were analyzed by comparison to the consensus sequence of the *RB1* gene (GenBank L11910.1) using Chromas software (South Brisbane, QLD, Australia). Additional information about mutations and variants was obtained from the GenBank database.

Table 1 List and sequences of the primers used for RB1 exons

	Sequence (5->3′)
RB1F	TTTTGTAACGGGAGTCGGG
RB1R	CATTCTGCAGACGCTCCG
RB2F	TGTTATGTGCAAACTATTGAAACAAG
RB2R	AGGTAAATTTCCTCTGGGTAATG
RB3F	TGCCATCAGAAGGATGTGTTAC
RB3R	TGGCAGTTCACTATTTGGTCC
RB4F	TGTAGAGCTGATAATCTTTTGAATTG
RB4R	AATTCCCAGAATCTAATTGTGAAC
RB5F	TTGGGAAAATCTACTTGAACTTTG
RB5R	CACAGGACTTAAATCTATGGGC
RB6F	TCTGGAAAACTTTCTTTCAGTGATAC
RB6R	TGGGGAATTTAGTCCAAAGG
RB7F	TCTACCCTGCGATTTTCTCTC
RB7R	CCACTAGACATTCAATAAGCAACTG
RB8F	CAGAGTAGAAGAGGGATGGC
RB8R	GGGAGAACTTACATCTAAATCTAC
RB9F	TTACCCTGCATTGTTCAAGAGTC
RB9R	CTTGGCTAGATTCTTCTTGGGC
RB10F	ATTGCATGCGAACTCAGTGT
RB10R	TCTACCTATATCAGTATCAACCT
RB11F	ATTTTCAGTATGTGAATGACTTC
RB11R	ATCTGAAACACTATAAAGCCATGA
RB12F	GGAGGCAGTGTATTTGAAGA
RB12R	GGATAACTACATGTTAGATAGGAGA
RB13F	CTGATTACACAGTATCCTCGAC
RB13R	TTATACGAACTGGAAAGATGCTGC
RB14-16F	CCCAGGAGTGTGAAGGCCA
RB14-16R	TCTCCCCCGACCAAAGAAAC
RB17F	TGTTTTCTTTGTCTGATAATAACTTCC
RB17R	TTCCCTATATGTTCTTGAGGTAGATG
RB18F	GCCACTGTCAATTGTGCCTA
RB18R	ATGCAAATCCTAGGTGATTCAG
RB19F	TGTATGTATAATCTGTGATTCTTAGCC
RB19R	CATGATTTGAACCCAGTCAGC
RB21F	TTTGTTCTTTAAACACACTTTGGG
RB21R	CATAATTACCCTTATCTTTCCAATTC
RB22-23F	CTTTATAATATGTGCTTCTTACCAG
RB22-23R	ATTCTTGGATCAAAATAATCCCC
RB24F	TCATCTCTGCAAAATTGTATATGG
RB24R	AGGTGTTTGAATAACTGCATTTGG
RB27F	CAGCCACTTGCCAACTTACC
RB27R	CAGTCACATCTGTGAGAGACAATG

Structure prediction of RB1 mutant proteins

Since all the novel mutations in this study were frameshifts, a combination of fold recognition and hidden markov model based methods was used to predict the 3D structures/models of the native and mutant RB1 proteins. Therefore, phyre2[26,27] a fold recognition algorithm and

SAM-T08[28] an HMM based algorithm were used. During these processes, 3D structures were predicted by the multi-templates method. Finally, a Meta prediction following rigid body assembly and energy minimization was performed using Modeller V 9.12 software,[29] and the minimized structures presented.

Results

The mean follow-up time for the RB patients in this study was 27.6 ± 19.2 months (6–138 months). The mean age at the time of diagnosis was 12.7 ± 10.5 and 22.1 ± 19.6 months for RB with and without a family history, respectively; these ages are not significantly different. The most common presenting sign was leukocoria and strabismus, in 65.9 and 17.7% of cases, respectively (Table 2).

We enrolled 106 patients with retinoblastoma for *RB1* germline mutation detection in this study. Clinically heritable disease occurred in 9 patients with and 64 without a history of bilateral involvement. The other 33 (31.1%) unilateral cases were classified as sporadic. The mean age at diagnosis was 19.2 ± 18.3 months for bilateral patients and 24.0 ± 17.3 months for unilateral cases. In total, we identified 38 different causative *RB1* mutations in 52 of 106 patients (global mutation detection rate of 49.1%; Table 2). The detection rate of *RB1* mutations was 65.8% (48/73) in bilateral/heritable, and 12.1% (4/33) in sporadic unilateral cases. Seven mutations (13.5%) were novel, and 45 (86.5%) had been previously reported (*RB1* gene database). The novel mutations included: c.755delC (p.Thr252AsnfsX16), c.862delG (p.Val288rmfsX1), heterozygous c.1195_1202delAAATCTGA (p.E398D; fsX4), heterozygous c.1535_1536delTT (p.Leu512V;fsX8), c.1703delC (p.Pro568LeufsX42), c.1831delA (p.Arg611AspfsX11), c.1887_1888delGA (p.Glu629AspfsX22). The 52 patients with detected mutations showed 31 single-base substitutions leading to 28 nonsense (eg p.R251X, p.R255X, p.R320X, p.R358X, p.R579X, p.R787X, p.S320X, p.Q344X, p.S443X, p.Q62X), 1 missense (R661W) amino acid change, and 2 splice site mutations (c.1499-2A > C, c.1216+1G > A) in introns 12 and 16. Of 31 point mutations, 23 occurred in the 11 *arginine* codons of *RB1* gene, resulted in 22 stop codons (R → X) and 1 missense mutation (R661W), respectively. The 19 other mutations were small deletions of one or two base pairs causing a frameshift and premature termination (eg p.Arg611AspfsX11, p.Thr252AsnfsX16, p.Pro568LeufsX42) of the open reading frame. All mutations are summarized in Table 3. The analysis of the splice site mutations (c.1499-2A > C;g.70330G > A, c.1216 +1G > A;g.78081A > C) with Human Splicing Finder software revealed the consensus value of the wild-type splice acceptor site was decreased by 96%, suggesting a complete disruption of the normal splicing event. In genetic analysis of bilateral familial RB, three out of four cases (#152, #172, and #182) showed the same causative mutations (c.1831delA, c.C1147T, and c.689C > A, respectively) in their sibs/parents, but no common mutation was found in the fourth one (#187) or any sibs.

Table 2 The frequency of major complaints of RB patients in this study

Symptom/major complaint	*Frequency*	*Percent*
Leukocoria	70	66
Strabismus	18	17
Red eye	7	6.6
Proptosis, cellulitis,..	5	4.7
FH+(EUA)[a]	6	5.7
Total	106	100

[a]Patients with positive family history and diagnosed under anesthesia.

Mutant RB1 proteins models

Nonsense-mediated decay likely has a more significant role in determining the phenotype than mis-folded truncated protein in novel frameshift mutations. However, to compare them together, the 3D structures/models of the native and mutant RB1 proteins were predicted utilizing a combination of phyre2, hidden markov, and SAM-T08 algorithms (Figure 1). Predicted structures were also assessed by Prosa *Z*-score (Table 4) to evaluate the effects of the identified mutations. The more negative the *Z*-scores, the more quality and less error in model prediction. The accuracy of computational results in predicting the protein structure by Prosa *Z*-score, was also evaluated. As the result, an equal quality to NMR and X-ray crystallography models of the wild and dysfunctional mutant forms of the RB1 protein has been detected (Figure 2). The exon 16 mutation, p.L512VfsX8, showed the most similarity to the wild-type RB1 protein (Figures 1d and 2d); the novel mutations in exons 18 and 19 showed much less similarity.

Discussion

This is the most comprehensive report of *RB1* mutations in Iranian patients with retinoblastoma, using ARMS, MLPA, and direct sequencing of 27 exons of *RB1* gene. The patients were referred to the clinic of genetic ophthalmology during a period of 2.5 years. In this study, we found a trend for the bilateral and unilateral retinoblastoma patients. The number of bilateral cases were much more than the unilateral ones (the ratio for bilateral/unilateral cases is more than 2:1). While the frequency of distribution of retinoblastoma might partly contribute to higher bilateral/unilateral ratio, this is mainly because the ophthalmology department in Rasoul Akram

Table 3 Retinoblastoma patients with detected *RB1* mutations

No	*code*	*Protein*	*DNA*	*cDNA*	*Exon/Intron*	*laterality*
Deletions (Large (6 single exon, 5 whole RB1 gene) and 7 small site nucleotide numbers)						
1	11	Exon 2 deletion			2	Bilateral
2	21	Exon 2 deletion			2	Unilateral
3	10	Exon 7 deletion			7	Bilateral
4	194	Exon 17 deletion			17	Bilateral
5	159	Exon 19 deletion			19	Bilateral
6	179	Exon 19 deletion			19	Bilateral
7	3	Whole gene deletion				Bilateral
8	34	Whole gene deletion				Bilateral
9	120	Whole gene deletion				Bilateral
10	139	Whole gene deletion				Bilateral
11	158	Whole gene deletion				Unilateral
12	167	p.Thr252AsnfsX16	g.59687delC	c.755delC	8	Bilateral
13	26	p.Val288rmfsX1	g.61730delG	c.862delG	9	Bilateral
14	45	p.E398D;fsX4 (Heterozygote)	c.1195_1202delAAATCTGA		12	Unilateral
15	49	p.Leu512V;fsX8 (Heterozygote)	c.1535_1536del TT		16	Unilateral
16	174	p.Pro568LeufsX42	g.150005delC	c.1703delC	18	Bilateral
17	152	p.Arg611AspfsX11	g.153224delA	c.1831delA	19	Bilateral
18	33	p.Glu629AspfsX22	g.153280_153281delGA	c.1887_1888delGA	19	Bilateral
(31 Nonsense, 1 missense, 2 Intron) Mutations						
19	153	p.Q62X	g.5470C>T	c.184C>T	2	Bilateral
20	13	p.L158X	g.42018T>A	c.473T>A	4	Bilateral
21	32	p.Q166X	g.42041G>T	c.496G>T	4	Bilateral
22	182	p.S230X	g.56935C>A	c.689C>A	7	Bilateral
23	2	p.R251X	g.59683C>T	c.751C>T	8	Bilateral
24	197	p.R251X	g.59683C>T	c.751C>T	8	Bilateral
25	30	p.R255X	g.59695C>T	c.763C>T	8	Bilateral
26	4	p.R320X	g.64348C>T	c.958C>T	10	Bilateral
27	40	p.R320X	g.64348C>T	c.958C>T	10	Bilateral
28	58	p.R320X	g.64348C>T	c.958C>T	10	Bilateral
29	178	p.R320X	g.64348C>T	c.958C>T	10	Bilateral
30	198	p.Q344X	g.64420C>T	c.1030C>T	10	Bilateral
31	22	p.R358X	g.65386C>T	c.1072C>T	11	Bilateral
32	165	p.R358X	g.65386C>T	c.1072C>T	11	Bilateral
33	172	p.Q383X	g.70261C>T	c.C1147T	12	Bilateral
34	149	IVS12+1G>T	g.70330G>A	c.1216+1G>A	Intron 12	Bilateral
35	121	p.S443X	g.73865C>A	c.1328C>A	13	Bilateral
36	23	p.R445X	g.76430C>T	c.1333C>T	14	Bilateral
37	138	p.R445X	g.76430C>T	c.1333C>T	14	Bilateral
38	189	p.R445X	g.76430C>T	c.1333C>T	14	Bilateral
39	52	p.R455X	g.76460C>T	c.1363C>T	14	Bilateral
40	140	p.R467X	g.76898C>T	c.1399C>T	15	Bilateral
41	17	p.R467X	g.76898C>T	c.1399C>T	15	Bilateral
42	25	p.R467X	g.76898C>T	c.1399C>T	15	Bilateral
43	27	IVS16	g.78081A>C	c.1499-2A>C	Intron 16	Bilateral
44	80	p.R556X	g.78250C>T	c.1666C>T	17	Bilateral
45	193	p.R579X	g.150037C>T	c.1735C>T	18	Bilateral
46	18	p.Q631X	g.153284C>T	c.1891C>T	19	Bilateral
47	15	p.R661W	g.156713C>T	c.1981C>T	20	Bilateral
48	12	p.Y728X	g.160807C>G	c.2184C>G	21	Bilateral
49	6	p.R787X	g.162237C>T	c.2359C>T	23	Bilateral
50	24	p.R787X	g.162237C>T	c.2359C>T	23	Bilateral
51	177	p.R787X	g.162237C>T	c.2359C>T	23	Bilateral
52	184	p.R787X	g.162237C>T	c.2359C>T	23	Bilateral

Hospital has been the primary referral center (among 2 centers) for the treatment and study of retinoblastoma and also the *RB1* genetic study has been offered to patients there as the only and unique center in Iran. Therefore, all ophthalmologists in the country used to refer only their 'bilateral retinoblastoma' to this center.

The screening of 106 patients identified 38 different germline mutations in 52 cases. Our overall mutation

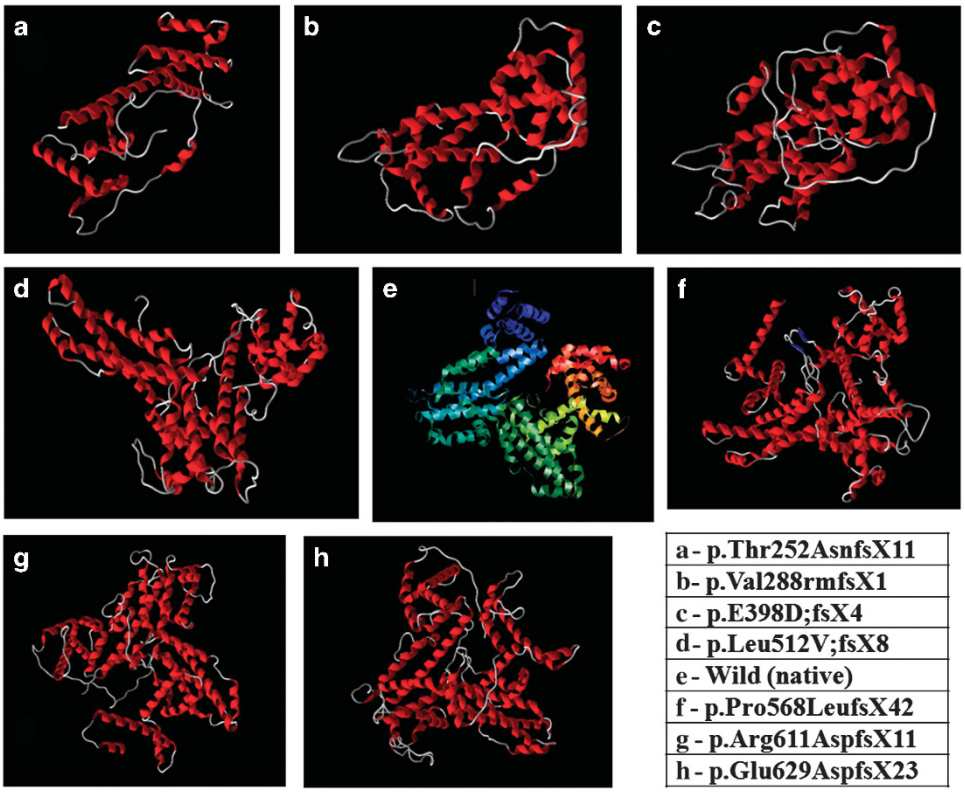

Figure 1 Predicted 3D structure models of the RB1 wild (e) and novel mutant (a, b, c, d, f, g, h) proteins. (a) Truncated protein contains an alpha helix; RB1 dysfunction of this mutant type is due to frameshift and change in amino acids. (b) Truncated mutant; frameshift caused disrupted structure in this protein which is mostly loop. (c) Truncated form contains mostly native structure but the function has been lost upon frameshift. (d) This mutant protein contains mostly native structure but mutation and frameshift made the structure incomplete and caused RB1 dysfunction. (e) Native type. (f) This mutant has high similarity with the native structure but an alpha helix structure was transformed to a beta sheet, which indicates a major and significant RB1 structural variation in comparison with wild type. (g) and (h): Most of the wild-type RB1 protein exists but a frameshift resulted in protein dysfunction at the terminal residues.

Table 4 Novel mutations found in the present study and their Prosa *Z*-scores

No.	*RB1 isoforms/mutants*	*Prosa Z-score*
1	p.Thr252AsnfsX11	−6.37
2	p.Val288rmfsX1	−7.85
3	p.E398D;fsX4	−9.22
4	p.Leu512V;fsX8	−11.34
5	p.Pro568LeufsX42	−9.96
6	p.Arg611AspfsX11	−10.75
7	p.Glu629AspfsX23	−10.69
	Wild RB1 protein (native)	−12.21

detection rate was 49.1%, with 66% in the heritable/bilateral cases and 12% in the sporadic unilateral cases; published studies have reported rates ranging from 19 to 72%.[3,12,14,15,17,25] A recent study by Rushlow *et al* on 1024 patients[17] reported 95% detection. However, only 10–12% of sporadic unilateral cases are caused by germline mutations.[1] This variability in mutation detection rate might be explained by the different techniques employed and sample sizes recruited in these studies. We have already described MLPA and ARMS as rapid techniques for *RB1* mutation detection in a great number of RB cases.[21–23] Limitations of the mutation detection methods likely largely account for the moderate mutation detection rate in this study. Another important reason for the lower rate of detection in this study for the bilateral RB (66%) compared to some other publications is the shortage of budget. The study was mainly supported by ASHK charity rather than the payment by the patients and the health insurance companies. Recruiting more budgets for utilizing some other techniques including FISH, cytogenetic, quantitative multiplex PCR, testing for promoter mutations, and methylation studies, might enhance the power for detecting the gene alteration and/or large rearrangements leading to retinoblastoma. However, the authors may also not rule out the possible more roles of the gene modifiers in retinoblastoma in Iranian population. To increase the sensitivity of *RB1* mutation detection, a combination of two or more different methods has been recommended by The European Molecular Quality Network (EMQN). In turn,

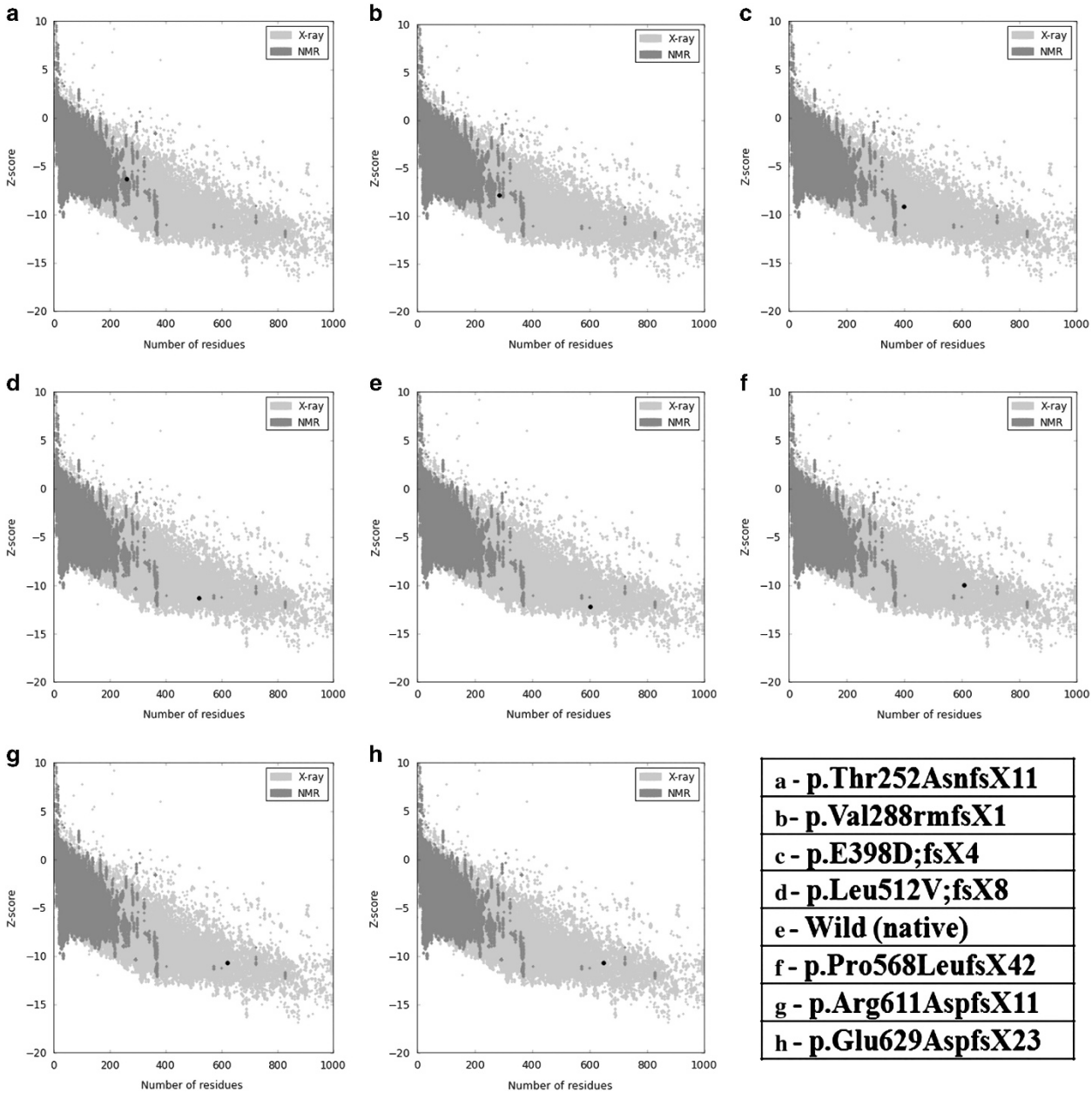

Figure 2 Predicted X-Ray and NMR histograms for the wild-type and novel mutant forms of the RB1 proteins. Dark blue dots indicate the NMR ray quality, while the *x* axis is the number of residues and the *y* axis determines Prosa *Z*-score. a, b, c, d, f, g, and h indicate mutant histograms while e represent the native form of the protein. The mutant protein in d has the greatest similarity to the native (wild) structure. A full-colour version of this figure is available at *EYE Journal* Online.

our results in detection of *RB1* mutation are consistent with previous reports utilizing two different techniques,[12,14,16,17] and are significantly higher than reported results utilizing only PCR sequencing.[30,31] Therefore, mutation detection technologies that are sensitive to short and large rearrangements as well as epigenetic alterations might be employed to maximize the *RB1* detection rate. In our patients with no detected *RB1* mutation, 16 were unilateral sporadic cases, either bearing two somatic mutations or having an *RB1* mutation in a mosaic state. In bilateral cases, the absence of a mutation can be explained either by *RB1* inactivation through mutations in non-coding regions, by an epigenetic mechanism, and the role of some modifiers for RB1. Meanwhile, some cases of bilateral sporadic RB are carriers of somatic mosaicism and therefore not detectable in blood samples,[14,17,25,32] while others may fail to be detected due to technological limitations.

The most prevalent *RB1* mutations reported in our study are nonsense and frameshift mutations; all of the 7

novel mutations we identified were frameshifts. This novel pattern of *RB1* mutation may suggest the independency of the *RB1* mutations causing RB in most cases. Nonsense and frameshift mutations mostly result in multifocal bilateral tumors; however intronic splice mutations showing low expressivity and incomplete penetrance due to minimal RB function [16,33–36] that can be associated with less invasive disease.

The distribution of *RB1* mutations in this study showed no alterations in exons 3 and 22, most mutations were found in exons 10 and 19 followed by exons 8, 14, and 23. Meanwhile, deletions of five exons (8, 10, 14, 19, and 23) (out of the twenty-seven) of *RB1* were observed in 52% of retinoblastoma cases in this study, considering the 5 cases of the whole gene deletion. Considering functional importance of pocket A (exons 12–17) and pocket B (exons 20–23) domains of the RB protein,[37] 27 of 52 patients (52%) had an alteration of the relevant 10 exons. However, *RB1* exon 19 is the most prevalent for the occurrences of both overall and novel mutations, and it may indicate the presence of a hot spot for *RB1* mutations in Iranian patients with retinoblastoma.

In this study, we investigated the effects of the 7 novel *RB1* mutations on the function of the proteins. For p. Thr252AsnfsX11, p.Val288rmfsX1 and p.E398D;fsX4, an immediate stop codon is located just after mutation. This makes a truncated protein with loss of function. For other mutations, the resultant protein is more similar to native form. The 3D structures of RB protein in all these 7 mutant types demonstrated a clear misfolding which is consistent with the pathogenesis of retinoblastoma.

In conclusion, although molecular analysis of the *RB1* gene is technically difficult and its clinical implementation is complex, *RB1* mutation screening allows for clinical prognosis of the disease and earlier management of patients with retinoblastoma. This analysis can also predict risk for families with germline mutations, which in turn may affect their family planning decision, and it may also result in reducing the number of unnecessary examinations under anesthesia for children in succeeding generations.

Summary

What was known before

- This is the most comprehensive report of RB1 screening in Iranian retinoblastoma patients.
- In this study distinctive mutational spectrum and some novel RB1 mutations have been observed, although it is comparable to those reported previously.

What this study adds

- Novel rare variations might be present in Iranian population, thus, RB1 genetic screening is crucial for prenatal diagnosis of retinoblastoma.

Conflict of interest

The authors declare no conflict of interest.

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