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# New Frameshift in PAX6 And Missense Mutation in EYA1 Gene Found by Whole Exome Sequencing Associated with Severe Eye Impairments in an Iranian Family

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## 1. Abstract

**1.1. Introduction:** Previous studies have examined the impacts of PAX6 mutations on a wide range of eye impairments. Because of the PAX6 protein's binding functions, alterations in its structure may prevent it from correctly connecting with the DNA molecule.

**1.2. Materials and Methods:** Whole Exome Sequencing [WES] was used to perform in vitro analysis on a 30-year-old woman who sent to a genetic laboratory for PND. Her spouse had clear serious vision problems. To determine the genotypes, both her spouse and her husband's sister underwent mutation confirmation tests. In silico predictions were also conducted using SWISS-MODEL, ProSA, Molprobity, and SuperPose.

**1.3. Results:** WES analysis revealed a new frameshift of the PAX6 gene [exon5:c.11delG:p.S4fs] and a missense mutation in the EYA1 gene [exon14:c.C1309T:p.R437C] in a 30-year-old woman. Mutation conformations represented the heterozygosity for the PAX6 mutation in both the husband and his sister. Further in silico predictions showed a distinct deleted part of PAX6 resulted from the frameshift mutation compared with the normal allele.

**1.4. Conclusion:** Altogether, for the first time, our report introduced two new mutations in the PAX6 and EYA1 genes associated with severe signs of anterior segmental dysgenesis with cataract and corneal dystrophy in an Iranian family based on an autosomal dominant pattern of genetic inheritance.

## 2. Introduction

PAX6 is a transcriptional regulator with a paired-type DNA-binding domain which identifies target genes. The paired-type domain is formed by two distinct DNA-binding subdomains, the N-terminal subdomain [NTS] and the C-terminal subdomain [CTS], each of which matches a different DNA sequence. The human PAX6 gene produces two alternatively spliced isoforms with the unique structure of the paired domain. An insertion of 14 additional amino acids encoded by exon 5a into the NTS inhibits the NTS's DNA-binding function while masking the CTS's DNA-binding capability. Exon 5a appears to have a molecular switching function which selects target genes [1].

As a member of the EYA family, EYA1 has protein phosphatase functions, and the enzymatic activity of EYA is essential for genes that encode growth controllers and signaling molecules by regulating precursor cell proliferation [2]. A prior report on Drosophila showed that the Eya gene is involved in the development of compound eyes. Flies with Eya loss-of-function mutations have no eyes and produce ectopic eyes in the antennae and the ventral zone of the head on target expression. It has been indicated that a highly conserved homologous gene in various invertebrates and vertebrates has some impacts on eye formation [3].

Based on severe visual problems in a pregnant woman's wife and a similar phenotype in the man's sister, whole exome sequencing was first performed on the man, and a novel frameshift in PAX6 and a new missense in EYA1 genes were found.

## 3. Material and Methods

### 3.1. Case Presentation

A 30-year-old pregnant woman sought genetic counseling for her husband's anterior segment dysgenesis. During genetic counseling, it was determined that the man's sister also suffered from a similar disease; However, the marriage of their parents was not consanguineous, and none of the parents of this sibling showed any signs of this disease. So in the first step, the geneticist requested a WES test for the woman's husband, and after reviewing the results, he found two mutations in the two genes. Among these mutations were a heterozygous frameshift in the PAX6 gene and a missense mutation in the EYA1 gene. For additional examination, confirmation of mutations in loci discovered for both genes in her husband, husband's sister, and fetus was performed. The results indicated that, like the patient's spouse, the husband's sister and the fetus were heterozygotes, but the case possesses a normal allele in these positions. An abortion order was issued as a consequence of the studies and autosomal inheritance of the PAX6 mutation in Anterior segment dysgenesis, as well as the detection of the mutation in the fetus.

### 3.2. In vitro Diagnosis

WES used to identify possibly harmful mutations in the woman's husband: A filter-based method utilized to extract and purify genomic deoxyribonucleic acid [gDNA] from a case blood sample, which subsequently examined. During the DNA preparation process, 1.0g of gDNA used. The Agilent SureSelect Human All ExonV7 Kit [Agilent Technologies, CA, USA] used to create sequencing databases, and x-index codes applied to the sample attribute sequences. Using a hydrodynamic shear device, DNA fragmented into 180-280 bp fragments [Covaris, Massachusetts,

USA]. Exonuclease/polymerase activities blunted the remaining overhangs, and enzymes were eliminated in the nest. After adenylation of the 3' ends of the DNA fragments, adapter oligonucleotides ligated; DNA fragments with adaptor molecules attached at both ends preferentially selected in a PCR reaction. Captured libraries enriched in a PCR reaction to add index tags for hybridization preparation. The products were purified by the AMPure XP system [Beckman Coulter, Beverly, USA] and quantified through the Agilent High Sensitivity DNA Assay on the Agilent Bioanalyzer 2100 System. Qualified libraries fed into the NovaSeq 6000 Illumina sequencer. Quality control of data, analysis, and interpretation was then carried out on Generation G9 from the HP server by a Unix-based operating system.

### 3.3. In Silico Analysis

In silico investigations were carried out to have a better understanding of the frameshift impacts on the normal structure. Hence, in the first phase, the 11th nucleotide [G] was deleted from the amino acid length, and a fresh translation using ExPasy TRANSLATE revealed multiple stop and start codons with Methionine. After that, homology modeling for PAX6 protein structures in wild-type and frameshift [mutant] models was done using the PDB ID 6PAX structure. SWISS-MODEL was used to model the Paired box protein Pax-6 [422 amino acids] [UniProt ID: P26367], and it was also evaluated using Molprobity and ProSA online tools [available at https://prosa.services.came.sbg.ac.at/prosa.php and https://prosa.services.came.sbg.ac.at/prosa.php]. Surprisingly, the superimposition was accomplished using SuperPose version 1.0 [http://superpose.wishartlab.com/].

## 4. Results

### 4.1. Molecular Reports

The results of the WES test for the case revealed a new deletion in exon 10th of the PAX6 gene [c.11delG:p.S4fs] associated with Anterior segmental dysgenesis with cataract and corneal dystrophy [OMIM: 106210] and a new missense in 14th exon of EYA1 gene [c.C1309T:p.R437C]. Screening of PAX6 by WES represented heterozygosity for the position [G/deletion], and further mutation confirmation by Sanger sequencing indicated genotype G/deletion for his sister and unfortunately, for the fetus (Figure 1). The father and his sister were both heterozygotes for the EY1 missense mutation, but the fetus was a normal homozygote. As a result, the disruptive impacts of PAX6 deletion are responsible for the lack of suitable eye formation. Overall, previous professional genetic counseling is strongly advised for the interpretation of test findings or the choice to conduct genetic testing in the case of prenatal diagnosis for future pregnancies such as this one.
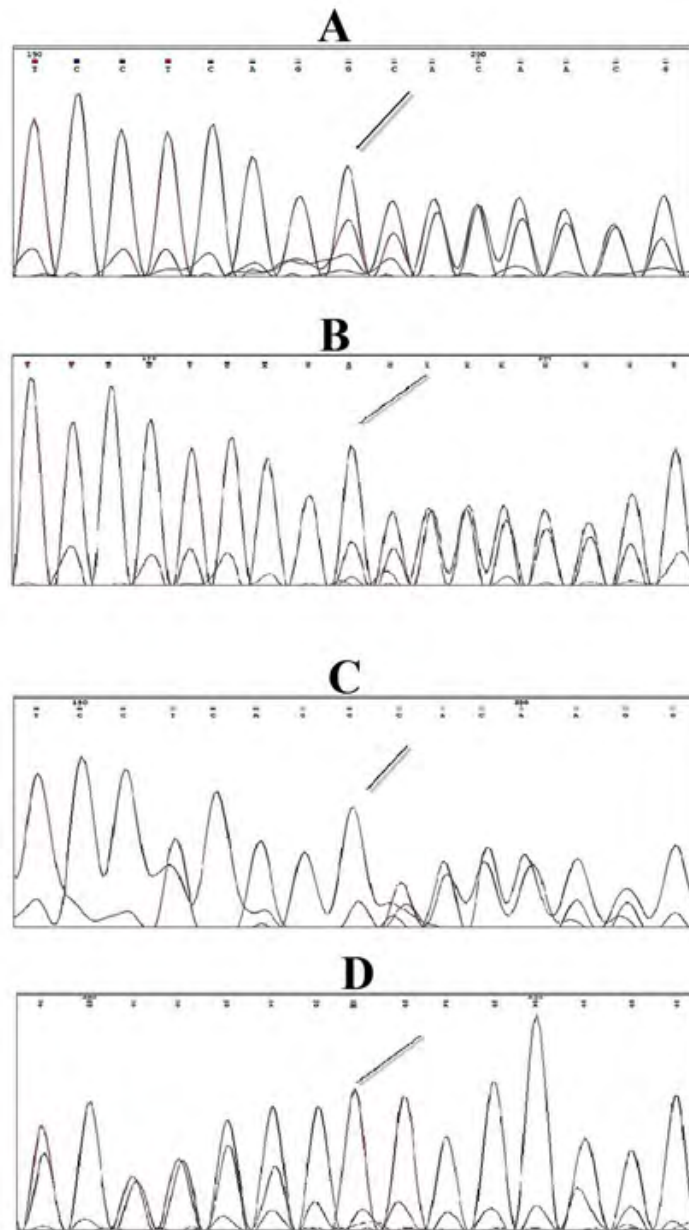
**Figure 1:** Mutation confirmation by Sanger sequencing including the genotype of case's husband (A), his sister (B), and forward and reverse sequencing of aborted fetus (C and D, respectively).

### 4.2. In Silico Results

The mutant structure was obtained from the largest possible frame from ExPasy Tranlate result and modeled by SWISS-MODEL. Ramachandran favored rotamers for the final energy minimized structure was 96.83 percent. Also, further validations by ProSA indicated z-scores of -4.04. SuperPose showed a significant truncated part which binds with the DNA in the wild-type model (Figure 2).
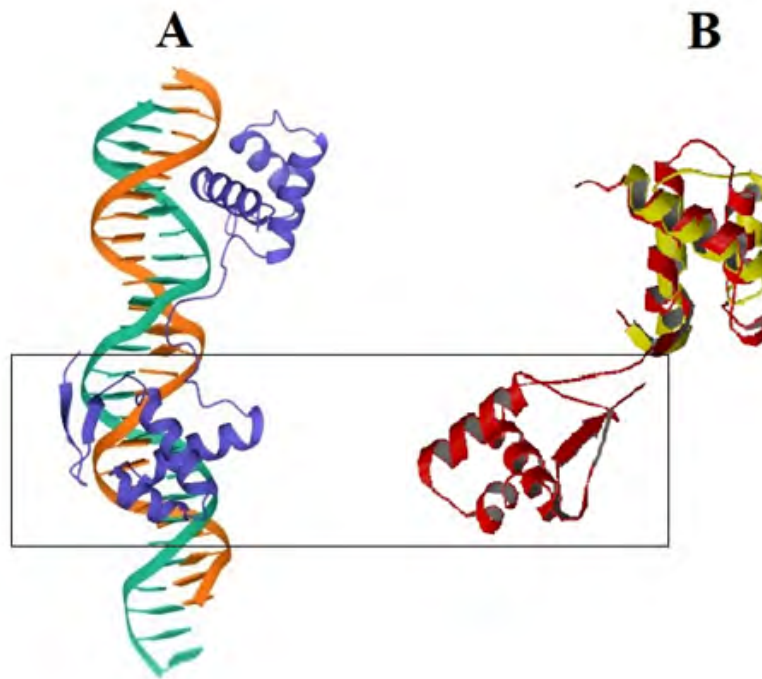
**Figure 2:** Modeling analysis of PAX6 in the X-rayed crystallographic image (A; PDB ID: 6PAX) compared with superimposed result of wild-type and new deletion resulting the truncated PAX6 (B).

## 5. Discussion

In the current case report, we reported a new mutation in the fifth exon of the PX6 gene [Ser4fs] in an aborted fetus, her father, and aunt with severe eye impairments using the WES test, Sanger sequencing confirmation, and in silico predictions. Because of the fetus's father's different phenotype of disease, mutation conformations were exposed to the mutation in both the fetus's father and aunt. This mutation is inherited in an autosomal dominant pattern.

There is not enough study showing the association of PAX6 mutations with eye impairments. However, Hanson studied the mutational spectrum of the human PAX6 gene and stated that 71% of the mutations might be in a premature termination codon [37% nonsense, 23% frameshift, and 11% splice site], while 4% were anti-termination mutations. Insertions or deletions included 7% of PAX6 mutations. Missense mutations accounted for 18% of PAX6 mutations and half of them showed associations with aniridia and resulted in significant loss of functions. Since missense mutations cause a partial loss or gain of function, the author hypothesized that this might explain why other missense mutations have been connected to other eye phenotypes such as ectopia pupillae, isolated foveal hypoplasia, and Peters anomaly [4]. Functional studies on eight missense and two nonsense disease-causing mutations in PAX6 and its exon 5a variant revealed unanticipated pleiotropic effects on gene regulation, which crystallography of PAX6 bound with DNA did not reveal. The position of the mutation would determine the transactivation of PAX6 and 5a isoforms, the type of

DNA-binding site, and the cellular environment [5]. According to Chauhan et al., activation of PAX6 and the 5a isoform, as well as minor symptoms associated with PAX6 missense mutations, may result from unnatural protein function in a limited number of ocular cell types [6]. Azuma et al. discovered the first splice variant mutation, a heterozygous mutation [Val54Asp] in exon 5a. Val-54Asp was found in four families suffering from Peters anomaly, Axenfeld anomaly, congenital cataract, and/or foveal hypoplasia. According to the functional experiments, the Val54Asp mutation increased NTS binding somewhat but reduced CTS transactivation activity by almost half. All four pedigrees originated in Japan and were located in or around Tokyo. One of the four patients signified a sporadic case, meanwhile, neither of her parents had the mutation [1]. Dominguez et al. revealed that Notch signaling did not increase development in Drosophila eyes by the two PAX6 transcription factors 'eyeless' [ey] or 'twin of eyeless' [twin of eyeless] [toy]. It instead functions via 'eyegone' [eyg], which has a truncated paired domain that only includes the C-terminal sub-region. The single PAX6 gene in humans and mice creates the exon 5a isoform by alternative splicing; like eyegone, this isoform interacts with DNA via the C-terminal of the paired domain. Overexpression of the human PAX6 exon 5a isoform causes strong overgrowth in vivo, but the canonical PAX6 variant does not affect growth. These findings highlighted the significance of growth and eye development in independent modulation and presented a new look at hyperplasia [7]. Overexpression of the exon 5a PAX6 isoform in embryonic chick eye can promote ectopic differentiation

of retina-like structure, according to Azuma et al. These ectopic retina-like structures could not be induced by 5a PAX6 isoform point mutations in individuals with foveal hypoplasia. According to the researchers, PAX6 [+5a] can likely initiate a developmental cascade in the prospective fovea, area centralis, or visual streak region, resulting in the formation of a retinal design with completely packed optical cells [8].

In conclusion, PAX6 mutations, notably this new frameshift mutation [exon5:c.11delG:p.S4fs] with an autosomal dominant genetic inheritance pattern, may have effects on eye development. The current study's in silico predictions can strongly define the cause of severe phenotypes of eye impairments in patients with this mutation if more molecular confirmations are obtained in future investigations.

## 6. Acknowledgement

## 7. Conflict of Interest

The authors have no conflict of interest to disclose.

### References

1. Azuma N, Yamaguchi Y, Handa H, Hayakawa M, Kanai A, Yamada M, et al. Missense mutation in the alternative splice region of the PAX6 gene in eye anomalies. The American Journal of Human Genetics. 1999; 65(3): 656-63.

2. Li X, Oghi KA, Zhang J, Krones A, Bush KT, Glass CK, et al. Eya protein phosphatase activity regulates Six1-Dach-Eya transcriptional effects in mammalian organogenesis Nature. 2003; 426: 247-54.

3. Azuma N, Hirakiyama A, Inoue T, Asaka A, Yamada M. Mutations of a human homologue of the Drosophila eyes absent gene (EYA1) detected in patients with congenital cataracts and ocular anterior segment anomalies. Human molecular genetics. 2000; 9(3): 363-6.

4. Hanson IM. PAX6 and congenital eye malformations. Pediatric research. 2003; 54(6): 791-6.

5. Chauhan BK, Yang Y, Cveklová K, Cvekl A. Functional properties of natural human PAX6 and PAX6 (5a) mutants. Investigative ophthalmology & visual science. 2004; 45(2): 385-92.

6. Dominguez M, Ferres-Marco D, Gutierrez-Aviño FJ, Speicher SA, Beneyto M. Growth and specification of the eye are controlled independently by Eyegone and Eyeless in Drosophila melanogaster. Nature genetics. 2004; 36(1): 31-9.

7. Azuma N, Tadokoro K, Asaka A, Yamada M, Yamaguchi Y, Handa H, et al. The Pax6 isoform bearing an alternative spliced exon promotes the development of the neural retinal structure. Human molecular genetics. 2005; 14(6): 735-45.