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Assessment of PAX6 alleles in 66 families with aniridia

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Abstract

We report on *PAX6* alleles associated with a clinical diagnosis of classical aniridia in 81 affected individuals representing 66 families. Allelic variants expected to affect *PAX6* function were identified in 61 families (76 individuals). Ten cases of sporadic aniridia (10 families) had complete (8 cases) or partial (2 cases) deletion of the *PAX6* gene. Sequence changes that introduced a premature termination codon into the open reading frame of *PAX6* occurred in 47 families (62 individuals). Three individuals with sporadic aniridia (3 families) had sequence changes (1 deletion, 2 run-on mutations) expected to result in a C-terminal extension. An intronic deletion of unknown functional significance was detected in one case of sporadic aniridia (1 family), but not in unaffected relatives. Within these 61 families, single nucleotide substitutions accounted for 30/61 (49%), indels for 23/61 (38%), and complete deletion of the *PAX6* locus for 8/61 (13%). In five cases of sporadic aniridia (5 families), no disease-causing mutation in the coding region was detected. In total, 27 unique variants were identified that have not been reported in the LOVD database. Within the group assessed, 92% had sequence changes expected to reduce *PAX6* function, confirming the primacy of *PAX6* haploinsufficiency as causal for aniridia.

Keywords

Absent Iris; Eye Abnormalities; Eye Disease; Irideremia; chromosome 11p13

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INTRODUCTION

Aniridia is a congenital, progressive disorder for which the majority of cases are caused by heterozygous loss-of-function mutations of the *PAX6* gene (1, 2). Aniridia occurs in approximately 1/64,000 to 1/96,000 live births and is primarily characterized by iris hypoplasia that is clinically detectable at birth (2, 3). Associated foveal hypoplasia, indicated by early infancy nystagmus, causes reduced visual acuity. The progressive nature of the disease frequently leads to multiple ocular abnormalities such as keratopathy, corneal vascularization and opacification, glaucoma, anterior chamber fibrosis, and cataracts (2, 4–7). Along with distinct ocular characteristics, the condition is associated with a number of other irregularities spanning sensory, neural, cognitive (8) and pancreatic phenotypes (9, 10). These include auditory processing deficits (11, 12), deficient pituitary function (13) and olfactory dysfunction (14). Previous studies have identified a correlation between *PAX6*-mediated aniridia and a number of other disorders, including diabetes (15) and autism spectrum disorders (16). Additionally, structural abnormalities in major fiber tracts and subcortical structures in the brain including the corpus callosum, anterior and posterior commissures, pineal gland and probst bundles have been observed (14, 17). A functional understanding of the *PAX6* gene in context of the manifestation of aniridia-related clinical traits continues to be the focus of much research.

Functional mutations that occur only in the *PAX6* gene or associated regulatory regions give rise to aniridia, which can be sporadic or familial. WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies and mental retardation) is associated with large heterozygous genomic deletions at the 11p13 chromosome that include deletion of the *PAX6* locus and the *WT1* locus (18, 19). In addition to clinical symptomologies associated with aniridia, patients with WAGR syndrome present with Wilms tumor and, depending on the size of the causal chromosomal deletion, often exhibit cognitive delays and disabilities in addition to developmental genitourinary defects (19). Aniridia is not comorbid with WAGR syndrome; however, it can be further classified at the cellular and molecular level by the specific mutation affecting the *PAX6* gene.

The *PAX6* gene encodes a transcription factor critical for normal ocular and neural development. The gene is highly conserved and is expressed in the developing eye, brain, spinal cord and pancreas (20). *PAX6* is required for various aspects of anatomical and functional development. The mechanistic role of *PAX6* in eye development has been investigated and its involvement has been demonstrated in initial lens development (21), cell differentiation (22–24), and cell proliferation and adhesion/migration (25). *PAX6* has also been identified as having critical maintenance functions in corneal homeostasis (26, 27). Additionally, in the central nervous system *PAX6* is thought to be involved in brain patterning and regionalization and the formation of neural circuits, particularly in the forebrain (25, 28–30). In these ways, *PAX6* is an integral upstream regulator involved in numerous developmental gene networks. It follows that the broad developmental and progressive phenotypic outcomes caused by *PAX6* mutations are a consequence of the extensive roles of the gene and its products.

The PAX6 protein is composed of four functional domains: a paired domain and a homeodomain (involved in DNA binding) joined by a linker domain, and a proline-serine-threonine (PST) rich transactivation domain (31). Should products of mutant alleles undergo translation, disruptions in the coding sequence of different functional domains will alter the efficacy of PAX6 as a transcriptional regulator, which has been shown in molecular and phenotypic studies of missense and run-on *PAX6* mutations in aniridia and other related disorders (32–39). However, the majority of *PAX6*-mediated aniridia cases are caused by mutations that give rise to premature termination codons (PTCs) leading to haploinsufficient levels of wild-type PAX6 protein.

Prior to the current study, the Human *PAX6* Allelic Variant Database (LOVD database) (http://lsdb.hgu.mrc.ac.uk/home.php?select_db=PAX6) has identified 361 unique variants of *PAX6* (833 total reported). The current study sought to further examine and classify mutations of *PAX6* in patients diagnosed with classical aniridia, as well as examine our data in the context of previously identified mutations of the gene. This line of investigation provides insights towards identifying locations on the *PAX6* gene that are more susceptible to mutations, and seeks to explore individual-specific mutation characteristics which could benefit from new therapeutics within the aniridia population.

MATERIALS & METHODS

Participants

DNA samples were collected from 81 individuals with a clinical diagnosis of aniridia and 77 unaffected genetic relatives from 66 families (Supplementary Table 1). Participants were recruited through the Aniridia Foundation International Conferences in 2007, 2009, and 2011 through affiliation and direct physician referral for participation in the study. The study protocol was in keeping with the tenets of the Declaration of Helsinki and was reviewed, approved, and overseen by the Institutional Review Boards (IRBs) at the University of Tennessee Health Science Center and the University of Georgia. All samples were collected after written informed consent had been obtained from each participant.

DNA Preparation

Genomic DNA from individuals with aniridia and their relatives was prepared from peripheral venous whole blood. Briefly, 10mL volume blood samples were collected from each adult participant, and 2mL blood samples were collected from each infant. Buffy coat preparation was performed on each sample and genomic DNA (gDNA) prepared by use of a Genra Puregene Blood Kit (Qiagen). Buccal cell lysates were prepared from saliva or swab samples (Oragene-DNA OG-575, DNA Genotek, Inc; buccal swabs and QuickExtract DNA extraction solution, Epicentre). All gDNA samples were stored in 10 mM Tris-HCl (pH 7.5), 1 mM EDTA buffer at -20°C .

Molecular Analysis

The *PAX6* gene (11p13) [OMIM: 607108] is composed of sixteen exons (Supplementary Table 2) (20), of which eleven (4 to 13 and 5a) contribute to the protein-coding regions of the mRNA transcripts. The other exons (0, 1, 2, 3, and *alpha*) contribute to the non-coding

regions of the mRNA transcripts. For this study, *PAX6* exons 1–13 and 5a were individually amplified in a polymerase chain reaction (PCR) with primers located in the introns as previously described (40, 41). Mutation screening was performed on the PCR products by direct bi-directional sequencing using either the same PCR primers or more internal primers (40, 41). Bi-directional sequence was analyzed and compared to *PAX6* reference sequences. Reference sequences for *PAX6* cDNA and protein are contained in GenBank entry NM_000280 (<http://www.ncbi.nlm.nih.gov/Genbank>; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD). The February 2009 human reference sequence GRCh37 produced by the Genome Reference Consortium (<http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/>) was used as the *PAX6* genomic reference after first validating against genomic sequences obtained from cosmids FAT5 and A1280 (Genbank accession numbers Z95332 and Z83307, respectively), which together encompass all *PAX6* exons. Supplementary Table 2 lists intron-exon boundary sequences for human *PAX6* and all exon sizes. Potential mutations were identified as differences relative to the appropriate reference sequence. Results were confirmed by repeat PCR, which in most cases was performed using an independent, second gDNA sample prepared from either blood or buccal cells. Changes from the reference sequence were identified, classified and are documented in Table 1 using the nomenclature system recommended by the Human Genome Variation Society (42), with the first base of the ATG initiation codon in the *PAX6* cDNA reference sequence NM_000280 denoted as nucleotide 1.

Deletion/duplication testing was performed either by array-comparative genomic hybridization (CGH) analysis (43) (GeneDx or Emory Genetics Laboratory), fluorescence in situ hybridization (44), or physical mapping (45).

Individuals for which no *PAX6* pathogenic mutations were detected were retested using fresh samples by GeneDx or the Denver Genetic Laboratories (University of Colorado Anschutz Medical Campus).

RESULTS

A total of 158 individuals (81 affected, 77 unaffected genetic relatives) from 66 families were evaluated in this study (Supplementary Table 1). All affected individuals had a clinical diagnosis of aniridia (Figure 1, data not shown). In the context of this study, “family” was used to group genetically related individuals, and includes instances of sporadic aniridia (a single individual within the family) or familial aniridia (multiple individuals). Of the eighty-one affected individuals, 53 were female and 28 were male. Fifty-eight cases were sporadic (44 female, 14 male), with no affected relatives at the time of birth. Of these, five females subsequently had children (2 female, 4 male) with aniridia. Fifteen additional cases (6 female, 9 male) were familial from 4 separate families. The parents for two cases (1 female, 1 male) were unknown, and so it is not possible to determine if these two individuals were sporadic or not.

The *PAX6* gene was characterized for all individuals. Sequence changes relative to the appropriate *PAX6* reference sequence (see Methods) were identified and classified (Table 1, Supplementary Table 1). Potential aniridia-causing changes were identified as differences

relative to the reference sequence and sequences from unaffected genetically related family members. Sequence variants were identified in individuals with aniridia in 64 of the 66 families (79 out of 81 individuals). One of these variants (c.766-12C>T) was detected in both affected and unaffected individuals in three families (3 individuals with sporadic aniridia) and is therefore likely to be a benign sequence change (Table 1C), as has been previously suggested (46–48). No sequence changes were detected in unaffected relatives in the remaining 74 families. Thus aniridia-specific alterations in the *PAX6* gene were identified in 61 families (76 individuals).

Sequence alterations predicted to cause loss-of-function of one copy of the *PAX6* gene were identified in 57 families (72 individuals). Whole gene deletions were detected in 8 cases of sporadic aniridia (8 families); seven of these deletions also included the *WT1* gene. A partial deletion of *PAX6* was detected in 2 cases of sporadic aniridia (2 families). In one case, the region extending from the P1 promoter to exon 4 was deleted, and in the other, a region encompassing exons 6 and 7 was deleted. Nonsense mutations were identified in 18 families (24 individuals), and frameshifting deletions or insertions were identified in 16 families (23 individuals). Changes expected to disrupt normal splicing were identified in 13 families (15 individuals). Of these, 9 were changes to the dinucleotides in the 5' and 3' splice sites. Also included were a C to T transition at the -3 position in the 5' flanking sequence of exon 10 (c.766-3C>T, family 173) and a T to G transversion at the +6 position in the 3' flanking sequence of exon 11 (c.1032+6T>G, family 130). These two variants were included because changes in these positions are known to affect splicing in other human genes (49) and there is a high degree of conservation in the sequences immediately flanking *PAX6* exons between humans, dogs, mice, chickens, *Xenopus tropicalis*, and zebrafish (data not shown). In families 117, 143, 163, and 169, where deletions included both exonic and intronic sequences, the deletion was scored as “frameshifting” if the deletion originated within the exon and included the 3' flanking sequence (families 143 and 163) or “splice junction” if the deletion originated in the intron and included the 5' end of the subsequent exon (families 117 and 169).

Mutations predicted to result in a *PAX6* protein with a C-terminal extension were identified in three cases of sporadic aniridia. Two cases had an A to T transversion (c.1268A>T) that converts the stop codon (TAA) to a leucine codon (TTA). This mutation is predicted to result in the addition of 14 residues to the C-terminal portion of the *PAX6* protein.

One case had a single nucleotide deletion (c.1256delC) that is predicted to result in a frameshift at amino acid 419 and the addition of 105 residues to the C-terminal portion of the *PAX6* protein. One case of sporadic aniridia (family 128) had a heterozygous deletion of the 18th – 20th nucleotides in the intronic sequence just 3' to exon 5 (c.141+18_+20delGCC). This deletion was not present in unaffected family members and is, therefore, likely to be causal for aniridia in this individual. Although the molecular effect of this change is not known, its position within the intron may affect splicing or alter a regulatory element.

The exon-by-exon distribution of single nucleotide substitutions and small indels (< 150 bp) associated with aniridia that were found in 51 families is shown in Figure 2. Mutations were

detected in all exons except for 4, 5a and 9. Twenty-one variants (41%) occurred within the paired domain, 11 variants (22%) occurred within the homeodomain, 8 (16%) within the linker domain, and 11 variants (22%) occurred within the C-terminal transactivation domain (Figure 3). In total, 27 unique variants were identified which have not been previously reported in the LOVD database (Table 1: variants in bold).

DISCUSSION

In the group of individuals with a diagnosis of classical aniridia analyzed here, aniridia-associated alterations in the *PAX6* gene were identified in 61 out of 66 families (76 out of 81 individuals). These changes included complete (8 instances) or partial deletion (2 instances) of the *PAX6* gene, introduction of a premature termination codon into the open reading frame of *PAX6* (47 instances), and likely addition of residues to the C-terminal end of the PAX6 protein (3 instances). These changes are all predicted to reduce PAX6 function. Additionally, an intronic deletion of unknown functional significance was detected in one case of sporadic aniridia (1 family), but not in unaffected relatives. Overall, the distribution of the variants identified in the current study demonstrates similar characteristics when compared with the LOVD database (Figure 2a, 2b), except that the comparative frequency of frame-shifting insertions or deletions is higher in this study. These results confirm the primacy of *PAX6* haploinsufficiency as causal for aniridia.

No pathological sequence changes were observed in 5 families with aniridia. At this time, these individuals appear clinically indistinguishable from others with aniridia who have known *PAX6* mutation (Figure 1, data not shown). Three of these individuals are children and it is possible that clinical differences will emerge as they get older. These persons could harbor mutations in other genes involved in anterior eye development, such as *FOXC1* or *PITX3*, among others, or they may have mutations in *PAX6* regulatory elements. We are currently exploring these possibilities.

The major mutational mechanisms underlying genetic variation are single nucleotide substitutions (50–52), small DNA insertions and deletions (indels) ranging from 1 to 10,000 bp in length (53–56), and large scale (>10 kb) genome rearrangements (57–63). For the 61 families with aniridia-specific alterations in the *PAX6* gene, single nucleotide substitutions accounted for 30/61 (49%), indels accounted for 23/61 (38%), and gross deletion of the *PAX6* locus accounted for 8/61 (13%) of instances. These results demonstrate that single nucleotide substitutions and indels in *PAX6* are a significant source of aniridia-causal mutations. Of the single nucleotide substitutions, 13/30 (43%) occurred at CpG dinucleotides, a structure known for its high mutability in the human genome (64, 65) and to be associated with significant mutation hotspots in *PAX6* (66, 67). Consistent with previous reports (66, 67), nonsense mutations in exons 8, 10 and 11 occurred at CpG locations that created arginine CGA codons Arg203 (c.607C>T, 2 instances), Arg261 (c.781C>T, 4 instances) and Arg317 (c.949C>T, 3 instances), respectively. Additionally, a CpG located at the 3' exon/intron junction of exon 6 was mutated in four families (c.357+1G>A, 3 instances; c.357+1G>T, 1 instance). The frequency of indels is notable as it is about twofold more than has been reported for the genome at large (56). Since indels result from nonhomologous end joining as a result of DNA break repair, the high frequency of indels in

PAX6 suggests that the 11p13 region of human chromosome 11 may be unstable relative to other regions of the genome. Alternatively, the frequency of indels in the human genome may currently be underreported due to technical issues with detecting these types of changes throughout the genome (68). Indels have been reported to occur preferentially in the male germline (69), and may be useful in tracking inheritance.

Analysis of the different types of mutations found in this study, in conjunction with those previously reported in the LOVD database can help to differentiate possible functional implications of the nature of mutations, in the context of phenotype analysis as well as potential genetic treatments. This requires consideration of individual mutations in the context of molecular and cellular mechanisms that act on mutant gene products. Mutations that lead to a PTC give rise to mRNA transcripts, which are putative targets of nonsense-mediated mRNA decay (NMD). NMD is an RNA-surveillance pathway which recognizes and degrades transcripts with PTCs upstream of the last exon-junction complex (EJC) during the primary round of translation (70). This mechanism serves to prevent the translation of truncated proteins with possible dominant negative effects, and is expected to target all transcripts with a PTC upstream of the last EJC regardless of the nature of the causal mutation. In patients with mutations fitting these criteria, degradation of transcripts from the mutant *PAX6* allele should result in *PAX6* haploinsufficiency. However, *PAX6* transcripts harboring mutations upstream of the coding region of *PAX6*, those causal for PTCs near or downstream of the last EJC, or missense mutations would likely evade NMD and could produce dysregulated or truncated dominant negative *PAX6* protein products from the mutant allele. Three cases in the current study previously mentioned as causal for C-terminus extensions of the open reading frame would fall into this category, leaving the vast majority of cases subject to this RNA surveillance pathway and ultimately leading to *PAX6* haploinsufficiency. Dominant negative *PAX6* proteins could interfere with proteins produced from the wild-type allele, especially if the DNA binding domain structures remain intact. Future studies will seek to compare phenotypic differences at the levels of both cellular/molecular differences and clinical presentation between patients with and without mutations identified as putative NMD targets.

In addition to contributing to our understanding of *PAX6* allelic differences in the context and implications of haploinsufficiency, knowledge about the different disease-causing mutations is necessary for the development of potential therapeutic approaches to treat individuals with aniridia. Approaches that could be used include: 1) those that are cell-based coupled with genome editing or manipulation; 2) those designed to increase *PAX6* expression from the normal allele, through the use of microRNAs for example; and 3) those targeted towards generating functional *PAX6* protein from the mutant transcripts.

Recently, a novel therapeutic known as ataluren (formerly PTC124) has been identified as a treatment for genetically-mediated diseases which are caused by mutations leading to a PTC (71). With successful clinical applications for other diseases (72–75) as well as evidence of the efficacy of ataluren in mouse models of aniridia (76), data from the current study could serve to identify potential candidates for this treatment based on specific genetic mutations of *PAX6*. Though ataluren has been demonstrated to effectively promote read-through of all PTCs, it shows the most significant increase in read-through when the PTC consists of the

UGA nucleotide sequence (followed in significance by UAG and then UAA) (71). The efficacy of ataluren on promoting read-through of PTCs (via START drug formulation) specifically in the *PAX6* gene has recently been demonstrated in a mouse model of aniridia possessing a nonsense mutation which induces a UGA in-frame premature stop codon (76). Further, administration of this drug to mice with a mutant *PAX6* allele provides demonstrable evidence for partial rescue of ocular phenotypes associated with aniridia, as a product of both systemic and topical postnatal application (76). These results are promising for the treatment of human aniridia with START therapy in patients with nonsense mutations, especially those consisting of a UGA in-frame premature stop codon. The current study has identified 14 families (20 individuals) with an UGA in-frame premature stop codon who may be good candidates for this therapy, followed by 3 families (3 individuals) with an UAG in-frame premature stop and 1 family (1 individual) with an UAA in-frame premature stop.

Results of the current study have identified new variants in the human *PAX6* gene causal for aniridia and, in conjunction with previously identified mutations, serve to further the understanding of *PAX6* mutations in this disorder as well as inform future studies of novel pharmacological treatments which may be beneficial for the treatment of aniridia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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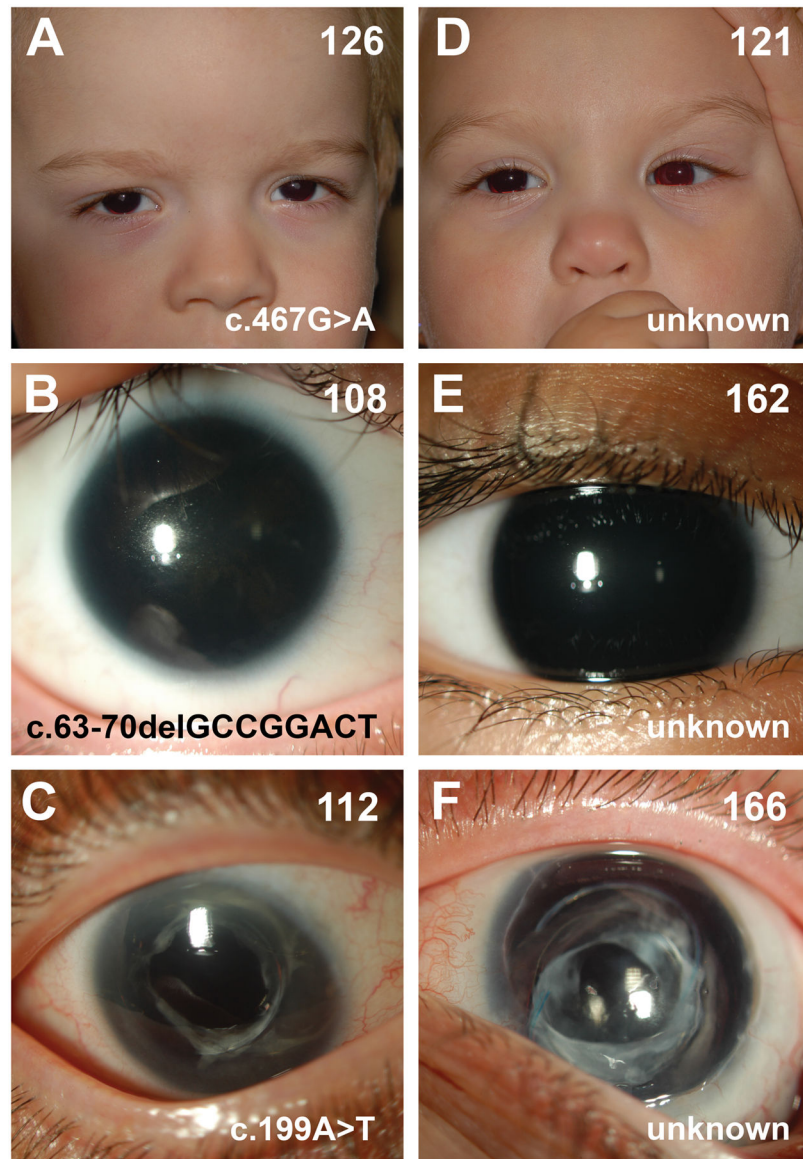


Figure 1. Ocular phenotypes associated with individuals harboring *PAX6* coding mutations (A–C) and those for whom no coding mutations were detected (D–F). Images from individuals with known *PAX6* mutations were chosen to facilitate comparison with panels D–F and do not capture the full range of ocular phenotypes observed in individuals with identified mutations of the *PAX6* gene. Family ID, upper right; causal sequence variant, lower right.

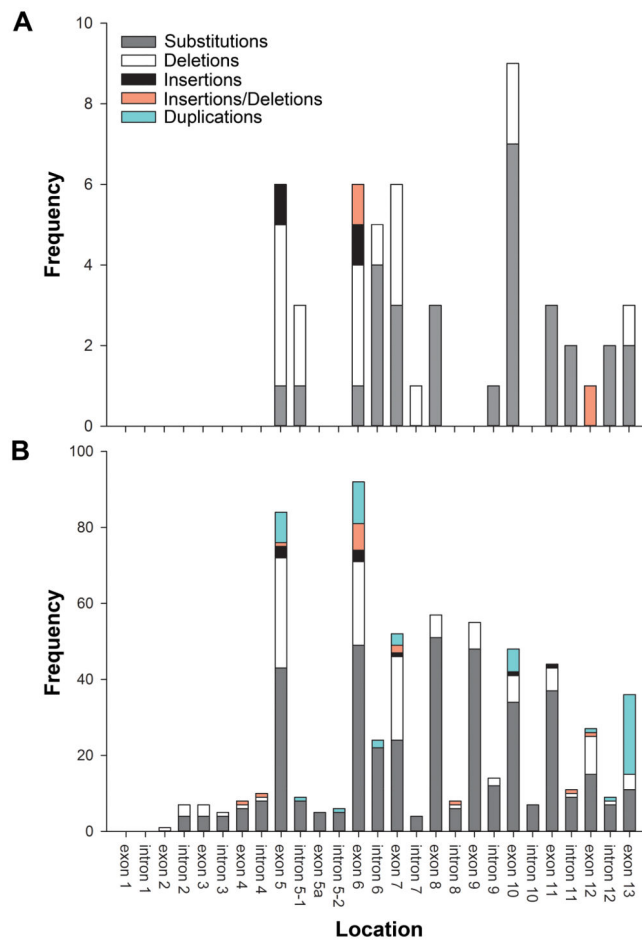


Figure 2. Distribution of *PAX6* variants. (A) Frequency of pathological variants identified in the current study located in each exon and intron of the *PAX6* gene (N=51). (B) Frequency of variants compiled from the PAX6 Allelic Variant Database as of June 2, 2015 (N=623). Variants classified based on nature of sequence alteration.

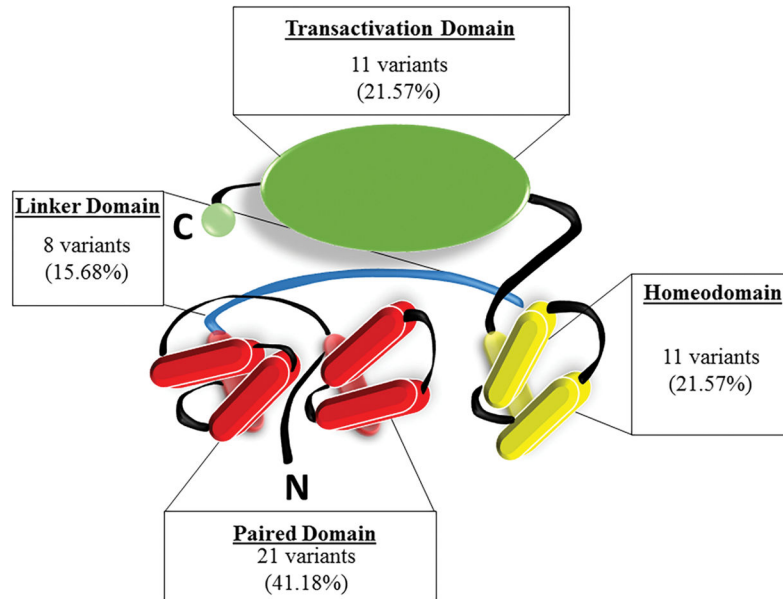


Figure 3. Summary of allelic variants predicted to affect each protein domain. Illustration of the PAX6 protein with summary statistics of the variants in the current study predicted to affect protein structure in (A) the paired domain, (B) the linker domain (C) the homeodomain, and (D) the transactivation (PST) domain.

Table 1
Identified variants in the current study

(A) 51 sequence variants (from 51 families) sorted by genomic location and are described by type of variant, intronic/exonic location, and the predicted effect on gene products. (B) Complete and partial deletions of *PAX6* from 10 families. (C) 5 families with no pathological change in *PAX6* detected. Variants in bold represent those not previously identified in the LOVD database.

Table 1A: PAX6 variants expected to cause pathology.

<i>FID</i>	<i>LOCATION</i>	<i>VARIANT</i>	<i>TYPE OF VARIANT</i>	<i>PREDICTED EFFECT</i>
157	Exon 5	c.28C>T	transition	nonsense
118	Exon 5	c.57delG	indel	frameshift deletion
108	Exon 5	c.63-70delGCCGACT	indel	frameshift deletion
133	Exon 5	c.112delC	indel	frameshift deletion
101	Exon 5	c.112-116delCGGCC	indel	frameshift deletion
135	Exon 5	c.121_122insGCGG	indel	frameshift insertion
138	Intron 5	c.141+1G>T	transversion	splice junction disruption
152	Intron 5	c.141+2_+30delTGATCCTCCCGGCGCCGCCCACTCGCCG	indel	splice junction disruption
128	Intron 5	c.141+18_+20delGCCC	indel	Unknown effect
150	Exon 6	c.179-185delATTACGAinsCTGAT	indel	frameshift deletion/insertion
112	Exon 6	c.199A>T	transversion	nonsense
147	Exon 6	c.204delC	indel	frameshift deletion
155	Exon 6	c.332insG	indel	frameshift insertion
1003	Exon 6	c.343delG	indel	frameshift deletion
163	Exon 6 & Intron 6	c.352-357+2delCCAAGCGT	indel	frameshift deletion
100	Intron 6	c.357+1G>A	transition	splice junction disruption
129	Intron 6	c.357+1G>A	transition	splice junction disruption
230	Intron 6	c.357+1G>A	transition	splice junction disruption
105	Intron 6	c.357+1G>T	transversion	splice junction disruption
169	Intron 6 & Exon 7	c.358-3_361delCAGGTGT	indel	splice junction disruption
168	Exon 7	c.365C>A	transversion	nonsense
141	Exon 7	c.401delA	indel	frameshift deletion

Table 1A: PAX6 variants expected to cause pathology.

<i>FID</i>	<i>LOCATION</i>	<i>VARIANT</i>	<i>TYPE OF VARIANT</i>	<i>PREDICTED EFFECT</i>
104	Exon 7	c.454C>T	transition	nonsense
126	Exon 7	c.467G>A	transition	nonsense
127	Exon 7	c.480delT	indel	frameshift deletion
114	Exon 7	c.482delG	indel	frameshift deletion
117	Intron 7 & Exon 8	c.524-101_534del112bp	indel	splice junction disruption
102	Exon 8	c.607C>T	transition	nonsense
160	Exon 8	c.607C>T	transition	nonsense
189	Exon 8	c.631C>T	transition	nonsense
173	Intron 9	c.766-3C>G	transversion	splice junction disruption
124	Exon 10	c.771delG	indel	frameshift deletion
144	Exon 10	c.781C>T	transition	nonsense
164	Exon 10	c.781C>T	transition	nonsense
170	Exon 10	c.781C>T	transition	nonsense
232	Exon 10	c.781C>T	transition	nonsense
110	Exon 10	c.794G>A	transition	nonsense
227	Exon 10	c.795G>A	transition	nonsense
191	Exon 10	c.799A>T	transversion	nonsense
103	Exon 10	c.802_806delGAAGA	indel	frameshift deletion
120	Exon 11	c.949C>T	transition	nonsense
153	Exon 11	c.949C>T	transition	nonsense
1001	Exon 11	c.949C>T	transition	nonsense
130	Intron 11	c.1032+6T>G	transversion	splice junction disruption
122	Intron 11	c.1033-2A>G	transition	splice junction disruption
143	Exon 12 & Intron 12	c.1174_+6delACTTCAACAGGTGAGC	indel	frameshift deletion
119	Intron 12	c.1183+1G>A	transition	splice junction disruption
1004	Intron 12	c.1183+1G>A	transition	splice junction disruption

Table 1A: PAX6 variants expected to cause pathology.

<i>FID</i>	<i>LOCATION</i>	<i>VARIANT</i>	<i>TYPE OF VARIANT</i>	<i>PREDICTED EFFECT</i>
185	Exon 13	c.1256delC	indel	frameshift deletion; C-terminal extension
115	Exon 13	c.1268A>T	transversion	run-on, C-terminal extension
140	Exon 13	c.1268A>T	transversion	run-on, C-terminal extension

Table 1B: Partial and gross deletions of PAX6.

<i>FID</i>	<i>LOCATION</i>	<i>VARIANT</i>	<i>TYPE OF VARIANT</i>	<i>PREDICTED EFFECT</i>
228		Deletion P1 promoter to exon 4	indel	
1002		Deletion exons 6 and 7	indel	
161		WT1-PAX6; 3' extent unknown	gross deletion	loss-of-function
174		WT1-PAX6; 3' extent unknown	gross deletion	loss-of-function
180		WT1-PAX6; 3' extent unknown	gross deletion	loss-of-function
182		WT1-PAX6; 3' extent unknown	gross deletion	loss-of-function
214		WT1-PAX6; 3' extent unknown	gross deletion	loss-of-function
219		WT1-PAX6; 3' extent unknown	gross deletion	loss-of-function
225		WT1-PAX6; 3' extent unknown	gross deletion	loss-of-function
501		Deletion of PAX6	gross deletion	loss-of-function

Table 1C: Families with no pathological mutations of PAX6 detected.

<i>FID</i>	<i>LOCATION</i>	<i>VARIANT</i>	<i>TYPE OF VARIANT</i>	<i>PREDICTED EFFECT</i>
121		No changes detected		N/A
162	Intron 9	c.766-12C>T	transition	Variant of unknown significance
166	Intron 9	c.766-12C>T	transition	Variant of unknown significance
190	Intron 9	c.766-12C>T	transition	Variant of unknown significance
231		No changes detected		N/A