Published in final edited form as:

Hum Mutat. 2018 April ; 39(4): 471–494. doi:10.1002/humu.23395.

Mutation Update of Transcription Factor Genes *FOXE3*, *HSF4*, *MAF* and *PITX3* Causing Cataracts and Other Developmental Ocular Defects

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Abstract

Mutations in the transcription factor genes *FOXE3*, *HSF4*, *MAF*, and *PITX3* cause congenital lens defects including cataracts that may be accompanied by defects in other components of the eye or in non-ocular tissues. We comprehensively describe here all the variants in *FOXE3*, *HSF4*, *MAF*, and *PITX3* genes linked to human developmental defects. A total of 52 variants for *FOXE3*, 18 variants for *HSF4*, 20 variants for *MAF*, and 19 variants for *PITX3* identified so far in isolated cases or within families are documented. This effort reveals *FOXE3*, *HSF4*, *MAF* and *PITX3* to have 33, 16, 18, and 7 unique causal mutations, respectively. Loss-of-function mutant animals for these genes have served to model the pathobiology of the associated human defects, and we discuss the currently known molecular function of these genes, particularly with emphasis on their role in ocular development. Finally, we make the detailed *FOXE3*, *HSF4*, *MAF*, and *PITX3* variant information available in the Leiden Online Variation Database (LOVD) platform at <http://www.LOVD.nl/FOXE3>, <http://www.LOVD.nl/HSF4>, <http://www.LOVD.nl/MAF>, and <http://www.LOVD.nl/PITX3>. Thus, this article informs on key variants in transcription factor genes linked to cataract, aphakia, corneal opacity, glaucoma, microcornea, microphthalmia, anterior segment mesenchymal dysgenesis, and Ayme-Gripp syndrome, and facilitates their access through web-based databases.

Keywords

cataract; aphakia; anterior segment mesenchymal dysgenesis; Ayme-Gripp syndrome; microcornea; LOVD

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Disclosure statement: The authors have no conflicts of interest to declare.

Background

Perturbation of early eye development, specifically during the morphogenesis of the anterior segment tissues – the lens and cornea – can result in congenital cataract, aphakia, anterior segment mesenchymal dysgenesis, coloboma (defined as missing tissue in or around the eye; can affect lens, macula, optic nerve, uvea, retina, choroid, iris and eyelids), microphthalmia and microcornea, among other ocular defects. Here we focus on four transcription factor-encoding genes *FOXE3* (MIM# 601094), *HSF4* (MIM# 602438), *MAF* (MIM# 177075), *PITX3* (MIM# 602669), mutations in which lead to ocular defects with a subset of shared features, but commonly exhibiting defects in the lens, including cataract. Cataract is an eye disease arising due to the loss of ocular lens transparency, and depending on the age of onset can be classified as congenital or age-related. Age-related cataract is among the leading causes of blindness worldwide (Shiels and Hejtmancik, 2017). Congenital cataract can present either as the primary phenotype (isolated or non-syndromic) or one among multiple phenotypes (syndromic), and between 25–50% of congenital cataract cases are estimated to be caused by an underlying genetic alteration (Shiels and Hejtmancik, 2015, 2017). Predominantly, congenital cataract defects are linked to mutations in genes that encode various crystallin proteins, cytoskeletal proteins, or gap junction proteins (Shiels et al., 2010). Additionally, mutations in several regulatory molecules, including transcription factor-encoding genes, are linked to anterior eye and lens defects (Sowden, 2007; Reis and Semina, 2011; Shiels and Hejtmancik, 2017). However, there is no recent article comprehensively summarizing variants in the transcription factor genes *FOXE3*, *HSF4*, *MAF*, and *PITX3* in human, especially with regards to genotype-phenotype correlation and informing on the significance of specific variants.

We provide here a detailed mutation update of *FOXE3*, *HSF4*, *MAF* and *PITX3*. We present a graphical representation of the location of all currently known variants in these transcription factor genes on the genomic, cDNA, and protein levels, which serve to highlight their significance in context of the protein structure, especially in relation to conserved regions and functional domains. Further, we also provide a map for these genes that informs on the conservation of the mutated amino acids across several vertebrate species. We then discuss in detail the various animal resources, based on both targeted gene deletions as well as spontaneous mutations, that have been used to model the human defects and to gain insights into the molecular regulatory function of these proteins. Finally, we make the compiled variant profiles including DNA and protein changes, mutation type (germline, somatic or *de novo*), segregation and frequency for these genes publicly available by depositing and updating this information to the Leiden Online Variation Database (LOVD) platform. Using this freely available database, end-users can readily access variant information, as well as graphical summary through UCSC Genome Browser, Ensembl or NCBI sequence viewer.

Genotype-Phenotype Correlation

In the following sections, we focus on summarizing all the currently known variants (both mutations and polymorphisms as of December 19, 2017) in the four transcription factor genes *FOXE3*, *HSF4*, *MAF* and *PITX3* that have key function in eye development and are

linked to cataract, among other defects, in human patients. We also discuss the functional and clinical significance of the variations. Sequence variations in these genes are described by following the mutation nomenclature guidelines outlined in <http://varnomen.hgvs.org> (den Dunnen et al., 2016).

Disease-Causing *FOXE3* Variants

The forkhead box (FOX) gene family encodes transcription factor proteins that contain a DNA-binding region called the “forkhead” domain (Weigel et al., 1989). Mutations in the FOX family gene *FOXE3* are associated with distinct ocular phenotypes, commonly involving microphthalmia, aphakia, and glaucoma, and predominantly affecting the anterior segment tissues, the lens and cornea. These are: microphthalmia (47 affected individuals), aphakia (18 affected individuals), glaucoma (17 affected individuals), corneal opacities (13 affected individuals), coloboma with or without affecting the optic disc (13 affected individuals), sclerocornea (18 affected individuals), anterior segment dysgenesis (5 affected individuals), microcornea (5 affected individuals), Peters anomaly (7 affected individuals), nystagmus (4 affected individuals), congenital and early childhood onset cataracts (3 affected individuals), anophthalmia (2 affected individuals), corneal ectasia with central scarring (2 affected individuals), absence of iris (2 affected individuals), myopia (2 affected individuals), retinal dysplasia (2 affected individuals), corneal limbal insufficiency (1 affected individual), corneal neovascularization (1 affected individual), and aniridia (1 affected individual) in reported cases (Table 1). Interestingly, in addition to ocular phenotypes, mutations in *FOXE3* have recently been associated with thoracic aortic aneurysms and acute aortic dissections (TAADs) (Kuang et al., 2016). Over the past two decades, 52 studies have reported 33 unique *FOXE3* mutations – classified as 6 frameshift, 23 missense, 3 nonsense and 1 nonstop mutation– in 110 affected individuals from diverse populations. Of the 33 unique *FOXE3* mutations, 15 present in the forkhead domain are missense variants, predicted as damaging or experimentally validated as non-functional or low affinity proteins (Fig. 1, Table 1). Of these fifteen, 10 are associated with eye defects, which include c.232G>A (p.Ala78Thr), c.244A>G (p.Met82Val), c.269G>T (p.Arg90Leu), c.289A>G (p.Ile97Val), c.292T>C (p.Tyr98His), c.307G>A (p.Glu103Lys), c.310C>T (p.Arg104Cys), c.351C>G (p.Asn117Lys), c.358C>G (p.Arg120Gly) and c.472G>C (p.Gly158Arg), whereas 5 are associated with thoracic aortic aneurysms and acute aortic dissections (TAADs), which include c.334C>T (p.Pro112Ser), c.410G>A (p.Gly137Asp), c.457G>C (p.Asp153His), c.466G>A (p.Asp156Asn), and c.490C>A (p.Arg164Ser). Further, protein sequence alignment reveals that amino acids residues are conserved among vertebrates for all of the 15 mutations present in the forkhead domain (Fig. 2). Thus, it is interesting to note that specific mutations in the forkhead DNA-binding domain have specific outcomes with regards to either ocular or aortic defects. However, of the other 17 mutations present outside of the forkhead domain, only the amino acid encoded at position c.Ins(G)943 (p.Leu315AlafsX117) is conserved among vertebrates. Further, of these 17 mutations in non-conserved amino acids, majority were predicted to be damaging/deleterious or to encode non-functional proteins, as reported in the original publications (Table 1). One particular *FOXE3* mutation c.720C>A (p.Cys240X) – located downstream of the forkhead domain reported in ten unrelated individuals to occur in the homozygous state – is frequently associated with aphakia and microphthalmia, among other eye defects. *FOXE3*

variants (c.146G>C (p.Gly49Ala), c.601G>A (p.Val201Met), c.898A>G (p.Ser300Gly), c.618C>G (p.Ala206Ala)) have been identified in both affected and control populations at similar frequencies and are therefore unlikely to be disease-associated (Iseri et al., 2009; Reis et al., 2010; Jimenez et al., 2011). Interestingly, the frequency of these variants are found to be different in Caucasian, Hispanic, African American and Asian population controls. Additionally, three silent (c.510T>C (p.Ala170Ala), c.618C>G (p.Ala206Ala), c.828C>G (p.Pro276Pro)) and two missense (c.587G>C (p.Gly196Ala), c.898A>G (p.Ser300Gly)) *FOXE3* variants have been identified at similar frequencies in the anterior segment disorder patient population as well as in the control population (Semina et al., 2001). A complete list of *FOXE3* variants, including nucleotide and predicted amino acid changes are given in Table 1 and further details on probands are given in Supp. Table S1. The location of these changes, relative to important domains, are indicated in a schematic representation of the *FOXE3* gene and protein (Fig. 1). The updated *FOXE3* gene variants information is deposited in the Leiden Online Variation Database (LOVD) platform at <http://www.LOVD.nl/FOXE3>.

Disease-Causing *HSF4* Variants

The heat shock factor (HSF) family of genes encode transcriptional regulators that have a conserved winged helix-turn-helix domain that binds DNA in a sequence-specific manner (Akerfelt et al., 2010). Mutations in the HSF family protein HSF4 are predominantly associated with congenital and early childhood onset cataract, and have been found to be inherited in both autosomal dominant and recessive modes. *HSF4* mutation-linked cataracts are commonly of the lamellar sub-type, wherein the opacity is localized between the inner nuclear and outer cortical lens fiber cell layers (Table 2). In two reported cases, in addition to cataract, the eye defect nystagmus was observed (Behnam et al., 2016). Further, one *HSF4* exonic sequence variant was associated with age-related cataract (Shi et al., 2008). Sixteen studies have reported a total of 14 unique *HSF4* mutations in 136 affected individuals (Fig. 3, Table 2). Majority of the *HSF4* mutations described to date are missense (13), but frameshift mutations in 2 cases, and a nonsense mutation in 1 case, have also been described. Of the 16 unique mutations, 11 located in the HSF4 DNA-binding domain are conserved among vertebrates: c.57C>A (p.Ala19Asp), c.69G>T (p.Lys23Asn), c.103C>T (p.His35Tyr), c.179C>A (p.Pro60His), c.182A>G (p.Gln61Arg), c.190A>G (p.Lys64Glu), c.218G>A (p.Arg73His), c.256A>G (p.Ile86Val), c.331C>T (p.Arg110Cys), c.341T>C (p.Leu114Pro), c.355C>T (p.Arg119Cys) (Table 2) (Fig. 3). Three mutations (c.521T>C (p.Leu174Pro), c.524G>C (p.Arg175Pro), c.595_599delGGCC (p.Gly199GlufsX5)) are located in hydrophobic repeat (HR- A/B) region and two mutations (c.1213C>T (p.Arg405X), c.1327+4A>G (p.Met419GlyfsX29)) are located in downstream of hydrophobic repeat (DHR) region. Protein sequence alignment shows that more than 50% of the following reported *HSF4* mutations cause a change of an amino acid that is conserved across different vertebrate species: c.57C>A (p.Ala19Asp), c.69G>T (p.Lys23Asn), c.103C>T (p.His35Tyr), c.179C>A (p.Pro60His), c.190A>G (p.Lys64Glu), c.218G>A (p.Arg73His), c.256A>G (p.Ile86Val), c.341T>C (p.Leu114Pro), c.355C>T (p.Arg119Cys), c.521T>C (p.Leu174Pro) c.524G>C (p.Arg175Pro) and c.1327+4A>G (p.Met419GlyfsX29) (Fig. 4). Additionally, a missense variant (c.347G>A (p.Arg116His), originally described as c.1243G>A) was reported for *HSF4* in an age-related cataract population ($n=150$; 3/150) as

well as in a control group ($n=100$; 2/100) (Shi et al., 2008). Upon evaluation of all the *HSF4* variants, it is interesting to note that although all mutations identified in the DNA binding domain are missense variants, they are inherited autosomal dominantly, whereas mutations identified in the HR-A/B and DHR regions are inherited autosomal recessively. Based on these correlations, it can be speculated that perturbation of the DNA binding domain affecting only one allele is sufficient to result in early onset-cataract, in turn suggesting that this domain is integral to HSF4 function. On the contrary, no autosomal dominant cases have been reported for mutations affecting other functional domains of HSF4, suggesting that in such cases both alleles have to be perturbed in order to observe a clinical phenotype. A complete list of *HSF4* variants, including nucleotide and predicted amino acid changes are given in Table 2 and further details on probands are given in Supp. Table S2. The updated *HSF4* gene variants information is deposited in the Leiden Online Variation Database (LOVD) platform at <http://www.LOVD.nl/HSF4>.

Disease-Causing *MAF* Variants

The musculoaponeurotic fibrosarcoma (*MAF*) gene family encodes transcription factors that contain a basic-leucine zipper (bZIP) domain, which functions in protein dimerization for binding to DNA (Kataoka, 2007). Mutations in the *MAF* gene are reported in eighty individuals that exhibit various ocular defects including congenital cataract. Mutations in *MAF* are also linked to Ayme-Gripp syndrome, which presents with congenital cataract in addition to flat facial features resembling Down syndrome, as well as brachycephaly, deafness, intellectual disability, and seizures. Besides these, *MAF* mutations are associated with other defects such as iris coloboma (13 affected individuals), glaucoma (3 affected individuals), microcornea (15 affected individuals), microphthalmia (3 affected individuals), myopia (3 affected individuals), nystagmus (8 affected individuals), and Peters anomaly (3 affected individuals). Twenty studies have reported eighteen unique mutations in 83 affected individuals in *MAF* that includes 1 translocation event t(5;16)(p15.3;q23.2) and 17 missense mutations (Table 3). Interestingly, a significant subset (seven) of the *MAF* mutations associated with eye defects are located within the bZIP domain-encoding region c.864G>C (p.Arg288Pro), c.880C>T (p.Arg294Trp), c.890A>G (p.Lys297Arg), c.895C>A (p.Arg299Ser), c.908A>C (p.Gln303Leu), c.915C>T (p.Cys305Trp), c.958A>G (p.Lys320Glu)), but no mutations have been identified to lie within the transactivation domain-encoding region in human (although such a mutation is reported in mouse, see the animal model section below) (Fig. 5). However, eight mutations are within the N-terminal upstream of the transactivation domain (c.161C>T (p.Ser54Leu), c.172A>G (p.Thr58Ala), c.173C>T (p.Thr58Ile), c.176C>G (p.Pro59Arg), c.176C>A (p.Pro59His), c.176C>T (p.Pro59Leu), c.185C>G (p.Thr62Arg), c.206C>G (p.Pro69Arg)) are associated with Ayme-Gripp syndrome (Fig. 5). Protein sequence alignment shows that all the amino acids affected in *MAF* missense mutations are conserved in vertebrates (Fig. 6). While the transactivation domain activates transcription by forming a dimer, the bZIP domain facilitates dimerization that results in recognition of the Maf recognition element (MARE) on the target genes activated by Maf dimer complexes. Based on the genotype-phenotype correlations drawn from the *MAF* mutation spectrum in affected individuals, we speculate that the transactivation domain is functionally critical, and thus mutations in this region lead to lethality. Furthermore, mutations in the N-terminal region upstream of the transactivation

domain, may disrupt dimerization, protein stability, and/or protein function, resulting in the syndromic clinical phenotypes observed. Lastly, mutations in the bZIP domain that result in exclusively eye related-defects suggest that the MAF bZIP domain may have unique functions in the eye in addition to its known regulatory roles in other tissues. These speculations warrant further work on specifically investigating the significance of individual domains in MAF in the eye and other tissues of interest using animal models. Furthermore, while proteins in the small MAF family, MafG and MafK, have been associated with cataract in animal models (Agrawal et al., 2015), no variants have been identified in human patients yet. Interestingly, although small MAFs lack the transactivation domain present in large MAFs, they share the bZIP leucine zipper region, which may harbor potential causative variants in patients with cataract and other tissue-defects. A complete list of *MAF* variants, including nucleotide and predicted amino acid changes are given in Table 3 and further details on probands are given in Supp. Table S3. The updated *MAF* gene variants information is deposited in the Leiden Online Variation Database (LOVD) platform at <http://www.LOVD.nl/MAF>.

Disease-Causing *PITX3* Variants

The pituitary homeobox (*PITX*) family of proteins are transcription factors that contain two domains, the homeobox domain and the OAR (otp, aristaless, rax) domain that function in DNA-binding and potentially transactivation, respectively. Mutations in the *PITX* family gene *PITX3* cause congenital cataract and anterior segment mesenchymal dysgenesis (ASMD) (Semina et al., 1998). Presently, nineteen studies have reported seven unique mutations with only 1 missense, and 6 frameshift *PITX3* mutations in 168 individuals and are associated with the following defects: ASMD (87 affected individuals), congenital or early childhood onset cataract (155 affected individuals), corneal opacity (9 affected individuals), microphthalmia (3 affected individuals), microcornea (4 affected individuals), nystagmus (3 affected individuals) and glaucoma (1 affected individual) (Table 4). The missense *PITX3* mutation (p.Ser13Asp) reported (Semina et al., 1998) is inherited autosomal dominantly, and is the only mutation altering Exon 2 of *PITX3*. Exon 2 is the first coding exon, and based on the serine (polar) to aspartic acid (acidic) amino acid substitution, it can be inferred that this alteration likely affects the protein structure/function significantly, even though the mutation is in-frame and the protein is likely still expressed. Furthermore, majority of the *PITX3* mutations identified thus far are in exon 4 and interestingly no mutation has yet been described in the homeodomain- or the OAR domain-encoding region (Fig. 7). Sequence alignment demonstrate that the Ser13 residue in *PITX3*, which is affected by the c.94G>C mutation, is conserved in vertebrates (Fig. 8). A complete list of *PITX3* variants, including nucleotide and predicted amino acid changes are given in Table 4 and further details on probands are given in Supp. Table S4. The updated *PITX3* gene variants information is deposited in the Leiden Online Variation Database (LOVD) platform at <http://www.LOVD.nl/PITX3>.

Functional Insights from Animal Models and Biochemical Assays

Various animal models such as zebrafish, *Xenopus*, chicken, and mouse have been applied to investigate the function of *Foxe3*, *Hsf4*, *Maf* and *Pitx3* in development and organogenesis.

Notably, multiple gene-specific perturbation mouse mutants have been derived for all four genes. The characterization of these animal models has informed on the pathobiology of the underlying defects, in turn elucidating the molecular function of these transcription factors in ocular and non-ocular tissue development. These various animal models and the principle findings from their studies are discussed below for each of these genes.

FOXE3 Animal Models and Mechanistic Insights into the Pathology of Anterior Segment Defects

FOXE3 expression in the developing ocular lens is conserved in vertebrates as revealed from studies on fish (zebrafish, *Danio rerio*), frog (*Xenopus*) and mammals (mouse, human) (Kenyon et al., 1999; Blixt et al., 2000; Brownell et al., 2000; Semina et al., 2001; Shi et al., 2006; Swindell et al., 2008). *Foxe3* expression in mouse lens development is initiated early at the lens placode stage and in later stages is restricted to the anterior epithelium of the lens (AEL). In addition, *Foxe3* is also expressed in the forebrain, midbrain and the pharyngeal arches in mouse development (Blixt et al., 2000; Brownell et al., 2000; Kuang et al., 2016). Several distinct types of *Foxe3* perturbation mouse models – exhibiting either gain-of-function or loss-of-function – have been characterized. These include mice carrying spontaneous mutations, namely *dy1* (dysgenetic lens) (Sanyal and Hawkins, 1979; Blixt et al., 2000; Brownell et al., 2000) and *rct* (Rinshoken cataract) (Wada et al., 2011), two independent mouse targeted gene knockout mouse models (Medina-Martinez et al., 2005; Blixt et al., 2007), as well as a transgenic mouse with *Cryaa*-promoter driven *Foxe3* over-expression in lens fiber cells (Landgren et al., 2008). In the *dy1* mouse mutant, two closely located co-segregating missense mutations (Phe93Leu, Phe98Ser) were identified in the DNA-binding domain encoding region of the *Foxe3* gene, which compromise its DNA-binding function. Mice homozygous for the *dy1* locus exhibit fully penetrant anterior segment defects characterized by the failed separation of the lens vesicle from the surface ectoderm (that contributes to the future corneal epithelium) (Sanyal and Hawkins, 1979; Blixt et al., 2000; Brownell et al., 2000). Interestingly, *dy1* heterozygous mice also exhibit lens and cornea defects with features of Peters anomaly, albeit at reduced penetrance, and therefore have been suggested as an animal model for this eye disorder (Ormestad et al., 2002). Homozygous *rct* mutant mice carry a 22-bp deletion in a putative *Foxe3* cis-regulatory element that results in reduced *Foxe3* expression in the lens, and causes cataract and microphthalmia (Wada et al., 2011).

Targeted deletion of *Foxe3* in mice causes eye defects similar to those observed in homozygous *dy1* mice, although there are some minor differences between two independently developed gene-knockout models (Medina-Martinez et al., 2005; Blixt et al., 2007). Marker gene expression analysis of the various *Foxe3* null mice has indicated that FOXE3 is required for promoting proliferation of cells of the AEL (explaining the reduced size of the mutant lens) while preventing their pre-mature differentiation into fiber cells. Ablation of FOXE3 results in a progressive loss of AEL cells, defective fiber differentiation and induction of apoptosis, which causes abnormal spaces in the posterior region of the lens (Blixt et al., 2000; Brownell et al., 2000; Medina-Martinez et al., 2005; Blixt et al., 2007). Notably, FOXE3 negatively regulates an inhibitor of cyclin-dependent kinase (CDKN1C, p57^{KIP2}) as well as a transcription factor (PROX1) that is required for proper fiber cell

differentiation. In support of this, the expression of the fiber cell enriched crystallin genes, namely α - and β -Crystallins, are abnormally up-regulated in *Foxe3* null mouse lens AEL (Brownell et al., 2000). In addition to the lens defects, *Foxe3* deficiency in mice causes a defect in the differentiation of the periocular mesenchyme into corneal endothelium, trabecular meshwork and the different cell types of the iris and the ciliary body stroma, and therefore offers an explanation for the human ocular phenotypes involving the iris and cornea observed in patients with *FOXE3* mutations (Blixt et al., 2007). Further, *Foxe3* heterozygous mice exhibit a partial closure of the filtration angle by iridocorneal tissue, which may serve to explain the observed glaucoma defects in a subset of human patients (Blixt et al., 2007).

The molecular consequence of ectopic *Foxe3* expression in lens fiber cells, which results in severe lens defects in mice, has been investigated by expression profiling microarrays (Landgren et al., 2008). These microarray data indicate that *FOXE3* interferes with fiber cell differentiation by mis-regulating genes involved in maintenance of various cellular properties including its shape, cytoskeleton, organelle degradation and extracellular matrix. For example, genes associated with fiber cell differentiation (*Capn3*, *Casp7*, *Bcl2l13*) are down-regulated, while those associated with AEL (*S100a1*, *Sdpr*) are up-regulated upon *FOXE3* over-expression in fiber cells. Further, expression of a Peters anomaly associated truncated *FOXE3* mutant protein in human cell lines results in the down-regulation of *DNAJB1* on both mRNA and protein levels, suggesting that *FOXE3* may directly control autophagy factors in ocular development (Khan et al., 2016). In addition to the mouse models that compromise *Foxe3* function, a mouse mutant (“*vacuolated lens*”, *vl*) – carrying a *Foxe3* polymorphism that results in amino acid change Leu23Pro and a *Gpr161* truncation mutation – exhibit cataract, in turn suggesting *Foxe3* as a genetic modifier of the cataract phenotype (Matteson et al., 2008).

Further, various biochemical assays have been used to investigate the different human *FOXE3* variants with regards to alterations in their DNA-binding properties and transactivation of reporter gene expression (Islam et al., 2015). The protein products encoded by the *FOXE3* variants linked to primary aphakia in humans c.21_24del, c.358C>G, and c.705del showed loss of DNA-binding and reduced activation of luciferase reporter. Interestingly, while the protein product of the *FOXE3* variant c.269G>T retained its DNA-binding property, it showed reduced activation in luciferase reporter assays. This study provides evidence that specific *FOXE3* variants result in functionally defective proteins, thus suggesting altered *FOXE3* function, rather than reduced dosage, as the pathogenic mechanism for *FOXE3* dominant mutations (Islam et al., 2015).

Finally, the necessity of *FOXE3* for proper lens development is conserved in vertebrates, although some differences in its function are observed between fish and mice. In zebrafish, *foxo3* knockdown using antisense morpholinos causes a small lens phenotype that exhibits multiple layers of epithelial cells and abnormal fiber cells (Shi et al., 2006; Swindell et al., 2008). Thus, while *Foxe3* mutant mice exhibit an epithelial cell proliferation defect, zebrafish *foxo3* morphants harbor an undifferentiated population of epithelial cells. However, both mouse and fish *Foxe3* mutants exhibit fiber cell defects and a down-regulation of the platelet-derived growth factor receptor *pdgfra*, similar to that observed in

Foxe3 mutant mice (Blixt et al., 2000; Medina-Martinez et al., 2005; Swindell et al., 2008). Interestingly, the zebrafish *foxe3* morphant lenses exhibited mis-expression of genes (*e.g.* rhodopsin) normally expressed in the retina, indicating its requirement for cell-specific gene regulation in lens development (Shi et al., 2006). Together, these studies on animal models provide mechanistic insights into FOXE3 gene regulatory network in eye development and into the underlying pathology of ocular defects associated with its deficiency.

HSF4 Animal Models and Mechanistic Insights into the Pathology of Cataract

Apart from the DNA-binding domain near the amino-terminal, HSF proteins also contain leucine zipper-like heptad repeat A and B (HR-A/B) domains that are required for HSF monomer trimerization and binding to DNA. Among the HSF factors, HSF4 is unique because it does not contain an additional leucine zipper-like HR-C domain (a hydrophobic repeat at the carboxyl-terminal), which normally functions in HSF1 and HSF2 to inhibit the formation of active trimers (Nakai et al., 1997). This suggests that HSF4 may be able to bind to HSEs under non-stress conditions and therefore may participate in developmental events. Further, HSF4 is expressed as two splice forms, HSF4a and HSF4b (HSF4b has an additional 30 amino acids and is expressed in the lens), that function as a transcriptional repressor and a transcriptional activator, respectively (Tanabe et al., 1999; Smaoui et al., 2004). Finally, a motif for a unique phosphorylation-dependent sumoylation (PDSM) translational modification has been identified in the regulatory domain of HSF4, which may control its function (Hietakangas et al., 2006).

Hsf4 expression has been reported at high levels in embryonic and adult mouse lens (Fujimoto et al., 2004; Min et al., 2004; Lachke et al., 2012) and its expression in the human brain, heart, lung, pancreas and skeletal muscle is also documented (Nakai et al., 1997; Fujimoto et al., 2004). So far, HSF4 function in development has been investigated using multiple different *Hsf4* mouse mutant alleles that were generated either by targeted inactivation, BAC transgenesis, or were identified as a spontaneous mutation (Fujimoto et al., 2004; Jablonski et al., 2004; Min et al., 2004; Talamas et al., 2006; Shi et al., 2009; He et al., 2010; Liang et al., 2011; Gangalum et al., 2014; Jing et al., 2014). Targeted *Hsf4* deletion in mice results in lens fiber cell defects and cataract (Fujimoto et al., 2004; Min et al., 2004; Shi et al., 2009). Further, a spontaneous mutation in *Hsf4* intron 9 (termed “lens opacity 11” locus (*lop11*)) has been identified in the RIIS/J mouse strain (Talamas et al., 2006). *Hsf4^{lop11}* mice carry a 61-bp insertion of early transposable element (ETn) in intron 9 that functions as a pseudo-exon. This insertion generates a chimeric *Hsf4* transcript, which encodes a truncated, non-functional HSF4 protein with a 132 amino acid deletion in the carboxyl terminus (Talamas et al., 2006; Liang et al., 2011). The same mutation was identified in the mouse termed “lens disrupter 1” (*ldis1*) that exhibits cataract (Jablonski et al., 2004). *Hsf4^{lop11}* mice exhibit severe fiber cell defects and cataract at postnatal day (P) 12 and involve fiber cell denucleation defects (Talamas et al., 2006; Liang et al., 2011). In addition to these mutant mice, BAC transgenesis-derived mice, which model the lamellar opacity observed in human childhood cataract, have been described previously (Gangalum et al., 2014). This approach has led to specifically modelling the lamellar cataract observed in human *HSF4* p.Arg116His mutation (Jing et al., 2014).

Gene expression profiling studies on *Hsf4* mouse mutant lenses have identified several potential target genes that may be involved in the cataract pathobiology. For example, *Hsf4* targeted knockout mouse early postnatal (P2) lens exhibits downregulation of γ -crystallin genes (*Cryga*, *Crygb*, *Crygc*, *Cryge*, *Crygf*), all of which carry HSEs in their promoter regions (Fujimoto et al., 2004). Down-regulation of γ -crystallin genes is also exhibited by *Idis1* mouse mutants (Jablonski et al., 2004). Further, the heat shock protein HSPB1 (also known as HSP25, HSP27), which is known to interact with α A-crystallins, is down-regulated in *Hsf4* null mouse lens (Fujimoto et al., 2004; Min et al., 2004). In an independently generated *Hsf4* germline knockout mouse mutant, the cataract-linked and fiber cell expressed genes *Bfsp1*, *Bfsp2* and *Crygs* are down-regulated in the lens at age 8-weeks (Shi et al., 2009). Further, transcriptional activity assays suggest that *Bfsp1* and *Bfsp2* are direct targets of HSF4. A two-dimensional (2-D) electrophoretic analysis identifies a post-translational modification defect in α A-Crystallin and shows that CRYGS was also reduced at the protein-level in the *Hsf4* mutant lens, as were the calcium activated proteases LP82 and CALPAIN2, which are responsible for post-translational modifications in differentiating fiber cells (Shi et al., 2009). Finally, microarray-based genome-level transcriptional profiling has been performed on lenses of two independent *Hsf4* targeted knockout mouse mutants at postnatal stages P0 and P10 (Min et al., 2004; He et al., 2010). Newborn *Hsf4* null lenses exhibit 1428 differentially expressed genes, a subset of which are commonly regulated by other transcription factors in the lens (He et al., 2010). Microarray analysis at P10 stage *Hsf4* null lens revealed that the range of HSF4-target genes is well beyond those associated with stress-response and also involves genes functioning in lens development (He et al., 2010), which is also independently suggested by chromatin-immunoprecipitation (ChIP) assays for HSF4 in stage P2 mouse lens (Fujimoto et al., 2008). Both microarray studies identified *Dnase2b* – known to be required for fiber nuclear degradation (Nishimoto et al., 2003) – to be significantly reduced in *Hsf4* null mutant lenses, providing an explanation for the de-nucleation defect associated with Hsf4 deficiency. Interestingly, Hsf4 has been shown to regulate transcription of Dnase2b by direct binding to its promoter (Cui et al., 2013; He et al., 2016).

Thus, these cellular, biochemical and molecular studies using animal models are beginning to define a HSF4-regulatory network operational in differentiating lens fiber cells that when perturbed causes cataract.

MAF Animal Models and Mechanistic Insights into the Pathology of Eye Defects

MAF (*c-MAF*) encodes a leucine zipper transcription factor that exhibits conserved expression in vertebrate eye development (Yang and Cvekl, 2007). In mice, *Maf* is expressed in the lens placode, and subsequently in the lens vesicle and lens fiber cells (Kawauchi et al., 1999; Kim et al., 1999; Ring et al., 2000). Outside of the eye, MAF expression is detected in the cartilage (of basioccipital bone, limb, rib), chondrocytes, cochlea, dorsal root ganglia neurons, dorsal spinal cord, forebrain, heart, intestine, kidney, liver, lung, muscle, perichondrium of the skeleton, skin, spleen, uterus, and in the T helper (Th) cells – Th2 and Th17 (Sakai et al., 1997; Ring et al., 2000; Sato et al., 2011; Wende et al., 2012; Niceta et al., 2015). In mice, targeted deletion of *Maf* (homozygous null) results in early postnatal mortality and ocular developmental defects including microphthalmia as well as lens defects

(Kawauchi et al., 1999; Kim et al., 1999; Ring et al., 2000). In *Maf*^{-/-} mutant mouse, lens fiber cells exhibit normal development until E12.5, but beyond this stage, they fail to elongate. Further, *Maf*^{-/-} mutant lenses exhibit reduced expression of several Crystallin genes (e.g. Crystallins α A-, β B2-, β A3/A1-, β A4-, and γ D-). In agreement with this, MAF-binding motifs (termed MAF response elements (MAREs) are identified in the *cis*-regulatory regions of several Crystallin genes (Cui et al., 2004; Rajaram and Kerppola, 2004; Yang et al., 2004; Yang and Cvekl, 2005; Yang et al., 2006). Together these data show indicate that MAF is an important regulator of the Crystallin gene regulatory network in the lens. Further, besides the ocular phenotypes, *Maf*^{-/-} mice also show defects in terminal differentiation of chondrocytes during bone development (MacLean et al., 2003) – a phenotype which is not entirely surprising, as MAF is shown to be expressed in chondrocytes (Sakai et al., 1997).

In addition to targeted deletion, spontaneous – as well as ENU-induced – *Maf* mutant alleles have been identified in mice. Interestingly, the mouse spontaneous *Maf* missense mutation c. 1803G>A (p.Arg291Gln), termed “opaque flecks in lens” (*Ofl*), lies near the human *MAF* mutations p.Arg288Pro and p.Arg294Trp, which are in the basic domain of the encoded protein (Lyon et al., 2003; Sun et al., 2014; Ma et al., 2016). While in human, the p.Arg288Pro mutation causes cortical or nuclear cataract, microcornea and iris coloboma, and the p.Arg294Trp causes nuclear cataract (Table 3), in mouse, the p.Arg291Gln mutation causes cataract accompanied by anterior segment abnormalities, which is influenced by the genetic background (Lyon et al., 2003). Interestingly, heterozygous *Ofl* mutant mice exhibit pulverulent cataract and homozygous *Ofl* mutants exhibit – in addition to ocular defects – renal tubular nephritis and early postnatal lethality (Lyon et al., 2003). Biochemical assays indicate that the mouse *Ofl* p.Arg291Gln mutation alters the DNA-binding affinity of MAF, and thus reduces its transactivation potential at target promoters, similar to the human p.Arg288Pro mutation (Perveen et al., 2007). This finding offers a molecular explanation for the observed dominant negative effect of the *Ofl* mutant allele that causes the cataract phenotype in heterozygous state in these mouse mutants, which is not observed in *Maf* targeted knockout mice. Another *Maf* gene mouse mutant, designated *Maf*^{ENU424}, has been identified in an N-ethyl-N-nitrosourea (ENU) mutagenesis screen (Perveen et al., 2007). This *Maf* missense mutation (c.269A>T, p.Asp90Val) affects a conserved aspartate residue in the transactivation domain of the protein, which in homozygous state, results in isolated cataract. Further, biochemical studies show that the p.Asp90Val mutation causes MAF-mediated transactivation to be non-receptive to inhibition by the protein kinase A pathway, and therefore results in elevated promoter activity, which interesting includes the *Pitx3* promoter (Perveen et al., 2007). These studies show that specific changes in conserved regions of the MAF protein can result in loss-of-function (e.g. mouse p.Arg291Gln, human p.Arg288Pro) or gain-of-function (p.Asp90Val) variants.

In addition to these mutants, a mouse line overexpressing *Maf* in has been developed (Ho et al., 1998). Using these mice, it has been demonstrated that MAF negatively regulates Th1 differentiation while positively regulating Th2 differentiation. Finally, in addition to these various loss-of-function and gain-of-function *Maf* mouse mutant alleles, a recent study has generated a targeted conditional *Maf* mouse mutant line (Wende et al., 2012). In conjunction with a *Isl1*Cre detector mouse, this conditional *Maf* mouse line has been utilized to

demonstrate an unprecedented function for MAF in mechanoreceptors (Wende et al., 2012). Together, these findings suggest that the range of phenotypes associated with MAF deficiency may be broader than previously anticipated.

PITX3 Animal Models and Mechanistic Insights into the Pathology of Eye Defects

In mice, PITX3 expression is detected in the lens placode starting at E9.75, the entire lens vesicle at E10.5, and in both the anterior epithelial cells and the posteriorly localized fiber cells at E11.5 through E12.5, before being restricted in the epithelium at E14.5 and later stages (Semina et al., 1997; Ho et al., 2009; Medina-Martinez et al., 2009). In addition to the lens, PITX3 is also strongly expressed in mouse midbrain mesodiencephalic dopaminergic neurons starting from E11.5 and this expression is conserved between mouse, rat, and human (Smidt et al., 1997; Zhao et al., 2004). Further, PITX3 is detected in tongue, incisors, sternum, vertebrae and limbs (Semina et al., 1998) and in skeletal muscle cells (Coulon et al., 2007). Both spontaneous and targeted knockout mouse mutants have been described for *Pitx3* (Semina et al., 1997, 2000; Ho et al., 2009; Rosemann et al., 2010).

The spontaneous mutation *aphakia* (*ak*) is recessive and causes a small eye phenotype with the absence of lens and eyelids (Varnum and Stevens, 1968; Grimm et al., 1998). *Pitx3* was mapped near the *ak* locus on mouse chromosome 19 and its expression was absent in *ak/ak* homozygous mutant lens (Semina et al., 1997, 2000). While the *Pitx3* coding sequence is intact in *ak* mice, these animals carry a double deletion – a distant intergenic deletion plus another removing exon 1 and part of intron 1 – that segregates with the *ak* allele (Semina et al., 2000; Rieger et al., 2001). The deletion of the *Pitx3* upstream sequence carrying potential regulatory elements (for transcription factors such as MAF) is considered the basis of the *ak* eye-related phenotypes (Semina et al., 2000). Subsequent analysis has shown that *ak* mutant lenses exhibit reduced proliferation and elevated apoptosis, as well as defects in fiber differentiation as indicated by reduced expression of Prox1, p57^{Kip2} and various crystallins, including the abnormal distribution of γ -crystallin transcripts (Medina-Martinez et al., 2009). While one study found no genetic interaction between *Pitx3* and *Foxe3* (Medina-Martinez et al., 2009), other findings suggest that PITX3 transcriptionally controls *Foxe3* expression (Ahmad et al., 2013). Interestingly, *pitx3* knockdown in zebrafish results lens and retina defects and in reduced *foxe3* lens expression (Shi et al., 2005, 2006), suggesting that PITX3 mediated regulation of FOXE3 is conserved in vertebrates. In addition to the ocular defects, and as expected from PITX3 expression pattern, *ak* mice also exhibit significantly reduced numbers of dopaminergic neurons (Hwang et al., 2003).

Besides *ak*, another spontaneous recessive mutation, *Pitx3^{eyl}* (c.416insG), has been identified in mouse, which results in a frameshift that is predicted to include 121 different amino acids downstream of the homeodomain (Rosemann et al., 2010). Homozygous *Pitx3^{eyl/eyl}* mutants have ocular and neuronal defects similar to *ak* mutants, and additionally exhibit defects in the spleen and liver. Further, these mutants have behavioral defects resembling human Parkinson patients (Rosemann et al., 2010). Recently, yet another new spontaneous nonsense recessive *Pitx3* mutation (c.444C>A; p. Tyr148>X) called *miak* (microphthalmia and aphakia) has been identified in the Japanese mouse strain *Mus musculus molossinus*, which causes aphakia and microphthalmia (Wada et al., 2014).

Homozygous *Pitx3^{miak/miak}* mice exhibit an over-expression of the mutant PITX3 protein that is predicted to have a C' 155 amino acid truncation. These mutants have delayed differentiation of lens fiber cells and showed reduction in important lens proteins such as FOXE3, MIP, $\alpha\beta$ -, β -, and γ -Crystallins. Finally, germline targeted *Pitx3* deletion mouse mutants have been generated. Interestingly, while FOXE3 is reduced in the *Pitx3* null mouse lenses in agreement with other *Pitx3* mouse mutant models, PROX1 expression does not appear to be reduced as in *ak* lenses, but rather is up-regulated in the lens anterior epithelium, suggesting that PITX3 functions to repress PROX1 in these cells (Ho et al., 2009). Further, β - and γ -crystallin expression was abnormally detected in early stages of lens development in *Pitx3* null mice. These findings suggest that PITX3 has distinct functions in epithelial cell maintenance and initiation of fiber differentiation.

Interestingly, a genome level search of PITX3 binding motifs has led to the identification of MIP as a direct target of this protein. Indeed, chromatin immunoprecipitation, reporter assays and knockdown in zebrafish has demonstrated that PITX3 is necessary for transcriptionally activating MIP expression by binding to a regulatory site that is conserved in human and fish (Sakazume et al., 2007; Sorokina et al., 2011). Moreover, functional analysis by electrophoretic mobility shift assay (EMSA) and luciferase reporter assays revealed defective DNA-binding and transactivation rendered by specific mutations in human PITX3 proteins (p.11Lys>Glu, p.13Ser>Asn, p.Ser192AlafsX117, p.Gly220ProfsX95) (Sakazume et al., 2007; Sorokina et al., 2011; Verdin et al., 2014).

Future Prospects

In this article, we provide a comprehensive and updated summary of variants in four transcription factors genes *FOXE3*, *HSF4*, *MAF* and *PITX3* that are commonly associated with ocular defects, including cataract, in addition to other developmental defects. Schematic of the variants in relation to the cDNA/gene are depicted at the nucleotide level as is their effect on the predicted protein. This serves to inform on variant distribution and frequency in the context of the protein and its domain(s), while the tables convey clinical details. Additionally, the conservation of the amino acid residues, which are affected by these mutations, across various vertebrate species is presented, and biochemical assays delineating the functional relevance of the variants are described. Importantly, this update reveals the frequently identified mutations and their correlation with specific phenotypes. For example, the *FOXE3* gene has distinct variants that are linked to either ocular defects or thoracic aortic aneurysms and acute aortic dissections. Thus, this mutation update for *FOXE3*, *HSF4*, *MAF* and *PITX3* provides key information to clinicians and developmental geneticists that may impact genetic investigation as well as diagnosis and prognosis of patients with these ocular defects.

In particular, this update highlights how animal models have provided rich information on understanding the function of these proteins in normal development and the effect of their mutation/deficiency on pathology. In some cases, the mouse mutant models have revealed an unexpected function, for example MAF's role in mechanoreceptor cell function. Thus, these animal models may help investigate new subtle/previously undiagnosed defects in human patients. Also, some of the spontaneous or ENU mutations in mice have shed light into the

function of these proteins. For example, while no variant in the transactivation domain of MAF has been described so far in human, a mutation in this region of the protein has been identified in the mouse. Additionally, mutations in the regulatory regions of transcription factor genes such as *PAX6* are linked to human ocular defects (Kleinjan et al., 2001; Kleinjan and van Heyningen, 2005). Efforts will be needed to characterize the transcriptional regulation of *FOXE3*, *HSF4*, *MAF*, and *PITX3* in animal models, which will facilitate the identification of regulatory mutations in these genes in human. Further, additional molecular studies on these mouse mutants employing chromatin immunoprecipitation followed by next-gen sequencing (ChIP-seq) and RNA-sequencing in combination with the bioinformatics tools such as iSyTE (integrated Systems Tool for Eye gene discovery) (Kakrana et al. 2018) will provide comprehensive information on the range of target genes regulated by *FOXE3*, *HSF4*, *MAF* and *PITX3*. These studies will also inform on the inter-connectedness of the gene regulatory network of these transcription factors, in support of which there is already some evidence. For example, *FOXE3* may be regulated by *PITX3*, which itself may be downstream of *MAF*. Finally, although majority of this research has used germline targeted gene deletion in mouse, generation of conditional mutant alleles and the advent of CRISPR technology (Cong et al., 2013) will expedite the process of generating customized mouse mutant models for specific human mutations identified in these genes, in turn leading to a comprehensive understanding of their function in development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Research in the Lachke laboratory is supported by the National Eye Institute of the National Institutes of Health under Award Number R01EY021505 and a University of Delaware Research Foundation, Inc. Strategic Initiatives (UDRF-SI) Grant to SAL. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. SAL is a Pew Scholar in Biomedical Sciences.

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Figure 1. Schematic of *FOXE3* gene, protein domains, and position of mutations

(A) The human *FOXE3* gene (top) spans a single exon (E1) that encodes the FOXE3 protein (bottom), which contains the forkhead DNA-binding domain (pink). Nucleotide positions for cDNA and genomic DNA in the reference sequence (hg38) and the corresponding amino acid positions in the protein sequence are given. The UCSC genome browser (<https://genome.ucsc.edu>) was used to obtain cDNA, genomic and protein position information from *FOXE3* human GRCh38/hg38 assembly (reference sequence NM_012186, uc001crk.3 at chr1: 47,416,072–47,418,052). Total 52 mutation studies, of which 33 represent unique mutations identified in independent studies are indicated as flags. These include 6 frameshift, 23 missense, 3 nonsense and 1 nonstop mutation. Nucleotide changes in DNA and amino acid changes in protein sequence are indicated. Changes in cDNA that are not documented in original article are represented as (*) or (**). Variants linked to eye defects are shown above the gene (and below the protein) and those associated with thoracic aortic aneurysms and acute aortic dissections are shown below the gene (and above the protein).

Figure 2. Multiple sequence alignment of FOXE3 protein sequences

Protein sequences for FOXE3 downloaded from the UCSC browser for *Homo sapiens*, *Mus musculus*, *Gallus gallus*, *Xenopus tropicalis* and *Danio rerio* shows amino acid conservation across different vertebrate species. Amino acid changes caused by specific human mutations are indicated by yellow arrowheads. The sequence consensus is presented for each amino acid and the forkhead domain is indicated by green arrow.

Figure 3. Schematic of *HSF4* gene, protein domains, and position of mutations

(A) The human *HSF4* gene spans fifteen exons (E1-E15) that encode the HSF4 protein (bottom). While there are multiple HSF isoforms, the specific isoform (HSF4 isoform b) that has high expression in the lens is described here because of its relevance to the cataract defect. Exons 3 through 6 encode the DNA binding domain (pink), exons 6 through 8 encode an oligomerization domain termed hydrophobic repeat (HR- A/B) (blue) and exons 12 through 14 encode a region termed as downstream of hydrophobic repeat (DHR) (purple). HSF4b is derived by alternative mRNA splicing of exons 10 and 11 (exon 8 and 9 in the originally reported study). Nucleotide positions for cDNA and genomic DNA in the reference sequence (hg38) and the corresponding amino acid positions in the protein sequence are given. The UCSC genome browser (<https://genome.ucsc.edu>) was used to obtain cDNA, genomic and protein position information from *HSF4* human GRCh38/hg38 assembly (reference sequence NM_001040667.2 at chr16:67163385–67169945). Total 18 mutations, of which 16 represent unique mutations identified in independent studies are indicated as flags. These include 2 frameshift, 13 missense and 1 nonsense mutation. Nucleotide changes in DNA and amino acid changes in protein sequence are indicated. In Table 2 * indicates nucleotide and amino acid positions as indicated in the original article

Figure 4. Multiple sequence alignment of HSF4 protein sequences

Protein sequences for HSF4 downloaded from UCSC browser for *Homo sapiens*, *Mus musculus*, *Gallus gallus*, *Xenopus tropicalis* and *Danio rerio* shows amino acid conservation across different vertebrate species. Amino acid changes caused by specific human mutations are indicated by yellow arrowheads. The sequence consensus is presented for each amino-acid. HSF-DNA binding domain and vertebrate heat shock transcription factor domains are indicated by green arrow.

MAF

Figure 5. Schematic of *MAF* gene, protein domains, and position of mutations

(A) The human *MAF* gene (top) spans two exons (E1-E2) that encode the HSF4 protein (bottom). Exon 1 encodes the transactivation domain (pink) and the bZIP domain (blue). Nucleotide positions for cDNA and genomic DNA in the reference sequence (hg38) and the corresponding amino acid positions in the protein sequence are given. The UCSC genome browser (<https://genome.ucsc.edu>) was used to obtain cDNA, genomic and protein position information from *MAF* human GRCh38/hg38 assembly (reference sequence NM_005360, uc002ffm.4 at chr16:79,593,838–79,600,714). Total 20 mutations, of which 18 represent unique mutations identified in independent studies are indicated as flags. These include 17 missense and 1 translocation mutation. Nucleotide changes in DNA and amino acid changes in protein sequence are indicated.

PITX3

Figure 7. Schematic of *PITX3* gene, protein domains, and position of mutations

(A) The human *PITX3* gene (top) consists of four exons (E1-E4), of which exons 2 through 4 encode the PITX3 protein. Exons 2 through 4 encode the homeodomain (pink) and exon 4 encodes the OAR domain (blue). Nucleotide positions for cDNA and genomic DNA in the reference sequence (hg38) and the corresponding amino acid positions in the protein sequence are given. The UCSC genome browser (<https://genome.ucsc.edu>) was used to obtain cDNA, genomic and protein position information from *PITX3* human GRCh38/hg38 assembly (reference sequence NM_005029, uc001kuu.2 at chr10:102,230,186–102,241,474). Total 19 mutations, of which 7 represent unique mutations identified in independent studies are indicated as flags. These include 6 frameshift and 1 missense mutation.

PITX3

Figure 8. Multiple sequence alignment of PITX3 protein sequences

Protein sequences for PITX3 downloaded from UCSC browser for *Homo sapiens*, *Mus musculus*, *Gallus gallus*, *Xenopus tropicalis* and *Danio rerio* shows amino acid conservation across different vertebrate species. Amino acid changes caused by specific human mutations are indicated by yellow arrowheads. The sequence consensus is presented for each amino-acid. Homeodomain and OAR domain are indicated by green arrow.

Table 1

FOXE3 human mutations

Nuc. Pos.	AA Pos.	Vt	AD/ AR	Prot. Func.	A A C on .	P at h o.	A p	A sd	A no	A n	C	C o	FT AA Ds	G	C o l	M c	M t	N y	P a	Sc	V d	Pt. Info.	Ref.
c.21_24del	p.Met7IlefsX216	fs	AR	nr	n	u	y	n	n	n	n	y	n	n	y	n	y	n	n	n	n	Proband	(Iseri et al., 2009)
c.21_24del	p.Met7IlefsX216	fs	AR	nr	n	u	y	n	n	n	n	y	n	y	n	n	n	n	n	n	n	Cousin of proband	
c.21_24del	p.Met7IlefsX216	fs	AR	Exp- d	n	p	y	n	n	n	n	y	n	y	n	n	n	n	n	n	n	Proband	(Islam et al., 2015)
c.146G>C	p.Gly49Ala	ms	AD	Pre-nd	n	p	n	n	n	n	n	n	n	n	y	y	y	n	n	n	n	Proband	(Iseri et al., 2009)
c.146G>C	p.Gly49Ala	ms	AD	Pre-nd	n	p	n	n	n	n	y, cr	n	n	n	n	n	n	n	n	n	n	Mother of proband	
c.146G>C	p.Gly49Ala	ms	AD	Pre-nd	n	p	n	n	n	n	y	n	n	n	n	n	n	n	n	n	n	Maternal grandmother of proband	
c.232G>A	p.Ala78Thr	ms	NA	Pre-d	y	m	n	n	n	n	n	n	n	n	n	n	y	n	n	y	n	Second affected case	(Plaisancié et al., 2017)
c.232G>A	p.Ala78Thr	ms	NA	Pre-d	y	m	n	n	n	n	n	n	n	n	n	n	y	n	y	n	n	Two affected family (2) members (siblings)	(Plaisancié et al., 2017)
c.244A>G	p.Met82Val	ms	NA	nr	y	u	n	n	n	n	n	n	n	n	n	n	y	n	y	y	n	First affected case	(Plaisancié et al., 2017)
c.244A>G	p.Met82Val	ms	NA	nr	y	u	n	n	n	n	n	n	n	n	n	n	y	n		y	n	Two affected family (1) members (siblings)	(Plaisancié et al., 2017)
c.244A>G	p.Met82Val	ms	AR	Pre-d	y	p	y	n	n	n	n	n	n	y	n	n	n	n	n	y	n	Proband	(Iseri et al., 2009)
c.244A>G	p.Met82Val	ms	AR	Pre-d	y	p	n	y	n	n	n	n	n	y	n	n	n	n	n	n	n	Second affected family member (25 years age)	
c.244A>G	p.Met82Val	ms	AR	Pre-d	y	p	n	n	n	n	n	n	n	n	n	n	y	n	n	y	n	Third affected family member (24 years age)	
c.244A>G	p.Met82Val	ms	AR	Pre-d	y	p	n	y	n	n	n	n	n	y	n	n	n	n	n	n	n	Fourth affected family member	
c.244A>G	p.Met82Val	ms	AR	Pre-d	y	p	n	n	n	n	n	n	n	n	n	n	n	n	n	y	n	Fifth affected family member	
c.244A>G	p.Met82Val	ms	AR	Pre-d	y	p	n	n	n	n	n	n	n	n	n	n	n	n	n	y	n	Sixth affected family member	
c.244A>G	p.Met82Val	ms	AR	nr	y	p	n	n	n	n	n	n	n	n	n	n	n	n	n	y	n	Proband	(Reis et al., 2010)
c.269G>T	p.Arg90Leu	ms	NA	Exp- d	y	m	n	n	n	n	n	y	n	y	n	n	n	n	y	n	n	One affected case	(Ormestad et al., 2002)
c.269G>T	p.Arg90Leu	ms	NA	nr	y	u	n	n	n	n	n	n	n	n	n	n	y	n	y	y	n	First affected case	(Plaisancié et al., 2017)
c.269G>T	p.Arg90Leu	ms	AR	Exp- nd	y	m	y	n	n	n	n	y	n	y	n	n	n	n	n	n	n	Proband	(Islam et al., 2015)
c.289A>G	p.Ile97Val	ms	NA	Pre-d	y	m	n	n	n	n	y, eg	n	n	n	n	n	n	n	n	n	n	Proband	(Gillespie et al., 2014)
c.289A>G	p.Ile97Val	ms	AR	Pre-d	y	u	n	y	n	n	n	y	n	n	n	n	y	n	n	n	n	Proband	(Ullah et al., 2016)
c.289A>G	p.Ile97Val	ms	AR	Pre-d	y	u	n	y	n	n	n	y	n	n	n	n	y	n	n	n	n	Five affected family member	
c.292T>C	p.Tyr98His	ms	AR	Pre-d	y	p	y	n	n	n	n	y	n	n	y	n	y	n	n	n	n	Proband	(Ali et al., 2010)

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Nuc. Pos.	AA Pos.	Vt	AD/ AR	Prot. Func.	A A C C on .	P at h o.	A p	A sd	A no	A n	C	C o	FT AA Ds	G	C o I	M c	M t	N y	P a	Sc	V d	Pt. Info.	Ref.
c.292T>C	p.Tyr98His	ms	AR	Pre-d	y	p	y	n	n	n	y	y	n	n	y	n	y	n	n	n	n	Eight affected family member	
c.307G>A	p.Glu103Lys	ms	AR	nr	y	m	n	n	n	y	n	n	n	n	n	n	n	n	n	n	n	Proband	(Khan et al., 2016)
c.307G>A	p.Glu103Lys	ms	AR	Pre-d	y	m	n	n	n	y	n	n	n	n	n	n	n	n	n	n	n	Five affected family member	(Chen et al., 2017)
c.310C>T	p.Arg104Cys	ms	NA	Pre-d	y	m	n	n	n	n	n	n	n	n	n	y	y	n	n	y	n	Second affected case	(Plaisancié et al., 2017)
c.334C>T	p.Pro112Ser	ms	NA	Pre-nd	n	m	n	n	n	n	n	n	y	n	n	n	n	n	n	n	n	Proband	(Kuang et al., 2016)
c.345G>A	p.Trp115X	ns	NA	nr	y	u	n	n	n	n	n	n	n	n	n	n	y	n	n	y	n	Thirld affected case	(Plaisancié et al., 2017)
c.351C>G	p.Asn117Lys	ms	AR	nr	y	m	n	n	n	n	y	y	n	n	n	y	n	y	n	n	n	Proband	(Khan et al., 2016)
c.358C>G	p.Arg120Gly	ms	AR	Exp-d	y	p	y	n	n	n	y	y	n	y	n	n	n	n	n	n	n	Proband	(Islam et al., 2015)
c.410G>A	p.Gly137Asp	ms	NA	Pre-d	y	m	n	n	n	n	n	n	y	n	n	n	n	n	n	n	n	Proband	(Kuang et al., 2016)
c.457G>C	p.Asp153His	ms	AD	Pre-d	y	m	n	n	n	n	n	n	y	n	n	n	n	n	n	n	n	Eight affected family member	(Kuang et al., 2016)
c.466G>A	p.Asp156Asn	ms	NA	Pre-d	y	m	n	n	n	n	n	n	y	n	n	n	n	n	n	n	n	Proband	(Kuang et al., 2016)
c.472G>C	p.Gly158Arg	ms	NA	nr	y	u	n	n	n	n	n	n	n	n	n	n	y	n	n	y	n	Thirld affected case	(Plaisancié et al., 2017)
c.472G>C	p.Gly158Arg	ms	AR	nr	y	m	y	n	n	n	n	n	n	n	n	n	y	n	n	y	n	First affected family member (10 years age)	(Saboo et al., 2017)
c.472G>C	p.Gly158Arg	ms	AR	nr	y	m	y	n	n	n	y	y	n	n	n	n	n	n	n	y	n	Second affected family member (9years age)	
c.472G>C	p.Gly158Arg	ms	AR	nr	y	m	y	n	n	n	n	n	n	n	n	n	n	n	n	y	n	Third affected family member (55 years age)	
c.490C>A	p.Arg164Ser	ms	NA	Pre-d	y	m	n	n	n	n	n	n	y	n	n	n	n	n	n	n	n	Proband	(Kuang et al., 2016)
c.520G>A	p.Ala174Thr	ms	NA	Pre-nd	n	u	n	n	y	n	n	n	n	n	n	n	n	n	n	n	n	Proband	(Jimenez et al., 2011)
NA	p.Gly196Ala	ms	NA	Pre-nd	n	m	n	n	n	n	n	n	y	n	n	n	n	n	n	n	n	Proband	(Kuang et al., 2016)
c.557delT	p.Phe186SerfsX38	fs	AR	nr	n	p	y	y	n	n	n	n	n	n	n	n	n	n	n	y	n	Proband	(Reis et al., 2010)
c.557delT	p.Phe186SerfsX38	fs	AR	nr	n	p	n	n	n	n	y	y	n	n	n	n	n	n	n	n	n	Mother of proband	
c.571_579dup	p.Tyr191_Pro193dup	fs	NA	nr	n	n	n	n	n	y	y	y	n	n	n	n	n	y	n	n	n	Proband	(Brémond-Gignac et al., 2010)
c.685_686insTCCGGAGC	p.Ala230Argfs*2	fs	NA	nr	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	y	n	Proband	(Chassaing et al., 2014)
c.601G>A	p.Val201Met	ms	NA	nr	n	n	n	n	y	n	n	n	n	n	n	y	y	n	n	n	n	Proband	(Jimenez et al., 2011)
c.605C>T	p.Pro202Leu	ms	NA	Pre-nd	n	m	n	n	n	n	n	n	y	n	n	n	n	n	n	n	n	Proband	(Kuang et al., 2016)
c.705delC	p.Glu236SerfsX71	ms	AR	nr	n	p	n	n	n	n	n	n	n	n	n	n	y	n	n	y	n	Proband	(Reis et al., 2010)
c.705del	p.Glu236Serfs71	fs	AR	Exp-d	n	p	y	n	n	n	y	n	n	y	n	n	n	n	n	n	n	Proband	(Islam et al., 2015)
c.720C>A	p.Cys240X	ns	AR	nr	n	m	y	n	n	n	y	y	n	n	n	n	y	n	n	y	n	Seven affected family member	(Ali et al., 2010)

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Nuc. Pos.	AA Pos.	Vt	AD/ AR	Prot. Func.	A A C on .	P at h o.	A p	A sd	A no	A n	C	C o	FT AA Ds	G	C o l	M c	M t	N y	P a	Sc	V d	Pt. Info.	Ref.
c.720C>A	p.Cys240X	ns	AR	nr	n	m	y	n	n	n	n	n	n	n	n	n	n	n	n	n	n	Seven affected family member	(Anjum et al., 2010)
c.720C>A	p.Cys240X	ns	AR	nr	n	m	y	n	n	n	n	n	n	n	n	n	n	n	n	n	n	Proband	(Anjum et al., 2010)
c.720C>A	p.Cys240X	ns	AR	nr	n	n	y	n	n	n	y	n	n	y	n	n	y	n	n	n	n	Proband	(Reis et al., 2010)
c.720C>A	p.Cys240X	ns	AR	nr	n	n	n	n	n	n	y	n	y	y	n	y	y	y	n	n	n	Proband	(Reis et al., 2010)
c.720C>A	p.Cys240X	ns	AR	nr	n	n	n	n	n	n	y	n	y	y	n	n	y	y	n	n	n	Proband	(Reis et al., 2010)
c.720C>A	p.Cys240X	ns	AR	nr	n	n	y	y	n	n	n	n	n	n	n	n	y	n	n	n	n	Three affected family member	(Valleix et al., 2006)
c.720C>A	p.Cys240X	ns	NA	nr	n	n	n	y	n	n	y	n	n	y	n	n	n	n	y	n	n	Proband	(Khan et al., 2016)
c.720C>A	p.Cys240X	ns	NA	nr	n	n	n	n	n	n	n	n	n	n	n	n	y	n	n	n	n	Proband	(Jimenez et al., 2011)
c.720C>A	p.Cys240X	ns	NA	nr	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	y	n	Proband	(Chassaing et al., 2014)
c.Ins(G)943	p.Leu315AlafsX117	fs	NA	nr	y	m	n	n	n	n	y	n	n	n	n	n	n	n	n	n	n	Proband	(Semina et al., 2001)
c.Ins(G)943	p.Leu315AlafsX117	fs	NA	nr	y	m	n	n	n	n	y	n	n	n	n	n	n	n	n	n	n	Mother of proband	
c.958T>C	p.X320AargextX72	ms	AD	nr	NA	p	y	n	n	n	y	n	n	n	y	n	y	n	y	y	n	Proband	(Iseri et al., 2009)
c.958T>C	p.X320AargextX72	ms	AD	nr	NA	p	n	n	n	n	y	n	n	n	n	n	n	n	n	n	n	Mother of proband	
c.958T>C	p.X320AargextX72	ms	AD	nr	NA	p	n	n	n	n	y	n	n	n	y	n	n	n	n	n	n	Maternal uncle of proband	
c.958T>C	p.X320AargextX72	ms	AD	nr	NA	p	n	n	n	n	y	n	n	n	n	n	n	n	n	n	n	Maternal auttof proband	
c.958T>C	p.X320AargextX72	ms	AD	nr	NA	p	y	n	n	n	n	n	n	n	n	n	n	n	n	n	n	Maternal grandmother of proband	
c.959G>T	p.X320L	nt	AD	nr	NA	m	n	n	n	n	y	n	n	n	n	n	n	n	y	n	n	Proband	(Doucette et al., 2011)
c.959G>T	p.X320L	nt	AD	nr	NA	m	n	n	n	n	y	y	n	n	n	y	n	n	n	n	n	Father of proband	
c.959G>T	p.X320L	nt	AD	nr	NA	m	n	n	n	n	y	n	n	n	n	y	n	n	n	n	n	Three paternal aunts of proband	
c.959G>T	p.X320L	nt	AD	nr	NA	m	n	n	n	n	y, cg	n	n	n	n	n	n	n	n	n	n	Three paternal cousins of proband	
c.959G>T	p.X320L	nt	AD	nr	NA	m	n	y	n	n	y	y	n	n	n	n	n	n	n	n	n	Two affected family members of proband	
c.959G>C	p.X320SerextX72	ns	AD	nr	NA	n	n	n	n	n	y, cg	n	n	n	n	n	n	n	n	n	n	Proband	(Brémond-Gignac et al., 2010)
NA	p.Ser300Gly	ms	NA	Pre-nd	NA	m	n	n	n	n	n	n	y	n	n	n	n	n	n	n	n	Proband	(Kuang et al., 2016)

Notes: NA, not reported in original article; Nuc. Pos., nucleotide position in CDS; AA Pos., amino acid position; Vt, variant type; fs, frameshift; ms, missense; ns, nonsense; nt, nonstop; AD, autosomal dominant; AR, autosomal recessive; NA, not available; Prot. Func., protein function; nr, not reported; Exp-d, experimental-damaging; Exp-nd, experimental-not damaging; Pre-d, predicted-damaging; Pre-nd, predicted-not damaging; n, no; y, yes; AA con., amino acid conservation; Patho., pathogenicity; Ap, Aphakia; Asd, Anterior segment dysgenesis; Ato, Anophthalmia; An, Aniridia; C, Cataract; cr, cerulean; eg, congenital; Co, Corneal opacity/abnormal cornea; Fe, Facial characteristics; FTAADs, Familial thoracic aortic aneurysms and acute aortic dissections; G, Glaucoma; Col, Iris coloboma/coloboma/abnormal iris; Mc, Microcornea; Mt, Microphthalmia; Ny, Nystagmus; Pa, Peters anomaly; Sc, Sclerocornea; Vd, vitreoretinal dysplasia; Pt. Info., patient information; Ref., reference

HSP4 human mutations

Table 2

Nuc. Pos.	AA Pos.	Vt	AD/AR	Prot. Func.	AA Con.	Patho.	C	Ny	Pt. info.	Ref.
c. 57C>A (c. 53C>A *)	p.Ala19Asp (p.Ala20Asp *)	ms	AD	nr	y	m	y, la	n	Sixty-nine sporadic cases	(Bu et al., 2002)
c. 57C>A (c. 53C>A *)	p.Ala19Asp (p.Ala20Asp *)	ms	AD	nr	y	m	y, la	n	One affected family member	
c.69G>T	p. Lys23Asn	ms	AD	Pre-d	y	p	y, eg, cr	n	Proband	(Lv et al., 2014)
c.69G>T	p. Lys23Asn	ms	AD	Pre-d	y	p	y, ct	n	Eight affected family members of proband	
c.103C>T	p.His35Tyr	ms	NA	Pre-d	y	m	y, eg	y	Proband	(Gillespie et al., 2014)
c.103C>T	p.His35Tyr	ms	NA	Pre-d	y	m	y, eg	y	Mother of proband	
c.179C>A	p.Pro60His	ms	NA	Pre-d	y	m	y, nu	n	One non-familial sporadic affected	(Li et al., 2016)
c.182A>G (c. 1078A>G *)	p.Gln61Arg	ms	NA	nr	n	m	y, se	n	one out of one-fifty cases	(Shi et al., 2008)
c.190A>G	p.Lys64Glu	ms	AD	Pre-d	y	m	y, la	n	Seven affected family members	(Berry et al., 2017)
c.218G>A (c.221G>A *)	p.Arg73His (p.Arg74His *)	ms	AD	nr	y	p	y, eg	n	Seven affected family members	(Ke et al., 2006)
c. 256A>G	p.Ile86Val (p.Ile87Val *)	ms	AD	nr	y	m	y, la	n	Proband	(Bu et al., 2002)
c. 256A>G	p.Ile86Val (p.Ile87Val *)	ms	AD	nr	y	m	y, se	n	Father of proband	
c.331C>T	p.Arg110Cys (p.Arg111Cys *)	ms	AD	nr	n	m	y, eg	n	Six affected family members	(Liu et al., 2015)
c.341T>C (c.348T>C *)	p.Leu114Pro (p.Leu115Pro *)	ms	AD	nr	y	m	y, la	n	Proband	(Bu et al., 2002)
c.341T>C (c.348T>C *)	p.Leu114Pro (p.Leu115Pro *)	ms	AD	nr	y	m	y, la	n	Thirty affected family members	
c.341T>C	p.Leu114Pro	ms	NA	nr	y	m	y, la	n	Four affected family members	(Hansen et al., 2009)
c.341T>C	p.Leu114Pro	ms	NA	nr	y	m	y, po	n	One affected family member	
c.355C>T (c.362C>T *)	p.Arg119Cys (p.Arg120Cys *)	ms	AD	nr	y	m	y, ma	n	Proband	(Bu et al., 2002)
c.355C>T	p.Arg119Cys	ms	NA	nr	y	m	y, la	n	Two affected family members	(Hansen et al., 2009)
c.521T>C	p.Leu174Pro	ms	AR	Pre-d	y	m	y, eg	y	Proband	(Behnam et al., 2016)
c.521T>C	p.Leu174Pro	ms	AR	Pre-d	y	m	y, eg	y	Brother of proband	
c.524G>C	p.Arg175Pro	ms	AR	nr	y	m	y, eg, ct	n	Five affected family members (siblings)	(Forsheaw et al., 2005)
c.595_599delGGGCC	p.Gly199GlufsX15	fs	AR	nr	n	m	y, eg	n	Five affected family members	(Forsheaw et al., 2005)
c.1213C>T	p.Arg405X	ns	AR	nr	n	m	y, eg, ec	n	Eight affected family members	(Sajjad et al., 2008)

Nuc. Pos.	AA Pos.	Vt	AD/AR	Prot. Func.	AA Con.	Patho.	C	Ny	Pt. info.	Ref.
c.1327+4A>G	p.Met419GlyfsX29	fs	AR	nr	y	m	y, eg, to	n	Twenty-two affected family members	(Smaoui et al., 2004)

Notes: NA, not reported in original article;

* indicates nucleotide and amino acid positions as indicated in the original article; Nuc. Pos., nucleotide position in CDS; AA Pos., amino acid position; Vt, variant type; fs, frameshift; ms, missense; ns, nonsense; nt, nonstop; AD, autosomal dominant; AR, autosomal recessive; NA, not available; Prot. Func., protein function; nr, not reported; Pre-d, predicted-damaging; n, no; y, yes; AA con., amino acid conservation; Patho., pathogenicity; p, pathogenic; m, may be pathogenic; m, mamer; nu, nuclear; po, polar; se, senile; to, total; Ny, Nystagmus; Pt. Info., patient information; Ref., reference

Table 3

MAFhuman mutations

Nuc. Pos.	AA Pos.	Vt	AD/ AR	Prot. Func.	AA Con.	Pat ho.	Ap	C	C o	F c	G	H	C o l	M c	M t	N y	P a	Sy	Pat. Info.	Ref.
c.161C>T	p.Ser54Leu	ms	NA	Pre-d	y	m	n	y	n	y	n	y	n	n	n	n	n	Ayme-Gripp	Proband (age 39 years, female)	(Aymé and Philip, 1996; Niceta et al., 2015)
c.161C>T	p.Ser54Leu	ms	NA	Pre-d	y	m	n	y	n	y	n	y	n	n	n	n	n	Ayme-Gripp	Proband (age 43 years, female)	(Niceta et al., 2015)
c.172A>G	p.Thr58Ala	ms	NA	Pre-d	y	m	n	y	n	y	n	y	n	n	n	n	n	Ayme-Gripp	Proband (age 27 years, female)	(Gripp et al., 1996; Niceta et al., 2015)
c.173C>T	p.Thr58Ile	ms	NA	Pre-d	y	m	n	y	n	y	n	y	n	n	n	n	n	Ayme-Gripp	Proband (age 22 monthss, male)	(Nakane et al., 2002; Niceta et al., 2015)
c.176C>G	p.Pro59Arg	ms	NA	Pre-d	y	m	y	y, eg, nu	n	y	y	y	n	n	n	n	n	Asperger	Proband	(Javadiyan et al., 2017)
c.176C>G	p.Pro59Arg	ms	NA	Pre-d	y	m	n	y	n	y	n	y	n	n	n	n	n	n	Mother of proband	
c.176C>A	p.Pro59His	ms	NA	Pre-d	y	m	n	y	n	y	n	y	n	n	n	n	n	Ayme-Gripp	Proband (age 9 years, female)	(Keppler-Noreuil et al., 2007; Niceta et al., 2015)
c.176C>A	p.Pro59His	ms	NA	Pre-d	y	m	n	y	n	y	n	y	n	n	n	n	n	Ayme-Gripp	Proband (age 5 years, female)	(Niceta et al., 2015)
c.185C>G	p.Thr62Arg	ms	NA	Pre-d	y	m	n	y	n	y	n	y	n	n	n	n	n	Ayme-Gripp	Proband (age 21 months, male)	(Niceta et al., 2015)
c.206C>G	p.Pro69Arg	ms	NA	Pre-d	y	m	n	y	n	y	n	y	n	n	n	n	n	Ayme-Gripp	Proband (age 21 months, male)	(Gripp et al., 1996; Niceta et al., 2015)
c.809C>A	p.Ser270Tyr	ms	AD	Pre-d	y	m	n	y, nu	n	n	n	n	n	y	n	y	n	n	Proband	(Dudakova et al., 2017)
c.809C>A	p.Ser270Tyr	ms	AD	Pre-d	y	m	n	y, eg, nu	n	n	n	n	n	y	n	y	n	n	Half brother of proband	
c.819G>C	p.Glu273Asp	ms	AD	Pre-d	y	m	n	y, cr	n	n	n	n	n	n	n	n	n	n	Two affected family members (father and son)	(Ma et al., 2016)
c.864G>C	p.Arg288Pro	ms	AD	nr	y	m	n	y, ct, nu	n	n	n	n	y	y	n	n	n	n	Two affected family members	(Jamieson et al., 2002)
c.880C>T	p.Arg294Trp	ms	AD	Pre-d	y	m	n	y, eg	n	n	n	n	n	n	n	n	n	n	Probands from thirty families	(Ma et al., 2016)
c.880C>T	p.Arg294Trp	ms	AD	Pre-d	y	m	n	y, nu	n	n	n	n	n	n	n	y	n	n	Seven affected family members	(Sun et al., 2014)
c.890A>G	p.Lys297Arg	ms	AD	nr	y	m	n	y, cr	n	n	n	n	n	n	n	n	n	n	Proband (8 years age)	(Vanita et al., 2006)
c.890A>G	p.Lys297Arg	ms	AD	nr	y	m	n	y	n	n	n	n	n	n	n	n	n	n	Five affected family members	
c.890A>G	p.Lys297Arg	ms	AD	nr	y	m	n	y	n	n	n	n	y	y	n	n	n	n	Six affected family members	
c.895C>A	p. Arg299Ser	ms	NA	nr	y	m	n	y, eg, po, nu	n	n	n	n	y	n	n	n	n	n	Four affected family members	(Hansen et al., 2007, 2009)
c.908A>C	p.Gln303Leu	ms	NA	Pre-d	y	m	n	y, eg	n	n	n	n	n	n	n	n	n	n	Proband	(Narumi et al., 2014)
c.908A>C	p.Gln303Leu	ms	NA	Pre-d	y	m	n	y, eg	n	n	n	n	n	n	n	n	n	n	Mother of proband	
c.908A>C	p.Gln303Leu	ms	NA	Pre-d	y	m	n	y	n	n	n	n	n	n	n	n	n	n	Maternal grandmother of proband	
c.908A>C	p.Gln303Leu	ms	NA	Pre-d	y	m	n	y, la	n	n	n	n	y	y	n	n	n	n	First maternal cousin of proband	
c.908A>C	p.Gln303Leu	ms	NA	Pre-d	y	m	n	y, nu	n	n	y	n	n	n	n	n	n	n	Second maternal cousin of proband	

Nuc. Pos.	AA Pos.	Vt	AD/ AR	Prot. Func.	AA Con	Pat ho.	Ap	C	C o	F c	G	H	C o l	M c	M t	N y	P a	Sy	Pat. Info.	Ref.	
c.908A>C	p.Gln303Leu	ms	NA	Pre-d	y	m	n	y	n	n	n	n	n	y	n	n	n	n	Mother and sister of second maternal cousin of proband		
c.915C>T	p.Cys305Trp	ms	AD	Pre-d	y	m	n	y, eg	n	n	y	n	n	n	n	n	n	n	Proband	(Ma et al., 2016)	
c.958A>G	p.Lys320Glu	ms	NA	nr	y	m	n	y, eg	n	n	n	n	n	n	n	n	n	n	Proband	(Hansen et al., 2009)	
c.958A>G	p.Lys320Glu	ms	NA	nr	y	m	n	n	n	n	n	n	n	y	n	n	n	n	One affected family member		
t(5;16)(p15.3;q23.2)	-	-	NA	nr	y	m	n	y	n	n	n	n	n	n	n	n	n	n	Two affected family member	(Jamieson et al., 2002)	
t(5;16)(p15.3;q23.2)	-	-	NA	nr	y	m	n	y, eg	y	n	n	n	y	n	y	n	y	n	Other affected family members		

Notes: NA, not reported in original article; Nuc. Pos., nucleotide position in CDS; AA Pos., amino acid position; Vt, variant type; fs, frameshift; ms, missense; ns, nonsense; nt, nonstop; AD, autosomal dominant; AR, autosomal recessive; NA, not available; Prot. Func, protein function; nr, not reported; Pre-d, predicted- damaging; n, no; y, yes; AA con., amino acid conservation; Patho., pathogenicity; m, maybe pathogenic; Ap, Aphakia; C, Cataract; cr, cerulean; eg, congenital; ct, cortical; la, lamellar; nu, nuclear; po, polar; Co, Corneal opacity/ abnormal cornea; Fc, Facial characteristics; G, Glaucoma; H, Hearing loss; Col, Iris coloboma/coloboma/abnormal iris; Mc, Microcornea; Mt, Microphthalmia; Ny, Nystagmus; Pa, Peters anomaly; Sy, Syndrome; Pt. Info., patient information; Ref., reference

Table 4

PITX3 human mutations

Nuc. Pos.	AA Pos.	Vt	AD/AR	Prot. Func.	AA Con.	Patho.	Ap	Asd	C	Co	G	Mc	Mt	Ny	Sc	Pat. Info.	Ref.
c.94C>A	p.Ser13Asp	ms	AD	nr	y	m	n	n	y, cg	n	n	n	n	n	n	Proband	(Semina et al., 1998)
c.542delC	p.Pro181LeufsX127	fs	AD	nr	y	m	n	n	y, po	n	n	n	n	n	n	Proband	(Berry et al., 2011)
c.542delC	p.Pro181LeufsX127	fs	AD	nr	y	m	n	n	y, po	n	n	n	n	n	n	Eight affected family members	
c.573del	p.Ser192AlafsX117	fs	AD	Exp-d	n	m	n	n	y, cg	y	n	y	n	n	n	Proband	(Verdin et al., 2014)
c.573del	p.Ser192AlafsX117	fs	AD	Exp-d	n	m	n	n	y, cg	y	n	y	n	n	n	Brother of proband	
c.573del	p.Ser192AlafsX117	fs	AD	Exp-d	n	m	n	n	y, cg	n	n	y	n	n	n	Father of proband	
c.608delC	p.Ala203fs	fs	NA	nr	n	m	n	n	y, cg	n	n	n	n	n	n	Seven affected family members	(Liu et al., 2017)
c.640_656del	p.Ala214ArgfsX42	fs	AR	nr	y	m	n	y	y, cg	n	n	n	y	n	y	Proband	(Aldahmesh et al., 2011)
c.650delG	p.Gly217AlafsX91	fs	AD	nr	n	m	n	n	y, po	n	n	n	n	n	n	Six affected family members	(Berry et al., 2004)
c.650delG	p.Gly217AlafsX91	fs	AD	nr	n	m	n	n	y, po	y	n	n	y	n	n	Twenty-eight affected family members	Bidinosti et al 2006
c.657_673dup17	p.Gly220ProfsX95	fs	NA	nr	n	m	n	y	n	n	n	n	n	n	n	Eight affected family members	(Semina et al., 1998)
c.657_673dup17	p.Gly220ProfsX95	fs	AD	nr	n	m	n	n	y, cg, po, ec	n	n	n	n	n	n	Nine affected family members	(Berry et al., 2004)
c.657_673dup17	p.Gly220ProfsX95	fs	AD	nr	n	m	n	y	y, cg, po, ec	n	n	n	n	n	n	One affected family members	
c.657_673dup17	p.Gly220ProfsX95	fs	AD	nr	n	m	n	n	y, po	n	n	n	n	n	n	Seven affected family members	(Berry et al., 2004)
c.657_673dup17	p.Gly220ProfsX95	fs	AD	nr	n	m	n	y	y, po	n	n	n	n	n	n	Four affected family members	
c.657_673dup17	p.Gly220ProfsX95	fs	AD	nr	n	m	n	n	y, po	n	n	n	n	n	n	Seventeen affected family members	(Berry et al., 2004)
c.657_673dup17	p.Gly220ProfsX95	fs	AD	nr	n	m	n	n	y, po	n	n	n	n	n	n	Eighteen affected family members	(Berry et al., 2004)
c.657_673dup17	p.Gly220ProfsX95	fs	AD	nr	n	m	n	n	y, po	n	n	n	n	n	n	Twenty affected family members	(Finzi et al., 2005)

Nuc. Pos.	AA Pos.	Vt	AD/AR	Prot. Func.	AA Con.	Patho.	Ap	Asd	C	Co	G	Mc	Mt	Ny	Sc	Pat. Info.	Ref.
c.657_673dup17	p.Gly220ProfsX95	fs	AD	nr	n	m	y	n	y, po	n	n	n	n	y	n	One affected family member	(Burdon et al., 2006)
c.657_673dup17	p.Gly220ProfsX95	fs	AD	nr	n	m	n	n	y, po	n	n	n	n	n	n	Twenty eight affected family member	
c.657_673dup17	p.Gly220ProfsX95	fs	AD	nr	n	m	n	n	y	y	n	n	n	n	n	Proband	(Summers et al., 2008)
c.657_673dup17	p.Gly220ProfsX95	fs	AD	Exp-d	n	m	n	n	y	y	n	n	n	n	n	Proband	(Verdin et al., 2014)
c.657_673dup17	p.Gly220ProfsX95	fs	AD	Exp-d	n	m	n	y	y, cg	n	n	n	n	n	n	Brother of proband	
c.657_673dup17	p.Gly220ProfsX95	fs	AD	Exp-d	n	m	n	n	y, cg	n	n	n	n	n	n	Father of proband	
c.657_673dup17	p.Gly220ProfsX95	fs	AD	Exp-d	n	m	n	n	y, cg	y	y	y	n	y	n	Proband	(Verdin et al., 2014)
c.657_673dup17	p.Gly220ProfsX95	fs	AD	Exp-d	n	m	n	n	y, cg	n	n	n	n	n	n	Five affected family members	
c.657_673dup17	p.Gly220ProfsX95	fs	AD	Exp-d	n	m	n	y	y, cg	y	n	n	n	n	n	Proband	(Verdin et al., 2014)
c.657_673dup17	p.Gly220ProfsX95	fs	AD	Exp-d	n	m	n	n	y, cg	n	n	n	n	n	n	Brother of proband	
c.657_673dup17	p.Gly220ProfsX95	fs	AD	Exp-d	n	m	n	n	y, cg	n	n	n	n	n	n	Mother of proband	
c.657_673dup17	p.Gly220ProfsX95	fs	AD	Exp-d	n	m	n	n	y, cg	y	n	n	n	y	n	Proband	(Verdin et al., 2014)
c.657_673dup17	p.Gly220ProfsX95	fs	AD	Exp-d	n	m	n	n	y, cg	n	n	n	n	n	n	Five affected family members	

Notes: NA, not reported in original article; Nuc. Pos., nucleotide position in CDS; AA Pos., amino acid position; Vt, variant type; fs, frameshift; ms, missense; nt, nonsense; nr, not reported; Prot. Func., protein function; nr, not available; Exp-d, experimental-damaging; n, no; y, yes; AA con., amino acid conservation; Patho., pathogenicity; m, maybe pathogenic; Ap, Aphakia; Asd, Anterior segment dysgenesis; C, Cataract; cg, congenital; ec, early childhood; po, polar; Co, Corneal opacity/abnormal cornea; G, Glaucoma; Mc, Microcornea; Mt, Microphthalmia; Ny, Nystagmus; Sc, Sclerocornea; Pt, patient information; Ref., reference