FOXE3 mutations: genotype-phenotype correlations

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ABSTRACT

Microphthalmia and anophthalmia (MA) are severe developmental eye anomalies, many of which are likely to have an underlying genetic cause. More than 30 genes have been described, each of which is responsible for a small percentage of these anomalies. Amongst these, is the *FOXE3* gene, which was initially described in individuals with dominantly inherited anterior segment dysgenesis and, subsequently, associated with recessively inherited primary aphakia, sclerocornea and microphthalmia. In this work, we describe 8 individuals presenting with a MA phenotype. Among them, 7 are carrying biallelic recessive *FOXE3* mutations and 2 of these have novel mutations: p.(Ala78Thr) and p.(Arg104Cys). The last of our patients is carrying in the heterozygous state the recessive p.(Arg90Leu) mutation in the *FOXE3* gene. To further understand *FOXE3* involvement in this wide spectrum of ocular anomalies with two different patterns of inheritance, we reviewed all individuals with ocular abnormalities described in the literature for which a *FOXE3* mutation was identified. This review demonstrates that correlations exist between the mutation type, mode of inheritance and the phenotype severity. Furthermore, understanding the genetic basis of these conditions will contribute to overall understanding of eye development, improve the quality of care, genetic counseling and, in future, gene based therapies.

Keywords: anophthalmia, anterior segment dysgenesis, aphakia, cataract, eye development, *FOXE3*, genotype-phenotype correlations, microphthalmia.

INTRODUCTION

Eye development is driven by a complex network of molecular interactions, which depends on the precise dosage of numerous transcription factors acting via several signaling pathways. This process has been highly conserved throughout the evolution of vertebrates and any disruption to these events has the potential to generate an ocular anomaly. The different parts of the eye can be affected, resulting in anterior and/or posterior segment dysgenesis, as well as the entire globe, leading to microphthalmia or anophthalmia (MA).

Anterior segment dysgeneses encompass a wide spectrum of developmental disorders affecting the cornea, iris and lens. They are generally associated with ~50% risk for glaucoma (1). Some specific diagnostic entities exist, such as Peters' anomaly, which refers to the association of corneal opacity, defects in the posterior layers of the cornea and iridolenticulocorneal adhesions.

MA malformations represent the most severe ocular anomalies and their prevalence is estimated to be between 0.6 and 3.2/10,000 infants (2). Anophthalmia refers to complete absence of the globe in the presence of ocular adnexa, whereas microphthalmia refers to a reduced total axial length of the globe that is at least 2 standard deviations below the mean for age. Microphthalmia is classified as simple, or complex, when associated with anterior and/or posterior segment dysgenesis, and graded depending on the degree of axial length reduction. Microphthalmia is often associated with coloboma which corresponds to the failure of closure of the optic fissure, extending inferonasally from the anterior to the posterior portion of the eye, during embryogenesis. Coloboma can affect the vision especially when located in the posterior segment of the eye, and encroaching on the visual axis or involving the optic nerve.

The visual consequences of these ocular developmental defects are highly dependent on the associated ocular anomalies and unilateral or bilateral involvement. For example, Shah *et al.* (3) reported that 81% of microphthalmic eyes and 93% of microphthalmic eyes with coloboma were considered to have reduced vision. In a cohort of 98 patients with congenital corneal opacities (3/4 of whom had Peters' anomaly), the best corrected visual acuity was

impaired in 81% of affected eyes of bilaterally affected patients (4). In addition, these ocular anomalies can be isolated or associated with extraocular anomalies (5).

Although diverse, genetic origin appears to be the predominant cause of congenital ocular anomalies and recent advances in DNA sequencing technologies have significantly increased our knowledge of the underlying MA genes (6). The importance of their recognition and understanding is undeniable in terms of genetic counselling and prenatal diagnosis. Nevertheless, despite the identification of more than 30 genes in MA, mutations in each of these genes explain the symptoms in only a small percentage of patients. Indeed, overall the genetic cause is determined in around 30% of all MA patients, higher in severe MA cases (5- 7). The major gene still remains the *SOX2* gene (MIM#184429), contributing to the cause in 10-15% of individuals usually with a severe MA phenotype, then *OTX2* (MIM#600037) (2- 5%), *RAX* (MIM#601881) (3%), *PAX6* (MIM#607108) (2%) and *FOXE3* (MIM#601094) (2.5%) (6). The main genes involved in ASD are *PAX6* (MIM#607108), *CYP1B1* (MIM*601771) *PITX2* (MIM*601542), *FOXC1* (MIM*601090) and also *FOXE3* (MIM#601094) (1).

Only a few studies have determined the mutation detection rate using whole exome sequencing (WES). Deml B *et al.* (8) screened 28 probands affected by microphthalmia, anophthalmia and coloboma with no mutations in *SOX2* or *FOXE3*. They analyzed 83 genes involved in ocular development and identified mutations in 3 patients (11%). Weh E *et al.* (9) screened 27 patients affected by Peters' anomaly by WES and found 6 causative mutations (22%). Thus, to date, WES approach did not demonstrate a much higher mutation detection rate in patients with ocular developmental defects compared with the targeting sequencing of the main known genes. This approach had mainly led to the identification of new genes in particular pedigrees (10-12).

The *FOXE3* gene encodes a transcription factor of 319 amino acids with a DNA-binding domain, the forkhead domain, located from amino acid 71 to 165 (13). *FOXE3* is specifically expressed during lens development in human, mouse and zebrafish (14-16). It participates in lens vesicle formation and allows the maintenance of the lens cells in a proliferative state, thus preventing their early differentiation into lens fiber cells (14, 17). *FOXE3* is known to be one of the master gene *PAX6* targets (18) and, recently, Khan SY (19) demonstrated that FOXE3 acts through a downstream transcriptional target named *DNAJB1*, which has a crucial

role in the development and maintenance of lens transparency.

Homozygous *Foxe3* mutations were first identified in *dyl/dyl* mice displaying spontaneous microphthalmia associated with anterior segment abnormalities analogous to Peters' anomaly and cataract in the human (14). The biallelic mutation identified in this *dyl* murine model is located in the *Foxe3* forkhead domain and prevents the binding of the Foxe3 protein to its DNA targets (13). In humans, mutations in *FOXE3* were initially described in individuals with dominantly inherited anterior segment dysgeneses (13, 15). Subsequently, biallelic mutations were associated with primary congenital aphakia (20) and microphthalmia (21). In addition, Kuang SQ *et al*. (22) recently suggested that specific mutations located in a particular region of the forkhead domain (amino acids 137-164) may be responsible for familial forms of thoracic aorta dilatation (TAAD).

Thus, the potential role of *FOXE3* in a wide range of phenotypes with different patterns of inheritance prompted us to review all the individuals with ocular anomalies for which a *FOXE3* mutation was identified in our laboratory and to compare this with published cases, to determine any genotype-phenotype correlation associated with mutations of this gene.

METHODS

This study was designed in compliance with the tenets of the Declaration of Helsinki and patient enrollment had approval by our local Ethics Committees (Cambridgeshire 1 Ethics Committee 04/Q0104/129 and CPP Sud-Ouest et Outre-Mer II). DNA from 8 new individuals presenting with a MA phenotype underwent Sanger sequencing of the coding regions and splice sites, combined with the investigation of exonic rearrangements (by semi-quantitative methods) of major genes responsible for non-syndromic MA. Of note, the *SOX2* gene was first analyzed and then, if no mutation was identified, the analysis of the *OTX2, PAX6, RAX* and *FOXE3* genes was performed. Segregation analysis of any identified mutation was performed in each family (parents and other affected family members when DNA was available).

We then assembled all the clinical and genetic data available on individuals with ocular anomalies for which a *FOXE3* mutation was identified and their relatives, from an exhaustive review of the literature using the *PubMed* database. Ocular phenotypes were classified into

two groups: (i) a so-called “severe” phenotype for microphthalmia (complex or not) and/or primary congenital aphakia and (ii) a so-called “mild” phenotype grouping anomalies of the anterior segment of the eye (such as posterior embryotoxon, Peters’ anomaly and congenital cataract).

We categorized panocular involvement, such as microphthalmia and primary aphakia, as more severe in terms of developmental anomaly, compared to anomalies limited to the anterior segment, which were therefore considered as milder anomalies.

After phenotypic categorization, we performed an analysis of the relationship between, (i) the type of mutation and the mode of transmission, (ii) the severity of the phenotype and the mode of transmission and (iii) the severity of the phenotype and the type of mutation.

For studying the link between the mutation type and the severity of the phenotype, we used a logistic regression model. Since some subjects belonged to the same family, we also performed a logistic regression using the index cases alone. We present the marginal effects with their confidence intervals, i.e., the average predicted probabilities for each group. Concerning the link between the position of the truncating mutations and the severity of phenotype, we use a Fisher's exact test, as the conditions for using chi-square test or logistic regression were not met. Since some patients belonged to the same family, we also performed an analysis on the index cases only.

P-values <0.05 were considered as significant (Wald test). Statistical analyses were performed with STATA release 14.

RESULTS

From our cohort and the published literature, this work includes a study of the phenotype and the genotype of 123 patients (38 index cases and 85 family members) with a total of 20 different *FOXE3* mutations (Fig.1). Of these, 102 patients have been identified with a biallelic mutation of *FOXE3* (compound heterozygous or homozygous) and 21 patients with heterozygous mutations.

Nature and location of the *FOXE3* mutations

Mutations of various types have been reported: stop, frameshift, missense and no-stop. The position of these mutations is summarized in Figure 1. It appears that truncating mutations (stop or frameshift) are distributed throughout the gene, unlike missense mutations, which are all located in the forkhead domain. Mutations affecting the stop codon (p.(Ter320Argext*72), p.(Ter320Leuext*72) and p.(Ter320Serext*72)) are predicted to lead to a C-terminal extension of the protein, while preserving the entire FOXE3 sequence. Of note, a 3'end frameshift mutation (p.(Leu315AlafsTer117)) described here (Table 1) also results in a C-terminal extension with a conserved FOXE3 sequence (excepted for the 5 last amino acids of the protein) while all the other frameshift mutations described here are otherwise truncating.

Monoallelic *FOXE3* mutations (Table 1)

Among the 21 patients described with a unique *FOXE3* mutation, 17 have a no-stop mutation and 2 cases (an index case and a parent of that case) have the frameshift mutation p.(Leu315AlafsTer117), which similarly results in a C-terminal extension of the protein. Then, two individuals, one from our cohort and one described by Ormestad M. *et al.* (13) demonstrated the heterozygous p.(Arg90Leu) missense mutation that had previously always been associated with another *FOXE3* pathogenic allele and associated with no phenotype in heterozygous subjects. Moreover, the individual from our cohort, who presented with bilateral anophthalmia, was carrying, in addition to the heterozygous p.(Arg90Leu) in the *FOXE3* gene (inherited from her asymptomatic father), a *de novo* truncating mutation (p.(Ala198IlefsTer63)) in the *OTX2* gene. The ocular phenotype observed in this patient is attributed to the *OTX2* mutation rather than to the *FOXE3* mutation (although a modifying effect of the p.(Arg90Leu) in *FOXE3* cannot be completely excluded). As the p.(Arg90Leu) mutation is thought to be associated with recessive inheritance, these two latter patients were excluded from our genotype-phenotype correlation studies.

Of the 19 patients analyzed, 18 showed isolated, but variable anterior segment anomalies. Only one patient reported in the literature (21) with a "dominant" mutation in the *FOXE3* gene displayed a severe ocular phenotype. This patient had the no-stop p.(Ter320Argext*72) mutation and bilateral complex microphthalmia whereas her affected relatives

demonstrated milder ocular phenotypes (anterior segment anomalies) (Table 1).

Thus, the vast majority of patients with a dominant form (18/19, namely 95%) have isolated anterior segment dysgeneses, and all harbored a mutation leading to a C-terminal extension of the protein.

Biallelic *FOXE3* mutations (Table 2)

A total of 102 patients with a biallelic *FOXE3* mutation are reported here. Clinical and genetic data for all these patients and their relatives are summarized in Table 2. Among them, 7 are new patients from our cohort and 2 of these have novel mutations: c.232G>A (p.(Ala78Thr)) and c.310C>T (p.(Arg104Cys)). Eye picture of patient 1 from our cohort (Table 2) is shown in Figure 2.

The c.232G>A mutation (rs377669670) is listed solely as heterozygote in the gnomAD database with an overall occurrence of 8 in 216582 alleles which includes 97090 alleles from European population. The low frequency of the p.(Ala78Thr) mutation in gnomAD and its segregation in two unrelated pedigrees reported here (patient 2 and family 2) strongly argues the pathogenic nature of this mutation in the homozygous (family 2) or compound heterozygous (patient 2) state. Moreover, this novel missense mutation affects a conserved amino acid located in the functional forkhead domain and is predicted pathogenic by *in silico* analyses (PolyPhen-2, MutationTaster, SNP&GO and SIFT software).

The c.310C>T (p.(Arg104Cys)) is also listed solely as heterozygote in the gnomAD database with an overall occurrence of 1 in 244576 alleles which includes 110856 alleles from European population. This variant is therefore extremely rare. This novel missense mutation affects a conserved amino acid located in the functional forkhead domain and is predicted pathogenic by *in silico* analyses (PolyPhen-2, MutationTaster, SNP&GO and SIFT software). These data strongly argue the pathogenic nature of thisvariant.

Thus, p.(Ala78Thr) and p.(Arg104Cys) are categorized as “likely pathogenic” variants according to the ACMG guidelines (23). These mutations were entered into the ClinVar database (respectively SCV000579340 and SCV000579341).

Out of all the individuals with biallelic mutations, 77% (79/102) presented with a severe ocular phenotype (eye growth anomalies with or without anterior segment dysgenesis, or primary congenital aphakia) and 23% (23/102) exhibited isolated anterior segment anomalies. The presence of an extraocular phenotype (Arnold-Chiari malformation, global developmental delay, autism, hypertrichosis, polycystic ovary syndrome, ventricular septal defect, renal pelvic dilatation and umbilical hernia) was only seen in individuals who also had severe ocular phenotypes.

When considering the nature of the mutation, 37% (38/102) of the patients have 2 truncating mutations while 60% (61/102) have 2 missense mutations in the *FOXE3* gene. Three of them (3%) are compound heterozygote for a missense mutation and a truncating mutation (Table 3). It is worth noting that all relatives heterozygous for these mutations are asymptomatic, suggesting a recessive mode of inheritance.

The analysis of the mutation type and the phenotype severity in the recessive forms (Table 3) shows that 90% of patients with at least one truncating mutation (37/41) displayed a severe ocular phenotype, sometimes (22%) associated with extraocular features, while 69% (42/61) with biallelic missense mutations had a severe ocular phenotype which was always isolated. In addition, only 4 of the 23 (17%) patients with anterior segment dysgenesis have 2 truncating mutations while this phenotype was observed in 19 of the 61 patients (31%) with 2 missense mutations.

Statistical analyses of the data above (Table 4) show that the *FOXE3* mutation type has a strong influence on the severity of the ocular and extraocular phenotype ($p<0.05$). Indeed, when considering all the patients, the average predicted probability of them presenting with a severe ocular phenotype was 88.2% (95% CI=[77.4;99.1]) for patients with at least one truncating mutation and 68.9% (95% CI=[57.2;80.5]) for patients with 2 missense mutations ($p=0.016$). When we performed the same analysis on index cases alone (32 families) in order to see if we could separate the mutation effect and the family effect, we observed exactly the same tendency, but the difference between the groups was no longer statistically significant ($p=0.208$). Indeed, the rarity of the disease meant that there was an insufficient number of index cases to analyze.

Thus, truncating *FOXE3* mutations are more frequently associated with a severe phenotype than missense mutations. In addition to the severe ocular phenotype, truncating mutations are associated with a phenotype that includes extraocular features in one quarter of patients.

Furthermore, given that truncating mutations, unlike missense mutations, are distributed throughout the *FOXE3* gene, we studied a possible correlation between their position in relation to the forkhead domain and the phenotypic severity. No significantly statistical correlation could be established (Table 5).

DISCUSSION

We report here 8 new individuals with mutations in the *FOXE3* gene, a gene that has been involved in a wide spectrum of ocular anomalies with different patterns of inheritance. Thus, in order to bring appropriate genetic counselling to these families, we reviewed all the ocular phenotype of individuals with *FOXE3* mutations reported in the literature, to determine if genotype-phenotype correlations could be drawn.

This meta-analysis demonstrates that recessive cases are more frequent than dominant (84% versus 16%) and are associated with a more severe phenotype (77% versus 5%). Inferences can be drawn from the nature of the mutation. Truncating mutations are recessively inherited and generally lead to a severe phenotype, both ocular and extraocular features (Table 2). Missense mutations are also recessively inherited and associated with an isolated ocular phenotype, which can be mild in one third of cases. Dominant forms are associated with the presence of mutations leading to a C-terminal extension of the protein and responsible for an isolated and relatively mild ocular phenotype.

Finally, association of truncating and missense mutations with recessive inheritance in contrast to the “elongating” mutations that appear exclusively dominant suggests that pathogenic effects are driven by distinct mechanisms. Recent work (24) has attempted to explore further the mechanisms underlying the possible relationship between the nature and position of the *FOXE3* mutations, the mode of transmission and the severity of the phenotype. Their *in vitro* functional studies showed that the recessive mutations tested (2 missense mutations affecting the forkhead domain and 2 frameshift mutations one before

and one after the forkhead domain) led to reduced ability to bind DNA and activate transcription. A “loss-of-function” mechanism therefore seemed probable for these “recessive” truncating and missense mutations.

For the “dominant” mutations, a dominant negative effect was initially proposed wherein FOXE3 mutants would bind to their DNA target without transactivation capacity and thus prevent the action of the wild-type FOXE3 proteins. Islam L. *et al.* (24) investigated this hypothesis by testing two "dominant" mutations (1 no-stop and 1 frameshift leading to a C-terminal extension). However, they showed that, although the mutant proteins retained their DNA binding capacity with a reduction in their transactivation capabilities, they did not interfere with the wild-type protein after *in vitro* modelling of the heterozygous state. They suggested rather a gain-of-function mechanism for these “elongating” mutations.

In this situation, the mutant FOXE3 protein could exhibit a new property that would affect the development of the eye; the presence of the wild-type allele would then presumably allow the synthesis of a functional protein, explaining why the phenotype associated with the "dominant" mutations is less severe. Furthermore, this hypothesis could explain why heterozygous carriers of recessive genetic mutations are not symptomatic. Thus, the different modes of transmission observed for these mutations do not seem to be linked to a simple gene dosage effect, but rather to a difference in the mechanisms involved, related to the nature and position of the mutations in *FOXE3*.

Phenotypic variability and mode of inheritance may be compounded by another level of complexity, involving the position of the missense mutations. As mentioned above, mutations in the *FOXE3* gene have recently been implicated in dominant familial forms of thoracic aorta dilatation (TAAD) without ocular abnormalities (22). These mutations are all heterozygous missense mutations located at the 3' end of the forkhead domain (Fig.1). A mechanism different from that observed with mutations responsible for an ocular phenotype (depending mainly on the position of these missense mutations) has been suggested (22). Thus, it has been hypothesized that the missense mutations position in the forkhead domain could have an impact on both the mode of transmission and the phenotype: a recessive ocular versus a dominant vascular phenotype. However, we can see that several individuals (7 in total) reported by Saboo US *et al.* (25) and in our study (mother of patient 5, Table 2) were heterozygous for the missense mutation p.(Gly158Arg)

located in the 3’ region of the forkhead domain (Fig.1), but did not demonstrate any aortic dilatation.

In conclusion, this review demonstrates the presence of genotype-phenotype correlations for *FOXE3* mutations in congenital ocular abnormalities. These correlations will assist both in interpreting the molecular data of *FOXE3* analysis when the phenotype is known, and in predicting phenotype and mode of inheritance when knowing the genotype in a family. Moreover, these correlations lay the foundations for exploring in more detail the effects of potential modifying factors that could influence the ocular phenotype, improving our understanding of eye morphogenesis and ocular genetic diseases in general.

URLs

gnomAD: http://gnomad.broadinstitute.org
MutationTaster: http://www.mutationtaster.org
Polyphen-2: http://genetics.bwh.harvard.edu/pph2
SIFT: http://sift.jcvi.org
SNPs&GO: http://snps-and-go.biocomp.unibo.it/snps-and-go

LEGENDS

Figure 1: Schematic representation of the FOXE3 protein. The grey part corresponds to the forkhead domain (amino acids 71 to 165). The upper section shows the position of the recessive (biallelic) mutations. The lower section shows the position of the dominant (monoallelic) mutations. The truncating mutations are shown in red, the missense mutations in green and the no-stop mutations in blue. The red box indicates the 4 missense mutations described by Kuang SQ *et al*. in the dominant forms of aortic dilation (TAAD) without associated ocular phenotype (22). For each mutation, allele frequency from the gnomAD database is given in parentheses (a dash is indicated when unknown).

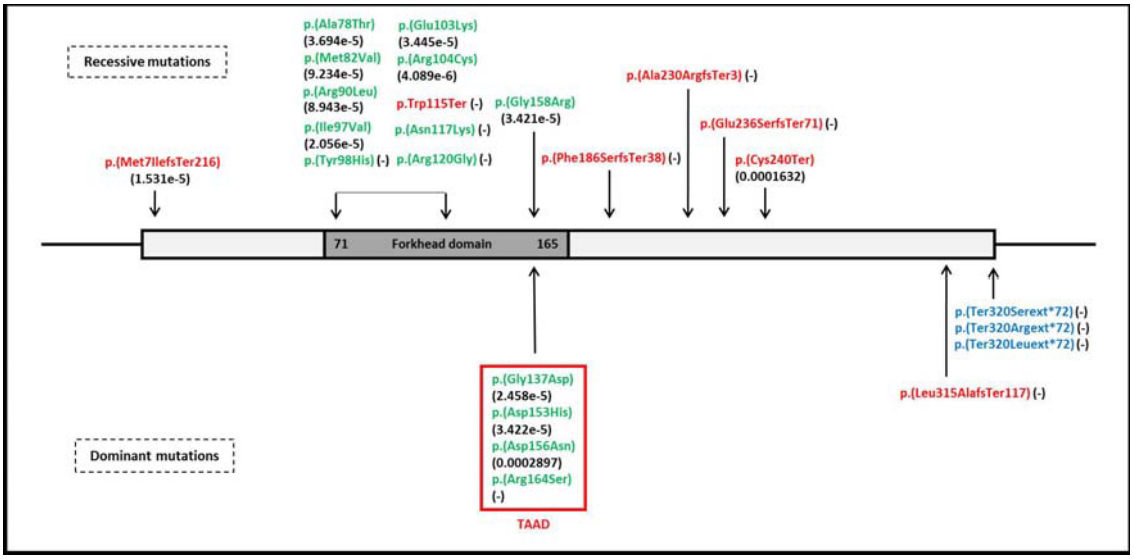

Figure 2: Eye picture of patient 1 from our cohort (Table 2) displaying normal external appearance of pseudophakic right eye, and left microphthalmia with sclerocornea and right Peters' anomaly.

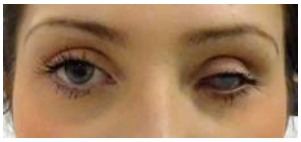

Table 1: Summary of monoallelic *FOXE3* mutations and associated phenotypes.

Table 2: Summary of biallelic *FOXE3* mutations and related phenotypes.

ACM = Arnold-Chiari malformation; ASD = anterior segment dysgenesis; ICA = irido-corneal adhesions; GDD = global developmental delay; ODC = optic disc coloboma; PCOS = polycystic ovary syndrome; RD = retinal detachment; VSD = ventricular septal defect. In bold, individuals with a missense mutation on one allele and a truncating mutation on the other one. *Sclerocornea can present as total opacification of the cornea (sclerocornea totalis) or as a peripheral corneal opacification (scleralization) leaving a central clear corneal area. For these patients, the clinical description was corneal opacity without any additional information.
**Abnormal folds of the retina in which the retinal lamination is disturbed (20). ***Mild cortical and blue dot lens opacities visible on dilated eye examination.

Table 3: Phenotype of patients in relation to *FOXE3* mutations in the recessive forms.

Table 4: Statistical analyses of the mutation type and the phenotype severity correlations in the recessive forms. *Mg*: Margins, i.e. average predicted probabilities of having a severe phenotype*

Table 5: Statistical analyses of the correlation between the position of truncating mutations in relation to the forkhead domain (before/after) and the phenotypeseverity.

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Table 1: Summary of monoallelic *FOXE3* mutations and associated phenotypes.

Publication	Patients	Monoallelic mutations	Ocular phenotype	Extraocular phenotype	Father	Mother
Doucette L. 2011	11 patients	c.[959G>T];[=] p.[(Ter320Leuext*72)];[=]	ASD			
Bremond-Gignac D. 2010	Patient 1	c.[959G>C];[=] p.[(Ter320Serext*72)];[=]	Congenital cataract		Genetic data not available asymptomatic	Genetic data not available asymptomatic
Iseri SU. 2009	Family 3 (5 patients)	c.[958T>C];[=] p.[(Ter320Argext*72)];[=]	1/5 with bilateral complex microphthalmia 4/5 with ASD or early onset cataract			
Ormestad M. 2002	Patient 1	c.[269G>T];[=] p.[(Arg90Leu)];[=]	Peters' anomaly			
Semina EV. 2001	Patient 1	c.[942dup];[=] p.[(Leu315AlafsTer117)];[=]	Cataract, posterior embryotoxon, myopia		Genetic and clinical data not available	c.[942dup];[=] Cataract, posterior embryotoxon

Table 2: Summary of biallelic *FOXE3* mutations and related phenotypes

Publication	Patients	Biallelic mutations	Ocular phenotype	Extraocular phenotype	Father	Mother
Chen J. 2017	Family 1 (5 patients)	c.[307G>A];[307G>A] p.[(Glu103Lys)];[(Glu103Lys)]	Congenital cataract		c.[307G>A];[=] asymptomatic	c.[307G>A];[=] asymptomatic
Saboo US. 2016	Family 1 (3 patients)	c.[472G>C];[472G>C] p.[(Gly158Arg)];[(Gly158Arg)]	Bilateral microphthalmia with aphakia, sclerocornea and anterior staphyloma		c.[472G>C];[=] asymptomatic	c.[472G>C];[=] asymptomatic
Ullah E. 2016	Family MA115 (2 patients)	c.[21_24del];[21_24del] p.[(Met7IlefsTer216)];[(Met7Ilefs Ter216)]	Bilateral complex microphthalmia with aphakia and corneal opacity*		c.[21_24del];[=] asymptomatic	c.[21_24del];[=] asymptomatic
	Family MA119 (3 patients)	c.[21_24del];[21_24del] p.[(Met7IlefsTer216)];[(Met7Ilefs Ter216)]	Bilateral complex microphthalmia with aphakia and corneal opacity*		Genetic data not available. asymptomatic	c.[21_24del];[=] asymptomatic
	Family MA143 (3 patients)	c.[289A>G];[289A>G] p.[(Ile97Val)];[(Ile97Val)]	Bilateral complex microphthalmia with aphakia and corneal opacity*		c.[289A>G];[=] asymptomatic	c.[289A>G];[=] asymptomatic
Khan YS. 2016	Family PKCC139 (4 patients)	c.[720C>A];[720C>A] p.[(Cys240Ter)];[(Cys240Ter)]	Peters' anomaly glaucoma		c.[720C>A];[=] asymptomatic	c.[720C>A];[=] asymptomatic
	Family PKCC009 (5 patients)	c.[351C>G];[351C>G] p.[(Asn117Lys)];[(Asn117Lys)]	Congenital cataract		c.[351C>G];[=] asymptomatic	c.[351C>G];[=] asymptomatic
	Family PKCC039 (7 patients)	c.[307G>A];[307G>A] p.[(Glu103Lys)];[(Glu103Lys)]	Congenital cataract		c.[307G>A];[=] asymptomatic	c.[307G>A];[=] asymptomatic
Islam L. 2015	Patient 1	c.[358C>G];[(358C>G)] p.[(Arg120Gly)];[(Arg120Gly)]	Bilateral primary aphakia, corneal opacity*, ICA, RD microphthalmia, and glaucoma		Genetic data not available. Asymptomatic	c.[358C>G];[=] asymptomatic
	Patient 2	c.[21_24del];[21_24del] p.[(Met7IlefsTer216)];[(Met7Ilefs Ter216)]	Bilateral primary aphakia with congenital corneal opacity*, ICA, RD glaucoma, corneal perforation		c.[21_24del];[=] asymptomatic	c.[21_24del];[=] asymptomatic
	Patient 3	**c.[269G>T];[705delC] p.[(Arg90Leu)];[(Glu236SerfsTer 71)]**	Bilateral primary aphakia with congenital corneal opacity* and glaucoma		c.[705delC];[=] asymptomatic	c.[269G>T];[=] asymptomatic
Garcia-Montalvo AI. 2014	Patient 1	c.[292T>C];[(292T>C)] p.[(Tyr98His)];[(Tyr98His)]	Bilateral complex microphthalmia with sclerocornea		Genetic and clinical data not available	Genetic and clinical data not available
Chassaing N. 2014	Patient 28	c.[685_686insTCCGGAGC];[(685_686insTCCGGAGC)] p.[(Ala230ArgfsTer3)];[(Ala230ArgfsTer3)]	Bilateral complex microphthalmia with sclerocornea	GDD	Genetic data not available asymptomatic	c.[685_686insTCCGGAGC];[=] asymptomatic
	Family 1 (Patient 29 and sibling)	c.[720C>A];[(720C>A)] p.[(Cys240Ter)];[(Cys240Ter)]	Bilateral complex microphthalmia with sclerocornea	PCOS	Genetic data not available asymptomatic	Genetic data not available asymptomatic
Pantoja-Melendez C. 2013	17 patients	c.[292T>C];[292T>C] p.[(Tyr98His)];[(Tyr98His)]	Bilateral complex microphthalmia, sclerocornea, aphakia, ODC		c.[292T>C];[=] asymptomatic	c.[292T>C];[=] asymptomatic

Publication	Patients	Biallelic mutations	Ocular phenotype	Extraocular phenotype	Father	Mother
Lopez Jimenez N. 2011	Patient 1	c.[720C>A];[(720C>A)] p.[(Cys240Ter)];[(Cys240Ter)]	Bilateral microphthalmia	ACM	Genetic and clinical data not available	Genetic and clinical data not available
Reis ML. 2010	Patient 1	**c.[244A>G];[705delC] p.[(Met82Val)];[(Glu236SfsTer71)]**	Bilateral severe microphthalmia sclerocornea	Renal pelvis dilatation	c.[705delC];[=] asymptomatic	c.[244A>G];[=] asymptomatic
	Patient 2	c.[557delT];[557delT] p.[(Phe186SerfsTer38)];[(Phe186SerfsTer38)]	Bilateral microphthalmia, sclerocornea, aphakia, dysplastic irides, probable glaucoma		c.[557delT];[=] asymptomatic	c.[557delT];[=] asymptomatic
	Patient 3	c.[720C>A];[(720C>A)] p.[(Cys240Ter)];[(Cys240Ter)]	Bilateral microphthalmia, aphakia, corneal opacity*, glaucoma, possible optic disc drusen and right ODC	Autism GDD	Genetic data not available asymptomatic	Genetic data not available asymptomatic
	Patient 4	c.[720C>A];[720C>A] p.[(Cys240Ter)];[(Cys240Ter)]	Bilateral microphthalmia, sclerocornea, coloboma, corneal ectasia and glaucoma	ACM, Umbilical hernia	c.[720C>A];[=] asymptomatic	c.[720C>A];[=] asymptomatic
	Patient 5	c.[720C>A];[720C>A] p.[(Cys240Ter)];[(Cys240Ter)]	Bilateral microphthalmia, sclerocornea, corneal ectasia and glaucoma	Hyper -trichosis	c.[720C>A];[=] asymptomatic	c.[720C>A];[=] asymptomatic
Anjum I. 2010	Family 1 (5 patients)	c.[720C>A];[720C>A] p.[(Cys240Ter)];[(Cys240Ter)]	Bilateral primary aphakia		c.[720C>A];[=] asymptomatic	c.[720C>A];[=] asymptomatic
Ali M. 2010	Family MEP54 (7 patients)	c.[292T>C];[292T>C] p.[(Tyr98His)];[(Tyr98His)]	Microphthalmia, aphakia, sclerocornea		Genetic data not available asymptomatic	Genetic data not available asymptomatic
	Mexican family (9 patients)	c.[720C>A];[720C>A] p.[(Cys240Ter)];[(Cys240Ter)]	Microphthalmia, aphakia, sclerocornea and ODC		c.[720C>A];[=] asymptomatic	c.[720C>A];[=] asymptomatic
Iseri SU. 2009	Family 1 (6 patients)	c.[244A>G];[244A>G] p.[(Met82Val)];[(Met82Val)]	4/6 with complex microphthalmia and/or primary aphakia with sclerocornea 2/6: ASD and glaucoma		c.[244A>G];[=] asymptomatic	c.[244A>G];[=] asymptomatic
	Family 2 (3 patients)	c.[21_24del];[21_24del] p.[(Met7IlefsTer216)];[(Met7IlefsTer216)]	Complex microphthalmia, bilateral primary aphakia, sclerocornea	GDD	c.[21_24del];[=] asymptomatic	c.[21_24del];[=] asymptomatic
Valleix S. 2006	Siblings (3 patients)	c.[720C>A];[720C>A] p.[(Cys240Ter)];[(Cys240Ter)]	Bilateral primary aphakia, aniridia, microphthalmia and retinal dysplasia**		c.[720C>A];[=] asymptomatic	c.[720C>A];[=] asymptomatic
Patients in this study	Patient 1	c.[244A>G];[269G>T] p.[(Met82Val)];[(Arg90Leu)]	Left microphthalmia with sclerocornea and right Peters' anomaly		c.[244A>G];[=] asymptomatic	c.[269G>T];[=] asymptomatic***
	Patient 2	c.[310C>T];[232G>A] p.[(Arg104Cys)];[(Ala78Thr)]	Unilateral microphthalmia with bilateral sclerocornea		c.[310C>T];[=] asymptomatic	c.[232G>A];[=] asymptomatic
	Patient 3	**c.[345G>A];[472G>C] p.[(Trp115Ter)];[(Gly158Arg)]**	Bilateral complex microphthalmia with sclerocornea	GDD VSD	c.[345G>A];[=] clinical data not available	c.[472G>C];[=] clinical data not available
	Family 1 (2 sisters)	c.[244A>G];[244A>G] p.[(Met82Val)];[(Met82Val)]	Bilateral complex microphthalmia with sclerocornea		c.[244A>G];[=] asymptomatic	c.[244A>G];[=] asymptomatic
	Family 2 (2 sisters)	c.[232G>A];[232G>A] p.[(Ala78Thr)];[(Ala78Thr)]	Bilateral complex microphthalmia with Peters' anomaly		c.[232G>A];[=] asymptomatic	c.[232G>A];[=] asymptomatic

Table 3: Phenotype of patients in relation to *FOXE3* mutations in the recessive forms

Mutation type	Mild ocular phenotype	Severe ocular phenotype	Severe ocular phenotype with extraocular features	Total	Distribution
2 missense	19	42	0	61	60%
1 missense + 1 truncating	0	1	2	3	3%
2 truncating	4	27	7	38	37%
Total	23	70	9	102	
Distribution	23%	69%	8%		

Table 4: Statistical analyses of the mutation type and the phenotype severity correlations in the recessive forms

	Population sizes			Probability of having a severe phenotype (Logistic regression)			
	N	n	%	n	Mg*	95% CI	p-value
All patients	**102**						
No truncating		61	59.8	42	68.9	57.2 ; 80.5	**0.016**
At least one truncating		41	40.2	37	88.2	77.4 ; 99.1	
Index cases only	**32**						
No truncating		14	43.8	11	78.6	57.1 ; 1.00	**0.208**
At least one truncating		18	56.2	17	94.4	83.9 ; 1.00	

Table 5: Statistical analyses of the correlation between the position of truncating mutations in relation to the forkhead domain and the phenotype severity

		Population sizes			Probability of being severe (Exact test)		
		N	n	%	n	%	**p-value**
All patients		**41**					
	Before		10	24.4	10	100.0	**0.556**
	After		31	75.6	27	87.1	
Index cases only		**18**					
	Before		5	27.8	5	100.0	**1.000**
	After		13	72.2	12	92.3	