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Authors
Bardakjian, Tanya
Krall, Max
Wu, Di
et al.

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Case report

A recurrent, non-penetrant sequence variant, p.Arg266Cys in Growth/Differentiation Factor 3 (GDF3) in a female with unilateral anophthalmia and skeletal anomalies

Tanya Bardakjian, Max Krall, Di Wu, Richard Lao, Paul Ling-Fung Tang, Eunice Wan, Sarina Kopinsky, Adele Schneider, Pui-yan Kwok, Anne Slavotinek

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A B S T R A C T

Purpose: The genetic causes of anophthalmia, microphthalmia and coloboma remain poorly understood. Missense mutations in Growth/Differentiation Factor 3 (GDF3) gene have previously been reported in patients with microphthalmia, iridial and retinal colobomas, Klippel-Feil anomaly with vertebral fusion, scoliosis, rudimentary 12th ribs and an anomalous right temporal bone. We used whole exome sequencing with a trio approach to study a female with unilateral anophthalmia, kyphoscoliosis and additional skeletal anomalies.

Observations: Exome sequencing revealed that the proposita was heterozygous for c.796C>T, predicting p.Arg266Cys, in GDF3. Sanger sequencing confirmed the mutation and showed that the unaffected mother was heterozygous for the same missense substitution.

Conclusions and importance: Although transfection studies with the p.Arg266Cys mutation have shown that this amino acid substitution is likely to impair function, non-penetrance for the ocular defects was apparent in this family and has been observed in other families with sequence variants in GDF3. We conclude p.Arg266Cys and other GDF3 mutations can be non-penetrant, making pathogenicity more difficult to establish when sequence variants in this gene are present in patients with structural eye defects.

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1. Introduction

Anophthalmia (absent eyes) and microphthalmia (small eyes) are important birth defects because of the medical significance of severely reduced vision. Coloboma, or a failure of the optic fissure to close, is a common accompaniment to microphthalmia. Mutations in SOX2 and genes coding for other transcription factors can cause microphthalmia, anophthalmia and coloboma (MAC), but there is high genetic heterogeneity and more than 50% of affected individuals remain without a molecular genetic diagnosis for their birth defect after sequencing of the currently known causative genes. Mutations in the Growth/Differentiation Factor 3 (GDF3) gene were first described as a cause of microphthalmia and coloboma after five, heterozygous sequence variants that cause single amino acid substitutions were identified in seven individuals from a cohort of 472 patients with MAC. This frequency of GDF3 sequence variants (7/472 or 1.5%) was significantly increased in MAC patients compared to controls (p = 0.007) for that study. Affected individuals exhibited a spectrum of ocular and skeletal findings comprising unilateral or bilateral microphthalmia and coloboma with involvement of the iris and retina, vertebral defects with Klippel-Feil syndrome and thoracic and lumbar scoliosis, rudimentary 12th ribs and an anomalous right temporal bone. There was high intrafamilial and interfamilial variability.

One recurrent mutation in GDF3, c.796C>T, predicting p.Arg266Cys, segregated with four affected members of a three-generation pedigree, two affected members of a two-generation pedigree, and was present in an individual with no affected relatives. p.Arg266Cys affects a well-conserved arginine residue...
located in the mature TGF-β domain (Table 1) and the mutation introduces a cysteine residue that is predicted to enable the formation of an aberrant disulfide bridge between residues C266 and C329 of the GDF3 protein to prevent stabilization of the TGF-β fold. Transfection of COS-7 cell lysates and a SOX9-based reporter assay revealed an appreciable reduction in mature, secreted GDF3 ligand on Southern blot, with diminished expression of the downstream SOX9 gene for mutant p.Arg266Cys GDF3 compared to wildtype GDF3.

As part of a study into the genetic etiology of anophthalmia and microphthalmia, we sequenced a female with unilateral microphthalmia and skeletal defects comprising pectus excavatum, scoliosis, enhanced kyphosis and finger contractures. We identified the recurrent GDF3 sequence variant, c.796C>T, predicting p.Arg266Cys, that was inherited from the proposita’s healthy mother. We report this patient and review the evidence that this and other reported missense variants in GDF3 are pathogenic in MAC.

2. Case report

The pregnancy with the proposita was normal and she was delivered using forceps for failure to progress. She was noted to have unilateral right anophthalmia/severe microphthalmia, with a small residual eye remnant described as the size of a ‘raisin.’ Congenital hip dislocation was treated with a cast for 2 weeks and resolved at 3–4 months of age. She started talking at one year of life and walked at 18 months of age. She had normal intelligence and was an excellent student who learned to read at 4–5 years of age. She is currently 20 years of age and is active and able to play sports and continue her studies. Her depth perception is reportedly resolved at 3 months of age.

She was diagnosed with pectus excavatum, scoliosis and bilateral finger contractures at 11 years of age. She developed chest asymmetry with a smaller right breast, but has not required surgery for her pectus deformity. Her other medial history included severe dry skin, asthma treated with inhalants, and headaches. She had menarche at 11 years of age. At 20 years of age, her height was 170 cm (50–75th centile) and weight was 53 kg (10–25th centile). She had clubbing of the fingernails that was most marked for her second fingers and less evident for her fifth digits. A karyotype and microarray have not been performed.

Both of the proband’s (IV–1) parents (III–2 and III–3) were healthy and there was no known consanguinity (Fig. S1). Ethnicity was Caucasian. A maternal great-uncle (II–5) was blind in one eye, but remembered having a small amount of vision in that eye as a young child and has not had known structural eye defects; ‘degeneration’ was mentioned by the physician but he has not received a diagnosis. He had a maternal aunt (I–4) and a paternal great-aunt (not shown) with a similar condition by report. A maternal aunt had normal vision, but her son (IV–2) reportedly had anophthalmia/microphthalmia, although further details are unknown. A maternal cousin (III–1) had amblyopia by report. DNA testing on these relatives has not been performed.

2.1. Exome sequencing

After obtaining written, informed consent, venous blood samples were obtained and DNA was extracted from the proposita and both parents. Exome sequencing was performed as previously described. We utilized wAnnovar (http://wannovar.usc.edu/) with default parameters to generate an annotated variant call file (.vcf file). We analyzed the .vcf file using Station X GenePool software (http://www.stationxinc.com). We used SnpEff (http://snpeff.sourceforge.net/SnpEff_manual.html) to filter for highly deleterious and moderately deleterious variants that had a European allele frequency of less than 1% according to 1000 Genomes Database (http://www.1000genomes.org/). The variant list was then filtered for eye disease genes based on disease ontology annotations. The potential deleteriousness of novel sequence variants was assessed using SIFT (http://sift.jcvi.org/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and Mutation Taster (http://mutationtaster.org/MutationTaster/).

A mean target coverage of 96X was obtained, with 94.6% of the target sequence covered at 10X (Table S1). We observed a recurrent sequence variant that has previously been published as an autosomal dominant mutation for patients with anophthalmia/microphthalmia, c.796C>T predicting p.Arg266Cys (NM_020634.1). We used Sanger sequencing to verify the

Table 1

| Nucleotide alteration | Amino acid alteration | Protein domain | SIFT<sup>a</sup> | Polyphen-2<sup>b</sup> | Mutation Taster<sup>c</sup> | 1000 Genomes<sup>d</sup> | ExAC Browser<sup>e</sup> | dbSNP<sup>f</sup> |
|-----------------------|----------------------|----------------|-----------------|------------------|----------------------|-------------------|----------------|------------------|
| c.584G>A              | p.Arg195Gln          | Pre-pro domain | 0.54            | 0.001; B<sup>g</sup> | PM<sup>h</sup>; p = 0.999 | 1/1000; 0.001 | 65/121,408; 0.00005354; | rs146973734; 0.001 ± 0.002 |
| c.796C>T              | p.Arg266Cys          | Transforming growth factor-β, C-0.01 terminal | 0.24; PM<sup>i</sup> | DC<sup>j</sup>; p = 0.999 | PM; p = 0.995 | 2/1000; 0.002; 246/121,398; 0.002026; | rs140926412; 0.993 ± 0.0042 |
| c.820C>T              | p.Arg274Trp          | Transforming growth factor-β, C-0 terminal | 0.99 | DC<sup>j</sup>; p = 0.999 | PM; p = 0.995 | 2/1000; 0.002; 30/121,400; 0.0002471; | rs387906946; 0.000 ± 0.0016 |
| c.914T>C              | p.Leu305Pro          | Transforming growth factor-β, C-0 terminal | 1.0 | DC<sup>j</sup>; p = 0.999 | DC<sup>j</sup>; p = 0.999 | 8/1000; 0.008; 62/121,408; 0.0006754; | rs387906945; 0.001 ± 0.00266 |
| c.974C>T              | p.Pro325Leu          | Transforming growth factor-β, C-0.0002 0.0002 terminal | 1.0 | DC<sup>j</sup>; p = 0.999 | DC<sup>j</sup>; p = 0.999 | 1/1000; 0.001; 14/121,404; 0.0001153; | rs566097767; 0.000 ± 0.0011 |

<sup>a</sup> SIFT – Sorting Intolerant from Tolerant, http://sift.jcvi.org/.
<sup>b</sup> Polyphen-2 – http://genetics.bwh.harvard.edu/pph2/
<sup>c</sup> Mutation Taster – http://www.mutationtaster.org.
<sup>d</sup> 1000 Genomes – http://www.1000genomes.org.
<sup>e</sup> ExAC Browser – http://exac.broadinstitute.org.
<sup>f</sup> dbSNP – http://www.ncbi.nlm.nih.gov/SNP/.
<sup>g</sup> B = benign.
<sup>h</sup> PM = polymorphism.
<sup>i</sup> het. = heterozygous.
<sup>j</sup> DC = disease causing.
<sup>k</sup> Hz = homozygous.
sequence variant in the proposita and to test her parents, revealing that her unaffected mother carries the same variant (Fig. S2) and that her father does not have this variant. GDF3 encodes a single transcript that yields a protein of 364 amino acids; Arg266 is located in the Transforming Growth Factor-\(\beta\), C-terminal domain. Software predictions regarding p.Arg266Cys were mixed, with SIFT and MutationTaster predicting that the substitution was deleterious, whereas PolyPhen-2 predicted that the amino acid change was a benign polymorphism (Table 1). ExAC browser and dbSNP listed p.Arg266Cys as having an estimated frequency of 0.002 – 0.003 in normal controls, whereas the frequency in 1000 Genomes database was estimated at 0.0399361 (Table 1). Conservation of the Arg266 residue was noted in several species, including P. troglodytes, M. mulatta, F. catus, M. musculus and C. elegans, but the residue was not preserved in G. gallus, T. rubripes, D. rerio or X. tropicalis (Table 2). The mutation was not predicted to have an effect on splicing (MutationTaster).

In view of the inheritance of p.Arg266Cys from the proposita’s unaffected mother, we hypothesized that genetic burden for deleterious sequence variants in additional genes for MAC could explain the difference in penetrance.15 We used the same Station-X Gene-Pool filtering parameters to compare the number of predicted deleterious sequence variants in the listed genes associated with MAC1,2 in the proposita and both parents. We did not observe an increased burden of deleterious sequence variants in these genes in the proposita compared to her mother (Table 3), although this analysis was limited and does not include sequence variants in novel genes or copy number variants that were undetected with our exome analysis. Further testing of additional family members was not possible.

3. Discussion

We identified a sequence variant in GDF3, c.796C>T predicting p.Arg266Cys, in a female with unilateral anophthalmia and skeletal anomalies comprising pectus excavatum, scoliosis, kyphosis and bilateral finger contractures. This sequence variant has previously been published as a causative mutation for both structural eye defects and skeletal anomalies; however, non-penetrance has been described for this variant and was present in this family, assuming this variant is pathogenic.7 GDF3 is a member of the TGF-\(\beta\) superfamily and is classified as a BMP/GDF ligand, although it is missing the fourth of seven canonical cysteine residues that define the cysteine knot for members of the TGF-\(\beta\) superfamily.16 GDF3 has been shown to inhibit bone morphogenetic protein (BMP) signaling in human and mouse embryonic stem (ES) cells and frog embryos.16,17 The inhibition of BMP in these studies was executed by processed and unprocessed forms of GDF3, and it has been hypothesized that the “missing cysteine” residue in GDF3 was a critical component for the inhibitory activity of BMP signaling.16,18 However, the “missing” fourth cysteine motif is required for GDF3 function only if the cleavage of the prepro domain is also mutated to impair maturation of the GDF3 protein.16 At high doses, GDF3 has been shown to act as a Nodal-like ligand, stimulating activity of Smad 2/3.16

We reviewed the frequency and predicted deleteriousness of published sequence variants in GDF3 that are predominantly found in one publication (Table 4). In studies of MAC in which GDF3 was sequenced, five missense sequence variants found in a cohort of 472 patients accounted for 1.7% of individuals screened19 and GDF3 variants thus remain rare as putative causes of MAC.20 Three of the above amino acid substitutions in GDF3 were predicted to be benign or a polymorphism by at least one software prediction program (Table 1); all of the published missense variants have been detected in control databases, with two (c.820C>T, predicting p.Arg274Trp, and c.914T>C, predicting p.Leu305Pro) being present in a homozygous state in these databases (Table 1). No nonsense or frameshift mutations in GDF3 have been published in association with eye defects.20 Incomplete penetrance was also present in our family and in a family with a different mutation in the GDF3 gene. c.914T>C, predicting p.Leu305Pro, in which two individuals carried this missense substitution, but only one individual manifested...
unilateral microphthalmia. To explain the non-penetrance, it has been hypothesized that sequence variations in different BMP ligands can contribute additively to the ocular or skeletal phenotype. However, we did not find any increased genetic burden in the proposita compared to unaffected parents. One patient showed heterozygosity in GDF3 for c.974C>T, predicting p.(Pro325Leu), a sequence variant that was predicted to be highly conserved and damaging, but that was nevertheless inherited from the patient’s unaffected father. The child with p.(Pro325Leu) had microphthalmia and coloboma, but was also a heterozygote for two missense mutations in CYP1B1. Both genes were considered relevant to pathogenesis of the eye defects in view of the role of CYP1B1 in retinoic acid synthesis that regulates choroid fissure closure. Similarly, a child with horizontal and rotary nystagmus, bilateral iris colobomas with severe colobomatous microphthalmia, bilateral foveal hypoplasia, abnormally small optic discs with reduced optic nerve diameters and severely compromised vision (20/200) had p.Arg266Cys in GDF3, the same mutation as our proband, together with p.Ala199Thr in GDF6, whereas her parent carrying only the GDF3 p.Arg266Cys variant had mild bilateral iris colobomata and microphthalmia, normal optic discs and mildly affected vision (20/40) in each eye. As transfection of mutant GDF3 containing p.Arg266Cys together with GDF6 containing p.Ala199Thr resulted in greater reduction of activation of GDF6, another member of the TGF family. In that gene, the sequence variant c.746C>A, predicting p.(Ala249Glu), was present in patients with eye findings, but also in one individual with normal eyes. On two occasions, this mutation was inherited from an unaffected parent. In a study that examined 11 patients with developmental eye defects using exome sequencing, one patient showed heterozygosity in GDF3 for c.974C>T, predicting p.(Pro325Leu), a sequence variant that was predicted to be highly conserved and damaging, but that was nevertheless inherited from the patient’s unaffected father. The child with p.(Pro325Leu) had

### Table 3

| Gene          | Nucleotide | Protein     | Zygosity | 1000 Genomes | Predicted impact |
|---------------|------------|-------------|----------|--------------|------------------|
| GCN22: NM_145649 | c.305C>G   | p.Thr102Ser | Het      | 0.658946     | Moderate         |
| FANCDD2: NM_001018115 | c.1214A>G   | p.Asn405Ser | Het.     | –            | moderate         |
| GLI2: NM_005270 | c.3943C>T   | p.Pro1315Ser | Het.     | 0.998403     | moderate         |
| GDF3: NM_020634.1 | c.796C>T    | p.Arg266Cys | Het.     | 0.0399361    | moderate         |

### Table 4

Previously reported sequence variants in Growth/Differentiation Factor 3 (GDF3).

| Phenotype                      | Mutation | Penetration/Segregation | Reference |
|--------------------------------|----------|-------------------------|-----------|
| Ocular                         |          |                         |           |
| Microphthalmia, coloboma       |          |                         |           |
| Bilateral coloboma, microphthalmia, nystagmus | c.974C>T, p.Arg266Cys | Unaffected father | Prokudin et al., 2014 |
| Bilateral coloboma, mild microphthalmia, | c.979C>T, p.Arg266Cys | Family #3; Segregation in 2 generations | Ye et al., 2010 |
| Unilateral microphthalmia       | c.914T>C, p.Leu295Pro | Family #2; Incomplete | Ye et al., 2010 |
| Bilateral iris coloboma         | c.584G>C, p.Ala199Thr | Patient 1 | Ye et al., 2010 |
| Unilateral microphthalmia       | c.914T>C, p.Leu295Pro | Family #1; Segregation in 3 generations | Ye et al., 2010 |
| Bilateral microphthalmia and coloboma | c.820C>T, p.Arg274Trp | Family 1; Segregation in 3 generations | Ye et al., 2010 |
| Skeletal                       |          |                         |           |
| Scoliosis                      |          |                         |           |
| Klippel-Feil and vertebral fusion | c.979C>T, p.Arg266Cys | Family #1; Segregation in 3 generations | Ye et al., 2010 |
| Klippel-Feil and vertebral fusion | c.979C>T, p.Arg266Cys | Family #1; Segregation in 3 generations | Ye et al., 2010 |
| Combined                       |          |                         |           |
| Unilateral iris and retinal coloboma, rudimentary 12th ribs, mild scoliosis | c.796C>T, p.Arg266Cys | Family #1; Segregation in 3 generations | Ye et al., 2010 |
| Unilateral coloboma, microphthalmia, Anomalous right temporal bone | c.796C>T, p.Arg266Cys | Family #1; Segregation in 3 generations | Ye et al., 2010 |

1. In the first family, the proposita had both ocular and skeletal involvement, with unilateral iridal and retinal coloboma, rudimentary 12th ribs and a mild scoliosis. Two other affected relatives had Klippel-Feil and vertebral fusion and one affected relative had scoliosis (Ye et al., 2010; Pedigree #1).
models of loss of function for Gdf3 that have had eye defects. In the mouse, Gdf3 homozygous null embryos have early morphological defects, with aberrant anterior visceral endoderm induction and migration, processes that are regulated by both BMP and Nodal signaling, although neither pathway has been studied in the context of the null mice. These early developmental defects have precluded study of later developmental roles for this gene and conditional knock-outs have not yet been generated. The Danio rerio orthologue of gdf3/gdf1, namely dvr1 has also been studied. In-situ hybridization showed modest expression of dvr1 in the head region at 14–18 hours post fertilization (hpf) and injections with antisense morpholinos (MOs) targeting the translation start site of gdf3 recapitulated the human ocular phenotypes with reduced eye size, colobomas, and defective development of the lens and retina, including retention of nuclei in the lens and shortening or missing photoreceptor outer segments. Injection of a translational MO targeting gdf1/gdf3 also resulted in misexpression of the ventral retinal patterning marker, foxg1, suggesting a role in eye formation. However, these studies could have been confounded by decreased gdf1 expression in addition to reduced gdf3 expression.

Lastly, functional studies have been performed for the sequence variants in GDF3 and do provide some evidence in favor of pathogenicity. In addition to the reduced levels of mature GDF3 seen with p.Arg266Cys, western blot analysis of separately transfected COS-7 cell lysates detected mildly reduced amounts of full-length p.Leu305Pro-mutant protein and p.Arg195Gln-mutant protein in the cytosol, with striking reductions in the amount of mature p.Leu305Pro-mutant protein and p.Arg195Gln-mutant protein in the media. The p.Arg274Trp mutation changed a conserved hydrophilic residue to a hydrophobic residue and was not present in 480 control DNA samples.

4. Conclusions

The sequence variant p.Arg266Cys in GDF3 has previously been reported as a causative mutation for eye defects. However, in view of the non-penetrance observed with this variant and the lack of direct evidence from animal models for the involvement of Gdf3 loss of function in eye defects, we consider it plausible that p.Arg266Cys and other GDF3 sequence variants may act as modifiers, rather than causative genes, in combination with other genetic and possibly environmental factors. A greater number of reported variants and phenotypic analysis are still needed to classify this gene and the p.Arg266Cys variant.

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Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

Conflict of interest

The following authors have no financial disclosures: TB, MK, DW, RL, FT, EW, SK, ADs, PK, AS.

Patient consent

Written consent to publish the report was obtained from the patient.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ajoc.2017.06.006.

References

1. Deml B, Reis LM, Maheshwari M, Grifffis C, Rick D, Semina EV. Whole exome analysis identified dominant COLA1 mutations in patients with complex ocular phenotypes involving microphthalmia. Clin Genet. 2014;86:475–481.
2. Prokudin I, Simons C, Grigg JR, et al. Exome sequencing in developmental eye disease leads to identification of causal variants in GJA8, CRYGC, PAX6 and CYP1B1. Eur J Hum Genet. 2014;22:907–915.
3. Fantes J, Ragge NK, Lynch SA, et al. Mutations in SOX2 cause anophthalmia. Nat Genet. 2003;33:461–463.
4. Verma AS, FitzPatrick DR. Anophthalmia and microphthalmia. Orphanet J Rare Dis. 2007;2:47.
5. Bardakjian T, Weiss A, Schneider AS. Anophthalmia/microphthalmia overview. In: Pagon RA, Bird TC, Dolan CR, Stephens K, eds. GeneReviews [Internet], Seattle (WA), Seattle: University of Washington; 1993-2004. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20301552.
6. Slavotinek AM. Eye development and known syndromes. Mol Genet Metab. 2011;104:448–456.
7. Ye M, Berry-Wynne KM, Asai-Coakwell M, et al. Mutation of the bone morphogenetic protein GDF3 causes ocular and skeletal anomalies. Hum Mol Genet. 2010;19:287–298.
8. Slavotinek AM, Mehrotra P, Nazarenko I, et al. Focal facial dysplasia, type IV, is caused by mutations in CYP26C1. Hum Mol Genet. 2013;22:696–703.
9. Slavotinek AM, Garcia ST, Chandrattilake G, et al. Exome sequencing in 32 patients with anophthalmia/microphthalmia and developmental eye defects. Clin Genet. 2015;88:468–473.
10. Wang K, Li M, Hakonarson H. Functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38:e164.
11. Chang X, Wang K. wANNOVAR: annotating genetic variants for personal genomes via the web. J Med Genet. 2012;49:433–436.
12. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009;4:1073–1081.
13. Adzhubei IA, Schmidt S, Peshkin I, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010;7:248–249.
14. Schwarz JM, Rodelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods. 2010;7:575–576.
15. Gonzaga-Jauregui C, Harel T, Gambin T, et al. Exome sequence analysis suggests that genetic burden contributes to phenotypic variability and complex neu-ropathy. Cell Rep. 2015;12:1109–1113.
16. Levine AJ, Levine ZJ, Brivanlou AH. GDF3 is a BMP inhibitor that can activate Nodal signaling only at very high doses. Dev Biol. 2009;325:43–48.
17. Levine AJ, Brivanlou AH. GDF3 at the crossroads of TGF-beta signaling. EMBO J. 2007;26:4744–4755.
18. Williamson KA, FitzPatrick DR. The genetic architecture of microphthalmia, anophthalmia and coloboma. Eur J Med Genet. 2011;54:369–380.
19. Garcia-Montalvo IA, Pelackt-Luna E, Nelson-Mora J, Buentello-Volante B, Miranda-Duarte A, Zenteno JC. Mutational screening of FOXE3, GDF3, ATO7, and ALDH1A3 in congenital ocular malformations. Possible contribution of the FOXE3 p.VAL201MET variant to the risk of severe eye malformations. Ophthalmic Genet. 2014;35:190–192.
20. Ragge NK, Brown AG, Poleschek CM, et al. Heterozygous mutations of OTX2 cause severe ocular malformations. Am J Hum Genet. 2005;76:1008–1022.
21. Reis LM, Tyler RC, Schilter KF, et al. BMP4 loss-of-function mutations in developmental eye disorders including SHORT syndrome. Hum Genet. 2011;130:495–504.
22. Vu PQ, Tian JL, Sadun AA. Genetic incidence in ophthalmology. JAMA Ophthalmol. 2016;134:123–124.
23. Asai-Coakwell M, French CR, Ye M, et al. Incomplete penetrance and pheno-typic variability characterize Gdf6-attributable oculo-skeletal phenotypes. Hum Mol Genet. 2009;18:1110–1121.
24. den Hollander AI, Byunwilla J, Kovach P, et al. Genetic defects of CDF5 in the zebrafish out of sight mutant and in human eye developmental anomalies. BMC Genet. 2010;11:102.