Array comparative genomic hybridization analysis in patients with anophthalmia, microphthalmia, and coloboma

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Purpose: The goal of our study was to determine whether genomic copy number abnormalities (deletions and duplications) affecting genes involved in eye development contributed to the etiology of anophthalmia, microphthalmia, and coloboma. **Methods:** The affected individuals were evaluated for the presence of deletions and duplications in genomic DNA by a very high-resolution array comparative genomic hybridization. **Results:** Array analysis of 32 patients detected one case with a deletion encompassing the renal-coloboma syndrome associated gene *PAX2*. Nonpolymorphic copy number changes were also observed at several candidate chromosomal regions, including 6p12.3, 8q23.1q23.2, 13q31.3, 15q11.2q13.1, 16p13.13, and 20q13.13. **Conclusions:** This study identified the first patient with the typical phenotype of the renal-coloboma syndrome caused by a submicroscopic deletion of the coding region of the *PAX2* gene. The finding suggests that *PAX2* deletion testing should be performed in addition to gene sequencing as a part of molecular evaluation for the renal-coloboma syndrome. Array comparative genomic hybridization testing of 32 affected individuals showed that genomic deletions and duplications are not a common cause of nonsyndromic anophthalmia, microphthalmia, or coloboma but undoubtedly contribute to the etiology of these eye anomalies. Therefore, array comparative genomic hybridization testing represents an important and valuable addition to candidate gene sequencing in research and diagnostics of ocular birth defects. ***Genet Med* 2011:13(5):437–442.**

Key Words: *array CGH, deletions, duplications, microphthalmia, anophthalmia, coloboma*

Three related ocular birth defects—anophthalmia, microphthalmia, and coloboma—are important contributors to childhood visual impairment and blindness, impacting 2 of 10,000 newborns annually.[1–3] More than 20 different genetic loci have been implicated in congenital eye malformations, with most associated genes having a role in eye development.[4–6] However, each known gene is responsible for only a small percentage of cases, and many additional causative genetic factors still await identification.[5,6] Mutations in the known genes account for approximately 15% of cases of anophthalmia, microphthalmia, and coloboma.[7] For the remaining patients, the lack of a specific molecular diagnosis prevents prediction of long-term outcomes, anticipation of systemic complications, and estimation of the recurrence risk in their families.

Studies have shown that gene deletions and duplications may comprise up to 15% of mutations underlying monogenic disease.[8] High-resolution whole genome array comparative genomic hybridization (aCGH) testing of patients with genetic diseases can therefore detect copy number abnormalities in genes responsible for their clinical phenotypes. Application of aCGH not only detects abnormalities in known disease-causing genes but also can identify new candidate genes for specific disorders.[8] Multiple examples exist of successful application of aCGH in disease gene discovery. These include implication of the *TCF2* gene in the etiology of multicystic dysplastic kidneys,[9] identification of *PORCN* as the causative gene for focal dermal hypoplasia (Goltz syndrome),[10] and discovery of loci for congenital diaphragmatic hernia.[11] aCGH has also contributed to identification of genes associated with congenital eye anomalies. For example, CHARGE syndrome is characterized with a specific set of birth defects, which includes coloboma, with or without microphthalmia.[12] Vissers et al.[13] implicated the *CHD7* gene in the etiology of CHARGE syndrome by aCGH testing that detected a de novo microdeletion of the *CHD7* locus at 8q12 in an affected individual. The roles of the *GDF6* gene at 8q21.2-q22.1, *TFAP2A* gene at 6p24.3, and *TMX3* gene at 18q22.1 in causing ocular developmental anomalies have also been discovered by testing patients who carried deletions of these genes.[14–16]

Although causative copy number changes have been reported in isolated cases of ocular birth defects, no one has systematically tested large numbers of affected individuals for deletions and duplications in genomic DNA. We hypothesized that ocular birth defects frequently result from copy number abnormalities involving critical genes. To test this hypothesis, we examined a cohort of patients with anophthalmia, microphthalmia, and coloboma for submicroscopic deletions and duplications by whole genome high-resolution oligo aCGH.

MATERIALS AND METHODS

Patients were enrolled through an ongoing, Institutional Review Board-approved research study "Genetics of Microphthalmia, Anophthalmia, and Coloboma" at the Division of Genetics and Metabolism, Department of Pediatrics, University of Minnesota. Written informed consent was obtained from all participants and/or their parents, as appropriate. Thirty-two patients with either isolated anophthalmia, microphthalmia, and coloboma (23 cases) or with anophthalmia, microphthalmia, and coloboma in asso-

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Table 1 Patients with syndromic and nonsyndromic anophthalmia, microphthalmia, and coloboma tested by aCGH

Sample ID	Ocular phenotype	Other phenotypes (if not nonsyndromic)	Previous genetic testing (if known)
1	Bilateral iris coloboma		No information
2	Bilateral optic pits	Hypothalamic hypogonadism, mild cognitive disability, hypoplastic mullerian derivatives, absent ovary, left leg spasticity	Normal karyotype
3	Bilateral iris coloboma		No information
4	L iris coloboma		No information
5	L iris, retinal, and optic nerve coloboma		No information
6	Bilateral colobomatous microphthalmia	Developmental delay	No information
7	R retinal coloboma, L microphthalmia		No information
8	L anophthalmia, R microphthalmia	UPJ obstruction and hydronephrosis	No information
9	Bilateral colobomatous microphthalmia	Kidney reflux	No information
10	Bilateral iris coloboma		No information
11	Bilateral iris coloboma		No mutations detected in *PAX6*, *SHH*, normal karyotype
12	Bilateral iris coloboma		Sibling of 4071
13	Bilateral colobomatous microphthalmia, PHPV		No mutations detected in *PAX6*, *SHH*, *SIX6*, *SOX2*, normal karyotype
14	Bilateral iris coloboma		No information
15	Bilateral coloboma		No information
16	Unknown coloboma, grandchildren with aniridia		No information
17	Unilateral microphthalmia, morning glory		No previous testing
18	Bilateral iris coloboma		No information
19	Unilateral severe microphthalmia, inferonasal coloboma, and cataract		No information
20	Unilateral right complete coloboma with microphthalmia		No previous testing
21	Bilateral iris, retinal coloboma		Normal Agilent 44K array, normal karyotype
22	Coloboma unspecified		No information
23	Bilateral colobomatous microphthalmia		TORCH titres
24	Unilateral right iris coloboma		No information
25	Bilateral chorioretinal coloboma		No information
26	Bilateral colobomatous microphthalmia	Renal failure	No information
27	Coloboma unspecified		No information
28	Bilateral retinal/iris coloboma	Shawl scrotum and glandular hypospadias	No information
29	Coloboma unspecified		No information
30	Right unilateral microphthalmia	Multicystic kidney	No information
31	Morning glory anomaly	Bilateral renal failure with renal hypoplasia	No previous testing
32	Optic nerve hypoplasia	Abnormal ears, renal hypoplasia	No previous testing

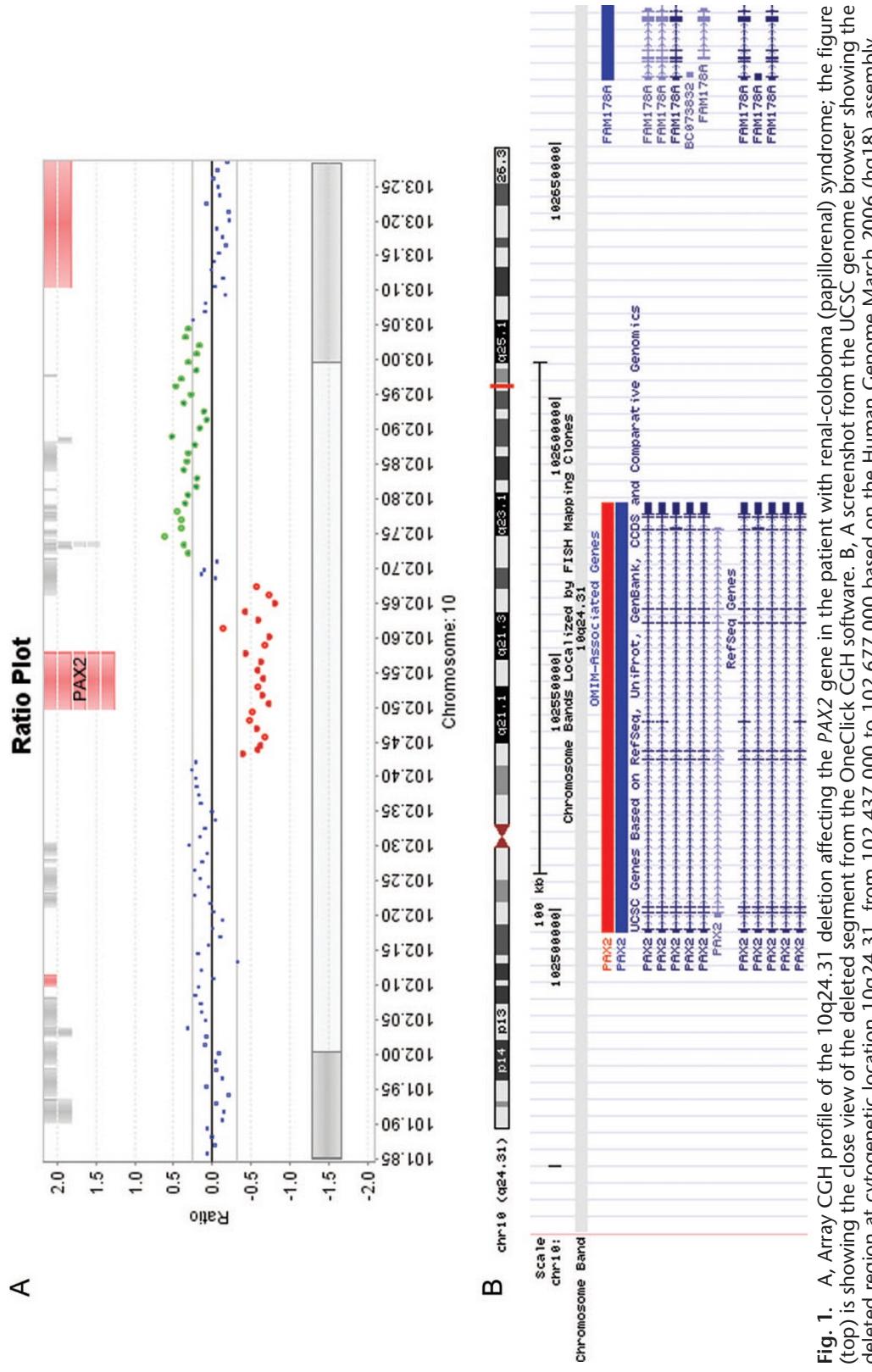

Fig. 1. A, Array CGH profile of the 10q24.31 deletion affecting the *PAX2* gene in the patient with renal-coloboma (papillorenal) syndrome; the figure (top) is showing the close view of the deleted segment from the OneClick CGH software. B, A screenshot from the UCSC genome browser showing the deleted region at cytogenetic location 10q24.31, from 102,437,000 to 102,677,000 based on the Human Genome March 2006 (hg18) assembly.

ciation with other congenital anomalies (cases 2, 6, 8, 9, 26, 28, 30, 31, and 32) were selected for testing (Table 1). All patients were evaluated by a clinical geneticist or an ophthalmologist and were enrolled in the study if they were lacking genetic diagnosis after clinical examination and standard of care testing. Molecular tests performed in each patient before the study (if known) are listed in Table 1. Array CGH analysis was performed with a commercially available HD2 human whole-genome CGH array (Roche NimbleGen Systems Inc., Madison, WI), which has approximately 2 million probes at a median interprobe distance of 1169 bp. This array can detect small genomic imbalances (deletions and duplications) at the resolution of individual genes (~5–10 kb). Specimen labeling, array hybridization, washing, and scanning were performed at NimbleGen service laboratory in Iceland. Data analysis was performed with NimbleScan and SignalMap software from Roche NimbleGen and OneClickCGH software from Infoquant, at the UW Cytogenetic Services Laboratory at the University of Wisconsin-Madison. Regions with copy number changes detected by aCGH were compared against the database of Genomic Variants (http://projects.tcag.ca/variation/), which catalogues known benign copy number polymorphisms in the human genome. Only imbalances that do not correspond to known polymorphisms were evaluated further. Nonpolymorphic copy number changes were confirmed by separate aCGH experiments, with a different aCGH platform (EmArray Cyto6000 array Agilent Technologies, Santa Clara, CA).

RESULTS

High-resolution aCGH analysis of 32 patients with ocular defects revealed approximately 240 kb deletion on chromosome 10 in a patient with clinical features of renal-coloboma (papillorenal) syndrome.[17] The deletion included the entire *PAX2* coding region and a portion of the *FAM178A (C10orf6*) gene (Fig. 1). This finding provided the molecular confirmation of the patient's clinical diagnosis and showed that, in addition to point mutations, deletions in the *PAX2* gene contribute to the etiology of the renal-coloboma syndrome.

A 5.38 Mb duplication was detected on the long arm of chromosome 15 (cytogenetic location 15q11.2q13.1) in a patient with microphthalmia (R), anophthalmia (L), ureteropelvic junction obstruction, and hydronephrosis. The following 14 genes were affected by the duplication: *ATP10A*, *C15orf2*, *GABRA5*, *GABRB3*, *GABRG3*, *GOLGA8E*, *HERC2*, *MAGEL2*, *MKRN3*, *NDN*, *OCA2*, *SNRPN*, *SNURF*, and *UBE3A*. Deletions of the same region, when inherited maternally, result in the phenotype of Prader-Willi syndrome, whereas paternal deletions of this region lead to the Angelman syndrome phenotype. Duplications of the 15q11.2 region, particularly when inherited on the maternal chromosome, are associated with hypotonia, autistic behavior, developmental delay, mental retardation, seizures, and mild dysmorphic features.[18] However, eye anomalies have not previously been reported in patients with the 15q11.2q13.1 duplications.

In addition to the deletion of the *PAX2* gene at 10q24.31 and duplication of the Prader-Willi/Angelman syndrome critical region at 15q11.2q13.1, nonpolymorphic copy number changes were detected at several candidate regions including 6p12.3, 8q23.1q23.2, 13q31.3, 16p13.13, and 20q13.13. Based on the potential function of the genes in the regions, deletions at 13q31.3 and 8q23.1q23.2 were selected for further follow-up.

The 13q31.3 deletion was detected in a patient with bilateral iris coloboma. The deletion was approximately 240 kb in size and included only one gene, glypican 5 (*GPC5*). The *GPC5* gene was a plausible positional and functional candidate gene for causing congenital eye defects in our patient; however, the 13q31.3 deletion was not present in the patient's maternal first cousin who was also affected with bilateral iris coloboma (parental samples were not available).

The 8q23.1q23.2 deletion was observed in a patient with unilateral severe microphthalmia. The deletion was 1.5 Mb in size and affected the following four known genes: *PKHD1L1*, *KCNV1*, *EBAG9*, and *GOLSYN*. Parental samples were tested by aCGH for the presence of the deletion detected in the proband, and the unaffected father was found to carry the same deletion on chromosome 8.

A summary of all detected nonpolymorphic copy number changes including their genomic location, gene content, presence in additional family members, and likely clinical significance is provided in Table 2.

DISCUSSION

Array CGH testing of 32 individuals with ocular birth defects detected one deletion responsible for the eye phenotype in the tested individual, one disease associated duplication that was unlikely the cause of the patient's eye anomalies, two deletions affecting strong candidate genes for eye anomalies, and three changes of completely unknown clinical significance.

The causative deletion was detected in a patient with clinical features of renal-coloboma (papillorenal) syndrome and it affected the known gene for this disorder, *PAX2*. The deletion also included a portion of the *FAM178A (C10orf6*) gene, which codes for a hypothetical protein of unknown function. The partial deletion of the *FAM178A* gene most likely did not significantly contribute to the patient's phenotype. The patient was a 9-year-old boy with typical features of the renal-coloboma syndrome, including optic nerve hypoplasia, secondary strabismus, mild deafness, dysplastic ear helices, and renal hypoplasia. *PAX2* gene sequencing had been performed previously and no point mutations had been found. To our knowledge, renal-coloboma syndrome due to a deletion of the *PAX2* gene has been reported in only one other patient who had a large interstitial 10q deletion encompassing the *PAX2* locus detected by high-resolution chromosome analysis.[19] We report the first submicroscopic deletion affecting the coding region of the *PAX2* gene. No other genes likely to contribute to the patient's phenotype were affected by the rearrangement. Identification of this deletion stresses the importance of incorporating deletion/duplication testing together with the *PAX2* gene sequencing into molecular diagnostics of the renal-coloboma syndrome.

Our study detected a duplication of the Prader-Willi syndrome critical region on chromosome 15 in a patient with multiple eye and kidney anomalies. Duplications of the 15q11.2q13.1 region have been well described in the literature; when inherited on the maternal chromosome, they are associated with hypotonia, autistic behavior, developmental delay, mental retardation, seizures, and mild dysmorphic features.[18] Eye anomalies have not been reported in patients with 15q11.2q13.1 duplications, although a locus for the autosomal dominant colobomatous microphthalmia has been mapped to an overlapping but more distal region on chromosome 15.[20] Therefore, this duplication may not be the cause of the eye anomalies in our patient, but its detection helps to explain his other clinical findings, such as developmental delay and behavioral issues.

Among the detected nonpolymorphic copy number changes, two seemed to involve likely candidate genes for eye anomalies: the 13q31.3 deletion and the 8q23.1q23.2 deletion. Deletion of the 13q31.3 region was initially considered as clinically significant based both on the chromosomal position and the function of the

Table 2 Summary of copy number abnormalities detected by aCGH testing

Sample	Copy number change	Chromosome	Start	Stop	Size in bp	Genes/proteins	Associated phenotype	Presence in other family members	Significance for ocular defects
7	Deletion	20	47,586,000	47,610,000	24,000	*PTGIS*-prostaglandin I2 (prostacyclin) synthase	Unknown	Unknown	Unknown
8	Duplication	15	20,850,000	26,298,000	5,448,000	*GOLGA8E*-golgi autoantigen, golgin subfamily a, 8E, *C15orf2*-hypothetical protein LOC23742, *SNRPN*-small nuclear ribonucleoprotein polypeptide N, *UBE3A*-ubiquitin protein ligase E3A, *AP10A*-ATPase, class V, type 10A, *GABRG3*-gamma-aminobutyric acid (GABA) A receptor, gamma, *MKRN3*-makorin ring finger protein 3, *MAGEL2*-MAGE-like protein 2, *NDN*-necdin, *GABRB3*-gamma-aminobutyric acid (GABA) A receptor, beta, *GABRA5*-gamma-aminobutyric acid (GABA) A receptor, alpha, *OCA2*-oculocutaneous albinism II	Maternal deletions: Prader-Willi syndrome; Paternal deletions: Angelman syndrome; Maternal duplications: autism, developmental delay, behavioral issues	Unknown	Unknown (likely unrelated)
10	Deletion	13	90,870,000	91,098,000	228,000	*GPC5*-glypican 5	Unknown	Not present in affected cousin	Unknown (likely unrelated)
15	Deletion	16	12,018,000	12,030,000	12,000	*RUNDC2A*-RUN domain containing 2A	Unknown	Not present in affected daughter	Unknown (likely unrelated)
19	Deletion	8	110,454,000	110,898,000	444,000	*PKHD1L1*-fibrocystin L, *KCNV1*-potassium channel, subfamily V, member 1, *EBAG9*-estrogen receptor binding site associated, *GOLSYN*-Golgi-localized syntaphilin-related protein	Unknown	Present in unaffected father	Unknown (possibly unrelated)
31	Deletion	6	45,258,000	45,378,000	120,000	*SUPT3H*-suppressor of Ty 3 homolog isoform 2, hsa-miR-586	Unknown	Unknown	Unknown
32	Deletion	10	102,438,000	102,678,000	240,000	*PAX2*-paired box protein 2 isoform c	Renal-coloboma Syndrome	Unknown	Causative

affected gene. Coloboma, microphthalmia, and anophthalmia have previously been reported in association with deletions at q31-q33 region of the long arm of chromosome 13.[21] The deletion detected in our patient included the *GPC5* gene, which belongs to a family of glycosylphosphatidylinositol-anchored, membrane-bound heparan sulfate proteoglycans. *GPC*s play a role in modulating the activity of heparan sulfate-binding growth factors.[22] Their involvement in developmental morphogenesis and growth regulation has been shown by Drosophila mutants, and human genetic disorders such as Simpson-Golabi-Behmel syndrome[23] and autosomal-recessive omodysplasia.[24] *Dally*, drosophila ortholog of the gene deleted in our patient *GPC5*, is known to affect cell division patterning in developing eye.[22] The *GPC5* gene was therefore considered a plausible candidate gene for causing the congenital eye defects in our patient, but testing additional family members did not detect the 13q31.3 deletion in the patient's cousin with the identical ocular defect. Because it does not segregate with the eye anomalies, the deletion is unlikely the cause of the ocular defects in this family. However, this deletion has not been reported as a benign variant (http://projects.tcag.ca/variation/) and has been detected by another laboratory in two unrelated individuals with developmental delay and cognitive impairment (personal communication). Therefore, the clinical significance of the 13q31.1 deletion requires further investigation.

The 8q23.1q23.2 deletion was observed in a patient with unilateral severe microphthalmia and small inferonasal coloboma. He also had a dense posteriorly subluxated crystalline lens and Persistent Hyperplastic Primary Vitreous. His head magnetic resonance imaging and development have been normal. The deletion affected four known genes, including *GOLSYN*, which is known to play an important role in neuronal development. The unaffected father was found to carry the same deletion. It is possible that the detected deletion has incomplete penetrance and that some of the carriers do not express abnormal phenotype. Alternatively, cases have been reported where benign copy number variants contribute to abnormal phenotypes by unmasking mutations in nondeleted alleles.[25,26]

The 13q31.1 and 8q23.1q23.2 deletion cases illustrate difficulties in interpreting clinical significance of copy number abnormalities detected by high-resolution aCGH testing. For example, the causative role cannot be assumed solely based on the function of the affected genes. Although plausible candidate genes mapped within both deleted regions, family studies reduced the possibility of the role of the 13q31.1 and 8q23.1q23.2 deletions in causing eye anomalies in the probands. These cases demonstrate the value of having clinical information and DNA samples available from patients' parents and other affected and unaffected members of their families.

Our study showed that aCGH could detect deletions and duplications associated with ocular birth defects. However, copy number abnormalities did not seem to be a common cause of isolated anophthalmia, microphthalmia, and coloboma. Although gene deletions and duplications significantly contribute to pathogenesis of genetic disorders, the majority of disease-causing mutations are nucleotide changes in genomic DNA. We propose that a combination of aCGH analysis with high-throughput sequencing methods that allow detection of base changes (point mutations) in a large number of candidate genes for eye malformations will be the most successful strategy for identification of new genetic causes of anophthalmia, microphthalmia, and coloboma.

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REFERENCES

1. Källén B, Robert E, Harris J. The descriptive epidemiology of anophthalmia and microphthalmia. *Int J Epidemiol* 1996;25:1009–1016.
2. Morrison D, FitzPatrick D, Hanson I, et al. National study of microphthalmia, anophthalmia, and coloboma (MAC) in Scotland: investigation of genetic aetiology. *J Med Genet* 2002;39:16–22.
3. Stoll C, Alembik Y, Dott B, Roth MP. Congenital eye malformations in 212,479 consecutive births. *Ann Genet* 1997;40:122–128.
4. Gregory-Evans CY, Williams MJ, Halford S, Gregory-Evans K. Ocular coloboma: a reassessment in the age of molecular neuroscience. *J Med Genet* 2004;41:881–891.
5. Fitzpatrick DR, van Heyningen V. Developmental eye disorders. *Curr Opin Genet Dev* 2005;15:348–353.
6. Chang L, Blain D, Bertuzzi S, Brooks BP. Uveal coloboma: clinical and basic science update. *Curr Opin Ophthalmol* 2006;17:447–470.
7. Gonzalez-Rodriguez J, Pelcastre EL, Tovilla-Canales JL, et al. Mutational screening of CHX10, GDF6, OTX2, RAX and SOX2 genes in 50 unrelated microphthalmia–anophthalmia–coloboma (MAC) spectrum cases. *Br J Ophthalmol* 2010;94:1100–1104.
8. Vissers LE, Veltman JA, van Kessel AG, Brunner HG. Identification of disease genes by whole genome CGH arrays. *Hum Mol Genet* 2005;14:R215–R223.
9. Mefford HC, Clauin S, Sharp AJ, et al. Recurrent reciprocal genomic rearrangements of 17q12 are associated with renal disease, diabetes, and epilepsy. *Am J Hum Genet* 2007;81:1057–1069.
10. Wang X, Reid Sutton V, Omar Peraza-Llanes J, et al. Mutations in X-linked PORCN, a putative regulator of Wnt signaling, cause focal dermal hypoplasia. *Nat Genet* 2007;39:836–838.
11. Zayed H, Chao R, Moshrefi A, et al. A maternally inherited chromosome 18q22.1 deletion in a male with late-presenting diaphragmatic hernia and microphthalmia-evaluation of DSEL as a candidate gene for the diaphragmatic defect. *Am J Med Genet A* 2010;152A:916–923.
12. Sanlaville D, Verloes A. CHARGE syndrome: an update. *Eur J Hum Genet* 2007;15:389–399.
13. Vissers LE, van Ravenswaaij CM, Admiraal R, et al. Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. *Nat Genet* 2004;36:955–957.
14. Asai-Coakwell M, French CR, Berry KM, et al. GDF6, a novel locus for a spectrum of ocular developmental anomalies. *Am J Hum Genet* 2007;80:306–315.
15. Milunsky JM, Maher TA, Zhao G, et al. TFAP2A mutations result in branchio-oculo-facial syndrome. *Am J Hum Genet* 2008;82:1171–1177.
16. Chao R, Nevin L, Agarwal P, et al. A male with unilateral microphthalmia reveals a role for TMX3 in eye development. *PloS ONE* 2010;5:e10565.
17. Eccles MR, Schimmenti LA. Renal-coloboma syndrome: a multi-system developmental disorder caused by PAX2 mutations. *Clin Genet* 1999;56:1–9.
18. Browne CE, Dennis NR, Maher E, et al. Inherited interstitial duplications of proximal 15q: genotype-phenotype correlations. *Am J Hum Genet* 1997;61:1342–1352.
19. Benetti E, Artifoni L, Salviati L, et al. Renal hypoplasia without optic coloboma associated with PAX2 gene deletion. *Nephrol Dial Transplant* 2007;22:2076–2078.
20. Michon L, Morlé L, Bozon M, et al. Physical and transcript map of the autosomal dominant colobomatous microphthalmia locus on chromosome 15q12–q15 and refinement to a 4.4Mb region. *Eur J Hum Genet* 2004;12:574–578.
21. Lansink PJ, Moll AC, Imhof SM, Schouten-van Meeteren AY, Goverts ST. Variable expression of ophthalmological findings in the 13q deletion syndrome. *Arch Ophthalmol* 2005;123:127–128.
22. Filmus J, Selleck SB. Glypicans: proteoglycans with a surprise. *J Clin Invest* 2001;108:497–501.
23. DeBaun MR, Ess J, Saunders S. Simpson Golabi Behmel syndrome: progress toward understanding the molecular basis for overgrowth, malformation, and cancer predisposition. *Mol Genet Metab* 2001;72:279–286.
24. Campos-Xavier AB, Martinet D, Bateman J, et al. Mutations in the heparan-sulfate proteoglycan glypican 6 (GPC6) impair endochondral ossification and cause recessive omodysplasia. *Am J Hum Genet* 2009;84:760–770.
25. Flipsen-ten Berg K, van Hasselt PM, Eleveld MJ, et al. Unmasking of a hemizygous WFS1 gene mutation by a chromosome 4p deletion of 8.3 Mb in a patient with Wolf-Hirschhorn syndrome. *Eur J Hum Genet* 2007;15:1132–1138.
26. Coman DJ, Gardner RJ. Deletions that reveal recessive genes. *Eur J Hum Genet* 2007;15:1103–1104.

