

ARTICLE

Genomic imbalances detected by array-CGH in patients with syndromal ocular developmental anomalies

Andrée Delahaye^{*,1,2,3,15}, Pierre Bitoun^{4,15}, Séverine Drunat⁵, Marion Gérard-Blanluet⁵, Nicolas Chassaing⁶, Annick Toutain⁷, Alain Verloes^{3,5,8}, Frédérique Gatelais⁹, Marie Legendre¹⁰, Laurence Faivre¹¹, Sandrine Passemard^{3,5,8}, Azzedine Aboura⁵, Sophie Kaltenbach^{5,12}, Samuel Quentin¹³, Céline Dupont^{1,5}, Anne-Claude Tabet⁵, Serge Amselem⁹, Jacques Elion^{3,5,8,14}, Pierre Gressens^{3,8}, Eva Pipiras^{1,2,3,16} and Brigitte Benzacken^{1,2,3,5,16}

In 65 patients, who had unexplained ocular developmental anomalies (ODAs) with at least one other birth defect and/or intellectual disability, we performed oligonucleotide comparative genome hybridisation-based microarray analysis (array-CGH; 105A or 180K, Agilent Technologies). In four patients, array-CGH identified clinically relevant deletions encompassing a gene known to be involved in ocular development (*FOXC1* or *OTX2*). In four other patients, we found three pathogenic deletions not classically associated with abnormal ocular morphogenesis, namely, del(17)(p13.3p13.3), del(10)(p14p15.3), and del(16)(p11.2p11.2). We also detected copy number variations of uncertain pathogenicity in two other patients. Rearranged segments ranged in size from 0.04 to 5.68 Mb. These results show that array-CGH provides a high diagnostic yield (15%) in patients with syndromal ODAs and can identify previously unknown chromosomal regions associated with these conditions. In addition to their importance for diagnosis and genetic counselling, these data may help identify genes involved in ocular development.

European Journal of Human Genetics (2012) 20, 527–533; doi:10.1038/ejhg.2011.233; published online 11 January 2012

Keywords: ocular developmental anomaly; array-CGH; *OTX2*; *FOXC1*; 16p11.2 deletion; *YWHAE*

INTRODUCTION

Ocular developmental anomalies (ODAs) are severe structural defects of the eye caused by disruption of the complex process of ocular morphogenesis during early gestation.¹ ODAs occur in about 1 in 3000 to 4000 neonates and have been estimated to account for at least 25% of cases of childhood visual impairment worldwide.^{2,3} ODAs include anophthalmia, microphthalmia, coloboma (failure of the optic fissure to close), congenital cataract (opacity of the lens fibres), and dysgenesis of the anterior segment (iris and cornea). These anomalies can occur separately or in combination and can be accompanied with other birth defects and/or with intellectual disability. The broad clinical spectrum of syndromal ODAs reflects the complexity of the pathways involved in ocular development. Many genes are known to be involved in ocular development, including several genes initially identified in chromosomal rearrangements.⁴

Chromosomal abnormalities are found in 7.7 to 10% of neonates with ocular anomalies and other birth defects.^{5,6} The introduction of microarray technology has shown a very high rate of rearrangements undetectable with standard or high-resolution karyotyping. Submicroscopic copy number variations (CNVs) account for a substantial proportion of normal and pathogenic genetic variation in humans.⁷

Few studies have specifically addressed the role for submicroscopic chromosomal imbalances in ODAs. In a case-series study of 32 patients with non-syndromal anophthalmia, microphthalmia, or coloboma, a single causal deletion was found, suggesting a limited causal role for CNVs in non-syndromal ODAs.⁸ Another study found pathogenic CNVs in 5 (13%) of 37 patients with ocular birth defects.⁹

The objective of this study was to determine the prevalence of genomic imbalances identified using comparative genomic hybridisation-based microarray analysis (array-CGH) in patients with syndromal ODAs.

PATIENTS AND METHODS

Informed consent to participation was obtained from all patients and/or parents before study inclusion. Parents also gave informed consent for their own tests.

PATIENTS

A total of 65 unrelated patients (42 males and 23 females) were included. They were born to non-consanguineous parents and had unexplained syndromal ODAs with normal karyotypes. All patients were examined by experienced ophthalmologists and clinical

¹AP-HP, Hôpital Jean Verdier, Service d'Histologie, Embryologie, et Cytogénétique, Bondy, France; ²Université Paris-Nord, Paris 13, UFR SMBH, Bobigny, France; ³Inserm, U676, Paris, France; ⁴AP-HP, Hôpital Jean Verdier, Consultations de génétique médicale, Service de Pédiatrie, Bondy, France; ⁵AP-HP, Hôpital Robert Debré, Département de Génétique, Paris, France; ⁶CHU Toulouse, Hôpital Purpan, Service de Génétique Médicale, Toulouse, France; ⁷CHU de Tours, Hôpital Bretonneau, Département de Génétique, Tours, France; ⁸Université Paris Diderot, Faculté de Médecine, Paris, France; ⁹CHU d'Angers, Service d'endocrinologie diabétologie pédiatrique, Angers, France; ¹⁰Inserm, U933, Université Paris 6, AP-HP, Hôpital Armand-Trousseau, Paris, France; ¹¹CHU de Dijon, Centre de Génétique, Dijon, France; ¹²AP-HP, Hôpital Necker-Enfants-Malades, Service de Cytogénétique, Université Paris Descartes, Paris, France; ¹³AP-HP, Hôpital Saint-Louis, Plateforme génomique, Institut Universitaire d'Hématologie, Paris, France; ¹⁴Inserm, U763, Paris, France
*Correspondence: Dr A Delahaye, AP-HP, Hôpital Jean Verdier, Service d'Histologie, Embryologie, et Cytogénétique, Avenue du 14 Juillet, 93140 Bondy, France. Tel: +33 148 026674; Fax: +33 148 026737; E-mail: andree.delahaye@jvr.aphp.fr

¹⁵These authors contributed equally to this work.

¹⁶These authors contributed equally to this work.

geneticists, and had negative routine diagnostic work-up results. There were 38 patients with micro-anophthalmia and coloboma, 7 with optic nerve hypoplasia, 2 with aniridia, 8 with anterior segment anomalies, 8 with congenital cataract, and 2 with other ocular defects (Duane syndrome and buphthalmos, respectively). Only patients with intellectual disability and/or at least one extraocular birth defect were included. Individual patient characteristics are reported in Supplementary Table 1.

Array-CGH

Blood samples were obtained from the study patients and, when possible, from their parents. Genomic DNA was isolated from blood samples using standard protocols. Oligonucleotide array-CGH was performed using the Human Genome CGH Microarray Kit 105A or SurePrint G3 Human CGH Microarray Kit, 4×180K (Agilent Technologies, Santa Clara, CA, USA). In the 105A kit, the arrays include a total of 105 750 probes, with an overall median probe spacing of 22 kb, and in the 180K kit a total of 180 880 probes, with an overall median probe spacing of 13 kb. Experiments were performed according to the standard Agilent protocol (Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis, version 6.3). Commercially available genomic DNA (Promega, Madison, WI, USA) was used as the control. Hybridised slides were scanned with a microarray scanner (G2505B SureScan High-Resolution Technology Agilent), and the image data were extracted and converted to text files using Agilent Feature Extraction software. The data were graphed and analysed using Agilent CGH Analytics software (statistical algorithm: ADM-2; sensitivity threshold: 6.1). Only gains or losses that encompassed at least three consecutive oligomers on the array were considered. CNVs previously identified in unaffected individuals according to the Database of Genomic Variants (<http://projects.tcag.ca/variation/>) were excluded. The validation method was chosen based on the imbalance type (deletion or duplication), size, and sample availability (DNA and/or metaphase spreads). Then, the clinical relevance of observed chromosomal aberrations was estimated according to data found in the scientific literature and databases for each of the regions and genes involved, using the DECIPHER database (<http://www.sanger.ac.uk/PostGenomics/decipher/>) for known microdeletion and microduplication syndromes and the Online Mendelian Inheritance in Man (OMIM, www.ncbi.nlm.nih.gov/Omim/getmorbidity.cgi) for known disease-causing genes, gene functions, and inheritance patterns. Whenever possible, to discriminate between *de novo* and inherited anomalies, the parents were tested using fluorescence *in situ* hybridisation (FISH), multiplex ligation-dependent probe amplification (MLPA), or real-time quantitative PCR technology (qPCR). When there was a family history of ODA or X-linked anomaly, additional family members were studied to evaluate the familial segregation of the inherited anomalies. DNA copy alterations were considered possibly pathogenic when they involved regions known to be associated with microdeletion or microduplication syndromes, involved known dosage-sensitive genes, involved known eye development genes, consisted in *de novo* imbalances, or exhibited a pattern of family segregation consistent with pathogenicity.

Fluorescence *in situ* hybridisation

FISH was performed using standard protocols with commercially available probes. BAC clones from the RPCI human BAC library were selected in the chromosomal region of interest using the UCSC Genome Browser (<http://genome.cse.ucsc.edu>). DNA was labelled with Spectrum Green™-11-dUTP or Spectrum Orange™-11-dUTP (Vysis, Downers Grove, IL, USA) by nick translation, using a commercial kit (Roche Diagnostics GMBH; <http://www.rochediagnostics.com>)

according to the manufacturer's instructions. All BAC clones used for confirmation are described in the online Supplementary Information file (Supplementary Table 2).

Multiplex ligation-dependent probe amplification

The microdeletion syndrome-specific MLPA kit SALSA P297-B1 was used with MRC-Holland reagents (MRC-Holland, Amsterdam, the Netherlands) according to the manufacturer's protocol. Amplification products were analysed using capillary electrophoresis in the DNA Analyser 3130XL and GeneMapper software v3.7 (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). This kit contains probes targeting the 16p11.2 region (*MAZ*, *MVP*, *HIRIP3*, *DOC2A*, and *MAPK3* genes).

Real-time qPCR

Real-time qPCR was performed on a StepOnePlus Real-Time PCR System (Applied Biosystems, Life Technologies) with fluorescent SYBR Green dye (Power SYBR green PCR master mix, Applied Biosystems, Life Technologies). Gene-specific primers for the target gene and endogenous control genes (*F9* and *PTEN*) were designed using Primer Express Software (Applied Biosystems, Life Technologies). The BLAST program from the NCBI browser (<http://www.ncbi.nlm.nih.gov/BLAST>) was used for *in silico* specificity analysis. Amplification efficiencies were calculated based upon the generation of standard curves using serial dilutions of genomic DNA. Assays with amplification efficiencies between 85 and 120% were considered acceptable. Each sample was run in triplicate for target gene quantification compared with endogenous control genes. Data were processed using the StepOne software v.2 (Applied Biosystems, Life Technologies), with the comparative $\Delta\Delta$ threshold cycle-number method.¹⁰ All the primers and probes used for qPCR are described in the online Supplementary Information file (Supplementary Table 2).

Microsatellite analysis

Microsatellites were selected from UCSC Genome Browser microsatellites or simple repeat tracks, and primers were designed using the NCBI Primer-BLAST program. For a single reaction, a master mix of 2.5 μ l 10× PCR buffer with 25 mM MgCl₂, 1 μ l 5 mM dNTP, 0.125 μ l AmpliTaq Gold enzyme (Applied Biosystems, Life Technologies), 0.25 μ l 20 pM primers (forward and reverse), and 16 375 μ l sterile H₂O was prepared. A volume of 1 μ l DNA (50 ng/ μ l) was added to each reaction. The PCR reaction was run in Eppendorf Thermocyclers (Eppendorf, Hamburg, Germany) using the following conditions: hot start at 95 °C for 10 min, 95 °C for 10 s, 50 °C for 10 s for 36 cycles, followed by a final extension step at 72 °C for 4 min. Samples were analysed on an ABI 3730 XL DNA sequencing analyser and processed using GeneMapper 3.7 software (Applied Biosystems, Life Technologies).

RESULTS

Molecular karyotyping

Array-CGH identified 15 DNA CNVs involving segments larger than 100 kb in 14 patients (Table 1). These alterations were consistently confirmed by FISH, MLPA, or qPCR. The altered segments ranged in size from 0.04 to 5.68 Mb (Figure 1). In four patients, we identified clinically relevant deletions encompassing a gene known to be involved in ocular development (*FOXC1* or *OTX2*; Table 1, patient no. 1–4). In four other patients, three pathogenic deletions not classically associated with ODAs were found, namely, del(10)(p14p15.3), del(17)(13.3p13.3), and del(16)(p11.2p11.2) (Table 1, patient no. 5–8). Microsatellite analyses showed that the deletions were *de novo* and paternally derived for patient no. 1, 2, and 5, and *de novo*

Table 1 DNA copy alterations identified using array-CGH

Patient	Imbalance	Size (Mb)	ISCN description ^a	Inheritance	Major genes involved	DECIPHER patient
<i>Possibly pathogenic chromosomal anomalies</i>						
Patient 1	Del	5.68	6p25.1p25.3(107,883-5,684,125)x1	Pat (dn)	FOXC1	PAR251592
Patient 2	Del	1.95	6p25.2p25.3(477,352-2,472,573)x1	Pat (dn)	FOXC1	PAR251563
Patient 3	Del	0.61	4q34q34(176,398,264-177,004,339)x1	Pat (inh)	GPM6A	PAR251586
Patient 4	Del	2.25	14q22.2q23.1(54,287,767-56,543,234)x1	Mat (inh)	OTX2, GCH1	PAR254661
	Del	0.04	11p15.4p15.4(3,765,195-3,809,332)x1	U	RHOG	
Patient 4	Del	0.11	14q23.1q23.1(56,326,564-56,433,789)x1	U	OTX2	PAR251587
	Del	5.55	10p14p15.3(2,911,242-8,457,638)x1	Pat (dn)	AKRIC2, GATA3	
Patient 5	Del	5.55	10p14p15.3(2,911,242-8,457,638)x1	Pat (dn)	AKRIC2, GATA3	PAR251587
Patient 6	Del	0.5	17p13.3p13.3(1,105,199-1,605,301)x1	U	YWHAE	PAR251562
Patient 7	Del	0.6	16p11.2p11.2(29,500,084-30,106,254)x1	Mat (dn)	SEZ6L2	PAR251593
Patient 8	Del	0.55	16p11.2p11.2(29,560,300-30,106,254)x1	Non-mat	SEZ6L2	PAR256688
Patient 9	Dup	0.18	11p15.4p15.4(7,283,552-7,466,165)x3	Pat (inh)	SYT9, OLFML1	PAR251548
Patient 10	Del	0.36	20p12.1p12.1(15,003,653-15,360,378)x1	U	MACROD2	PAR251588
<i>Probably non-pathogenic variant</i>						
Patient 11	Del	0.41	Xq25q25(127,268,216-127,679,006)x1	Mat (inh)	Pseudogenes	PAR251582
Patient 12	Del	0.77	2q23.3q23.3(153,511,511-154,277,644)x1	Mat (inh)	RPRM	PAR251584
Patient 13	Dup	0.2	6p11.2p11.2(27,767,070-27,969,040)x3	Mat (inh)	tRNA genes, histone gene cluster	PAR251546
Patient 14	Del	0.2	8p23.2p23.3(3,079,016-3,276,030)x1	Pat (inh)	CSMD1	PAR251596

Abbreviations: Del, deletion; dn, *de novo*; Dup, duplication; inh, inherited; ISCN, International System for Human Cytogenetic Nomenclature (2009); mat, maternal; pat, paternal; U, unavailable.
^aOn NCBI human genome Build 36 (UCSC hg18).
Bold denotes gene known to be involved in ocular development.

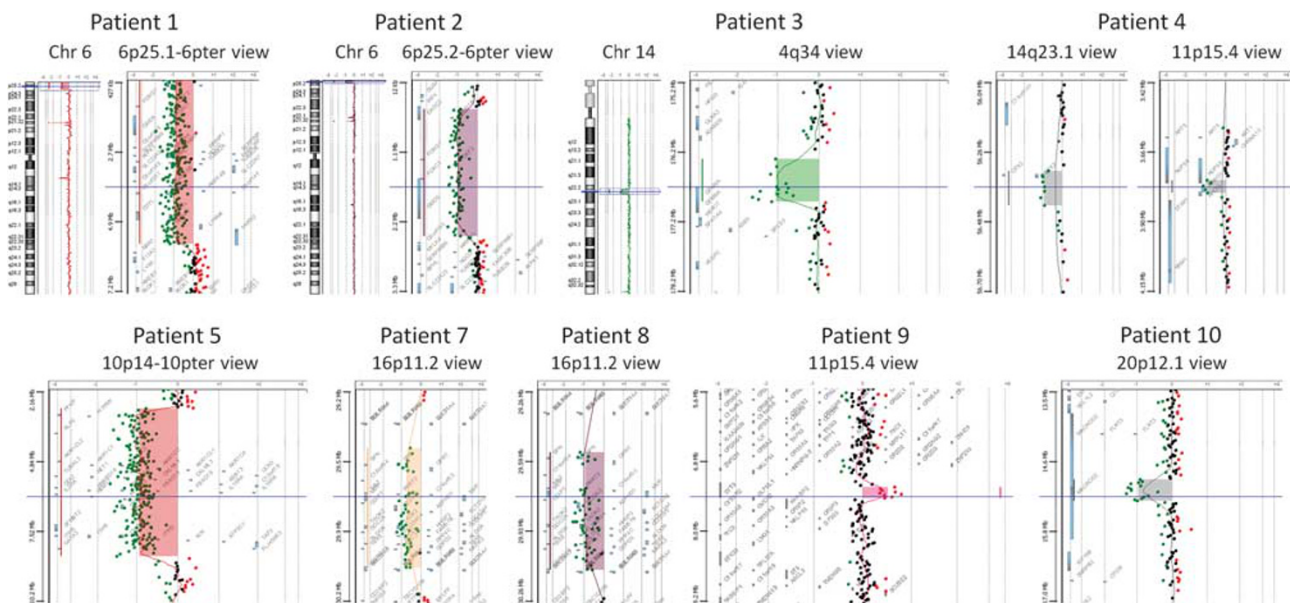


Figure 1 Possibly pathogenic chromosomal anomalies identified in patients with syndromal ODAs. Chromosome views and/or gene views of the affected chromosome or chromosome band produced by the Agilent CGH Analytics software and showing the aberrant region, which is highlighted in colour. On gene views, the dots correspond to the array targets, arranged on the y axis according to their genomic position and on the x axis according to their log₂ intensity ratio value.

and maternally derived for patient no. 7. In patient no. 9, the inherited 11p15.4 duplication involving genes not known to be associated with disease was interpreted as possibly pathogenic because the pattern of family segregation suggested an autosomal dominant defect with incomplete penetrance and variable expressivity (Figure 2, Family A). In patient no. 10, we provisionally classified the 360-kb 20p12.1 deletion as possibly pathogenic, although no known disease-associated gene, microdeletion, or microduplication syndrome was found. We are seeking to obtain samples from the parents and sisters to clarify the clinical significance of this deletion. In a male patient

(patient no. 11) with an Xq25 deletion involving genes not known to be associated with disease and inherited from a healthy mother, the family study identified this Xq25 deletion in an asymptomatic maternal uncle and was therefore probably a non-pathogenic variant (Figure 2, family B, and Table 1). In three patients (patient no. 12, 13, and 14), DNA copy alterations were inherited from a phenotypically normal parent with no family history of ODA and were interpreted as likely non-pathogenic variants (Table 1). All 14 patients have been submitted for registration in the DECIPHER database (<http://www.sanger.ac.uk/PostGenomics/decipher/>).

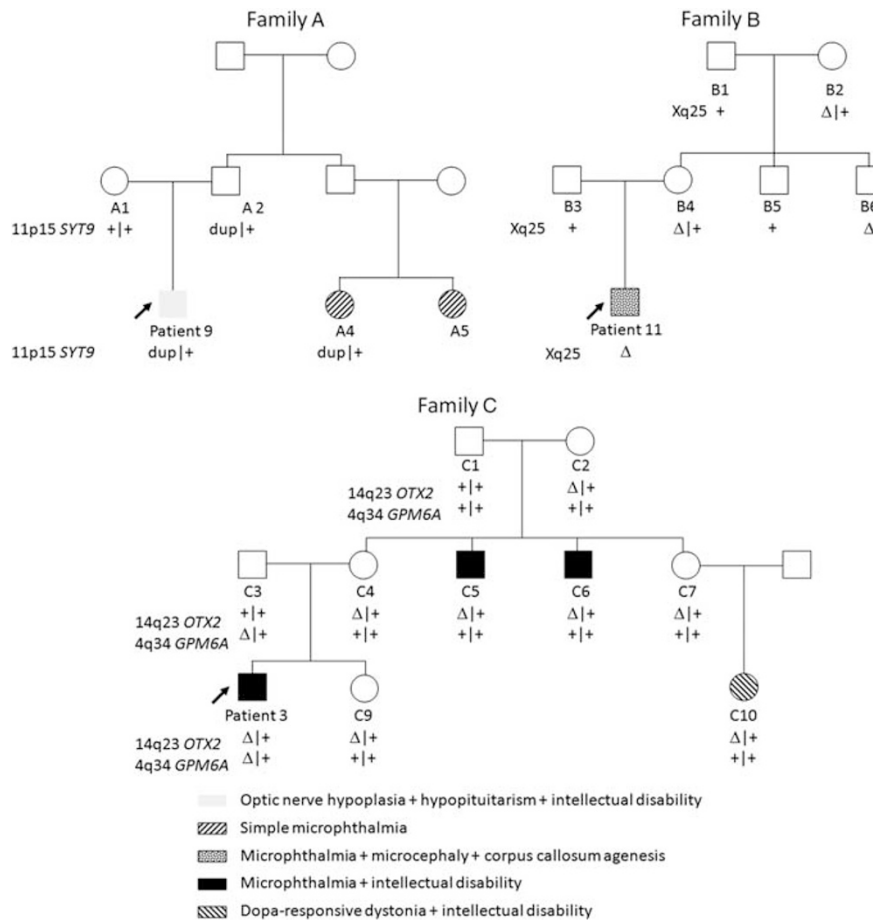


Figure 2 Family segregation of possibly pathogenic chromosomal anomalies. Family studies were performed for patient no. 9 (family A), 11 (family B), and 3 (family C). In family A, segregation of the 11p15 duplication was consistent with an autosomal dominant disorder with incomplete penetrance and variable expression. In family B, the Xq25 deletion was present in an asymptomatic maternal uncle, suggesting that it was a non-pathogenic variant devoid of clinical significance. Family C demonstrates the incomplete penetrance and variable expressivity of phenotypes associated with *OTX2* defects. The additional paternally inherited imbalance in patient no. 3 suggests that *GPM6A* haploinsufficiency may modify the phenotype associated with *OTX2* defects. dup, duplication; Δ, deletion; +, wild type.

Clinical data of patients with clinically relevant chromosomal abnormalities

Patient no. 1. This girl with a 6p25 deletion encompassing the *FOXC1* gene was referred to a clinical geneticist at 1 month of age for dilated cardiomyopathy and facial dysmorphism. She was the first and only child of healthy non-consanguineous parents. The pregnancy was uneventful. She was born at 39 weeks of gestation after a normal vaginal delivery. At birth, she had normal values for weight (2845 g), length (47.5 cm), and occipitofrontal circumference (34 cm). Dysmorphic features included a prominent forehead, hypertelorism, down-slanting palpebral fissures, a broad nasal bridge, and ocular proptosis. The combination of dilated cardiomyopathy and facial dysmorphism prompted further investigations. The conventional cytogenetic analysis was normal. The ophthalmologist found bilateral Axenfeld's anomaly with iridocorneal adhesions and corectopia of the right eye. Magnetic resonance imaging (MRI) of the brain showed a short corpus callosum and mega cisterna magna. During follow-up, mild motor delay developed and the cardiomyopathy remained stable. At last follow-up, she was 2 years of age and unable to walk alone; her weight was 10.2 kg (−1.5 SD), her height 81 cm (−1 SD), and her OFC 46 cm (−1 SD).

Patient no. 2. This 27-year-old man with a 6p25 deletion encompassing the *FOXC1* gene was born to non-consanguineous parents. He had Rieger syndrome with low vision and bilateral Rieger ocular anomaly (iris hypoplasia, iridocorneal adhesions, and posterior embryotoxon) complicated by bilateral glaucoma. Other abnormalities were hypotrophy, hypodontia, and autistic spectrum disorder. Cerebral MRI could not be performed.

Patient no. 3. This 24-year-old male belonging to family C (Figure 2) and his two maternal uncles (C5 and C6) had various combinations of colobomatous microphthalmia, palate anomalies, facial dysmorphism, renal malformation, microcephaly, and intellectual disability. They had a 14q23 deletion encompassing the *OTX2* gene. The carrier females had mild features (strabismus, nystagmus, low-normal intelligence, and speech problems), except one (C7), who was asymptomatic. Cousin C10 had developmental delay, strabismus, marked scoliosis, mild dysmorphism, and dopa-responsive dystonia. No other family members carrying the deletion had neurological symptoms suggesting dystonia.

Patient no. 4. This 14 year-old girl with a 14q23 deletion encompassing the *OTX2* gene was the first of two children of healthy

non-consanguineous parents. There was no family history of ODA. She was born at full term after a normal vaginal delivery. Her birth weight (2400 g) and length (46 cm) were at -2 SD (third percentile). She had severe bilateral microphthalmia, facial dysmorphism (prominent forehead and depressed nasal bridge), and developmental delay. When she was 4 years old, her weight was 10 kg (-3 SD), her height was 87 cm (-3.5 SD), and her skeletal bone age was 2 years. Investigations showed growth hormone deficiency and she was started on growth hormone replacement therapy. Brain MRI showed anterior pituitary hypoplasia and an ectopic posterior pituitary gland. At 10 years of age, after more than 5 years of growth hormone treatment, her weight was 21 kg (-2 SD) and her height 124 cm (-2 SD).

Patient no. 5. This 9-year-old boy with 10p14p15 deletion was the first and only child of healthy non-consanguineous parents. He was born at full term after a normal vaginal delivery, with growth parameters at -2 SD: weight 2380 g (fifth percentile); length 45 cm (third percentile), and OFC 32 cm (fifth percentile). He had multiple birth defects consisting of choanal atresia, bilateral coloboma of the iris, malrotation of the midgut, grade III vesicoureteral reflux, facial dysmorphism (blepharophimosis with down-slanting palpebral fissures), and microcephaly (OFC at -4 SD). During follow-up, epilepsy and developmental delay were diagnosed, followed by bilateral hearing impairment and hypoparathyroidism.

Patient no. 6. This 9-year-old boy previously described by Schiff *et al*¹¹ as patient D had a 17p13.3. deletion. He was the second child of healthy non-consanguineous parents. He had prenatal-onset growth retardation, bilateral chorioretinal coloboma with lens coloboma and right microcornea, developmental delay, and facial dysmorphism (large face, hypertelorism, down-slanting palpebral fissures, ptosis, short nose, pointed chin, and everted lower lip). Multidrug-resistant epilepsy developed during follow-up.

Patient no. 7. This 4-year-old girl with a 16p11.2 deletion had septo-optic dysplasia, with pituitary deficiency and intellectual disability. The ophthalmological examination was abnormal, with colobomatous microphthalmia of the left eye, persistent hyperplastic primary vitreous, and cataract of right eye. Brain MRI showed agenesis of the septum pellucidum and olfactory bulb and hypoplasia of the optic tract.

Patient no. 8. This 6-year-old boy with a 16p11.2 deletion was the second of two children of healthy non-consanguineous parents. There was no family history of ODA. He was born at full term after a normal vaginal delivery, with growth parameters at -2 SD: weight 2735 g (5–10th percentile); length 48 cm (10th percentile); and OFC 33 cm (10th percentile). Congenital nasolacrimal duct obstruction was repaired surgically. Chorioretinal coloboma with microcornea was found in the left eye; the right eye was normal. During follow-up, mild psychomotor delay with a speech defect developed. Brain MRI was not performed.

Patient no. 9. This 10-year-old boy with a 11p15.4 duplication was the first and only child of healthy non-consanguineous parents and belonged to family A (Figure 2). He had optic nerve hypoplasia, growth hormone and TSH deficiency, sleep disorder, and intellectual disability. Brain MRI showed anterior pituitary hypoplasia and ectopia of the posterior pituitary gland without other septal anomalies. His two paternal female cousins had simple microphthalmia.

Patient no. 10. This girl with a 20p12.1 deletion was the first-born child of healthy non-consanguineous parents. Abnormalities at birth consisted of bilateral anophthalmia, cleft lip and palate, deafness, and

bilateral inguinal hernia. Her psychomotor development and stature were normal. She had a sister with unilateral microphthalmia and cleft lip and palate.

DISCUSSION

Using whole-genome array-CGH, we found 10 potentially pathogenic chromosome imbalances among 65 patients with ODAs. This diagnostic yield of 15% is consistent with the findings from oligonucleotide-based array studies in patients with unexplained intellectual disability or developmental delay.^{12,13} Our study's arrays resolutions are theoretically lower than those used in the two previous array-CGH studies of patients with ODA.^{8,9} However, our diagnostic yield is similar or better. This is certainly explained by differences in the inclusion criteria; unlike us, these two studies included patients with isolated ODAs.

The chromosomal abnormalities identified in patient no. 1–8 were considered causal. In four patients (no. 1–4), we found deletions encompassing a gene known to be involved in ocular development (*FOXC1* or *OTX2*), in keeping with an earlier study.⁹ Adding our patients to those of this previous study, 8% of patients with ODAs had deletions encompassing *OTX2* ($n=3$), *FOXC1* ($n=3$), *COH1* ($n=1$), or *PAX6* ($n=1$). *FOXC1* alterations (mutations or 6p25.3 deletions/duplications) are involved in Axenfeld–Rieger anomaly or syndrome (MIM 601090, 602482, and 601631) and were recently shown to cause cerebellar vermis hypoplasia and to contribute to the Dandy–Walker continuum.¹⁴ *OTX2* deletions and heterozygous mutations cause syndromal micro-anophthalmia (MIM 600037 and 610125)¹⁵ and developmental anomalies of the pituitary gland,¹⁶ and *OTX2* mutations can cause septo-optic dysplasia.¹⁷ Family C and several previously described families were reported with *OTX2* alterations in patients presenting micro-anophthalmia and/or pituitary deficiency but inherited from an asymptomatic parent,^{9,16,18,19} suggesting the incomplete penetrance of *OTX2*-associated phenotypes. Therefore, a CNV encompassing *OTX2* transmitted by an unaffected parent can be pathogenic for his child. Intrafamilial heterogeneity of *OTX2* defects may be explained, in some cases, by an additional imbalance. The *GPM6A* gene included in the paternally inherited 610-kb deletion at 4q34 of our patient no. 3 and the *RHOG* gene in the 11p15 deletion of patient no. 4 have both been implicated in neurite outgrowth.^{20,21} RHO–ROCK signalling is involved in regulating the microfilament system, which is a key regulator of epithelial morphogenesis²² and controls the early phases of optic cup morphogenesis.²³ These data support a role for *RHOG* in ODAs. We therefore suggest that *RHOG* haploinsufficiency may modulate the *OTX2*-related phenotype of patient no. 4.

Patient no. 5 and no. 6 carry pathogenic deletions that are not classically associated with ODAs. In both patients, the extraocular phenotype is consistent with the deletion. Their ODA could be related to a separate unknown cause or explained by the incomplete penetrance of an unknown ocular developmental gene included in their deletion. In patient no. 5, the deletion includes *GATA3*, which is involved in the HDR syndrome (hypoparathyroidism, deafness, and renal dysplasia syndrome, MIM 146255), and the phenotype is consistent with HDR syndrome. The additional anomalies are possibly due to the other genes included in the large 10p14p15 deletion in this patient. In keeping with earlier data,^{11,24} ocular coloboma was a feature in our patient no. 6, pointing to a critical 0.67-Mb region for coloboma, with incomplete penetrance.

CNVs associated with partially penetrant phenotypes raise challenges, as seen also in our patient no. 7 and 8, who had a 16p11.2 deletion. The 16p11.2 deletion syndrome (OMIM 611913) has been

described in at least 100 individuals. The common deleted or duplicated region is 555 kb in length and is flanked by two low copy repeats of about 147 kb, suggesting that its pathogenic effects may be mediated by non-allelic homologous recombination. Recurrent microdeletions and microduplications at 16p11.2 have been shown to confer susceptibility to autistic spectrum disorder and have been identified in up to 1% of patients with this diagnosis.²⁵ The 16p11.2 deletion frequently co-segregates with severe early-onset obesity.^{26,27} ODA is not a common feature of 16p11.2 deletions. In a neonate with multiple anomalies, including right ocular coloboma and chorioretinitis, array-CGH detected a 16p11.2 deletion on the G-banded karyotype; the size of the deletion was not estimated, and the break points were not reported.²⁸ More recently, microphthalmia and optic nerve coloboma were reported in a patient with a *de novo* 16p11.2 microdeletion.²⁹ Our finding of a 16p11.2 deletion in two patients with ODAs supports a causal link between 16p11.2 haploinsufficiency and low-penetrance coloboma. A less likely hypothesis is that of an autosomal recessive disorder with loss of one of the alleles, allowing the mutation-carrying allele to cause the ODA. In this chromosomal region, *SEZ6L2* may be involved in ocular development, as it is expressed in the forebrain during development³⁰ as well as in the eye (BioGPS, <http://biogps.org/>; GeneHub-GEPIS, <http://www.cgl.ucsf.edu/Research/genentech/genehub-gepis/genehub-gepis-search.html>). Further studies are needed to evaluate the potential role for *SEZ6L2* or another 16p11.2 gene in ocular development.

The clinical relevance of the CNV detected by array-CGH was unclear in two of our patients. Criteria for pathogenic CNVs, as opposed to non-pathogenic CNVs, have been suggested (see Table 1 of Lee *et al*³¹). Nevertheless, it may be difficult to draw a definitive conclusion,^{7,32} most notably when samples cannot be obtained from both parents (and, in some cases, other family members). Unavailability of family samples is probably the greatest challenge raised by the use of array-CGH as a routine diagnostic tool. In our patient no. 10, the absence of family samples hindered our ability to interpret the 360-kb deletion at 20p12.1 in exon 6 and intron 6 of *MACROD2*. This deletion is not registered among the non-pathogenic CNVs in the DGV database. The function of *MACROD2* (previously *c20orf133*) is unknown. Genome-wide association studies found significant associations of *MACROD2* single-nucleotide polymorphisms with autistic spectrum disorder,³³ brain infarction,³⁴ and brain volume in multiple sclerosis.³⁵ The 360-kb deletion is located close to a hotspot of *de novo* and inherited CNVs with variable and non-recurrent break points, involving exon 5 and/or intron 5 of *MACROD2*. CNVs of this hotspot were found in control individuals in the DGV database and in individuals with schizophrenia,³⁶ holoprosencephaly,³⁷ and Kabuki syndrome,^{38,39} suggesting limited or absent clinical relevance.⁴⁰ The phenotype in patient no. 10 does not resemble Kabuki syndrome. These facts argue against a role for *MACROD2* in ODAs.

The clinical relevance of the 11p15 microduplication detected in patient no. 9 is uncertain. This 180-kb duplication partially encompasses two genes, *SYT9* (OMIM 613528) and *OLFML1*. Confirmation using qPCR cannot distinguish a tandem/inverted duplication from an insertion at a distance. The *SYT9* gene is specifically expressed in the mouse brain and may have a role in calcium-sensitive synaptic neurotransmitter release,⁴¹ but neither *SYT9* nor *OLFML1* is known to be associated with clinical disease. The 11p15 duplication was also found in a cousin of patient no. 9 who has a different ODA. Microphthalmia, optic nerve hypoplasia, and hypopituitarism belong to the growing spectrum of anomalies known to occur in septo-optic dysplasia.¹⁷ The pattern of family segregation is consistent with an autosomal dominant defect with incomplete penetrance and variable

expression. In the absence of similar cases, we can suggest, but not confirm, that this variant is pathogenic.

In our study, two-thirds of the patients were males, in keeping with the known male predominance among patients with ODA (and intellectual disability). No pathogenic CNVs were found in the X chromosome in our study or in earlier work. Therefore, the male preponderance can probably not be ascribed to genomic rearrangements involving developmental genes on the X chromosome. However, we cannot rule out inadequate distribution of oligonucleotide probes on the X chromosome.

In conclusion, our results emphasise the benefits of whole-genome array-CGH for the diagnosis of ODA syndromes. Detecting the genetic defect has important consequences for genetic counselling of the families and follow-up of the patients. Detailed molecular analysis of the rearranged regions may help to identify the genes involved in ocular development.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the patients and their families who participated in this study and the medical staff involved in the diagnosis of syndromal ocular development anomalies.

- 1 Fitzpatrick DR, van Heyningen V: Developmental eye disorders. *Curr Opin Genet Dev* 2005; **15**: 348–353.
- 2 Hornby SJ, Gilbert CE, Rahi JK *et al*: Regional variation in blindness in children due to microphthalmos, anophthalmos and coloboma. *Ophthalmic Epidemiol* 2000; **7**: 127–138.
- 3 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.
- 4 Mihelec M, St Heaps L, Flaherty M *et al*: Chromosomal rearrangements and novel genes in disorders of eye development, cataract and glaucoma. *Twin Res Hum Genet* 2008; **11**: 412–421.
- 5 Kallen B, Tornqvist K: The epidemiology of anophthalmia and microphthalmia in Sweden. *Eur J Epidemiol* 2005; **20**: 345–350.
- 6 Stoll C, Alembik Y, Dott B, Roth MP: Congenital eye malformations in 212,479 consecutive births. *Ann Genet* 1997; **40**: 122–128.
- 7 Lee C, Scherer SW: The clinical context of copy number variation in the human genome. *Expert Rev Mol Med* 2010; **12**: e8.
- 8 Raca G, Jackson CA, Kucinskas L *et al*: Array comparative genomic hybridization analysis in patients with anophthalmia, microphthalmia, and coloboma. *Genet Med* 2011; **13**: 437–442.
- 9 Balikova I, de Ravel T, Ayuso C *et al*: High frequency of submicroscopic chromosomal deletions in patients with idiopathic congenital eye malformations. *Am J Ophthalmol* 2011; **151**: 1087–1094, e1045.
- 10 Winer J, Jung CK, Shackel I, Williams PM: Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes *in vitro*. *Anal Biochem* 1999; **270**: 41–49.
- 11 Schiff M, Delahaye A, Andrieux J *et al*: Further delineation of the 17p13.3 microdeletion involving YWHAЕ but distal to PAFAH1B1: four additional patients. *Eur J Med Genet* 2010; **53**: 303–308.
- 12 Koolen DA, Pfundt R, de Leeuw N *et al*: Genomic microarrays in mental retardation: a practical workflow for diagnostic applications. *Hum Mutat* 2009; **30**: 283–292.
- 13 Xiang B, Zhu H, Shen Y *et al*: Genome-wide oligonucleotide array comparative genomic hybridization for etiological diagnosis of mental retardation: a multicenter experience of 1499 clinical cases. *J Mol Diagn* 2010; **12**: 204–212.
- 14 Aldinger KA, Lehmann OJ, Hudgins L *et al*: FOXC1 is required for normal cerebellar development and is a major contributor to chromosome 6p25.3 Dandy-Walker malformation. *Nat Genet* 2009; **41**: 1037–1042.
- 15 Nolen LD, Amor D, Haywood A *et al*: Deletion at 14q22-23 indicates a contiguous gene syndrome comprising anophthalmia, pituitary hypoplasia, and ear anomalies. *Am J Med Genet A* 2006; **140**: 1711–1718.
- 16 Dateki S, Kosaka K, Hasegawa K *et al*: Heterozygous orthodenticle homeobox 2 mutations are associated with variable pituitary phenotype. *J Clin Endocrinol Metab* 2010; **95**: 756–764.
- 17 McCabe MJ, Alatzoglou KS, Dattani MT: Septo-optic dysplasia and other midline defects: the role of transcription factors: HESX1 and beyond. *Best Pract Res Clin Endocrinol Metab* 2011; **25**: 115–124.

- 18 Ragge NK, Brown AG, Poloschek CM *et al*: Heterozygous mutations of OTX2 cause severe ocular malformations. *Am J Hum Genet* 2005; **76**: 1008–1022.
- 19 Schilter KF, Schneider A, Bardakjian T *et al*: OTX2 microphthalmia syndrome: four novel mutations and delineation of a phenotype. *Clin Genet* 2011; **79**: 158–168.
- 20 Alfonso J, Fernandez ME, Cooper B, Flugge G, Frasch AC: The stress-regulated protein M6a is a key modulator for neurite outgrowth and filopodium/spine formation. *Proc Natl Acad Sci USA* 2005; **102**: 17196–17201.
- 21 Katoh H, Negishi M: RhoG activates Rac1 by direct interaction with the Dock180-binding protein Elmo. *Nature* 2003; **424**: 461–464.
- 22 Sawyer JM, Harrell JR, Shemer G, Sullivan-Brown J, Roh-Johnson M, Goldstein B: Apical constriction: a cell shape change that can drive morphogenesis. *Dev Biol* 2010; **341**: 5–19.
- 23 Eiraku M, Takata N, Ishibashi H *et al*: Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* 2011; **472**: 51–56.
- 24 Nagamani SC, Zhang F, Shchelochkov OA *et al*: Microdeletions including YWHAE in the Miller-Dieker syndrome region on chromosome 17p13.3 result in facial dysmorphisms, growth restriction, and cognitive impairment. *J Med Genet* 2009; **46**: 825–833.
- 25 Fernandez BA, Roberts W, Chung B *et al*: Phenotypic spectrum associated with *de novo* and inherited deletions and duplications at 16p11.2 in individuals ascertained for diagnosis of autism spectrum disorder. *J Med Genet* 2010; **47**: 195–203.
- 26 Bochukova EG, Huang N, Keogh J *et al*: Large, rare chromosomal deletions associated with severe early-onset obesity. *Nature* 2010; **463**: 666–670.
- 27 Walters RG, Jacquemont S, Valsesia A *et al*: A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. *Nature* 2010; **463**: 671–675.
- 28 Hernando C, Plaja A, Rigola MA *et al*: Comparative genomic hybridisation shows a partial *de novo* deletion 16p11.2 in a neonate with multiple congenital malformations. *J Med Genet* 2002; **39**: E24.
- 29 Bardakjian TM, Kwok S, Slavotinek AM, Schneider AS: Clinical report of microphthalmia and optic nerve coloboma associated with a *de novo* microdeletion of chromosome 16p11.2. *Am J Med Genet A* 2010; **152A**: 3120–3123.
- 30 Kumar RA, Marshall CR, Badner JA *et al*: Association and mutation analyses of 16p11.2 autism candidate genes. *PLoS One* 2009; **4**: e4582.
- 31 Lee C, Iafrate AJ, Brothman AR: Copy number variations and clinical cytogenetic diagnosis of constitutional disorders. *Nat Genet* 2007; **39**: S48–S54.
- 32 Rodriguez-Revenga L, Mila M, Rosenberg C, Lamb A, Lee C: Structural variation in the human genome: the impact of copy number variants on clinical diagnosis. *Genet Med* 2007; **9**: 600–606.
- 33 Anney R, Klei L, Pinto D *et al*: A genome-wide scan for common alleles affecting risk for autism. *Hum Mol Genet* 2010; **19**: 4072–4082.
- 34 Debette S, Bis JC, Fornage M *et al*: Genome-wide association studies of MRI-defined brain infarcts: meta-analysis from the CHARGE Consortium. *Stroke* 2010; **41**: 210–217.
- 35 Baranzini SE, Wang J, Gibson RA *et al*: Genome-wide association analysis of susceptibility and clinical phenotype in multiple sclerosis. *Hum Mol Genet* 2009; **18**: 767–778.
- 36 Xu B, Woodroffe A, Rodriguez-Murillo L *et al*: Elucidating the genetic architecture of familial schizophrenia using rare copy number variant and linkage scans. *Proc Natl Acad Sci USA* 2009; **106**: 16746–16751.
- 37 Bendavid C, Rochard L, Dubourg C *et al*: Array-CGH analysis indicates a high prevalence of genomic rearrangements in holoprosencephaly: an updated map of candidate loci. *Hum Mutat* 2009; **30**: 1175–1182.
- 38 Maas NM, Van de Putte T, Melotte C *et al*: The C20orf133 gene is disrupted in a patient with Kabuki syndrome. *J Med Genet* 2007; **44**: 562–569.
- 39 Kuniba H, Yoshiura K, Kondoh T *et al*: Molecular karyotyping in 17 patients and mutation screening in 41 patients with Kabuki syndrome. *J Hum Genet* 2009; **54**: 304–309.
- 40 Bradley WE, Raelson JV, Dubois DY *et al*: Hotspots of large rare deletions in the human genome. *PLoS One* 2010; **5**: e9401.
- 41 Xu J, Mashimo T, Sudhof TC: Synaptotagmin-1, -2, and -9: Ca(2+) sensors for fast release that specify distinct presynaptic properties in subsets of neurons. *Neuron* 2007; **54**: 567–581.

Supplementary Information accompanies the paper on European Journal of Human Genetics website (<http://www.nature.com/ejhg>)