Neurological features of congenital fibrosis of the extraocular muscles type 2 with mutations in PHOX2A

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Congenital fibrosis of the extraocular muscles type 2 (CFEOM2) is a complex strabismus syndrome that results from mutations in the homeodomain transcription factor PHOX2A. To define the clinical and neuro-imaging features of patients with this autosomal recessive syndrome, we studied 15 patients with genetically defined CFEOM2. All patients underwent full neurological, neuro-ophthalmological and orthoptic assessments. Twelve patients had pupillary pharmacological testing and nine had 3.0 tesla MRI of the brain, brainstem and orbits. Patients were born with severe bilateral ptosis and exotropia with almost complete bilateral absence of adduction, elevation, depression and intorsion. Variable abduction was present prior to strabismus surgery in 14 patients, and central ocular motility reflexes (smooth pursuit, saccades, vestibulo-ocular reflex and optokinetic reflex) were intact except for convergence. Pupillary light and near reflexes were not present, but irises were anatomically normal and responded to pupillary pharmacology. Neuroimaging of brain and brainstem was remarkable for the anatomical absence of cranial nerve (CN) 3 and probably CN 4 bilaterally. Therefore, the CFEOM2 phenotype and neuroimaging are both consistent with the congenital absence of CNs 3 and 4. Additional features included presence of most central ocular motility reflexes, a central lack of pupillary responsiveness of uncertain aetiology and modest phenotypic variability that does not correlate with specific PHOX2A mutations. Clinical presentation, neuroimaging and Phox2a-/- animal models all support the concept that CFEOM2 is a primary neurogenic abnormality with secondary myopathic changes.

Keywords: brain imaging; brain development; ocular motor nerve; congenital ophthalmoplegia

Abbreviations: CCDDs = congenital cranial dysinnervation disorders; CFEOM2 = congenital fibrosis of the extraocular muscles type 2; CN = cranial nerve; EOM = extraocular muscle


Introduction

Congenital strabismus in humans can result from mutations in a number of genes, including ROBO3 (Jen et al., 2004), PHOX2A (Nakano et al., 2001), SALL4 (Al-Baradie et al., 2002), HOXA1 (Tischfield et al., 2005) and KIF21A (Yamada et al., 2003) that are essential to the normal development of brainstem motor neurons or axons. We now refer to these syndromes as congenital cranial dysinnervation disorders (CCDDs) (Gutowski et al., 2003).

The first insight into the genetics of the CCDDs came from studies of congenital fibrosis of the extraocular muscles type 2 (CFEOM2; OMIM 602078), in which affected...
individuals usually had bilateral severe congenital exotropia, severe ptosis and small, unreactive pupils (Wang et al., 1998; Yazdani et al., 2003). CFEOM2 was mapped to human chromosome 11q13 in 1998 (Wang et al., 1998), and was found to result from homozygous loss of function mutations of the PHOX2A gene in 2001 (Nakano et al., 2001).

Phox2a and its close relative, Phox2b, are homeodomain transcription factor genes with identical homeodomains and similar expression patterns restricted to several classes of differentiating neurons in the central and peripheral nervous system (Tiveron et al., 1996; Pattyn et al., 1997). Phox2a is expressed at E9 in the proliferating oculomotor and trochlear motor neuron precursors in mouse (Pattyn et al., 1997), supporting the hypothesis that CFEOM2 results from maldevelopment of the oculomotor and, possibly, trochlear nerves (Nakano et al., 2001). Both the oculomotor and trochlear nuclei are absent in mouse Phox2a−/− null mutants (Morin et al., 1997) and in homozygous soulless zebrafish with point mutations in the Phox2a homeodomain (Guo et al., 1999). In contrast to individuals with CFEOM2, however, the zebrafish and mice die soon after birth and have additional abnormalities including absence of the locus coeruleus (LC), atrophy of cranial sensory ganglia and, in the mouse, absence of parasympathetic ganglia of the head (Morin et al., 1997).

Initial reports of the CFEOM2 phenotype suggested defective development of oculomotor and trochlear motor neurons without autonomic abnormalities, but the phenotype has not been described in detail. The discovery that CFEOM2 results from mutations in PHOX2A now permits an opportunity to define the phenotypic spectrum of genetically defined CFEOM2 patients. Therefore, we evaluated patients with CFEOM2 and PHOX2A mutations to document neurological examinations and ocular motility, to study pupillary characteristics, and to carefully image the brainstem and cranial nerves (CNs).

### Material and methods

#### Patients

Table 1 details 15 affected patients (12 male and 3 females, aged 4–44 years) from 7 nuclear families who were homozygous for either the 386C→T (A72V) or IVS2.G→A,−1 splice site mutation in PHOX2A (Nakano et al., 2001). Thirteen were patients cared for at the King Khaled Eye Specialist Hospital (KKESH) for 3–19 years, all of whom had been genotyped as part of a previous genetic study (Nakano et al., 2001). Two participants (Patients 2 and 8) were affected members of previously genotyped families who had not been examined previously. CFEOM2 is fully penetrant, and their genotypes were assumed. One heterozygous family member was examined and underwent neuroimaging and pupillary pharmacological studies. All individuals signed informed consent approved by the Institutional Review Board. Eleven individuals had strabismus surgery (Patients 1, 4–7, 9–11 and 13–15) and seven individuals had at least unilateral ptosis surgery (Patients 1, 4, 5 and 8–11).

#### Clinical evaluation

All patients underwent neurological, neuro-ophthalmological and orthoptic examinations, including near and distance visual acuity (VA), refractive status and near point of accommodation.

Ocular alignment was observed in all positions of gaze. The angle of ocular misalignment (strabismus) was estimated by the prism reflex test (Krimsky test) which utilizes prisms to centre penlight reflections off the cornea that are displaced by misalignment of the visual axes. A complete description of this method can be found elsewhere (Pratt-Johnson and Tillson, 1994). The direction of misalignment could be accurately determined with this method; however, the strabismus size could only be estimated given the extent of exotropia and severe limitation of ocular motility in most patients.

Ocular movements were observed in all positions of gaze by two clinicians (T.M.B., D.T.O.), and all patients had video recording to confirm the characteristics of globe movement. Resting globe position was always eccentric and all eye movements were severely restricted, with the exception of abduction in a few individuals, necessitating a modified ocular motility grading system. Therefore, the size of eye movement in each cardinal meridian was scored as
minimal (1–5°), mild (6–10°), modest (11–20°) or moderate (21–30°). Central ocular motility reflexes, including smooth pursuit, saccades, vestibulo-ocular response (VOR, assessed in a rotating chair), optokinetic nystagmus (OKN) and convergence were observed and video recorded in most individuals. Smooth pursuit was assessed both directly and by inhibition of the VOR (Ansons and Davis, 2003).

Pupil testing
Pupils were evaluated for size, shape and reactivity to light and near stimuli by observation and by slit lamp photography. The ORBSCAN IIz (Bausch & Lomb, Rochester, NY, USA) was used to document pupil shape and size to within 0.1 mm diameter through a computerized slit lamp scanning technique. Digital photography of the iris, including iris retro-illumination, was obtained with a Nikon D1X camera mounted to a Topcon 8Z slit lamp using IMAGEnet 2000 digital imaging software (Topcon, Tokyo, Japan).

In order to acquire as much pupillary pharmacological data as possible during a limited examination period, pharmacological testing was performed according to the following protocol: (i) baseline observations were made of pupil size and shape that included photographs and ORBSCAN recordings; (ii) cyclopentolate 1% (cholinergic blocker) was applied to the right eye and dapiprazole 0.5% (adrenergic blocker) to the left, and repeat observations and recordings were made after 1 h; (iii) phenylephrine 2.5% (adrenergic stimulator) was then applied to the right eye and pilocarpine 2% (cholinergic stimulator) to the left eye, and repeat observations and recordings were made after 40 min. Pupillary responses of CFEOM2 patients were compared with seven controls studied with the same protocol.

Neuroimaging
MR images were obtained in nine patients on a 3.0 tesla Siemens Magneton Allegra scanner (Siemens Medical Systems, Germany) including sagittal T1-weighted (624/7), axial fluid-attenuated inversion recovery (8000/80) and axial MP-RAGE (2500/4.4, 1 mm thickness) through the brain, and axial 3DFT constructive interference in steady-state (cis) of the entire brainstem (11/5.6, 1 mm thickness). These images were compared with similar images in 15 individuals without CFEOM2. Coronal and parasagittal reformatted images of the cis data were performed for each patient and control.

Results
General and neurological examinations
All patients were alert, cooperative and seemed cognitively and emotionally normal. No patient reported a somatic developmental abnormality outside of the eyes and eyelids or another major general medical or neurological problem except for resolved unilateral iridocyclitis in one patient (Patient 14). All patients achieved normal developmental milestones and performed at an average level in school despite visual limitations. Patients reported dry eyes, particularly after ptosis surgery, but they denied dry mouth, difficulty with taste, nightmares, symptoms of
orthostatic hypotension, constipation or other obvious autonomic dysfunction. Some patients had difficulty in associating with peers, possibly because of their appearance. General neurological examination was normal, including speech, muscle tone and power, coordination, deep tendon reflexes, sensation and gait. The heterozygous family member had a normal neurological and neuro-ophthalmological examination.

**Afferent examination**

Visual acuities were at least mildly reduced in all patients (Table 2), while colour vision and fundoscopic appearance were normal except that one individual (Patient 13) had a pale optic disc noted in early childhood. Eight patients had severely reduced VA on one side because of stimulus deprivation amblyopia due to congenital ptosis. This problem was treated successfully in only one patient, as compliance with wearing glasses and/or a patch and outcome of ptosis surgery were typically poor.

Eleven individuals were prescribed spectacles for a range of refractive errors (Patients 2, 4–7 and 10–15); however, only one individual wore glasses with any frequency (Patient 15). Glasses did not significantly improve vision, in general, because eyes fixed in abduction made spectacle correction problematic. Accommodation was not demonstrable, and no patient reported appreciating a 'blur' point to an approaching accommodative target. Near VA equaled or exceeded distance VA, presumably because of a pinhole effect from miotic pupils.

**Eyelids**

Table 2 details the characteristics of ptosis in these patients. Levator palpebrae superioris (LPS) function was absent bilaterally and frontalis effort was used by all patients. Ptosis was frequently asymmetric and varied in severity between patients (Fig. 1). Five patients (Patients 1, 4 and 9–11) had bilateral occlusion of the visual axis in primary position; six patients (Patients 3, 5, 7, 8, 12 and 15) had unilateral severe ptosis that occluded the visual axis in one eye with partial occlusion of the contralateral eye; and four patients (Patients 2, 6, 13 and 14) had bilateral partial occlusion of the visual axis in primary gaze. Most patients required compensatory overaction of the frontalis and a slight chin-up head position to clear at least one pupillary axis. Some individuals, particularly children, used a finger to lift the upper lid unilaterally when they wished to attend to a visual scene.

Seven patients had ptosis surgery, including eyelid suspension in four by a sling procedure with care to avoid excessive widening of the palpebral fissure. Unfortunately, ptosis generally recurred (Tillett and Tillett, 1966; Manners et al., 1994).

**Pupils**

Pupil size was fixed in all patients and did not alter in dim or bright room illumination and did not change to direct or consensual light testing. Pupillary near response was absent. Only two patients had normal pupil size and shape (Patients 1 and 8). Eight patients (Patients 2, 3, 6, 7, 9, 10, 12 and 15) had miotic pupils (<2.5 mm diameter) bilaterally, and two additional individuals (Patients 4 and 5) had unilateral miosis. The only patient with anisocoria >2 mm had an unilateral mydriatic pupil after iridocyclitis (Patient 14). Pupil shape was irregular in nine patients (Patients 2–7, 9, 11 and 13), usually with an oval outline. Figure 2 illustrates the variability of pupil size and shape. Transillumination showed no evidence of either generalized or segmental atrophy of the iris stroma or the posterior pigment epithelium, and sector palsy of the iris sphincter was not apparent.

Despite being unreactive to environmental stimuli, pupils of CFEOM2 patients responded to topical pharmacological stimulation and blockade in a generally normal fashion, as detailed in Fig. 3 and illustrated in Fig. 4. Pupillary response was similar between CFEOM2 patients and controls for phenylephrine (CFEOM2 mean pupillary change 0.72 ± 0.42 mm, 95% CI 0.41–1.06; control mean 0.58 ± 0.67, 95% CI −0.12–1.28; P = 0.64), dapiprazole (CFEOM2 mean −0.26 ± 0.38, 95% CI −0.52 to −0.01; control
mean \(0.51 \pm 0.57, 95\% \text{ CI } -1.04\text{–}0.02; P = 0.33\) and pilocarpine (CFEOM2 mean \(-0.44 \pm 0.42, 95\% \text{ CI } -0.74\text{–}0.14; \text{ control mean } -0.74 \pm 0.35, 95\% \text{ CI } -1.07\text{–}0.42; P = 0.13\), but CFEOM2 patients reacted significantly less to cyclopentolate than controls (CFEOM2 mean 2.61 \pm 0.59, 95\% CI 2.22\text{–}3.00; control mean 3.82\pm 1.03, 95\% CI 2.74\text{–}4.89; \(P = 0.03\)). The absence of iris atrophy and the presence of pharmacological responsiveness suggest iris innervation of unknown source.

**Ocular alignment**

Table 3 contains details of ocular alignment together with the results of extraocular muscle (EOM) surgery. Clinical evaluation of unoperated patients and review of preoperative records in operated patients documented bilateral large angle exotropia in 14 out of 15 patients. Exotropia was estimated between 50 prism diopters (PD) and 120 PD, with eight individuals (Patients 2 and 9–15) having angles of 90 PD or greater (Fig. 5). Patients with an exotropia adopted a face turn away from the fixing eye. Patient 1 had no horizontal deviation from birth.

Eight patients had no vertical strabismus with eyes fixed at the vertical midline, while seven had a vertical misalignment of the eyes. This included bilateral infraduction (Patient 1), bilateral supraduction (Patients 7, 11 and 13) or unilateral vertical displacement with one eye supraducted (Patient 10) or infraducted (Patient 3). Patient 4 had supraduction of one eye and infraduction of the other eye.

Most EOM surgery included extirpation of the lateral rectus with or without resection of the medial rectus, often with traction sutures to hold the eyes in adduction during the immediate postoperative period. Postoperative alignment varied between a small esotropia and a large exotropia, and no surgical outcome permitted the development of fusion.

**Ocular motility**

Table 4 summarizes ocular motility. Adduction was absent in 13 patients with each eye fixed in an abducted position. Patients 4 and 5 had mild adduction of one eye, but not to midline, while Patient 14 developed modest adduction ability in one eye after lateral rectus extirpation. He was...
the only person to develop an esotropia following ocular motility surgery.

Vertical eye movement was almost absent. Four individuals (Patients 1, 3, 5 and 7) had minimal voluntary vertical movement of at least one eye, and others had minimal vertical movement of one eye during abduction (Patients 4 and 11), convergence (Patients 1 and 4), or blinking (Patients 1 and 3). Three patients developed minimal, unsustained abduction of an eye during attempted down gaze (Patients 4, 5 and 15). Bell’s phenomenon was absent in all patients.

The extent of abduction varied widely. Abduction was full bilaterally in only two individuals (Patients 2 and 5), and absent bilaterally in another (Patient 1), with the majority of individuals having reduced or unsustained abduction whether eye movement was assessed by smooth pursuit, Doll’s head manoeuvre or OKN. The only person with complete lack of abduction (Patient 1) was also the only individual orthophoric from birth. In other individuals, however, degree of lateral rectus function appeared unrelated to size of exotropia. Abduction ability varied between siblings and even between eyes in the same individual. For example, Patient 8 (Fig. 6) had very good abduction OD and reduced abduction OS. Patient 2 had full abduction OU while his brother (Patient 3) had markedly reduced abduction OU.

Smooth pursuit was apparent in every eye that had good acuity and that was able to abduct at least a modest amount. The OKN drum was able to elicit abducting saccades. Yoking of the medial rectus to the contralateral lateral rectus was evident on attempted smooth pursuit into adduction with optokinetic drum or VOR. Although the eye could not maintain fixation in the direction of adduction, the effort to do so elicited abducting movements contralaterally. Repetitive saccades were observed into lateral gaze in patients

Fig. 3 Graphs of pupillary response to pharmacological agents. Each line in a graph traces the size of an individual pupil before and after pharmacological manipulation. Pre = pupillary size before instilling pharmacological agent; Post = pupillary size 40–60 min after instilling pharmacological agent (see Material and methods). Change in pupillary size was significant for phenylephrine (mean pre 5.4 ± 1.33, post 6.1 ± 1.25, P = 0.003), cyclopentolate (mean pre 2.7 ± 1.54, post 5.2 ± 1.24, P < 0.0001) and pilocarpine (mean pre 2.4 ± 1.04, post 2.0 ± 0.92, P = 0.021), but not for dapiprazole (mean pre 2.5 ± 1.18, post 2.4 ± 1.04, P = 0.176). Pupillary response was similar between CFEOM2 patients and controls for phenylephrine, dapiprazole and pilocarpine, but CFEOM2 patients reacted significantly less to cyclopentolate than controls (see Results).
having abduction ability present. Clinical observation and review of video recordings revealed no gross abnormalities of saccadic speed or latency. Convergence was not apparent in any patient. Repetitive abducting saccades of the contralateral eye were present in seven individuals attempting convergence to a near target (Patients 2–6, 8 and 9), suggesting absence of vergence ability and an effort by the horizontal gaze centre to obtain fixation.

EOMs were described in surgical reports of six patients. In each patient the medial, superior and inferior recti were described as atrophic, tight, ‘thin strips of tissue’. The lateral recti were also described as inelastic in three patients, and one individual (Patient 5) did not have a right superior oblique muscle observed at surgery. Forced ductions were positive in all 10 tested patients in both horizontal and vertical directions. All patients adopted an abnormal head position to allow uniocular fixation because of strabismus, and all moved their heads to achieve horizontal and vertical refixation of their largely immobile eyes. Nevertheless, the frequent use of marked head movements required for fixation or to maintain fixation during body movement or movement of fixation objects was not a complaint of any patient.

Neuroimaging

The oculomotor nerve was easily visualized bilaterally in the cisternal space of all control individuals (Fig. 7A and C), but could not be demonstrated in any of nine affected individuals (Fig. 7B and D). Similarly, the trochlear nerve could not be visualized in any affected individual, but it could only be identified in the cisternal space of a subset of controls. The abducens nerve as well as the optic, trigeminal, facial and auditory nerves could be seen routinely in both patients and controls (data not shown). MRI through the orbits revealed bilaterally normal to large lateral recti muscles with reduction in the size of all other EOMs (Fig. 7F). Orbital fat was unremarkable, and supratentorial structures and brainstem appeared normal. The heterozygous family member had normal neuroimaging.

Discussion

We evaluated 15 genetically defined CFEOM2 patients and documented a phenotype similar to that reported previously (Wang et al., 1998; Nakano et al., 2001). Nine patients had high-resolution MRI imaging, and CN 3 was anatomically absent in all with reduced size of CN 3-innervated EOMs (LPS, superior, medial and inferior recti, inferior oblique). CN 4 was not visualized in any patient, and the muscle this nerve innervates, the superior oblique, was also small or absent in all patients. Conversely, CN 6 was visualized bilaterally in all imaged CFEOM2 patients, and lateral rectus muscles were normal or large compared to controls. These observations support the hypothesis that CNs 3 and 4 and their corresponding midbrain motor neurons are absent in CFEOM2, just as in Phox2a<sup>-/-</sup> mice and zebrafish (Morin et al., 1997; Guo et al., 1999).

Anatomic absence of CNs 3 and 4 explains the major lid and ocular motility features of CFEOM2. All patients had bilateral severe ptosis with no apparent LPS action, causing the major symptomatic problem of the syndrome. Ptosis ranged from bilateral total occlusion of the visual axis, to unilateral occlusion in primary position, to bilateral partial occlusion. All patients employed maximal frontalis effort in an attempt to clear the visual axis, but only limited chin-up head posture could be used because down gaze was absent. Most patients adopted compensatory strategies such as manually raising a lid. Dense stimulus deprivation amblyopia in eight patients was testimony to the ineffectiveness of this strategy in many patients.

A large angle exotropia was the principal ocular alignment, probably resulting from the unopposed action of the lateral rectus muscle innervated by CN 6. Elevation, depression and adduction were very limited or absent in all eyes, and ocular...
intorsion was not apparent during attempted vertical gaze. Ocular motility was mildly variable between individuals and between eyes of an individual. Some patients had minimal abduction, and minor vertical movements or vertical misalignments were common. Abduction was present in all but one patient, but it was frequently incomplete, unsustained or associated with small amplitude vertical or retraction movements. Patients assumed anomalous head positions in order to fixate with one globe at a time and used head movements rather than eye movements to reposition the globe. Patient 1 was the only individual who had absent abduction bilaterally with globes aligned horizontally but fixed in infraduction. His examination appeared more typical of congenital fibrosis and was not as severe as other presentations. Other patients had multiple anomalous head positions consisting of anomalous movements of the upper lid or Marcus Gunn jaw winking, and globe retraction. Many patients had at least one eye that was incompletely adducted, failed to come to the midline during attempted horizontal gaze, and/or had globe retraction. The mechanisms leading to vertical malpositioning of the globe were probably due to tethering of the globe by stiff, inelastic muscles and/or cocontraction of aberrant EOMs. This hypothesis is also supported by positive forced duction testing and tight muscles found at surgery. Although some anomalous eye movements were observed, no CFEOM2 patient had synergistic convergence or divergence, anomalous movements of the upper lid, or Marcus Gunn jaw winking, all of which are found in CFEOM1.

Several patients had anomalous eye movements, including mild vertical movements of an eye during abduction, convergence or blinking; abduction of an eye on attempted down gaze; and globe retraction. The mechanisms leading to vertical malpositioning of the globes, abnormal abduction and aberrant residual eye movements probably include tethering of the globe by stiff, inelastic muscles and/or cocontraction of aberrant innervation of CN 3- and CN 4-innervated EOMs by abducens axons. This hypothesis is also supported by positive forced duction testing and tight muscles found at surgery.

Patients assumed anomalous head positions in order to fixate with one globe at a time and used head movements rather than eye movements to reposition the globe. Patient 1 was the only individual who had absent abduction bilaterally with globes aligned horizontally but fixed in infraduction. His examination appeared more typical of congenital fibrosis of the EOMs type 1 (CFEOM1) (Yamada et al., 2003) except that his pupils were unreactive to light, highlighting the potential for these two syndromes to overlap clinically (Engle et al., 2002).

### Table 3 Ocular alignment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Preoperative globe position</th>
<th>Strabismus surgery</th>
<th>Globe position at final visit</th>
<th>Compensatory strategy for fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Infraducted OS &gt; OD</td>
<td>Inf rec recession OU with traction suture</td>
<td>Infraducted OU; OS in adduction</td>
<td>Chin up</td>
</tr>
<tr>
<td>2</td>
<td>Marked XT OU</td>
<td>None</td>
<td>Moderate XT OU; vertical misalignment eliminated</td>
<td>AHP</td>
</tr>
<tr>
<td>3</td>
<td>Marked XT OU; OS infraducted</td>
<td>Lat rec recession OU + med rec resection OU + sup rec recession OS + med rec resection OD + med rec resection OD with traction suture</td>
<td>Both eyes anchored in left gaze; no vertical misalignment</td>
<td>AHP</td>
</tr>
<tr>
<td>4</td>
<td>Marked XT OU; OS supraducted</td>
<td>Lat rec recession OU + med rec resection OU with traction suture; sup rec recession OD + inf rec recession OD with traction suture; sup rec extirpation OD</td>
<td>Both eyes anchored in right gaze; induced vertical misalignment with OD supraducted and OS infraducted</td>
<td>AHP</td>
</tr>
<tr>
<td>5</td>
<td>Marked XT OU</td>
<td>Lat rec extirpation OU + med rec resection OU with traction suture</td>
<td>Moderate XT; both eyes supraducted symmetrically</td>
<td>AHP</td>
</tr>
<tr>
<td>6</td>
<td>Marked XT OU</td>
<td>Lat rec extirpation OU + med rec resection OU with traction suture</td>
<td>Moderate XT OU; OS supraducted</td>
<td>AHP</td>
</tr>
<tr>
<td>7</td>
<td>Marked XT OU; supraducted OD &gt; OS</td>
<td>Lat rec recession OU + med rec resection OU with traction suture; sup rec recession OD + infraduction OD</td>
<td>Moderate XT OU; vertical misalignment eliminated</td>
<td>AHP</td>
</tr>
<tr>
<td>8</td>
<td>Marked XT OU</td>
<td>Lat rec recession OU + med rec resection OU; lat rec recession OD + sup rec recession OD</td>
<td>Marked XT OU</td>
<td>AHP</td>
</tr>
<tr>
<td>9</td>
<td>Marked XT OU</td>
<td>Lat rec recession OU; lat rec recession OD + sup rec recession OD</td>
<td>Marked XT OU; OD supraducted</td>
<td>AHP</td>
</tr>
<tr>
<td>10</td>
<td>Marked XT OU; OD supraducted</td>
<td>Lat rec recession OU + med rec resection OS + sup rec recession OD</td>
<td>Moderate XT OU; vertical misalignment eliminated</td>
<td>AHP</td>
</tr>
<tr>
<td>11</td>
<td>Marked XT OU; supraducted OD &gt; OS</td>
<td>Lat rec recession OU + med rec resection OU with traction suture; sup rec recession OD + infraduction OD</td>
<td>Moderate XT OU; vertical misalignment eliminated</td>
<td>AHP</td>
</tr>
<tr>
<td>12</td>
<td>Marked XT OU</td>
<td>Lat rec recession OU + med rec resection OU with traction suture; sup rec recession OD + med rec resection OD</td>
<td>Marked XT OU</td>
<td>AHP</td>
</tr>
<tr>
<td>13</td>
<td>Marked XT OU; supraducted OD &gt; OS</td>
<td>Lat rec recession OU + med rec resection OU with traction suture; sup rec recession OD + med rec resection OD</td>
<td>Marked XT OU</td>
<td>AHP</td>
</tr>
<tr>
<td>14</td>
<td>Marked XT OU</td>
<td>Lat rec recession OU + med rec resection OU with traction suture</td>
<td>Slightly adducted OD &gt; OS</td>
<td>AHP</td>
</tr>
<tr>
<td>15</td>
<td>Marked XT OU</td>
<td>Lat rec extirpation OU + med rec release OS</td>
<td>Small, symmetric XT</td>
<td>AHP</td>
</tr>
</tbody>
</table>

Multiple entries in ‘Strabismus surgery’ column separated by semicolon indicate separate surgeries; lat = lateral; med = medial; sup = superior; inf = inferior; rec = rectus; OD = right eye; OS = left eye; OU = both eyes; XT = exotropia; AHP = anomalous head position consisting of face turn away from fixing eye; AHP* = anomalous head position consisting of face turn toward fixing eye.
and PHOX2B mutations do not explain this dichotomy between anterograde axonal transport that probably plays an important role in axonal targeting of EOMs (Marszalek et al., 1999; Yamada et al., 2003). In contrast, Phox2a is essential to early motor neuron development, and it is likely that CN 3 and CN 4 axons never form in Phox2a−/− mice (Morin et al., 1997; Pattyn et al., 1997). Phox2a is not known to affect supranuclear ocular motility circuitry, and central horizontal gaze mechanisms were intact in CFEOM2 patients.

Pupils of CFEOM2 patients were generally small but varied considerably in size and shape. Remarkably, these pupils were unresponsive to light and accommodation, but generally responsive to pharmacological agents, suggesting some iris innervation tone. CFEOM2 patients responded less well to a cholinergic blocker than did control subjects, and this may reflect the abnormalities of CN 3 innervation in these patients. The source of cholinergic and adrenergic innervation may reflect the abnormalities of CN 3 innervation in these patients. The protein result in the congenital central hypoventilation syndrome (CCHS) (Goldberg and Ludwig, 1996). In contrast to the Phox2b−/− mouse model, most CCHS patients have miotic, poorly reactive pupils with abnormal pupillary response to light (Amiel et al., 2003). Because of the similarity of CCHS pupils to CFEOM2 pupils and because subsets of CCHS patients have ocular motility abnormalities, the mutant protein products of PHOX2B were hypothesized to cause a dominant negative effect on PHOX2A function (Amiel et al., 2003); however, this observation still does not anatomically localize the pupillary abnormality in CFEOM2 patients.

Twelve of the participating CFEOM2 patients harboured a homozygous PHOX2A amino acid substitution, while three harboured a homozygous splice site mutation. None of the phenotypic variability found among these patients segregated with a specific mutation, suggesting lack of a mutation-based phenotype–genotype correlation. This observation, coupled with the same phenotype in another CFEOM2 non-sense mutation (Yazdani et al., 2003), is consistent with the hypothesis that CFEOM2 results from complete loss of PHOX2A function (Nakano et al., 2001). These patients do not have major somatic anomalies or other autonomic, cognitive or focal neurological abnormalities. This is particularly interesting because Phox2a−/− mice lack the oculomotor nucleus, the trochlear nucleus, the LC and the parasympathetic ganglia of the head; have small superior cervical ganglia and other cranial sensory ganglia; and die soon after birth (Morin et al., 1997; Pattyn et al., 1997). These observations suggest that the spatial and temporal expression pattern of PHOX2A/Phox2a differs between human and mouse, or that PHOX2B or another protein may compensate for the lack of PHOX2A in humans.

Developmental absence of CNs 3 and 4 has been assumed in CFEOM2 based on the clinical phenotype and features of the mouse model, but MRI confirmation of this fact now places CFEOM2 unequivocally among the CCDDs. Since PHOX2A was identified as the first isolated strabismus gene in 2001, other CCDDs have been recognized and

**Fig. 5** Variability of ocular alignment in primary gaze. (A) Patient 1 with no horizontal strabismus at birth, but both eyes infraducted. (B) Patient 2 with eyes in severe exotropia with no vertical deviation. (C) Patient 3 with marked exotropia and OS in infraduction. These patients were all from the same nuclear family with only inferior rectus surgery OS in A.
### Table 4 Ocular motility

<table>
<thead>
<tr>
<th>Patient</th>
<th>Horizontal</th>
<th>Vertical</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adduction</td>
<td>Abduction</td>
<td>Elevation</td>
</tr>
<tr>
<td>1</td>
<td>None OU</td>
<td>None OU</td>
<td>Orthophoric; no horizontal strabismus surgery</td>
</tr>
<tr>
<td>2</td>
<td>None OU</td>
<td>Moderate OU</td>
<td>No horizontal strabismus surgery</td>
</tr>
<tr>
<td>3</td>
<td>None OU</td>
<td>Modest OU</td>
<td>No horizontal strabismus surgery</td>
</tr>
<tr>
<td>4</td>
<td>Modest OS</td>
<td>Modest OU</td>
<td>Residual abduction OU following surgery</td>
</tr>
<tr>
<td>5</td>
<td>Min OS</td>
<td>Moderate OU</td>
<td>Residual abduction OD following surgery; OS moved up and in with attempted abduction</td>
</tr>
<tr>
<td>6</td>
<td>None OU</td>
<td>Moderate OU</td>
<td>Residual abduction OU following surgery</td>
</tr>
<tr>
<td>7</td>
<td>None OU</td>
<td>Moderate OU</td>
<td>Residual abduction OU following surgery</td>
</tr>
<tr>
<td>8</td>
<td>None OU</td>
<td>Moderate OD; Mild OS</td>
<td>No horizontal strabismus surgery; smooth abduction OD and jerky OS</td>
</tr>
<tr>
<td>9</td>
<td>None OU</td>
<td>Moderate OU</td>
<td>Residual abduction OU following surgery</td>
</tr>
<tr>
<td>10</td>
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<td>Moderate OU</td>
<td>Residual abduction OU following surgery</td>
</tr>
<tr>
<td>11</td>
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<td>Moderate OU</td>
<td>Abduction OU following surgery</td>
</tr>
<tr>
<td>12</td>
<td>None OU</td>
<td>Moderate OU</td>
<td>No horizontal strabismus surgery</td>
</tr>
<tr>
<td>13</td>
<td>None OU</td>
<td>Mild OD; Modest OS</td>
<td>Residual abduction OU with unsustained abduction movement following surgery</td>
</tr>
<tr>
<td>14</td>
<td>None OU</td>
<td>Moderate OU</td>
<td>No abduction OU and modest abduction OD following surgery</td>
</tr>
<tr>
<td>15</td>
<td>None OU</td>
<td>Moderate OU</td>
<td>Residual abduction OU following surgery</td>
</tr>
</tbody>
</table>

Globe movements from resting position were scored as minimal (1–5°), mild (6–10°), modest (11–20°) or moderate (21–30°). Globe resting position was usually eccentric (see Table 3), so globe movement in any direction was <30°. Abduction column refers to preoperative abduction ability when lateral rectus surgery was performed. OD = right eye; OS = left eye; OU = both eyes.
Fig. 6 Ocular motility. Ocular motility montage of Patient 8 with no strabismus surgery. He had marked bilateral exotropia (B) with neither eye being able to fix in the straight ahead position. Up gaze (A) and down gaze (C) revealed complete absence of vertical eye movement with no intorsion of either globe during attempted depression. Right gaze (D) and left gaze (E) showed complete lack of adduction OU with almost full abduction OD but moderately reduced abduction OS.

Fig. 7 MRI of the oculomotor nerve and orbits. (A, C and E) Control individual. (B, D and F) CFEOM2 patient harbouring two mutated copies of PHOX2A. (A and B) Axial cist MR images at the level of the cisternal segment of the oculomotor nerve (arrows). Oculomotor nerve was not visible in the patient (B). (C and D) Coronal reformatted cist MR images ventral to the pons in the plane of the posterior cerebral arteries and superior cerebellar arteries and orthogonal to the oculomotor nerve (arrows). Oculomotor nerve was not visible in the patient (D). (E and F) Coronal MR images through the orbit posterior to the globe with the lateral rectus muscles and optic nerve labelled 'LR' and '*', respectively, in each image. The optic nerve is deviated medially in the patient (F) because of the exotropia. Superior, medial and inferior recti and superior oblique muscle are atrophic in the patient (F), but the lateral rectus muscle is somewhat large.
their genetic aetiologies defined. These disease genes are each involved in a specific stage of neuronal development in animal models and, likely, in humans as well. The first of the known CCDD genes to be expressed during development in mouse is \textit{Hoxa1}, and homozygous mutations result in abnormal hindbrain rhombomere segmentation and loss of the CN 6 nucleus and other brainstem structures. Next to act temporally is \textit{Phox2a}, which is expressed in proliferating motor neuron precursors immediately before they exit the cell cycle and migrate away from the ventricular zone. With loss of \textit{Phox2a}, these motor neurons fail to survive and the nuclei of CNs 3 and 4 do not develop. Finally, \textit{Kif21a} and \textit{Robo3} are involved in axonal pathfinding, and mutations in these genes probably cause selected axons to terminate incorrectly, with \textit{Kif21a} mutations resulting in aberrant innervation of EOMs and \textit{Robo3} mutations resulting in aberrant innervation of widespread neurological structures in the ipsilateral brain and spinal cord. Continued identification of CCDD phenotypes and their responsible genes should provide further insights into the development and function of the highly specialized human ocular motor system.

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