CONGENITAL ABNORMALITIES OF CRANIAL NERVE DEVELOPMENT: OVERVIEW, MOLECULAR MECHANISMS, AND FURTHER EVIDENCE OF HETEROGENEITY AND COMPLEXITY OF SYNDROMES WITH CONGENITAL LIMITATION OF EYE MOVEMENTS

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ABSTRACT

Purpose: The clinical and molecular genetic classification of syndromes with congenital limitation of eye movements and evidence of cranial nerve dysgenesis continues to evolve. This monograph details clinical and molecular genetic data on a number of families and isolated patients with congenital fibrosis of the extraocular muscles (CFEOM) and related disorders, and presents an overview of the mechanisms of abnormal patterns of motor and sensory cranial nerve development in these rare syndromes.

Methods: Clinical examination of one patient with CFEOM1, one family with clinical features of CFEOM2, one family with recessive CFEOM3, one family with horizontal gaze palsy and progressive scoliosis (HGPPS), and four patients with various combinations of congenital cranial nerve abnormalities. Genotyping of families with CFEOM and HGPPS for polymorphic markers in the regions of the three known CFEOM loci and in the HGPPS region, and mutation analysis of the *ARIX* and *KIF21A* genes in patients with CFEOM were performed according to standard published protocols.

Results: The patient with CFEOM1 had the second most common mutation in *KIF21A*, a 2861 G>A mutation that resulted in an R954Q substitution. The family with CFEOM2 phenotype did not map to the CFEOM2 locus. The family with recessive CFEOM3 did not map to any of the known loci. The HGPPS family mapped to 11q23-q25. One patient had optic nerve hypoplasia and fifth nerve dysfunction. Two patients had the rare combination of Möbius syndrome and CFEOM. One patient had Möbius syndrome and fifth nerve dysfunction.

Conclusions: There is genetic heterogeneity in CFEOM2 and CFEOM3. Abnormalities in sensory nerves can also accompany abnormalities of motor nerves, further substantiating the effect of individual mutations on developing motor as well as sensory cranial nerve nuclei.

Trans Am Ophthalmol Soc 2004;102:373-389

INTRODUCTION

Normal extraocular muscle (EOM) innervation depends on normal cranial nuclear motoneuron formation from neuronal precursors, normal axonal pathfinding from the cranial nuclei to developing EOMs, and the establishment and maintenance of normal connections between mature neurons and their target cells. A number of well-defined syndromes characterized by congenital limitation of eye movements from abnormal innervation or miswiring of the EOMs have recently been grouped as the "congenital cranial dysinnervation disorders," a term coined for congenital disorders resulting from aberrant innervation of the ocular and facial musculature.[1] In the most common of these conditions, Duane retraction syndrome (DRS) type I, there is absence of the motoneurons of the sixth nerve nucleus and abnormal innervation of the lateral rectus muscle by a distal branch of the third nerve. This leads to limited abduction of the affected eye and cocontraction of the medial and lateral rectus muscles on attempted adduction. Less common are the several types of congenital fibrosis of the extraocular muscles (CFEOM), in which there is severe restriction of eye movements and ptosis from abnormal oculomotor and trochlear nerve development, or from abnormalities of EOM innervation. Other conditions involving congenital limitation of eye movements include congenital third nerve palsy, congenital fourth nerve palsy, Möbius syndrome, double elevator palsy, and congenital horizontal gaze palsy. Abnormalities of cranial nerve development have been clearly demonstrated in some of these conditions using magnetic resonance imaging and/or post-

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mortem examination.[2-6]

The past few years have witnessed a significant expansion of our understanding of the genetics of CFEOM, predominantly through the work of Elizabeth Engle, MD, of Boston Children's Hospital.[1,7-26] In less than a decade, her group mapped and cloned the genes for two types of CFEOM and laid the foundation for the understanding of the pathophysiology of this group of disorders. A significant degree of genetic heterogeneity exists for some of the less common types of CFEOM, and work is under way to identify additional genes that cause these and other congenital disorders of ocular motility.

In addition to well-delineated syndromes, there exist a number of rare cases in which DRS or CFEOM is associated with congenital abnormalities of other cranial nerves. Some of these cases present well-defined clinical constellations of abnormalities and appear to result from abnormal development of multiple motor and sensory cranial nerves.[27-34]

Finally, some congenital cranial neuropathies are occasionally associated with recurrent and consistent patterns of craniofacial or systemic malformations, indicating a more generalized effect of the responsible genetic or teratogenic agent on the developing fetus, and leading to such well-defined conditions as thalidomide embryopathy[35] and Okihiro syndrome.[17,36,37]

The purpose of this monograph is to (1) review the current literature on congenital disorders of cranial nerve development; (2) present clinical and molecular genetic data on unreported patients and families with CFEOM, including evidence of genetic heterogeneity, supported by negative mutation analysis and linkage data; (3) describe an unreported family with autosomal recessive congenital horizontal gaze palsy with progressive scoliosis that confirms linkage to the recently identified HGPPS locus; (4) review the clinical data on a few rare patients with clinical findings that bridge some of the well-described syndromes; and (5) present an overview of the mechanisms of abnormal patterns of motor and sensory cranial nerve development that may lead to common and rare clinical syndromes encountered in clinical practice.

Embryology and Anatomy of Cranial Nerve Nuclei

Embryology

The hindbrain, or rhombencephalon, is composed of the cerebellum, pons, and medulla. With the exception of the oculomotor nuclei, cranial nerve nuclei are derived from rhombencephalic neuronal precursors. In early development, the hindbrain is segmented into five compartments called rhombomeres. Neuronal populations within individual rhombomeres display limited intermixing with neighboring compartments. As a result, the location and position of a neural progenitor during hindbrain segmentation determine its contribution to adult brainstem structures and axonal connections. For example, the trochlear, trigeminal, abducens, and facial neurons are generated in rhombomeres 1, 2-3, 5-6, and 4-5, respectively. Due to their compartmental identity, these neuronal progenitors display programmed migratory behaviors and send axons along defined trajectories toward their peripheral targets. While a neural cell progenitor's position along the anteroposterior axis determines its identity, the cell's position along the dorsoventral axis appears to influence its sensory or motor function.

Investigations into the molecular mechanisms of hindbrain development have yielded insights into the potential relationship between human developmental disorders and the molecular signals that determine neuronal identity and axonal guiding in the brainstem.[38] The sonic hedgehog (*SHH*) gene appears to be a key player in the process of neuronal class determination along the dorsoventral axis. Graded expression of SHH along this axis appears to generate domains conducive to either motor (ventral) or sensory (dorsal) neuron development. Using knockout mice generated by gene-specific homologous recombination, several families of homeobox transcription factors (*Hox, Kreisgler, Nkx, Phox,* and *Krox20*) and tyrosine kinase receptors (*Eph*) have been shown to play important roles in rhombomelic compartmentalization, neuronal precursor determination, and the establishment of specific brainstem axonal pathways. Other studies on the dorsoventral patterning of the rhombencephalon have implicated the homeobox genes *Pax6* and *Nkx2.2* in the early divergence of the transcriptional program of hindbrain somatic and visceral motor neuronal differentiation. *Pax6* is involved in the differentiation of the hindbrain somatic motoneurons and V1 interneurons in the hindbrain and/or spinal cord. In the mouse, Osumi and colleagues[39] have shown that *Pax6* is involved in the specification of hindbrain neurons through the regulation on *Islet2* and *Wnt7b* gene expression patterning. Takahashi and coworkers[40] demonstrated that *Pax6* regulates specification of the ventral neuron subtypes by establishing the correct progenitor domains. Pattyn and colleagues[41] provided genetic evidence that the paired-like homeodomain protein *Phox2b* is required for the formation of all branchial and visceral, but not somatic, motor neurons in the hindbrain. They used mice lacking *Phox2b* in which both the generic and subtype-specific programs of motoneuronal differentiation are disrupted at an early stage. Most motor neuron precursors died inside the neuroepithelium, whereas those that emigrated to the mantle layer failed to switch on early postmitotic markers and to down-regulate neuroepithelial markers.[41] *PHOX2A* or *ARIX* is another gene involved in differentiation of motor nuclei of the hindbrain, especially the nuclei of the third and fourth nerves.[42] The PHOX2A protein is

expressed widely throughout the midbrain pons and medulla in embryogenesis. Mutations in *PHOX2A* cause CFEOM2 in humans.[15,24]

Genes expressed in the developing rhombencephalon are good candidates for developmental disorders such as Möbius syndrome, DRS, and the CFEOM syndromes. Teratogenic insults or mutations in these developmental genes may lead to dysgenesis of cranial nerve nuclei, or in the processes involved in establishing normal connections to target muscles or end-organs. If a particular gene is involved and the pattern of dysgenesis consistent, a recurrent constellation of clinical findings appears and leads to development of a "syndrome." This concept has been identified in a number of clinical syndromes with congenital limitation of eye movements and has been expanded to a more global mechanistic and pathophysiologic scheme that explains common and rare abnormalities of ocular and oculomotor abnormalities and their associated clinical nonocular systemic manifestations.

Anatomy

Nuclei containing brainstem motor neurons form two discontinuous columns or sets (Figure 1). One set of motor nuclei is located dorsomedially in the brainstem, close to the ventricle. Its axons exit the brainstem in a ventromedial position. The second set of motor nuclei is located more laterally. Axons of this lateral set exit the brainstem in a lateral position, adjacent to the entrance of sensory fibers. Cranial nerves III in the rostral midbrain, IV in the caudal pons, and XII in the rostral medulla belong to the first set, called somatic motor because they innervate muscles derived from head somites. Their nuclei are located close to the midline and to the surface of the ventricle. Their axons exit the brainstem in a ventral position, close to the pyramidal tract: medial to it in the case of III, lateral to it in the cases of VI and XII. The trochlear, or fourth, nerve, located in the caudal midbrain, is also somatic motor, but its axons exit from the dorsal surface of the midbrain and are crossed, such that the right nucleus supplies the left superior oblique and vice versa. The other set of motor cranial nerves is called branchial motor because they innervate muscles associated with structures derived from branchial arch mesoderm. Their nuclei are located much more laterally. Motor 5, in the rostral pons, is close to the lateral angle of the fourth ventricle. Motor 7, in the caudal pons, is ventral to the descending sensory tract and nucleus of 5. The third nucleus of this set, nucleus ambiguus, contains many motoneurons which send their axons through the tenth nerve to skeletal muscles of the soft palate, pharynx, and larynx, and a smaller contingent, which send axons into the ninth nerve to innervate a single muscle, the stylopharyngeus. The axons of these three nuclei share the property that they exit in a lateral "dorsal root" position, adjacent to entering sensory axons of the same cranial nerves, rather than in a "ventral root" position like somatic motor cranial nerves. Axons of motor 7 travel medially and form a loop around the sixth nerve nucleus, and then travel ventrolaterally to exit the brainstem just medial to a special sensory nerve, the eighth nerve. The last branchial motor nerve, the spinal accessory, behaves partly like a spinal nerve, partly like a cranial nerve. Its motoneurons are in the caudal medulla and in the first five segments of the spinal cord, but the axons exit from the spinal cord in a "branchial motor" position just dorsal to the denticulate ligament, not with the ventral roots of those segments. The nerve then ascends to the cranial cavity, joins the vagus nerve briefly, then leaves it again and descends to the neck to innervate the two neck muscles, trapezius and sternocleidomastoid (see Figure 2 for the sites of exit of cranial nerves).

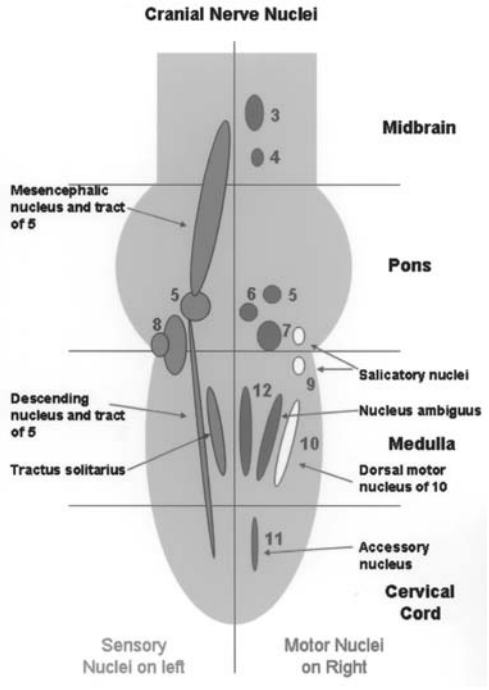

FIGURE 1
Diagram of motor and sensory cranial nerve nuclei location in hindbrain.

Congenital Syndromes With Motor and/or Sensory Cranial Nerve Abnormalities

It is beyond the scope of this paper to discuss all syndromes with congenital abnormalities of cranial nerve development. Table 1 highlights the salient clinical features of some of these syndromes and lists the identified genetic loci, individual genes, or evidence of genetic etiology or familial occurrence of each syndrome.

Congenital Abnormalities of EOM Cranial Nerve Nuclei Leads to Specific Syndromes

Hotchkiss and coworkers described the intracranial and orbital pathology of a clinically documented case of bilat-

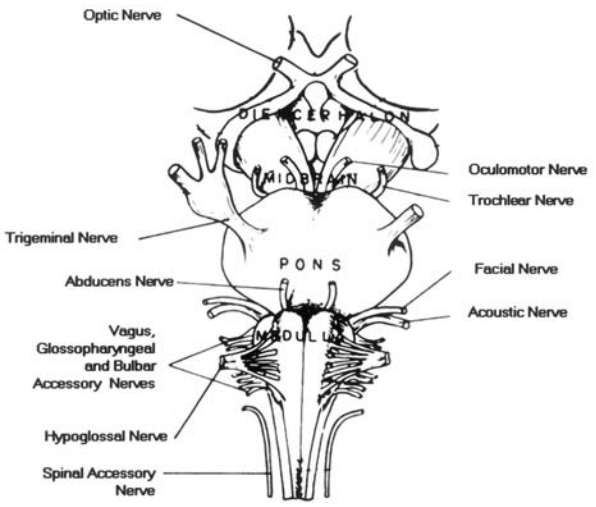

FIGURE 2
Sites of exit of cranial nerves from the hindbrain.

eral DRS.[5] Both abducens nuclei and nerves were absent from the brainstem, and the lateral rectus muscles were partially innervated by branches from the oculomotor nerves. Miller and colleagues[4] reported a case of unilateral DRS in which the right side of the brainstem, cavernous sinus, and orbit was completely normal. The left abducens nucleus contained no cell bodies from motor neurons, but contained in its rostral portion several small cell bodies compatible with internuclear neurons. The left abducens nerve was absent. The left lateral rectus muscle was partially innervated by branches from the inferior oculomotor nerve.

Engle and colleagues[9] reported the intracranial and orbital pathology of one and the muscle pathology of two other affected members of a family with CFEOM1. The superior division of the oculomotor nerve and its corresponding alpha motor neurons was absent, and there were abnormalities of the levator palpebrae superioris and the superior rectus, both innervated by the superior division of the oculomotor nerve. They also found increased numbers of internal nuclei and central mitochondrial clumping in other extraocular muscles, suggesting that the muscle pathology extends beyond the muscles innervated by the superior division of cranial nerve III. Their report presented the first tangible evidence that CFEOM results from an abnormality in the development of the extraocular muscle lower motor neuron system.

Radiologic and neuropathologic evidence of brainstem or cranial nerve nuclear abnormalities has also been presented in patients with Möbius syndrome[62] and horizontal gaze palsy.[23]

Congenital Fibrosis of the Extraocular Muscles

One of the earliest descriptions of CFEOM was given by Baumgarten in 1840,[63] but Heuck in 1879 gave the first account of the familial occurrence and the postmortem findings in one patient with this condition.[64] The term "general fibrosis syndrome" was coined by Brown in 1950 to describe patients with fibrosis of three or more extraocular muscles.[65] Brown proposed that strabismus disorders with congenital, nonprogressive ophthalmoplegia and active limitation and passive restriction of globe movement resulted from fibrous changes in the muscles or their tendon sheaths. He categorized them into five syndromes based on the putative muscles involved and the degree of paralysis: (1) typical and atypical (horizontal) retraction syndromes (medial and/or lateral recti), (2) strabismus fixus (medial and/or lateral recti), (3) vertical retraction syndromes (superior and/or inferior recti), (4) superior oblique tendon sheath syndromes (inferior and superior oblique muscles), and (5) general fibrosis syndrome (three or more of the extraocular muscles). We currently refer to Duane syndrome for the horizontal retraction syndromes and Brown syndrome for the superior oblique tendon sheath syndromes. The remaining conditions are globally called CFEOM.

The incidence and prevalence of the fibrosis syndromes is unknown. They have been reported as isolated or familial cases from around the globe. Dominant forms are more common in Western countries, and recessive forms are more commonly encountered in the Middle East.[66] The original clinical classification proposed by Harley and coworkers[67] that divided patients with CFEOM into five categories is now complemented by one that is based on the mode of inheritance and individual gene mutations (Table 2).[19] A correlation between phenotype and individual genetic types appears to exist.

The management of patients with CFEOM has been discussed in other publications and is beyond the scope of this article.[66,68,69]

CFEOM1

CFEOM1 is autosomal dominant, and its major determining gene maps to the centromere of chromosome 12 (12p11.2-q12).[7] Engle and colleagues[20] found evidence of genetic heterogeneity among 11 CFEOM1 pedigrees. All demonstrated autosomal dominant inheritance and nine were consistent with linkage to FEOM1. Two small CFEOM1 families were not linked to FEOM1, and both were consistent with linkage to FEOM3. Thus far, no *ARIX* mutations have been identified in any affected members of CFEOM1 pedigrees or in any sporadic cases of classic CFEOM. Individuals with CFEOM1 have congenital nonprogressive bilateral ptosis and external ophthalmoplegia. The eyes are fixed in 20 to 30 degrees of downgaze, and patients hold their heads in a chin-up position. Horizontal strabismus is common, and residual eye movements are often aberrant, with convergent or diver-

TABLE 1. SYNDROMES WITH CONGENITAL ABNORMALITIES OF CRANIAL NERVES I THROUGH VIII

CRANIAL NERVE	DISORDER	CLINICAL SIGNS	GENE
I	Kallman syndrome	Anosmia, hypopituitarism	*KAL1*[43-45]; *FGFR1*[46]
II	Optic nerve hypoplasia	Small optic nerve head, poor vision	None mapped. Familial cases have been reported[47]
	Septo-optic dysplasia (de Morsier syndrome)	Optic nerve hypoplasia, absent septum pellucidum	*HESX1*[48,49]; *PAX6*[50]
III	Congenital third nerve palsy	Ophthalmoplegia, ptosis	None mapped
	Congenital ptosis	Ptosis, isolated	PTOS1 mapped to 1p34.1-p32[10]; PTOS2 mapped to Xq24-q27.1[51]
	CFEOM1	Ophthalmoplegia, ptosis	*KIF21A*[25]
	CFEOM3	Ophthalmoplegia, ptosis	Gene maps to 16q24[12,22]
IV	Congenital fourth nerve palsy; isolated and familial cases	Ophthalmoplegia	None mapped Two reports of familial cases[52,53]
III, IV	CFEOM2	Ophthalmoplegia	*ARIX*[15,24]
V	Familial corneal hypesthesia/ anesthesia	Decreased corneal sensations	None mapped Two families reported[54,55]
VI	Duane syndrome type I	Ophthalmoplegia, cocontraction	DRS1, mapped to 2q31[56]
VI	Okihiro syndrome	Duane syndrome with radial ray defects	*SALL4*[17,36,37]
VI, II	Duane syndrome and optic nerve hypoplasia	Cocontraction, optic nerve hypoplasia, central incisor	No gene mapped[27,29]
VI	Horizontal gaze palsy with progressive scoliosis	Bilateral adduction deficit, progressive scoliosis	One gene mapped to 11q23-25[57]
VI (+/- VIII)	Duane syndrome variants, includes Wilderwank syndrome	Various ophthalmoplegia patterns, deafness, other systemic malformations	No genes mapped
VII	Congenital familial facial palsy	Unilateral or bilateral facial weakness	A few families reported[58]
VI, VII, other	Möbius syndrome	Ophthalmoplegia, facial weakness, other systemic malformations. Poland anomaly, flat foot, microdactyly, polydactyly, paralysis of lower legs with abnormal broad gait. Autism	Several familial instances with autosomal dominant inheritance.[59,60] One gene localized to 3q61 Another gene mapped to 10q62

gent movements on attempted upgaze. Families with CFEOM1 demonstrate full penetrance of the trait with little or no variability among or between families from around the world.[8,18] The evaluation of a Turkish family with CFEOM1 demonstrated a greater phenotypic heterogeneity at the FEOM1 locus than previously reported.[13] The phenotype in this family was marked by variable expression and overlap with both classic CFEOM1 and CFEOM3 phenotype (*vide infra*).

Yamada and coworkers[25] discovered that patients with CFEOM1 harbor heterozygous missense mutations in a kinesin motor protein encoded by *KIF21A*. They identified six mutations in 44 of 45 probands with CFEOM1. The primary mutational hotspots were in the stalk domain of the protein, highlighting an important new role for KIF21A in the formation of the oculomotor axis.[25] Neurons use kinesin and dynein microtubule-dependent motor proteins to transport essential cellular components along axonal and dendritic microtubules. Kinesins are mechanochemical motors that utilize the energy of ATP hydrolysis to walk along microtubules. Kinesin motors have been implicated in several types of motile processes, including transport of mitochondrial or vesicular cargoes along microtubules, intermicrotubule sliding, and the assembly and motility of mitotic and meiotic spindles. Ubiquitously expressed in all eukaryotic organisms, kinesin family members (KIFs) are defined by the extensive homology they share within a globular motor domain that contains both microtubule- and ATP-binding sites. Marszalek and coworkers[70] identified two mouse kinesins that provided insight into a unique intracellular kinesin-targeting mechanism in neurons. KIF21A and KIF21B share colinear amino acid similarity to each other but not to any previously identified kinesins outside of the motor domain, and localize differently to dendrites and axons. KIF21A protein is localized

TABLE 2. CLASSIFICATION OF CONGENITAL FIBROSIS SYNDROMES

TYPE	INHERITANCE	OMIM NO.*	GENE/LOCUS
CFEOM1	Autosomal dominant	135700	*KIF21A*/12p11-q12
CFEOM2	Autosomal recessive	602078	*ARIX*/11q13
CFEOM3	Autosomal dominant	600638	16q24.2-24.3
Unclassified	Autosomal recessive, unique phenotype	Family reported by Traboulsi and colleagues[14]	Possibly at 12p11-q12
Unclassified	Autosomal recessive with CFEOM2 phenotype	Present paper	No mutations in *ARIX* or *KIF21A*
Unclassified	Autosomal recessive, with CFEOM3 phenotype	Present paper	No mutations in *ARIX* or *KIF21A*

OMIM, Online Mendelian Inheritance in Man.
*http://www.ncbi.nlm.nih.gov/omim/.

throughout neurons, whereas KIF21B protein is highly enriched in dendrites.[70]

CFEOM2

CFEOM2 is autosomal recessive and its gene is located on chromosome 11q13.1.[11] Affected individuals are born with bilateral ptosis and restrictive ophthalmoplegia with eyes partially or completely fixed in an exotropic position. Ability to adduct or depress the globe is absent or severely reduced. Nakano and coworkers[15] identified *ARIX*, previously called *PHOX2A*, as the mutated gene in CFEOM2. They reported three mutations in four pedigrees. Yazdani and colleagues[24] found a fourth mutation in a recessive Iranian pedigree with two affected siblings who displayed bilateral ptosis and exotropic strabismus fixus. The 439C>T mutation in this last family was the first nonsense mutation to be identified, and confirmed *ARIX/PHOX2A* as the autosomal recessive CFEOM2 disease gene.

ARIX is a transcription factor essential for the development of oculomotor and trochlear nuclei in mice and zebra fish.[71] It is believed that CFEOM2 results from hypoplasia of the oculomotor and trochlear nerve nuclei as a result of mutations in both copies of *ARIX*.

Traboulsi and coworkers[14] reported a Yemenite family with autosomal recessive CFEOM consisting of two affected sisters, four unaffected siblings, and unaffected consanguineous parents. The investigators analyzed the family and excluded linkage to the CFEOM2 locus, as well as to the autosomal dominant CFEOM3 locus on chromosome 16q24. A lod score of 2.0 (the maximum possible, given the family size and structure) was obtained at the CFEOM1 locus. Haplotype analysis showed that the alleles at the CFEOM1 locus reduced to homozygosity in both affected daughters but none of the other children. This data was compatible with genetic heterogeneity in autosomal recessive CFEOM and suggested that a second recessive locus may be allelic to the autosomal dominant CFEOM1 locus at 12cen.[14]

Since the publication of that paper, and with identification of the CFEOM1 and CFEOM2 genes, mutation analysis of the *ARIX* and the *KIF21A* genes has failed to reveal any mutations in this family (E. Engle, MD, personal communication).

CFEOM3

CFEOM3 is autosomal dominant with variable expression and probable incomplete penetrance. The gene maps to markers on 16q24.2-q24.3.[12,22] Affected individuals are born with a nonprogressive eye movement disorder characterized by variable ptosis and restrictive external ophthalmoplegia. Severely affected individuals have ptosis, eyes fixed in a downward and exotropic position, and bilateral severe restriction of eye movements. Their phenotype resembles that of CFEOM1. Mildly affected individuals have normally positioned globes with limitation of vertical gaze. Moderately affected individuals have asymmetric involvement with one eye severely and one eye mildly affected. Mackey and colleagues[22] reported a second family that maps to the FEOM3 locus in which the phenotype is fairly uniform in 15 affected individuals and involves primarily the vertically acting EOMs, thereby expanding the manifestations of gene mutations at this locus.

Systemic Abnormalities in Patients With CFEOM

Patients with fibrosis of the extraocular muscles are generally healthy except for rare ocular or systemic problems in sporadic cases or in single family members of otherwise typical pedigrees (reviewed by Kalpakian and colleagues[72]). The associated ocular findings include, for example, bilateral optic nerve hypoplasia in one patient,[27,29,67] a chorioretinal coloboma in case XXII of Brown's patients,[65] and microphthalmia in one other

patient.[73] Optic nerve head dysplasia/coloboma was present in all affected members of one family reported by Khawam and coworkers[74]; this family, however, may have a syndrome distinct from classic CFEOM. Systemic associations include bilateral inguinal hernias and unilateral cryptorchidism,[75] a benign mesenchymoma of the ethmoid sinus that invaded the orbit,[76] Joubert syndrome,[77,78] and Prader-Willi syndrome.[68,72] Flaherty and colleagues[16] described a mother and two children with classic CFEOM and dilation of the left lateral ventricle secondary to hypoplasia of the body and tail of the ipsilateral caudate nucleus. There was fusion of an enlarged caudate nucleus head with the underlying putamen. Both children had widespread bilateral cortical dysplasia. Genetic analysis of the family was consistent with linkage to the CFEOM1 locus on chromosome 12. The investigators recommended neuroimaging of patients with CFEOM, particularly if there is developmental delay.[16] Uyama and coworkers[26] identified a Japanese family with CFEOM1 and spinal canal stenosis in the cervical spine in all affected family members who were examined.

The association of CFEOM with Joubert syndrome (cerebellar vermis hypoplasia and other CNS abnormalities) in some cases[77,78] indicates that genetic defects can involve the oculomotor nuclei as well as cerebellar structures.

Horizontal Gaze Palsy With Progressive Scoliosis

Congenital horizontal gaze palsy with early-onset progressive scoliosis is a rare autosomal recessive condition that probably results from brainstem maldevelopment and is probably caused by mutations in a gene necessary for the normal development of neurons in the abducens nuclei and caudal longitudinal fascicle. This leads to the development of "horizontal gaze palsy" and kyphoscoliosis. This condition is classified with the congenital cranial dysinnervation syndromes.[1] The gene for this condition has been recently mapped to chromosome 11q23-q25.[57]

Clinical as well as gene mapping and/or mutation analysis data on individual patients or families with typical or variants of CFEOM and with horizontal gaze palsy with progressive scoliosis (HGPPS) is presented in the following sections.

METHODS

Gene Mapping Studies

Patient and Pedigree Collection

Index patients and family members were examined. The Cleveland Clinic and Boston Children's Hospital institutional review boards approved the study, and all participants signed informed-consent forms. The methods adhere to the Declaration of Helsinki for research involving human subjects. Clinical information was recorded and pedigrees were drawn using the program Cyrillic (CyrillicSoftware, Reading, United Kingdom).

DNA Typing

Blood samples were collected from participating family members, and lymphocyte DNA was extracted using the Puregene kit (Gentra, Research Triangle Park, North Carolina). Linkage studies were conducted using polymorphic DNA microsatellite markers (di-, tri-, and tetranucleotides). To assess linkage to the dominant CFEOM1 region,[7] markers *D12S61*, *D12S87*, *D12S1584*, *D12S1621*, *D12S1648*, *D12S345*, *D12S1692*, *GATA63D01*, *AFM114yh2*, *D12S1029*, *D12S1048*, and *D12S1668* were analyzed. To assess linkage to the dominant CFEOM3 region,[12] markers *D16S498*, *D16S486*, *D16S3063*, *D16S689*, *D16S413*, *D16S3026*, *D16S303*, and *D16S671* were analyzed. To assess linkage to the recessive CFEOM2 region,[11] markers *D11S1337*, *D11S4139*, *D11S4196*, *D11S4162*, *D11S1314*, *D11S4184*, *D11S1369*, *D11S4207*, *D11S916*, and *D11S2371* were analyzed. To assess linkage to the dominant HGPPS region,[57] markers *D11S908*, *D11S925*, *D11S4464*, *D11S1328*, *D11S1896*, and *D11S415* were analyzed. All primer sequences are available from either the Genome Data Base (http://gdbwww.gdb.org) or the publications cited above. Primers were purchased from Research Genetics and Genosys Biotechnologies (http://www.genosys.com). Amplification and analysis of each repeat polymorphism were performed as reported elsewhere.[7,11,12]

Linkage Analysis

Haplotype analysis was done by inspecting the combination of alleles in any particular locus. If there was evidence of segregation of individual haplotypes with the disease trait, lod scores were calculated with the Fastlink version 3.0 package of programs[79] with the assumption of autosomal recessive or dominant inheritance with complete penetrance. Absence of cosegregation of a given haplotype with the disease trait was considered evidence of no linkage. Calculations involving the CFEOM3 markers were done with the assumption of autosomal dominant inheritance and reduced penetrance. Data on the population incidence of CFEOM mutations are not available; for purposes of lod-score calculations, a disease incidence of 1/1,000,000 births and 10 marker alleles of equal frequency was used.[7,11] Using standard convention, linkage was excluded based on a lod score of –2 or less across the entire critical region of a disease gene locus, and linkage was established based on a lod score of 3 or greater for marker(s) within the critical region. A lod score of –2 corresponds to an odds ratio of 1:100 that the disease gene and the marker are linked versus not linked, and a lod score of 3 corresponds to an

odds ratio of 1,000:1 that the disease gene and the marker are linked versus not linked.

Mutation Analysis

KIF21A mutation analysis was conducted by polymerase chain reaction amplification of the 38 *KIF21A* exons and flanking intron-exon boundaries from genomic DNA of each proband. The amplicons were subjected to analysis by denaturing high-performance liquid chromatography using the WAVE Nucleic Acid Fragment Analysis System (Transgenomic, Inc, Omaha, Nebraska) and/or to direct DNA sequencing on an ABI 377 DNA sequencer (PE-Applied Biosystems, Foster City, California) as previously described.[25] This was done in Dr Engle's laboratory.

The 3 *PHOX2A* (*ARIX*) exons and flanking intron-exon boundaries were similarly amplified using published primer sets,[15] and these amplicons were directly sequenced. Results were compared to normal control individuals. If a mutation was detected in a proband, the participating family members were subsequently screened for the mutation as well.

RESULTS

Representative CFEOM1 Case

A 32-year-old woman presented for genetic counseling about her strabismus syndrome, diagnosed as CFEOM at about age 3 years, and the risk of having an affected child. She was born with bilateral ptosis, severely restricted eye movements (Figure 3), and right Marcus-Gunn jaw winking.

She underwent three eye muscle surgeries at ages 3, 4, and 5 years and left frontalis suspension at age 6. Her visual acuity was 20/40 OD and 20/100 OS. She had a mild chin-up head position. There was almost no movement of her left eye; she could adduct and abduct her right eye about five degrees. There was no family history of a similar condition. Although her clinical features were most compatible with autosomal dominant CFEOM1, the possibility of a recessive variant of CFEOM3 could not be ruled out. She was told that she had a maximal risk of 50% of having an affected child. At the time of her first visit, the gene for CFEOM1 had not been identified. She contributed a blood sample to the research on CFEOM. She went on to have an unaffected daughter. Mutation analysis of the *KIF21A* gene in Dr Engle's laboratory revealed a 2861G>A mutation in the 21st exon of the *KIF21A* gene, resulting in an R954Q substitution (Figure 4).

Genetic Heterogeneity in CFEOM2

Traboulsi and colleagues studied an Iranian family (Figure 5, pedigree 869) with a phenotype identical to that of the family reported by Yazdani and colleagues.[24] There were two affected brothers, one affected sister, and two unaffected siblings. The parents were consanguineous. All three patients had bilateral ptosis and bilateral exotropic strabismus fixus (Figure 6).

Mutation analysis of *ARIX* and *KIF21A* failed to reveal any mutations, and haplotypes analysis at the CFEOM2 locus failed to show evidence of linkage (data not shown), indicating further genetic heterogeneity in CFEOM2.

A Presumed New Recessive Form of CFEOM3

A previously unreported Lebanese family (pedigree 205) with what appears to be a recessive form of CFEOM3 was examined. Clinical data was obtained and blood specimens were collected after informed consent. Two siblings and a cousin had unilateral EOM fibrosis and ptosis (Figure 7). Computed tomographic scanning in one of the patients revealed typical thinning of the EOMs on the affected side. The three children underwent a number of surgical procedures with moderate improvement in ocular alignment and ptosis. None of the parents were affected. Consanguinity was denied.

The family was studied in conjunction with Dr Engle's group for linkage to the CFEOM1, CFEOM2, and CFEOM3 loci and for mutations in the *ARIX* and *KIF21A* genes as outlined in the "Methods" section. Haplotype analysis of multiple markers in the three loci failed to suggest linkage to any of the three regions (Figure 8, A through C). Furthermore, there were no mutations in *ARIX or KIF21A*.

A New Family With Horizontal Gaze Palsy and Progressive Scoliosis

Five siblings of a United Arab Emirates family ranging in age from 4 to 17 years had an ocular motility disorder characterized by congenital bilateral esotropia and bilateral abduction deficits. Vertical eye movements were normal. All patients had convergence-like eye movements on attempted abduction. Visual acuity was normal in all patients. Pupils reacted normally to near viewing. Forced duction testing under general anesthesia revealed moderate tightness of the medial rectus muscles in all patients. All affected children had scoliosis (Figure 9).

Bilateral medial rectus recession resulted in good ocular alignment in primary position of gaze without any alteration of the abnormal ocular movement pattern. A clinical diagnosis of autosomal recessive horizontal gaze palsy with scoliosis (HGPPS) was made. Haplotype analysis revealed homozygosity for markers at the 11q23-q25 locus in affected individuals, and heterozygosity of the markers in parents or unaffected carrier siblings (Figure 10).

Since this thesis was accepted, the *ROBO3* gene responsible for HGPPS was discovered (Jen JC, et al, *Science* 2004;304:1509-1513). *ROBO3* is important in axonal guidance and required for hindbrain axon midline

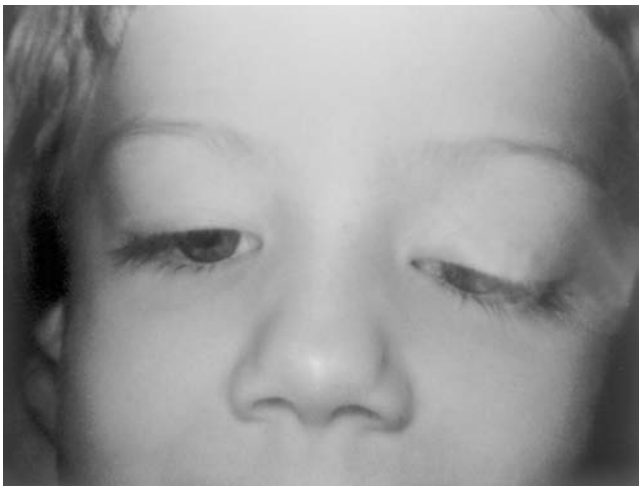

FIGURE 3
Patient with CFEOM1 preoperatively at age 3. There is bilateral ptosis and the eyes are fixed in downgaze.

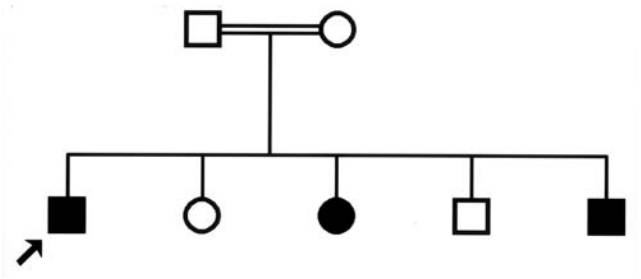

FIGURE 5
Pedigree of family with CFEOM2. The parents are consanguineous. There are two affected boys (solid black squares) and one affected girl (solid circle). The proband is indicated by an arrow. Clear symbols indicate unaffected individuals.

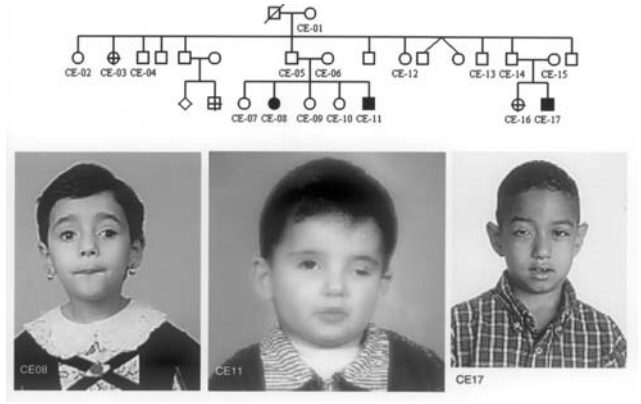

FIGURE 7
Two siblings (CE08 and CE11) and their cousin (CE17) with unilateral ptosis and ophthalmoplegia. Symbols with crosses indicate individuals with congenital cataracts. Solid symbols indicate individuals with CFEOM. Clear symbols indicate unaffected individuals.

crossing. Mutations in *ROBO3* result in uncrossed motor and sensory projections in patients with HGPPS, including sixth nerve fibers, resulting in bilateral gaze palsies. The present family has an IVS13 +1 G>A mutation in exon 13 of *ROBO3* that results in abnormal splicing (Jen

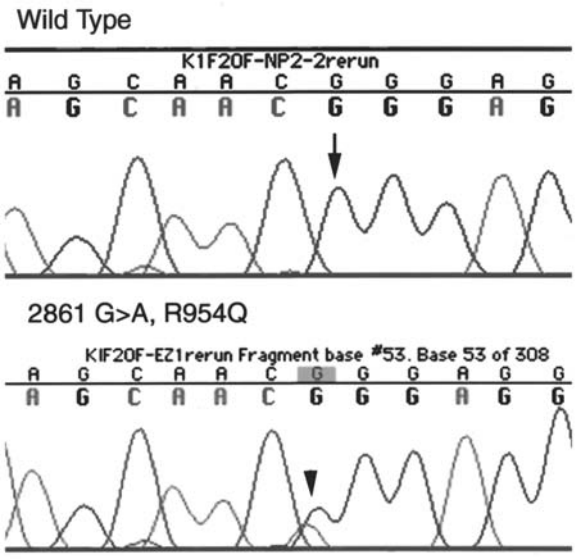

FIGURE 4
DNA sequence around codon 954. There is a G>A substitution at position 2861. Bottom sequence shows heterozygosity (G and A) for mutation (arrowhead), compared to homozygosity for a G in the same position in normal top sequence (arrow).

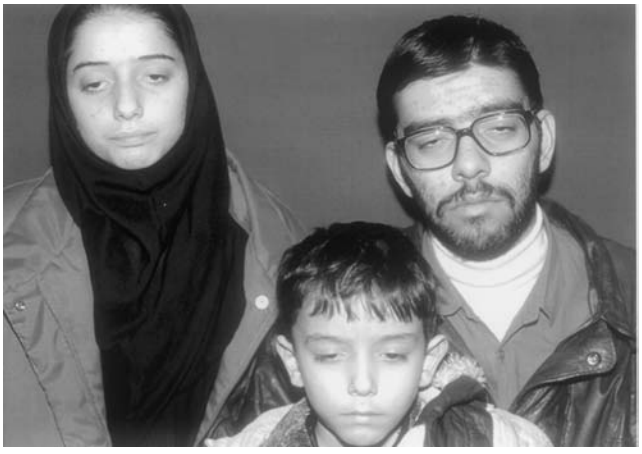

FIGURE 6
Three siblings with CFEOM2, ptosis, and exotropia.

JC, et al, *Science* 2004;304:1509-1513).

Patients With Rare Combinations of Congenital Cranial Nerve Abnormalities

Optic Nerve Hypoplasia and Fifth Nerve Dysfunction

A 2-year-old boy was brought in because of a self-induced scratch of his left eye. He had been poking himself in the left eye for a number of months and also had a habit of inserting foreign objects under his lid. Examination showed a central corneal abrasion of the left eye and ipsilateral optic nerve hypoplasia (Figure 11). His ocular movements were full. Corneal sensation was normal on the right but absent on the left. There was no developmental delay and no other medical problems or congenital malformations. The corneal abrasion was treated with

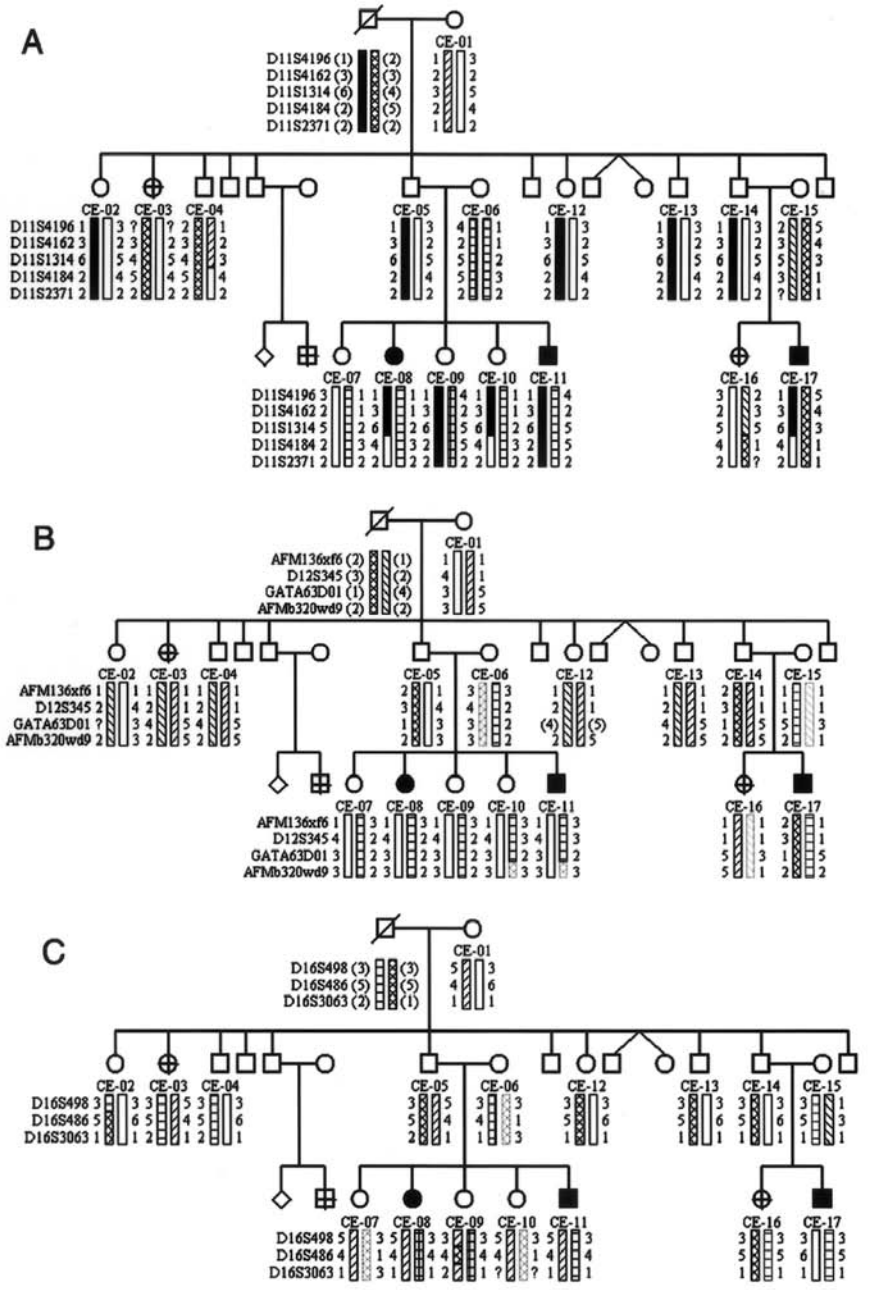

FIGURE 8

Genotyping data with markers on chromosomes 11 (A), 12 (B), and 16 (C) fails to show segregation of any specific haplotypes with the disease trait, indicating absence of linkage to the CFEOM1, 2, and 3 loci

patching and antibiotic ointment applications. The patient returned several times over the next 3 years with similar episodes. Vision was hand movements in the left eye, and he developed a large esotropia (Figure 12). The fundus examination remained unchanged.

Optic Nerve Hypoplasia and Duane Retraction Syndrome

Traboulsi and colleagues examined a patient who was born with a tracheo-esophageal fistula, tetralogy of Fallot with partial arteriovenous canal cardiac defect, bilateral type III DRS, and bilateral optic nerve hypoplasia. He was referred for neuroendocrinologic evaluation but failed to keep up with his appointments.

Fifth, Sixth, and Seventh Nerve Dysfunction

A 14-month-old boy presented with left congenital facial nerve palsy and an abduction deficit with esotropia of his left eye (Figure 13). There was mild developmental delay. The child had some difficulties with swallowing and was fed through a gastrostomy tube. There were no malformations

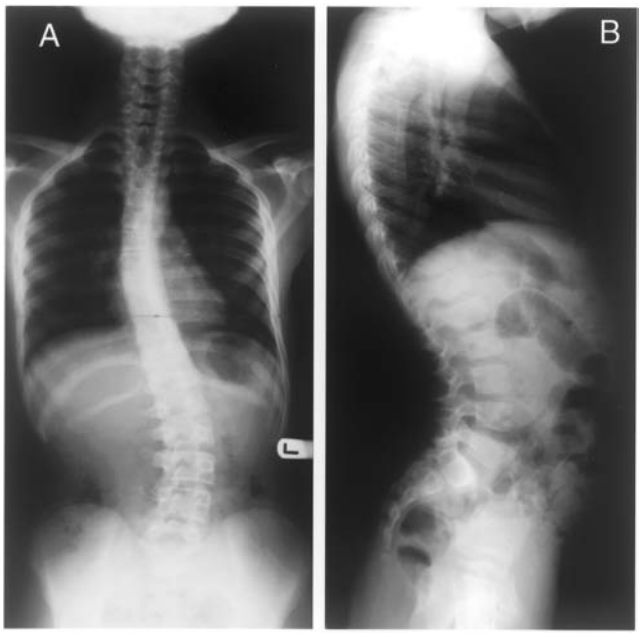

FIGURE 9
Severe scoliosis in one of the family members affected with congenital horizontal gaze palsy.

of the extremities. Vision was central, steady, and maintained OD, and he was able to fix and follow poorly OS. There was a hypermetropia of +5.00 diopters OU. The left cornea was anesthetic, and there were epithelial corneal defects and subepithelial scars. A diagnosis of Möbius syndrome with multiple cranial nerve involvement was made. Lubrication of the left eye was instituted, as well as patching for his amblyopia. He later underwent eye muscle surgery for his esotropia. On his latest visit at age 7 years, vision was 20/40 OD and 20/50 OS. His facial palsy, left sixth nerve palsy, and anesthetic and scarred cornea persisted. He continues to receive lubrication to his left eye.

Third, Seventh, and Eighth Nerve Dysfunction

Case 1. A 9-year-old patient with congenital bilateral facial nerve palsy, ptosis, and severe limitation of movements of both eyes was examined. He had been diagnosed with Möbius syndrome and strabismus. He had significant feeding difficulties as an infant and failed to thrive. He had bilateral hernia repair and was discovered to have bilateral mild hearing loss.

His visual acuity was 20/60 OU. There was latent nystagmus. He had bilateral moderate ptosis. Bell's phenomenon was present bilaterally. The eyes were positioned in 20 degrees of downgaze (Figure 14), with converging eye movements on attempted upgaze and an exotropia on attempted downgaze. He had mild limitation to abduction of both eyes. Ophthalmoscopy showed anomalous, slightly hypoplastic optic nerve heads. Strabismus and ptosis repair were refused. He was re-examined 4 years later, and there were no changes in vision, ptosis, or ocular motility.

Case 2. A 6-year-old girl with congenital bilateral ptosis, exotropia, and facial palsy had extremely limited ocular movements, and her eyes were fixed in a hypotropic position. Vision was 20/160 OU with a myopic correction of –20.00 + 2.00 × 180 bilaterally. Her fundi were severely myopic with tilted optic nerve heads but no macular degenerative changes.

DISCUSSION

The initial hypotheses that DRS and CFEOM are due to

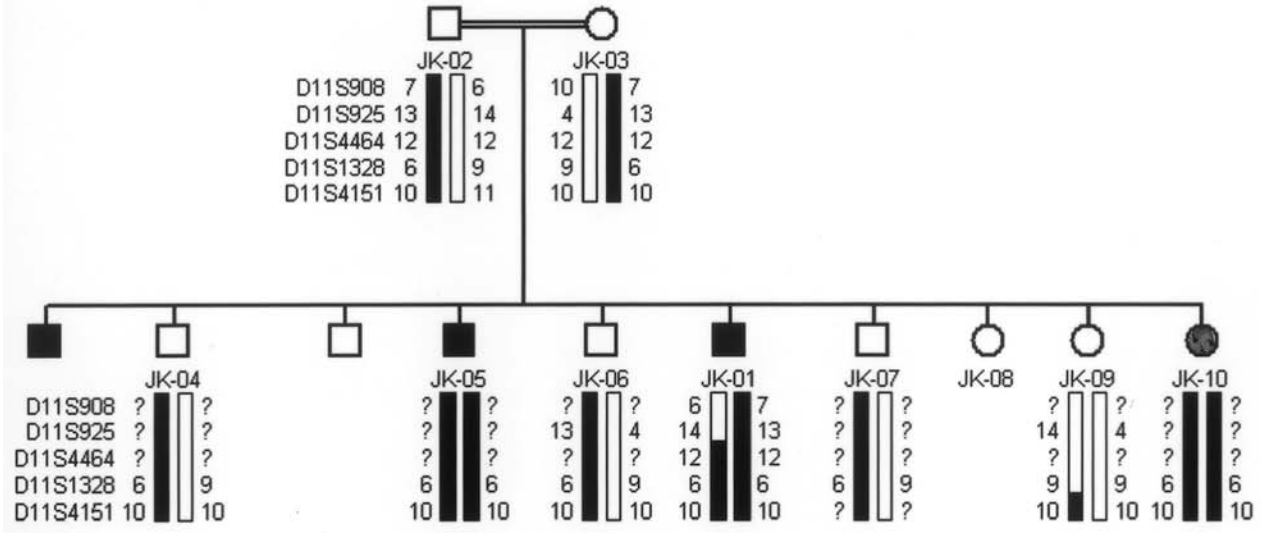

FIGURE 10
Haplotype analysis of markers in the 11q23-q25 region shows segregation with the disease trait in this autosomal recessive disease. The consanguineous parents and children JK-04, JK-06, and JK-07 are carriers.

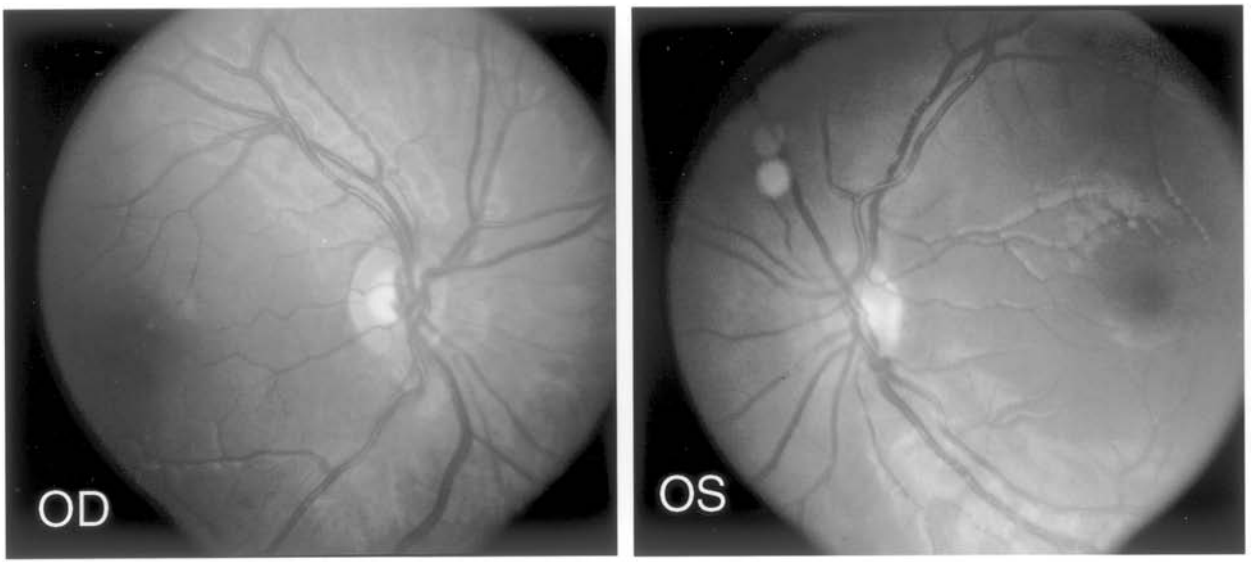

FIGURE 11
Left optic nerve hypoplasia. The left optic nerve is about one-third the size of the right one.

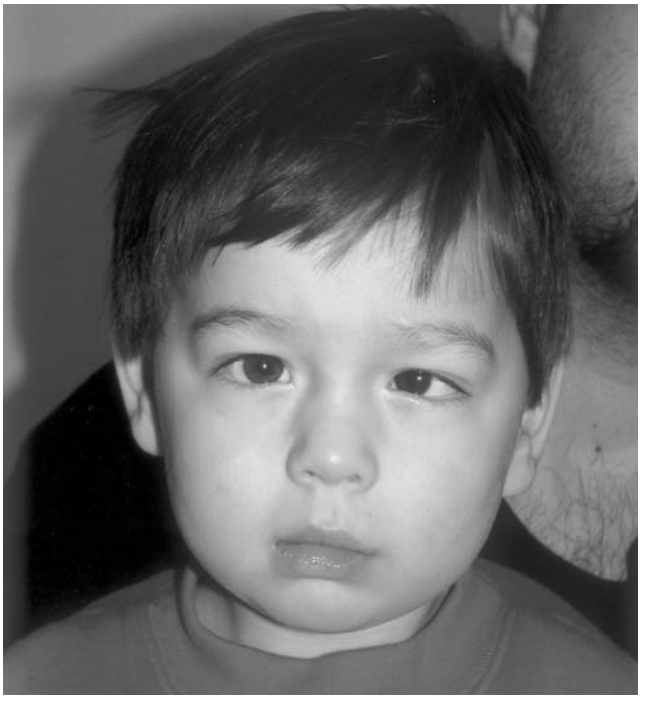

FIGURE 12
Left esotropia in patient with left optic nerve hypoplasia and left fifth nerve dysfunction.

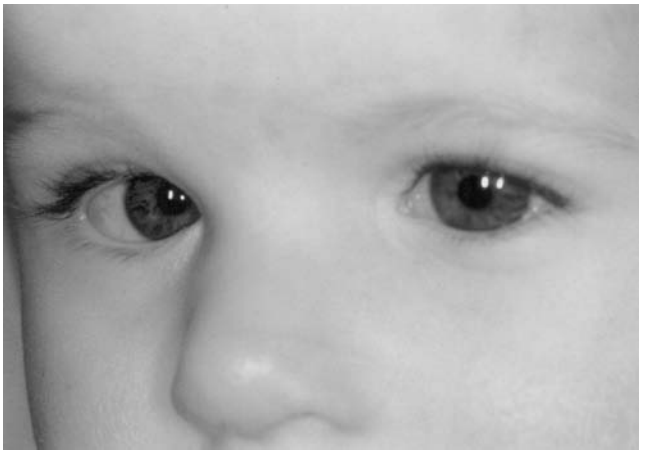

FIGURE 13
Left abduction deficit in patient with Möbius syndrome and left fifth nerve involvement

abnormalities in extraocular muscle development have been overturned by several lines of evidence of neurogenic etiologies.[19] Abnormalities of cranial nerve nuclear development have been established for conditions such as Möbius syndrome and its variants[80] and for familial horizontal gaze palsy. To date, there appear to be two main pathways through which congenital cranial nerve dysfunction occurs in patients with congenital ocular movement disorders. The first involves failure of the cranial nerve nuclei to develop normally and their motoneuron component cells to differentiate, aggregate, and establish proper neuronal connections; this could occur as a result of genetic factors or of teratogenic insults. Classic examples include the absence of the sixth nerve nucleus in DRS,[4,5] the presumed absence of the third nerve and fourth nerve nuclei in CFEOM2,[15,24] and the generalized abnormalities of midbrain, pons, and medulla in patients with Möbius syndrome and its variants. The second mechanism involves genetic defects that lead to abnormal axonal transport of molecules necessary for normal extraocular muscle function and development. This mechanism appears to operate in CFEOM1, in which a kinesin-related defect of axonal transport is caused by mutations in *KIF21A*.[25] The histopathologic studies in CFEOM1

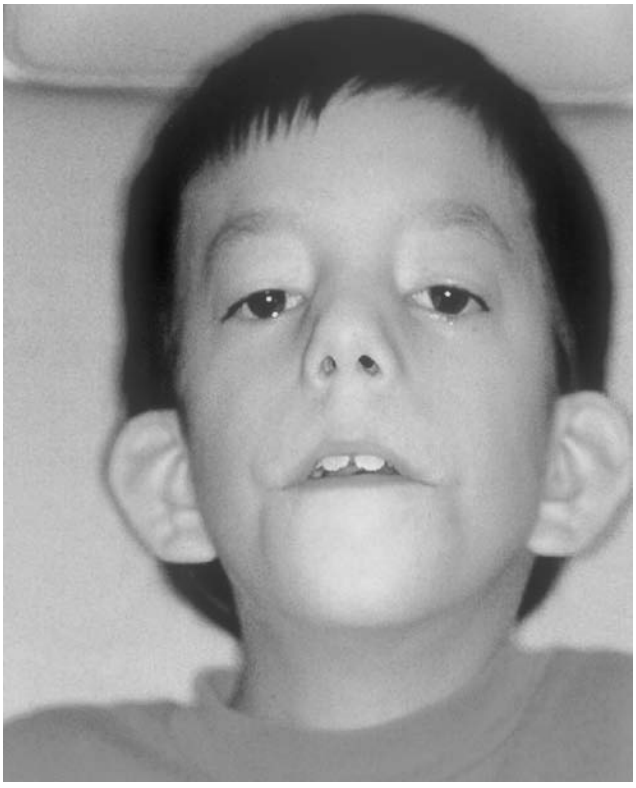

FIGURE 14
Bilateral facial nerve palsy and total ophthalmoplegia in 9-year-old boy.

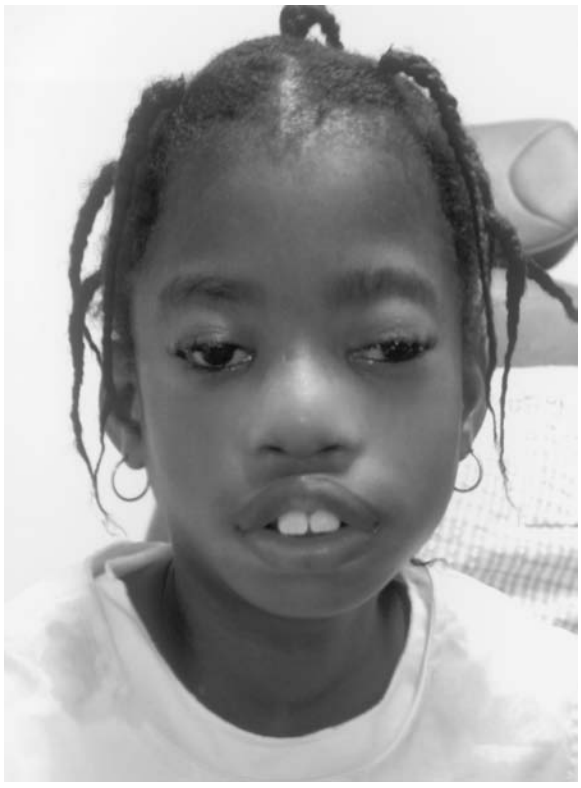

FIGURE 15
Six-year-old girl with bilateral facial palsy, ptosis, and ophthalmoplegia with the eyes fixed in a hypotropic and exotropic position.

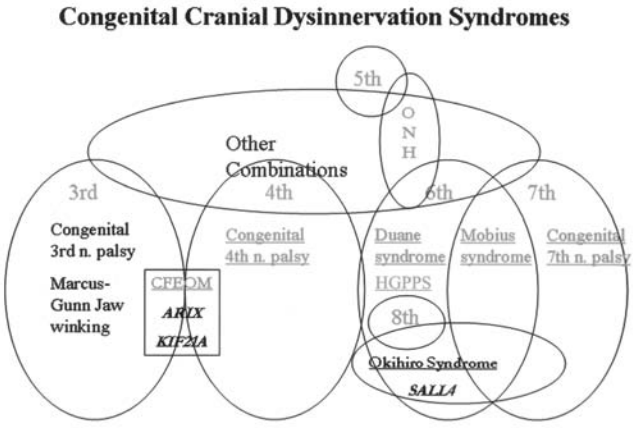

FIGURE 16
Diagram summarizing conditions with evidence of congenital cranial dysinnervation and their causative genes.

showing absence of the superior division of the oculomotor nerve and its corresponding alpha motor neurons, as well as abnormalities of the levator palpebrae superioris and rectus superior (the muscles innervated by the superior division of the oculomotor nerve), and suggesting that CFEOM1 results from an abnormality in the development of the extraocular muscle lower motor neuron system,[9] have to be reconciled with the more recent molecular genetic studies that favor an axonal transport defect. Because of the occasional presence of mild facial weakness, hypotonia, gross motor delay, and nonspecific abnormalities in quadriceps biopsies from affected CFEOM1 family members, Engle and coworkers[9] also proposed that the normal CFEOM protein plays at least a transient role in normal skeletal muscle development.

Additional evidence for a neurogenic etiology of the congenital cranial dysinnervation syndromes comes from the wide variety of cases in which CFEOM or DRS has been associated with other congenital abnormalities of motor and sensory cranial nerve development. Brodsky and associates[81] reported a 5-month-old boy with generalized CFEOM, oculocutaneous albinism, and neural misdirection resulting in synergestic divergence and Marcus-Gunn jaw winking phenomenon. They suggested that their patient's abnormalities provide evidence for a primary developmental defect precluding the establishment of normal neuronal connections in CFEOM, a theory certainly in line with current findings.[9,25] Brodsky[82] later presented three additional patients with an identical constellation of clinical findings. All displayed a variant of synergistic divergence characterized by simultaneous abduction with intorsion and depression of the synkinetically abducting eye. Three of the four patients had a variant of Marcus-Gunn jaw winking characterized by elevation of the ptotic eyelid during mouth opening. Brodsky

suggested that the patterns of neuronal misdirection implicated a regional innervational disturbance involving cranial nerves III through VI as the underlying cause of the generalized ophthalmoplegia in these patients. Another patient with unilateral fibrosis, enophthalmos, retraction, and Marcus-Gunn jaw winking phenomenon was included in the series of 24 patients reported by Traboulsi and coworkers,[68] suggesting that this phenomenon is present in about 5% of patients with CFEOM.

A patient was previously reported with de Morsier syndrome (septo-optic dysplasia), a developmental malformation complex characterized by optic nerve hypoplasia, dysgenesis of the septum pellucidum, hypothalamic-pituitary dysfunction, and DRS.[27] The patient had classic right Duane syndrome type I and bilateral optic nerve hypoplasia with double ring sign. He also had a superior central incisor, a finding usually associated with midline brain defects, an undescended testicle, and pituitary insufficiency with growth hormone deficiency and diabetes insipidus. It was postulated that DRS and de Morsier syndromes in this patient were due to an underlying genetically determined disturbance of neuronal development.[27] Parentin and coworkers[83] reported a 4-year-old Italian child with the association of a solitary median maxillary central incisor, growth hormone deficiency, DRS, and a duplicated thumb phalanx. The report did not mention the presence of optic nerve hypoplasia. Personal written communication with the authors revealed that a detailed ocular examination had been done and that optic nerve hypoplasia had been excluded. DRS and optic nerve hypoplasia can also occur in patients with fetal alcohol syndrome.[84] There was no history or evidence of exposure to teratogens in the two cases examined, suggesting a probable genetic etiology.

The second most common mutation in *KIF21A* was found in one patient with CFEOM1. This mutation has been identified in three other probands.[25] Five other mutations (M356T, M947V, M947R, R954W, and I1010T) also alter conserved amino acid residues within the KIF21A protein stalk region and are proposed to interfere with KIF21A dimerization, hence with the ability of KIF21A to carry its unidentified cargo from the oculomotor nucleus motoneurons toward the developing neuromuscular junction of the extraocular muscle.[25] These six mutations account for 98% of cases of CFEOM1.[25]

The present paper offers additional evidence of genetic heterogeneity in autosomal recessive CFEOM. One family with a clear-cut CFEOM2 phenotype did not link to the FEOM2 locus on 11q13 and did not have a mutation in *PHOX2A/ARIX* or in *KIF21A*. Genetic heterogeneity in CFEOM3 is also postulated in the previously unreported family in which two siblings and their cousin had a CFEOM3 phenotype, yet does not map to the three known CFEOM loci, nor has mutations in *PHOX2A/ARIX* or in *KIF21A*. The molecular genetic data suggests that this family has a third, yet-undescribed form of recessive CFEOM with clinical features that resemble those of CFEOM3 and are characterized by unilateral ptosis and severe restriction of extraocular movements. A genome-wide screen is under way to localize the responsible gene in these last two families.

In this paper, the localization of the HGPPS gene to 11q23-q25 in a family with five affected siblings and consanguineous parents was confirmed. The search for the responsible gene is under way in the family described in this thesis and in several others. It is hoped that discovery of the gene will be reported in the near future.

The occurrence of total external ophthalmoplegia in patients with Möbius syndrome, as observed in two cases in the present paper, has been previously reported in a few patients. Verzijl and coworkers[80] investigated the variable clinical picture of Möbius syndrome to further understand the pathogenesis of the disorder. They used a standardized questionnaire and examined 37 Dutch patients with Möbius syndrome. All patients underwent standardized neurologic examination with special attention to cranial nerve functions, motor skills, and facial and limb anomalies. All had facial paresis, and 97% had bilateral and 3% had unilateral ocular abduction weakness. Further analysis showed isolated abducens nerve palsy in 9%, a conjugated horizontal gaze paresis in 48%, features of DRS in 34%, and congenital fibrosis of the extraocular muscles in 9%. Other signs included lingual involvement (77%), dysfunction of palate and pharynx (56%), general motor disability (88%), poor coordination (83%), and respiratory abnormalities (19%). The presence of gaze palsies, DRS, feeding and respiratory problems, and poor motor development led these investigators to suggest that Möbius syndrome was the result of abnormal regional rhombencephalic development, involving predominantly motor nuclei and axons, as well as traversing long tracts.[80]

Although some cases of Möbius syndrome have been attributed to brainstem ischemia, genetic causes are most likely operative in others. Cytogenetic abnormalities in some patients have suggested genetic loci at 1p22 and 13q12.2-13.[85,86] Dutch investigators mapped two loci for dominant bilateral facial palsy, one on chromosome 3q21-q22 and the other on the long arm of chromosome 10.[61] The families in which these loci were mapped were considered to have Möbius syndrome, even though abducens palsy was not invariably present.

It has become evident that mutations in genes such as *ARIX*, *SALL4*, and *KIF21A* that are important in either motoneuronal development or in the integrity and normal function of cranial nerves can result either in well-delineated abnormalities of ocular motility or in complex syndromes in which multiple cranial nerves are dysfunctional from birth. These syndromes can combine abnor-

malities of sensory as well as motor nerves and can be associated with other neurologic abnormalities or other malformations. The description of additional cases similar to those in this report is needed to better define individual rare syndromes and to establish their molecular etiology.

Teratogenic insults to the fetal brain at the time of cranial nerve development have clearly been shown to cause well-defined clinical entities such as DRS, or complex neurologic and malformative syndromes with cranial nerve dysfunction.[35,84,87] A detailed maternal gestational history should be obtained in patients with congenital ocular motility defects, and teratogenic causes should be excluded before a genetic etiology is presumed.

Figure 16 illustrates the isolated as well as the complex syndromes that involve cranial nerves II through VIII. With time, additional genes will undoubtedly be identified that cause less common individual syndromes, and more cases with overlapping phenotypes will be described, allowing a better delineation of phenotype/genotype correlations.

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