

# Evidence of Neuronal Migration Disorders in Knobloch Syndrome: Clinical and Molecular Analysis of Two Novel Families

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**Knobloch syndrome is an autosomal recessive disease characterized by the early onset of severe myopia, vitreoretinal degeneration with retinal detachment, macular abnormalities, and midline encephalocele, mainly in the occipital region. Intra and interfamilial variability is present since the encephalocele is not found in all patients, and the degree of myopia is variable. Analysis of the associated malformations suggests alterations during early neuroectodermal morphogenesis. Only 24 cases have been reported. Recently, the gene responsible for the syndrome, mapped to 21q22.3, was identified. The present study reports on four new cases, revealing the existence of neuronal migratory defects associated with the disorder for the first time.** © 2003 Wiley-Liss, Inc.

**KEY WORDS:** Knobloch syndrome; ocular defects; neuronal migration disorders

## INTRODUCTION

Knobloch syndrome (KS) is an autosomal recessive disease characterized by the early onset of progressive, severe myopia, vitreoretinal degeneration with retinal detachment, macular abnormalities, elevated incidence of congenital cataract, midline encephalocele, mainly in the occipital region, and normal cognitive development [Cohen and Lemire, 1982].

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Received 13 March 2002; Accepted 26 September 2002  
DOI 10.1002/ajmg.a.20070

Knobloch and Layer [1971] were the first to describe a family in which five of ten sibs from unrelated parents were affected. Currently, only 24 cases are known [see Sniderman et al., 2000, for review]. Recently, a mutation in the collagen XVIII gene (*COL18A1* gene), mapped to 21q22.3, has been shown to cause the syndrome [Sertié et al., 1996, 2000].

The present study reports on four cases from two unrelated families, emphasizing neuronal migration disorders, not previously associated with the syndrome.

## CLINICAL REPORTS

### First Family

The parents are second degree cousins, the mother aged 40 years, and the father 36 years old. The mother suffered a miscarriage in the first trimester of pregnancy and has borne three children: two boys (patients 1 and 2) and a girl who is currently 12 years old and is physically, neurologically, and ophthalmologically normal. The parents state that no members of previous generations have worn glasses or exhibit ophthalmological alterations. However, we did not evaluate other family members.

**Patient 1.** This 11-year-old boy was born via vaginal delivery, at term, after an uneventful pregnancy. Birth weight was 3,200 g. The mother was aged 30 at the time of birth. Bilateral cataract and exotropia were noted at birth. The psychomotor milestones were normal but the patient never exhibited good visual acuity, and vision was lost in the left eye at the age of 1 year and 9 months. At that time, a diagnosis was made of retinal detachment in the left eye with anterior uveitis and bilateral cataract. At the age of 2 years, the patient suffered right retinal detachment. At the age of 3 years and 11 months, the child was submitted to lensectomy and posterior vitrectomy but never recovered his vision, remaining amaurotic with progressive atrophy of both eyeballs (phthisis bulbi).

At admission, the patient's physical and neurological examinations were normal. Weight was 32 kg, height 141.5 cm, and head circumference 54.5 cm without scalp

alterations. Cognitive development was normal and currently the patient is a gifted student attending a school for the visually handicapped.

Cranial radiography was normal revealing no alteration of the skull bones. Brain MRI, vertebral column radiography, and an ultrasound scan of the urinary tract gave normal results.

Ophthalmological evaluation revealed enophthalmos in the right eye with band keratopathy and a shallow anterior chamber. In the left eye, band keratopathy, a flat chamber, posterior synechiae, and a totally opaque lens were found. An ultrasound scan showed complete bilateral retinal detachment and phthisis bulbi; fundoscopic examination could not be performed owing to opacity of the humours.

**Patient 2.** This 7-year-old boy is the younger brother of patient 1. Pregnancy was normal with a vaginal delivery at term. Birth weight was 3,800 g. A "large head" was reported as there was a midline occipital bone defect which was confirmed by three-dimensional, CT scan reconstruction of the skull (Fig. 1A). At the age of 14 months severe myopia was detected and glasses were prescribed. Serial cranial CT scans performed annually revealed nonhypertensive, supratentorial, ventricular dilatation, a moderate and stable retrocerebellar arachnoid cyst, and a defect in the midline portion of the occipital bone. Psychomotor development was normal.

Height was 115 cm, weight 20 kg, and head circumference 55.5 cm (above the 98th centile). Physical and neurological examinations were normal. Cognitive level was adequate. Currently, the patient is already literate and is a gifted student.

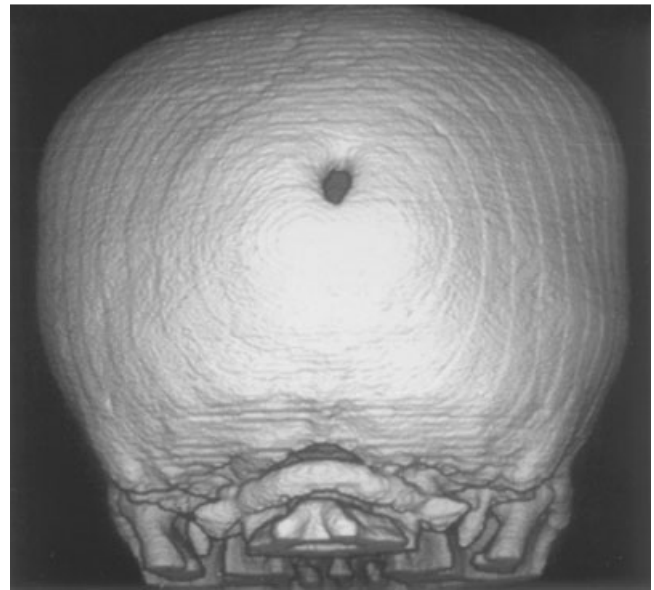
Ophthalmological evaluation revealed normal biomicroscopy. Fundoscopic examination of the right eye showed diffuse changes in the retinal pigment epithelium and macular coloboma with diffuse myopic degeneration. Fundoscopic examination of the left eye also revealed diffuse changes in the retinal pigment epithelium, and macular hypoplasia. Refraction evaluation showed myopia in the right eye (−8.50 sphere) while the left eye exhibited compound myopic astigmatism (−6.50 sphere; −2.00 cylinder at 180 degrees).

Cranial MRI revealed many subependymal, heterotopic nodules in the lateral ventricles, more evident on the left side (Fig. 1B). A urinary tract ultrasound scan and vertebral column radiography were normal.

## SECOND FAMILY

The parents are first degree cousins, the mother aged 37 and the father 40 years old. Both are healthy and their physical and neurological examinations were unremarkable. The mother's ophthalmological examination revealed compound hypermetropic astigmatism, pterygium of the right eye, and emmetropia. The father's examination showed mild myopia in both eyes (−0.50 sphere). Fundoscopic examinations and biomicroscopy were normal. The mother reported three pregnancies, giving birth to three children: two girls (patients 3 and 4) and a boy who died at the age of 7 months due to an acute infectious process. At the time,

**A**



**B**

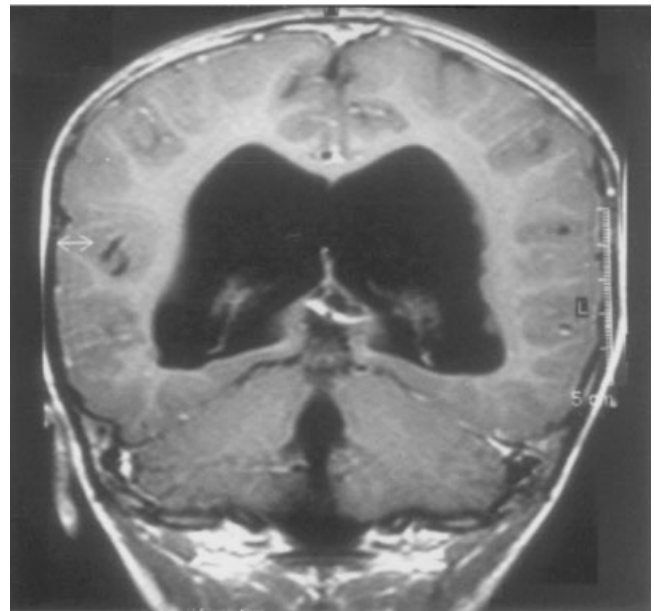


Fig. 1. Patient 2. **A:** A three-dimensional CT scan reconstruction of the skull revealing a discontinuity at the midline of the occipital bone. **B:** T1-weighted MRI (coronal section) showing bilateral, subependymal, heterotopic nodules, more evident on the left side.

his psychomotor development and visual contact were normal.

**Patient 3.** A 19-year-old girl, born at term by vaginal delivery when her mother was 18 years old. Pregnancy was normal. Birth weight was 3,100 g. Bilateral horizontal nystagmus and early bilateral exotropia, more intense during the visual fixation periods, were noted at birth. A small area of alopecia with a flat, wine-colored hemangioma also was observed at the

midline of the occipital region at birth. At the age of 2 years, severe myopia was diagnosed in both eyes ( $-9.0$  Diopters). At the age of 13 years, retinal detachment occurred in the patient's left eye with irreversible loss of vision.

Height was 166 cm, weight 53 kg, and head circumference 53 cm. Physical and neurological examinations were normal. Cognitive level was preserved and the patient is a gifted student. Cranial CT scan, EEG, vertebral column radiography, and a urinary tract ultrasound scan were normal. Cranial MRI showed a basilar impression. The three-dimensional CT scan reconstruction of the skull showed a depression at the midline of the occipital bone without discontinuity.

Ophthalmological evaluation revealed bilateral, horizontal nystagmus and exotropia, together with compound myopic astigmatism in the right eye ( $-12.25$  sphere;  $-3.75$  cylinder at 157 degrees), and amaurosis of the left eye.

**Patient 4.** This 13-year-old girl is the youngest sister of patient 3. Pregnancy and delivery at term were normal. Birth weight was 4,350 g. At birth, a slight prominence and alopecia were noted at the midline of the occipital region, together with bilateral, horizontal nystagmus and esotropia. A cranial CT scan performed at the age of 12 months showed moderate, nonhypertensive, supratentorial, ventricular dilatation. An ophthalmological evaluation diagnosed severe myopia, and the patient has worn glasses since then. Over the last 2 months the patient has suffered several, generalized grand-mal seizures, medicated with Phenobarbital (100 mg/day). Height was 148 cm, weight 51 kg, and head circumference 54 cm. Physical and neurological examinations were normal. The patient is a gifted student and her cognitive level is preserved.

EEG, vertebral column radiography, and a urinary tract ultrasound scan were normal. A three-dimensional reconstruction of the skull by cranial CT scan showed a small discontinuity at the midline of the occipital region (Fig. 2A), moderate, nonhypertensive, supratentorial, ventricular dilatation, and slight calcification of the right parietal lobe. Cranial MRI disclosed moderate ventricular dilatation and a pachygyric area in the right frontal lobe, together with a heterotopic nodule on the subependymal surface of the right lateral ventricle (Fig. 2B). Ophthalmological evaluation revealed severe myopia (OD:  $-14.50$  sphere; OS:  $-13.25$  sphere), bilateral, horizontal nystagmus and esotropia, iris pigments in the anterior lens capsule of the right eye, and diffuse changes in the retinal pigment epithelium with macular hypoplasia in both eyes, together with myopic degeneration.

#### METHODS AND RESULTS: MOLECULAR ANALYSIS

DNA was isolated from whole peripheral blood samples using standard techniques [Miller et al., 1988]. Segregation analysis was performed employing four markers (the microsatellite D21S1897 and 3 SNPs—dbSNP IDs: rs2236451, rs2236474, and rs7499, <http://www.ncbi.nlm.nih.gov/SNP>) located within or near the

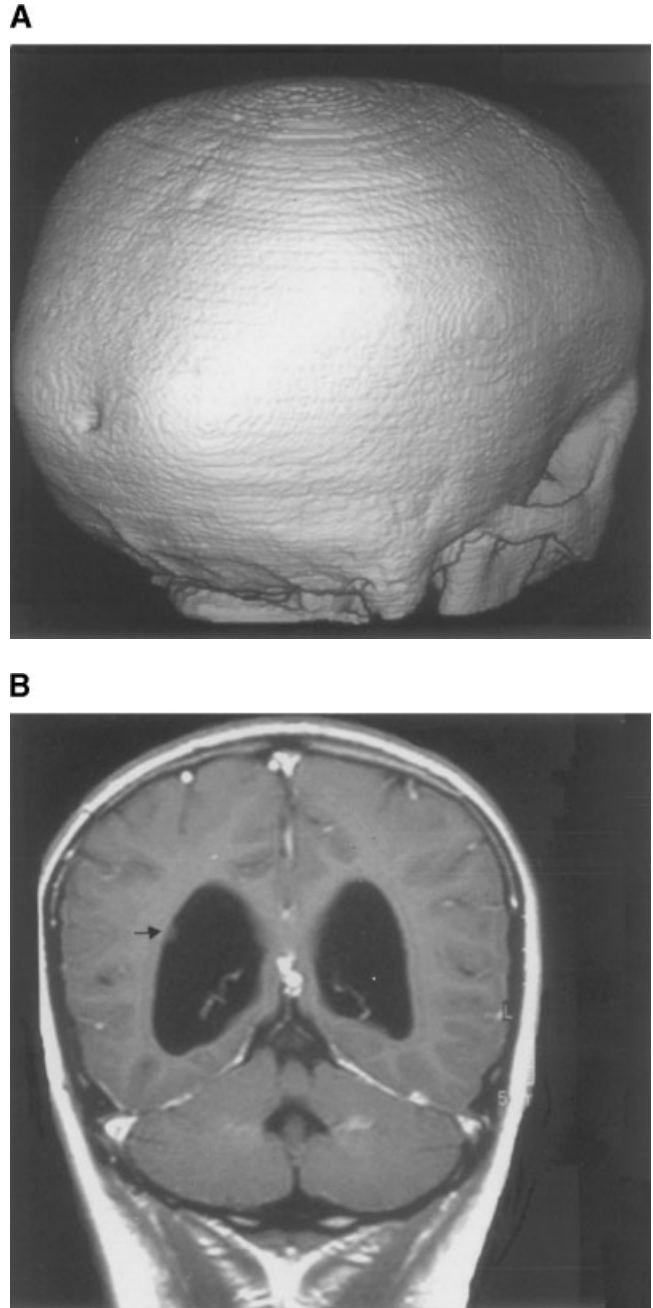


Fig. 2. Patient 4. **A:** A three-dimensional CT scan reconstruction of the skull showing a discontinuity at the midline of the occipital bone. **B:** T1-weighted MRI (coronal section) showing nodular, subependymal heterotopia on the right side (arrow).

*COL18A1* gene in the two families. Both parents, the two affected sibs from each family, and the normal sister from the first family were included in the analysis. Mutation screening in the 43 coding exons of *COL18A1* is being performed using PCR-SSCP as described elsewhere [Sertié et al., 2000]. PCR products displaying a mobility shift were sequenced in an automatic sequencer (ABIPrism377, PE Biosystems, Foster City, CA) according to the manufacturer's instructions.

The two affected sibs in each family share a common haplotype. These results corroborate the hypothesis that mutations in the *COL18A1* gene may be responsible for the patients' disease in the two families. A total of 27 and 25 of the 43 exons were screened by SSCP analysis in the first and second families, respectively. Twelve alterations, confirmed through sequence analysis, were found. All were shown to be polymorphisms, except for one; a nucleotide substitution from G to A at position c4181 (AF018081; gi: 2920534) (Fig. 3). This mutation (A1381T) was present in both alleles of the two affected sibs from the second family and it was not detected in 100 control chromosomes. We are currently performing functional studies to elucidate if this mutation is responsible or not for the KS phenotype. Presently no pathogenic change has been detected in the first family.

### DISCUSSION

Knobloch and Layer [1971] described five sibs showing early, progressive, severe myopia, associated with occipital encephalocele in four. Cognitive development was preserved in all patients. Since then, 19 further patients with similar manifestations have been reported in the literature [see Sniderman et al., 2000, for review]. The progressive nature of the ocular defects in KS is the most prominent symptom and such patients are usually referred to ophthalmologists. Vitreoretinal degenera-

tion with severe myopia of early onset and eventual retinal detachment are universal in this syndrome which has a poor prognosis, despite surgical treatment or prophylactic cryotherapy [Knobloch and Layer, 1971; Wilson et al., 1998]. Ophthalmological evaluation has been performed in 15 of the 24 reported patients, 12 of which presented retinal detachment. The five patients described by Knobloch and Layer [1971] were followed up for 10 years by Cook and Knobloch [1982], and unilateral or bilateral retinal detachment was found in all of them between the age of 7 and 11 years. Retinal detachment between the age of 2 and 5 years has been reported in two patients by Czeizel et al. [1992] and in another by Seaver et al. [1993]. The three remaining patients who did not show retinal detachment reported by Seaver et al. [1993], Wilson et al. [1998], and Sniderman et al. [2000] were only 2, 3, and 4 years old, respectively, at the time of the report. However, irreversible ocular damage can occur in the first month of life as found in the patient reported by Wilson et al. [1998]. One of our patients (patient 1) presented retinal detachment in the left eye at the age of 1 year and 9 months, and in the right eye at the age of 2 years, with irreversible amaurosis; the other patient (patient 3) presented retinal detachment in the left eye at the age of 13 years with irreversible, unilateral loss of vision.

Neural tube closure defects are prominent and have been described in 22 of the 24 reported cases. There is considerable controversy concerning their classification as scalp defects or true encephalocele. When present, encephalocele is mild and atretic. Pathological analysis was performed in five patients who underwent corrective surgery. Of these, the two patients reported by Czeizel et al. [1992] presented only meningocele. The histological examination of both patients reported by Seaver et al. [1993] revealed heterotopic neuronal tissue in the occipital scalp defect, with a descending column of fibrous tissue extending to the periosteum containing heterotopic hair shafts and sweat glands. In the single patient reported by Wilson et al. [1998], histologic examination revealed a cyst-like space lined by multilayered, unremarkable meningotheilium, containing small amounts of neuroglial tissue in the wall.

Several somatic malformations have been encountered in seven of the twenty four reported cases. It is unclear whether these features constitute part of the clinical spectrum of the syndrome. Knobloch and Layer [1971] reported a patient with Scimitar syndrome. Czeizel et al. [1992] found many somatic malformations like spina bifida occulta of L5-S3, carried teeth, asymmetrical chest, unusual palmar creases, finger nail hypoplasia, and bifid ureter in their two patients. Seaver et al. [1993] encountered short palpebral fissures, bilateral epicanthic folds, flat nasal bridge, midface hypoplasia, and generalized hyperextensibility of the joints in their two patients. Wilson et al. [1998] reported a patient with a single umbilical artery, patent ductus arteriosus, pyloric stenosis, micrognathism, and "lop" ears. The patient reported by Sniderman et al. [2000] presented hypertelorism, telecanthus, flat nasal bridge, and a high-arched palate. None of our patients exhibited

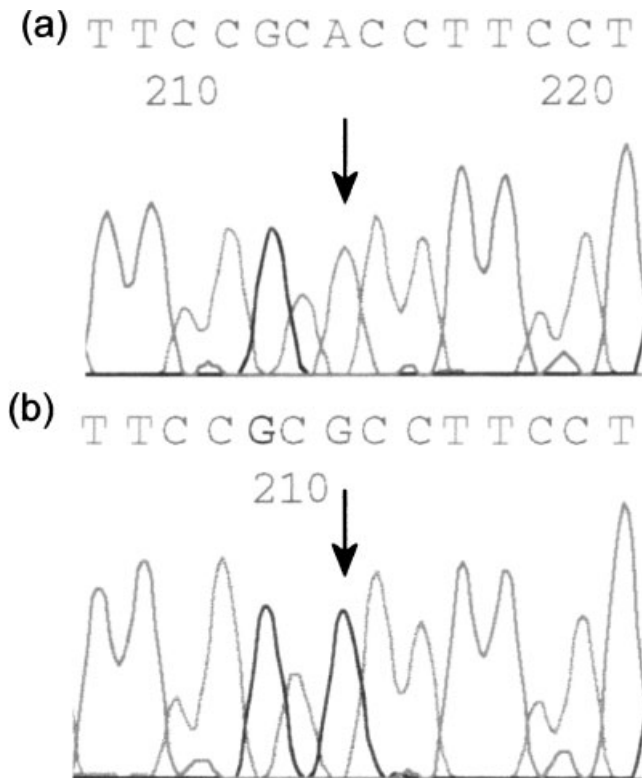


Fig. 3. G to A substitution at position c4181 (gi: 2920534) identified in patients from the second family: (a) mutated type; (b) wild type.

malformations, nor did the 12 patients reported by Passos-Bueno et al. [1994].

Ocular development and neural tube formation take place during the first 3 weeks of embryonic life. Thus, any pathological event occurring during this period may adversely affect the embryogenesis of both structures [Seaver et al., 1993].

The neuronal migration disorders found in two of our unrelated patients suggest that this cerebral malformation may constitute part of KS. Despite this cerebral defect, the psychomotor development and neurological examinations of both patients were normal. However, patient 4 presented epilepsy, not previously reported in the syndrome. This patient's imaging examinations showed a small calcification of the right parietal lobe associated with nodular heterotopia and moderate, lateral, ventricular dilatation. Whether the epilepsy in this case is related to the heterotopia or to the calcified lesion, itself possibly consequent to a calcified cysticercus, is a matter of speculation.

Migration disorders occur after neural tube closure during embryogenesis. Sertié et al. [2000] showed that KS is caused by mutations in the *COL18A1* gene, and its absence might impair embryonic cell proliferation and/or migration as a primary or secondary effect. These authors also stated that collagen XVIII plays a critical role in the maintenance of retinal structure and in neural tube closure based on the phenotype of this syndrome. In addition to a structural function of the collagen XVIII, its COOH-terminal domain (NC1) is proteolytic cleaved and produces endostatin (ES), a potent antiangiogenic agent [O'Reilly et al., 1997]. Ackley et al. [2001] have recently identified a type XVIII collagen homologue, designated as cle-1, in the nematode *Caenorhabditis elegans*. Deletion of the NC1 domain of the cle-1, containing the ES region, results in cell and axon migration defects. These authors also demonstrated that monomeric ES might inhibit the promigratory activity of the NC1 domain, indicating that the cle-1 NC1/ES domain regulates cell and axon migration in *C. elegans*. Kuo et al. [2001] also showed that the collagen XVIII NC1/ES induces motility of non-endothelial mammalian cells, as those from embryonic kidney. Therefore, the present report further supports a

role of collagen XVIII in migration of nonendothelial cells and it is showing for the first time a role of this collagen in neuronal migration in vivo.

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