Research Letter

CNS Malformations in Knobloch Syndrome With Splice Mutation in COL18A1 Gene

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To the Editor:

Knobloch syndrome (KS) is an autosomal recessive disorder associating severe early onset myopia, starting in most cases before 1 year of age, and an occipital defect. The first description of the disease was made in 1971 in five affected sibs by Knobloch and Layer [1972] and Cook and Knobloch [1982]. Fifteen families with a total of 39 affected patients have been reported [Czeizel et al., 1992; Seaver et al., 1993; Passos-Bueno et al., 1994; Wilson et al., 1998; Sniderman et al., 2000; Suzuki et al., 2002; Kliemann et al., 2003; Duh et al., 2004]. In 1996, the gene responsible for KS was mapped to 21q22.3 in a large Brazilian family by linkage analysis [Sertie et al., 1996]. A mutation at the AG consensus acceptor site of COL18A1 gene that predicts a stop codon in exon 4 was subsequently identified in this family [Sertie et al., 2000]. Suzuki et al. [2002] found mutations in 5/8 additional KS families, confirming that mutations in COL18A1 cause KS. Recently genetic heterogeneity in KS has been suggested [Suzuki et al., 2002; Menzel et al., 2004]. Knockout mice demonstrate abnormal retinal vessels in young Col18a1−/−, with irregular bending but no leakage, and disruption of the posterior iris pigment epithelial cell layer [Fukai et al., 2002; Marneros and Olsen, 2003, 2005; Rychkova et al., 2005]. Kliemann et al. [2003] reported the presence of abnormal neuronal migration in two unrelated patients who showed heterotopic nodules at neuroimaging. One of them had localized pachygria. We report here on two sibs with KS; a girl with mental retardation and severe supratentorial CNS anomalies, and a fetus with severe brain malformations, complete vermian agenesis, and mesencephalic hamartoma. This report confirms that endostatin (or full-length collagen XVIII) plays a role in neuronal migration. CNS anomalies in KS may be more severe than initially thought.

Patient 1 was the first child of healthy Algerian parents who were half first cousins, their fathers being half brothers. She has a younger brother in good health. Her mother was 27 years old at the time of her birth, and her father was 33. The mother had cystitis in the first 3 months of her pregnancy. She was treated with amoxicillin and nitroxolin, without any complications. The ultrasonographic investigations during late pregnancy term showed microcephaly and excessive femoral length. Birth was uneventful except for transient tachypnoea of the newborn which improved spontaneously after 1 day. Birth weight was 2,940 g, and birth length was 56 cm. Head circumference at birth was not recorded. At birth, an...
occipital meningocele was noted. There were no brain structures in the meningocele that initially had a diameter of 5 cm, and expanded progressively over time. Nystagmus was noted at the age of 6 months. The meningocele was removed surgically at 8 months of age. She had no seizures. She held her head at the age of 9 months and was able to stand at 12 months. When examined at the age of 24 months, her height was 91 cm (+2 SD), her weight was 14 kg (+2 SD), and her head circumference was 50 cm (+1 SD). She was able to say a few words and had difficulties in understanding simple orders. She showed mild facial dysmorphism: narrow face, high and large forehead, horizontal eyebrows, bilateral epicanthic folds, bulbous nasal tip and temporal narrowing (Fig. 1). Neurological examination and EEG were normal. Ophthalmologic examination showed horizontal nystagmus, severe rapidly worsening myopia: \(-15.25 (-1.75 \times 150) \text{ OD and } -15.75 (-1.25 \times 175) \text{ OS with best corrected visual acuity of 20/200 J3 both eyes. The ocular axial length was } 27.81 \text{ mm OD and } 28.18 \text{ mm OS by ultrasound. Anterior segment was normal. At the fundus, severe myopic retinopathy was observed. Early signs of vitreous degeneration with marked fibrillar aspect were noted. Unusual round-shaped chorioretinal pseudo-colobomatous lesions were present on both sides in the inferior temporal retina, extending to the paramacular area (Fig. 2). No retinal pigment was visible in the lesions or their surroundings. Vascular rigidity of retinal vessels was observed. Electroretinography (ERG) and visual evoked potentials (VEP) explorations were normal. Retinal detachment occurred at 5 years of age leading to legal blindness. Besides an occipital bone defect resulting from surgery, cranial radiographs, and magnetic resonance imaging (MRI) showed agenesis of the septum pellucidum, bilateral pachygyria/polymicrogyria of the entire frontal lobes, and heterotopic hypersignals (on TW2 images) along the radial migration tracts (Fig. 3). Psychometric evaluation at the age of 3 years and 11 months was complicated by visual impairment and distractibility. Using Brunet–Lezine and Vineland scales, and some tests of the WPPSI-III chart (Wechsler Preschool and Primary Scale of Intelligence), a developmental level between 2 years 2 months and 3 years 6 months was determined, corresponding to a Developmental Quotient of 75.

The mother's next pregnancy was marked by the discovery of a recurrence of the syndrome, with presence of a posterior encephalocele at 17 weeks of gestation. The pregnancy was terminated. Pathological analysis showed Patient 2, a male fetus, with retrognathia, enlarged neck, and a meningocele of 12 mm of diameter (and a head circumference of 130 mm) (Fig. 4). There was a defect in the skull, of 1.5 cm of diameter, in the occipital region. The left occipital lobe was attracted to the defect, but the meningocele contained no brain tissue. A severe cerebellar malformation was present, with complete agenesis of the vermis, and a poor differentiation of the hemispheres. A hamartomatous lesion of the mesencephalic roof was found on histology, with isthmic dysplasia and presence of buried ependymal cells (Figs. 5 and 6). No retinal anomalies were identified, but examination was made difficult because of tissular lysis.

All the coding regions of the gene, including the exon–intron splice junctions, were PCR amplified and directly sequenced in an automatic sequencer (MegaBACE, GE Healthcare) for mutation screening. The primer pairs used were published by Suzuki et al. [2002]. We identified a homozygosity single nucleotide change located near the donor splice site of intron 36. This mutation, \(c.3544 + 3A > C\) (numbering according to the GenBank sequence AF018081), was not detected in a 100-chromosome control samples and is located in a highly conserved site among humans and other species. Computational analysis of this base substitution predicts the abolition of the splice donor site (https://splice.cmh.edu) [Nalla and Rogan, 2005]. The third nucleotide of introns usually is a purine [Stephens and Schneider, 1992]. Therefore, this change is very likely to be pathogenic and it is possibly critical for the normal functions of the encoded protein.
processing of the RNA. The analysis of the fetus was technically very difficult, because of the poor quality of the post-termination DNA sample. The amplification failed, but both sibs were homozygous for \( \text{Col18A1} \) gene region by microsatellite analysis (not shown). DNA samples of the parents were not available.

A diagnosis of KS in Patient 1 is sustained by the combination of high-grade myopia due to excessive increasing of ocular axial length and midline skull defect; and confirmed by the presence of a mutation in the \( \text{COL18A1} \) gene. Ocular anomalies are a major feature in KS, usually with a poor prognosis and resulting most often in complete loss of vision. Most patients need an early correction of \(-10\) diopters or higher. As in this report, vitreoretinal degeneration, a non-specific consequence of severe myopia, is almost always present in the form of vitreous liquefaction and pigmentary lesions. Retinal detachment is the main complication and occurs before the age of 15 years in most cases. Preventive cryotherapy is often inefficient. Lens opacities are also frequently found in KS; they can lead to totally opaque lenses. Our patient did not show those lesions at age 5 years. Her fundi showed an unusual paramacular retinal coloboma. This lesion has not been associated with any particular disorder. The defect is different from the retinal aspect of Aicardi syndrome since there is no pigmentation either inside the lesion or in its surroundings. Kliemann et al. [2003] also reported a

![FIG. 2](image1.jpg)  
**FIG. 2.** Fundus of OD showing severe myopic retinopathy and paramacular colobomatous lesion without retinal pigment inside the lesion and in its surrounding (at the left side of the optic disc). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

![FIG. 3](image2.jpg)  
**FIG. 3.** MRI of Patient 1.  
**A:** Axial TW1 image showing the absence of the septum pellucidum and the pachygryria/polymicrogyria appearance of the frontal cortex.  
**B:** Coronal TW2 image illustrating the absence of the septum pellucidum with characteristic pointing down frontal ventricular horns.  
**C:** Axial TW1 image showing the occipital defect resulting from the surgically removed meningocele. Note the axial length of the eyes about 25 mm (normal for emmetropic eye = 24 mm).  
**D,E:** Axial TW1 and TW2 slices showing bilateral polymicrogyria frontal cortex and hypersignals along the radial migration tracts.

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retinal coloboma in a patient with KS. As the lesion is not precisely described in the latter, we ignore if the unusual appearance of the defect is really distinctive.

Midline scalp defect is the second most frequent sign of KS, but it does not have full penetrance [Knobloch and Layer, 1972; Wilson et al., 1998; Kliemann et al., 2003]. We can therefore suppose that sporadic KS can be underdiagnosed in patients presenting only with severe myopia. The nature of the occipital defect is still controversial. In most cases, the evaginated pouch does not seem to contain brain tissue at neuro-imaging or macroscopic examination, although histological examination showed heterotopic, poorly formed neural tissue in some cases [Czeizel et al., 1992; Seaver et al., 1993; Wilson et al., 1998]. For Wilson et al. [1998], such findings classify scalp defects in KS as encephalocele rather than meningocele. In Patient 1, the scalp lesion was macroscopically described as a meningocele, without histological confirmation, but the fetus had an empty meningocele, with an attraction of the left occipital lobe. The defect is always in the occipital midline region; this hypothesis has been further supported by the observation that a patient with a frontal defect and clinically classified as KS did not present mutations in the \textit{COL18A1} [Sniderman et al., 2000].

Until 2003, supratentorial CNS and development were considered to be normal in KS. Recently Kliemann et al. [2003] reported brain anomalies in two unrelated patients with KS. Both showed a neuronal migration disorder with heterotopic nodules scattered in the white matter. One of them had a unilateral area of pachygyria in the frontal lobe. These patients did not show any neurological symptoms, and their psychomotor development was normal. Our patient, with heterotopias and bilateral pachygyria of the frontal lobes gives an independent confirmation that neuronal migration is affected in KS. Agensis of the septum pellucidum has not been described before in KS. Usually this lesion is associated with septo-optic dysplasia (most often) or holoprosencephaly [Malinger et al., 2005]. In our patient, there were no signs of holoprosencephaly (even mild) and the papillae of the optic nerves were normal. Not surprisingly, our patient had borderline mental delay and learning disability, another feature which has not been reported insofar in association with KS.

\textit{COL18A1} gene has three characterized isoforms that differ only by their N-terminal [Saarela et al., 1998]. The longest transcript is expressed in the liver, lung, skeletal muscle, spleen, thymus and kidney;
the medium transcript is expressed almost exclusively in the liver, while the shorter variant is mainly expressed in kidney but it is also found in most of the other tissues of the body, including retina [Saarela et al., 1998; Sertie et al., 2000]. No hepatic or kidney lesion has ever been reported with KS. Endostatin, a proteolytic cleavage product of the C-terminal part of collagen XVIII, is a potent inhibitor of angiogenesis [O’Reilly et al., 1997]. All KS patients with CNS abnormalities bear mutations located in the C-terminal domain which is shared by all the isoforms; therefore, these mutations lead to deficiency of all the three collagen XVIII isoforms and endostatin. Considering that the original patients, who lacked only one of the collagen isoforms and were older than 40 years of age, did not present neuronal migration abnormalities [Sertie et al., 2000], we could speculate that major CNS abnormalities are related to deficiency of the medium and/or large isoforms or of endostatin.

In conclusion, this report contributes to a better characterization of the ocular alterations due to deficiency of collagen XVIII and adds new data on the effects of the deficiency of this collagen in CNS. Further reporting is needed to elucidate whether CNS involvement is dependent of specific mutations, and to evaluate the risk of recurrence of mental handicap. Both issues are of major importance for genetic counseling.

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