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Brain Malformations Associated with Knobloch Syndrome – Review of Literature, Expanding Clinical Spectrum and Identification of Novel Mutations

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Abstract

BACKGROUND—Knobloch syndrome is a rare, autosomal recessive, developmental disorder characterized by stereotyped ocular abnormalities with or without occipital skull deformities (encephalocele, bone defects, cutis aplasia). Although there is clear heterogeneity in clinical presentation, central nervous system malformations, aside from the characteristic encephalocele, have not typically been considered a component of the disease phenotype.

METHODS—Four patients originally presented for genetic evaluation of symptomatic structural brain malformations. Whole-genome genotyping, whole-exome sequencing, and confirmatory Sanger sequencing were performed. Using immunohistochemical analysis, we investigated the protein expression pattern of *COL18A1* in the mid-fetal and adult human cerebral cortex and then analyzed the spatial and temporal changes in the expression pattern of *COL18A1* during human cortical development using the Human Brain Transcriptome database.

RESULTS—We identified two novel homozygous deleterious frame-shift mutations in the *COL18A1* gene. Upon further investigation of these patients and their families, we found that many exhibited certain characteristics of Knobloch syndrome, including pronounced ocular defects. Our data strongly support an important role for *COL18A1* in brain development and this report contributes to an enhanced characterization of the brain malformations that can result from deficiencies of collagen XVIII.

CONCLUSIONS—This case series highlights the diagnostic power and clinical utility of whole-exome sequencing technology – allowing clinicians and physician scientists to better understand the pathophysiology and presentations of rare diseases. We suggest that patients who are clinically diagnosed with Knobloch syndrome and/or found to have *COL18A1* mutations via genetic screening should be investigated for potential structural brain abnormalities even in the absence of encephaloceles.

Keywords

Knobloch Syndrome; Cortical Development; COL18A1; Collagen XVIII; Whole-Exome Sequencing

Introduction

First described by Knobloch and Layer in 1971, Knobloch syndrome (OMIM: #267750, #608454) is a rare, autosomal recessive syndrome, characterized by stereotyped ocular with or without occipital skull abnormalities. [1] Ocular conditions traditionally include high myopia, lens subluxation, vitreoretinal degeneration, retinal detachment, and early-onset cataracts; occipital skull abnormalities can include encephalocele, bone defects, or cutis

aplasia. Since the original report, at least 85 cases of Knobloch syndrome from 44 families have been described, each with varying degrees of clinical heterogeneity. [2–5]

In 2000, Sertie et al. identified *COL18A1* (OMIM *120328) as the disease-causing gene for Knobloch syndrome by performing linkage analysis in a large consanguineous family. [6] The authors discovered a homozygous acceptor splice site mutation affecting the gene product of *COL18A1*. Since this original report, numerous mutations in *COL18A1* have been identified in unrelated families who have Knobloch syndrome, thus confirming its causal relation with the syndrome. [7] To date, more than 21 different mutations have been described in patients from various ethnicities. [4–6; 8–12]

In addition to the characteristic ocular and occipital skull defects, Knobloch syndrome can present with a spectrum of phenotypic findings – some patients have presented with lung hypoplasia, hyper-extensible joints, duplication of the renal collecting system, epilepsy, neuronal migration abnormalities, and dysmorphic findings such as mid-face hypoplasia, flat nasal bridge, dental abnormalities, high arched palate, or micrognathia. [10; 13]

Although neuronal migratory abnormalities and brain malformations have been previously reported in the literature (a total of seven patients from four case series, Table 1) [3; 9; 10; 14], central nervous system malformations (aside from the characteristic encephalocele) have been considered relatively rare and have not traditionally been considered a hallmark feature of Knobloch syndrome. Here, we describe four patients with structural brain malformations (with or without encephaloceles) who were found to possess frame-shift homozygous mutations in *COL18A1* via whole-exome and Sanger sequencing. When we further investigated these patients and their families, we discovered that these patients possessed some of the characteristic phenotypic features of Knobloch syndrome.

Methods

Participants

The study protocol was approved by the Yale Human Investigation Committee (HIC) (protocol number 0908005592). Institutional review board approvals for genetic and magnetic resonance imaging studies, along with written consent from all study subjects, were obtained by the referring physicians at the participating institutions. All fetal human tissues were collected under guidelines approved by the Yale HIC (protocol number 0605001466).

Human fetal brains at 20 and 22 weeks of gestation were obtained from the Human Fetal Tissue Repository at the Albert Einstein College of Medicine (CCI number 1993-042).

Genome-wide Genotyping

Samples were analyzed using an Omni 1M Quad V1-0 B SNP chip and 610K Quad Bead Chips according to the manufacturer’s protocol (Illumina).

Exome Capture and Sequencing

NimbleGen 2.1M human exome array (Roche Nimblegen, Inc.) was used to capture the exomes of all samples according to the manufacturer's protocol with certain modifications, as described previously. [15] Sequencing of the library was performed on HiSeq2000 using a barcoding technology allowing for 6 samples per lane. The Illumina pipeline version 1.8 was used for image analysis and base calling. These methods were previously described in detail. [16]

Exome Data Analysis

Analysis of the sequencing data was performed according to the previously described in-house written data analysis pipeline. [17] Briefly we used sequence reads that passed Illumina quality filter for analyzing whole exome data. We aligned reads to the human genome reference sequence (version GRCh37, the same as used in the phase 1 of 1000 Genomes Project) using Stampy (version 1.0.16) [18] in a hybrid mode with BWA (version 0.5.9-r16). [19] We detected variant sites (point mutations and small indels) using the Unified Genotyper algorithm from GATK [20]. We annotated variant alleles using Ensembl database (version 66) with the help of Variant Effect Predictor (v2.4) tool (http://useast.ensembl.org/info/docs/variation/vep/vep_script.html).

Sanger Sequencing

Coding regions and exon-intron boundaries of *COL18A1* were evaluated via Sanger sequencing using standard protocols. Amplicons were cycle sequenced on ABI 9800 Fast Thermocyclers, and post cycle sequencing clean up was carried out with CleanSEQ System (Beckman Coulter Genomics). The amplicons were analyzed on 3730×L DNA Analyzer (Applied Biosystems Inc.).

Immunohistochemistry

Cortices from human fetal brain (20 weeks post-conception) were fixed in 4% paraformaldyhyde and cut into sections using a vibratome. Free-floating sections were preincubated in 1% hydrogen peroxide solution (T.J. Baker) at room temperature for 20 minutes and recovered in PBS. Sections were then pre-blocked in blocking solution containing 5% normal donkey serum (Jackson Immuno Research Laboratories,), 1% bovine serum albumin, 0.1% glycine, 0.1% lysine and 0.3% Triton X-100 in PBS for one hour at room temperature. After pre-blocking, sections were incubated in anti-human Endostatin (goat, 1:40; R&D Systems, AF1098) diluted in blocking solution on a horizontal shaker at 4°C for 48 hours, and then washed in PBS at room temperature to remove excessive primary antibody. Sections were then incubated with biotinylated secondary antibody (donkey, anti-Goat IgG) (Jackson ImmunoResearch) diluted in blocking solution for 2 hr at room temperature. After washing in PBS, sections were further incubated in avidin-biotin-perosidase complex (Vectastain ABC Elite Kit, Vector Laboratories) for 1 hour at room temperature and washed three times in PBS. Peroxidase activity was developed using a DAB peroxidase substrate kit (Vector Laboratories). Sections were mounted on Superfrost Plus charged slides (Fisher Scientific), allowed to air-dry and then were coverslipped with Permount (Fisher Scientific) for later examination and imaging.

RESULTS

Patient Descriptions

Four patients, two males and two females, with a median age of 13.5 years (range: 13 – 22 years), and their parents and siblings from four distinct consanguineous Turkish families (NG133, NG1348, NG1426, NG159) were enrolled in our study in accordance with the policies of and following the approval by our institutional review board (Supplementary Figure 1). These four patients originally presented for genetic work-up for their symptomatic structural brain malformations. All four patients were confirmed to have structural brain malformations and various ocular abnormalities (Table 2). Representative MRI findings from these patients are shown in Figure 1.

Genotyping and Whole-Exome Sequencing

DNA was extracted from the blood samples obtained from the four patients and their parents and siblings. Whole-genome genotyping was performed for three of these four families (NG133, NG1348, NG1426) and identified shared homozygous segments (each > 2.5 centimorgans (cM)) and revealed an index of homozygosity consistent with a consanguineous union in each of these three families. We then performed whole-exome sequencing using NimbleGen SeqCap EZ and the Illumina HiSeq 2000 on the index patients from each of these three families (as previously described [16]). We achieved mean 20x coverage of 87%, 82%, and 84%, and for NG133-1, NG1348-1, and NG1426-1, respectively, of all targeted bases (Supplementary Table 1). This coverage was more than sufficient to identify homozygous variants with a high degree of specificity in each of these three patients (Supplementary Tables 2 – 4).

We identified two novel homozygous deleterious frame-shift mutations (c.2309insC [p.Gly773ArgfsX55] in NG1348-1 and c.5199_5202insTGCC [p.Ala1734CysfsX14] in NG1426-1) and one previously reported mutation (c.4768_4769delCT [p.Leu1590ValfsX72] in NG133-1) in the *COL18A1* gene (Table 3, Figure 1, and Supplementary Figure 2). [14] All variants were confirmed as homozygous in the affected patients and heterozygous in their respective parents via Sanger sequencing (Supplementary Figure 3). DNA extracted from patient NG159-2's blood underwent Sanger screening for the exons of *COL18A1* and was found to harbor the homozygous c.4768_4769delCT mutation (p.Leu1590ValfsX72). Like the other patients, NG159-2's parents were heterozygous for this variant (Supplementary Figure 4).

For two of the three patients who underwent whole-exome sequencing, we identified no other known disease causing mutations that may have been responsible for their phenotypes. In one patient NG1348-1, we found a non-conserved missense mutation in *PCNT* in addition to the *COL18A1* mutation (Supplementary Table 3). However, while the mutation in *PCNT* may be contributing to the phenotype, we believe that it is the *COL18A1* mutation that is predominantly responsible for abnormalities identified in this patient.

COL18A1 Expression in the Developing Brain

To better understand the potential for mutations in *COL18A1* to contribute to the pathogenesis of central nervous system malformations in these patients, we assessed the extent to which *COL18A1* was expressed in the developing brain. First, we performed immunohistochemistry using antibodies against endostatin, a naturally-occurring C-terminal fragment derived from *COL18A1*. We found that *COL18A1* protein is highly expressed in the pia and blood vessels of postmortem mid-fetal (Fig 2A) and adult (Fig 2B) human cerebral cortex, which is similar to the expression of *Col18a1* in the embryonic mouse brain (Fig 2C). We then analyzed the spatial and temporal changes in the expression pattern of *COL18A1* during human cortical development using the Human Brain Transcriptome database. [21] The Human Brain Transcriptome database is a public database containing genome-wide RNA expression data, and associated metadata set was generated from 1340 tissue samples collected from 16 brain regions including the cerebellar cortex, mediodorsal nucleus of the thalamus, striatum, amygdala, hippocampus, and 11 areas of the neocortex of 57 developing and adult postmortem brains of clinically unremarkable donors representing males and females of multiple ethnicities. The relative levels of RNA expression can be tracked over the course of development. We found that *COL18A1* mRNA expression is low-to-moderate ($6<\log^2$ intensity<7) in the cortical areas of human brains (Supplementary Fig 5).[21] We next performed coexpression analysis 21 and 22 on *COL18A1* and found that *COL18A1* is positively coexpressed with genes known to cause cortical malformation syndromes such as DOCK6 (responsible for Adams-Oliver syndrome 2), LAMC3 (responsible for occipital cortical malformations), and COL6A3 (responsible for Bethlem myopathy) (Supplementary Table 5).

We then analyzed the spatial and temporal changes in the expression pattern of *COL18A1* during human cortical development using the Human Brain Transcriptome (HBT) database. [21] The HBT database is a public database contains genome-wide RNA expression data and associated metadata set was generated from 1,340 tissue samples collected from 16 brain regions including the cerebellar cortex, mediodorsal nucleus of the thalamus, striatum, amygdala, hippocampus, and 11 areas of the neocortex of 57 developing and adult post-mortem brains of clinically unremarkable donors representing males and females of multiple ethnicities. The relative levels of RNA expression can be tracked over the course of development. We found that *COL18A1* mRNA is present in pyramidal neurons of all layers of the cortical plate shortly after conception; this expression persists, though decreases throughout fetal development until late fetal life (Supplementary Figure 5). [21] At this time, the level of expression again increases until infancy when expression plateaus and remains at this level throughout adult life. [21] These findings correlate with our immunohistochemical data demonstrating the presence of *COL18A1* in the cortical plate of the adult human cortex. We next performed co-expression analysis [21; 22] on *COL18A1* and found that *COL18A1* is positively co-expressed with genes known to cause cortical malformation syndromes such as *DOCK6* (responsible for Adams-Oliver syndrome 2), *LAMC3* (responsible for occipital cortical malformations), and *COL6A3* (responsible for Bethlem myopathy) (Supplementary Table 5).

Discussion

COL18A1 and Knobloch Syndrome

COL18A1 is located on the long arm of chromosome 21 (chr21q22.3) and is comprised of 43 exons. It encodes the collagen XVIII protein which has been shown to be an important component of basement membranes. [23] *COL18A1* has at least three distinct isoforms of different lengths; these isoforms arise through the use of at least two promoters and alternative splicing in the third exon. [24; 25 b] Although *COL18A1* is ubiquitously expressed, its isoforms have different tissue and developmental distribution. [7] In addition to these three isoforms, *COL18A1* can produce endostatin via proteolytic cleavage. Endostatin is a signaling molecule known to inhibit the migration and proliferation of endothelial cells and is capable of suppressing angiogenesis. [26] Genetic studies have identified exons 30 through 42 of *COL18A1* as being the most frequently mutated in Knobloch syndrome patients; however, there remains much heterogeneity in mutation site distribution. [4] Recently, germline compound heterozygous mutations were also described in patients with Knobloch syndrome. [8]

In our cohort of four patients, who presented with brain malformations, we identified two novel homozygous deleterious mutations and found one mutation that was previously reported[14]—all mutations were located in either the alpha-helix or the endostatin domains of *COL18A1* (Fig 1).

Aside from one patient (NG1348-1) who possesses a non-conserved missense mutation in *PCNT*, none of the four individuals described in this study possessed any other known disease causing mutations that may have been responsible for their phenotypes. Patient NG1348-1 displayed none of the characteristic phenotypical findings associated with *PCNT* mutations (stereotyped facial dysmorphisms, microcephaly, long-bone/axial skeleton aberrations, high-pitched voice, microdontia, hyperinsulinism, pigmentation abnormalities, etc.). [27] While the mutation in *PCNT* may be contributing to the phenotype, the clinical presentation of this patient is much more consistent with a Knobloch syndrome diagnosis and suggests that it is the mutation in *COL18A1* that is responsible for the observed brain malformations.

Clinical Features of Knobloch Syndrome

Ocular and occipital skull defects are the two hallmark features of Knobloch syndrome. Ocular defects often appear before one year of age and include progressive high myopia, lens subluxation, vitreoretinal degeneration, retinal detachment, and cataracts. [1; 2; 4] All four patients in our cohort were found to have ocular abnormalities consistent with the Knobloch syndrome phenotype.

Occipital skull defects range from encephaloceles to purely bone lesions to scalp abnormalities such as cutis aplasia.[1, 2 and 4] Studies have found that there is no correlation between the size or the severity of occipital abnormality and the site of the mutation in *COL18A1*; our findings are consistent with these data. Further, variability in the size of the occipital alteration is commonly observed in both intrafamilial and interfamilial cases, and sometimes, it is noticed only through computed tomographic scan. [2 and 7] Interestingly, only

one of the four patients in our cohort was evident to have an occipital defect characteristic of the Knobloch syndrome phenotype.

Although both early-onset high myopia and the presence of an occipital skull defect are the two hallmarks clinical features of patients with Knobloch syndrome, a spectrum of phenotypic expressions clearly exists. Due to the variable presentation of occipital defects, we can assume that a certain percentage of patients who harbor mutations in *COL18A1* will present without any occipital skull abnormalities.

This may explain the lack of occipital skull phenotype in our patients. Further, a number of sporadic Knobloch syndrome patients who present with only ocular defects may go undiagnosed. [9; 28; 29] Given the consanguinity, a second disorder might also be possible though this should have been evident on exome sequencing with homozygous changes.

A recent study by Khan et al., 2012 also demonstrated that ophthalmic findings are sufficient to accurately diagnose to Knobloch Syndrome. [3] Therefore, an experienced ophthalmologist is capable of properly diagnosing patients with this condition. This highlights the importance of the ocular phenotype and offers further evidence of the phenotypic heterogeneity of this disorder. Interestingly, all of our cases have cognitive decline and/or developmental delay, neither of which is common in Knobloch syndrome patients (Table 2). One possible explanation is previous studies have documented the high rates of concomitant developmental and neurological disability that are associated with structural brain disorders. [30] Severity and types of behavioral and cognitive outcomes of the structural brain disorders seem to depending on the location of the cortical abnormality. [31] However we believe that generally a precise diagnostic prediction of symptoms associated with specific anomalies has not been possible, and this issue requires further studies.

We observed additional aberrations in our patients including atrial septal defects, seizures, and minor dysmorphic findings. These findings highlight the wide phenotypic spectrum that Knobloch syndrome can encompass and also further illustrates the importance of type XVIII collagen in the normal development of multiple organ systems.

Brain Malformations in Knobloch Syndrome

To date, only four case series describing a total of seven patients reported brain malformations associated with Knobloch syndrome (Table 1). [3; 9; 10; 14] As with other phenotypic manifestations of Knobloch syndrome, it appears that structural brain abnormalities due to mutations in *COL18A1* have marked heterogeneity. An additional possibility is that, due to methodological limitations, we are failing to detect the other causative variants driving the brain malformation phenotype. Given our findings, we remain confident that mutations in *COL18A1* can result in a brain malformation phenotype. The mutations we identified were located in either the alpha-helical or the endostatin domains. It appears that mutations in either of these domains are sufficient to result in a brain malformation phenotype.

Possible Role of *COL18A1* in Human Neurodevelopment

Using immunohistochemical and expression analyses we have demonstrated *COL18A1* expression in the pia and blood vessels of the developing human cerebral cortex. In *C. elegans*, inactivation of collagen XVIII results in improper neuronal cell migration. [30]

These findings may suggest a potential role for *COL18A1* in human neurodevelopment. Furthermore, aberrations in neuronal migration that result from *COL18A1* mutations may contribute to the pathogenesis and phenotypes observed in these patients.

Conclusions

In summary, we describe a cohort of four patients from four consanguineous families who demonstrated phenotypic characteristics consistent with Knobloch syndrome and who were found to possess mutations in *COL18A1* via whole-exome and Sanger sequencing. All four of these patients presented with various structural brain malformations aside from encephaloceles, an unusual finding for Knobloch syndrome. This study contributes to the reports demonstrating significant clinical variability in Knobloch syndrome while simultaneously highlighting the importance and persistence of the ocular phenotype. Further, our data illustrates the intra- and interfamilial phenotypic heterogeneity that can result from mutations in *COL18A1* – our genetic analysis and review of previously reported cases demonstrated no specific *COL18A1* isoform or mutation effect on the resulting phenotype. This report contributes to a better characterization of the brain malformations associated with deficiency of collagen XVIII, further elucidates potential Knobloch syndrome phenotypes, and suggests a role for *COL18A1* in cortical development. Finally, we propose that patients diagnosed with Knobloch syndrome and found to have *COL18A1* mutations should be investigated for potential CNS lesions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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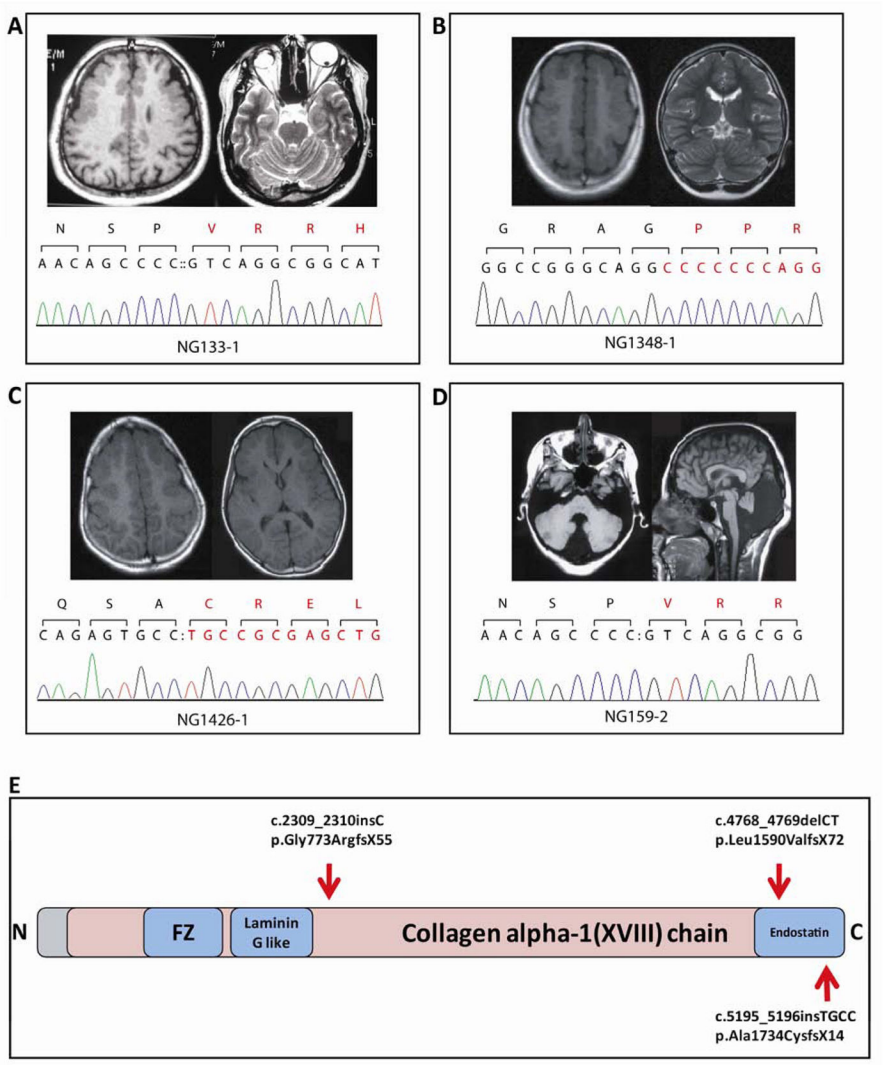

Figure 1.

MRI findings for our 4 patients and schematic representation of the *COL18A1* gene and the mutation locations were given. For each mutation, representative chromatographs obtained via Sanger sequencing analysis of *COL18A1* patients.

A) Panels left and right – Axial T1- and T2-weighted images respectively, of patient NG133-1 demonstrating polymicrogyria of the bilateral frontal lobes and evidence of vitreo-retinal degeneration in the left eye globe with phthisis bulbi in the right eye globe, respectively.

B) Panels left and right – Axial T1-weighted and coronal T2-weighted images respectively, of patient NG1348-1 demonstrating polymicrogyria involving the bilateral frontal and parietal lobes.

C) Panels left and right – Axial T1-weighted images of patient NG1426-1 indicating polymicrogyria in the bilateral frontal lobes.

D) Panels left and right – Axial and sagittal T1-weighted images respectively, of NG159-2 illustrating a Dandy-Walker Malformation (cerebellar vermian atrophy). There is also an

occipital bony defect present without any visible herniation of the meninges or brain parenchyma.

E) Schematic representation of the *COL18A1* gene and the mutations identified in the described patients.

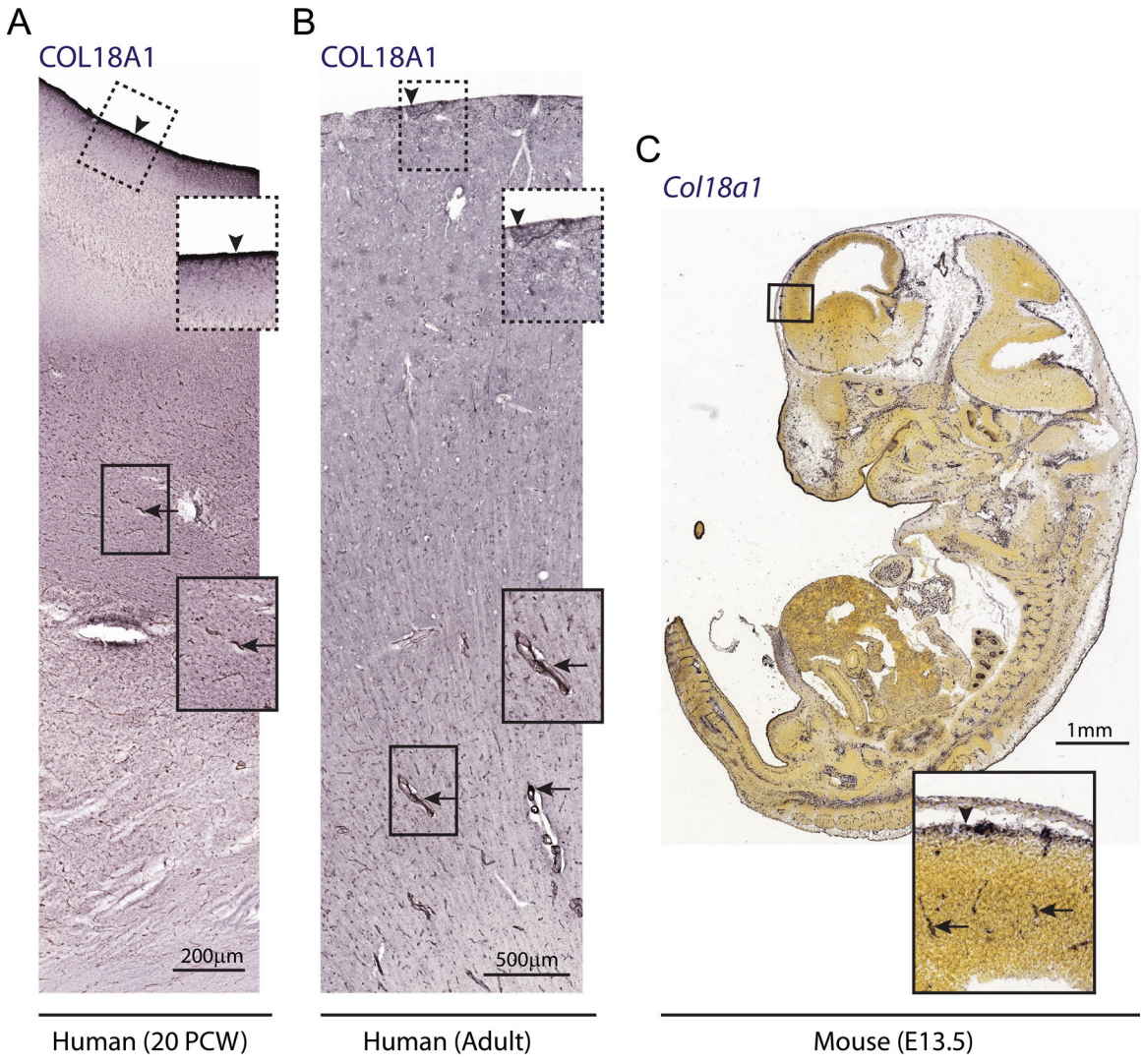

Figure 2.

In the mid-fetal (A) and adult (B) human cerebral cortex, *COL18A1* protein is expressed in the pia (arrow heads) as well as the blood vessels (arrows). In the mouse embryo (C), mouse Col1Ba1 mRNA is also highly expressed in the pia and blood vessels within the cerebral cortex. Mouse images were obtained from the publicly available Allen Developing Mouse Brain Atlas (http://developingmouse.brain-map.org/). PCW, postconception weeks; E, embryonic day. Rectangles represent area displayed in magnified panels. (The color version of this figure is available in the online edition.)

Table 1

Summary of the seven previously reported patients with Knobloch syndrome and associated brain malformations.

		Kliemann et al. [2003], Patient 2	Kliemann et al. [2003], Patient 4	Paisán-Ruiz et al. [2009], Patient 1	Paisán-Ruiz et al. [2009], Patient 2	Khan et al. [2012], Patient 1	Keren et al. [2007], Patient 1	Keren et al. [2007], Patient 2
Gender/Age (years)		M/7	F/13	F/47	F/41	M/12	F/5	M/17 weeks gestation
Characteristic Knobloch Syndrome Findings	**High myopia/Severe visual loss**	Yes	Yes	Yes	Yes	Yes	Yes	Not accessed; pregnancy terminated
	Vitreoretinal/Myopic degeneration	Yes	Yes	Yes	Yes	Yes	Yes	Not appreciated
	Lens subluxation	No	No	Yes	Yes	Yes	Yes	No
	Occipital bone defect/Encepahlocele	Yes	Yes	No	No	Yes	Yes	Yes
Neurologic Findings	**Mental status**	Normal	Normal	Developmental delay; cognitive decline	Developmental delay; cognitive decline	Developmental delay	Mild developmental delay	Not accessed; pregnancy terminated
	Seizures	No	Yes	Yes	Yes	Yes	No	No
MRI Findings		Multiple subepednymal nodules in the lateral ventricles; retrocerebellar arachnoid cyst; ventricular dilation	Pachygyria (right frontal lobe); subependymal nodule (right lateral ventricle); calcification of right parietal lobe; ventricular dilation	Polymicrogyria (bilateral frontal and temporal lobes); marked cerebellar atrophy; brainstem and supratentorial volume loss; slender spinal cord	Polymicrogyria (bilateral frontal and temporal lobes); cerebellar atrophy; brainstem and supratentorial volume loss; slender spinal cord	Heterotropic grey matter in lateral ventricles	Pachygyria and polymicrogyria (bilateral frontal lobes); agenesis of the septum pellucidum; heterotopic hypersignals (on T2 weighted images) along radial migration tracts	Complete agenesis of cerebellar vermis; abnormal formation of cerebellar hemispheres; hamartomatous lesion of the mesencephalic roof
***COL18A1* Mutation**		c.4181G>A	c.4181G>A	c.3514-3515 delCT	c.3514-3515 delCT	c.355delG	c.3544+3A>C	c.3544+3A>C

Table 2

Summary of the clinical findings for each our four patients. M – male; F – female; yo – years old; (R) – right; (L) – left; (BL) – bilateral.

Patient ID			**NG133-1**	**NG1348-1**	**NG1426-1**	**NG159-2**
Gender/Age			M/22yo	M/14yo	F/13yo	F/13yo
Consanguinity	**Patient's parents were**		First cousins	First cousins	First cousins	First cousins
General	**Developmental delay**		No	Yes	Yes	No
	Facial dysmorphisms		No	No	Synorphys	No
Head and Neck	**Microcephaly**		No	No	No	No
	Eyes	**High myopia**	No	No	Yes	No
		Vitreoretinal degeneration	Yes (L)	No	Yes	No
		Retinal detachment (childhood)	No	No	No	Yes (BL)
		Congenital aphakia	No	Yes (BL)	No	No
		Optic discs	Normal	Normal	Atrophic (R)	Normal
		Glaucoma	No	Yes (R)	Yes (R)	No
		Lens subluxation	Yes (L)	Aphakia (BL)	No	
		Vitreous attachment at disc	No	No	Yes (R)	No
		Loss of vision	Yes (L)	Yes (BL)	Yes (R)	Yes (BL)
		Nystagmus	No	No	Yes	No
		Other	Phthisis bulbi (R)	Leukoma (R)	No	No
Skeletal, Hair, and Skin	**Skull**	**Midline occipital bone defect**	No	No	No	Yes
	Hair	**Alopecia at the occipital defect**	No	No	No	No
	Skin	**Occipital dermal sinus tract**	No	No	No	Yes
Neurologic		**Cognitive decline**	Yes	Yes	Yes	Yes
		Cerebellar signs	No	No	Yes	No
		Seizures	Yes	Yes	Yes	No
Cardiovascular System	**Cardiac structural abnormalities**		No	Atrial septal defect	No	No
Genitourinary System	**Renal**	**Duplication of collecting system**	No	No	No	No
MRI Findings			Polymicrogyria	Polymicrogyria	Polymicrogyria	Cerebellar Vermian Atrophy
***COL18A1* Mutation**			c.4768_4769delCT p.Leu1590ValfsX 72	c.2309_2310ins C p.Gly773Argfs X55	c.5195_5196insT GCC	c.4768_4769del CT p.Leu1590Valfs X72

Patient ID	**NG133-1**	**NG1348-1**	**NG1426-1**	**NG159-2**
			p.Ala1734CysfsX14	
***COL18A1* Domain Affected**	Endostatin	Alpha-helix	Endostatin	Endostatin

Table 3

Summary of the mutations identified in *COL18A1* and the resulting protein alterations in each of our four patients. CDS – coding DNA sequence; Chr – chromosome.

Patient ID	Chr	Position Start	Position End	Position in CDS	Mutation	Result	Highest-Impact Protein Change	COL18A1 Domain
NG133-1	21	46930004	46930005	c.4768_4769 delCT	“CT” Deletion	Frame-shift	p.Leu1590Val	Endostatin
NG1348-1	21	46897722	46897722	c.2309insC	“C” Insertion	Frame-shift	p.Gly773 Arg	Alpha-helix
NG1426-1	21	46932246	46932250	c.5199_5202 insTGCC	“TGCC” Insertion	Frame-shift	p.Ala1734Cys	Endostatin
NG159-2	21	46930004	46930005	c.4768_4769 delCT	“CT” Deletion	Frame-shift	p.Leu1590Val	Endostatin