Original Investigation

Molecular and Clinical Findings in Patients With Knobloch Syndrome

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IMPORTANCE Knobloch syndrome is a rare, recessively inherited disorder classically characterized by high myopia, retinal detachment, and occipital encephalocele, but it is now known to have an increasingly variable phenotype. There is a lack of reported electrophysiologic data, and some key clinical features have yet to be described.

OBJECTIVE To expand on current clinical, electrophysiologic, and molecular genetic findings in Knobloch syndrome.

DESIGN, SETTING, AND PARTICIPANTS Twelve patients from 7 families underwent full ophthalmic examination and retinal imaging. Further investigations included electroretinography and neuroradiologic imaging. Bidirectional Sanger sequencing of *COL18A1* was performed with segregation on available relatives. The study was conducted from July 4, 2013, to October 5, 2015. Data analysis was performed from May 20, 2014, to November 3, 2015.

MAIN OUTCOMES AND MEASURES Results of ophthalmic and neuroradiologic assessment and sequence analysis of *COL18A1.*

RESULTS Of the 12 patients (6 males; mean age at last review, 16 years [range, 2-38 years]), all had high myopia in at least 1 eye and severely reduced vision. A sibling pair had unilateral high myopia in their right eyes and near emmetropia in their left eyes from infancy. Anterior segment abnormalities included absent iris crypts, iris transillumination, lens subluxation, and cataract. Two patients with iris transillumination had glaucoma. Fundus characteristics included abnormal collapsed vitreous, macular atrophy, and a tesselated fundus. Five patients had previous retinal detachment. Electroretinography revealed a cone-rod pattern of dysfunction in 8 patients, was severely reduced or undetectable in 2 patients, and demonstrated cone-rod dysfunction in 1 eye with undetectable responses in the other eye in 2 patients. Radiologic imaging demonstrated occipital encephalocele or meningocele in 3 patients, occipital skull defects in 4 patients, minor occipital changes in 2 patients, and no abnormalities in 2 patients. Cutaneous scalp changes were present in 5 patients. Systemic associations were identified in 8 patients, including learning difficulties, epilepsy, and congenital renal abnormalities. Biallelic mutations including 2 likely novel mutations in *COL18A1*, were identified in 6 families that were consistent with autosomal recessive inheritance with a single mutation identified in a family with 2 affected children.

CONCLUSIONS AND RELEVANCE This report describes new features in patients with Knobloch syndrome, including pigment dispersion syndrome and glaucoma as well as cone-rod dysfunction on electroretinography. Two patients had normal neuroradiologic findings, emphasizing that some affected individuals have isolated ocular disease. Awareness of the ocular phenotype may aid early diagnosis, appropriate genetic counseling, and monitoring for potential complications.

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Knobloch syndrome is a rare, recessively inherited disorder first described in 1971 in a family affected by vitreoretinal degeneration, retinal detachment, high myopia, occipital encephalocele, and lens subluxation.[1] Additional ocular features have since been identified, including cataract, smooth irides, and persistent fetal vasculature.[2-4] Limited electrophysiologic characterization has been reported[5,6] in 3 patients. Neuroradiologic imaging has revealed a variety of developmental brain anomalies including occipital skull defects with or without encephalocele, polymicrogyria, subependymal nodules, and cerebellar vermis atrophy.[5,7-10] Additional systemic findings include epilepsy, developmental delay, and renal abnormalities.[2-4,7,10-14]

The Knobloch locus was mapped to 21q22.3 in 1996 and *COL18A1* was subsequently identified as the causative gene.[15,16] The *COL18A1* gene encodes the collagen α-1(XVIII) chain, ubiquitously expressed in vascular and epithelial basement membranes.[17,18] Collagen XVIII has multiple functions in ocular and neurologic development, including angiogenesis, basement membrane maintenance, and in the Wnt/β-catenin signaling pathway.[17] The present report describes the detailed phenotypes and molecular genetic findings in 12 affected individuals of 7 families with Knobloch syndrome.

Key Points

Question What potentially novel clinical and molecular features are identified from an investigation of 12 patients with Knobloch syndrome?

Findings In this case series, the features identified included pigment dispersion syndrome and glaucoma, cone-rod dysfunction on electroretinography, and 2 mutations that, to our knowledge, have not been previously reported. All patients had severe visual impairment, myopia, and macular atrophy; in addition, 5 individuals had retinal detachment and 2 had normal findings of neuroradiologic imaging.

Meaning Awareness of the clinical features of Knobloch syndrome might facilitate diagnosis and monitoring for potential complications.

Methods

Families in which 1 or more member had clinical features of Knobloch syndrome were identified from the inherited retinal clinics at Moorfields Eye Hospital and the Oregon Health & Science University Casey Eye Institute. Each affected individual underwent a full clinical examination including visual acuity and dilated fundus examination. Retinal imaging was obtained by 35° (Topcon Great Britain Ltd), or 60° color fundus photography (Canon USA Inc), ultra-widefield confocal scanning laser imaging (Optos plc), RetCam imaging (Clarity Medical Systems Inc), 30° or 55° fundus autofluorescence imaging (Spectralis, Heidelberg Engineering Ltd), and spectral domain optical coherence tomography scans (Spectralis). Full field electroretinography (ERG) was performed in older children and adults to incorporate the International Society for Clinical Electrophysiology of Vision standards using gold foil electrodes (Moorfields Eye Hospital) or Burian Allen electrodes (Oregon Health & Science University); recording in infants and young children was performed with surface electrodes as previously described.[19-23] Pattern ERG was performed in the patients from Moorfields Eye Hospital.[24] Eleven patients underwent radiologic imaging of the brain, either by magnetic resonance imaging or computed tomography.

The study protocol adhered to the tenets of the Declaration of Helsinki[25] and was approved by the Research Management Committee at Moorfields Eye Hospital, London, England, and the institutional review board at Oregon Health & Science University, Portland. Written informed consent was obtained from all participants prior to study inclusion, with parental written consent provided on behalf of the children involved. There was no financial compensation.

The study was conducted from July 4, 2013, to October 5, 2015. Data analysis was performed from May 20, 2014, to November 3, 2015.

Molecular Biology

Bidirectional Sanger sequencing of all 41 exons and intron-exon boundaries of the medium isoform of *COL18A1* (GenBank NM_030582.3) was performed in an affected proband from families 1 to 4 and segregation confirmed in the affected sibling and available relatives. The DNA was amplified using specifically designed primers by polymerase chain reaction, and the resulting fragments were sequenced using standard protocols (eTable 1 in the Supplement). Patients 1.1 and 5 had previously undergone arrayed primer extension microarray (Asper Biotech Ltd), performed using a genotyping microarray containing more than 700 disease-causing variants for 28 retinal dystrophy genes.[26]

Patients 5, 6.1, 6.2, and 7 underwent whole-exome sequencing (WES) with identified variants then confirmed by bidirectional Sanger sequencing in the affected probands and available relatives. A diagnosis of Knobloch syndrome was clinically suspected in patients 6.1, 6.2, and 7 prior to WES but not in patient 5. For patient 5, WES was performed at AROS Applied Biotechnology using a solution-phase Agilent SureSelect 38 Mb exome capture (SureSelect Human All Exon Kit, Agilent Technologies Inc) and the Illumina HiSeq 2000 sequencer (Illumina Inc). Reads were aligned to the hg19 human reference sequence using Novoalign (Novocraft), version 2.05. The ANNOVAR tool (OpenBioinformatics.org) was used to annotate single-nucleotide polymorphisms and small insertions/deletions. Patients 6.1, 6.2, and 7 underwent WES at Baylor College of Medicine using NimbleGenSeqCap EZ hybridization and wash kit (NimblegenSeqCap EZ human exome library, version 2.0) according to the manufacturer's instructions. Illumina HiSequation 2000 (Illumina) was used to sequence captured libraries. Bioinformatics analysis was performed as described previously.[27] Further filtering of variants was performed based on a frequency higher than 0.5% in a series of public and internal databases, including Exome Aggregation Consortium (ExAC).

Table. Key Clinical Features of Affected Patients

Patient No./Sex/ Ethnic Origin/ Genetic Database No.	Age at Last Review, y	Visual Acuity, logMAR (Snellen equivalent)	Refraction, Under Cycloplegia[a]	Anterior Segment Features	Posterior Segment Features	Neurologic and/or Systemic Features
1.1/M/Indian/GC14449	23	R NPL, L CF	R −17.50 DS, L +1.0 DS (atropine 1998)	Absent iris crypts, poor pupillary dilatation	Retinal detachment, R paracentral, circumscribed CR atrophy, L generalized retinal and RPE atrophy	Subgaleal fat pad, polymicrogyria; epilepsy, developmental delay
1.2/F	21	R 1.3 (20/400), L PL	R −13.5/−1.00 × 180, L +0.75/−1.00 × 180 (subjective 2002)	Absent iris crypts, persistent pupillary membrane, poor pupillary dilatation	R paracentral, circumscribed CR atrophy, L generalized retinal and RPE atrophy	Midline occipital defect, atretic encephalocele, polymicrogyria; none
2.1/M/British, white GC19526	14	R 1.9 (20/1600), L NPL	R −18.00/−2.00 × 15, L +9.00 DS	Persistent pupillary membrane, poor pupillary dilatation, iris transillumination	Retinal detachment, generalized, ill-defined macular CR atrophy BE	Normal; hypermobile joints
2.2/M	11	R 1.6 (20/800), L 1.4 (20/500)	R −24.00/−2.00 × 10L +3.00/−1.50 × 15	Persistent pupillary membrane, poor pupillary dilatation, iris transillumination, lens subluxation	Paracentral well-defined CR atrophy BE	Resected occipital encephalocele, polymicrogyria; hypermobile joints
3.1/M/Slovak/GC20422	17	R 1.6 (20/800), L 1.4 (20/500)	R +12.00/−2.00 × 100, L −10.00/−6.00, 0 × 110 (subjective 2014)	Absent iris crypts, poor pupillary dilatation, cortical lens opacity, lens subluxation	Paracentral ill defined retinal and RPE atrophy BE	Midline occipital defect; learning difficulties
3.2/F	11	R 1.2 (20/320), L 1.4 (20/500)	R −20.00/−2.00 × 100, L −19.00/−2.00 × 180	Absent iris crypts, poor pupillary dilatation, cortical lens opacity, lens subluxation	Paracentral CR atrophy, well defined on R, ill-defined on L	Occipital lobe corticated channel; none
4.1/F/Arab/GC20693	4	R 2.1 (<20/2000), L 2.1 (<20/2000)	R −12.00/−2.00 × 10, L −12.50 DS	None	Central, ill-defined CR atrophy BE	Midline occipital defect; congenital hydronephrosis, hypermobile joints
4.2/M	2	R PL, L NPL	Not performed	Cortical lens opacity	Retinal detachment, central, ill-defined CR atrophy BE	Midline occipital defect; hypermobile joints
5/F/British, white/GC18840	38	R 1.06 (20/250), L 1.06 (20/250)	R −35 DS, L −28 DS (before lens removal)	Poor pupillary dilatation, iris transillumination, cortical lens opacity, lens subluxation	Paracentral, well defined, deep large CR atrophy on R, smaller and ill-defined on L	Normal; unilateral duplex kidney/bifid ureter, hamstring sarcoma
6.1/F/Northern European	19	R CF, L CF	R −13.00 DS, L −14.00 DS (subjective 2006)	Iris transillumination, cortical lens opacity	Central ill-defined CR atrophy BE	Resected occipital meningocele; vertigo
6.2/M	24	R 1.4 (20/500), L CF	R −10.00 DS, L −10.00 DS (subjective 2008)	Poor pupillary dilatation, iris transillumination cortical lens opacity	Retinal detachment, paracentral, well-defined CR atrophy BE	None known; occipital cutaneous alopecia
7/F/African	4	R 1.6 (20/800), L 1.5 (20/600)	R −1.50/+1.00 × 87, L −0.50/+1.25 × 89 (subjective 2011)	Poor pupillary dilatation	Retinal detachment, R paracentral, well-defined CR atrophy, L detached with macular hole	Resected occipital encephalocele; none

Abbreviations: BE, both eyes; CR, chorioretinal; CF, counting fingers; DS, diopter sphere; L, left; NPL, no perception of light; PL, perception of light R, right; RPE, retinal pigment epithelium.

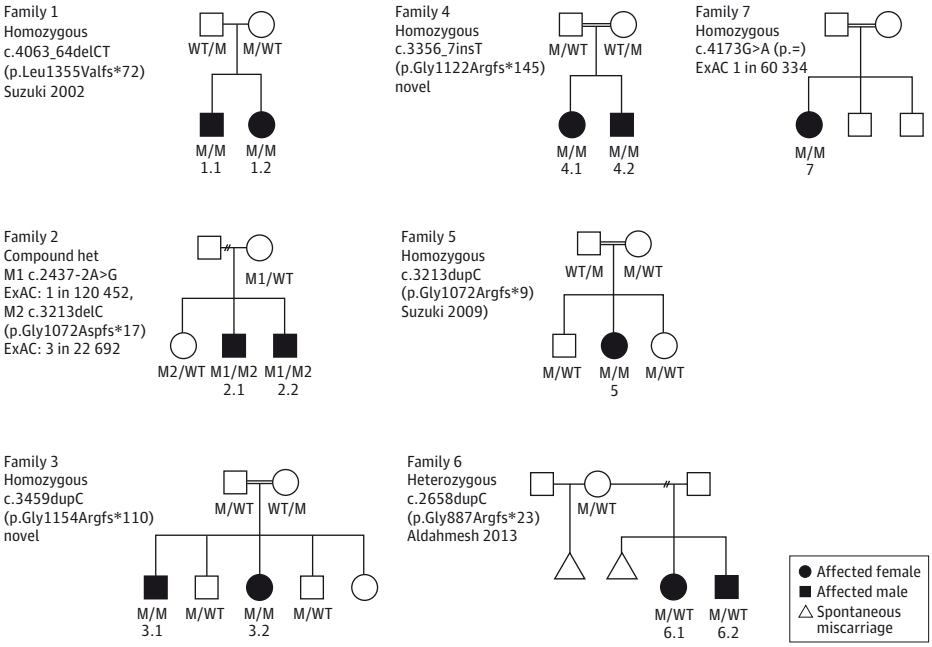
[a] Parenthetical information indicates the use of a different refraction method.

Mutation nomenclature was assigned in accordance with GenBank accession number NM_030582.3, with nucleotide position 1 corresponding to the A of the ATG initiation codon. Variants were identified as novel if not previously reported in the literature and if absent from dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), NHLBI GO Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), 1000 Genomes Project (http://www.1000genomes.org/), and ExAC (http://exac.broadinstitute.org); all sites were accessed December 29, 2015.[28] These databases were also interrogated using the mutations transcribed for the alternate short isoform also reported in the literature (GenBank NM_130445.2). Variants likely to affect function were assessed for segregation in available family members. Potential splice variants were assessed using Splice Site Prediction by Neural Network (http://www.fruitfly.org/seq_tools/splice.html) and Human Splicing Finder (http://www.umd.be/HSF3/).[29,30]

Results

Key clinical features are summarized in the **Table**. Twelve patients from 7 families (**Figure 1**) were evaluated; their ages at the last review ranged from 2 to 38 years (mean [SD], 16 [9.9]; median, 15.5 years). All patients had presented in infancy with nystagmus and variable convergent or divergent squints except for patient 3.2, who was orthophoric. All patients had severe visual impairment with best-corrected visual acuity in

Figure 1. Pedigrees of Families Affected by Knobloch Syndrome With Segregation Analysis and First Report of the Mutation



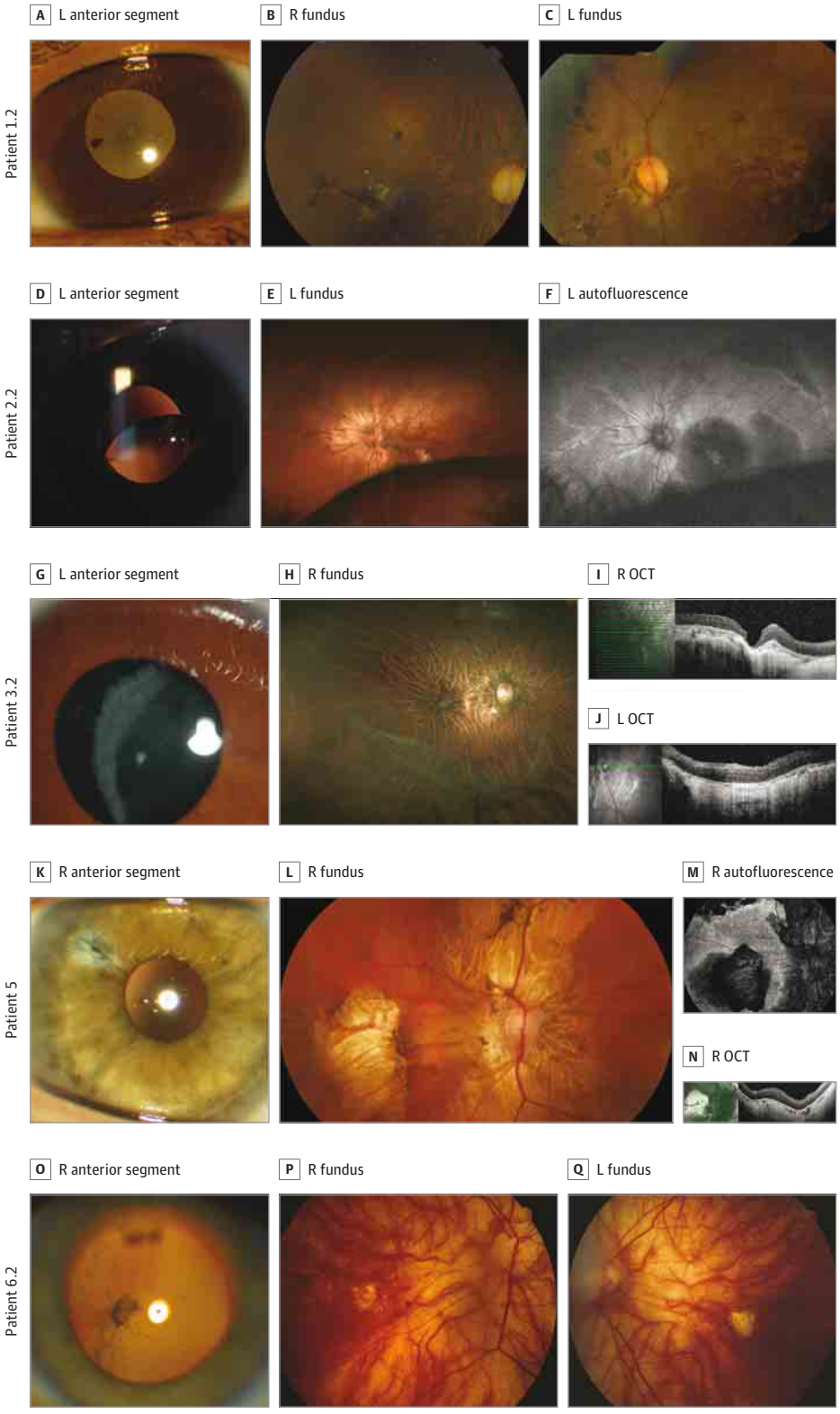
M indicates mutation; WT, wild-type.

each eye at last review ranging from 1.06 logMAR (Snellen equivalent 20/250) to no perception of light. Patient 1.2 had stable vision of 1.3 logMAR (Snellen equivalent 20/400) OD from the first review at age 9 years to the last review at age 21 years; patient 5 also had stable vision of 1.06 logMAR (Snellen equivalent 20/250) in each eye over a 36-year review period. Patient 6.2 had stable vision of 1.4 logMAR (Snellen equivalent 20/500) OD over an 8-year review, with the left eye developing a chorioretinal scar after detachment surgery that reduced his vision. All patients had high myopia (−6 diopters or more) in at least 1 eye, with high myopia reported in patients 4.2 and 5 before cataract extraction. Five patients were anisometropic with high myopia in 1 eye and hyperopia in the other. This anisometropia was related to lens subluxation in 2 patients (2.2 and 3.1), unilateral retinal detachment with hyperopic shift in 1 patient (2.1), and unilateral high myopia in the right eye with near emmetropia in the left eye from infancy in 2 siblings unrelated to retinal detachment (1.1 and 1.2). These siblings had asymmetrical axial lengths as measured by B-scan ultrasonography at age 8 years for patient 1.1 and 19 years for patient 1.2 when the length of the myopic eyes were 26.7 and 27.9 mm and that of the emmetropic eyes were 20.7 and 21.1 mm, respectively. These siblings were previously reported[31] when children; new data in this report, 15 years later, include visual acuity, additional ERG tests, anterior segment and fundus features, and neuroradiologic findings.

Anterior segment abnormalities were present in all participants except patient 4.1, who was examined at age 4 years. Abnormalities included poor pupillary dilatation (8 patients), absence of crypts associated with a featureless iris (5 patients), iris transillumination (5 patients), and persistent pupillary membrane (3 patients) (**Figure 2**). Seven patients had cataracts with 2 requiring cataract extraction. Three patients had lens subluxation in the inferotemporal direction with patient 5 reported to have lens subluxation prior to cataract extraction. All 5 patients of Northern European origin (families 2, 5, and 6), had iris transillumination with all but patient 6.1 developing raised intraocular pressure. Glaucomatous disc cupping was identified in 1 eye of patients 2.1 at age 11 years and 6.2 at 22 years; neither had lens subluxation. Patient 5, in addition to iris transillumination, had endothelial pigment (Krukenberg spindles), pigment on the lens capsule and heavily pigmented angles on gonioscopy, all consistent with a diagnosis of pigment dispersion syndrome. Endothelial pigment was also noted in patient 6.2.

All highly myopic eyes had disc pallor, attenuated vessels, a markedly tessellated appearance with prominent choroidal vessels, peripapillary atrophy, and occasional pigmented spots (Figure 2); 2 eyes had staphylomas. The emmetropic left eyes of patients 1.1 and 1.2 were heavily pigmented. Abnormal vitreous condensations were noted in 9 patients. Macular atrophy was present in 23 of 24 eyes, with the left eye of patient 7 having detached centrally with a macular hole at initial assessment (Table). The atrophy was chorioretinal in most patients, involving the outer retina, retinal pigment epithelium, and choroid. In 13 eyes the atrophy was paracentral, being well circumscribed in 9 of 13 eyes; in 10 eyes it was central and ill defined (Figure 2). In patients without central macular atrophy, poor foveal reflexes were noted. Three patients developed bilateral retinal detachments and 2 patients developed unilateral detachment. Optical coherence tomography findings of the posterior pole was available in 6 patients. All scans showed a lack of foveal pits, extensive loss of outer retinal structure and, in 4 patients, additional atrophy

Figure 2. Anterior Segment and Retinal Imaging in Knobloch Syndrome

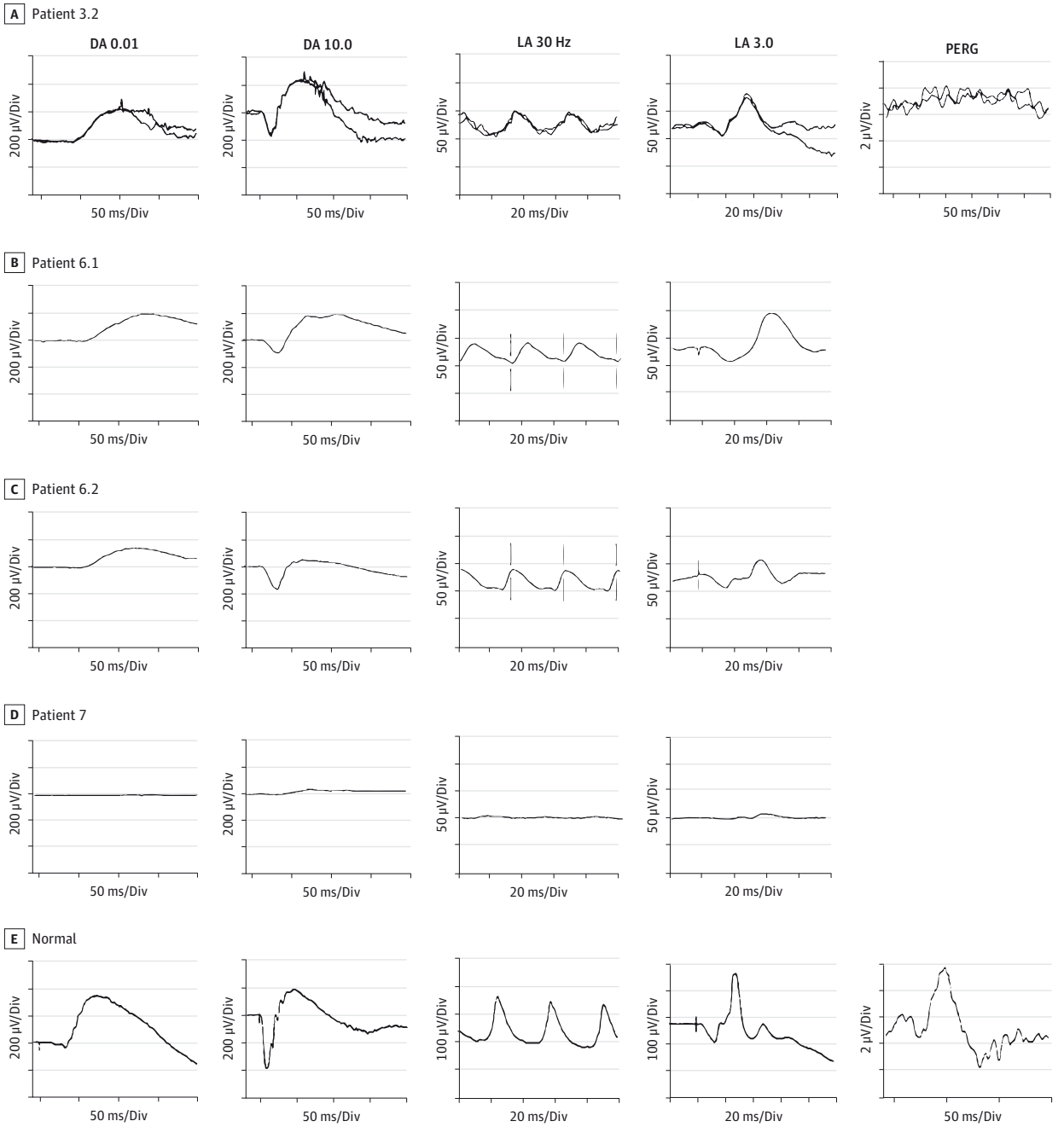


Patient 1.2 at age 21 years: left (L) anterior segment photograph (A), right (B) and left (C) color fundus photographs (Topcon Great Britain Ltd). Patient 2.2 at age 14 years: left anterior segment photograph (D), left Optos wide field color (E), and left Optos autofluorescence imaging (Optos plc) (F). Patient 3.2 at age 11 years: left anterior segment photograph (G), right wide field imaging (Optos plc) (H), and right (I) and left (J) optical coherence tomography (OCT) (Spectralis). Patient 5 at age 38 years: right anterior segment photograph (K), right fundus photograph (Topcon Great Britain Ltd) (L), right autofluorescence imaging (Spectralis) (M), and right OCT (N). Patient 6.2 at age 11 years: right anterior segment photograph (O) to demonstrate vitreous clumping, and right (P) and left (Q) fundus color photographs.

of the retinal pigment epithelium and choroid. Fundus autofluorescence imaging findings, available in 5 patients, demonstrated well-circumscribed loss of posterior pole autofluorescence.

Electroretinography showed cone-rod dysfunction in 18 eyes (10 patients) and severely reduced or undetectable responses in 6 eyes (4 patients) (**Figure 3**). Patients 1.1 and 1.2 at initial testing in 2002 had undetectable ERGs in their

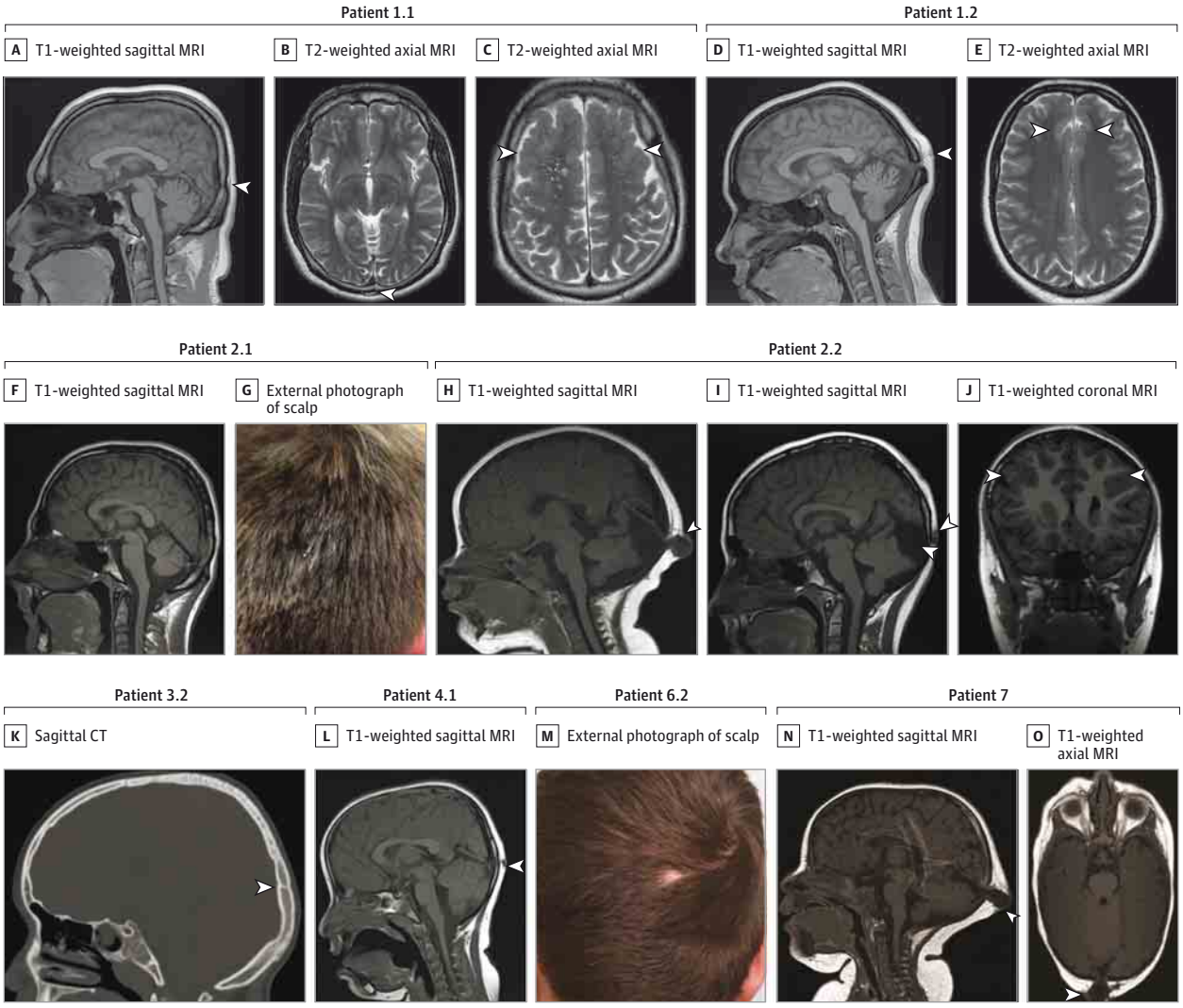
Figure 3. Electroretinogram (ERG) of 1 Eye of Patients 3.2, 6.1, 6.2, and 7



In patient 3.2 (A), rod-specific (dark-adapted [DA] 0.01) ERG is mildly subnormal; bright flash (DA 10.0) a-wave amplitude is subnormal; cone flicker (light-adapted [LA] 30 Hz) and single-flash (LA 3.0) ERGs are markedly subnormal and delayed (note the differences in calibration compared with the normal); pattern ERG (PERG) is undetectable. In patients 6.1 (B) and 6.2 (C), rod-specific and bright flash amplitudes are subnormal, cone flicker and single flash are markedly subnormal and delayed; abnormalities are more severe for patient 6.2, partly related to sedation required for the ERG. Patients 3.2, 6.1, and 6.2 show a cone-rod pattern of dysfunction; patient 7 (D) has severe loss of both rod and cone responses. Normal ERG (E) is shown for comparison. Div indicates division.

emmetropic left eyes with cone dysfunction more than rod dysfunction in their myopic right eyes. Additional ERG in 2014 in patient 1.1 showed undetectable ERGs in both eyes, with the right having developed a total retinal detachment and the left showing a peripheral detachment. Additional ERG in patient 1.2 showed marked deterioration, particularly of cone responses in the right eye, but with stable visual acuity. Electroretinography was undetectable in patient 4.2 who was tested under general anesthesia with silicone oil in both vitreous cavities. Patient 7

Figure 4. Neuroradiologic and Cutaneous Occipital Findings in Knobloch Syndrome



Patient 1.1: subgaleal fat pad (arrowheads) overlying occipital bone (A and B) and extensive bifrontal polymicrogyria (arrowheads) (C) on magnetic resonance imaging (MRI). Patient 1.2: small occipital bone defect (arrowhead) with atretic encephalocele/meningocele (D) and medial bifrontal polymicrogyria (arrowheads) (E) on MRI. Patient 2.1: no abnormalities on MRI (F) and occipital patch of white hair (G). Patient 2.2: aged 6 months, occipital encephalocele (arrowhead) on MRI (H), age 11 years, occipital scarring and retained retrocerebellar arachnoid cyst at site of previous surgery (arrowhead) (I), and age 11 years, bilateral inferior frontal polymicrogyria (arrowhead) (J) on MRI. Patient 3.2: small, well-corticated channel in the midline of the occipital lobe (arrowhead) (K) on computed tomographic scan. Patient 4.1: small bony occipital defect (arrowhead) with meningeal tissue communicating to subcutaneous tissue through the defect (L) on MRI. Patient 6.2: cutaneous alopecia overlying occiput (M). Patient 7: age 4 months, occipital encephalocele (arrowhead) (N and O) on MRI. The MRIs were intracranial sagittal and coronal T1-weighted and axial T2-weighted images.

had severely reduced ERG in both eyes, with retinal detachment in the left eye.

Neuroradiology was performed in 11 patients (**Figure 4**). Imaging results were normal in 2 patients. Four patients had occipital skull defects and 3 had occipital encephalocele or meningocele. Minor abnormalities in 2 patients comprised an occipital subgaleal fat pad and a corticated (covered in cortical bone) small channel in the occipital lobe, possibly representing an atretic encephalocele. Three patients had polymicrogyria. The imaging results differed between siblings in all families except family 4. Cutaneous occipital abnormalities were present in 5 patients, including palpable swelling, alopecia, and a patch of white hair. Systemic abnormalities included learning difficulties, epilepsy, congenital hydronephrosis due to a ureteric abnormality, and duplex kidney with bifid ureters in patient 5, who also had undergone treatment for a hamstring sarcoma.

Biallelic variants in *COL18A1* predicted to be pathogenic were identified in 5 families, with a single heterozygous variant in the sixth family and a homozygous, synonymous change in the seventh family. Segregation was confirmed in available family members (Figure 1). To our knowledge, 2 novel mutations were identified with 3 additional mutations not previously reported in an affected patient but found at a very low

mean allele frequency in the ExAC database (Figure 1). Eight mutations, 6 that create premature termination codons, a splice site mutation, and a synonymous variant were identified. The splice site mutation, c.2437-2 A>G, which is very rare on ExAC (1 in 120 452), disrupts the canonical acceptor site for exon 17. The heterozygous variant identified in family 6, c.2658dupC (p.Gly887Argfs*23), has been previously reported.[32] The synonymous variant, c.4173G>A, was found homozygously in patient 7 and is very rare on ExAC (1 in 60 334 alleles). The in silico splice predictions for this variant were equivocal; the Human Splicing Finder algorithm suggesting that an exonic cryptic acceptor site may have been created was not replicated by other algorithms (Splice Site Prediction by Neural Network). Whole-exome sequencing in patients 6.1, 6.2, and 7 identified no other potentially pathogenic variant in *COL18A1* or any other known retinal dystrophy genes.

Discussion

The classic description of Knobloch syndrome includes myopia, retinal detachment, and occipital encephalocele, but more recent publications[5,10,32] have described an increasingly variable ocular and systemic phenotype. The present report, with detailed retinal imaging, ERG, and neuroradiology in a large series, has allowed a detailed assessment of the clinical phenotype. To our knowledge, this is the first report of the electrophysiologic finding of cone-rod dysfunction and first description of childhood-onset glaucoma associated with iris transillumination.

All patients presented in infancy with nystagmus and had high myopia in at least 1 eye. Three patients had inferotemporal lens subluxation consistent with a previous report.[5] Iris abnormalities were also common. Absence of iris crypts and a single case of iris atrophy have been described previously.[3-5,32] In a knockout mouse model *COL18a1−/−*, there was disruption of the posterior iris pigment epithelial cell layer and release of melanin granules that resembled the human pigment dispersion syndrome.[33] Two patients in our series with clinical features of pigment dispersion syndrome had associated glaucoma.[34] This association suggests an increased risk of pigmentary glaucoma in white patients with Knobloch syndrome.

Many of the retinal changes noted in this series are consistent with high myopia and are not specific to the syndrome.[35] These changes include peripapillary atrophy and the tessellated fundus appearance with prominent choroidal vessels. Although vitreous abnormalities are seen in high myopia, the collapsed abnormal vitreous present in our patients from a young age may relate to the underlying disorder. Macular atrophy was identified in all patients. This abnormality can also occur in high myopia with diffuse atrophy or focal areas of atrophy; the latter was shown to develop in the fifth decade in a large natural history study.[36] The young age of the patients in the present study suggests that the atrophic lesions are likely to be a consequence of mutations in *COL18A1*. Macular atrophic lesions and abnormal vitreous condensations have been previously reported[5,7,32] and may be key features of the disorder.

Previous electrophysiology reports on Knobloch syndrome are limited; delayed and depressed photopic and scotopic ERGs were observed in 2 children in 1 report,[5] but few details on technique or amplitude of responses were given, and a severely diminished ERG was described in 1 patient in another report.[6] The present series with detailed electrophysiologic testing of all affected patients demonstrates both cone and rod dysfunction. Additional ERG in patient 1.2 showed deterioration but stable visual acuity. It therefore remains unclear whether Knobloch syndrome represents a progressive dystrophy of photoreceptors or a stable dysfunction; however, progression, if present, appears to be asymptomatic and slow. In our patients with long-term follow-up, there was little deterioration in visual acuity unless complicated by retinal detachment.

COL18A1 encodes the collagen α-1(XVIII) chain, which is highly expressed throughout the human eye including the iris, ciliary body, trabecular meshwork, Schlemm canal, the inner limiting membrane, retinal vessels, basement membrane of the retinal pigment epithelium, and Bruch membrane, but not in photoreceptors.[18] The inner limiting membrane and vitreous body are important regulators of eye size in a chick embryo model with disruption of these structures leading to eye enlargement. This finding could explain the high myopia seen in Knobloch syndrome as evidenced by the high axial length measured in the myopic eyes of family 1.[37] In mice, lack of type XVIII collagen causes abnormal vitreous separation, consistent with the abnormal vitreous and retinal detachment found in human disease.[38] The underlying pathogenesis of photoreceptor dysfunction is not clear from animal models but the abnormal Bruch's membrane, retinal pigment epithelium, and inner limiting membrane would be predicted to have secondary effects on the photoreceptors.[38] Alternatively the distribution and function of type XVIII collagen may differ in the human eye.

Occipital encephalocele or meningocele is reported[39] to be common in Knobloch syndrome but was identified in only 3 patients in the present series. Normal neuroimaging has previously been reported[7,12] in 2 patients; to our knowledge, the additional minor abnormality of a subgaleal fat pad is a new observation. Five patients had externally observable occipital findings, ranging from soft-tissue swellings to hair abnormalities, emphasizing the importance of occipital scalp examination if Knobloch syndrome is suspected.[7,10,13,30] In 1 patient, a cutaneous scalp abnormality was identified in the absence of neuroradiologic abnormality. This abnormality has been previously reported in a single patient.[12] Polymicrogyria was identified on magnetic resonance imaging in 3 patients (1.1, 1.2, and 2.2); this feature has now been reported in several patients with Knobloch syndrome.[3,8-10]

Type 18 collagen is found in many different tissues and it is unsurprising that mutations in *COL18A1* may result in a varied systemic phenotype. Systemic associations in the present series include epilepsy, learning difficulties, congenital hydronephrosis, and unilateral duplex kidney with bifid ureter. There have been several reports[5,7,10,14,32,40] of epilepsy in patients with Knobloch syndrome. Renal abnormalities in

Knobloch syndrome are unusual, with 2 previous reports[2,13] of congenital duplex kidney and bifid ureter. Sarcoma, present in 1 patient, has not been previously reported in Knobloch syndrome, although there has been 1 case of acute lymphoblastic leukemia.[41] Those reports and the present series highlight the importance of systemic assessment.

The *COL18A1* gene consists of 43 exons with 3 main alternate isoforms produced.[17,42] There have been 22 previously reported likely pathogenic mutations in *COL18A1* leading to recessively inherited disease, 17 of which create premature termination codons, 2 large deletions encompassing at least a whole exon, and 3 splice site mutations, which may indicate that this syndrome represents a null phenotype (eTable 2 in the Supplement). The c.4063_4064delCT mutation is the most common, found in 14 families to date.[9,10,14,32,40,41,43,44] The diverse ethnic origins of the reported families include Indian, Brazilian, North American, Saudi, Irish, Pakistani, and Turkish in keeping with a mutational hotspot—not a founder effect. A further 5 disease-causing mutations were identified in the present series. A single mutation was identified in family 6 in this report; there may be a large structural variant, such as an exonic deletion on the second allele, that is not identifiable by direct sequencing. The synonymous variant found in patient 7 may affect splicing, but further confirmatory functional studies are needed.

Conclusions

Knobloch syndrome is a systemic disorder with variable neurologic involvement and severe visual impairment from early childhood. The syndrome may be undiagnosed without careful examination of the anterior segment and awareness of the potential lack of scalp and/or intracranial occipital abnormalities. The diagnosis might be considered in any patient with infantile-onset high myopia, developmental abnormalities of the anterior segment, and evidence of cone-rod dysfunction on ERG. A timely diagnosis not only ensures that patients are aware of the potential complications of the disorder, such as lens subluxation, retinal detachment, and glaucoma, but may facilitate targeted molecular sequencing and informed genetic counseling.

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