Published in final edited form as:

Am J Med Genet A. 2010 November ; 152A(11): 2875–2879. doi:10.1002/ajmg.a.33621.

Collagen XVIII Mutation in Knobloch Syndrome with Acute Lymphoblastic Leukemia

Vinit B. Mahajan, MD, PhD^{1,2}, Ann Haskins Olney, MD³, Penny Garrett⁴, Ajit Chary⁵, Ecaterina Dragan⁶, Gary Lerner, MD⁷, Jeffrey Murray, MD⁶, and Alexander G. Bassuk, MD, PhD⁶

¹ Department of Ophthalmology and Visual Sciences, The University of Iowa Hospitals & Clinics, Iowa City, IA

² Omics Laboratory, The University of Iowa Hospitals & Clinics, Iowa City, IA

³ Munroe-Meyer Institute, University of Nebraska Medical Center, Omaha NE

⁴ Iowa Education Agency

⁵ Department of Pediatrics, Northwestern University, Chicago IL

⁶ Departments of Pediatrics, University of Iowa, Iowa City IA

⁷ Children's Hospital, Omaha NE

Abstract

Knobloch syndrome (KNO) is caused by mutations in the collagen XIII gene (*COL18A1*) and patients develop encephalocele and vitreoretinal degeneration. Here we report an El Salvadorian family where two sisters showed features of KNO. One of the siblings also developed acute lymphoblastic leukemia. DNA sequencing of *COL18A1* revealed a homozygous, 2-base pair deletion (c3514-3515delCT) in exon 41, which leads to abnormal collagen XVIII and deficiency of its proteolytic cleavage product endostatin. KNO patients with mutations in *COL18A1* may be at risk for endostatin-related conditions including malignancy.

INTRODUCTION

Knobloch syndrome (KNO) is characterized by occipital scalp defects or encephalocele and ophthalmic findings that include high myopia, vitreoretinal degeneration, and retinal detachment. (Capstick 1975; Knobloch and Layer 1971; Seaver, et al. 1993; Sniderman, et al. 2000) Intellect is typically normal, with only a few reports of CNS anomalies and developmental delay. Knobloch syndrome is caused by DNA mutations in the collagen 18A gene (*COL18A1*, KNO1, chromosome 21q22.3), (Kliemann, et al. 2003; Sertie, et al. 1996) and has been found in families from Brazil, North America, Europe, India, and Algeria (Paisan-Ruiz, et al. 2009; Passos-Bueno, et al. 1994). Two other loci, KNO2 (unlinked) and KNO3 (linkage to chromosome 17q11.2) have also been implicated (Khaliq, et al. 2007; Menzel, et al. 2004; Wilson, et al. 1998).

COL18A1 codes for type XVIII collagen, a proteoglycan component of basement membranes including those of the iris, vitreous, and retina. In the eye, collagen XVIII plays

Correspondence: Alexander Bassuk MD, PhD, Department of Pediatrics, The University of Iowa Carver College of Medicine, Iowa City, IA 52242 (alexander-bassuk@uiowa.edu). Phone 319-384-4648. Fax- 319-356-4855.

Financial Disclosure: None.

a key role in ocular development, maintenance of visual function, and neuronal migration. Collagen XVIII is also cleaved near its C-terminus to produce endostatin, a potent inhibitor of angiogenesis and tumor growth (Digtyar, et al. 2007; Menzel, et al. 2004; Sertie, et al. 2000). Angiogenesis may be important in the pathogenesis of hematological cancer, and Collagen XVIII is associated with malignancy [(Feldman, et al. 2002; Hoffmann, et al. 2008; Iizasa, et al. 2004a; Iizasa, et al. 2004b; Lurje, et al. 2010; Ni, et al. 2009; Ohlund, et al. 2008; Ren, et al. 2003; Suzuki, et al. 2002a; Woo, et al. 2006)].

Over 48 patients in 16 families have been reported with this autosomal recessive condition, but there are no reports of patients with both Knobloch syndrome and malignancy. In this study, we describe a KNO1 family with a unique genotype where one affected sibling developed acute lymphoblastic leukemia (ALL).

METHODS

The study protocol was approved by the Institutional Review Board for Human Subjects Research (IRB) at the University of Iowa, was HIPPA compliant, and adhered to the tenets of the Declaration of Helsinki. The IRB waived informed consent for this retrospective chart study. Standard ophthalmological examinations were performed and fundus images were captured with the RetCam imaging system (Clarity Medical Systems, CA). Gene sequencing involved the exons and flanking 100bp intronic or 200bp promoter regions of the proband DNA amplified using the Platinum Pfx PCR kit (Invitrogen). Primer sequences are available upon request. PuriWedge PCR amplification products were electrophoresed on ABI 3100 and 3730 DNA sequencers by the Genomics Core.

RESULTS

The pedigree of an El Salvadorian family was represented by two parents (I-1, I-2) without known consanguinity, two daughters, and one son (Figure 1). The proband (II-3) was a 4-year-old female reported to have midline occipital soft tissue swelling and alopecia, hypotonia, mild developmental delay, and normal growth and OFC. A Brain MRI at 13 months of age showed an occipital dermal sinus tract, frontal cortical dysplasia, white matter loss, and mild ventriculomegaly. She had mild developmental delay. The proband's 7 year old sister (II-2) had excision of a small occipital encephalocele at 1 month of age. She also had an occipital scalp defect, but normal growth and development. Brain MRI at age 4 showed an occipital dermal sinus tract and multiple punctuate areas of high signal along the supratentorial sulci. She was then diagnosed with acute lymphoblastic leukemia (ALL), pre-B type. Following treatment using the "AALL0031" regimen (Schultz, et al. 2009), she successfully went into remission, was on maintenance, and completed her treatment at age 6-years. She remains in remission. The 11-year-old brother (II-1) was healthy. The proband and her sister had infantile nystagmus, high myopia, and poor vision, and they were referred for ophthalmic evaluation.

Ophthalmic Findings

In the proband (II-3), the visual acuity measured 5/80 (20/320) OD and 5/63 (20/250). Cycloplegic refraction was -15.25 diopters OD and -14.75 0.50 090 diopters OS. A-scan echography confirmed high myopia by measuring axial eye lengths of 27.02 mm OD and 27.08 mm OS. The anterior segment exam was unremarkable. The intraocular pressures were 16 mmHg OU. A dilated fundus exam revealed vitreous attachment at the disc, syneresis, and small round condensations in sheets of anterior vitreous (Figure 2). These may represent persistent fetal hyaloid vasculature that has failed to regress completely (Duh, et al. 2004; Fukui, et al. 2002). The optic discs were small with peripapillary atrophy. There were visibly prominent choroidal vessels in the posterior pole and atrophic changes in the

central macula. These white areas in the macula have been described as having the appearance of a macular coloboma. Examination of the peripheral retinas showed 360 degrees of irregular white dots and lines at the vitreoretinal interface. There were a few vitreoretinal adhesions temporally, but no lattice, retinal tears or detachment were found. There was a single sclerotic vessel in the right eye but no neovascularization.

Similar exam findings were present in her older sister (II-2). The visual acuity measured 20/100 OD and 20/250 OS. Cycloplegic refraction was -18.00 diopters OU. A-scan echography confirmed high myopia by measuring axial eye lengths of 28.83 mm OD and 28.09 mm OS. There were vitreous sheets anteriorly with small round condensations and syneresis with attachment at the disc (Figure 3). There was an opacity on the left posterior lens capsule. The intraocular pressures were 11 mmHg OU. The posterior poles had staphylomatous changes with chorioretinal atrophy involving the fovea OD and a dense area of atrophy involving the fovea OS. In the peripheral retina there were the previously described irregular white dots and lines at the vitreoretinal interface. There were a few vitreoretinal adhesions temporally, but no lattice, retinal tears or detachment.

Mutation Analysis

The combination of ophthalmic findings and encephaloceles in the daughters suggested Knobloch syndrome. The *COL18A1* gene was sequenced in the two affected subjects and parents. A homozygous, 2-base pair deletion (c3514-3515delCT) was detected in exon 41 of both daughters (Figure 4). The identical heterozygous mutation was identified in each of the parents. This creates a frameshift at near amino acid 167 of the collagen XVIII protein, which is upstream from the proteolytic cleavage site of endostatin (O'Reilly, et al. 1997)

DISCUSSION

This is the first Knobloch family originating from El Salvador, and the mutation represents a unique homozygous deletion on the *COL18A1* that was also recently reported in a family from India. (Paisan-Ruiz, et al. 2009) Affected individuals in the Indian family presented with predominantly neurological symptoms, such as ataxia, epilepsy, cognitive decline, and no leukemia, which emphasizes the emerging variability in the Knobloch disease phenotype. Although there was no known consanguinity, both parents were from the same region and there may be a founder effect. This mutation was previously reported in several unrelated KNO families. In these cases, however, affected subjects were compound heterozygotes with a different *COL18A1* mutant companion. (Suzuki, et al. 2002a) None of the affected in these subjects developed ALL.

The homozygous mutation we describe causes a frameshift mutation that would eliminate the downstream proteolytic cleavage site for endostatin, suggesting that these patients may have no functional endostatin (Menzel, et al. 2004; Sertie, et al. 2000; Suzuki, et al. 2002b). Collagen XVIII is a structural protein, and might explain the connective tissue abnormalities of scalp defects and vitreoretinal degeneration found in Knobloch syndrome. The antiangiogenic properties of endostatin may be relevant to eye development, where persistent fetal vasculature has been reported in knockout mice, in a previously described family human family, and is suggested in images we present. (Duh, et al. 2004) The systemic effect of endostatin deficiency in Knobloch syndrome, however, is not known. (Digtyar, et al. 2007)

Based on the function of endostatin, it is interesting to speculate whether abnormal endostatin production might have contributed to the development of ALL in this family. Although ALL has not been previously reported, mutations in the terminal portion of the *COL18A1* have raised the possibility of defective endostatin in KNO patients. Abnormal

endostatin levels are found in a variety of human cancers (Digtyar, et al. 2007) and the antiangiogenic properties of endostatin may be therapeutic in some cases (Feldman, et al. 2002; Hoffmann, et al. 2008; Iizasa, et al. 2004a; Iizasa, et al. 2004b; Lurje, et al. 2010; Ni, et al. 2009; Ohlund, et al. 2008; Ren, et al. 2003; Schuch, et al. 2005; Suzuki, et al. 2002a; Woo, et al. 2006). Elevated plasma endostatin levels in some children with ALL have been detected, however, the mechanisms regulating angiogenesis and the role of endostatin in childhood ALL are poorly understood (Pui 1998; Schneider, et al. 2007). We recommend all Knobloch syndrome patients, particularly those with *COL18A1* mutations affecting endostatin, have careful monitoring for the development of leukemia and other cancers.

Acknowledgments

Funding/Support: NIH 1R01 NS064159-01

References

- Capstick N. Depressive reactions in the course of clomipramine therapy used in the treatment of obsessional conditions. *Scott Med J*. 1975; 20(1 suppl):45–8. [PubMed: 1154013]
- Digtyar AV, Pozdnyakova NV, Feldman NB, Lutsenko SV, Severin SE. Endostatin: current concepts about its biological role and mechanisms of action. *Biochemistry*. 2007; 72(3):235–46. [PubMed: 17447877]
- Duh EJ, Yao YG, Dagli M, Goldberg MF. Persistence of fetal vasculature in a patient with Knobloch syndrome: potential role for endostatin in fetal vascular remodeling of the eye. *Ophthalmology*. 2004; 111(10):1885–8. [PubMed: 15465551]
- Feldman AL, Alexander HR Jr, Yang JC, Linehan WM, Eyler RA, Miller MS, Steinberg SM, Libutti SK. Prospective analysis of circulating endostatin levels in patients with renal cell carcinoma. *Cancer*. 2002; 95(8):1637–43. [PubMed: 12365010]
- Fukai N, Eklund L, Marneros AG, Oh SP, Keene DR, Tamarkin L, Niemela M, Ilves M, Li E, Pihlajaniemi T, Olsen BR. Lack of collagen XVIII/endostatin results in eye abnormalities. *EMBO J*. 2002; 21(7):1535–44. [PubMed: 11927538]
- Hoffmann S, Wunderlich A, Lingelbach S, Musholt PB, Musholt TJ, von Wasielewski R, Zielke A. Expression and secretion of endostatin in thyroid cancer. *Ann Surg Oncol*. 2008; 15(12):3601–8. [PubMed: 18818971]
- Iizasa T, Chang H, Suzuki M, Otsuji M, Yokoi S, Chiyo M, Motohashi S, Yasufuku K, Sekine Y, Iyoda A, Shibuya K, Hiroshima K, Fujisawa T. Overexpression of collagen XVIII is associated with poor outcome and elevated levels of circulating serum endostatin in non-small cell lung cancer. *Clin Cancer Res*. 2004a; 10(16):5361–6. [PubMed: 15328173]
- Iizasa T, Chang H, Suzuki M, Otsuji M, Yokoi S, Chiyo M, Motohashi S, Yasufuku K, Sekine Y, Iyoda A, Shibuya K, Hiroshima K, Fujisawa T. Overexpression of collagen XVIII is associated with poor outcome and elevated levels of circulating serum endostatin in non-small cell lung cancer. *Clinical Cancer Research*. 2004b; 10(16):5361–6. [PubMed: 15328173]
- Khaliq S, Abid A, White DR, Johnson CA, Ismail M, Khan A, Ayub Q, Sultana S, Maher ER, Mehdi SQ. Mapping of a novel type III variant of Knobloch syndrome (KNO3) to chromosome 17q11.2. *Am J Med Genet A*. 2007; 143A(23):2768–74. [PubMed: 17975799]
- Kliemann SE, Waetge RT, Suzuki OT, Passos-Bueno MR, Rosemberg S. Evidence of neuronal migration disorders in Knobloch syndrome: clinical and molecular analysis of two novel families. *Am J Med Genet A*. 2003; 119A(1):15–9. [PubMed: 12707952]
- Knobloch WH, Lauer JM. Retinal Detachment and Encephalocele. *J Pediatr Ophthalmol*. 1971; 8(3):181–184.
- Lurje G, Husain H, Power DG, Yang D, Groshen S, Pohl A, Zhang W, Ning Y, Manegold PC, El-Khoueiry A, Iqbal S, Tang LH, Shah MA, Lenz HJ. Genetic variations in angiogenesis pathway genes associated with clinical outcome in localized gastric adenocarcinoma. *Ann Oncol*. 2010; 21(1):78–86. [PubMed: 19622587]

- Menzel O, Bekkeheien RC, Reymond A, Fukai N, Boye E, Kosztolanyi G, Aftimos S, Deutsch S, Scott HS, Olsen BR, Antonarakis SE, Guipponi M. Knobloch syndrome: novel mutations in COL18A1, evidence for genetic heterogeneity, and a functionally impaired polymorphism in endostatin. *Hum Mutat.* 2004; 23(1):77–84. [PubMed: 14695535]
- Ni Q, Ji H, Zhao Z, Fan X, Xu C. Endostar, a modified endostatin inhibits non small cell lung cancer cell in vitro invasion through osteopontin-related mechanism. *Eur J Pharmacol.* 2009; 614(1–3):1–6. [PubMed: 19389394]
- O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell.* 1997; 88(2):277–85. [PubMed: 9008168]
- Ohlund D, Ardnor B, Oman M, Naredi P, Sund M. Expression pattern and circulating levels of endostatin in patients with pancreas cancer. *Int J Cancer.* 2008; 122(12):2805–10. [PubMed: 18360823]
- Paisan-Ruiz C, Scopes G, Lee P, Houlden H. Homozygosity mapping through whole genome analysis identifies a COL18A1 mutation in an Indian family presenting with an autosomal recessive neurological disorder. *Am J Med Genet B Neuropsychiatr Genet.* 2009; 150B(7):993–7. [PubMed: 19160445]
- Passos-Bueno MR, Marie SK, Monteiro M, Neustein I, Whittle MR, Vainzof M, Zatz M. Knobloch syndrome in a large Brazilian consanguineous family: confirmation of autosomal recessive inheritance. *Am J Med Genet.* 1994; 52(2):170–3. [PubMed: 7802003]
- Pui CH. Recent advances in the biology and treatment of childhood acute lymphoblastic leukemia. *Curr Opin Hematol.* 1998; 5(4):292–301. [PubMed: 9747636]
- Ren B, Hoti N, Rabasseda X, Wang YZ, Wu M. The antiangiogenic and therapeutic implications of endostatin. *Methods and Findings in Experimental and Clinical Pharmacology.* 2003; 25(3):215–24. [PubMed: 12743627]
- Schneider P, Vasse M, Corbiere C, Legrand E, Marie-Cardine A, Boquet C, Cazin L, Vannier JP. Endostatin variations in childhood acute lymphoblastic leukaemia--comparison with basic fibroblast growth factor and vascular endothelial growth factor. *Leuk Res.* 2007; 31(5):629–38. [PubMed: 17011029]
- Schuch G, Oliveira-Ferrer L, Loges S, Laack E, Bokemeyer C, Hossfeld DK, Fiedler W, Ergun S. Antiangiogenic treatment with endostatin inhibits progression of AML in vivo. *Leukemia.* 2005; 19(8):1312–7. [PubMed: 15931265]
- Schultz KR, Bowman WP, Aledo A, Slayton WB, Sather H, Devidas M, Wang C, Davies SM, Gaynon PS, Trigg M, Rutledge R, Burden L, Jorstad D, Carroll A, Heerema NA, Winick N, Borowitz MJ, Hunger SP, Carroll WL, Camitta B. Improved early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: a children's oncology group study. *Journal of Clinical Oncology.* 2009; 27(31):5175–81. [PubMed: 19805687]
- Seaver LH, Joffe L, Spark RP, Smith BL, Hoyme HE. Congenital scalp defects and vitreoretinal degeneration: redefining the Knobloch syndrome. *Am J Med Genet.* 1993; 46(2):203–8. [PubMed: 8484411]
- Sertie AL, Quimby M, Moreira ES, Murray J, Zatz M, Antonarakis SE, Passos-Bueno MR. A gene which causes severe ocular alterations and occipital encephalocele (Knobloch syndrome) is mapped to 21q22.3. *Human Molecular Genetics.* 1996; 5(6):843–7. [PubMed: 8776601]
- Sertie AL, Sossi V, Camargo AA, Zatz M, Brahe C, Passos-Bueno MR. Collagen XVIII, containing an endogenous inhibitor of angiogenesis and tumor growth, plays a critical role in the maintenance of retinal structure and in neural tube closure (Knobloch syndrome). *Hum Mol Genet.* 2000; 9(13):2051–8. [PubMed: 10942434]
- Sniderman LC, Koenekoop RK, O'Gorman AM, Usher RH, Sufrategui MR, Moroz B, Watters GV, Der Kaloustian VM. Knobloch syndrome involving midline scalp defect of the frontal region. *Am J Med Genet.* 2000; 90(2):146–9. [PubMed: 10607954]
- Suzuki M, Iizasa T, Ko E, Baba M, Saitoh Y, Shibuya K, Sekine Y, Yoshida S, Hiroshima K, Fujisawa T. Serum endostatin correlates with progression and prognosis of non-small cell lung cancer. *Lung Cancer.* 2002a; 35(1):29–34. [PubMed: 11750710]

- Suzuki OT, Sertie AL, Der Kaloustian VM, Kok F, Carpenter M, Murray J, Czeizel AE, Kliemann SE, Rosemberg S, Monteiro M, Olsen BR, Passos-Bueno MR. Molecular analysis of collagen XVIII reveals novel mutations, presence of a third isoform, and possible genetic heterogeneity in Knobloch syndrome. *Am J Hum Genet.* 2002b; 71(6):1320–9. [PubMed: 12415512]
- Wilson C, Aftimos S, Pereira A, McKay R. Report of two sibs with Knobloch syndrome (encephalocele and vitreoretinal degeneration) and other anomalies. *Am J Med Genet.* 1998; 78(3):286–90. [PubMed: 9677068]
- Woo IS, Kim KA, Jeon HM, Hong SH, Rho SY, Koh SJ, Lee MA, Byun JH, Kang JH, Hong YS, Lee KS, Cho CS, Choi MG, Chung IS. Pretreatment serum endostatin as a prognostic indicator in metastatic gastric carcinoma. *Int J Cancer.* 2006; 119(12):2901–6. [PubMed: 16998835]

Figure 1.

Figure 2.

Figure 3.

Figure 4.