COL4A1

Tutorial with Parents (May 20, 2019)

The *COL4A1* tutorial took place on the afternoon of May 20, 2019, with both parents present as well as their 4-year old daughter who was held in the mother's lap throughout the tutorial. Dr. Walkley hosted the meeting which occurred in the IDDRC office in Kennedy. The child's pediatrician, Dr. Wasserstein, began with a review of her patient and the general information about the severe clinical consequences evident with mutations in this gene. The parent, particularly the mother, offered additional comments on her daughter's development. This was followed by a lay scientific summary of the gene and its protein and possible explanations for some of the brain clinical signs by Dr. David Spray's lab, with postdoc, Sean McCutcheon providing most of the details. Another postdoc, Antonio Cibelli also attended. The Spray lab is currently funded by a 2019 IDDRD pilot grant to pursue an understanding of how this gene defect could undermine the integrity of the blood brain barrier. The parents, with the mother living in Queens and the father in the Bronx, expressed considerable gratitude for the explanations of their daughter's condition.

Patient Description:

This is a 4-year-old girl with severe failure to thrive (she currently weighs 14 lb, the typical weight of a six-month-old), microcephaly, global developmental delay, bilateral congenital cataracts (s/p removal at 8 and 9 months of age), glaucoma, seizures and spasticity. She was born at 27 weeks gestation with IUGR (Intrauterine Growth Restriction); the prematurity, however, does not explain her symptoms. At four years of age, she is legally blind, unable to sit alone largely due to spasticity, and has several single words. She continues to have complex medical needs focused on ophthalmologic care, seizure control, addressing spasticity, and her nutritional needs. Of note, an MRI of the brain was performed in December of 2015 with the following results—periventricular leukomalacia, a small right caudate lacunar infarct, a thin corpus callosum, asymmetry, and the suggestion of several "prominent venous structures".

Follow-up Tutorial with Parents (February 17, 2023)

This follow-up took place in the morning of February 17th, 2023. Both parents, maternal aunt, and daughter (patient) were present. The patient was in the father's lap playing on her iPad for the duration of the visit. The meeting, hosted by IDDRC co-director Dr. Steven Walkley, took place in the IDDRC office in Kennedy Center. Co-director Dr. Sophie Molholm, and IDDRC administrator Julie Mota were also present. The meeting proceeds began with Dr. Melissa Wasserstein, the patient's former pediatrician, introducing the patient and the family and providing a summary of events since they were last seen by Dr. Wasserstein. The mother supplemented information regarding her daughter's medical and social history. This was followed by an open floor for the family to address additional comments regarding the patient's development. Lastly, a scientific presentation on *COL4A1* tailored to the family was given by MSTP graduate student, Amira Millette, in Dr. David Spray's lab. Dr. Spray and visiting graduate student, Tagore Gonzalez de Morais,

were also present as representatives of the Spray lab. The Spray lab is interested in how this specific gene defect can alter brain structure and function, particularly in the blood-brain barrier and extracellular matrix. The mother and patient currently live in Staten Island with the patient's aunt. The father lives in the Bronx. The family expressed eagerness and gratitude for the care that the IDDRC Gene Team has in their daughter. Mother and aunt expressed considerable knowledge about the condition since the last visit. Mother and aunt shared thoughts on moving to North Carolina in the near future for improved quality of life for patient, and express interest in remaining in contact with the IDDRC.

Patient Description (February 17, 2023):

Patient is a 7-year-old girl with severe failure to thrive (she currently weighs 25lb, consistent with a developing two-year-old), microcephaly, global developmental delay, bilateral congenital cataracts (s/p removal at 8 and 9 months of age), glaucoma, seizures, and spasticity. She was born at 27 weeks' gestation with IUGR (Intrauterine Growth Restriction); the prematurity, however, does not explain her symptoms. At 7 years old, she is still legally blind, but wears contact lenses as corrective vision. She is still unable to sit alone largely due to spasticity and has difficulty holding her head up without support. She relies on a stroller/wheelchair for mobility. Her fine motor skills include pressing large buttons with her fist and nose. She can navigate her iPad and her 9-panel assisted device. She can speak five words, among which include, "hi", "mom", "hello".

Patient was diagnosed with cerebral palsy since her last visit to the IDDRC. She had a gastric feeding tube that was displaced in 2020 and was not replaced until August 2022. During that time, mother spoon-fed the patient, who at the time was able to eat soft baby foods. But she has since gained 3lb post g-tube re-placement, which was also partially due to a new diet. She is currently seen by a specialist team of ophthalmology, neurology, gastroenterology, and pulmonology at another institution for continued complex medical care. Most recent neurology visit considered surgical intervention on her ankles, which are "growing inward", so that she may start to practice supporting herself standing/walking. Prior to that visit, she has been receiving Botox injections in her ankles and casting to stabilize the ankles. Other surgical indications include future eye surgery to provide permanent corrective vision. Her intraocular pressure has been stable since last ophthalmology visit. She is currently taking levetiracetam (Keppra) as an anti-seizure medication, and polyethylene glycol (Miralax) for digestion. She is also on a regimen of other medications, not listed here.

Patient lives with mother and aunt. She speaks to father nightly on the phone and has regular visits with him. She currently attends a special needs school in NYC, which focuses on educating students from ages 5-21 with multiple disabilities. She takes a school bus that picks her up from Staten Island Mon-Fri. She enjoys attending school, and mom describes daughter's attitude as independent.

Patient Specific Genetics:

A heterozygous de novo pathogenic variant in COL4A1, c.2987 G>A (p.G996D), was detected in this patient, c.2987 G>A (p.G996D). This variant has not been reported in

large populations, nor has it previously been reported to be either pathogenic or benign. The non-conservative amino acid substitution caused by this mutation, based on in-silico analyses, supports the pathogenicity of this mutation (see below Protein/Mechanisms).

Disease/Syndrome Features:
Pathogenic mutations in COL4A1 are usually highly penetrant and manifest clinically as a spectrum of disorders which have been described in a total of less than 100 families of American, Spanish, Chinese, Japanese, Dutch, Italian, French, and German descent.

Despite usually being inherited in an autosomal dominant fashion, more than twenty seven percent of patients with diseases related to this gene harbor a de novo mutation with variable age of onset within and between families. Dosing defects of this gene are felt to be lethal since to date no deletions or duplications causative of COL4A1-related disorders have been found.

Manifestations of COL4A1 mutations in individual organs:

Brain--small-vessel brain disease (as periventricular leukomalacia, porencephaly, lacunar infarcts, microhemorrhages, dilated perivascular spaces, deep intracerebral hemorrhages--all presenting either antenatally, neonatally, or recurrently), large-vessel disease (as cerebral aneurysms)
--clinically presenting as infantile hemiparesis, seizures, single/recurrent hemorrhagic stroke, ischemic stroke, isolated migraine with aura, intellectual/developmental delay, dementia.

Eye-- retinal arterial tortuosity, Axenfeld-Reiger anomaly (iris abnormalities, posterior embryotoxon, microcornea, increased ocular pressure/glaucoma), cataracts, micro/anophthalmia —can clinically present as transient visual loss due to retinal hemorrhage

Kidney-- hematuria, unilateral renal atrophy, renal cysts

Muscle-- cramps, elevated creatine kinase

Peripheral vascular--Raynaud phenomenon

Cardiac-- supraventricular arrhythmia, mitral valve prolapse

Erythrocytes--hemolytic anemia

COL4A1-related Diseases (OMIM 120130):
Collagen of Basement Membrane, Alpha-1 chain

Porencephaly Type 1/Autosomal dominant familial porencephaly (OMIM 175780): Porencephaly with varying levels of periventricular leukomalacia, microbleeds, lacunar infarcts, and intracerebral calcifications. Neurologic symptoms include infantile hemiparesis, seizures, intellectual deficits, dystonia, strokes, and migraines. Frequently associated with congenital cataracts and anterior segment abnormalities, rarely with retinal artery tortuosity, occasionally with hematuria or muscle cramping.

Brain small-vessel disease with or without ocular anomalies/ Autosomal dominant brain small vessel disease with hemorrhage (OMIM 607595): Diffuse periventricular leukomalacia, lacunar infarcts, microbleeds, dilated perivascular spaces, deep intracerebral hemorrhages, intracerebral calcifications. Neurologic symptoms—ranges from none to migraine with aura or infantile hemiparesis. Variably associated with retinal artery tortuosity, congenital cataracts, anterior segment anomalies, renal atrophy, and renal cysts. Occasionally associated with hematuria, hemolytic anemia, or muscle cramping.

HANAC (hereditary angiopathy with nephropathy, aneurysms and muscle cramps) (OMIM 611733): Asymptomatic small-vessel brain disease asymptomatic with subcortical, periventricular or pontine leukoencephalopathy, dilated perivascular spaces, lacunar infarcts, microbleeds, and carotid siphon aneurysms. Gross or microhematuria, bilateral cortical or medullary renal cysts. Muscle cramps, elevated creatine kinase levels and bilateral retinal arteriolar tortuosity in all patients. Variably associated with Raynaud's phenomenon, supraventricular arrhythmia and liver cysts.

Isolated retinal vessel tortuosity (OMIM 180000): Manifests as second/third order retinal artery involvement with normal first order retinal arteries/veins. Spontaneous or stress/trauma induced retinal hemorrhage leading to transient visual

PADMAL (Pontine Autosomal Dominant Microangiopathy and Leukoencephalopathy) (OMIM: 618564): Characterized by cerebral small-vessel disease, resulting in onset of recurrent ischemic strokes in ages 30-40s. Progressive, variable cognitive and motor impairment, consistent with multi-infarct dementia. Focal infarcts in the pons with diffusive leukoencephalopathy in various regions of the brain.

Susceptibility to intracerebral hemorrhage (OMIM: 614519): Adult-onset hemorrhagic stroke

Nonsyndromic autosomal dominant congenital cataracts

Schizencephaly (OMIM 269160)

Clinical mimics involving other genes:

COL4A2-related Diseases (OMIM: 120090): Collagen of Basement Membrane, Alpha-2 chain characterized by cerebral hemorrhage or porencephaly with periventricular leukomalacia.

Autosomal dominant Walker-Warburg syndrome/Muscle-brain-eye disease (Labelle-Dumais et al. 2011) (OMIM: 236670): Brain and eye malformations include cobblestone lissencephaly, cerebellar malformations, ventricular dilatation, midline hypoplasia, and macrocephaly or microcephaly, and retinal detachment. Also presents with profound mental retardation, congenital muscular dystrophy, and early death.

CARASIL/CADASIL (Cerebral Arteriopathy, Autosomal Recessive/Dominant, with Subcortical Infarcts and Leukoencephalopathy) (OMIM: 600142/125310): AR features are characterized by non-hypertensive cerebral small vessel arteriopathy with alopecia, spondylosis, and progressive motor dysfunction and dementia in the ages 20-30s. AD features are characterized by small vessel disease manifested by migraine, strokes, white matter lesions, and resultant cognitive impairment.

RVCLS/HERNS/HVR (Retinal, Vasculopathy, with Cerebral Leukoencephalopathy and Systemic Manifestations (OMIM: 192315): Adult-onset micro-vasculopathy with CNS degeneration, progressive vision loss, stroke, motor impairment, and cognitive decline. Systemic vascular involvement includes Raynaud phenomenon, micronodular cirrhosis, and glomerular dysfunction.

Micro/anophthalmia with coloboma spectrum (OMIM: 6155145): microphthalmia with or without coloboma, developmental delays, and motor impairments. Additional ocular anomalies include ptosis, microcornea, sclerocornea, anterior segment dysgenesis, and keyhole-shaped pupils.

Synonyms of COL4A1/A2-Related Disorders: COL4A1/A2 syndrome, Gould syndrome

COL4A1 Gene/Protein:
Type IV collagen is the main component of basement membranes. Other components of which are laminins, proteoglycans and entactin/nidogen. Type IV collagen is made up of three trimers consisting of varying proportions of six variants of type IV alpha chains (alpha-1/alpha-1/alpha-2; alpha-3/alpha-4/alpha-5; alpha-5/alpha-5/alpha-6). Commonalities between the six different alpha chains are: 1) the 7S amino-terminal domain and 2) the large collagenous domain formed by Gly-X-Y repeats. Interactions between non-collagenous NC1 carboxy-terminals of theses chains (**arresten-** *see below*) determine the types and proportions of alpha chains ultimately undergoing assembly in the above configurations. The glycine residues are crucial for the stabilization of the triple helices that form, and the distribution of the three isoforms are tissue and developmentally specifically expressed, with the first of the three being the most ubiquitously expressed.

The gene for the alpha-1 chain of Type IV collagen is located on chromosome 13q34, is 158 kilobases long and contains 52 exons. There are too few pathogenic variants in this gene to be able to outline true genotype/phenotype relationships, but certain patterns

have emerged. Firstly, the most common pathogenic variants involve missense alterations to glycine residues within the collagenous domain (exons 24-51) which most closely correlate with brain disease (Jeanne et al 2016). Exon 41 deletion has been most characterized in animal models (Gould et al. 2005). Similarly, the HANAC form of COL4A1 disease involves variants in exons 24 and 25 exclusively affecting glycine residues in a specific proximal 30-amino acid region of the protein. Only six pathogenic variants in the arresten domain have been found to date.

Putative molecular mechanisms for COL4A1-related disease:
Sudhakar et al. 2005 found that arresten binds to alpha-1/beta-1 integrins and plays a role in angiogenesis in the context of low oxygen tension environments, specifically inhibiting migration, proliferation, and tube formation by endothelial cells. Maeshima et al. 2000 also suggests that the NC1 domain of the COL4A1 exhibits anti-tumor properties, by inhibiting angiogenesis. *Could mutations in the collagenous domains of the COL4A1 allow for unmitigated arresten activity, perhaps explaining why vascular seqeulae follow the malformation of Type 4 alpha 1 chains, particularly in microvascular beds during development?*

Certain missense mutations in Col4a1 in animals causes focal detachment of vascular endothelium from its underlying media. Additionally, by reducing intrinsic endothelial-based nitric oxide synthase activity, hypotension and reduced red cell volume was induced in the areas fed by the Col4a1-defective vessels (Van Agtmael et al 2010; Wang et al. 2011). *Could the perturbation in Col4a1 induce nitric oxide synthase activity in endothelial cells to further exacerbate symptoms of an already weakened vascular basement membrane?*

In Drosophila embryo and ovary, type IV collagen extracellular matrix proteins were shown to bind Dpp, a bone morphogenetic protein (BMP) signaling molecule, and regulate its signaling by modulating its presence in the extracellular space during development (Wang et al, 2008). The authors predicted that this role of type IV collagens is likely to be conserved. *Perhaps an early disruption in BMP gradient formation during early development is an underlying mechanism contributing to the profound growth failure observed in our patient?*

Databases used: Online Inheritance in Man & **PubMed**

Publications:
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Support Groups and Information:

Gould Syndrome Foundation: https://gouldsyndromefoundation.org

- Nonprofit dedicated to providing hope and help for children and adults with Gould Syndrome, affecting COL4A1 and COL4A2 genes. Mission includes education, advocacy, and forming connections between doctors and researchers to patient global registry to bring research and medical therapeutic options to those affected.
- Contact: info@col4a1foundation.org

The Arc: https://thearc.org

- Disability rights organization dedicated to “promoting and protecting the human rights of people with intellectual and developmental disabilities” Core values include promoting the value, individuality, rights, equity and diversity of the IDD community and supporting independent decision making through advocacy.
- Contact: info@nysarc.org for the NY chapter

- Bronx Developmental Center, 2400 Halsey St, Bronx, NY 10461

Facebook groups:

- Col4a1/Col4a2 Gould Syndrome Family Support Group (private facebook group, >300 members)
- Col4a1 Gene mutation – GOULD SYNDROME (private facebook group, >180 members)
- Gould Syndrome Foundation Col4a1/Col4a2 Group (private facebook group, family support and advocacy site for Gould Syndrome Foundation, >150 members)
- Gould Syndrome Awareness- COL4a1/COL4a2 Gene Mutation, >300 members)

Facebook pages:

- Little Braveheart (Facebook community page, profile of a child with a COL4A1 mutation)

Instagram pages:

- @logans_sidekicks (Instagram page, profile of a child with a COL4A1 mutation and infantile spasms)

Last updated 5/9/2020, MKR
Last updated 2/20/2023, A. Millette