



HAL
open science

Expanding the phenotypic spectrum of ARCN1-related syndrome

Alyssa L. Ritter, Jessica Gold, Hiroshi Hayashi, Amanda M. Ackermann, Stephanie Hanke, Cara Skraban, Sanmati Cuddapah, Elizabeth Bhoj, Dong Li, Yukiko Kuroda, et al.

► **To cite this version:**

Alyssa L. Ritter, Jessica Gold, Hiroshi Hayashi, Amanda M. Ackermann, Stephanie Hanke, et al.. Expanding the phenotypic spectrum of ARCN1-related syndrome. *Genetics in Medicine*, 2022, 24 (6), pp.1227-1237. 10.1016/j.gim.2022.02.005 . hal-04219146

HAL Id: hal-04219146

<https://hal.science/hal-04219146>

Submitted on 26 Sep 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Expanding the Phenotypic Spectrum of *ARCN1*-related syndrome

Alyssa L. Ritter MS CGC¹, Jessica Gold MD¹, Hiroshi Hayashi MD¹, Amanda M. Ackermann MD PhD², Stephanie Hanke MD MS², Cara Skraban MD¹, Sanmati Cuddapah MD¹, Elizabeth Bhoj MD PhD¹, Dong Li PhD³, Yukiko Kuroda MD PhD¹, Jessica Wen MD MSc⁴, Ryojun Takeda MD⁵, Audrey Bibb MS CGC⁶, Salima El Chehadeh MD⁷ Amélie Piton, MD PhD⁸, Jeanine Ohl, MD⁹, Mary K Kukulich MD¹⁰, Keisuke Nagasaki MD PhD¹¹, Kohji Kato MD PhD¹², Tomoo Ogi PhD¹², Tricia Bhatti MD¹³, Pierre Russo MD¹³, Bryan Krock PhD FACMG¹⁴, Jill R Murrell PhD¹⁴, Jennifer A. Sullivan MS CGC¹⁵, Vandana Shashi MD¹⁵, Nicholas Stong PhD¹⁶, Hakon Hakonarson MD PhD³, Kentaro Sawano MD¹¹, Erin Torti MS CGC¹⁷, Rebecca Willaert MS MA CGC¹⁷, Yue Si MD PhD¹⁷, William Ross Wilcox MD PhD⁶, Katrine Verena Wirgenes MD PhD^{18,19}, Kristian Thomassen MD²⁰, Katherine Carlotti MS CGC²¹, Angelika Erwin MD PhD²¹, Joanna Lazier MD²², Thorsten Marquardt PhD²³, Miao He PhD²⁴, Andrew C. Edmondson MD PhD¹, and Kosuke Izumi MD PhD¹

¹Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, PA, USA

²Division of Endocrinology and Diabetes, Department of Pediatrics, The Children's Hospital of Philadelphia, PA, USA

³Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA

⁴Division of Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, The Children's Hospital of Philadelphia, PA, USA

⁵Division of Genetics, Nagano Children's Hospital, Nagano, Japan

⁶Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA

⁷Service de Génétique Médicale, Hôpitaux Universitaires de Strasbourg, Institut de Génétique Médicale d'Alsace (IGMA), Strasbourg, France. Laboratoire de Génétique Médicale, UMRS_1112, Institut de Génétique Médicale d'Alsace (IGMA), Université de Strasbourg et INSERM, Strasbourg, France

⁸Department of Translational Medicine and Neurogenetics, Institut Génétique Biologie Moléculaire Cellulaire, 67400 Illkirch, France; Laboratoire de Diagnostic Génétique, Hôpitaux Universitaires de Strasbourg, 67000 Strasbourg, France.

⁹Service d'assistance médicale à la procréation, CMCO (Centre médico-chirurgical et obstétrical), Schiltigheim, France

¹⁰Department of Genetics, Cook Children's Medical Center, Fort Worth, TX, USA

¹¹Department of Pediatrics, Niigata University Medical & Dental Hospital, Niigata, Japan

¹²Department of Genetics, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan

¹³Division of Anatomic Pathology, The Children's Hospital of Philadelphia, PA, USA

¹⁴Division of Genomic Diagnostics, Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, PA, USA

¹⁵Department of Pediatrics, Division of Medical Genetics, Duke University Medical Center, Durham, NC, USA

¹⁶Institute of Genomic Medicine, Columbia University, New York, NY, USA

¹⁷GeneDx, Gaithersburg, MD, USA

¹⁸Department of Medical Genetics, Oslo University Hospital, Oslo, Norway

¹⁹Institute of Clinical Medicine, University of Oslo, Oslo, Norway

²⁰Department of Radiology and Nuclear Medicine, Oslo University Hospital, Oslo, Norway

²¹Genomic Medicine Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

²²Department of Medical Genetics, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada

²³Department of Pediatrics, University Hospital of Muenster, Muenster, Germany

²⁴Metabolic and Advanced Diagnostics, The Children's Hospital of Philadelphia, PA, USA

Corresponding Author: Kosuke Izumi, MD PhD, Division of Human Genetics, Children's Hospital of Philadelphia, Pennsylvania, USA, izumik1@chop.edu

Conflict of Interest

The following authors have no conflicts of interest to declare for this manuscript: ALR, JG, HH, AMA, SH, CS, SC, EB, DL, YK, MA, AB, SEC, MKK, KN, KK, TB, PR, BK, JRM, JAS, VS, NS, HH, KS, RT, WRW, KVV, KT, KC, AE, JL, TM, MH, ACE and KI.

JW receives research support from Gilead, Abbvie, and Alexion and consulting relationship with Gilead.

ET, RW, and YS are employees of GeneDx, Inc.

Abstract

Purpose: Describe the phenotypic and molecular characteristics of *ARCN1*-related syndrome.

Methods: Patients with *ARCN1* variants were identified, and clinicians were connected using GeneMatcher and physician referrals. Clinical histories were collected from each patient.

Results: Nine newly identified cases of *ARCN1*-related syndrome, 5 fetal cases from 3 families with severe manifestations, and previous cases were identified for a total of 20 cases.

Intrauterine growth restriction (IUGR), micrognathia, and short stature were present in all patients. Other common features included prematurity (11/15, 73.3%), developmental delay (10/14, 71.4%), genitourinary malformations in males (6/8, 75%), and microcephaly (12/15, 80%). Novel features of *ARCN1*-related syndrome included transient liver dysfunction and specific glycosylation abnormalities during illness, giant cell hepatitis, hepatoblastoma, cataracts and lethal skeletal manifestations. Developmental delay was seen in 73% of patients, but only 3 patients had intellectual disability (ID), which is less common than previously reported.

Conclusions: *ARCN1*-related syndrome presents with a wide clinical spectrum ranging from a severe embryonic lethal syndrome to a mild syndrome with IUGR, micrognathia, and short stature without ID. Patients with *ARCN1*-related syndrome should be monitored for liver dysfunction during illness, cataracts, and hepatoblastoma. Additional research to further define the phenotypic spectrum and possible genotype-phenotype correlations are required.

Keywords: *ARCN1*, *COPI*, micrognathia

INTRODUCTION

ARCN1-related syndrome [MIM: 617164] was first described by Izumi, et al in 2016 and is characterized by dysmorphic facial features, severe micrognathia, mild developmental delay, and short stature with rhizomelic shortening¹. Functional studies demonstrated defective type I collagen transport and reduction of collagen secretion in individuals with loss-of-function variants in *ARCN1* [MIM: 600820], consistent with the known function of *ARCN1* as the delta subunit of coat protein 1 (COPI) related intracellular protein transport. Two additional patients have been described since the original publication with transient N-glycosylation and liver function abnormalities in addition to features similar to those previously reported^{2,3}. The clinical phenotype of previously reported cases is relatively conserved; however, the small number of previously reported cases limited our ability to understand the clinical and variant spectrum of *ARCN1*-related syndrome^{1,2}. The purpose of this article is to delineate the phenotypic spectrum of *ARCN1*-related syndrome through the report of 9 additional patients and 5 fetuses (from 3 families) with *ARCN1*-related syndrome. Herein, we describe updates to the genotypic and phenotypic features of *ARCN1*-related syndrome, including novel phenotypes.

MATERIALS AND METHODS

Patients were identified to have *ARCN1* variants (NM_001655.4) via testing at various institutions: clinical exome sequencing in 8 cases, research based exome sequencing with clinical confirmation in 3 cases, and large multi-gene next generation sequencing panel in 1 case at various institutions. Variants were classified using the ACMG/AMP classification of sequence variants. GeneMatcher and physician referral were used to identify patients and connect researchers⁴. Comprehensive medical records were obtained and reviewed when available. All individuals were appropriately consented for publication as per respective institutional protocol. Informed consent was obtained from families for participation in this report

and for publication of photographs. Plasma/serum N-glycan profiling were performed at the Metabolic and Advanced Diagnostics, Children's Hospital of Philadelphia as previously described⁵. Data from this previously published study was collected in compliance with appropriate principles of research ethics and received IRB approval (Children's Hospital of Philadelphia IRB #14–011223)⁵.

RESULTS

Nine new patients with *ARCN1*-related syndrome were identified through collaboration. The diagnosis was identified via clinical or research exome sequencing in eight cases and large multi-gene panel performed as a trio in one case. In the majority of cases, clinical exome sequencing was done as a trio with both parents. In addition to six patients previously reported^{1,2,3}, we report genotypic and phenotypic data for 9 patients and summarized data for a total of 15 patients in Table 1. In addition, five fetuses from three families were also identified, and their phenotypes are reported separately in Table 2. Clinical exome sequencing was performed due to fetal anomalies in all fetal cases. Only one patient had an additional variant identified through testing (variant of uncertain significance in *INVS* which does not have significant overlap with reported medical history). All variants were classified as likely pathogenic or pathogenic, and variant types included nonsense, frameshift, and splice-site variants⁶. Nine novel variants were identified (Figure 1A). One of the variants (c.192_193delGT; p.M66Gfs*26) was reported as a variant of uncertain significance by a clinical laboratory. However, we consider this variant pathogenic based on ACMG/AMP classification criteria (PVS1, PM2, PM6, PP3)⁶. A recurrent nonsense variant (c.934C>T, p.Arg312*) was identified in three patients, and a recurrent splice variant (c.654-15A>G) was identified in two patients.

Patients ranged in age from nine months to 36 years of age. All 15 patients were noted to have intrauterine growth restriction (IUGR), micrognathia, and short stature. The majority (11/15, 73.3%) of patients were born prematurely (<37 weeks gestation), with an average gestational age of 34 weeks (range 26 6/7 weeks to 40 weeks). The skeletal manifestations of *ARCN1*-related syndrome mainly involved the appendicular skeleton, and over half of patients (8/15, 53%) had rhizomelic shortening. One patient and one fetal case also had mesomelia. Short stature was universal, most with heights between -2 and -4 SD for age, while weight tended to become more age-appropriate over time (Figure 2A). Facial dysmorphisms commonly seen included: downslanting palpebral fissures, bulbous nasal tip, and large or prominent ears (Figure 1B). While micrognathia was seen in all patients, 6 (40%) patients had cleft palate, and 2 (13.3%) had bifid uvula. Three of fifteen (20%) patients required tracheostomy due to respiratory failure, often complicated by both micrognathia and prematurity. At least two patients required mandibular distraction for the management of micrognathia. Septal defects were the most common congenital heart disease reported, with patent foramen ovale (PFO), atrial septal defect (ASD), and/or ventricular septal defect (VSD) seen in a total of 5 patients (33%). Males had a high frequency of genitourinary anomalies (6/7, 85%), most commonly hypospadias and small or microphallus. One patient with significant microphallus, hypospadias, bifid scrotum, and cryptorchidism was diagnosed as having ambiguous genitalia.

Developmental delays were common, but not universal (11/15, 73%). For five newly identified patients with developmental milestone data available, the average age of sitting was 6.6 months (range 3-12). The average age of walking was 19.2 months (range 12-24). The average age of first words was 15.4 months (range 8-26 months). Of those with neuropsychological testing, only 3/13 (23.1%) had intellectual disability, which was most commonly reported as mild. While 73% have developmental delay, on 23.1% are reported to

have intellectual disability, presenting a unique neurodevelopmental phenotype possibly influenced by the high rate of prematurity in this cohort.

Novel features seen in multiple individuals, primarily in the previously unreported cohort, included: cataracts (4/15, 27%), transient liver dysfunction (6/15, 40%), glycosylation abnormalities (including carbohydrate deficient transferrin (CDT) and/or plasma N-glycan profile) seen in 4 patients, and giant cell hepatitis (reported in 3 patients)⁵. Cataracts were diagnosed at 9 months, 6 years, and 13 years old and included bilateral central cortical cataracts and a Mittendorf dot (congenital vascular malformation) vs posterior polar cataract. One patient was found to have hepatoblastoma, and another patient was also found to have significant neuromyelitis optica.

Giant cell hepatitis, which subsequently resolved, was seen in two of the three patients reported to have hepatitis. All three patients with hepatitis initially presented with cholestasis. On pathology, there were more marked portal infiltrates in patient C3 than patient C2 (Figure 2B). Patient N1 was diagnosed with hepatoblastoma at 15-months-old after presenting with circulatory collapse and hemoperitoneum due to rupture of the tumor (Figure 2C). She underwent resection of the tumor with pre- and post-operative chemotherapy and is currently in remission (Supplemental Data). Of note, her variant was the recurrent variant that was also seen in patients C1 and C3, both with histories of transient liver function abnormalities and CDT abnormalities.

We previously reported mild undergalactosylation and mild increases of Man₅GlcNAc₂ in plasma N-glycan profile of two patients⁵. Subsequently, transient CDT abnormalities detectable with isoelectric focusing in a patient with *ARCN1* variant during viral illness were also reported by Reunert et al. which was thought to be related to increases of protein synthesis rate². To understand the impact of *ARCN1* variants in glycosylation profile, we evaluated N-glycan profiles among five cases (four cases (C1, P1, C2 and C3) and a case reported by Reunert et

al.) (Supplemental table 1a). N-glycan profiles of C1 and P1 were partly reported in our previous publication⁵. Among these 5 patients, in addition to undergalactosylation similar to PGM1-CDG, we detected different degrees of increases in Man₅GlcNAc₂ in the plasma N-glycans, a pattern that is specific for *ARCN1*-related syndrome. During acute illness, the increase of Man₅GlcNAc₂ in plasma N-glycan is profound in both the case reported by Reunert et al. and our patient (C2, 14 months old female, admitted after several weeks of vomiting and worsening conjugated hyperbilirubinemia and transaminitis). This pattern distinguished *ARCN1*-related syndrome from those of PGM1-CDG. CDT was abnormal for a mixed type I and type II pattern with both hypoglycosylation and undergalactosylation, a pattern that is the same as that in PGM1-CDG patients, though overall CDT changes in three *ARCN1*-related syndrome patients during their acute illness were milder than those in typical PGM1-CDG patients (Supplemental table 1a & b). Both CDT and N-glycan abnormality in these patients normalized rapidly upon recovery, CDT normalized as soon as within two weeks post the acute episode while plasma N-glycan may normalize in one month (Supplemental table 1a & b). The similar transient and mild N-glycan abnormalities also were detected in third male patient (C3) , at the age of 10 months old during a routine clinic without obvious viral illness but was on acetaminophen for fever or pain (Supplemental table 1a & b), two weeks later he was admitted with cough, fever, difficulty breathing and influenza positive.

Five fetuses, including three males and two females, were also identified with *ARCN1* pathogenic variants via clinical exome sequencing (Table 2). Outcomes included one spontaneous miscarriage at 14 weeks gestation, two intrauterine fetal demises (25 and 26 weeks gestation), and two terminations of pregnancy based on fetal anomalies. Fetal anomalies were suspected with ultrasound and confirmed with postmortem evaluation including x-rays. Similar to the other patients, all fetuses shared the core features of IUGR and micrognathia. Additionally, all had rhizomelic shortening and one had mesomelia (Figure 3). Males all had

genitourinary anomalies including, microphallus, hypospadias, bifid scrotum, and/or cryptorchidism. On the severe end of the phenotypic spectrum, one fetus presented with agenesis of the corpus callosum, hindbrain abnormalities, 1-2 and 4-5 syndactyly of both hands, hypoplastic nails on the 3rd digits on hands and feet, and short 3rd toes bilaterally. Exome sequencing in this case did not identify other likely pathogenic or pathogenic variants that could explain the skeletal findings. Another case also presented severely with skeletal abnormalities, brachycephaly, hypertelorism, and ventriculomegaly in addition to the core features (Figure 3). Three of the fetal cases are part of a familial case of *ARCN1*-related syndrome whose mother is included in this report (F1) and has milder features, demonstrating intra-familial variability in disease severity (Supplemental Figure 1). This mother also had another fetus suspected to be affected, but for which genetic testing was not able to be performed to confirm the diagnosis.

DISCUSSION

The additional nine patients and five fetuses identified with *ARCN1*-related syndrome after the original publication have provided insight into the wide clinical spectrum of the disorder and novel associated phenotypes. Our data confirm that micrognathia and growth retardation are the core clinical features of *ARCN1*-related syndrome and expand upon the phenotypic spectrum of the disorder. Additional clinical features commonly seen in patients with *ARCN1*-related syndrome include microcephaly, preterm birth, liver dysfunction, GU anomalies such as cryptorchidism and hypospadias in male patients, a variable neurodevelopmental phenotype and rhizomelic shortening. Rare, but potentially important, complications seen in patients include transient liver enzyme elevations and glycosylation abnormalities in the setting of illness, cataracts and hepatoblastoma.

Growth profile: Individuals with *ARCN1*-related syndrome demonstrate IUGR with persistent postnatal short stature. Contrary to height measurements, the weight measurements of some patients are in the normal range, suggesting that they may be prone to develop overweight and obesity. Patients C1 and C2 received growth hormone therapy in early childhood, with initial improved height velocity.

Pierre-Robin complications: While all 15 patients have micrognathia, 3 patients required tracheostomy due to complications of their Pierre-Robin sequence and at least two patients required mandibular distraction. Feeding difficulties were common, with at least 5 patients requiring surgical gastrostomy tube placement. It is likely that the combination of prematurity and Pierre-Robin sequence results in high levels of medical support required for some patients.

Liver dysfunction and abnormalities: While not universal, 6 patients were found to have abnormal liver function tests. These abnormalities tended to be transient and occur more often in periods of illness, such as with accompanying fever. Histologically, neonatal giant cell hepatitis (NGCH) was identified in three patients presenting with cholestasis (Figure 2B). NGCH is a common biopsy finding in neonates that have cholestasis with normal biliary tree architecture and may be related to pituitary abnormalities⁷. In our cohort, the combination of genitourinary anomalies and short stature responsive to growth hormone suggests deficient pituitary activity, which may play a role in causing NGCH. Additionally, most patients with *ARCN1* pathogenic variants are born prematurely and with low birth weights, both of which are known risk factors for NGCH. More information is needed to determine if NGCH is specific to *ARCN1*-related syndrome or a common finding for cholestatic disease due to impaired Golgi transport. Of note, one patient was found to have hepatoblastoma, which was treated by resection and chemotherapy who remains in remission. It remains unclear whether this is a rare co-occurrence or truly associated with pathogenic variants in *ARCN1*; thus, further studies and

clinical monitoring in *ARCN1*-related syndrome in patients exhibiting similar symptoms are warranted.

Neurocognitive development: While in our previous report, all four patients had developmental delay and/or intellectual disability, four of nine newly identified patients with *ARCN1*-related syndrome presented without any developmental delay or intellectual disability. This observation indicates a wide range of neurodevelopmental defects can be associated with *ARCN1*-related syndrome. It remains unknown what determines the severity of developmental disabilities. Since many patients with *ARCN1*-related syndrome required prolonged neonatal hospitalization due to micrognathia and prematurity, prenatal or perinatal insults represent one of the possible environmental factors influencing the developmental outcome of *ARCN1*-related syndrome.

Fetal presentations of *ARCN1*-related syndrome: The identification of multiple fetuses with *ARCN1* pathogenic variants and severe phenotypes, often resulting in intrauterine fetal demise, raises the possibility of a wider clinical spectrum than previously acknowledged. These fetuses all shared the core features of *ARCN1*-related syndrome but had a higher proportion of rhizomelia and skeletal anomalies than liveborn cases. The two cases without a family history of *ARCN1* variants had additional genetic testing including chromosomal microarray and exome sequencing which did not identify additional variants which could explain the severity of these presentations. It is possible that an unknown dual diagnosis or other genetic modifiers could play a role in the severity of these fetal cases. Variant type or location does not seem to correlate with severity of phenotype either in fetuses or living patients (Fig 1a), though further work is warranted to determine any possible genotype-phenotype correlations. Three of the fetal cases were offspring of an affected mother who had much milder features of the disorder. Intrafamilial variability has been previously reported in this diagnosis by Izumi et al., suggesting variable expressivity with families¹. This highlights the importance of appropriate

genetic counseling for affected individuals who may have offspring with more significant complications.

Glycosylation abnormalities: We identified specific CDT and N-glycan profile changes in some of our patients either when they were well or when they were sick, confirming the association between glycosylation abnormalities and *ARCN1*-related syndrome, as previously reported^{2,5}. *ARCN1*-related syndrome's glycosylation abnormalities include features of both Type I (loss of entire glycan chains) and Type II (truncated glycan chains) changes on transferrin specifically lacking galactose, in a pattern that is well recognized in PGM1-CDG⁸. So far CDT abnormalities in patients with *ARCN1* variants have only been observed during acute illness. Different from PGM1-CDG, there is often a prominent increase of Man₅GlcANc₂ in the *ARCN1*-related syndrome by plasma N-glycan profiling, while undergalactosylation is relatively mild. Mild changes in plasma N-glycan sometimes can be detected when the child is well, though not in all the affected patients. Therefore, CDG screening labs such as CDT and N-glycan profile may serve as a clinical functional test to evaluate the possible diagnosis of *ARCN1*-related syndrome. Indeed, in one of our patients (C2), the profound and specific plasma N-glycan and CDT abnormalities during her initial presentation facilitated the diagnosis of *ARCN1*-related syndrome. However, it is important to note that CDT/N-glycan abnormalities were only apparent before or during times of illness. During well visits, multiple plasma N-glycan tests are necessary to evaluate whether mild changes in N-glycan profile are chronic changes specific for *ARCN1*-related syndrome, Hence, the results must be interpreted with caution.

The overlap of glycosylation profile and clinical features (micrognathia, bifid uvula/cleft palate, growth retardation, hepatopathy) with PGM1-CDG suggests that fetal glycosylation abnormalities could be a shared pathomechanism explaining the skeletal features. Furthermore, since liver dysfunction is occasionally seen in individuals with congenital disorders of

glycosylation, glycosylation defects may also mediate the observed transient liver phenotype seen in the *ARCN1*-related syndrome.

Disease-causing variant spectrum of *ARCN1*: The classes of variants identified in *ARCN1*-related syndrome include nonsense, frameshift, and splice-altering variants, confirming haploinsufficiency of *ARCN1* as a mechanism of *ARCN1*-related syndrome. Additionally, two recurrent variants were identified in several unrelated patients: c.934C>T (p.Arg312*) and c.654-15A>G. A previous study by Tidwell et al. demonstrated this variant induces an exon extension and then leads to a frameshift isoform by using RT-PCR³. In this study, we also identified characteristic glycosylation changes in one patient who carries this recurrent intronic variant, further supporting it as a pathogenic change at the functional level.

Multiple patients with varying phenotypic severity have been identified to have the same *ARCN1* variant, making genotype-phenotype correlations difficult in this cohort. This is further exemplified by the wide phenotypic spectrum seen in the two familial cases of *ARCN1*-related syndrome including a case of mildly affected female with *ARCN1*-related syndrome (F1) gave birth to 4 affected fetuses resulting in fetal demise (Supplemental Figure 1). These observations suggest the possible contribution of genetic modifiers or environmental effects in determining phenotypic severity of *ARCN1*-related syndrome.

While we demonstrated the association between *ARCN1* loss-of-function variants and micrognathia, short stature and developmental delay of varying degree, it remains to be determined whether *ARCN1* missense variants cause a similar clinical phenotype. In gnomAD, missense variants identified in *ARCN1* are less than expected⁹. Therefore, it is possible that *ARCN1* missense variants cause milder *ARCN1*-related syndrome phenotype.

Molecular mechanism of *ARCN1*-related syndrome: *ARCN1* encodes the coatomer subunit delta protein, which is one of the components of COPI (coat protein complex I). Other

COPI components include coatomer subunit alpha (encoded by *COPA*) and coatomer subunit beta prime (encoded by *COPB2*). Heterozygous pathogenic variants in *COPA* cause autoimmune interstitial lung, joint and kidney disease¹⁰. Biallelic variants in *COPB2* cause primary microcephaly¹¹. Very recently, heterozygous *COPB2* loss-of-function variants are associated with developmental delay and osteoporosis¹². Therefore, the clinical consequences of *ARCN1*, *COPA*, and *COPB2* variants are distinctively different, despite all encoding components of COPI. These observations raise the possibility that these COPI subunits have non-canonical functions outside of COPI. Further research is warranted to understand the mechanism of not only *ARCN1*-related syndrome, but also other COPI-related disorders.

In summary, herein we report updates to the clinical and genotypic spectrum of *ARCN1*-related syndrome. Notably, transient liver function and specific glycosylation abnormalities often can be obvious during times of illness, normal developmental and neurocognitive outcomes, and severe prenatally lethal manifestations are novel findings in patients with *ARCN1*-related syndrome. We recommend close monitoring of liver function during illnesses, routine eye exams to identify cataracts. Further studies to investigate possible environmental or genetic factors responsible for the wide phenotypic spectrum and to establish the presence or absence of genotype-phenotype correlations are warranted in order to better understand this rare diagnosis.

Data Availability

Data supporting this work is available upon request. Clinical data sets have been de-identified.

Acknowledgments

The authors greatly appreciate the patients and their families for their participation in our study.

Funding

This work was supported by NIH grant U54 NS115198 (A.C.E. and M.H.). Data for patient D1 was obtained by the Duke Genome Sequencing clinic supported by the Duke University Health System.

Author Information

Conceptualization: A.L.R., J.G., A.C.E., K.I.; Data curation: A.L.R., K.I.; Formal Analysis: A.L.R., K.I.; Project Administration: A.L.R.; Resources: A.L.R., J.G., H.H., A.M.A., S.H., C.S., S.C., E.B., D.L., Y.K., M.A., A.B., S.E.C., M.K.K., K.N., K.K., T.B., P.R., B.K., J.R.M., J.A.S., V.S., N.S., H.H., K.S., R.T., W.R.W., K.V.W., K.T., K.C., A.E., J.L., M.H., A.C.E., J.W., E.T., R.W., R.S., T.M., and K.I.; Supervision: K.I.; Visualization: J.G., A.C.E., A.L.R., K.I.; Writing - original draft: A.L.R., J.G., C.S., J.W., K.I.; Writing - review & editing: J.G., J.W., A.C.E., A.L.R., K.I.

Ethics Declaration

This study was reviewed and approved by the Children's Hospital of Philadelphia Institutional Review Board (#16-013231) for patients identified through the institution. Each patient was enrolled in appropriate local IRB studies per their institutional requirements. Informed consent was obtained from all participants as required by the IRB and local institutional requirements. Clinical data has been de-identified. Written consent for inclusion in this publication was obtained for all participants, including for publication of participant photographs. This study adhered to the principles set out in the Declaration of Helsinki. Data from a previously published

study was collected in compliance with appropriate principles of research ethics and received IRB approval (Children's Hospital of Philadelphia IRB #14-011223).

Figure Titles & Legends

Figure 1. A) Variant Spectrum in *ARCN1*-related Syndrome. Variants depicted per NM_001655.4 transcript. Blue bars represent exons, and black lines represent introns. Bold variants represent recurrent variants in unrelated patients. Italicized variants represent previously reported variants. **B)** Facial Photographs of Patients with *ARCN1*-related Syndrome. Common dysmorphic features include downslanting palpebral fissures, bulbous nasal tip, and large or prominent ears.

Figure 2. A) Growth Parameters of Patients with *ARCN1*-related Syndrome. The top portion of each box represents the first quartile and the bottom portion of each box represents the third quartile, which are separated by the median. Box-and-whisker plots display mean, 95% confidence interval, and range. **B)** Liver Biopsy Histology in Patients with *ARCN1*-related Syndrome and Giant Cell Hepatitis. Images at 40x original magnification. Scale bar represents 50 microns. Hematoxylin and eosin stained section of liver biopsy showing giant cell transformation of hepatocytes with cellular enlargement and multinucleation. Foci of intracellular bile and canalicular cholestasis are also present. Portal areas contain a mild chronic inflammatory infiltrate, and there are scattered foci of lymphocytic lobulitis (original magnification x200). **C)** Hepatoblastoma with Tumor Rupture. Coronal contrast enhanced CT image (i) shows a large tumor on the inferior aspect of the right liver lobe. An ill-defined border on the inferior aspect of the tumor (white arrowhead) and adjacent high attenuating fluid in the peritoneal cavity (white arrow) indicate hemoperitoneum secondary to tumor rupture. Low attenuating areas within the tumor (black arrowhead) most likely represent areas of tumor necrosis, while regions of higher attenuation indicate viable tumor tissue (black arrow). Sagittal contrast enhanced CT image (ii) shows the tumor pedunculated from the inferior aspect of the right liver lobe (arrowheads).

Figure 3. X-rays of Two Fetuses with *ARCN1*-related syndrome. A) X-rays of fetus with c.307_311delTCTGA (p.S103AfsX4) variant following termination of pregnancy at 21 weeks gestation. There is generalized decreased bone density of the skull with brachycephaly. The thorax is narrow with short and narrow ribs. Bilateral upper and lower extremity are short with foreshortened humeri, radius, ulna, femori, and tibia. B) X-rays of fetus with c. 1241+1GA>A variant following intrauterine fetal demise at 25 weeks gestation. Notable findings include shortened humeri, forearms, femurs, and tibula/fibula; micrognathia; and bell-shaped thorax.

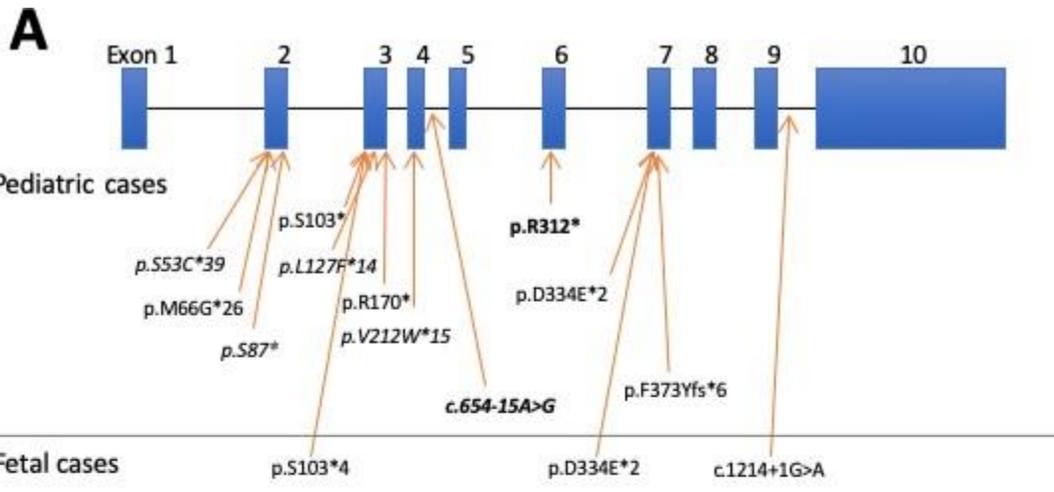
Table Titles & Legends

Table 1. Clinical Features of Patients with *ARCN1*-related Syndrome. Legend: CDT (carbohydrate deficient transferrin), GU (genitourinary), IUGR (intrauterine growth restriction), n/a (not applicable). *Patient D4 has elevated liver function tests due to treatment for significant neuromyelitis optica.

Table 2. Clinical Features of Fetal Cases with *ARCN1*-related syndrome. Legend: GU (genitourinary), IUGR (intrauterine growth restriction).

References

1. Izumi K, Brett M, Nishi E, et al. ARCN1 Mutations Cause a Recognizable Craniofacial Syndrome Due to COPI-Mediated Transport Defects. *Am J Hum Genet.* 2016;99(2):451-459.
2. Reunert J, Rust S, Grüneberg M, et al. Transient N-glycosylation abnormalities likely due to a de novo loss-of-function mutation in the delta subunit of coat protein I. *Am J Med Genet A.* 2019;179(7):1371-1375.
3. Tidwell T, Deshotel M, Palumbos J, Miller C, Bayrak-Toydemir P, Carey JC. Novel de novo ARCN1 intronic variant causes rhizomelic short stature with microretrognathia and developmental delay. *Cold Spring Harb Mol Case Stud.* November 2020. doi:10.1101/mcs.a005728
4. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. *Hum Mutat.* 2015;36(10):928-930.
5. Chen J, Li X, Edmondson A, et al. Increased Clinical Sensitivity and Specificity of Plasma Protein N-Glycan Profiling for Diagnosing Congenital Disorders of Glycosylation by Use of Flow Injection–Electrospray Ionization–Quadrupole Time-of-Flight Mass Spectrometry. *Clinical Chemistry.* 2019;65(5):653-663. doi:10.1373/clinchem.2018.296780
6. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-424.
7. Torbenson M, Hart J, Westerhoff M, et al. Neonatal giant cell hepatitis: histological and etiological findings. *Am J Surg Pathol.* 2010;34(10):1498-1503.
8. Tegtmeyer LC, Rust S, van Scherpenzeel M, et al. Multiple phenotypes in phosphoglucomutase 1 deficiency. *N Engl J Med.* 2014;370(6):533-542.
9. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature.* 2020;581(7809):434-443.
10. Watkin LB, Jessen B, Wiszniewski W, et al. COPA mutations impair ER-Golgi transport and cause hereditary autoimmune-mediated lung disease and arthritis. *Nat Genet.* 2015;47(6):654-660.
11. DiStasio A, Driver A, Sund K, et al. Copb2 is essential for embryogenesis and hypomorphic mutations cause human microcephaly. *Hum Mol Genet.* 2017;26(24):4836-4848.
12. Marom R, Burrage LC, Venditti R, et al. COPB2 loss of function causes a coatopathy with osteoporosis and developmental delay. *Am J Hum Genet.* 2021;108(9):1710-1724.

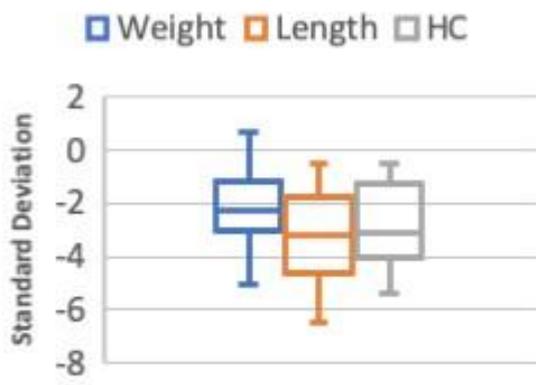


B

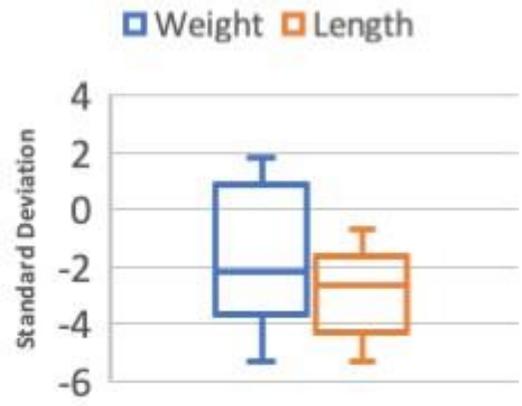


A

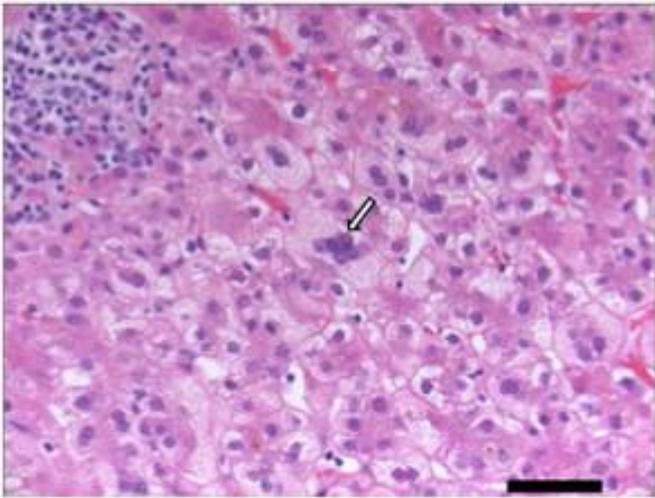
BIRTH PARAMETERS



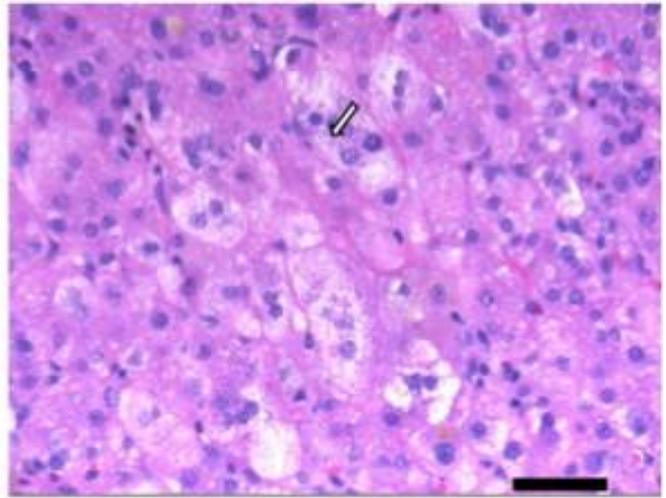
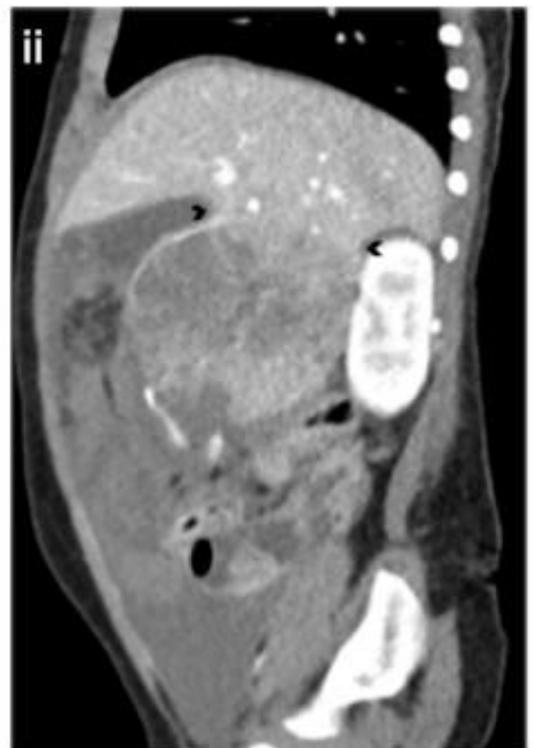
CURRENT GROWTH

**B**

P2



P3

**C**

Patient	C1	C2	C3	C4	D1	T1	F1	N1	J1		
Demographics										Previously Reported Cases	Total (%)
Sex	M	F	M	F	M	F	F	F	F		
Gestational Age (weeks, days)	32w 5d	37w 2d	30w 6d	26w 6d	38w	37w	36w	30w 0 d	31w1d		
Age at follow-up	4y	3y	9m	3y	7y5m	14y4m	36y	2y	15y		
ARCN1 variant [NM_001655.4]	c.934C>T; p.Arg312*	c.654-15A>G; p?	c.934C>T; p.Arg312*	c.192_193del; p.Met66Glyfs *26	c.508C>T; p.Arg170*	c.305_306insCTGAGAG, p.Ser103*	c.1001dup; p.Asp334Glu fs*2	c.934C>T; p.Arg312*	c.1118_1119del; p.Phe373Tyrfs*6		
Classification	pathogenic	likely pathogenic	pathogenic	likely pathogenic	pathogenic	pathogenic	pathogenic	pathogenic	pathogenic		
ACMG/AMP Criteria	PVS1, PM2, PP3, PP5	PS3, PM2, PP5	PVS1, PM2, PP3, PP5	PVS1, PM2, PP3	PVS1, PM2, PP3	PVS1, PM2, PP3	PVS1, PM2, PP3	PVS1, PM2, PP3, PP5	PVS1, PM2, PP3		
Inheritance	de novo	de novo	de novo	de novo	de novo	de novo	unk	de novo	unk		
Test Type	trio research exome	trio clinical exome	trio clinical exome	trio clinical exome	trio research exome	trio clinical exome	clinical exome	trio NGS panel	research exome		
Core Features											
IUGR	+	+	+	+	+	+	+	+	+	6/6	15/15 (100)
Micrognathia	+	+	+	+	+	+	+	+	+	6/6	15/15 (100)
Microcephaly	+	+	+	+	+	+	—	—	+	5/6	12/15 (80)
Cleft palate	+	+	—	—	—	+	+ (bifid uvula)	—	—	2/6	6/15 (40)
Tracheostomy	—	—	—	—	—	+	—	—	—	2/6	3/15 (20)
Congenital Anomalies											
Congenital heart disease	—	—	+ (PFO v. ASD)	—	+ (PFO, PDA)	—	—	—	—	3/6	5/15 (33)
GU anomalies	+ (hypospadias, bifid scrotum, cryptorchidism)	n/a	+ (ambiguous genitalia, hypospadias)	n/a	+ (small penis)	n/a	n/a	n/a	n/a	3/4 males	6/7 males (85)
Cataract	—	—	—	+ (bilateral central cortical cataract)	+ (mittendorf dot v. posterior polar cataract)	—	—	—	+ (unspecified type)	1/6	4/15 (27)
Musculoskeletal											
Short stature	+	+	+	+	+	+	+	+	+	6/6	15/15 (100)
Rhizomelic shortening	—	—	—	+	+	—	+	—	+	4/6	8/15 (53)
Joint laxity	—	—	—	—	—	+	—	—	unk	3/6	4/15 (27)
Neurologic/Development											
Developmental delay/ID	+	—	+	+ (mild)	+	—	—	+ (speech delay)	+	5/6	11/15 (73)
Autism	—	—	—	—	—	—	—	—	—	2/6	2/15 (13)
Seizure	—	—	—	—	—	—	—	—	—	2/6	2/15 (13)
Novel Features											
CDT abnormalities	+	+	+	normal	normal	—	unk	unk	unk	1/6	4/15 (27)
Giant Cell hepatitis	unk	+	+	+	—	—	unk	—	—	0/6	3/15 (20)
Liver function abnormalities	+	+	+	+	+	—	unk	+	—	0/6	6/15 (33)
Hepatoblastoma	—	—	—	—	—	—	—	+	—	0/6	1/15 (7)

Table 2. Clinical Features of Fetal Cases with ARCN1-related syndrome.

Clinical Feature	Positive/Total	Percent (%)
Original Core Features		
IUGR	5/5	100
Micrognathia	5/5	100
Microcephaly	5/5	78.6
Cleft Palate	1/2	50
Congenital Anomalies		
Congenital Heart Disease	0/2	0
GU Anomalies	3/3	100
Rhizomelic Shortening	5/5	100
Brain Anomalies	2/5	40