HHS Public Access

Author manuscript

*Am J Med Genet A*. Author manuscript; available in PMC 2024 May 01.

Published in final edited form as:

*Am J Med Genet A*. 2023 May ; 191(5): 1227–1239. doi:10.1002/ajmg.a.63130.

# A mutational hotspot in *AMOTL1* defines a new syndrome of orofacial clefting, cardiac anomalies and tall stature

**Alanna Strong**[1,2,3], **Soumya Rao**[4], **Sandra von Hardenberg**[5], **Dong Li**[1,2,3], **Liza L. Cox**[4], **Paul C. Lee**[6], **Li Q. Zhang**[4], **Waheed Awotoye**[7], **Tamir Diamond**[3,8], **Jessica Gold**[1], **Catherine Gooch**[6], **Lord Jephthah Joojo Gowans**[9], **Hakon Hakonarson**[1,2,3,10], **Anne Hing**[11], **Kathleen Loomes**[3,8], **Nicole Martin**[12,13], **Mary L. Marazita**[14,15], **Tarja Mononen**[16], **David Piccoli**[3,8], **Rolph Pfundt**[17], **Salmo Raskin**[18], **Stephen W. Scherer**[19,20], **Nara Sobriera**[21], **Courtney Vaccaro**[2], **Xiang Wang**[2], **Deborah Watson**[2], **Rosanna Weksberg**[13,22], **Elizabeth Bhoj**[1,2,3,23], **Jeffrey C. Murray**[24], **Andrew C. Lidral**[25], **Azeez Butali**[26], **Michael F. Buckley**[27], **Tony Roscioli**[27,28,29], **David A. Koolen**[17], **Laurie H. Seaver**[30,31], **Cynthia A. Prows**[32], **Rolf W. Stottmann**[32,33,34,35], **Timothy C. Cox**[4,36]

[1]The Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

[2]The Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

[3]Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania

[4]Department of Oral & Craniofacial Sciences, School of Dentistry, University of Missouri-Kansas City Kansas City, Missouri

[5]Department of Human Genetics, Hannover Medical School, Hannover, Germany

[6]Division of Genetics and Genomic Medicine, Department of Pediatrics, Washington University School of Medicine, St Louis, Missouri

[7]Department of Orthodontics, College of Dentistry, University of Iowa, Iowa City, Iowa

**Corresponding Authors:** Timothy C. Cox, Department of Oral & Craniofacial Sciences, School of Dentistry, University of Missouri-Kansas City, 625 East 25th Street, Kansas City, Missouri, 64108, USA, coxtc@umkc.edu, Phone: 816-235-2068; Alanna Strong, The Division of Human Genetics, Children's Hospital of Philadelphia, 3615 Civic Center Blvd, Philadelphia, Pennsylvania, 19104, USA, strong.alanna@gmail.com, Phone: 215-590-2920.

**Author Contribution Statement:** Conceptualization (A.S., L.H.S, C.A.P., R.S., T.C.C) Data Curation (A.S., S.R, S.H., D.L, L.L.C., P.C.L., L.Q.Z., W.A., T.D., J.G., C.G., J.J.G., H.H., A.H., K.L., N.M., M.L.M., T.M., D.P., R.P., S.R., S.W.S., N.S., C.V., X.W., D.W., R.W., E.B., J.C.M., A.L., A.B., M.B., T.R., D.A.K., L.H.S., C.A.P., R.S., T.C.C). Formal Analysis (A.S., S.R., S.H., D.L., L.L.C, L.Q.Z, C.G., C.V., X.W., D.W., D.A.K., L.H.S., C.A.P., T.C.C). Writing-original draft (A.S, L.H.S., C.A.P., R.W.S., T.C.C). Writing-reviewing and editing (A.S., S.R. S.H., P.L., C.G., D.A.K., L.H.S, C.A.P., R.W.S., T.C.C).

**Ethics Approval Statement**: The Institutional Review Board of the Children's Hospital of Philadelphia approved this study (Protocol number 16-013278). All other institutions involved in human participant research received local IRB approval and signed appropriate consent forms.

**Patient Consent Statement:** Informed consent was obtained from all participants and/or their legal guardians. All studies were performed in accordance with the Declaration of Helsinki. Permission for use of clinical photographs was given separately. All clinical data was deidentified as part of this study.

**Conflict of Interest Disclosure:** S.W.S. is on the Scientific Advisory Committee of Population Bio and serves as a Highly Cited Academic Advisor for the King Abdulaziz University. The authors declare no financial or competing interests related to this manuscript.

[8]Division of Gastroenterology, Hepatology and Nutrition. Children's Hospital of Philadelphia, Philadelphia, PA

[9]Department of Biochemistry and Biotechnology, Kwame Nkurumah University of Science and Technology, Kumasi, Ghana

[10]Division of Pulmonary Medicine, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

[11]Division of Craniofacial Medicine, Department of Pediatrics, University of Washington, Seattle, Washington

[12]Division of Clinical & Metabolic Genetics and Department of Genetic Counselling, The Hospital for Sick Children, Toronto, Ontario, Canada

[13]Institute of Medical Sciences and Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada

[14]Department of Oral and Craniofacial Sciences, Center for Craniofacial and Dental Genetics School of Dental Medicine, Pittsburgh, Pennsylvania

[15]Department of Human Genetics, School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

[16]Department of Clinical Genetics, Kuopio University Hospital, Kuopio, Finland

[17]Department of Human Genetics, Radboud university medical center, Nijmegen, The Netherlands

[18]Assistance Center for Cleft Lip and Palate (CAIF), Curitiba-PR, Brazil

[19]The Centre for Applied Genomics and Department of Genetics & Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada

[20]McLaughlin Centre and Dept. of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada

[21]McKusick-Nathans Department of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland

[22]Division of Clinical & Metabolic Genetics, Department of Pediatrics, and Genetics and Genome Biology Program, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada

[23]Division of Genomic Diagnostics and Department of Pathology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

[24]Department of Pediatrics, University of Iowa, Iowa City, Iowa

[25]Lidral Orthodontics, Rockford, Michigan

[26]Departments of Oral Pathology, Radiology and Medicine, College of Dentistry & Pediatrics, Carver College of Medicine, University of Iowa, Iowa City, Iowa

[27]NSW Health Pathology Genomics Laboratory, Prince of Wales Hospital, Randwick, NSW, Australia

[28]Centre for Clinical Genetics, Sydney Children's Hospital, Randwick, NSW, Australia

[29.]Neuroscience Research Australia and Prince of Wales Clinical School, University of New South Wales, Kensington, NSW, Australia

[30.]Spectrum Health Helen DeVos Children's Hospital, Grand Rapids, Michigan

[31.]Department of Pediatrics and Human Development, Michigan State University College of Human Medicine, Grand Rapids, Michigan

[32.]Divisions of Human Genetics and Patient Services, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

[33.]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

[34.]Steve & Cindy Rasmussen Institute for Genomic Medicine, Nationwide Children's Hospital, Columbus, Ohio

[35.]Department of Pediatrics, The Ohio State University School of Medicine, Columbus, Ohio, USA

[36.]Department of Pediatrics, School of Medicine, University of Missouri-Kansas City Kansas City, Missouri, 64108, USA

## Abstract

*AMOTL1* encodes angiomotin-like protein 1, an actin-binding protein that regulates cell polarity, adhesion, and migration. The role of *AMOTL1* in human disease is equivocal. We report a large cohort of individuals harboring heterozygous *AMOTL1* variants and define a core phenotype of orofacial clefting, congenital heart disease, tall stature, auricular anomalies, and gastrointestinal manifestations in individuals with variants in *AMOTL1* affecting amino acids 157 – 161, a functionally undefined but highly conserved region. Three individuals with *AMOTL1* variants outside this region are also described who had variable presentations with orofacial clefting and multi-organ disease. Our case cohort suggests that heterozygous missense variants in *AMOTL1*, most commonly affecting amino acid residues 157 – 161, define a new orofacial clefting syndrome, and indicates an important functional role for this undefined region.

### Keywords

orofacial clefting; YAP; congenital heart disease; cleft lip; cleft palate; exome sequencing; genome sequencing

## Introduction:

Cleft lip with or without cleft palate (CL/P) is one of the most common birth defects with an incidence of 1 in 700 – 1000 births (Tolarova and Cervenka, 1998; Genisca et al., 2009; Mai et al., 2019). The etiology of CL/P is heterogenous and includes monogenic, polygenic, and environmental causes (Leslie, 2022). CL/P can occur in isolation; however, 30 – 50% of cases represent an underlying genetic syndrome and carry high risk of multi-organ involvement (Jugessur and Murray, 2005). Syndromes commonly associated with CL/P include Wolf-Hirschhorn syndrome, Opitz GBBB syndrome, and Van der Woude syndrome as well as Trisomy 13 (Schutte and Murray, 1999). Frequent comorbidities include developmental delay, congenital heart disease, limb anomalies, and urogenital

defects (Burg et al., 2016). Multiple signaling pathways have been implicated in the pathogenesis of CL/P, including BMP, Wnt, SHH and Hippo signaling (Jugessur et al., 2009). Despite advances in next generation sequencing and our understanding of biological pathways, a definitive molecular diagnosis currently cannot be made in greater than 60% of cases with clear evidence of heritability and in greater than 90% of simplex cases, highlighting our incomplete understanding of the genetics of CL/P (Cox et al., 2018).

Human *AMOTL1* maps to chromosome 11q21 and encodes the protein angiomotin-like protein 1 (AMOTL1), one of three members of the AMOT protein family that exhibit some functional redundancy (Bratt et al., 2002). AMOTL1 is a cytosolic protein in mesenchymal cells but localizes to tight junctions and adherens junctions in polarized epithelia (Hirate et al, 2013). Targeted studies reveal a critical role for *AMOTL1* in tubulogenesis and angiogenesis; however, a function in other tissues has not been widely examined (Wang et al., 2011; Zhao et al, 2011; Huang et al., 2018). AMOTL1, like the two other related proteins AMOT and AMOTL2, has been shown to bind to and regulate nucleocytoplasmic shuttling and turnover of YAP, a key component of the Hippo signaling pathway. *AMOTL1* overexpression also inhibits Wnt signaling by promoting the degradation of β-catenin (Wang et al., 2011; Zhao et al, 2011; Huang et al., 2018). Animal models have so far not proven successful in dissecting the role of *AMOTL1* during development, perhaps in part due to functional redundancy among AMOT family members. Furthermore, the extent of *AMOTL1*-related phenotypes in humans has yet to be fully appreciated (Zheng et al., 2009; Zheng et al., 2016; Liegel et al., 2019).

To date, two heterozygous *AMOTL1* variants – one affecting amino acid position 157 and another affecting amino acid position 160 – have been reported in individuals with some phenotypic overlap, including CL/P and congenital heart disease (Liegel et al., 2019; Rips et al., 2020). Here we describe 16 individuals from 12 families with heterozygous variants in *AMOTL1*. From these cases, we propose that pathogenic variants in *AMOTL1*, most commonly missense variants affecting amino acid 157, underlie a novel orofacial clefting syndrome.

## Materials and Methods:

### Ethical Compliance:

The Institutional Review Board of the Children's Hospital of Philadelphia approved this study (Protocol number 16-013278). All other institutions involved in human participant research received local IRB approval and signed appropriate consent forms.

### Editorial Policies and Ethical Considerations:

Informed consent was obtained from all participants and/or their legal guardians. All studies were performed in accordance with the Declaration of Helsinki. Permission for use of clinical photographs was given separately. All clinical data was deidentified as part of this study.

### Primary Cohort:

Patients were referred for evaluation by clinical geneticists due to multiple congenital anomalies. All patients were diagnosed through exome or genome sequencing. Patients 1 and 12 were evaluated at The Children's Hospital of Philadelphia. Trio exome sequencing for Patient 1 was initially performed at The Children's Hospital of Philadelphia Genetics laboratory (2019). Repeat duo exome sequencing was performed by GeneDx. Proband-only exome sequencing for Patient 12 was performed in The Center for Applied Genomics at The Children's Hospital of Philadelphia. Patients 2 - 4 and Patient 16 were evaluated at Radboud University Medical Center. Trio exome sequencing was performed following routine diagnostic procedures as described previously (Neveling et al., 2013). Patient 5 was evaluated at Kuopio University Hospital. Patients 6 – 8 (Family ID #6104) were ascertained at Seattle Children's Craniofacial Center and subject to exome sequencing through the Center for Mendelian Genomics at the University of Washington (Seattle) as part of a large study of 72 multi-affected, multigenerational families presenting with CL/P (Cox et al., 2018). Patient 9 was evaluated at Washington University in St. Louis. Duo exome sequencing was performed by GeneDx. Patient 10 was evaluated at The Hospital for Sick Children (SickKids) in Toronto, Canada. The *AMOTL1* variant was identified via research genome sequencing at the Center for Applied Genomics/SickKids as part of the Autism Speaks MSSNG project (research.mss.ng). Patient 11 was evaluated at Spectrum Health Helen DeVos Children's Hospital, with trio exome sequencing performed by GeneDx. Patient 13 was ascertained from the NIH Gabriella Miller Kids First cohort (see Secondary Validation Cohort section below). Patient 14 was ascertained from a related consortium of CL/P studies from multiple Centers under the auspices of the Baylor-Hopkins Center for Mendelian Genomics. Patient 15 was evaluated at Medizinische Hochschule Hannover. Exome sequencing was performed by Hannover Medical School. Center-specific bioinformatic pipelines were applied to respective cases. The majority of cases were brought together into the cohort described here through GeneMatcher (Sobreira et al., 2015). Other cases were assembled through clefting cohorts (Seattle Children's Craniofacial Center, Center for Applied Genomics/SickKids Autism Speaks MSSNG project, NIH Gabriella Miller Kids First cohort, and Baylor-Hopkins Center for Mendelian Genomics). The previously published patients with *AMOTL1* variants are included in Table 1. Numbering of all reported variants in *AMOTL1* are relative to RefSeq transcript NM_130847.3. The AMOTL1 protein structure prediction was generated using i-TASSER (https://zhanggroup.org/I-TASSER/).

### Secondary Validation Cohort:

A variant search was also performed on three CL/P cohorts available as part of the NIH Gabriela Miller Kid's First program, representing genome sequencing data from 876 trios: dbGaP accession numbers - phs001420.v1.p1 (262 trios, Latin American ancestry); phs001168.v2.p2 (376 trios, European ancestry); phs001997.v1.p1 (238 trios, African and Asian ancestry). Initial searches were performed in the first two datasets using the variant explorer in the Kid's First portal (https://portal.kidsfirstdrc.org/variant) and analyzed using the Cavatica platform. To select only rare, potentially high impact variants, we initially used an allele frequency cutoff of $1x10^{-4}$ in gnomAD v3.1, and a CADD score >15. Searchable summary data on the African-Asian cohort was not available through the Kid's First portal

at the time of these analyses. Furthermore, the identity of the individual(s) harboring each variant cannot be determined on the Cavatica platform at this time. Consequently, access to the genome sequencing data for all 876 trios was requested and approved, and then the entire datasets were downloaded to determine the trios and the individuals within each trio that harbored identified *AMOTL1* variants.

## Results:

### Variants Affecting Amino Acids 157 and neighboring residues:

We identified a hotspot for variants in *AMOTL1* affecting amino acids 157 - 161. Together with two previously reported cases (Liegel et al., 2019; Rips et al., 2021), these constitute 3 recurring variants: p.(Arg157Cys) in 3 new families plus 1 previously-reported case), p.(Arg157His) in 4 families, and p.(Pro160Leu) in 1 new family plus 1 previously-reported individual. We additionally identified a variant affecting the adjacent amino acid (p.(Gln161Arg)) in a similarly affected adult. The most common clinical features in the 16 patients from these families are orofacial clefting (15/16; 94%), large and dysplastic ears (10/16; 62.5%), congenital heart disease (8/16; 50%), tall stature (7/16; 44%), hearing loss (5/16; 31%), liver disease (5/16; 31%), and neurodevelopmental disease (5/16; 31%). Additional findings are presented below in brief case descriptions and are summarized in Table 1 and Supplemental Tables 1 and 4.

### Patient 1 (Family A):

Patient 1 (male) presented with bilateral CL/P, bilateral coronal craniosynostosis, myopia, bilateral mild hearing loss, recurrent otitis media, constipation, elevated aminotransferases, unilateral cryptorchidism, recurrent fevers, easy bruising, attention deficit/hyperactivity disorder, and global developmental delay. Growth parameters at last examination at 6 years of age were notable for a weight of 40.9 kg (>98%), a height of 127.8 cm (94%), and a head circumference of 53.5 cm (75%). Physical examination was notable for 2 hair whorls (anterior and posterior/temporal), a round face with flattened midface, hypertelorism, proptosis, blue sclera, flattened and deviated nasal tip, inverted nipples, and tapered fingers (Figure 1). Research-based exome reanalysis within the Center for Applied Genomics with emphasis on ciliopathy-associated genes was unrevealing. Repeat duo exome sequencing through GeneDx was notable for a missense variant of uncertain significance (VUS) in *AMOTL1* (c. 469 C>T; p.(Arg157Cys)) that was not maternally-inherited and a missense VUS in *CAMK2G* (c.269 T>C; p.(Phe90Ser)), also not maternally-inherited.

### Patients 2 – 4 (Family B):

Patient 2 (male) was born at 32 + 4 weeks gestation and has a history of intraventricular hemorrhage with post-hemorrhagic ventricular dilation, unilateral left cleft lip, bilateral cleft palate, congenital mixed bilateral hearing loss requiring cochlear implants, atrial septal defect, premature adrenarche, recurrent candida infections, neutropenia, and global developmental delay. His sister (Patient 3) has bilateral CL/P, preauricular skin tag, congenital mixed bilateral hearing loss, atrial septal defect, feeding difficulties requiring G-tube placement, recurrent candida infections, and global developmental delay. His mother (Patient 4) has severe myopia, high palate, cholestasis, and scoliosis. Quad

exome sequencing demonstrated a shared missense VUS in *AMOTL1* (c. 469 C>T; p.(Arg157Cys)). Patient 2 was also found to harbor a heterozygous nonsense variant in *KANSL1* (c.3169C>T; p.(Gln1057*)) and a heterozygous splice variant in *SMURF2* (c.773-2A>C (r.spl?)). DNA methylation profile was not consistent with Koolen-de Vries syndrome.

**Patient 5 (Family C):**

Patient 5 (male) presented with submucosal cleft, bifid uvula, atrial septal defect, mitral valve prolapse, and scoliosis. Physical examination was notable for tall stature, prominent eyes, maxillary hypoplasia, and large ears. Exome sequencing showed a *de novo* variant in *AMOTL1* (c.469 C>T; p.(Arg157Cys)).

**Patients 6 – 8 (Family D):**

Patient 6 (female) was diagnosed at birth with bilateral cleft lip/alveolus and cleft palate. Mother (Patient 7) has unilateral right-sided cleft lip, cleft palate, hypodontia, atrial septal defect, and pulmonic stenosis. Maternal uncle (Patient 8) has unilateral left-sided cleft lip, cleft palate, hypodontia (missing upper right #1, left # 1 and 2), an auricular anomaly, and large ears. Exome sequencing demonstrated a shared missense VUS in *AMOTL1* (c.470 G>A; p.(Arg157His)) in the patient, her mother and maternal uncle. The grandfather was deceased, was not described by family as having clinical features of note, and no sample was available for testing. The proband's maternal grandmother presented with myopia and eczema but without CL/P. Subsequent genetic testing on DNA isolated from whole blood failed to identify the familial *AMOTL1* variant in the grandmother, indicating possible genetic mosaicism or subclinical disease in the grandfather.

**Patient 9 (Family E):**

Patient 9 (male) presented at 20-years of age with a history of right CL/P, dysmorphic features, eustachian tube dysfunction, poor dentition, atrial septal defect, and left sided congenital diaphragmatic hernia. Development had been normal. Duo exome sequencing was notable for a missense VUS in *AMOTL1* (c.470 G>A; p.(Arg157His)) that was not maternally-inherited. Follow-up testing of the father showed the variant to be *de novo* in the proband.

**Patient 10 (Family F):**

Patient 10 (female) was seen at 7-years of age due to dysmorphic features, bifid uvula, atrial septal defect, portocaval fistula, liver fibrosis, advanced bone age, tall stature, autism, and developmental delay. Birth history was notable for large size and NICU admission for three weeks for feeding difficulties requiring NG tube supplementation. Growth parameters at 7 years of age were notable for a height of 136 cm (97%) and a weight of 36.7 kg (99%). Physical examination was notable for a high anterior hairline, broad forehead, prominent metopic suture, bitemporal narrowing, sparse temporal hair, hypoplastic supraorbital ridges, epicanthal folds, telecanthus, left ptosis, anisocoria, downslanting palpebral fissures, ectropion lower eyelids, broad and depressed nasal bridge with bulbous tip, a small nose with a hooked appearance, bifid uvula, short and poorly formed philtrum,

thin upper lip vermilion with a midline ridge in the upper lip and downturned corners of the mouth, microretrognathia, widely spaced and inverted nipples, umbilical hernia, long tapered fingers, proximally placed thumbs, bilateral fifth digit clinodactyly, clinodactyly of multiple digits, and pes planus. Brain MRI was notable for subtle pituitary abnormalities, narrow optic chiasm, dysplastic corpus callosum, and severe thinning and flattening of cervicomedullary junction. Genetic testing included a normal karyotype, SNP microarray, Fragile X testing, and *EZH2* sequencing. Methylation testing revealed hypomethylation at imprinting center 2 (IC2) on chromosome 11p15.5, consistent with a diagnosis of Beckwith-Wiedemann syndrome. Given the severity of the patient's presentation and the multiple features not explained by Beckwith-Wiedemann syndrome trio genome sequencing was performed, which identified a *de novo* VUS in *AMOTL1* (c.470 G>A; p.(Arg157His)).

**Patient 11 (Family G):**

Patient 11 (male) had bilateral CL/P, tetralogy of Fallot, bilateral aplasia/hypoplasia of the distal nasolacrimal duct, right sensorineural hearing loss, obstructive sleep apnea, feeding difficulties requiring G-tube placement and Nissen fundoplication, elevated aminotransferases, chronic constipation, dysmotility, Chilaiditi syndrome (colon abnormally placed between the liver and diaphragm), hydronephrosis, global developmental delay, attention deficit hyperactivity disorder, autism, and severe intellectual disability. Patient never acquired fluent speech. Physical examination at 18 years of age was notable for asymmetric posterior plagiocephaly, two posterior parietal hair whorls, proptosis, hypertelorism, telecanthus, ectropion, prominent ears with mild underfolding, midface hypoplasia, and prognathism. Chromosomal microarray was non-diagnostic. At last evaluation at 18-years of age height was 186 cm (90%) and weight was 55.8 kg (10%). Trio exome sequencing detected a *de novo* variant in *AMOTL1* (c.479 C>T; p.(Pro160Leu)) and a maternally-inherited VUS in *TRIP12*. Patient died at 19 years of age from complications of abdominal compartment syndrome.

**Patient 12 (Family H):**

Patient 12 is a 26-year-old female with a history of bifid uvula, submucosal cleft palate, myopia, posterior embryotoxon, ptosis, sensorineural hearing loss, neonatal cholestasis with persistently elevated aminotransferases, bile duct paucity, vesicoureteral reflux, dysphagia, esophageal dysmotility, and digit contractures. Patient had mild speech delay secondary to undiagnosed hearing loss. Growth parameters at last examination at 26 years of age were notable for a weight of 74.8 kg (90 – 95%), a height of 174.4 cm (97%), and a head circumference of 55 cm (50%). Physical examination was notable for ptosis, bifid uvula, micrognathia, prominent ears with thin helices, curved toes, and bilateral contractures of the fifth upper extremity digits (Figure 1). Prior clinical genetic testing included *JAG1* sequencing (normal), *FLNA* sequencing (normal) and FISH for 17p11.2 and 22q11.2 deletion (normal). Research-based exome sequencing performed at the Center for Applied Genomics was notable for a missense VUS in *AMOTL1* (c. 482 A>G; p.(Gln161Arg)). There were no variants identified in *NOTCH2*. Parental samples were not available.

### Variants Affecting Other Amino Acids:

We identified 3 additional patients (Patients 14 – 16) carrying rare, potentially pathogenic missense variants in *AMOTL1*. One of these variants (c.1102 C > G; p.(Pro368Ala)), identified in a patient presenting with unilateral CL/P, is predicted to disrupt one of the two PPEY motifs that are reported to be important for AMOTL1 function (Chan et al., 2011) (Figure 2A, Supplemental Figure 2). The inheritance status of this variant is unknown. The remaining 2 variants, which were both *de novo*, involve side chain charge changes (c.1519 G > A; p.(Glu507Lys), and c.1735 G > A; p.(Glu579Lys)) and localize to the actin-binding BAR domain in close proximity to each other in the predicted tertiary structure (Supplemental Figure 2). The patient carrying the *de novo* p.(Glu579Lys) variant presented with bifid uvula, constipation, obsessive behavior and developmental delay, whereas the patient carrying the *de novo* p.(Glu507Lys) variant had a complex presentation including cardiac, neurological, kidney and pulmonary findings but did not present with CL/P or related phenotypes. Additional findings are summarized in Table 2 and Supplemental Table 2. Each of these variants impact invariant residues across the AMOT protein family and across species (see Figure 2B).

### Variants Identified in the NIH Gabriela Miller Kid's First CL/P Cohort:

Genome sequencing data from the NIH Gabriella Miller Kid's First orofacial cleft collections (total 876 trios) were queried as a validation cohort. Five variants were identified in affected probands (Supplemental Table 3) that met the filtering criteria (allele frequency <1×10-4 and CADD >15). The CADD scores for these variants ranged between 20.9 and 27.8. Two variants were seen between 2 and 4 times in the combined gnomAD v2.1 and v3.1 databases, respectively, and were absent from TOPMED. The other three represent novel rare variants (Supplemental Table 3). Among these five variants was the *AMOTL1* c.470G>A; p.(Arg157His) variant, the same variant identified in our primary cohort, supporting the biological relevance of this residue to the pathogenesis of CL/P. The individual harboring this variant was of European ancestry and presented with isolated bilateral CL/P and is presented in Table 1 as Patient 13. The variant was inherited from his reportedly unaffected mother. CL/P, although variable in severity, was the only presentation noted in all five cases (Supplemental Table 3).

## Discussion:

*AMOTL1* encodes the protein angiomotin-like protein 1, which plays a critical role in cell polarity, adhesion, and migration. To date, two heterozygous variants in *AMOTL1* affecting amino acids 157 and 160 have been reported in patients with some clinical overlap including orofacial clefting and multi-organ disease (Liegel et al., 2019; Rips et al., 2021); however, the pathogenicity and the full spectrum of clinical features associated with *AMOTL1* variants remains ill-defined. Here, we present a total of 16 cases of children and adults across 12 families with heterozygous missense *AMOTL1* variants (Tables 1 and 2, Supplementary Tables 1, 2, 3, and 4, Supplemental Figure 1 and 2). Of significance, this expanded cohort together with the previously published cases suggest a novel syndromic clefting disorder predominantly caused by heterozygous missense variants affecting amino acid residues 157, 160 and 161 of *AMOTL1*. Further highlighting a pathogenic role for

*AMOTL1* variants in orofacial clefting was the finding of rare missense *AMOTL1* variants in a large cohort of patients with isolated CL/P, which included one of the same missense variants, p.(Arg157His), seen in our primary cohort. The remaining 3 individuals from our primary cohort have heterozygous *AMOTL1* missense variants outside this hotspot region but in highly conserved residues within known functional domains. This cohort shares some features seen in individuals with variants affecting amino acids 157 – 161 (clefting, constipation, developmental delay) but also includes an individual with severe multi-organ disease, which is rarer in the 157 – 161 cohort (congestive heart failure, respiratory insufficiency, consumptive coagulopathy). Further cases will be needed to better define the phenotypic spectrum of *AMOTL1* variants that localize outside the hotspot region.

Our cohort includes only *AMOTL1* missense variants; individuals with specific *AMOTL1* deletions or nonsense and frameshift variants have not been described. Indeed, *AMOTL1* has a pLI score of 0, suggesting some tolerance to loss-of-function. *AMOTL1* maps to 11q21, and individuals with copy number variations within this interval that include the *AMOTL1* locus have been described, with many probands presenting with orofacial clefting and/or upper lip anomalies (Hertz et al., 1995; Li et al., 2006; Kirk et al., 2020; Firth et al, 2009). Intriguingly, *YAP*, which encodes an AMOT-family binding protein, is located ~7.5 Mb from *AMOTL1* on chromosome 11 and is also deleted in many, although not all, of these patients. Heterozygous nonsense variants in *YAP* have previously been associated with CL/P (Williamson et al., 2014). Further studies are needed to clarify the relative contributions of *AMOTL1* and *YAP* to these phenotypes. It is, however, noteworthy that duplications of 11q21 spanning *AMOTL1*, including some that exclude *YAP*, are associated with tall stature, bifid uvula, intellectual disability, and lip anomalies (Decipher database, Firth et al, 2009). This observation and the hypothesized tolerance of *AMOTL1* to loss-of-function variants (pLI of 0; LOEUF of 0.47) support the notion of a gain-of-function impact for some of our described variants, most likely those affecting amino acid residues 157 – 161, although further studies aimed at elucidating molecular mechanisms are required to resolve this.

## Proposed Mechanism of Pathogenesis in *AMOTL1*-related Disease:

The precise mechanism by which pathogenic *AMOTL1* variants cause disease is currently unknown. AMOTL1 disruption impacts cellular adhesion and cell migration (Huang et al., 2018), cellular processes that are critical for almost all embryonic tissue morphogenetic events, including development of the lip, palate, and heart (Ohashi et al., 2017; Antiguas et al., 2022). AMOTL1 is an actin-binding scaffold protein that interacts with multiple protein partners to control cell polarity and cellular mechano-responsiveness. In epithelia and endothelia, AMOTL1 localizes to both tight junctions and adherens junctions (Wang et al., 2011; Zhao et al, 2011). *AMOTL1* is a negative regulator of Wnt/β-catenin signaling (Li et al., 2012) and has been directly implicated as a regulator of the YAP/Hippo signaling pathway through its interaction with a number of key effector proteins of this pathway. The role of *AMOTL1* in YAP signaling is complex: in some contexts *AMOTL1* serves as a YAP inhibitor by activating the YAP-inhibitor LATS1/2, preventing YAP nuclear translocation, leading to the sequestering of YAP in the cytoplasm for degradation (Chan et al., 2011; Wang et al, 2011; Mana-Capelli et al., 2014; Mana-Capelli and Paramasivam, 2018). In

other contexts, *AMOTL1* has been identified as an activator of YAP signaling, required for YAP nuclear translocation and activity of the YAP-TEAD transcription factor complex (Ragni et al., 2017; Zhou et al., 2020; Xu et al., 2021). Further work is needed to resolve these seemingly dichotomous roles.

Notably, both Wnt/β-catenin and YAP signaling are implicated in the pathogenesis of orofacial clefting (Jugessur et al., 2009). Loss of *Wnt9b* in mice results in an incompletely penetrant and variably expressive spectrum of CL/P that mirrors that seen in humans, and variants in *WNT9B* and *WNT3* have both been associated with CL/P in humans. Similarly, heterozygous nonsense variants in *YAP* cause a multiorgan syndrome that includes CL/P (Williamson et al., 2014). Pathogenic variants in genes encoding components of the Wnt signaling pathway, including *WNT10A*, *WNT10B*, *LRP6* and *AXIN2*, are among the most common causes of hypodontia and oligodontia (Zhu et al., 2017; Lu et al., 2019; Yu et al., 2019; Diaz-Cuadros et al., 2020), which is also seen in at least three individuals from our cohort. Wnt and YAP pathways are also involved in skeleton and bone formation and homeostasis, and dysregulation of these pathways may contribute to the cranioskeletal features seen with *AMOTL1* variants. YAP and Wnt/β-catenin signaling are also critical for proper development of many tissues and organs, including the brain, liver, and heart, which could explain the multi-organ disease seen in individuals with *AMOTL1* missense variants (Figure 3).

The phenotypes of individuals with *AMOTL1* variants affecting amino acids 157 and neighboring residues overlap with other well-known syndromes such as Hardikar syndrome [MIM #301068] caused by pathogenic nonsense and frameshift variants in *MED12* (Li et al., 2021), Opitz GBBB syndrome [MIM #300000] caused by loss of function variants in MID1 (Cox et al., 2000), Teebi hypertelorism syndrome [MIM #145420] caused by pathogenic variants in *SPECC1L* and *CDH11* (Bhoj et al., 2015; Li et al., 2021), and Simpson-Golabi Behmel syndrome [MIM #312870] caused by pathogenic variants in *GPC3* (Pilia et al., 1996). Of note, many of these genes are similarly implicated in YAP signaling, β-catenin signaling, adherens junctions, cytoskeletal organization, and cell migration, consistent with a shared mechanism of disrupted cell signaling and migration at the heart of clefting syndromes (Saadi et al., 2011; Galli et al., 2015; Wilson et al., 2016; Bhoj et al., 2019; Kolluri and Ho, 2019).

Missense variants affecting amino acids 157 and 160 are most commonly represented in our cohort and in previous reports, suggesting a mutational hot-spot and specific importance of these amino acid residues to the function of AMOTL1 (Figure 2A). These residues map within the larger glutamine-rich N-terminus of AMOTL1 within a stretch of amino acids that is one of the most highly conserved regions shared across all species and amongst all members of the AMOT protein family. No function has yet been ascribed to this specific region. An experimentally-derived structure of AMOTL1 is currently not available and no interacting proteins have been identified that bind to this region (Figure 2B). Consequently, a clear pathogenic mechanism, especially for the variants affecting the conserved residues 157-161, cannot be illuminated at this time. Generation of a predicted AMOTL1 structure (using i-TASSER) indicates that the two variants that localize to the BAR domain may reside in close proximity and thus have similar functional impact (Supplemental Figure

2). We nevertheless hypothesize that the described *AMOTL1* variants, particularly those affecting residues 157 – 161, may have a gain-of-function or dominant negative effect in contrast to missense variants affecting other domains and residues, explaining the disparate phenotypes. Further experimental work will be needed to better understand both the normal function of AMOTL1 during embryonic development and the functional impact of these variants.

### *AMOTL1* and neuropsychiatric phenotypes

Seven probands described in our cohort have neurological phenotypes, including 5 individuals with missense variants affecting residues 157 and 160 (Patients 1, 2, 3, 10 and 11) and 2 probands with other missense variants (Patients 15 and 16). Patient 1 has a VUS in *CAMK2G* that was not maternally inherited. Paternal testing could not be performed. Pathogenic variants in *CAMK2G* are associated with autosomal dominant intellectual disability, and so it is plausible that Patient 1's global developmental delay reflects a dual diagnosis and is unrelated to his *AMOTL1* variant. Patient 2 was born prematurely and had an intraventricular hemorrhage, which may be responsible for his developmental delay. He was also found by exome sequencing to have a nonsense variant in *KANSL1*, although the DNA methylation profile was not consistent with Koolen-de Vries syndrome. Patient 11 has a VUS in *TRIP12*, which is associated with autosomal dominant intellectual disability; however, this variant was maternally-inherited and the mother has no neurological phenotype. Patient 11 also had a complex post-operative course after cardiac repair, which may have contributed to his global delays. For Patients 3, 10, 15 and 16 no etiology other than the identified *AMOTL1* variant was reported that could explain the neuropsychiatric phenotypes and structural brain differences.

*De novo* variants in *AMOTL1* have also recently been reported in large exome studies of patients with intellectual disability and autism (Kaplanis et al., 2020; Satterstrom et al., 2020). Of note, at least one of the individuals identified in the Kaplanis intellectual disability cohort – a patient with the recurrent p.(Pro160Leu) variant – also reportedly has orofacial clefting (Dr. P. Kruszka, personal communication). Intellectual disability, developmental delay and autism are also frequent features in patients with copy number variants covering *AMOTL1*. Further work is required to clarify the role of missense *AMOTL1* variants in neurological disease and the penetrance of neurological phenotypes in *AMOTL1*-related disease.

### Animal Models of *AMOTL1*

To date, data from animal models have not completely recapitulated the phenotypes identified in our human cohort. Specifically, murine models of *AMOTL1* deficiency and variant-specific mouse models (p.(Arg157Cys)) did not exhibit the overt cardiac or craniofacial phenotype described in humans (Liegel et al., 2019). Interestingly, the p.(Arg157Cys) mouse mutant exhibited extremely reduced survival through unknown mechanisms while two deletion alleles presumably leading to complete *AMOTL1* loss of function did not affect survival. These data and the heterozygous inheritance of the alleles reported here again may reflect either a gain-of-function or dominant negative mechanism for variants affecting amino acids 157 and 160 of AMOTL1. As mentioned earlier, a gain-of-

function or dominant negative mechanism for *AMOTL1*-associated clefting may explain the disparate phenotypes seen with distinct *AMOTL1* variants (Supplemental Table 2).

The mechanism of lethality in the p.(Arg157Cys) mouse model is unknown. Of note, during embryogenesis YAP is supposed to be excluded from the nucleus of cells that form the inner cell mass, and this is critical for proper embryo development (Tremblay and Camargo, 2012). It is possible that reduced viability reflects dysregulated YAP nuclear exclusion due to disruption of *AMOTL1* function and consequent failure of embryo development. Regarding the absence of clefting and congenital heart disease, it is possible that these are reduced-penetrance traits, with the threshold of activity in mice being different to humans as seen with other CL/P gene mouse models. Indeed, in our cohort not all individuals harboring *AMOTL1* variants manifested clefting or congenital heart disease, with marked variability even within families.

### Clinical Considerations of *AMOTL1*-related Disease

Two previously published cases reported variants in amino acids 157 and 160 of *AMOTL1* and were associated with orofacial clefting, congenital heart disease and multiple congenital anomalies. Our expanded patient cohort strengthens evidence for this association and emphasizes additional features such as distinct dysmorphisms (abnormal head shape, craniosynostosis, hypertelorism, large ears), myopia, hearing loss, micrognathia, immune dysfunction, scoliosis, tall stature, chronic constipation, liver dysfunction and global developmental delay. We recommend that individuals with *AMOTL1* variants, especially those affecting amino acid residues 157 – 161, be screened by: 1) careful examination for evidence of orofacial clefting and velopharyngeal insufficiency, 2) regular eye examinations to evaluate for myopia, 3) regular audiology examinations to screen for hearing loss, 4) echocardiogram and electrocardiogram to screen for congenital heart disease and arrhythmia, 5) screening and aggressive treatment of constipation, 6) evaluation of liver function, 7) scoliosis evaluation and 8) developmental assessment. We anticipate refinement of these recommendations as additional probands are identified.

## Conclusion

In this study, we report 16 additional individuals with *AMOTL1* variants, bringing the total in the literature to 19 individuals. Caveats of the study include limited clinical information for some individuals, the lack of confirmed inheritance pattern in 6 patients and unresolved mechanism of disease. Although our data indicate decreased penetrance of CL/P in individuals with missense *AMOTL1* variants affecting amino acids 157 and 160, incomplete penetrance is a common feature of most monogenic causes of CL/P. Nevertheless, orofacial clefting is the most common feature seen in our cohort, although this may in part be due to selection bias. Despite these limitations, we propose that *AMOTL1* variants affecting amino acids 157, 160 and 161 cause a recognizable phenotype of orofacial clefting, congenital heart disease, and tall stature with variable dysmorphic features, gastrointestinal involvement, and developmental delay. We further suggest that variants affecting other residues of *AMOTL1* cause multiorgan disease with or without

CL/P. Increased characterization of the spectrum of *AMOTL1*-related disease is critical for anticipatory guidance and appropriate screening for comorbidities.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements:

The authors wish to acknowledge the participating families for their time and contribution to this study and to the generosity of all donors who supported this program. The authors also wish to acknowledge the resources of MSSNG (www.mss.ng), Autism Speaks, The Cleft Palate Foundation (AL), and The Centre for Applied Genomics at The Hospital for Sick Children, Toronto, Canada. Special thanks to Drs Lina Moreno Uribe and Aline Petrin, University of Iowa, for sample management and shipment.

**Grant Support:**

The authors also wish to acknowledge their funding sources: NIH - K08DK128606 (AS), R01 DE027091 (RWS), R01 DE027879 (TCC), R01-DE014667 (ACL), R37-DE008559 (JCM), R01-016148, X01-HL132363, X01-HL136465 (MLM), eMERGE, U01HG011172, and R01HG010166 (CP), the Australian National Health & Medical Research Council - AU/1/BA51117 (TR, MFB, TCC, JCM, and ACL), the March of Dimes Basil O'Connor award #FY 98-0718 and research grant #6-FY01-616 (ACL), and the Stowers Family Foundation Endowment (TCC)

## Data Availability Statement:

Individual genomic data summaries may be provided upon request where individual consent permits. Genomic and summary clinical data from the NIH Gabriella Miller Kid's First orofacial cleft cohorts is freely available upon request to the GMKF Program (https://kidsfirstdrc.org/).

## References:

Whitney G, Wang S, Chang H, Cheng KY, Lu P, Zhou XD, Yang WP, McKinnon M, & Longphre M (2001). A new siglec family member, siglec-10, is expressed in cells of the immune system and has signaling properties similar to CD33. European journal of biochemistry, 268(23), 6083–6096. [PubMed: 11733002]

Bhoj EJ, Li D, Harr MH, Tian L, Wang T, Zhao Y, Qiu H, Kim C, Hoffman JD, Hakonarson H, & Zackai EH (2015). Expanding the SPECC1L mutation phenotypic spectrum to include Teebi hypertelorism syndrome. American journal of medical genetics. Part A, 167A(11), 2497–2502. [PubMed: 26111080]

Bhoj EJ, Haye D, Toutain A, Bonneau D, Nielsen IK, Lund IB, Bogaard P, Leenskjold S, Karaer K, Wild KT, Grand KL, Astiazaran MC, Gonzalez-Nieto LA, Carvalho A, Lehalle D, Amudhavalli SM, Repnikova E, Saunders C, Thiffault I, Saadi I, … Verloes A (2019). Phenotypic spectrum associated with SPECC1L pathogenic variants: new families and critical review of the nosology of Teebi, Opitz GBBB, and Baraitser-Winter syndromes. European journal of medical genetics, 62(12), 103588. [PubMed: 30472488]

Bratt A, Wilson WJ, Troyanovsky B, Aase K, Kessler R, Van Meir EG, & Holmgren L (2002). Angiomotin belongs to a novel protein family with conserved coiled-coil and PDZ binding domains. Gene, 298(1), 69–77. [PubMed: 12406577]

Burg ML, Chai Y, Yao CA, Magee W 3rd, & Figueiredo JC (2016). Epidemiology, Etiology, and Treatment of Isolated Cleft Palate. Frontiers in physiology, 7, 67. [PubMed: 26973535]

Chan SW, Lim CJ, Chong YF, Pobbati AV, Huang C, & Hong W (2011). Hippo pathway-independent restriction of TAZ and YAP by angiomotin. The Journal of biological chemistry, 286(9), 7018–7026. [PubMed: 21224387]

Cox LL, Cox TC, Moreno Uribe LM, Zhu Y, Richter CT, Nidey N, Standley JM, Deng M, Blue E, Chong JX, Yang Y, Carstens RP, Anand D, Lachke SA, Smith JD, Dorschner MO, Bedell B, Kirk E, Hing AV, Venselaar H, … Roscioli T (2018). Mutations in the Epithelial Cadherin-p120-Catenin Complex Cause Mendelian Non-Syndromic Cleft Lip with or without Cleft Palate. American journal of human genetics, 102(6), 1143–1157. [PubMed: 29805042]

Diaz-Cuadros M, Wagner DE, Budjan C, Hubaud A, Tarazona OA, Donelly S, Michaut A, Al Tanoury Z, Yoshioka-Kobayashi K, Niino Y, Kageyama R, Miyawaki A, Touboul J, & Pourquié O (2020). In vitro characterization of the human segmentation clock. Nature, 580(7801), 113–118. [PubMed: 31915384]

Firth HV, Richards SM, Bevan AP, Clayton S, Corpas M, Rajan D, Van Vooren S, Moreau Y, Pettett RM, & Carter NP (2009). DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. American journal of human genetics, 84(4), 524–533. [PubMed: 19344873]

Galli GG, Carrara M, Yuan WC, Valdes-Quezada C, Gurung B, Pepe-Mooney B, Zhang T, Geeven G, Gray NS, de Laat W, Calogero RA, & Camargo FD (2015). YAP Drives Growth by Controlling Transcriptional Pause Release from Dynamic Enhancers. Molecular cell, 60(2), 328–337. [PubMed: 26439301]

Genisca AE, Frías JL, Broussard CS, Honein MA, Lammer EJ, Moore CA, Shaw GM, Murray JC, Yang W, Rasmussen SA, & National Birth Defects Prevention Study (2009). Orofacial clefts in the National Birth Defects Prevention Study, 1997–2004. American journal of medical genetics. Part A, 149A(6), 1149–1158. [PubMed: 19441124]

Hertz JM, Tommerup N, Sørensen FB, Henriques UV, Nielsen A, & Therkelsen AJ (1995). Partial deletion 11q: report of a case with a large terminal deletion 11q21-qter without loss of telomeric sequences, and review of the literature. Clinical genetics, 47(5), 231–235. [PubMed: 7554347]

Hirate Y, Hirahara S, Inoue K, Suzuki A, Alarcon VB, Akimoto K, Hirai T, Hara T, Adachi M, Chida K, Ohno S, Marikawa Y, Nakao K, Shimono A, & Sasaki H (2013). Polarity-dependent distribution of angiomotin localizes Hippo signaling in preimplantation embryos. Current biology : CB, 23(13), 1181–1194. [PubMed: 23791731]

Huang T, Zhou Y, Zhang J, Cheng ASL, Yu J, To KF, & Kang W (2018). The physiological role of Motin family and its dysregulation in tumorigenesis. Journal of translational medicine, 16(1), 98. [PubMed: 29650031]

Jugessur A, Murray JC. Orofacial clefting: recent insights into a complex trait. Curr Opin Genet Dev. 2005;15(3):270–278. [PubMed: 15917202]

Jugessur A, & Murray JC (2005). Orofacial clefting: recent insights into a complex trait. Current opinion in genetics & development, 15(3), 270–278. [PubMed: 15917202]

Kaplanis J, Samocha KE, Wiel L, Zhang Z, Arvai KJ, Eberhardt RY, Gallone G, Lelieveld SH, Martin HC, McRae JF, Short PJ, Torene RI, de Boer E, Danecek P, Gardner EJ, Huang N, Lord J, Martincorena I, Pfundt R, Reijnders MRF, … Retterer K (2020). Evidence for 28 genetic disorders discovered by combining healthcare and research data. Nature, 586(7831), 757–762. [PubMed: 33057194]

Kirk B, Kharbanda M, Bateman MS, Hunt D, Taylor EJ, Collins AL, Bunyan DJ, Collinson MN, Russell LM, Bowell S, & Barber JCK (2020). Directly Transmitted 12.3-Mb Deletion with a Consistent Phenotype in the Variable 11q21q22.3 Region. Cytogenetic and genome research, 160(4), 185–192. [PubMed: 32316019]

Kolluri A, & Ho M (2019). The Role of Glypican-3 in Regulating Wnt, YAP, and Hedgehog in Liver Cancer. Frontiers in oncology, 9, 708. [PubMed: 31428581]

Leslie EJ (2022). Genetic models and approaches to study orofacial clefts. Oral diseases, 28(5), 1327–1338. [PubMed: 34923716]

Li D, Strong A, Shen KM, Cassiman D, Van Dyck M, Linhares ND, Valadares ER, Wang T, Pena SDJ, Jaeken J, Vergano S, Zackai E, Hing A, Chow P, Ganguly A, Scholz T, Bierhals T, Philipp D, Hakonarson H, & Bhoj E (2021). De novo loss-of-function variants in X-linked MED12 are associated with Hardikar syndrome in females. Genetics in medicine : official journal of the American College of Medical Genetics, 23(4), 637–644. [PubMed: 33244166]

Li D, March ME, Fortugno P, Cox LL, Matsuoka LS, Monetta R, Seiler C, Pyle LC, Bedoukian EC, Sánchez-Soler MJ, Caluseriu O, Grand K, Tam A, Aycinena ARP, Camerota L, Guo Y, Sleiman P,

Callewaert B, Kumps C, Dheedene A, … Bhoj EJ (2021). Pathogenic variants in CDH11 impair cell adhesion and cause Teebi hypertelorism syndrome. Human genetics, 140(7), 1061–1076. [PubMed: 33811546]

Li P, Zhang HZ, Huff S, Nimmakayalu M, Qumsiyeh M, Yu J, Szekely A, Xu T, & Pober BR (2006). Karyotype-phenotype insights from 11q14.1-q23.2 interstitial deletions: FZD4 haploinsufficiency and exudative vitreoretinopathy in a patient with a complex chromosome rearrangement. American journal of medical genetics. Part A, 140(24), 2721–2729. [PubMed: 17103440]

Li Z, Wang Y, Zhang M, Xu P, Huang H, Wu D, & Meng A (2012). The Amotl2 gene inhibits Wnt/β-catenin signaling and regulates embryonic development in zebrafish. The Journal of biological chemistry, 287(16), 13005–13015. [PubMed: 22362771]

Liegel RP, Finnerty E, Blizzard L, DiStasio A, Hufnagel RB, Saal HM, Sund KL, Prows CA, & Stottmann RW (2019). Using human sequencing to guide craniofacial research. Genesis (New York, N.Y. : 2000), 57(1), e23259. [PubMed: 30375152]

Lu X, Yang J, Zhao S, & Liu S (2019). Advances of Wnt signalling pathway in dental development and potential clinical application. Organogenesis, 15(4), 101–110. [PubMed: 31482738]

Mai CT, Isenburg JL, Canfield MA, Meyer RE, Correa A, Alverson CJ, Lupo PJ, Riehle-Colarusso T, Cho SJ, Aggarwal D, Kirby RS, & National Birth Defects Prevention Network (2019). National population-based estimates for major birth defects, 2010–2014. Birth defects research, 111(18), 1420–1435. [PubMed: 31580536]

Mana-Capelli S, Paramasivam M, Dutta S, & McCollum D (2014). Angiomotins link F-actin architecture to Hippo pathway signaling. Molecular biology of the cell, 25(10), 1676–1685. [PubMed: 24648494]

Mana-Capelli S, & McCollum D (2018). Angiomotins stimulate LATS kinase autophosphorylation and act as scaffolds that promote Hippo signaling. The Journal of biological chemistry, 293(47), 18230–18241. [PubMed: 30266805]

Neveling K, Feenstra I, Gilissen C, Hoefsloot LH, Kamsteeg EJ, Mensenkamp AR, Rodenburg RJ, Yntema HG, Spruijt L, Vermeer S, Rinne T, van Gassen KL, Bodmer D, Lugtenberg D, de Reuver R, Buijsman W, Derks RC, Wieskamp N, van den Heuvel B, Ligtenberg MJ, … Nelen MR (2013). A post-hoc comparison of the utility of sanger sequencing and exome sequencing for the diagnosis of heterogeneous diseases. Human mutation, 34(12), 1721–1726. [PubMed: 24123792]

Ohashi K, Fujiwara S, & Mizuno K (2017). Roles of the cytoskeleton, cell adhesion and rho signalling in mechanosensing and mechanotransduction. Journal of biochemistry, 161(3), 245–254. [PubMed: 28082721]

Pilia G, Hughes-Benzie RM, MacKenzie A, Baybayan P, Chen EY, Huber R, Neri G, Cao A, Forabosco A, & Schlessinger D (1996). Mutations in GPC3, a glypican gene, cause the Simpson-Golabi-Behmel overgrowth syndrome. Nature genetics, 12(3), 241–247. [PubMed: 8589713]

Ragni CV, Diguet N, Le Garrec JF, Novotova M, Resende TP, Pop S, Charon N, Guillemot L, Kitasato L, Badouel C, Dufour A, Olivo-Marin JC, Trouvé A, McNeill H, & Meilhac SM (2017). Amotl1 mediates sequestration of the Hippo effector Yap1 downstream of Fat4 to restrict heart growth. Nature communications, 8, 14582.

Rips J, Mor-Shaked H, Erdin S, Yanovsky-Dagan S, Eventov-Friedman S, & Harel T (2021). De novo variant in AMOTL1 in infant with cleft lip and palate, imperforate anus and dysmorphic features. American journal of medical genetics. Part A, 185(1), 190–195. [PubMed: 33026150]

Saadi I, Alkuraya FS, Gisselbrecht SS, Goessling W, Cavallesco R, Turbe-Doan A, Petrin AL, Harris J, Siddiqui U, Grix AW Jr, Hove HD, Leboulch P, Glover TW, Morton CC, Richieri-Costa A, Murray JC, Erickson RP, & Maas RL (2011). Deficiency of the cytoskeletal protein SPECC1L leads to oblique facial clefting. American journal of human genetics, 89(1), 44–55. [PubMed: 21703590]

Satterstrom FK, Kosmicki JA, Wang J, Breen MS, De Rubeis S, An JY, Peng M, Collins R, Grove J, Klei L, Stevens C, Reichert J, Mulhern MS, Artomov M, Gerges S, Sheppard B, Xu X, Bhaduri A, Norman U, Brand H, … Buxbaum JD (2020). Large-Scale Exome Sequencing Study Implicates Both Developmental and Functional Changes in the Neurobiology of Autism. Cell, 180(3), 568–584.e23. [PubMed: 31981491]

Schutte BC, & Murray JC (1999). The many faces and factors of orofacial clefts. Human molecular genetics, 8(10), 1853–1859. [PubMed: 10469837]

Sobreira N, Schiettecatte F, Valle D, & Hamosh A (2015). GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Human mutation, 36(10), 928–930. [PubMed: 26220891]

Tolarová MM, & Cervenka J (1998). Classification and birth prevalence of orofacial clefts. American journal of medical genetics, 75(2), 126–137. [PubMed: 9450872]

Tremblay AM, & Camargo FD (2012). Hippo signaling in mammalian stem cells. Seminars in cell & developmental biology, 23(7), 818–826. [PubMed: 23034192]

Wang W, Huang J, & Chen J (2011). Angiomotin-like proteins associate with and negatively regulate YAP1. The Journal of biological chemistry, 286(6), 4364–4370. [PubMed: 21187284]

Williamson KA, Rainger J, Floyd JA, Ansari M, Meynert A, Aldridge KV, Rainger JK, Anderson CA, Moore AT, Hurles ME, Clarke A, van Heyningen V, Verloes A, Taylor MS, Wilkie AO, UK10K Consortium, & Fitzpatrick DR (2014). Heterozygous loss-of-function mutations in YAP1 cause both isolated and syndromic optic fissure closure defects. American journal of human genetics, 94(2), 295–302. [PubMed: 24462371]

Wilson NR, Olm-Shipman AJ, Acevedo DS, Palaniyandi K, Hall EG, Kosa E, Stumpff KM, Smith GJ, Pitstick L, Liao EC, Bjork BC, Czirok A, & Saadi I (2016). SPECC1L deficiency results in increased adherens junction stability and reduced cranial neural crest cell delamination. Scientific reports, 6, 17735. [PubMed: 26787558]

Xu G, Seng Z, Zhang M, & Qu J (2021). Angiomotin-like 1 plays a tumor-promoting role in glioma by enhancing the activation of YAP1 signaling. Environmental toxicology, 36(12), 2500–2511. [PubMed: 34480788]

Yu M, Wong S-W, Han D, & Cai T (2019). Genetic analysis: Wnt and other pathways in tooth agenesis. Oral Diseases, 25(3), 646–651. [PubMed: 29969831]

Zhao B, Li L, Lu Q, Wang LH, Liu CY, Lei Q, & Guan KL (2011). Angiomotin is a novel Hippo pathway component that inhibits YAP oncoprotein. Genes & development, 25(1), 51–63. [PubMed: 21205866]

Zheng Y, Vertuani S, Nyström S, Audebert S, Meijer I, Tegnebratt T, Borg JP, Uhlén P, Majumdar A, & Holmgren L (2009). Angiomotin-like protein 1 controls endothelial polarity and junction stability during sprouting angiogenesis. Circulation research, 105(3), 260–270. [PubMed: 19590046]

Zheng Y, Zhang Y, Barutello G, Chiu K, Arigoni M, Giampietro C, Cavallo F, & Holmgren L (2016). Angiomotin like-1 is a novel component of the N-cadherin complex affecting endothelial/pericyte interaction in normal and tumor angiogenesis. Scientific reports, 6, 30622. [PubMed: 27464479]

Zhou Y, Zhang J, Li H, Huang T, Wong CC, Wu F, Wu M, Weng N, Liu L, Cheng ASL, Yu J, Wong N, Lo KW, Tang PMK, Kang W, & To KF (2020). AMOTL1 enhances YAP1 stability and promotes YAP1-driven gastric oncogenesis. Oncogene, 39(22), 4375–4389. [PubMed: 32313226]

Zhu Z, Xu L, Leung-Sang Tang N, Qin X, Feng Z, Sun W, Zhu W, Shi B, Liu P, Mao S, Qiao J, Liu Z, Sun X, Li F, Chun-Yiu Cheng J, & Qiu Y (2017). Genome-wide association study identifies novel susceptible loci and highlights Wnt/beta-catenin pathway in the development of adolescent idiopathic scoliosis. Human molecular genetics, 26(8), 1577–1583. [PubMed: 28334814]

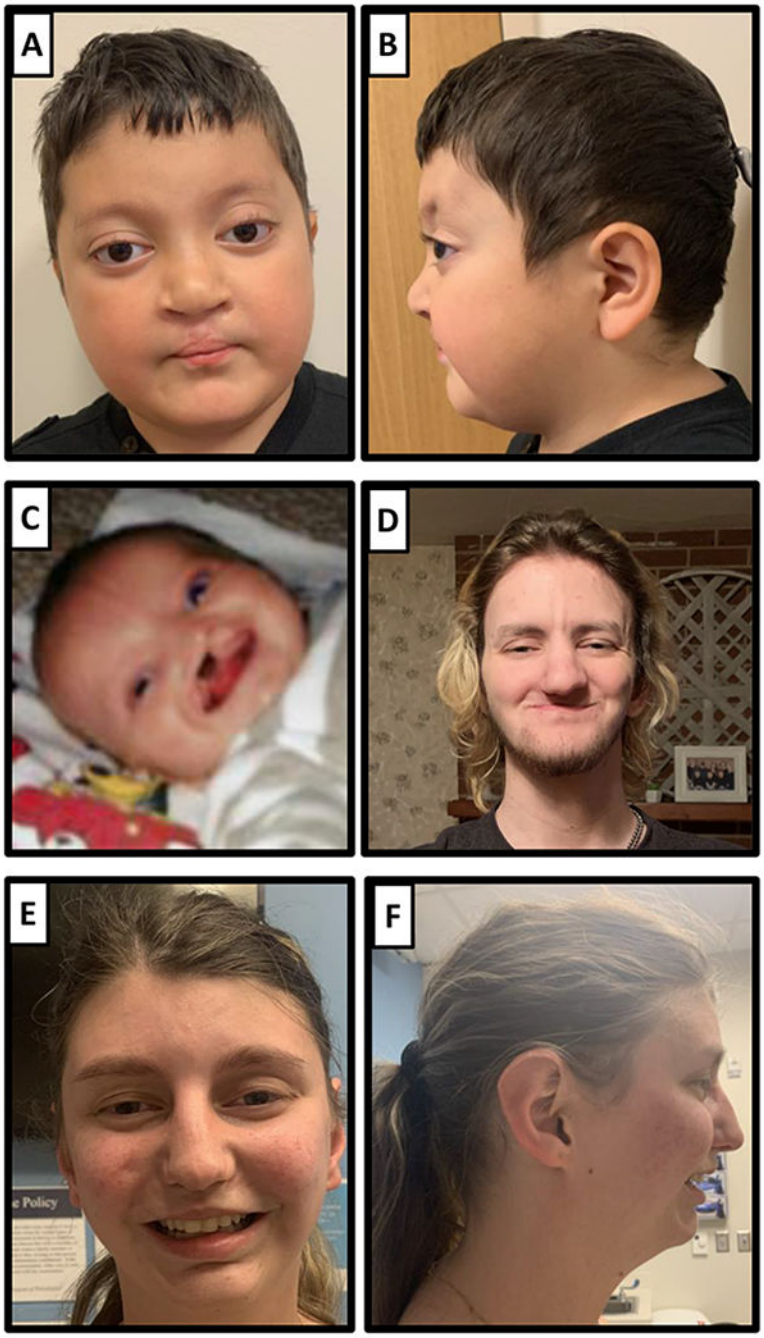


**Figure 1:**
Patient Photos. **A-B)** Patient 1 at 6-years of age **C-D)** Patient 9 as an infant and at 20 years of age **E-F)** Patient 12 at 26-years of age

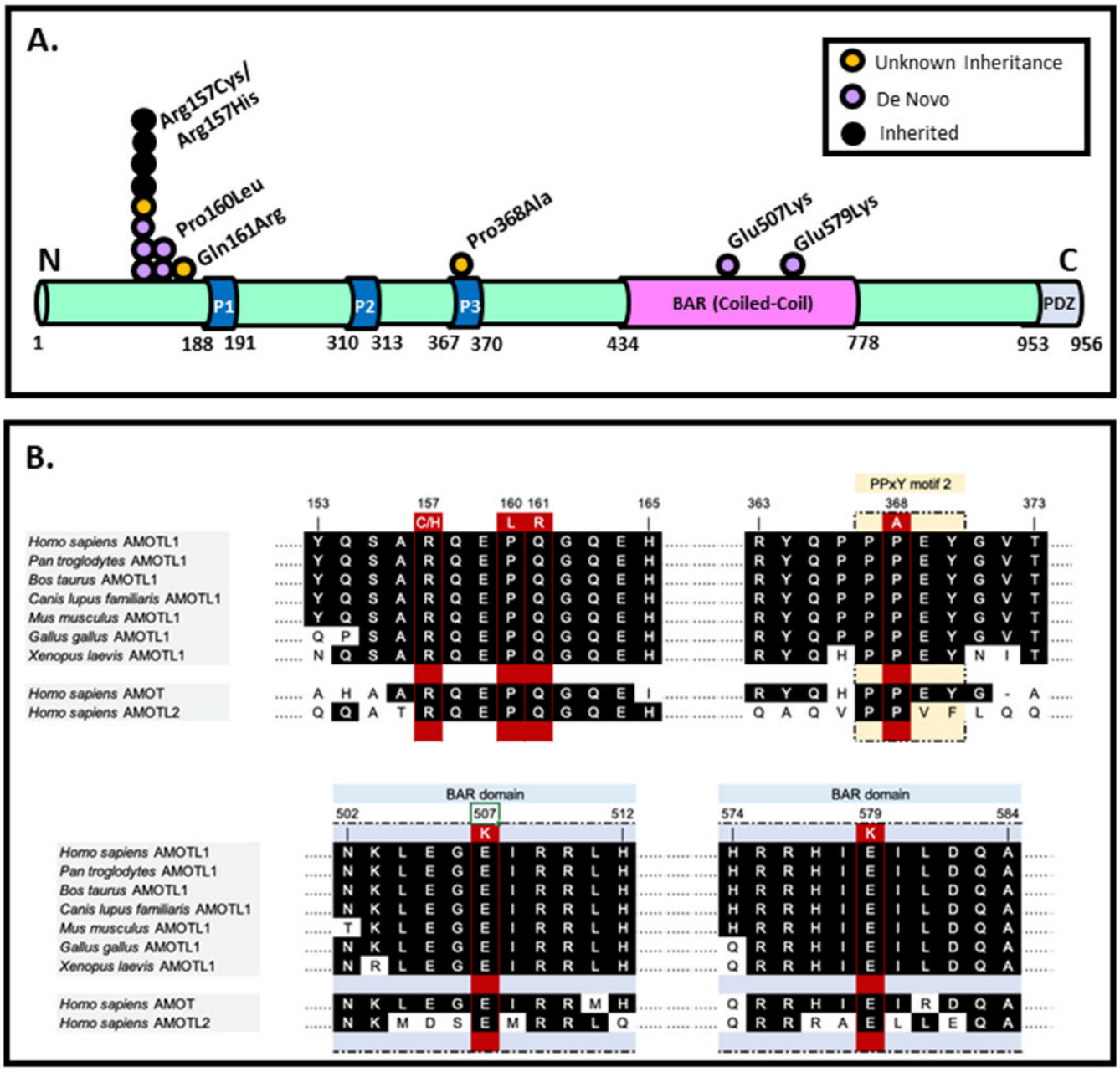


**Figure 2:**

**A**. Schematic of the *AMOTL1* protein with location of described variants. This includes the 12 new variants identified in the families described in this report plus the 2 previously reported variants. P1, P2 and P3 denote the LPTY and two PPxY domains (amino acids 188 – 191 [LPTY], 310 – 313 [PPxY], and 367 – 370 [PPxY], respectively). The pink box denotes the coiled-coil or BAR domain (amino acids 434 – 778). The blue-grey box denotes the PDZ-binding domain (EVLI, amino acids 953 – 956). **B**. Alignment of AMOT protein regions across species. Black shading with white text highlight residues conserved across the listed species and AMOT family members. The dark red columns mark the residues impacted by the patient variants, with the variant residues indicated at the top of each red

column. Numbering of the amino acid sequence and the domain impacted (if known) is shown above each alignment.

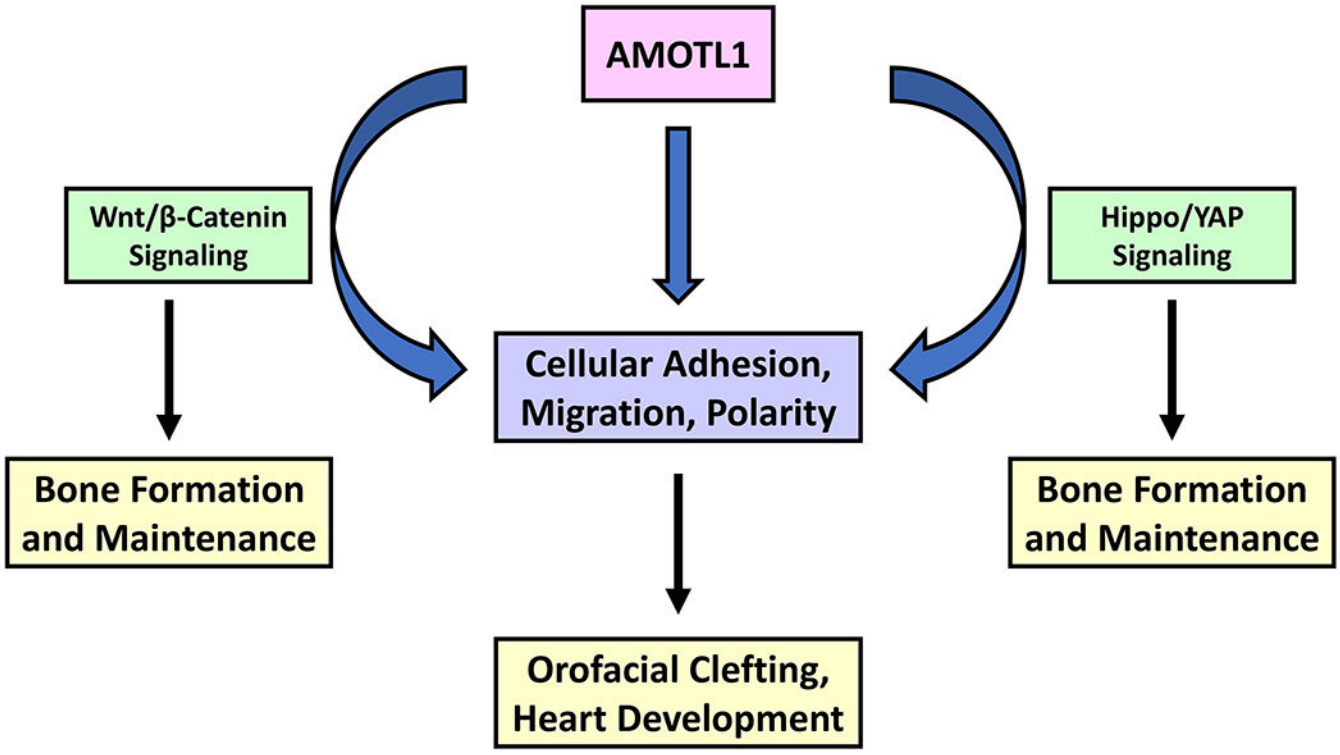


**Figure 3:**

Proposed mechanism for *AMOTL1*-related disease. *AMOTL1* plays a role in cell migration, adhesion and polarity either directly or through the Wnt/β-catenin and YAP pathways. *AMOTL1* variants may cause orofacial clefting and disrupt cardiac development through its effect on cellular dynamics. Additionally, the Wnt/β-catenin and YAP pathways are involved in bone formation, and disruption of these pathways due to variants in *AMOTL1* may cause the cranioskeletal phenotypes seen in patients.

**Table 1:**

Phenotypes for Individuals With Variants In Amino Acids 157 - 161

| Family | A | B | | | C | D | | | E | F | G | H | I | Liegel et al 2019 | | Rips et al, 2019 | Summary |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | | | | |
| Sex | Male | Male | Female | Female | Male | Female | Female | Male | Male | Female | Male | Female | Male | Male | Male | Female | 9M/7F |
| Base Pair Change | c.469 C > T | c.469 C > T | c.469 C > T | c.469 C > T | c.469 C > T | c.470 G > A | c.470 G > A | c.470 G > A | c.470 G > A | c.470 G > A | c.479 C > T | c.482 A > G | c.470 G > A | c.469 C > T | c.469 C > T | c.479 C > T | |
| Amino Acid Change | p. (R157C) | p. (R157C) | p. (R157C) | p. (R157C) | p. (R157C) | p. (R157H) | p. (R157H) | p. (R157H) | p. (R157H) | p. (R157H) | p. (P160L) | p. (Q161R) | p. (R157H) | p. (R157C) | p. (R157C) | p. (P160L) | |
| Inheritance | Unknown | Maternal | Maternal | Unknown | *De Novo* | Maternal | Unknown | Unknown | *De Novo* | *De Novo* | *De Novo* | Unknown | Maternal | *De Novo* | Paternal | *De Novo* | 4Mat, 1Pat, 5U, 6D |
| Dysmorphisms | + | | | | + | | | | + | + | + | | | | + | + | 7/16 |
| Clefting (CL/P) | + | + | + | | + | + | + | + | + | + | + | + | + | + | + | + | 15/16 |
| Myopia | + | | | + | | | | | | | | + | | | | | 3/16 |
| Large/ Dysplastic Ears | | + | + | | + | | | + | + | | + | + | | + | + | + | 10/16 |
| Hearing Loss | + | + | + | | | | | | | | + | + | | | | | 5/16 |
| Congenital Heart Disease | | + | + | | + | | + | | + | + | + | | | | + | | 8/16 |
| Constipation | + | | | | | | | | | | + | | | | | | 2/16 |
| Liver Disease | + | | | + | | | | | | + | + | + | | | | | 5/16 |
| Tall Stature | + | | | | + | | | | | + | + | + | | + | + | | 7/16 |
| Immune Involvement | + | + | | | | | | | | | | | | | | | 2/16 |
| Scoliosis | + | | | + | + | | | | | | | | | | | | 3/16 |
| Developmental Delay | + | + | + | | | | | | | + | + | | | | | | 5/16 |
| Psychiatric Diagnoses | | | | | | | | | | + | + | | | | | | 2/16 |

Abbreviations: M = male, F = female, Mat = maternal, P = paternal, U = unknown, D = de novo

**Table 2:**

Phenotypes for Individuals With Variants In Other Amino Acids

| **Family** | **J** | **K** | **L** | **Summary** |
|---|---|---|---|---|
| **Patient #** | **14** | **15** | **16** | |
| Sex | Male | Male | Female | 2M, 1F |
| Base Pair Change | c.1102 C > G | c.1519 G > A | c.1735 G > A | |
| Amino Acid Change | p.(Pro368Ala) | p.(Glu507Lys) | p.(Glu579Lys) | |
| Inheritance | Unknown | *De Novo* | *De Novo* | 1U, 2D |
| Clefting (CL/P) | Unilateral left cleft lip/palate | No | Bifid uvula | 2/3 |
| Cardiac/Vascular | | Congestive heart failure, vein of galen malformation | | 1/3 |
| Pulmonary | | ARDS, respiratory insufficiency, pulmonary hemorrhage | Asthma, allergies, snoring | 2/3 |
| Gastrointestinal | | | Constipation, gastroesophageal reflux disease | 1/3 |
| Genitourinary | | Acute kidney failure | | 1/3 |
| Hematological | | Consumptive coagulopathy | | 1/3 |
| Neurological | | Left hemispheric subdural and intracerebral parenchymal hemorrhage | Global developmental delay, obsessive behaviors | 2/3 |
| Dermatological | | | Skin tag | 1/3 |

Abbreviations: ARDS = acute respiratory distress syndrome; Pink = BAR/coiled-coil domain, Blue = PPxY domain; M = male, F = female, U = unknown, D = de novo