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CONFERENCE REPORT

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38th Annual David W. Smith Workshop on Malformations and Morphogenesis: Abstracts of the 2017 Annual Meeting

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The 38th Annual David W. Smith Workshop on Malformations and Morphogenesis occurred on August 26th – 29th, 2017 at the Stoweflake Resort and Conference Center in Stowe, VT. The Workshop, which honors the legacy of David W Smith, brought together clinicians and researchers interested in congenital malformations and their underlying mechanisms of morphogenesis. The Workshop highlighted four themes besides mechanisms of morphogenesis and new syndromes: Disorders of Transcriptional Regulation, Dysmorphology (Syndromes and Malformations) in Minority and Unique Populations, Syndromes and Isolated Birth Defects Involving Malformations of the Developing Foregut, and the Natural History of Syndromes. This Conference Report includes the abstracts presented at the 2017 Workshop.

KEYWORDS
congenital malformations, foregut, morphogenesis, natural history, transcription, underrepresented minority populations

The annual David W. Smith Workshop on Malformations and Morphogenesis occurred on August 26th–29th, 2017 at the Stoweflake Resort and Conference Center in Stowe, VT. The conference convened over 130 clinicians and researchers interested in human congenital malformations and their underlying mechanisms of morphogenesis. This year's Workshop, which annually honors the legacy of David W. Smith, widely recognized as the father of dysmorphology, was the 38th consecutive meeting. As is the annual tradition, the Workshop highlighted four themes besides mechanisms of morphogenesis and new syndromes; these included: Disorders of Transcriptional Regulation, Dysmorphology (Syndromes and Malformations) in Minority and Unique Populations, Syndromes and Isolated Birth Defects Involving Malformations of the Developing Foregut, and the Natural History of Syndromes. The invited keynote speakers: Drs. Judith G. Hall, Dale Dorsett, Edward Morrisey, Robert Krauss, Maximillian Muenke, and John C. Carey provided state-of-the-art lectures on these selected themes. The meeting was organized by Drs. Cathy Stevens and Ian Krantz, coauthors of this Conference Report, with the assistance of the Planning Committee.

The Workshop was cosponsored by the Centers for Disease Control and Prevention, the National Institute of Child Health and Development, and the March of Dimes.

Attached herein are the abstracts presented at the annual Workshop.

Workshop Session 1
DISORDERS OF TRANSCRIPTIONAL REGULATION I

DYSMORPHOLOGY HISTORY PALLISTER HALL SYNDROME A TALE OF BURIED TREASURE

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The evolution of a clinical syndrome with dysmorphic features or congenital anomalies often follows a circuitous route – giving rise (perhaps even to heroes and heroines) to legends, cardinal features, and many profound questions. Pallister-Hall Syndrome (PHS) is no exception. And we are nowhere near the end of the tale.

Procedures change over time as do ethical perspectives. The story of PHS began in winter of 1978. Between a conflicted family, a determined general practitioner, and another similarly affected child, an exhumation was undertaken. Would you be so bold?

Pathologists are heroes too! Detailed post-mortem studies from around North America revealed a pattern of anomalies not previously described. Many single cases, then familial cases, revealed a variable pattern of findings, inevitably leading to another set of heroes who identified the responsible gene and mutations 20 years later. And of course, more questions.

Over that 20 years, and the subsequent 20 years, new concepts of phenotype have evolved. Today we find ourselves with new methodologies for the unfolding of a clinical phenotype. Nevertheless, careful observation of clinical features, multiple individual descriptions, measurements of structures, changes with growth and development, ethnic variation, and natural history are every bit as important today in defining a clinical phenotype.

DROSOPHILA MELANOGASTER AS A MODEL FOR TRANSCRIPTIONAL DYSREGULATION SYNDROMES

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Multiple human dysmorphologies are caused by mutations affecting proteins that have broad roles in the regulation of gene transcription. Key examples are Rubinstein-Taybi, CHOPS, CHARGE, Coffin-Siris, and Cornelia de Lange Syndrome (CdLS). These syndromes have overlapping phenotypes, presumably reflecting similar effects on transcription during development. Most are characterized by distinctive facial dysmorphisms.

The roles of many of these proteins and genes in transcription and development have been intensively studied in Drosophila melanogaster. With strong genetic tools, and a significantly smaller genome, studies in Drosophila often provide unique and important insights that are more difficult to obtain in mammalian models.

As an example, much is known about how the proteins affected in CdLS influence transcription in Drosophila, which has guided studies on CdLS etiology. Most cases of CdLS are caused by dominant loss-of-function mutations affecting the Nipped-B- Like (NIPBL) protein that loads the cohesin complex onto chromosomes and a smaller number by mutations affecting cohesin subunits or other proteins that influence cohesin function. Drosophila with CdLS-like mutations in the Nipped-B gene display morphological abnormalities, and learning and memory deficits reminiscent of those that occur in CdLS. NIPBL and cohesin influence the transcription of hundreds of developmentally-important genes via multiple mechanisms. Some of these mechanisms involve functional interactions with proteins affected in other transcriptional syndromes.

Screens of FDA-approved compounds in Drosophila discovered related compounds that partially reverse some mutant phenotypes and increase Nipped-B RNA levels. These compounds also increase NIPBL mRNA levels in mouse and human cells. This suggests that Drosophila might be useful in screening for compounds could be therapeutic for syndromes caused by mutations affecting other general transcriptional regulators.

DISORDERS OF TRANSCRIPTIONAL REGULATION (DTRS) – A GROWING GROUP OF DISORDERS WITH PHENOTYPIC OVERLAP WITH CORNELIA DE LANGE SYNDROME

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Mutations in structural and regulatory cohesin proteins including NIPBL, SMC1A, SMC3, RAD21 and HDAC8 are causative of CdLS. Cohesin, a multiprotein complex, plays a canonical role in regulating sister chromatid segregation during mitosis. A non-canonical role for cohesin in regulating gene expression is the likely mechanism underlying developmental disorders. Growing evidence supports a "transcriptome disruption model" for cohesinopathies and related diagnoses, including the implication of key transcriptional regulators (Mediator, Polycomb, CTCF and others) with cohesin. Most recently our group identified mutations in the *AFF4* gene, a critical component of the Super Elongation Complex (SEC) in a CdLS-like disorder (CHOPS syndrome), demonstrating regulatory interactions between Cohesin, the SEC and transcriptional elongation. A large number of genes/proteins are involved in the complexity of transcriptional regulation (from initiation, general transcription, elongation, pausing, backtracking, processing, termination and associated epigenetic modification). Increasingly many of these genes, and the protein complexes they contribute to (cohesin, mediator, CTCF, TAF, SEC, CBP, SWI/SNF, ASX) are being implicated in human developmental disorders when disrupted. The term "transcriptomopathies" was coined by Yuan et al. (JCI, 2015), and more recently a more accurate moniker "Disorders of Transcriptional Regulation" or "DTRs" was coined by Izumi (Mol Syndromol, 2016). Interestingly, genetic disorders caused by mutations in components of the transcriptional machinery as well as in the proteins involved in epigenetic modification of the genome share many overlapping features

(e.g. the Cornelia de Lange, Rubinstein-Taybi, Coffin-Siris, Bohring-Opitz, CHOPS syndromes and others). Both reverse and forward genetic approaches have led to both insights into transcriptional regulation as well as the identification of novel disease genes. An overview of these disorders and their molecular and clinical interrelatedness will be discussed.

DIAS-LOGAN SYNDROME: DELINEATING A NEWLY RECOGNIZED DISORDER OF TRANSCRIPTIONAL REGULATION

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Dias-Logan syndrome is a recently described condition characterized by intellectual disability (ID) and persistence of fetal hemoglobin (HbF). It is caused by haploinsufficiency of *BCL11A*, on 2p16.1, encoding a transcription factor belonging to the SWI/SNF chromatin remodeling complex (Dias et al., 2016). Here we describe a newly diagnosed patient, and discuss additional clinical features and pathogenesis.

Our patient presented with severe ID, early-onset seizures, postnatal deceleration of head circumference resulting in microcephaly (−4 *SD*), short stature, scoliosis, small testes, and cutis marmorata. His distinctive facial gestalt included short forehead, deep-set eyes with strabismus and long palpebral fissures, wide mouth with tented vermillion of the upper lip and full and everted lower lip, and widely spaced teeth.

A possible diagnosis of Alpha-Thalassemia X-Linked Intellectual Disability syndrome (ATRX) had previously been proposed based on clinical features and facial gestalt, but molecular analysis of *ATRX* failed to identify a mutation and there were no erythrocyte HbH inclusion bodies. Of note, sequential hemoglobin (Hb) electrophoresis showed low HbA1 (72%, normal 95–98%) and normal HbA2 (2%) but persistence of fetal hemoglobin (26%; normal <2.1%). Exome sequencing identified a novel *de novo* pathogenic variant in *BCL11A* (c.1078dupC; p.Leu360fs), causative for Dias-Logan syndrome and consistent with his phenotype. However, our patient was more severely affected than previously reported individuals.

A total of 11 patients with point mutations have been described (Dias et al., 2016) and, by review of the literature, we found 19 additional individuals with 2p15p16.1 microdeletions involving *BCL11A*: only one of them had seizures, with onset at age 12 weeks (Hucthagowder et al., 2012). Given a positive family history of seizures (maternal uncle) and the paucity of patients with this syndrome and epilepsy in the literature, we reviewed the exome results looking for possible mutations in over 100 epilepsy genes. No pathogenic or likely pathogenic mutations were identified, suggesting that early onset seizures could represent an additional manifestation of Dias-Logan syndrome.

It is notable that the facial gestalt of our patient resembled ATRX more than Dias-Logan syndrome and, by review of available photos, 38% of previously described individuals had a facial gestalt consistent with the ATRX phenotype. BCL11A acts as a transcriptional repressor of fetal hemoglobin, and ATRX encodes a SWI/SNF-like protein that regulates the alpha-globin locus. Of note, STRING network analysis identified HDAC1 to play an important role in transcription regulation, as functionally associated with both ATRX and BCL11A proteins.

Interestingly, both gene products cause hemoglobin changes and two overlapping syndromes with ID, microcephaly and similar facial features, warranting further studies on their respective roles in chromatin remodeling in early development.

In conclusion, our report expands the phenotype of *BCL11A* mutations to include early onset seizures, and the overlapping manifestations between Dias-Logan syndrome and ATRX syndrome suggest convergence on a common pathway of transcription regulation of hemoglobin genes.

MUTATIONS IN H3F3A AND H3F3B ENCODING HISTONE 3.3 CAUSE THE FIRST REPORTED GERMLINE HISTONE SYNDROME: REPORT OF 23 PATIENTS WITH NEURODEVELOPMENTAL AND CONGENITAL MANIFESTATIONS

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Histones are nuclear proteins that associate with deoxyribonucleic acid (DNA) and allow DNA to be packaged into condensed chromatin. Histones are dynamically decorated with post- translational modifications (PTMs), which regulate such processes as DNA repair, gene expression, mitosis, and meiosis. The specific Histone 3 Family 3 (H3F3) histones (H3.3), encoded by H3F3A and H3F3B, mark active genes, maintain epigenetic memory, and maintain heterochromatin and telomeric integrity. Specific somatic mutations in H3F3A have been strongly associated with pediatric tumors, but no germline mutations in any histone proteins have been described in humans. Here we report 23 patients, ages 4 months to 32 years, with de novo missense germline mutations in H3F3A or H3F3B who share a core phenotype of progressive neurologic dysfunction and congenital anomalies, but no malignancies yet. These 23 patients were identified through exome sequencing performed for their neurodevelopmental delays and congenital anomalies. Notably, two of the six patients with H3F3B and one patient with H3F3A mutations have already demonstrated developmental regression, and the majority of patients have developed seizures beyond the neonatal period. As the majority of the patients are still quite young, it is unclear how universal the progression of disease will be. The additional clinical features in these patients include craniosynostosis, congenital heart disease, limb malformations, and urogenital anomalies. The core phenotype is similar to that described for patients with mutations affecting proteins that interact with H3.3, such as ATRX in X-

linked alpha- thalassemia mental retardation syndrome, which also is associated with developmental delay and cortical atrophy. These 16 mutations in 23 patients are all de novo and are not found in large datasets, such as the Exome Aggregation Consortium (ExAC). In ExAC, both H3F3A and H3F3B demonstrate higher than average constraint respective Z scores of 3.84 and 3.96, suggesting that genetic variation in these genes are not tolerated in the general population. There are three recurrent mutations in our cohort; the two variants p.R18G and p.A115G were found in two unrelated patients, and one variant (p.T46I) was found in four unrelated patients. We hypothesized that these missense mutations contribute to the shared patient phenotype through the induction of epigenetic dysregulation of histone PTMs. Histones PTMs within the nucleosome affect chromatin state, mitotic initiation, and gene expression. Therefore, histones from multiple tissues from several H3F3A and H3F3B patients were analyzed by mass spectroscopy (MS), which revealed that the mutant histone proteins are present at a level similar to that of wild-type H3.3. MS analysis allowed for quantitation of some of the PTMs on mutant histones, many of which showed strikingly aberrant patterns that suggested local, but not global, dysregulation of chromatin structure. These data suggest that the pathogenic mechanism of germline histone mutations is distinct from that of the cancer-associated somatic histone mutations. In addition, RNA-Seq on patient tissues showed a statistically significant upregulation of genes related to mitosis and cell division, which are being further explored. Characterization of the pathology behind this novel syndrome provides insight into novel therapeutic targets for the neurologic degeneration in these patients.

MUTATIONS IN *EBF3* DISTURB TRANSCRIPTIONAL PROFILES AND UNDERLIE A NOVEL SYNDROME OF INTELLECTUAL DISABILITY, ATAXIA AND FACIAL DYSMORPHISM

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From a GeneMatcher-enabled international collaboration, we identified ten individuals affected by intellectual disability, speech delay, ataxia, and facial dysmorphism and carrying a deleterious EBF3 variant detected by whole-exome sequencing. One 9-bp duplication and one splice-site, five missense, and two nonsense variants in EBF3 were found; the mutations occurred de novo in eight individuals, and the missense variant c.625C>T (p.Arg209Trp) was inherited by two affected siblings from their healthy mother, who is mosaic. EBF3 belongs to the early B cell factor family (also known as Olf, COE, or O/E) and is a transcription factor involved in neuronal differentiation and maturation. Structural assessment predicted that the five amino acid substitutions have damaging effects on DNA binding of EBF3. Transient expression of EBF3 mutant proteins in HEK293T cells revealed mislocalization of all but one mutant in the cytoplasm, as well as nuclear localization. By transactivation assays, all EBF3 mutants showed significantly reduced or no ability to activate transcription of the reporter gene CDKN1A, and in situ subcellular fractionation experiments demonstrated that EBF3 mutant proteins were less tightly associated with chromatin. Finally, in RNA-seq and ChIP-seq experiments, EBF3 acted as a transcriptional regulator, and mutant EBF3 had reduced genome-wide DNA binding and gene-regulatory activity. Our findings demonstrate that variants disrupting EBF3-mediated transcriptional regulation cause intellectual disability and developmental delay and are present in ~0.1% of individuals with unexplained neurodevelopmental disorders.

NSD1 OVEREXPRESSION ALTERS THE LEVELS OF INSULIN RECEPTOR (INR) AND TSC2

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Nuclear receptor binding SET domain protein 1, NSD1, encodes for a histone methyltransferase that transfers methyl groups to lysine residues at position 36 of histone 3 (H3K36). Deletions of or mutations in NSD1, located at 5q35.2-q35.3, are associated with Sotos syndrome, which is characterized by overgrowth with advanced bone ages, and heights and OFCs = 2 *SD*. In contrast, the duplication of 5q35.2-q35.3 is characterized by undergrowth with delayed bone ages, as well as heights and OFCs = −2 *SD*. We report eight new patients with a duplication of 5q35.2-q35.3, including a family of five affected individuals (an affected mother and four affected half-siblings). These patients contribute to the emerging phenotype of the 5q35.2-q35.3 duplication syndrome as reciprocal to Sotos syndrome, and illustrate the

contrasting features that occur in microdeletion-duplication syndromes. Our study documents the long term prognosis and penetrance of this recently delineated microduplication syndrome, and suggests the possibility of a differential diagnosis for children with delayed bone age and short stature. Focusing specifically on the growth phenotype, we have modeled the effects of NSD1 overexpression in Drosophila melanogaster. Overexpression of the fly homologue of NSD1 in the developing wing causes undergrowth associated with increased H3K36 methylation and increased apoptosis.

Altering the levels of insulin receptor (InR) and TSC2, a negative regulator of mTor signaling, rescues the apoptosis and the wing undergrowth phenotype, demonstrating alterations in PI3K/mTOR pathway signaling. Most interestingly, supplementing the diet with leucine also rescues the cell death, suggesting that this may represent a potential intervention for treating the growth phenotype.

Workshop Session 2
DISORDERS OF TRANSCRIPTIONAL REGULATION II

A HOMOZYGOUS SPLICE SITE VARIANT IN *TAF8* CAUSES INTELLECTUAL DISABILITY, BRAIN ABNORMALITIES AND *IN VITRO* EVIDENCE OF ABSENT TAF8 WITH ALTERATIONS IN THE TFIID COMPLEX

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The general transcription factor II D complex (TFIID), consisting of the TATA binding protein (TBP) and 12–14 TBP associated factors (TAFs) is required for preinitiation complex assembly, and subsequent transcription by RNA polymerase II. Recently intellectual disability and dysmorphic features have been reported associated with mutations in *TAFs* 1, 2 and 13 (Hellman-Aharony et al., 2013; O'Rawe et al., 2015; Tawamie et al., 2017) and *TAF6* mutations have been reported in patients with ID and a Cornelia de Lange (CDLS)-like phenotype (Yuan et al., 2015).

We report a patient with homozygous splice site variants in another TFIID component, *TAF8*, a gene not previously reported in human disease. First seen at age one with severe delay and chronic aspiration, she was born to non-consanguineous teenaged Hispanic parents. An extensive metabolic and genetic evaluation, including a normal SNP array, was negative. MRI revealed reduced myelination, small vermis and partial absence of the corpus callosum. Currently at age 4, she is G tube fed, has mild microcephaly ($-2-3$ *SD*), brachycephaly, dysmorphic features with a vague resemblance to CDLS and a picture of evolving lower extremity spasticity. She rolls and smiles but has no other developmental achievements. Exome sequencing revealed a c.781- 1G>A homozygous splice site variant carried by her parents.

Mice with homozygous germline deletions in the Taf8 gene die at the blastocyst stage of embryonic development (embryonic Day 4) (Voss et al., 2000). Deletion of Taf8 using the Nestin-Cre transgene, which is activated mid-gestation, results in embryos with massive cell death in the cerebral cortex, and reduced brain size as compared to control littermates. Embryos are born but do not survive beyond postnatal Day 2 because they fail to suckle (El Saafin unpubl).

Studies on this child's fibroblasts, performed by the French investigators, reveal that the *TAF8* c.781-1G>A intron 7 mutation results in *TAF8* mRNA with a new splice acceptor site and a frameshift in the open reading frame. Western blots utilizing anti-*TAF8* antibodies found NO detectable mutated TAF8 protein. Mass spectroscopy is underway to detect any residual mutant TAF8 activity. Other data suggest that *TAF1*,7 and TBP may form a partially active TFIID complex in this child's cells accounting for her survival and relative good general health.

Clinical delineation and *in vitro* studies of this rare phenotype has implications for our understanding of the role of *TAF* genes in the TFIID complex and their role in human intellectual disability. Studies are underway to better understand why this *TAF8* variant is viable despite an apparent global effect on DNA transcription.

DE NOVO, DELETERIOUS SEQUENCE VARIANTS IN *PBX1* ARE ASSOCIATED WITH INTELLECTUAL DISABILITY AND EAR, BRANCHIAL ARCH, RENAL, CARDIAC AND DIAPHRAGMATIC ABNORMALITIES

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We present six patients who are heterozygous for de novo, deleterious sequence variants in *PBX1* and establish mutations of this gene as causative in the etiology of a pleiotropic intellectual disability syndrome. The patients ranged in age from 9 months to 27 years of age.

Developmental delays were present in all, with independent walking at 18–36 months and single words first spoken at age 2 years. Microtia and/or dysplastic ear helices were noted in three patients and two had stenosis of the external auditory canals. One male had cartilaginous neck rests, a rare finding caused by aberrant development of the branchial arches. Renal anomalies comprised renal hypoplasia, left pyelocaliectasis and dilated fetal ureters. Three patients had a patent ductus arteriosus and one had Ebstein anomaly. Two males had unilateral eventration of the diaphragm with hypoplasia of the corresponding lung. The three males had cryptorchidism and one had intra-abdominal testes and retained Muellerian structures. All patients had de novo,

deleterious sequence variants in *PBX1*, including missense variants involving residues in or close to the homeobox domain (p.Met224Lys, p.Arg227Pro, p.Arg234Pro, and p.Arg235Gln) that may interrupt the interaction of the protein with DNA. Two patients had truncating variants, with c.783dupC, predicting p.Ser262Gln*fs**2, and c.862C>T, predicting p.Arg288*. Pbx1 encodes a three amino acid loop extension (TALE) homeodomain transcription factor that can dimerize with other TALE class homeodomain proteins from the Meis and Prep families to form nuclear complexes that enhance the binding specificity of Hox genes to DNA and regulate segment identity. *Pbx1*-deficient mice that are homozygous for a null allele (*Pbx1*$^{-/-}$) develop malformations, severe hypoplasia, or aplasia of multiple organs, including the ear pinnae, lungs, liver, stomach, gut, pancreas, diaphragm and kidneys, that are comparable to the human phenotype. Conditional mouse mutants have also revealed that *Pbx1* is important for regional identity and laminar patterning of the developing neocortex. Eight patients with congenital anomalies of the kidney and urinary tract and interstitial deletions encompassing *PBX1* were recently reported with extrarenal manifestations comprising outer ear anomalies, hearing impairment, developmental delays and cardiac defects (Le Tanno et al. (2017), *J Med Genet* Mar 7).

However, that report could not associate the observed abnormalities with haploinsufficiency for *PBX1* due to the inclusion of additional genes in the deletions. We conclude for the first time that deleterious sequence variants in *PBX1* are associated with a novel intellectual disability syndrome that manifests ear, branchial arch, renal, cardiac and diaphragmatic abnormalities.

A NEW ASHKENAZI JEWISH SYNDROME? NUP188 AND ITS ROLE IN A NEWLY DESCRIBED OCULO-FACIAL-NEURO SYNDROME

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Nuclear pore complexes are composed of approximately 30 distinct proteins termed nucleoporins (NUP). Alterations in NUP genes are linked to several human diseases including primary biliary cirrhosis, triple A syndrome (achalasia, Addison disease, alacrima), atrial fibrillation, and neoplastic disease. NUP188, with other nucleoporins, constitutes the NUP93 complex, the second largest nuclear pore complex structural unit. NUP188 and NUP93 form a barrier preventing membrane proteins from passing from the outer to inner nuclear membrane (Nofrini, 2016). Although NUP188 has never been directly implicated in human disease, NUP188 duplication has been suggested to contribute to heterotaxy. Here we present three patients from two unrelated Ashkenazi families with the same compound heterozygous truncating variants and a distinct phenotype, representing a new Ashkenazi Jewish syndrome. Patient 1 is a 4-month-old patient born prematurely who presented to our institution with multiple congenital anomalies found at birth. Family history was significant for maternal and paternal Ashkenazi Jewish descent and maternal history of Tetralogy of Fallot. The patient was born in breech presentation at 36 1/7 weeks to a 23-year-old G1P1 mother and presented with severe microcephaly, bitemporal narrowing, bilateral congenital cataracts, epicanthal folds, small palpebral fissures, wide nasal bridge and wide nose, micrognathia, cleft palate, generalized hypotonia, overlapping toes, and transitional palmar creases. Brain MRI findings showed marked volume loss of the cerebral and cerebellar white matter, ventriculomegaly, and deficient appearance of the corpus callosum, brainstem, and inferior cerebellar vermis. Echocardiogram showed trivial mitral regurgitation and a large PDA that closed by age 3 months. Renal, bladder, abdominal and pelvic ultrasounds were normal and EEG showed hypsarrhythmia starting at 3 months old. Patient was also noted to have pancytopenia early in life requiring filgrastim, platelet and RBC transfusions. Exome sequencing identified biallelic truncating variants in NUP188: c.904_907delATTT (p.I302VfsX7) and c.3144 C>G (p.Y1048X). Using GeneMatcher, we identified a collaborator with two affected Ashkenazi siblings. Both affected siblings have the same compound heterozygous truncating variants as patient 1. The phenotypes were also strikingly similar, presenting with thin corpus callosum, progressive microcephaly, severely delayed brain myelination, prenatal onset ventriculomegaly, gliosis at autopsy, congenital cataracts, partial anomalous pulmonary venous return, bicuspid aortic valve, large great toe, long gracile fingers, preaxial polydactyly, and streak ovaries. NUP188 has many described functions, including cilia formation and regulation of chromosome segregation. Del Viso (Cell, 2016) showed that knockdown of Nup188 or its binding partner Nup93 leads to a loss of cilia during embryonic development. This new syndrome shares phenotypes with other syndromes of cilia dysfunction, especially Lowe oculocerebrorenal syndrome. Additionally, Foerster (Development, 2017) presents data to suggest that primary cilia regulate ventricle morphogenesis by acting as a brake on the mTORC1 pathway; this may contribute to the ventriculomegaly in these patients. In the gnomAD database, the p.I302VfsX7 is present at an allele frequency of 0.077% and p.Y1048X at 0.0406% in the Ashkenazi population; neither variant is present in other populations.

Therefore, although there appears to be a low carrier frequency, this syndrome may be considered for inclusion on Ashkenazi carrier panels.

BICORNUATE UTERUS IN CORNELIA DE LANGE SYNDROME: OVERLAP WITH HAND-FOOT-GENITAL SYNDROME?

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Female genital tract malformations are rare, occurring in 0.4–4.3% of the fertile female general population (Grimbizis et al., 2001; Byrne et al., 2000), and higher if infertility and sex chromosome abnormalities are included. The Mullerian system is initiated and influenced by many genetic factors, and defects occur when there is failure or incomplete fusion of the two Mullerian ducts. Although genital malformations including cryptorchidism, micropenis and hypospadias are noted in over half of males with Cornelia de Lange syndrome (CdLS), a disorder of the cohesin protein complex, few genital deformities have been reported in females. As part of our long-term adolescent and adult multidisciplinary aging clinic, a pelvic and abdominal ultrasound is performed on all patients if tolerated. In addition to assessing for renal

malformations, the female genital tract is evaluated. Following ultrasounds of 24 females with CdLS, ages 12 to 54 years, notably six (25%) were found to have a bicornuate uterus. Some uteri measured small, but most ovaries were normal in appearance, and no other pathology was noted, including no ambiguous or intersex genitalia. Few genetic syndromes present with Mullerian fusion anomalies such as bicornuate uteri, which typically do not cause medical complications other than pregnancy difficulties. Bicornuate uterus has been seen as a distinct malformation in Fryns syndrome (Slavotinek, 2004), Bardet-Biedl syndrome (Stoler et al., 1995) and, interestingly, in Roberts syndrome (Freeman et al., 1974), another disorder of the cohesin protein complex. In addition, the Hand-Foot-Genital syndrome (HFGS) presents with genital malformations in both males and females, including Mullerian fusion defects. Specific extremity findings include small hands and feet, short first metacarpals/metatarsals, brachyclinodactyly of 5th fingers, and short great toes. Facial features have been infrequently reported. A recent female patient was found to have speech delay (Pezzani et al., 2015). Of note, one report includes a family with typical hand and foot findings, in which the mother has bicornuate uterus, and her son has feeding problems, poor weight gain, genital abnormalities, gastroesophageal reflux, obstructive sleep apnea, peripheral pulmonic stenosis, diaphragmatic hernia, and scoliosis (Tas et al., 2016). Many of the HFGS findings can also be seen in CdLS. Half of the females in our cohort with CdLS and bicornuate uteri have short great toes, and half have brachyclinodactyly of the 5th fingers. HFGS is due to mutations or deletions in HOXA13 in the HOXA gene cluster on 7p15. Cohesin is known to coordinate HOXA chromatin structure and gene expression (Wang et al., 2015) and maintain associated domains at HOXA (Nwigwe et al., 2015) during development. We hypothesize that chromatin-coordinating role of cohesin is related to proper expression of the HOXA cluster. If this activity is disrupted, this could lead to uterine fusion anomalies and limb features, as well as other features overlapping with those seen in CdLS.

Workshop Session 3
DISORDERS OF TRANSCRIPTIONAL REGULATION III

IDENTIFICATION OF A DEFINING PERIPHERAL BLOOD DNA METHYLATION SIGNATURE OF KABUKI SYNDROME ENABLES *KMT2D* VARIANT CLASSIFICATION

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Kabuki syndrome (KS, OMIM #147920) is a rare congenital disorder characterized by distinct dysmorphic facial features, intellectual disabilities, postnatal growth retardation, skeletal malformations and dermatoglyphic abnormalities. Up to 80% of cases described in the literature have *de novo* mutations. However, familial occurrence with autosomal dominant inheritance has also been reported. The major genetic cause of KS are mutations in the *KMT2D* (lysine-specific methyltransferase 2D; MIM #602113) gene, also known as *MLL2*, and, in up to 10% of the cases, mutations in the *KDM6A* (lysine-specific demethylase 6A; MIM #30012) gene. Most identified pathogenic mutations are truncating, likely leading to haploinsufficiency of the KMT2D protein. KMT2D is required for H3K4 di- and trimethylation, a hallmark of transcriptional activation. We hypothesized that the truncating mutations of *KMT2D* seen in KS could cause differential methylation, and that these differences may provide insight into the pathogenesis of this disorder, in addition to providing a tool for biomarker development and pathogenicity classification of novel mutations of *KMT2D* in KS patients. Using a discovery cohort (10 KS patients, 30 controls) and a validation cohort (13 KS patients, 142 controls), we assessed >450,000 CpGs across the genome and identified multiple genomic regions as well as 218 single CpGs which can accurately identify KS patients with known *KMT2D* gene mutations. Additionally, we demonstrated that this methylation signature distinguishes patients with benign variants from those with pathogenic variants thereby enabling classification of variants of unknown significance as benign or pathogenic. Gene network analysis identified HOX, immunological, and gonadal differentiation as the most significantly affected gene pathways.

These findings provide novel insights into the molecular etiology of KS, and demonstrate application of clinical epigenomics in the molecular diagnostics of KS. Lastly, the methods used in this study can be further expanded to other conditions resulting from the mutations in the genes that regulate the epigenetic machinery.

DELETION OF AN EVOLUTIONARILY CONSERVED CHROMATIN INSULATOR ELEMENT ASSOCIATED WITH ELEVATED RETINOID SIGNALING AS THE GENETIC BASIS FOR AN OAVS-LIKE PRESENTATION IN MICE

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Oculo-Auriculo-Vertebral Spectrum (OAVS) affects ~1:5,600 live births and is characterized by a variable array of features that includes microtia, maxillary and mandibular hypoplasia, pre-auricular or lateral facial tags, ear canal atresia with associated hearing deficits, and cervical vertebral anomalies. OAVS is also particularly noted for its frequent asymmetric presentation. Despite rare examples of familial inheritance, few genes have been definitively implicated. However, an increased risk has been associated with elevated Vitamin A exposure during pregnancy.

Here we report a unique mutant mouse line that presents almost all of the classic OAVS features, including the marked phenotypic variability and asymmetry. Using low-pass whole genome sequencing we identified a ~20kb inversion within the mapping interval that physically disrupted only a single gene. This gene encodes a zinc-finger

transcription factor, the expression of which was decreased by >90% in mutant tissue. However, complementation experiments demonstrated that loss of this gene is not responsible for the classic OAVS features. Re- evaluation of the chromosomal anomaly revealed that a small evolutionarily conserved intronic sequence was also deleted with the inversion. This deletion removes a validated chromatin insulator element, suggesting it could be disrupting local gene expression. Consistent with this, we demonstrate that the expression levels of two genes immediately adjacent to the transcription factor-encoding gene, which themselves are not physically disrupted, are upregulated more than 8 fold in mutant branchial arch tissue. Notably, these two genes are paralogs that encode relatively uncharacterized retinol dehydrogenase enzymes. Our data point to elevated retinoid signaling as a likely mechanism in this OAVS mouse model, providing the first direct evidence that genetic mutations disrupting retinoid signaling could underlie OAVS in patients.

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DE NOVO VARIANTS IN *KMT2E*, A CANDIDATE HAPLOINSUFFICIENT GENE, ARE A NOVEL CAUSE OF INTELLECTUAL DISABILITY

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Despite significant advances in genomic technologies, it is estimated that more than half of the genes that cause rare diseases remain undiscovered. Using large-scale human genomics of healthy individuals, we can identify genes that are depleted for loss-of-function or missense variation, known as constrained genes. The Exome Aggregation Database (ExAC) is a large- scale reference database with high quality, jointly processed exome data from 60,706 individuals. Leveraging the ExAC dataset, we have identified ~3,300 genes that are constrained for loss-of-function variation. Over two-thirds of these candidate haploinsufficient genes are not yet associated with any human disease phenotype. *KMT2E* (*MLL5*) is one such candidate haploinsufficient gene in which there are no high quality nonsense or canonical splice site mutations seen in ExAC, while 52 are expected based on the gene length and sequence. *KMT2E* has previously been implicated (but not definitely proven) as a candidate autism disease gene in exome sequencing studies but no further phenotype information is available. Loss of function in *KMT2E* is predicted to cause a disorder of transcriptional regulation. *KMT2E* encodes a histone methyltransferase epigenetic protein, a transcriptional regulator reported to play key roles in diverse biological processes, including cell cycle progression, genomic stability maintenance, adult hematopoiesis, and spermatogenesis.

We report here on the phenotype of heterozygous *KMT2E* predicted loss-of-function variants. Clinical exome sequencing performed on a 2-year-old male with macrocephaly, infantile epilepsy, and developmental regression identified a *de novo* Y284H missense variant in *KMT2E*. The proband had normal development until 6 months of age. He developed medically refractory epilepsy at 6 months, associated with developmental stagnation. At 14 months of age, a ketogenic diet was initiated and maintained for the subsequent 6 months, during which time he had severe developmental regression. Since the discontinuation of the ketogenic diet, he has started to make slow developmental gains but has not returned to his prior baseline.

Recent studies have shown that beta-hydroxybutyrate (ketone bodies) are natural inhibitors of histone deacetylases, leading to an altered brain epigenetic landscape. It is intriguing to hypothesize that the abnormal brain epigenetic landscape of individuals with loss of *KMT2E* function could have become detrimentally more abnormal as a result of the ketogenic diet.

Through GeneMatcher, we have identified eleven additional probands (ages 1–12 years) with *de novo* protein truncating variants in *KMT2E* to further delineate the phenotype. All of these children have developmental delay and intellectual disability, while 80% have hypotonia. Three have a formal diagnosis of autism. Two-thirds have relative macrocephaly. Several have a short nose and tented vermilion of the upper lip. Only one additional subject has childhood onset epilepsy. This case series supports *KMT2E* as a novel intellectual disability and autism disease gene.

SMC1A MUTATIONS CAUSE MECHANISTICALLY SEPARABLE ALLELIC DISORDERS: ATYPICAL CORNELIA DE LANGE SYNDROME AND A RETT-LIKE EPILEPTIC ENCEPHALOPATHY

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Mutations in *SMC1A* were first identified in 2006 in males with a variant presentation of Cornelia de Lange syndrome (CdLS; Musio et al., 2006). Subsequent efforts (Deardorff et al., 2007; Huisman et al., 2017) have identified a substantial number of males and females with CdLS-like presentations caused by mutations in *SMC1A*. These subjects demonstrate features that vary from classical CdLS that include broad full brows, more normal growth parameters and less frequent congenital malformations, although their intellectual disability can be as severe.

SMC1A mutations that cause CdLS-like features are missense mutations. Notably, *SMC1A* is on the X chromosome but not inactivated and expressed biallelically in females. The findings that females can be as severely affected as males suggests that mutations in *SMC1A*

that cause a CdLS-like presentation cause a dominant-negative effect, and potentially disrupt a specific function of the protein.

Recently, an allelic disorder has been emerging caused by *SMC1A* loss of function mutations. All individuals are girls who present, not with CdLS features, but with an early onset epileptic encephalopathy. We have identified over 15 girls with this clinical picture, some of whom have no dysmorphic features of CdLS, but are often described as "Rett-like". Their seizures typically have an onset in the first year of life, are generalized tonic-clonic, can be difficult to manage, but have resolved in a small number of cases.

SMC1A is a core component of the cohesin complex that forms a dimer with SMC3. Together SMC1A and SMC3 undergo a complex folding process to form an antiparallel coiled- coil dimer, attaching at their midportions to generate a bracelet-like structure. Cohesin is loaded onto chromatin and re-positioned to generally regulate chromatin dynamics and transcription. This process is regulated by a series of proteins, including NIPBL, the gene in which loss of function mutations have been identified in CdLS.

These phenotypic data, suggest that SMC1A mutations that cause CdLS-like features lead to disrupted loading, repositioning or chromatin regulation, in a manner similar to that noted for loss of NIPBL function. In contrast, loss of function mutations in SMC1A that result in X-linked female epileptic encephalopathy likely cause a haploinsufficiency of SMC1A, leading to specifically to reduced cohesin function, rather than altered cohesin function. Consistent with the clinical neurologic presentation, Drosophila and mouse models have also noted that neural cells, a post-mitotic cell type, are more sensitive to a loss of cohesin function than other cell types. These observations strongly suggest a dosage-dependent post-replicative role for cohesin in neural development. Elucidation of the specific mechanism of these likely chromatin and transcriptional-regulatory deficiencies will serve to provide insight into better understanding and management of these allelic clinical disorders.

NEUROLOGIC, NEUROMUSCULAR AND CRANIOFACIAL SYNDROMES

THE GENETIC LANDSCAPE OF CEREBELLAR MALFORMATIONS

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The cerebellum is a complex part of the brain that in humans accounts for only ~10% of total brain volume, but is densely packed and contains more than 80% of all neurons. It is best known for its function in motor coordination, but appears to coordinate cognition, emotion and attention as well. Malformations of the cerebellum are collectively common and difficult to classify birth defects that are associated with epilepsy, autism, intellectual disability, mood dysregulation and attention deficit disorder. The genetic basis of these developmental brain disorders is poorly understood, and few patients receive a genetic diagnosis.

We selected 104 children from 98 families diagnosed with cerebellar malformations from a large cohort of developmental brain disorder patients recruited between 1983 and 2012 with DNA available but without a known genetic diagnosis. The cohort includes common types of diffuse cerebellar (CBLH) and cerebellar vermis (CBVH) hypoplasia as well as Dandy-Walker malformation (DWM), but excluded several rare and specific malformations such as (near) complete cerebellar agenesis, giant tectum/absent vermis, and rhombencephalosynapsis. We performed exome sequencing in 98 families in which one or more children had CBLH-CBVH or DWM, using an in house analysis pipeline that calls sequence variants detected by both GATK and FreeBayes with depth of coverage $\geq$10, AAF $\leq$0.1%, and a functional change with CADD score $\geq$10 if applicable. Our final interpretation of variants often required searches of clinical and research variant databases plus recent literature, as well as the GeneMatcher website. We have so far found a genetic diagnosis in 40 of 98 (41%) families. Unexpectedly, we were able to solve 28 of 54 (52%) children with CBLH-CBVH, but only 11 of 44 (25%) with DWM. The difference was statistically significant ($p = .007$), which suggests greater causative complexity for DWM than for isolated CBLH-CBVH. Notably, several prenatal extrinsic factors are known to cause cerebellar hypoplasia, including prenatal hemorrhage, hypoxia-ischemia (especially in extremely low gestational age newborns), infections (i.e. Zika virus), and teratogens such as retinoid acid.

We identified single gene mutations in 32 different genes including 27 previously associated with developmental brain disorders, and 5 novel genes (for example, *FZD3*). For a majority of the known genes, cerebellar malformations had not been reported as part of the phenotype. The mutations were de novo heterozygous for 19, homozygous or compound heterozygous for 10, and X-linked for 3 genes. We found mutations in more than one child for 6 of the 32 genes (*CASK*, *BCL11A*, *DDX3X*, *FOXP1*, *STXBP1* and *TUBA1A*), but only *CASK* was seen in more than two children. All of the genes were expressed in fetal or adult cerebellum. Our results show that cerebellar malformations occur as a co-morbidity with numerous different developmental brain disorders, and that the yield of testing is higher for CBLH-CBVH that for classic DWM. Our results suggest that *CASK* may be the only "common" CBLH gene and that non-genetic causes are an important cause of cerebellar malformations.

TUBA1A: OUTCOME OF MOSAICISM AND PHENOTYPIC ANALYSIS

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TUBA1A encodes the microtubule protein, Tubulin-α-1A (TUBA1A). TUBA1A is expressed only in the brain and has a temporal expression

pattern peaking in fetal brain and decreasing in the post-natal period into adulthood. Abnormal TUBA1A protein is proposed to interfere with microtubule function, thus impairing neuronal migration. Pathogenic variants (PV) in *TUBA1A* cause a spectrum of brain malformations associated with disrupted neuronal migration including classic lissencephaly, lissencephaly with cerebellar hypoplasia and agenesis of the corpus callosum, cerebellar hypoplasia without lissencephaly, perisylvian asymmetrical polymicrogyria, and polymicrogyria-like cortical dysplasia among others.

Somatic mosaicism of PV is known to alter the phenotype in several genetic conditions. This article presents phenotypic findings in four individuals mosaic for PV in *TUBA1A*.

Case 1: A two-year-old girl presented with developmental delay, microcephaly, stereotypies and MR with decreased cortical volume but no structural abnormalities. Whole Genome Sequencing identified a de novo variant in the *TUBA1A* gene, c.167C>T (p.Thr56Met), which has been previously reported in three unrelated individuals associated with lissencephaly with cerebellar hypoplasia. The variant was present in only 16% of cells (confirmed by qPCR) and was not present in either parent.

Case 2: 2 siblings presented with cognitive impairment and structural brain anomalies consistent with a neuronal migration abnormality. Sequencing of the *TUBA1A* gene identified a PV, c.13A>C (p.Ile5Leu). The mother carries this PV in 5.6% of her fibroblasts. MR of the mother, who was described as asymptomatic, showed a thin corpus callosum, hypoplasia of the superior vermis, and a thin medulla.

Case 3: 2 siblings were referred for evaluation of developmental delay, microcephaly and structural brain anomalies. Whole exome sequencing in one sibling identified a heterozygous PV in the *TUBA1A* gene, c.1091C>G (p.P364R), which was confirmed in the sibling by single site testing. The mother has mosaicism for this PV in blood. She has mild cognitive impairment.

Case 4: A fetus was evaluated with MR at 30 weeks EGA due to intertwin discordant cephalic measurements. Multiple brain anomalies consistent with neuronal migration abnormality were found and subsequently confirmed postnatally. Infantile spasms and profound developmental delay were present. A next-generation brain malformation panel identified a heterozygous c.790C>G (p.R264G) PV in *TUBA1A*. The father was mosaic in blood for this PV. He had normal intellect, no history of seizures, and normal brain MRI.

Somatic mosaicism for PV in *TUBA1A* should be considered in the evaluation of children with developmental delay and intellectual disability particularly if structural brain anomalies are present and in parents of affected siblings. Parental testing should also be offered for recurrence counseling in isolated cases. Genetic and genomic testing should include assessment of mosaicism as part of the analytic pipeline.

PHENOTYPIC SPECTRUM OF FEMALES CARRYING MUTATIONS IN *ZC4H2*

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Arthrogryposis is a highly heterogeneous group of conditions defined as non-progressive congenital joint contractures of two or more different body areas. Arthrogryposis is a clinical feature of more than 500 rare conditions and can be caused by abnormalities of the central or peripheral nervous system, neuromuscular junction, or muscle. The distal arthrogryposes (DA) are a phenotypically and genetically heterogeneous subset of arthrogryposis conditions putatively caused by mutations in 15 genes. Distinguishing among the different DA early in life can difficult.

Analysis of exome sequence data from a large cohort of families putatively diagnosed with DA identified predicted loss-of-function (LOF) mutations in *ZC4H2* in six females, each with a different mutation – five of which were confirmed to be *de novo*. Three females with deletions of *ZC4H2* have been reported bringing the total number of females with putative LOF variants to nine.

Hemizygous mutations in *ZC4H2* cause Wieacker-Wolff syndrome, a X-linked recessive condition characterized by arthrogryposis, hypotonia, motor and developmental delay, and sometimes early death. Some carrier females are unaffected, while others are reportedly "mildly affected" with mild intellectual disability, camptodactyly, and clubfoot. In contrast, clinical characteristics of females with LOF variants in *ZC4H2* included moderate to severe intellectual disability, seizures, poor growth, scoliosis, and severe contractures.

Overall, females with predicted LOF mutations *ZC4H2* appear to be comparatively more severely affected than either females or males with missense mutations in *ZC4H2*. This suggests that LOF mutations in females leads to a phenotype than is more severe than missense mutations in *ZC4H2* in either males or females. LOF variants in *ZC4H2* have not been reported in males.

We hypothesize that most *ZC4H2* missense variants are hypomorphic whereas LOF variants result in haploinsufficiency and that haploinsufficiency in males is embryonic lethal and in females results in severe disease. The spectrum of disease severity in females with LOF variants in *ZC4H2* may be associated with the level of X-inactivation of the mutant allele.

POSTER PRESENTATIONS

KBG SYNDROME DUE TO MUTATIONS IN *ANKRD11*: COMPLEX CASES OF BLENDED PHENOTYPES, PARENTAL INHERITANCE, AND POTENTIAL PHENOTYPIC EXPANSION TO INCLUDE HEARING LOSS

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UAB's Undiagnosed Disease Program and HudsonAlpha's Clinical Sequencing Exploratory Research program have identified 5 cases of KBG syndrome due to *ANKRD11* variants. Each case has a unique phenotype that may explain why patients were not previously diagnosed and referred to these "last resort" programs.

Case 1: 3yo male with bilateral hearing deficits, developmental delay, and global parenchymal volume loss on MRI. Exam showed brachycephaly, short and upslanting palpebral fissures, widely spaced eyes, full cheeks, wide nasal bridge, upturned nasal tip with underdeveloped ala nasi, simple cupped ears, long flat philtrum, and thin upper lip. WGS revealed three diagnoses: Waardenberg syndrome (*PAX3* p.Arg271His), KBG syndrome (*ANKRD11* p.Lys1107fs) and CDK13-related disorder (*CDK13* p.Cys701Tyr). His facial dysmorphisms encompassed features of all three conditions.

Case 2: 10yo male with seizures, autism, speech delay, and moderate intellectual disability. He had a history of tremors, failure to thrive, gastroesophageal reflux, and dysmorphic facial features. He was found to have de novo *ANKRD11* p.Glu2158X.

Case 3: 12yo female with seizures, hearing impairment, myalgia, moderate intellectual disability, and dysmorphic features. Prior diagnoses considered included a mitochondrial disorder, as she had atypical Complex I, III, V activity and two mitochondrial variants of uncertain significance. WGS revealed de novo *ANKRD11* p.Ala2265fs. It is unclear if there is a second, undetected condition, or if the KBG phenotype could include myalgia or hearing loss.

Case 4: 12yo female with complex congenital heart defect (single ventricle, mitral atresia, restrictive ASD), esophageal varices, portal hypertension, short stature, hearing impairment, moderate intellectual disability, macrocephaly and dysmorphic facial features. She harbors a pathogenic *ANKRD11* variant (p.Lys803fs), inheritance unknown as the egg donor was unavailable for testing.

Case 5: 6yo male with seizures, developmental delay, and moderate intellectual disability. A nonsense variant in *ANKRD11* (p.R733X) was inherited from his mother with seizures, and testing is pending in a similarly affected brother.

Three of our cases have hearing impairment (one attributed to a blended phenotype), 1 with cardiac manifestations and 1 with myalgias. These may represent phenotypic expansion or unrelated conditions. Most interesting is the hearing loss, varying forms are reported in over 30 other patients – 1 of the original KBG patients of Herrmann (1975), also by Tekin (2004), Goldenberg (2016), Low (2016). *ANKRD11* is a member of ankryin-repeat cofactors that colocalizes with histone deacetylases to modify transcriptional activation and is expressed in the cerebral cortex neurons (Sirmaci, 2011). Auditory cortex, auditory neuronal, or cranial nerve involvement may potentially be a mechanism of hearing loss in KBG patients.

ANOTHER ETIOLOGY FOR COFFIN-SIRIS SYNDROME? *ARID2* CAUSES A CHROMATIN REMODELING TRANSCRIPTIONAL REGULATION DISORDER

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ARID2 is a component of the Polybromo and BRG1-associated factor (PBAF), which is a subunit of the SWI/SNF (switch/sucrose nonfermentable) complex that helps regulate chromatin remodeling. Coffin-Siris syndrome (CSS) is caused by mutations in other genes involved in the BAF subunit of SWI/SNF. *ARID2*, found in PBAF, was initially reported in 2015 as a pathogenic cause of intellectual disability and developmental delay in four cases. Two additional cases were subsequently reported in 2017, with the additional noting of similar clinical features to CSS.

An additional patient is presented with a de novo, nonsense mutation (c.2872C>T, p.Gln958Ter) in exome 15 of *ARID2* detected on whole exome sequencing. This mutation has not previously been reported but is predicted to cause loss of normal protein function. The patient was 26 months of age at the time of testing, had minimal words, and did not walk independently. The patient's delivery was complicated by nuchal cord times two, and there was resultant concern for a hypoxic event leading the patient to be diagnosed with cerebral palsy prior to genetic testing.x

Health concerns for this young man include difficulty gaining weight (weight less than 1%ile), shorter stature (height 3%ile), laryngotracheomalacia, hypotonia, and white matter loss on brain MRI. Physical features include: coarse face with prominent forehead, protruding and posteriorly rotated ears, thin upper lip, wide nasal bridge, as well as hypoplastic 5th digit nails of both fingers and toes. The patient is very jovial and often smiling broadly. Many of this patient's features are shared by those previously reported with *ARID2* mutations as well as those with Coffin-Siris syndrome.

The BAF subclass of the SWI-SNF complex contains the majority of the genes already associated with CSS. While BAF and PBAF are distinct entities, they share many core components and are major parts of SWI-SNF. *ARID2* is exclusively found in PBAF, yet it interacts with BAF during some developmental stages. It is expressed in all tissues and appears integral for tissue and cell-specific gene expression and maintenance. *ARID2* mutations likely cause haploinsufficiency, which is similar to *ARID1A* and *ARID1B*.

Though several genes are known to cause CSS, nearly 40% of clinical cases elude genetic diagnosis. With the similarity in clinical concerns and physical features, as well as the relationship between *ARID2* to the complex indicted in CSS, consideration should be made to including *ARID2* as a causative gene for CSS or at least a CSS-like condition. There is considerable clinical variability in CSS, and most of the *ARID2* patients seem to share the more common features. This may represent some ascertainment bias regarding *ARID2*. However, for the time being in clinical situations, if other genetic etiologies for CSS are not found, *ARID2* should be considered.

DE NOVO MISSENSE VARIANT IN UPF1, A KEY REGULATORY COMPONENT OF NONSENSE-MEDIATED DECAY, IS ASSOCIATED WITH A UNIQUE PHENOTYPE WITH GLOBAL DEVELOPMENTAL DELAY

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The nonsense-mediated decay (NMD) pathway is best known for its selective degradation of mRNA transcripts harboring premature termination codons (PTC). UPF1 (up-frameshift-1) is one of 12 known NMD factors, and is the key central component in PTC-containing mRNA recognition and degradation. When recruited to a translating ribosome by a PTC in an mRNA transcript and complexed with UPF2 and UPF3, UPF1 becomes phosphorylated and initiates enzymatic degradation of the transcript. Despite its central role in NMD (a highly evolutionary conserved process which has been in yeast through mammals), variants in UPF1 have not been associated with human Mendelian conditions. However, pathogenic variants in UPF3B (an X- linked paralogue of UPF3) are associated with a distinct facial gestalt with intellectual disability (Tarpey et al., 2007; Laumonnier et al., 2010). We recently identified a de novo missense variant in UPF1 in a toddler with global developmental delay and a distinctive facial gestalt. The proband was initially evaluated at 2 years and 3 months old for global developmental delay and non-familial facial features. He is the second child of non-consanguineous parents and there is no family history of X-linked intellectual disability. Pregnancy was uncomplicated and he was AGA at 37 weeks. He was noted to have "crunched up ears" at birth. He had poor weight gain on breast milk and skin sensitivity improved with switch to formula. He had gastric dysmotility and mild hydronephrosis. He had delayed head control and showed little developmental progress between 12 and 24 months. He sat independently at 12–15 months, crawled at 24 months, pulled to stand at 27 months, and babbles at 24 months with just a couple of word-like vocalizations. He is curious and described as "laid back". He had normal cytogenomic SNP microarray and brain MRI. On physical exam he is hesitant to stand and has poor balance. His length is 25th centile, weight is ~10th centile, and OFC is ~2nd centile. He has a long face with a high forehead, cupped ears with prominent lobes, myopathic face with intermittent ptosis, underdeveloped calves and puffy dorsum of the feet. Whole exome sequence analysis identified a de novo sequence variant of uncertain significance in UPF1 (c.1762T>C; p.Ser588Pro). Located in the RecA1 domain (RNA binding domain) of UPF1, the p.Ser588Pro variant is not listed in population databases, and serine 588 is highly conserved, including yeast and Drosophila. It is an exposed amino acid at the juncture of two alpha helix domains, but it is not a commonly phosphorylated serine residue. Lymphoblastoid cell lines have been generated from the proband and his parents to determine the efficiency of NMD (Tarpey et al., 2007). We hypothesize that this novel amino acid change in UPF1, the key regulatory component of nonsense-mediated mRNA decay, leads to less efficient NMD. We further speculate that similar to pathogenic variants in UPF3B, attenuated NMD due to this UPF1 variant leads to deregulation of neuronal- specific transcripts important in normal intellectual development.

BIALLELIC MUTATIONS IN THE TRANSCRIPTIONAL REGULATOR *MED17* CAUSE AN INTELLECTUAL DISABILITY SYNDROME WITH CEREBELLAR HYPOPLASIA AND POSSIBLE RISK FOR MYELODYSPLASIA

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Homozygosity for a *MED17* founder mutation in Caucasus Jews causes profound developmental delay, post-natal microcephaly, cerebral and cerebellar atrophy, seizures, and spasticity (Kaufmann et al., 2010). Japanese siblings with compound heterozygous mutations in *MED17* were reported with severe-profound developmental delay, hypotonia, choreiform movements, and cerebellar atrophy in one sibling (Hirabayashi et al., 2016). We report a 6 year- old girl of mixed European, Pacific Islander, and Native American ancestry with severe developmental delays, Duane anomaly, hematopoietic myelodysplasia, and cerebellar hypoplasia found to have compound heterozygous mutations in *MED17*.

The patient was born at term following an uncomplicated pregnancy and delivery.

Developmental delays were noted in the first 6 months. At 12 months she was diagnosed with bilateral Duane anomaly. At age 4 years she developed thrombocytopenia and bone marrow biopsy revealed myelodysplasia. She has remained severely delayed but with no developmental regression. She sat independently at 3 years and now rolls and scoots. She remains nonverbal without any specific communicative signing. She does finger feed. She has a friendly demeanor and frequently laughs without provocation. Her exam is notable for normal growth with head circumference at the 15th percentile, diffuse hypotonia, bilateral intention tremors, thick eyebrows with mild synophyrys, and a right preauricular tag.

Brain MRI at 17 months revealed moderate cerebellar hypoplasia involving the vermis more than the hemispheres, mild pons hypoplasia, and mild enlargement of the frontal horns. Clinical whole exome sequencing revealed compound heterozygous variants in *MED17*: maternally-inherited c.1299_1302del (p.Ala435SerfsTer5) and paternally-inherited c.1183C>G (p.Pro395Ala). The p.Ala435fs variant is expected to lead to non-sense mediated decay and has an allele frequency in the Exome Aggregation Consortium (ExAC) of 0.0045% in Europeans. The p.Pro395Ala variant is predicted to be damaging by computational analysis programs and is not listed in population frequency databases.

Mediator is a multi-protein variable complex that plays an essential role in eukaryotic transcriptional regulation through its control of RNA polymerase II activity. The human mediator complex is composed of up to 26 proteins and mutations in five of these genes have been associated with severe intellectual disability syndromes with variable brain malformations and neurologic findings. We believe that our patient's neurodevelopmental disabilities and cerebellar dysgenesis are a consequence of her biallelic *MED17* mutations and that mediator complex abnormalities may represent a particular category of transcriptional regulation disorders.

The views expressed are those of the author(s) and do not reflect the official policy of the Department of the Army, the Department of Defense or the U.S. Government.

AN N TERMINAL KAT6B MISSENSE VARIANT CAUSES A MILD ROBERTS SYNDROME PHENOTYPE, AND BRINGS INTO FOCUS PHENOTYPIC OVERLAPS ACROSS THE LYSINE ACETYL TRANSFERASE SYNDROMES

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Lysine acetyl transferases regulate transcription by regulating chromatin. Lysine acetyl transferase genes have been implicated in disease: *CREBBP* and *EP300* (the Rubinstein-Taybi syndromes), *ESCO2* (Roberts syndrome), *KAT6A* (ID), and *KAT6B* (genitopatellar and SBBYSS variant Ohdo syndromes). These latter syndromes are associated with exon 18 *KAT6B* variants, which are thought to escape nonsense mediated decay and act via a dominant negative effect[1]. A few individuals with more N-terminal or copy number *KAT6B* variants, and a non-specific developmental or "atypical Ohdo" phenotype have been described[2]. Given the rarity of such reports, the pathogenicity of non-exon 18 variants may be under called. We hypothesize that reverse phenotyping in instances of non-exon 18 variants is improved by thinking beyond the features seen in genitopatellar and Ohdo syndromes, and assessing for the features seen across the lysine acetyl transferases syndromes.

We describe a normocephalic, well grown 39 month boy with a left partial cleft lip, distinct from common clefts in that it is associated with more significant deficiency of regional tissues. He has shortening of the proximal and distal segments of his right leg. His right foot has four toes.

He has history of a small PFO and ASD. His facial features are neither genitopatellar nor Ohdo like. Further, his knees are normal without contracture, and the patellae are normal to palpate. The genitalia are normal. His development was normal until ~30 months when, over the course of a week, he lost his words and toilet training, behaviours changed. He has subsequently been formally diagnosed with autism. Exome sequencing identified one variant of interest, a *de novo* missense variant (c.458G>A; p.Arg153Gln), which has a CADD score of 25, and is not found in gene specific data bases or the population databases.

In comparing this boy to published and other cases we have cared for with lysine acetyl transferases syndromes, this boy's cleft, hypomelia, and foot malformation are similar to those of Roberts syndrome. Further, the acute loss of skills and development of autism is similar to that which we observed in an individual with EP300 syndrome. Careful description of this boy's features will support reverse phenotyping in other more subtly affected individuals where variants in lysine acetyl transferases are identified.

1. Campeau et al. (2012); *Human Mutation* 33: 1925–1929 2. Gannon et al. (2015); *European J of Human Genetics* 23: 1165–1170

Please consider the CAUSES Study an additional author. Investigators in the CAUSES Study include Shelin Adam, Christèle du Souich, Alison M. Elliott, Anna Lehman, Jill Mwenifumbo, Tanya N. Nelson, Clara van Karnebeek, and Jan M. Friedman.

HIDDEN PATHOLOGIES REVEALED BY STRUCTURE AND YEAST GENETICS: A LESSON FROM THE NAGER SYNDROME

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The spliceosome is a dynamic and flexible biological complex fundamentally involved in cell differentiation, homeostasis, and disease (Papasaikas and Valcarcel, 2016). Since the first draft of the human genome was published, over a dozen genes involved in the spliceosomal machinery have been reported in relation to the etiology of Mendelian diseases.

Nager syndrome (NS), the prototype for mandibulofacial dysostoses with preaxial limb defects, is one of such disorders due to mutations in SF3B4, a component of the U2 complex of the spliceosome (Bernier et al., 2012). SF3B4 encodes the spliceosome associated protein 49 (SAP49), a highly conserved protein with two RNA-recognition motifs (RRMs) in its N-terminal followed by a proline-glycine rich region domain in its C-terminal. During the pre-spliceosomal assembly of the U2 complex, SAP49 and SAP145 form a complex in which the two RRMs of SAP49 are required in the interaction (Champion-Arnaud and Reed, 1994). In Nager syndrome, approximately 60% of the patients have a heterozygous loss of function of SAP49 as a result of truncating mutations in SF3B4 (Bernier et al., 2012; Czeschik et al., 2013; Macpherson et al., 2014; Petit et al., 2014). We are reporting on a patient with a clinical diagnosis of NS who was found to have a novel missense substitution in exon 3 of SF3B4, in the RRM1 domain. The patient has a recognizable gestalt, normal growth parameters, repair for duodenal atresia, hearing loss, mild limb anomalies, and a neurobehavioral phenotype. The missense mutation, c.251T>G (I84R) is deemed pathogenic by all *in silico* analyses performed. Further, in order to test the effect of this mutation on viability, a plasmid shuffle in a yeast strain for I84R was performed and no viable transformants were identified. Following this results, we have surveyed the ExAC database for missense mutations in the SF3B4 gene and applied the same *in vitro* yeast model to test their pathogenicity. To date, we have identified one additional SF3B4 missense substitution L28P for which the yeast construct is lethal.

In this article, we bring evidence to support that pathogenicity of mutations in SF3B4 are not only related to the type of mutation but more importantly to the domain of the protein involved in specific spliceosomal function. We propose a rapid functional *in vitro* model that could be used particularly when studying the significance of mutations involved in spliceosomopathies and raise attention for potential pathogenic mutations reported in the ExAC database. This latter finding has particular implications for the counseling of patients in regards to disorders known to be underlined by incomplete penetrance including mandibulofacial dysostoses.

MUTATION C.925C>T (P.ARG309TRP) IN MECP2 CAUSES A SYNDROMIC FORM OF INTELLECTUAL DISABILITY THAT IS DISTINCT FROM RETT SYNDROME

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Mutations in MECP2 are associated with Rett syndrome characterized by initially normal progress followed by a period of regression, loss of functional use of the hands, feeding problems, seizures and severe intellectual disability in females. Affected males are rare with a high rate of early loss in male pregnancies. The function of MECP2 protein is incompletely understood. It binds to methylated DNA, and can then promote or repress transcription. We report a family with a mutation at c.925C>T (p.Arg309Trp) with multiple affected male and female family members. The initial presentation was a male infant with an apparently syndromic developmental disability. The proband was delivered to a G1P0 female at term; As a newborn he was noted to have hypotonia, macroglossia, macrocephaly, and mildly dysmorphic features.

Further evaluation identified congenital hypothyroidism, mild hepatomegaly, dilated aortic root (Z score 2.4 but has improved to 1.6 with growth), and severe developmental disability. He is nonverbal at age 9. He is able to walk and can follow a few one step commands. The family has had several additional children including 2 additional boys with presentation similar to the proband current ages 7 years and 22 months. The second boy also had aortic root dilation (z score 4.5 at age 7), and seizures. The oldest sister has developmental disability. She is verbal and ambulatory, but has not had movement disorder or regression. She has continued to gain skills.

The second sister has had normal development. The mother had a mild intellectual disability, but was able to communicate effectively and care for the children. She has a sister with similar disability who has 2 children with more severe developmental disability who reportedly are very similar to our proband. They have not been evaluated nor had genetic testing. Dysmorphic features have included macrocephaly, epicanthic folds, full lips with smooth philtrum, course facial features, and frontal cowlick. The affected sister was very mildly dysmorphic. Testing for X-linked intellectual disability reported no diagnostic mutations, but included 3 variants of unknown significance. Segregation within the family demonstrated that only the MECP2 c.925C>T change segregated with the phenotype. The mutation is in the C terminal end of a transcriptional repressor domain. The impact on protein function is not known. There are a total of 13 previous cases of MECP2 c.925C>T reported with clinical description. Two were described as unaffected females, 7 females with highly variable intellectual disability and variable dysmorphic features that overlap with our patients. In addition there are 3 additional males who are essentially similar to our patients except that hypothyroidism and dilation of the aortic root were not commented on. The discussion in the previous reports was based on comparison to Rett syndrome. On review of the available cases we believe that this mutation is associated with a syndromic X-linked intellectual disability that is distinct from Rett syndrome.

DE NOVO HETEROZYGOUS MISSENSE MUTATIONS IN *DDB1* CAUSE A NOVEL DISORDER OF TRANSCRIPTIONAL REGULATION MANIFESTING AS HYPOTONIA, INTELLECTUAL DISABILITY AND DYSMORPHIC FEATURES

Susan M White, Elizabeth Bhoj, Kym Boycott, Taila Hartley, Simon Sadedin, Broad, Dong Li, Jaime Barea, Paul Lockhart, Marjan Nezarati, Kristin Kernohan

The DNA damage binding protein DDB1 is part of the CUL4–DDB1 ubiquitin E3 ligase complex which is involved in the regulation of transcription, proteasome degradation, cell-cycle progression, replication and DNA damage response. Loss-of- function mutations in *CUL4* and in the gene encoding DDB1-CUL4 substrate receptor *PHIP1* have recently been identified to cause phenotypes of syndromic intellectual disability with hypotonia and obesity. We report three unrelated individuals with *de novo* heterozygous missense mutations in *DDB1* identified by whole exome sequencing. The individuals were ascertained through Matchmaker Exchange and have a remarkably similar phenotype comprising moderate hypotonia, mild to moderate intellectual disability, and strikingly similar facial features with wavy eyebrows, lateral extension of the palpebral fissures, mid-face hypoplasia and a small nose. The older affected child has truncal obesity.

The phenotypes caused by mutations in *CUL4*, *PHIP1* and *DDB1* have overlapping features of obesity, hypotonia, intellectual disability and dysmorphism, suggesting that this is a new family of conditions mediated by disruption of the ubiquitin ligase pathway. The CUL4-DDB1 ubiquitin E3 ligase complex binds with substrate receptor PHIP1 to target proteins for proteolysis and is involved in regulation of transcription, cell cycle and cell death. The CUL4–DDB1 ubiquitin ligase also interacts with several WD40-repeat proteins thereby regulating histone methylation. Studies of the functional impact of the *DDB1* mutations in patient lymphoblast cell lines will be presented.

HETEROZYGOUS *KAT5* MUTATIONS CAUSE A NEURODEVELOPMENTAL DISORDER WITH OVERLAPPING FACIAL DYSMORPHISMS, SHORT STATURE, AND BRAIN MALFORMATIONS

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Epigenetic regulation by histone acetylation is essential for proper development, and its role in human genetic diseases is increasingly being recognized. Notably, mutations in lysine acetyltransferase genes, such as *KAT6A* and *KAT6B*, have been identified in individuals with neurodevelopmental disorders characterized by intellectual disability

and malformations. *KAT5* (a.k.a. *TIP60*) mutations have not yet been associated with a syndrome. *KAT5* encodes a lysine acetyltransferase involved in DNA repair, chromatin remodeling, apoptosis and cell proliferation. *KAT5*-mediated acetylation of lysine 5 of histone H2A leads to chromatin relaxation and facilitates DNA double-strand break detection, DNA repair protein recruitment and transcriptional activation.

Here, we compare three individuals with *de novo* missense mutations in *KAT5* affecting highly conserved residues. All three individuals have short stature, cerebral malformations, seizures and global developmental delay or intellectual disability, with a significant speech disorder. Dysmorphisms each present in at least two individuals include a broad nasal bridge, downturned corners of the mouth, a prominent chin and a flat facial profile. Two individuals have microcephaly, and two have anomalies of the corpus callosum, with one also having cerebellar atrophy. One patient has focal polymicrogyria and cystic dilation of the fourth ventricle with inferior cerebellar vermis hypoplasia or atrophy. All three individuals present disruptive behavior and two individuals have a severe sleep disorder. All three individuals have genitourinary anomalies, including cryptorchidism, hypospadias, horseshoe kidneys and vesicoureteral reflux. One of them was born with unilateral cleft lip and palate.

Histone acetylation assays demonstrate that the two variants tested to date (NP_874369.1 Cys402Ser and Ser446Ala) impair the enzymatic function of the protein. Additional functional impacts, at the molecular, cellular and transcriptomic levels, are currently being investigated. In conclusion, *KAT5* mutations cause histone acetylation deficiency with likely transcriptional dysregulation of multiples downstream genes, thereby leading to a neurodevelopmental syndrome with overlapping dysmorphisms.

DETECTION OF GENES INVOLVED IN TRANSCRIPTIONAL PROCESSES UNDERLYING CONGENITAL DISORDERS IN 30 PATIENTS: RESULTS FROM 200 FAMILIES

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Whole exome sequencing (WES) offers a powerful tool to rapidly and efficiently sequence all coding genes in individuals presenting with phenotypically and genetically heterogeneous disorders, such as multiple congenital anomaly (MCA) with dysmorphic features, heart defects and/or neurological manifestations. We have undertaken a focused study of rare MCA using WES approach at Center for Applied Genomics at The Children's Hospital of Philadelphia. Thus far, we have sequenced 432 subjects reflecting 200 independent kindreds exhibiting a range of structural malformations of multiple organs - 70% of patients have craniofacial dysmorphisms (such as microcephaly, macrocephaly, hypertelorism, downslanted palpebral fissures, wide nasal bridge, microphthalmia, anophthalmia, cleft lip and/or palate, craniosynostosis, thick eyebrows, microtia, coloboma, and Pierre Robin Sequence); 59.5% have developmental delay/mental retardation/intellectual disability/autism; over one third (34.5%) have cardiovascular or abdominal defects (such as heterotaxy, dilated aorta, ASD, VSD, small ventricle, omphalocele, congenital diaphragmatic hernia, and urogenital anomalies); about one quarter (26%) have neuromuscular involvement; 17.5% have limb defects (such as syndactyly, ectrodactyly, polydactyly, clinodactyly, brachydactyly, triphalangeal thumb, absent radius, absent thumb, and club foot); 14.5% have short stature and/or skeletal dysplasia; and 6% have brain malformations (such as hypoplasia/agenesis of corpus callosum, and cerebellar hypoplasia). We have applied Mendelian filtering techniques, further supported by several lines of evidence, to successfully identify causal mutations in known and novel Mendelian genes for 31.5% of kindreds. The identified mutations were mostly de novo dominant (n = 51, 81%), consistent with the majority of cases of the study cohort arise sporadically without familial occurrence, but also X-linked or autosomal recessive (n = 12, 19%). Among them, we have identified and characterized a number of known and novel disease-causing genes in transcriptional processes and related epigenetic modification pathway important for human developmental disorders in 15% of patients in this cohort, such as *H3F3A*, *KMT5B*, *SMARCE1*, *U2AF2*, *STAG2*, *MED12*, *SMC1A*, *MAF*, *SATB2*, *ADNP*, *HIVEP2*, *HNPNPK*, *TP63*, *DDX3X*, *SF3B4*, *LEUTX*, *EFTUD2*, *FOXP1*, *FOXC1*, *MED13L*, *TFAP2A*, and *EYA1*. WES also identified likely pathogenic variants in about a dozen genes not previously implicated in rare multiple congenital anomalies, including but not limited to *DDB1*, *BMP2*, *MTOR*, *RICTOR*, *TUBA1B*, *SMG5*, and *SPECC1*. We will present an overview of our recent novel discoveries in MCA, discuss options to partner with laboratories that can provide follow up functional measurements, and explore novel mechanisms as therapeutic targets for part of symptoms representing in MCA.

TATTON-BROWN-RAHMAN SYNDROME, A DISORDER OF DNA METHYLATION, IS A DIAGNOSTIC CONSIDERATION IN INDIVIDUALS WITH MARFANIOD FEATURES

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Tatton- Brown- Rahman (TBR) syndrome is a recently described overgrowth syndrome with intellectual disability, associated with pathogenic variants in DNMT3A, which encodes DNA (cytosine-5)-methyltransferase 3A. Pathogenic mutations in DNMT3A alter residues in

functional domains of the protein, and protein modelling suggests they interfere with domain- domain interactions and histone binding, thereby disrupting DNA methylation. 13/152 individuals with overgrowth of unknown etiology after analysis for Sotos, Weaver, PTEN Hamartoma and Beckwith Wiedemann syndromes were identified to have pathogenic mutations in DNMT3A, suggesting it is relatively common (1). The series suggested association with features such as scoliosis and identified a distinctive facial appearance characterized by a round face, heavy horizontal eyebrows and narrow palpebral fissures (1,2). The purpose of this report is to expand the phenotype of TBR syndrome and highlight it as a diagnostic consideration in the differential diagnosis of Loeys Dietz and Marfan syndromes. We report a 12 year old female referred for query connective tissue disorder. She had tall stature 169cm (> 99th percentile), mitral valve prolapse, arachnodactyly, thoracic lordosis and scoliosis, straight heavy eyebrows, short palpebral fissures (<3rd centile), high arched palate, bifid uvula, droplet cortical lens opacities, failure and retention of some primary dentition and hirsutism. There was mild ID and a history of three nocturnal seizures. A de novo pathogenic missense mutation in DNMT3A was identified by whole exome sequencing. The presented case has some hallmarks of TBR syndrome such as the mild ID, distinctive facies, tall stature and scoliosis. Other features such as bifid uvula and droplet lens opacities have not been previously reported, but high arched palate, mitral valve prolapse, scoliosis each do appear > of the previously described cases. This case expands the TBR syndrome phenotype and suggests it should be considered a differential diagnosis of patients being assessed for Marfan (like) conditions. The novel mutation identified in this case is located in the C-terminal DNA methyltransferase domain of DNMT3A and so is expected to result in disruption of DNA methylation and transcriptional regulation. 1) Nature Genetics 2014;46:385–388 2) Clin Genet 2017: 91: 623–628

*Investigators in the CAUSES Study include Shelin Adam, Christèle du Souich, Alison M. Elliott, Anna Lehman, Jill Mwenifumbo, Tanya N. Nelson, Clara van Karnebeek, and Jan M. Friedman.

THE MANY FACES OF PEROXISOMAL DISORDERS: LESSONS FROM A LARGE COHORT

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Peroxisomes are single-membrane organelles that enclose various enzymes required for many important functions in human metabolism. Defects in the biogenesis and/or function of peroxisomes result in such phenotypes as Zellweger-spectrum, rhizomelic chondrodysplasia punctate, isolated fatty acid beta-oxidation deficiency, as well as less known phenotypes that were only recently described. In this study, we describe the largest cohort to date (75 families) of clinically, biochemically and molecularly characterized patients with peroxisomal disorders.

At the molecular level, we identified 41 disease-causing variants, more than half of which (22) are novel. Missense variants were the predominant class of variants accounting for 34% (14/41), followed by small indels 24% (10/41), nonsense 20% (8/41), splicing 12%, and large rearrangements in 10%. The founder nature of many of the variants identified in this study allowed us to make a minimum estimate of disease incidence of around 1 per 20,000, much higher than previous estimates in other populations.

Clinically, we found a strong genotype/phenotype correlation that is primarily driven by individual alleles rather than individual genes or mutations classes. Although Zellweger spectrum disorder is known for its severity that results in death in infancy, 39% (16/40) of the patients have documented survival at the time of reporting the study above one year of life.

Most unusual among the long-term survivors was a multiplex family in which the affected members had minimum to no elevation of VLCFA and presented as adults with nonspecific intellectual disability. Other unusual presentations include Usher syndrome-like phenotype, as well as the very recently described Peroxisomal fatty acyl-CoA reductase 1 disorder as well as CRSPW syndrome (cone-rod dystrophy, developmental delay, spastic paraparesis, and white matter disease).

We conclude that peroxisomal disorders are more heterogeneous in their clinical presentation than previously thought and should be considered in the differential diagnosis of a wide array of clinical presentations. Our data also demonstrate that milder forms cannot be ruled out by the "gold standard" VLCFA assay, which highlights the value of a genomics-first approach in these cases.

CELL CYCLE AND CILIOPATHY PATHWAYS - A PHENOTYPE APPROACH TO THE INVESTIGATION OF AUTOSOMAL RECESSIVE INTESTINAL ATRESIA

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The clinical presentation of fetal malformations or malformation syndromes, particularly if lethal in utero or perinatally, and their genetic causes often remain undefined for various reasons. Intestinal atresias have long been hypothesized to result from either failure of recanalization of the intestinal lumen or in utero vascular accidents, jejunal atresia being the most common in newborns. However, some animal models implicate disruptions in molecular pathways in atresia formation. Clinically, few familial hereditary forms have been reported. Apple peel atresia is most likely due to agenesis or maldevelopment of the superior mesenteric artery and its branches.

We have recently described autosomal recessive mutations in *CENPF* to be causal for Strømme syndrome in the original sibs presenting with the triad of apple peel intestinal atresia, ocular malformations and microcephaly and a novel family with two fetuses with duodenal and multiple jejunal atresias, hyperrotation of the midgut, histopathological signs of intestinal and cardiac myopathy and overrepresentation of bi-nucleated cells, additional organ anomalies including eye anomalies and preaxial polydactyly – phenotypic variability compatible with the CENPF regulatory role in cell proliferation and ciliary function. In

addition, autosomal recessive multiple intestinal atresia with or without immunodeficiency is due to mutations in *TTC7A*, recessive mutations in *RFX6* have been described in the clinically overlapping Mitchell-Riley and Martinez-Frias syndromes. Interestingly, autosomal dominant mutations in *ACTG2* were shown to cause familial visceral myopathy presenting with functional gastrointestinal obstruction even leading to death due to impaired function of enteric smooth muscle cells. Single case reports mention the association of jejunal atresia and polydactyly, biliary atresia or heterotaxy.

Since we systematically use exome sequencing for discovery of novel autosomal recessive conditions in non-consanguineous families with recurrent lethal fetal phenotypes after autopsy, we now report on a family with a first girl presenting with duodenal atresia and heterotaxy, the intrauterine death of the second boy at 21 gestational weeks with IUGR, anal atresia, malrotation, and cystic dysplastic kidneys, and a boy born and deceased at 32 weeks with arthrogryposis, hypotrophic skeletal and smooth muscle, intestinal string of pearls stenosis and hypoplastic genitalia. We hypothesize an autosomal recessive disorder due to mutations in a gene implied in an embryonic pathway affecting cell cycle and ciliary function leading to a variably expressed phenotype linking all three phenotypic findings - atresia, muscle impairment and clinical signs of ciliopathies. We will present data of the currently ongoing analysis.

Atresia phenotypes show great overlap, but also important even intrafamilial variability – in severity but also type of atresia as well as associated anomalies. Therefore, they likely represent different degrees of malformations of the same or linked embryologic process. Autosomal recessive inheritance may play a significant role. We discuss the degrees of phenotypes and linked pathways as well as the notion that genes involved in cell cycle control and division as well as ciliogenesis and -function may be considered important candidates when elucidating underlying pathways in intestinal atresia.

FOREGUT MALFORMATIONS: THE MIDLINE ASSOCIATED WITH LATERALITY DEFECTS

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Establishment of the various body axes, including left-right asymmetry, begins just before and during gastrulation with initiation of the primitive streak by *NODAL*, and results in a complex pattern of spatial and temporal gene expression to allow the development of both symmetric and asymmetric structures. Midline defects are reported in heterotaxy disorders, although foregut defects receive less attention than the asymmetric midgut. The purpose of this report is to highlight foregut malformation in two malformed fetuses with laterality defects and review the pertinent embryology of the trachea/esophagus.

Case 1 is a stillborn male delivered to a 26 yo G2P1sAb1 healthy mother at 33 wk gestation. Autopsy showed occipital encephalocele, cleft palate, right arm phocomelia with 2 digits, absent left thumb, dextrocardia, complex heart defect, abnormal lung lobation, **complete tracheal rings throughout**, accessory spleens, GI malrotation, multicystic right and absent left kidney, abnormal ribs and scoliosis. Karyotype and array CGH normal. WES revealed 2 inherited heterozygous variants considered likely pathogenic; a *NODAL* missense variant (mat) and a frameshift in *DYNC2H1* (pat), associated with Short Rib Thoracic Dysplasia 3. In addition were 2 more VUS, considered possibly related to the phenotype. Case 2 is a male delivered to a 21 yo G1P1 mother at 26 wk gestation. Autopsy showed alobar holoprosencephaly, bilateral microphthalmia, cleft lip, dysgnathia, microglossia,/glossoptosis, dextrocardia, marked LV dilation, bilateral unsegmented lungs and **esophageal atresia with tracheoesophageal (TE) fistula.** Array CGH was normal.

The developmental biology of the foregut derivatives, including patterning of the tracheal cartilaginous rings has just recently been studied in detail. *Shh*, a familiar gene in left-right signaling and patterning the ventral neural tube, has also been shown in a mouse model to be critical in foregut development. *Shh* signaling is required for the formation and patterning of tracheal cartilage by regulation of *Sox9* [Park et al. (2010); *Dev Dyn* 239: 514–526]. Antagonism in the spatial relationship between smooth muscle and cartilage in the mouse airway is also documented [Hines et al. (2013); *PNAS* 110: 19444–19449]. Complete tracheal rings may thus be the result of failure to maintain the ventral restricted pattern. Likewise, esophageal atresia/TE fistula may be the result of local disruption or abnormal dorsal/ventral positioning of the tracheoesophageal septum.

It is not certain whether the *NODAL* variant contributed to the defects in Case 1. The variant (R275H) involves the same codon as a previously reported case of heterotaxy, also inherited from unaffected mother. Heterotaxy disorders are typically sporadic, but rare familial variants in *NODAL* have been associated with heterotaxy and holoprosencephaly, and it has been suggested that these variants may interact with other genetic modifiers to result in malformation [Roessler et al. (2009); *Mol Genet Metab* 98: 225–234]. The combination of variants in Case 1 may thus be important. A similar genetic mechanism has been suggested for *OTX2* related microphthalmia and otocephaly-dysgnathia complex [Patat et al. (2013); *Mol Syndromol* 4: 302–305].

LIKELIHOOD OF VATER/VACTERL IN PATIENTS WITH ESOPHAGEAL ATRESIA

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Esophageal atresia (EA), with or without, Tracheo-esophageal fistula (TEF) (EA+/-TEF) are anomalies of the developing foregut occurring in ~1:5000 live births. They can be isolated anomalies or present in association with other malformations. EA+/-TEF is a part of VATER/VACTERL association as defined by the presence of: **V**ertebral defects, **A**nal atresia, **C**ardiac defects, **TE** fistula, **R**enal, and **L**imb anomalies. Defects comprising VATER/VACTERL vary somewhat among clinicians; however it is diagnosed clinically by the presence of at least three of the above listed malformations without evidence of alternate syndrome diagnoses such as Trisomies 18 and 21, 22q11.2 deletion, and CHARGE syndrome.

The purpose of our study was to evaluate cases of EA+/-TEF within the context of two standardized definitions of VATER/VACTERL to investigate how many cases meet formal criteria for diagnosis.

In our study we reviewed all the cases of EA+/-TEF as recorded into Texas Birth Defects Registry (TxBDR) from 1999 to 2014. We defined VATER as described by Quan and Smith in 1973, and VACTERL based on the 2011 review by Solomon. Cases were classified into five groups using National Birth Defects Prevention Study criteria – Isolated, EA+/-TEF with malformations meeting defined criteria for VATER and/or VACTERL, EA+/-TEF associated with multiple malformations not meeting the VATER/VACTERL definitions (unknown etiology), and those with known chromosomal or single gene disorders.

There were 174 cases of EA, and 1001 cases of EA with TEF. All cases that met the definition of VATER also met criteria for VACTERL: the columns are do not add to 100%.

	EA		EA with TEF		Total	
Total	174		1001		1175	
Isolated	43	24.7%	262	26.1%	305	25.9%
VATER	9	5.2%	164	16.3%	173	14.7%
VACTERL	20	11.5%	223	22.2%	243	20.6%
Multiple malformations	63	36.2%	379	37.7%	442	37.6%
Single gene/ syndromes	48	27.5%	136	13.5%	184	15.6%

Among the cases of EA+/-TEF in the TxBDR, 14.7% met our criteria for a diagnosis of VATER and 20.6% for a diagnosis of VACTERL. It is noted that the rates were higher for cases of EA with TEF. Conversely, identifiable genetic diagnoses were more likely in cases with EA without TEF. The two groups were roughly equal in isolated and multiple malformation cases that did not meet standardized VATER/VACTERL definition. This reinforces the need to consider alternate, possibly identifiable, diagnoses in the presence of these foregut defects, particularly EA without TEF.

CHOLEDOCHAL CYSTS: A FOREGUT MALFORMATION ASSOCIATED WITH TRISOMY 18

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Choledochal cysts are a rare congenital malformation resulting in cystic dilatation of the biliary tree due to abnormal development of the foregut. The incidence of choledochal cysts is approximately 1 in 100,000 to 150,000 infants though higher in some populations. They are 4 times more common in females. There are a number of types of choledochal cysts which are most commonly categorized according to the Todani Classification System. On the other hand, trisomy 18 is a common chromosomal abnormality occurring in approximately 1 in 5000 live births. Median survival is approximately 2 weeks, although 8% of children are alive at 12 months. Malformations in multiple organ systems are common, including gastrointestinal findings of omphalocele, Meckel's diverticulum, intestinal malrotation, and, less commonly, biliary atresia. The purpose of this report is to describe two previously unreported cases of choledochal cysts in patients with trisomy 18, review other previously reported cases in the literature, and to review the embryology and various types of this relatively rare malformation.

Case 1 was a female with trisomy 18 who was born at term weighing 2300 grams with cleft lip and palate, choanal atresia, microtia, a preauricular tag and VSD. At 21 months of age she had her cleft lip and palate repaired, and, while in the hospital, was found to have poor growth and a possible seizure. X-rays showed osteopenia, and a skeletal survey showed multiple fractures of the ribs, vertebral bodies and femurs. Radiographs also identified calcifications in the area of the liver, and a large mass overlying the liver. A CT scan revealed the calcifications to be stones within a choledochal cyst. A biopsy of the liver mass revealed a hepatoblastoma. She has undergone chemotherapy and subsequent resection of the residual mass. Case 2 was a female with trisomy 18 who was born at term weighing 2155 grams with VSD and tracheobronchomalacia. She had already undergone pulmonary banding and was ventilator- dependent with a tracheostomy. She developed severe hyperbilirubinemia and was found to have a large choledochal cyst. A drain was placed in the cysts at 5 months of age at an outside hospital. She presented at 8 months of age at our institution with severe hyponatremia due to drainage from the type I cyst. After stabilization, she was underwent a Roux-en-Y hepaticojejunostomy with resection of the cyst.

The gallbladder and biliary tree develop along with the liver at the distal end of the foregut beginning in the middle of 3rd week of gestation. The cystic duct is visible by the late 4th week budding from the side of the hepatic diverticulum. The embryologic origins of choledochal cysts are thought to most commonly involve an abnormal union of the pancreaticobiliary duct due to failure of migration of the choledopancreatic junction into the duodenal wall. While other intrahepatic abnormalities have been previously described with trisomy 18, a review of the literature found only 2 other cases in which choledochal cysts were incidentally mentioned. Our findings suggest that choledochal cysts, a rare foregut malformation, occur more commonly in trisomy 18, and expand the phenotype of this syndrome.

THE NATURAL HISTORY (PRENATAL, PERINATAL, POSTNATAL) OF TRISOMY 16 SUSPECTED BY NON-INVASIVE PRENATAL SCREENING

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Maternal uniparental disomy for chromosome 16 (matUPD16) was first reported in 1992.

However, whether matUPD16 causes a recognizable syndrome is controversial. One complication is that matUPD16 is almost always accompanied by mosaic trisomy 16, either confined to the placenta or also present mosaically in the fetus. The challenge lies in determining

whether mosaic trisomy 16 or matUPD16 (or both) are responsible for the subsequent phenotypic effects in the infant. Identification of pregnancies affected by trisomy 16 is becoming more common as select laboratories report on trisomy 16 in addition to trisomy 13, 18, and 21.

We report the prenatal, perinatal, and postnatal findings for seven maternal and fetal/infant pairs with trisomy 16 detected during pregnancy. Two mothers with elevated hCG levels on a quad screen were labeled with a risk for trisomy 21 that was >10 times their age-related risk. Average maternal age was 32 years (range 25–37). Maternal preeclampsia leading to eclampsia or HELPP syndrome was common, with six infants delivered by C-section between 26–35 weeks gestation. One pregnancy was electively terminated at 24 weeks. A two-vessel cord was noted in two cases. Echogenic bowel was noted in three fetuses prenatally. In almost all of the pregnancies, slowing of fetal growth was a concern. Although these fetuses were said to have IUGR (intrauterine growth restriction) during the pregnancies, values for birth weight, length and OFC were 2nd-60th centile for all newborns.

In three cases, the cell-free DNA (cfDNA) screen identified an increased risk of trisomy 16. Three mothers had normal cfDNA screening performed by laboratories that did not report trisomy 16. Homozygosity on chromosome 16, suggestive but not diagnostic of uniparental disomy, were detected in two cases by SNP microarray. The seventh case was diagnosed by prenatal amniocentesis which showed mosaic trisomy 16 (5/20 cells). All placental samples tested showed trisomy 16 (mosaic or full). Foreskin fibroblasts from two males had no evidence of mosaic trisomy 16, illustrating the limited diagnostic window of detection.

Postnatally, infants had complications related to prematurity. Congenital anomalies were also frequent. Cardiac malformations affected six of the fetuses/infants and included PFO, PDA, ASD, left-sided IVC, AV canal, hypoplastic left heart or single ventricle, and TAPVR. One female had a multicystic dysplastic kidney, while one male had bilateral moderate/severe pelvicaliectasis with ureteral dilation. One male had an imperforate anus. Two cases had limb anomalies: one with a shortened 3rd phalanx with a hypoplastic nail, the other noted prenatally to have a left absent radius, shortened ulna, abnormal left digits, and bilateral contracted/clenched hands.

The number of conditions included in non-invasive prenatal screening is expanding and can include trisomy 16. This presents a unique opportunity to identify confined placental mosaicism of trisomy 16 with or without uniparental disomy, a condition that can be difficult to detect postnatally. Clinically significant maternal and fetal issues can occur in the setting of placental and/or mosaic fetal trisomy 16 or UPD16. Analyzing the resultant clinical and molecular data improves our understanding of how to properly care for these families.

COMPLICATIONS FOLLOWING HEART TRANSPLANTATION IN PATIENTS WITH DANON DISEASE

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Danon disease is an X-linked disorder caused by mutations in *LAMP2* (lysosome-associated membrane protein 2) located at Xq24. Clinical features include cardiac and skeletal myopathy, cardiac conduction abnormalities, intellectual disability and retinal anomalies. Cardiomyopathy, primarily hypertrophic (HCM) and dilated (DCM), is often severe and progressive necessitating transplantation. The postoperative course, however, may be complicated by profound weakness secondary to the primary disorder. We present 5 patients with Danon disease (representing 3 families) that underwent cardiac transplantation. Postsurgical outcomes are herein described.

Pt. 1 was diagnosed with HCM at age 11 years. Despite ICD implantation and cardiac myectomy at age 13, he developed end-stage heart failure (HF) and subsequently underwent orthotopic heart transplant (HT) at age 16. His postoperative course was complicated by extreme muscle weakness necessitating several weeks of intensive outpatient rehabilitation. Gene testing identified a c.293 G>A (p.Trp98Stop) mutation in *LAMP2*. Pt. 2, the younger brother of Pt.1, had a history of WPW and ventricular tachycardia prior to being diagnosed with HCM at age 9. After developing end-stage HF, he underwent implantation of a left ventricular assist device (HVAD) at age 18 followed by HT one month later. His postoperative course was complicated by prolonged ventilation, hypotension and extensive muscle weakness necessitating several weeks of inpatient rehabilitation. Pt. 3 was diagnosed with L ventricular noncompaction after presenting with palpitations and syncope at age 14. He eventually developed DCM and underwent HT at 19. His postoperative course was notable for weakness, seizures and prolonged intubation. Muscle biopsy demonstrated vacuolar myopathy; a c.877 C>T (p.Arg293Ter) mutation was identified. Pts. 4 and 5 are aunt and niece with an extensive family history of cardiomyopathy. The niece was diagnosed with HCM at age 8, underwent HT at age 20 and died 18 months postoperatively of transplant vasculopathy. Her aunt was diagnosed with arrhythmia at age 40 and subsequently required HT by age 44. She died on POD#5 of cardiac arrest of unknown etiology. An IVS 7-1 G>A mutation causing abnormal splicing was identified in this family.

The *LAMP2* protein coats the inner surface of the lysosomal membrane where it protects against hydrolytic enzymes. It is composed of a glycosylated luminal domain, a transmembrane domain and a short carboxy-terminal cytoplasmic tail. Alternative splicing of the protein leads to 3 isoforms (2A, 2B, 2C), each of which is proposed to have a different role in the autophagy process; *LAMP2B* is thought to be expressed at higher levels in the heart, skeletal muscle and brain. Reduction of the protein leads to disruption of intracytoplasmic trafficking and ultimately to accumulation of autophagic vacuoles. How this pathophysiologic finding results in Danon disease is not fully understood. Genotype/phenotype correlations have not been described to date.

Danon disease is a severe and progressive condition with a grave prognosis. While HT may be the only option for cardiac rehabilitation, ongoing skeletal myopathy complicates postoperative recovery.

MYHRE SYNDROME INCLUDES NEOPLASIA, ESPECIALLY ENDOMETRIAL CARCINOMA

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Myhre syndrome (OMIM #139210) is due to specific *SMAD4* mutations, which cause short stature and a striking multisystem phenotype. The largest review[1]of 54 patients (pts) focusing on cardiovascular (CV) abnormalities included 3/54 (6%) with neoplasia, notable given the young age (20 yrs, all pts). Additional pts have prompted us to report a possible increase in endometrial cancer, in particular, which may have important clinical implications.

RESULTS: 6/61 (10%) pts with molecularly verified Myhre syndrome have neoplasia. Of those with cancer, the mean/median age was 31/29 yrs, compared to mean age of 20 yrs among all patients. Of note, 3/34 (9%) females have endometrial cancer. 3/6 (50%) with neoplasms have the rare p.Arg496C mutation which is present in only 10% of all pts.

CONCLUSIONS: Germline loss of function mutations in *SMAD4* cause autosomal dominant juvenile polyposis syndrome (JP) with hereditary hemorrhagic telangiectasia (HHT)[2]. Somatic mutations are common in multiple cancers. In contrast, the locus-limited *de novo* missense mutations that cause Myhre syndrome are considered gain-of-function mutations, currently not known to contribute to cancer susceptibility. Although current numbers are small, 1) there may be a predisposition to benign and malignant neoplasms especially endometrial cancer in Myhre syndrome. The mechanism is unclear, but not inconsistent with the role of TGF-ß signaling in tumorigenesis, though gain-of-function mutations may be novel. 2) It is unclear if traditional risk factors for endometrial cancer are relevant here, but limited reports suggest a tendency for menstrual irregularity. Patients may benefit from endocrinologic and gynecologic evaluations, and a heightened awareness for potential neoplasms. Surveillance with diagnostic testing is not yet indicated. 3) In addition, the three other reported neoplasms are, unexpectedly, part of the NFI/II spectrum. 4) The Arg mutation may confer increased risk. 4) Future studies should include genomic analysis of tumors.

1 Lin et al. (2016); *AJMG*; 2 Wain et al. (2014); *Gen in Med*; 4 Ikushima & Miyazono (2010); *Nat Rev/Cancer*

15q11.2 DEL SYNDROME: EXPANDING THE PHENOTYPE

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It is recognized that a pattern of dysmorphological and neurobehavioral abnormalities result from a 15q11.2 chromosomal microdeletion. However, because of the subtle physical features seen in affected individuals, this condition may escape recognition. Patients may not be referred to Medical Genetics for evaluation and testing. As more patients are recognized, the emerging phenotype suggests that this should be a recognizable pattern of abnormalities. The most common features include developmental, motor, and language delays; behavior and emotional problems; attention deficit disorders; and autism spectrum disorder. Other features may include congenital heart disease, clefting, broad forehead, ataxia and seizures.

We present data in a series of 16 patients that were evaluated in our center due to neurodevelopmental difficulties between 2005 and 2016. They were diagnosed with 15q11.2 microdeletion syndrome by microarray analysis. The group consisted of 10 males and 6 females between the ages of 3 and 15 years. The most common clinical findings included developmental delay (94%), hypotonia (88%), ADHD (75%), anxiety (50%), feeding difficulties (44%), autism (31%), dolicocephaly (25%), 2–3 toe syndactyly (19%), seizure activity (19%), and congenital heart disease (13%). Additional findings included prominence of the metopic suture, epicanthal folds, micrognathia, ankle torsion, incontinence, sleeping difficulties. There was tendency to become overweight with age. Our developmental evaluation by the Bayley Scales of Infant development indicated an average Mental Developmental Index of 75

TABLE 1 PATIENT TABLE: All known neoplasms reported in Myhre Syndrome

Type of cancer Patient, Author, Year	Age dx neoplasia/ last visit, sex	Type of testing; *SMAD4* mutation	Additional history
1Endometrioid adenoCA, II. (Lindor et al., '02, '12)	44/50 (53 death) F	Targeted gene p.Arg496C	Onset menses (12 y); irregular and infrequent
Endometrioid endometrial CA, Stage IV (New pt MGH)	32/35 F	Clinical exome seq Ile500 Val	Early menses (7y). PCOS, DM1, fatty liver. FH cancers.
Endometrial CA, Stage I (Kalia et al., ACMG poster 2017)	47/47 F	Cancer screening panel p.Arg496C	FH Lynch syndrome? Panel test reported only *SMAD4*. Daily bleeding 15–26 yrs
1Mesencephalic glioma (Caputo et al., 2012, pt T0_10)	13/13 M	Clinical exome seq Ile500 Thr	Fibrous dysplasia maxilla
Optic nerve sheath meningioma (Starr et al., 2016, pt 3)	23/26 (death) F	Targeted gene Ile500 Val	Dysfunctional uterine bleeding, hysterectomy.
Vestibular schwannoma (Kernis et al., 2014)	26/26 M	Targeted gene p.Arg496C	

(NI = 85) and Motor Developmental Index of 75 (NI = 85). Close to 43% of patients had OFC > or equal 97%ile which suggests the presence of macrocephaly in at least half of the patients evaluated. This finding should be taken cautiously as it was a retrospective review of the charts. About 86% of the cases required medical treatment of their neurobehavioral difficulties, all responded well to treatment showing improvement of their neurocognitive functioning as evidenced by school reports and resolution of symptoms.

Our findings confirm the need for all patients with ADHD to be evaluated by a medical geneticist as recognition of clinical patterns would allow the guidance for appropriate genetic testing. The combination of Hypotonia, ADHD/autism, Developmental Delay and mild dysmorphology should alert the clinicians to the diagnosis of 15q11.2 del syndrome.

GASTROINTESTINAL COMPLAINTS IN WILLIAMS BEUREN SYNDROME AND SUPRAVALVAR AORTIC STENOSIS SUGGEST A VASCULAR CONTRIBUTION

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Background: Elastin insufficiency mediated diseases, Williams Beuren syndrome (WS) and supravalvar aortic stenosis (SVAS), cause a range of vascular phenotypes including arterial stenosis, vascular stiffness and altered blood flow to end organs. In addition, decreased elastin increases risk of connective tissue dysfunction in the bowel wall. Many affected individuals describe symptoms of abdominal pain and it is unclear to what extent these symptoms can be ascribed to their vascular or connective tissue disease. Methods: Parents or caregivers of 155 affected children and adults (152 WS and 3 SVAS) filled out a screening questionnaire related to GI symptoms as part of our WS/SVAS DNA and Tissue Bank. 38 individuals filled out a follow up questionnaire with more extensive GI questions and 18 participated in a 4 day visit to the NIH during which evaluation for vascular disease and other etiologies for abdominal pain were undertaken in all subjects. Statistical analyses were performed to investigate the prevalence of abdominal phenotypes and subsequent deep phenotyping in the smaller cohort aimed to correlate patient symptoms with imaging findings and testing. Results: As expected, questionnaire data revealed slow weight gain and colic in the majority of WS/SVAS infants. Oral motor dysfunction was also common in infancy, however, only 5% receive a temporary feeding tube and less than 1% require chronic g-tube feeding. Feeding challenges persisted into childhood with more than 50% reporting difficulty transitioning to textured foods. Altered bowel motility was reported in the majority, with 66% endorsing constipation and 36% noting diarrhea or reflux. 7% reported rectal prolapse. A minority of patients (<5%) note a history of childhood bowel surgery, including pyloroplasty or bowel resection. 40% reported jaundice. While most hyperbilirubinemia occurred in infancy, 3% noted chronic liver dysfunction. Celiac disease is uncommon. Diverticular disease, however, is an important cause of abdominal pain in adults with WS and occurs earlier than in non-WS/SVAS individuals. Nearly 50% of individuals report abdominal pain occurring more than once a week. To assess for a role of elastin vasculopathy in patients' abdominal pain, we performed CT angiograms on subjects older than 5 years who visited the NIH. Of these, 50% were found to have stenosis of at least one abdominal vessel (abdominal aorta, celiac, SMA or IMA). Nearly all patients with significant abdominal pain exhibited stenosis, while those without symptoms were less likely to have narrowing. Anxiety and decreased parasympathetic tone were seen nearly universally and may contribute to abdominal symptomatology in some. Conclusion: Gastrointestinal symptoms are present in most patients with WS/SVAS and span all ages. Current data point to a contributing role for vascular disease in gastrointestinal discomfort. However, in most cases stenosis is seen in only one vessel. Work in animal models suggests that abdominal vasculature in Eln+/- is narrow, stiff and poorly reactive, even in the absence of focal stenosis, suggesting that medical treatments that open these vessels may improve GI discomfort in this population. Adjunct treatments that improve anxiety and parasympathetic response could represent useful treatment modalities in the future.

FETAL CERVICAL HYPEREXTENSION (IN ARTHROGRYPOSIS)

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Perhaps the most dramatic position of a newborn after delivery is when there is hyperextension of the neck and back, such that the feet touch the head! That position will have been present in utero and many of the affected infants have multiple congenital contractures (arthrogryposis).

This report is about 41 such infants among over 4,000 cases of arthrogryposis. They were divided into extended elbows, flexed elbows and upper limbs only.

An increased occurrence of abnormal presentation (breech, face, transverse lie, etc.), uterine anomalies, fibroids, monozygotic twinning, torticollis, congenital scoliosis, large head, micrognathia, trismus, large ears, hirsutism, GI vascular anomalies, digit loss, and males were observed among these 41 infants. Only 4 died. The rest did surprisingly well, "opening up" through slow stretching and physical therapy. Most appeared to have Amyoplasia when they returned to a "normal" position.

Cervical hyperextension is seen in 5–7% of breech presentations, and it has been learned that such pregnancies should be delivered by C-section without attempt at version prior to the onset of labour in order to avoid trauma to the spinal cord and brainstem.

EXPERIENCE OF THE FIRST MULTIDISCIPLINARY ADULT PRADER-WILLI SYNDROME CLINIC IN THE USA

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Multidisciplinary clinics are a good way to reduce healthcare costs, to help individuals receive complete care for complex disorders, and to enhance and update their understanding of diagnoses, possible

complications, surveillance, and management. Until recently, the only such care for individuals with Prader-Willi Syndrome (PWS)was in the pediatrics setting. Our Adult Prader-Willi Syndrome clinic opened at UC Irvine Medical Center in July, 2015 with the support of the Prader-Willi California Foundation, and herein we report on its progress in the first 18 months. We have had approximately 31 visits of individuals with Prader-Willi Syndrome. The initial plan was to schedule a clinic four times a year, but with increased awareness of the clinic and the need for follow-up appointments, we quickly increased to every other month. The clinic has a multidisciplinary approach and is staffed by an adult endocrinologist, dietician, clinical geneticist, and genetic counselor, who is the program coordinator. The intent was to maximize healthcare benefits for the patient and family in a "one stop" clinic. Ideally, we would like to have a psychiatrist, gastroenterologist, and social worker present in the clinic as well, but so far, this has not been possible due to limited clinic space. However, they are available for consultation referrals. Data collected so far includes sex, age, cytogenomic or molecular diagnosis (if adequate records are not available, diagnostic testing is sent), past medical and behavioral history, growth data, family history and pedigree, and information regarding past and/or present treatment with growth hormone. Physical examination includes vital signs and measurements, BMI, and careful documentation of the presence or absence of physical and behavioral features associated with PWS, including any evidence of skin picking and edema. Behavioral symptoms are given special attention, which improves the satisfaction of the patient and his/her family or caregivers. It is also important to give every patient some private time with the clinician to discuss issues they may not want their family or caregivers to know about, such as sexual relationships, contraception, avoidance of sexually-transmitted diseases, and possible suicidal ideation or other concerns. This is done with the safety of the individual as paramount. Several patients who carry a clinical diagnosis of PWS do not have a confirmed molecular diagnosis, and it has been difficult to get insurance companies to authorize confirmatory testing. One female, who has had an unconfirmed molecular diagnosis of PWS for 24 years, has had a negative DNA methylation analysis, a normal SNP-oligo microarray, and the results of additional genetic testing are pending. She has many of the features of PWS received growth hormone for three to four years until the epiphyses closed. She has benign intracranial pressure but does not have typical facial features. We will present data from the clinic and discuss the various medical problems we have encountered, such as the difficulty in bringing adult patients' BMI down to <40 so that we can recommend starting growth hormone and the advantages of initiating treatment in previously untreated adults.

NIJMEGEN BREAKAGE SYNDROME: FOLLOW UP OF SEVEN CASES IN CHILE

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Nijmegen Breakage Syndrome (NBS) is a rare autosomal recessive disease. It is characterized by congenital microcephaly, intrauterine growth retardation and short stature, immunodeficiency, chromosome instability and cancer proneness. The disease seems to occur worldwide, but has a much higher prevalence among Central and Eastern European Slavic populations. Over 90% of identified patients bear a common deletion. We report the natural history of seven patients from Chile, South America. Since June 2000, seven patients have been referred to our genetic clinic, primarily because of microcephaly. Their diagnosis workup established NBS. Four probands were male, the mean age of their first genetic evaluation was 6 years (range 10 mo to 14 y) Strikingly six of them came from one particular region of Chile; one had history of consanguinity. All had a typical face with receding forehead, and a receding mandible. One patient was referred with diagnosis of NBS, two had already manifest malignancy at diagnosis. During their follow up two other patients developed non-Hodgkin lymphoma or Leukemia. Three of the seven patients are dead. All of them were homozygous for the common Slavic mutation 657del5 in the *NBS1* gene. NBS can be suspected in clinical grounds, although there is considerably clinical overlap with other inherited conditions. Its confirmation is useful for an appropriate surveillance and for genetic counseling. The fact that 6/7 patients came from the same region let us to propose a founder effect. Although historical records tell us that the Chilean population was founded by the admixture of local Amerindian populations and colonizing Spaniards, it is probable that the genomic make up of current Chileans is much more complex, with multiple ethnic groups contributing at different times since the initial contact between Americans and Europeans.

A NEONATE WITH MULTIPLE AGGRESSIVE CARDIAC RHABDOMYOMAS AND BIRT-HOGG-DUBÉ SYNDROME

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Cardiac rhabdomyomas are the most common primary cardiac tumors in the pediatric age group and, when syndromic, are most often associated with tuberous sclerosis (TSC). 47%-67% of fetuses/neonates with TSC have cardiac rhabdomyomas, and 52%–86% of patients with rhabdomyomas have mutations in TSC1 or TSC2. Rhabdomyomas have been reported in other syndromes, e.g., the nevoid basal cell carcinoma syndrome and (once) Beckwith-Wiedemann syndrome, but these are non-cardiac lesions, usually found in the head and neck.

Recent reports have noted clinical overlap between TSC and Birt-Hogg-Dubé Syndrome (BHDS), primarily facial angiofibromas and renal angiomyolipomas in patients with BHDS and occasional BDHS-associated lesions in individuals with TSC. There are only two reports of rhabdomyoma in BHDS; one was in a 5-month-old boy who had a cardiac arrest and two large intracardiac rhabdomyomas (Bondavalli et al., 2014), and the other was only mentioned as "rhabdomyoma," without the patient's age or the anatomic location, in a clinical and molecular study of 50 BHDS patients (Toro et al., 2008). There is also a

report of cardiac rhabdomyoma in 15 of 125 heterozygote Nihon rats, which have heterozygous mutations in Bhd (Kouchi et al., 2006).

We report a boy who, at 6 days of age, had a seizure-like episode requiring CPR; evaluation revealed arrhythmias and multiple biopsy-proved cardiac rhabdomyomas. The tumors did not regress and, in fact, continued to grow. An internal defibrillator was placed at 1 month of age, and he was hospitalized from 4 months to 6 months of age, when he had a cardiac transplant. Microarray and TSC1 and TSC2 sequencing and dup/del analysis were negative. Clinical exome trio sequencing revealed a heterozygous pathogenic mutation in FLCN, the gene for BHDS, inherited from his father: c.1252dupC (p.L418fs) in exon 11 (chr17:17119741). At 23 months, he had a single small hypopigmented spot but no other lesions associated with either TSC or BHDS; renal ultrasound and brain MRI were normal. He had speech delay and normal motor development. He also has a diagnosis of Crohn disease.

BHDS is an important differential diagnosis in cases of suspected TSC. Folliculin (FLCN), hamartin (TSC1), and tuberin (TSC2) all have roles in the mTOR pathway. Interestingly, BHDS results from mTOR inhibition, and TSC results from mTOR activation, and the presence of cardiac rhabdomyomas, renal angiomyolipomas, and facial angiofibromas in both disorders suggests that both increased and decreased mTOR activity can cause the same kinds of tumor.

BHDS usually presents in adult life, and there is little to no information about affected infants and children. Bondavalli, et al., stated that two reported cases of cardiac rhabdomyomas did not provide enough evidence to recommend routine screening for the lesions in asymptomatic offspring of affected individuals. However, BHDS is uncommon, and the potential consequences of cardiac rhabdomyomas are severe enough that we believe such screening is both medically and economically prudent.

DEVELOPMENTAL MILESTONES IN CHILDREN WITH ANGELMAN SYNDROME– FINDINGS FROM THE ANGELMAN SYNDROME NATURAL HISTORY STUDY

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Angelman syndrome (AS) results from the loss of expression of the maternally-inherited copy of *UBE3A* in the brain due to one of four mechanisms, viz. a deletion on maternal chromosome 15q11q13, paternal uniparental disomy (UPD) of chromosome 15, an imprinting defect, or a mutation in the maternally-inherited copy of *UBE3A*. Although AS is a neurodevelopmental disorder that was described over 50 years ago, the ages at which these individuals achieve various developmental milestones have not been systematically studied.

The AS Natural History Study initiated in 2006 is a longitudinal study in which each participant is evaluated at a study site at regular, typically annual, intervals. At each study visit, we ask parents the ages at which the participants achieved specific motor and language skills. We have enrolled 302 individuals with a cytogenetic or molecular confirmation of AS (age range: 5 months to 40½ years old).

Information on the achievement of developmental milestones was available on 296 participants, 72% of whom had a deletion, 11% had a UBE3A mutation, and the remaining 17% had UPD, imprinting defect, or abnormal methylation not further classified. Our data revealed that the median age [interquartile range] of sitting unsupported was 10 [8 – 13] months, crawling on all 4 limbs was 20 [15 – 28] months, pulling to stand was 21 [15 – 27] months, and walking independently was 36 [26 – 47] months. The median age [interquartile range] of transferring objects between hands was 15 [9 – 24] months and having a pincer grasp was 24 [18 – 38] months. The median age [interquartile range] of pointing or gesturing to indicate their needs was 30 [18 – 48] months, of using sign language was 35½ [24 – 54] months, and (among the 110 participants with verbal language), of speaking first words was 36 [24 – 47] months. We also found that most participants who had a deletion in the chromosome 15 AS critical region achieved these milestones at a later age than those who had either UPD/imprinting defects. We will present analyses of other developmental milestones and provide further breakdown of the age of achievement of these milestones by different molecular subclasses.

Our findings should help clinicians provide parents with a more accurate prognosis on the developmental trajectory in AS children, and facilitate the ability of clinicians to determine whether an AS child is achieving milestones at the expected rate, failure of which may be a sign of subclinical seizures or other medical complications.

THE NATURAL HISTORY OF MELANOCORTIN-4 RECEPTOR DEFICIENCY

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JH was seen for an initial genetics consultation at 17 months of age due to macrosomia and motor delays (in particular to "rule out Prader-Willi syndrome"). JH was born at 38 weeks gestation by repeat C-section to a 31 year old G2P2L2 female with a birth weight of 10 lb 11oz (>98th%), length 21 inches (98th%), and head circumference 35.5 cm (75th%) with apgars of 7 and 8. The pregnancy was complicated by maternal gestational diabetes requiring insulin. JH was in the NICU for 1 week after delivery for treatment of tachypnea and hypoglycemia. The family noted a vigorous appetite since birth with no significant illness other than a current hospitalization for respiratory distress associated with an RSV infection. Motor delays were evident with crawling at 14 months and the recent achievement of cruising at 17 months. At the time of our evaluation he demonstrated a weight of 65 pounds (z-score 7.6 *SD*), length 37 inches (z-score 4.6), and HC 53.5 (z-score 5.3). This child demonstrated noisy breathing, his neurologic exam was normal except that movement was difficult due to his markedly increased weight, and his genitalia was normal for age (with the phallus buried in the suprapubic fat pad). We did not recognize an obvious underlying syndrome and pursued a chromosome study and a

DNA methylation study for Prader-Willi syndrome, both of which were normal. We made a referral to endocrinology and requested a follow up in 6 months; however, we did not see JH for four years, during which he was identified as having melanocortin-4 receptor (MC4R) deficiency.

MC4R deficiency will be discussed in the context of the melanocortin pathway and energy homeostasis. The natural history of MC4R deficiency will be explored through the examination of JH's now 15 year history and the medical literature, with current and possible future weight management interventions discussed.

WHOLE EXOME SEQUENCING OF ADULT PATIENTS SIGNIFICANTLY EXPANDS THE NATURAL HISTORY OF RARE CONGENITAL DISORDERS: IS PNPT1- RELATED HEARING LOSS EVER NON-SYNDROMIC?

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Whole exome sequencing (WES) has accelerated the pace of novel gene discovery, and has led to the identification of novel rare disorders in a 'genotype first' manner where the phenotype of the condition is then delineated in 'reverse'. As the majority of patients undergoing WES are children, the natural history, adult phenotype and phenotypic spectrum is often unknown.

We describe three adult siblings (two males, one female) born to non-consanguineous parents who presented originally with isolated severe congenital sensorineural hearing loss (SNHL). In their 40s, they each developed and then followed a nearly identical neurodegenerative course presenting with ataxia, dystonia, generalized weakness, and progressive cognitive decline. Now, in their 50s and 60s, all have developed optic nerve atrophy, spasticity, and incontinence, and have limited communication through signing. After a series of negative biochemical and molecular investigations, WES of the two brothers revealed novel (absent in ExAC) compound heterozygous mutations in the PNPT1 gene; one frame shift (p.A581FS), and one missense (p.M745T) at a 100% conserved residue predicted to be pathogenic by both SIFT and Polyphen.

PNPT1 (Polyribonucleotide nucleotidyltransferase 1) is involved in small RNA import into the mitochondria. To date, there have been 4 reports of families with recessive PNPT1 mutations. In three families, all probands presented with congenital or early childhood onset multisystem disease compatible with a mitochondrial disorder. All had optic atrophy, SNHL, cognitive developmental delay and other neurological concerns.[1,2,3] In the 4th family however, three siblings presented with apparently isolated congenital SNHL, with the oldest being last examined at age 24.[4] Recessive mutations in PNPT1 are currently associated with both combined oxidative phosphorylation deficiency (MIM 614932) and isolated autosomal recessive deafness (MIM 614934). While the latter remains possible, our family challenges the concept that mutations in PNPT1 cause a pure sensory phenotype. Identification of further families will be required to fully understand the natural history associated with mutations in PTPN1. However, given the large window of time (four decades) with only a single symptom, there may be a future therapeutic opportunity for similar families.

It is likely that other rare disorders presenting in childhood with single system involvement may ultimately be associated with other complications. Reporting such families, studying more undiagnosed adult patients, creating gene or disease-specific databases with natural history information, and working in partnership with patients and their families will be necessary to further our understanding of such rare disorders.

1. Alodaib et al. (2016); *Eur J Hum Genet*. 2. Slavotinek et al. (2015); *Clin Genet* 3. Verdenne et al. (2012); *Am J Hum Genet* 4. Von Amen et al. (2012); *Am J Hum Genet*

THE NATURAL HISTORY OF PMM2-CDG

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PMM2-CDG, previously known as CDG-Ia, is the most-common and first-described congenital disorder of glycosylation (CDG). The CDGs are an emerging group of disorders that are characterized by aberrant post-translational modification of glycoproteins. PMM2-CDG is an autosomal recessive condition caused by mutations in *PMM2*, an 8-exon gene encoded on chromosome 16p13. Mutations in this gene reduce phosphomannomutase enzyme activity and disrupt N-linked glycosylation. Classically characterized as a recognizable syndrome with abnormal fat distribution, inverted nipples, developmental delay, and cerebellar hypoplasia, PMM2-CDG is a multisystem disorder with a highly variable phenotype. Patients are typically identified through one of three presentations: (1) infantile multisystem presentation, (2) late-infantile/childhood ataxia-intellectual disability, and (3) adult stable disability. The infantile multisystem presentation can further be divided into a neurologic-multivisceral form, with reported mortality of approximately 20%, and a nonfatal neurologic form. Lack of genotype- phenotype correlation has been reported.

We retrospectively collected clinical observations of patients with PMM2-CDG who were cared for at the Clinical Genetics and Metabolism divisions of The Children's Hospital of Philadelphia and Hospital of the University of Pennsylvania. Using ICD-10 codes, we identified 11 PMM2-CDG patients, who ranged in age from 2 months to 67 years. Longitudinal data from the cohort were standardized with the Nijmegen Pediatric CDG Rating Scale (NPCRS). Among the 11 patients, 10 had developmental delay, 9 had cerebellar hypoplasia/atrophy (1 patient never had brain imaging), 7 had inverted nipples, and 5 had an abnormal fat distribution. Other frequent features included hypotonia (9/11), esotropia/strabismus (6/11), pericardial effusions (7/11), failure to thrive (7/11), and endocrinopathies (6/11) including hypothyroidism

(5/6), hypoglycemia (4/6), hyperinsulinism (2/6), and adrenal insufficiency (2/6).

In contrast to the reported mortality rate of 20% with the infantile multisystem presentation, we observed a mortality rate of 55% (5/9) in patients presenting in the first year of life. One patient with the childhood ataxia-intellectual disability presentation is now 21 years old and exhibits seizure disorder, retinitis pigmentosa, and progressive kyphoscoliosis. Another patient was diagnosed at age 67 years after evaluation of progressive ataxia and cerebellar atrophy. This patient had been thought to have cerebral palsy due to abnormal childhood gait.

Various hypotheses have been raised regarding phenotype prediction in PMM2-CDG. Homozygosity or compound heterozygosity for pathogenic mutations with virtually no residual activity appears to be incompatible with life. Individuals with compound heterozygous mutations have been proposed to have milder phenotypes due to mitotic intragenic recombination. Observations from our patient population suggest that residual enzyme activity is the best predicator of clinical severity. In our cohort, all patients with the lethal infantile multisystem presentation had heterozygous pathogenic mutations that previously were reported to be associated with high mortality rates. In contrast, our patient with homozygous mutations and greater residual enzyme activity (57% of lower limit of control) is alive at 15 months of age, with only moderate impairment on the NPCRS. Despite delays, he is making developmental progress.

THE AP-4 DEFICIENCY SYNDROME: AN UNDERDIAGNOSED CEREBRAL PALSY LIKE DISORDER?

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Hereditary spastic paraplegias (HSPs) are a family of progressive spasticity disorders characterized by neurodegeneration of the corticospinal and spinocerebellar tracts. There is enormous genetic heterogeneity in these disorders, with over 80 different HSP loci reported.

One HSP subtype, known as spastic paraplegia type 47 (SPG47), is caused biallelic loss- of-function mutations in the *AP4B1* gene. AP4B1 is a component of Adapter Protein complex 4, a heterotetrameric protein that regulates the transport of membrane proteins to their appropriate location in the cell membrane. Since 2011 a total of 11 individuals from 6 families have been reported with *AP4B1* mutations, 9 of which had homozygous mutations and were from consanguineous unions. The clinical characteristics of these individuals included severe intellectual disability (ID), a near absence of speech, some characteristic facial features, neonatal hypotonia, and progressive, early onset spasticity.

Trio based clinical exome sequencing of a 3-year old girl with moderate ID, near absence of speech, neonatal hypotonia, and progressive spasticity identified compound heterozygous mutations (p.R406* and p.L443P) in *AP4B1*. This child also had stable, asymmetric ventriculomegaly, thinning of the corpus callosum, and some dysmorphic facial features. Although thought to be a rare disorder, within 1 week of receiving this clinical exome result an additional 2 individuals with compound heterozygous *AP4B1* mutations were identified. This was accomplished primarily through Facebook.

Interestingly, loss of function of any of the 4 components of AP-4 (AP4B1, AP4S1, AP4E1, and AP4M1) disrupts the function of the tetrameric AP-4 complex, and individuals with biallelic mutations in AP4S1 (causing SPG52), AP4E1 (SPG51) and AP4M1 (SPG50) have all been reported, all with similar phenotype.

I will review the natural history of the published (n = 11) and new (n = 3) patients with *AP4B1* mutations, as well as the phenotypes of patients with AP4S1 (n = 9), AP4E1 (n = 6) and AP4M1 (n = 11). This "AP-4 deficiency syndrome" can be recognized by neonatal hypotonia that progresses to spasticity, a severe delay/absence of productive speech, a thinning of the corpus callosum, and some characteristic facial features. Adaptor protein 4 (AP-4) deficiency disorders should be suspected in children with spastic paraparesis and absent speech, particularly if MRI features are suggestive. Given the overlap of this phenotype with cerebral palsy, as well as the speed with which new patients are being ascertained, even in outbred populations, we believe this may be an under-diagnosed cerebral palsy mimic.

TORIELLO-CAREY SYNDROME: IS THERE REALLY SKIN IN IT?

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Toriello-Carey syndrome (TCS) was first described in 1988 as a combination of agenesis of the corpus callosum (ACC), a distinctive craniofacies including the Robin sequence with cognitive disability and variable other birth defects. The original report of 2 cases of both genders and included a sibship of 3; hence TCS was considered autosomal recessive. The core entity of TCS has been difficult to settle upon for a variety of reasons encompassing, the inherent variability of TCS itself, coupled with phenocopy admixture including unbalanced chromosome rearrangements. TCS, being such a fraught clinical assignment has unsurprisingly eluded gene identification so far.

A severely disabled female now aged 12 years has been followed for a decade for a diagnostically elusive combination of severe cognitive disability, postnatal growth delays, CNS dysgenesis with partial ACC and cerebellar hypoplasia with hypotonia and a progressive severe neuromuscular scoliosis, with distinctive external features, including facial asymmetry, a wide forehead, squared fleshy ears, telecanthus, tear-shaped philtrum and thin upper lip and unilateral 2/3 toe syndactyly. A prior epic workup including biochemical testing, array CGH, and de Lange gene sequencing was unrevealing.

Over the past 2 years, distinctive hyperkeratosis of the dorsum of both thumbs has appeared. As hyperkeratosis has been described in the LDDB as a feature of TCS this prompted re-consideration of TCS

as a candidate disorder. Thereafter, exome sequencing identified a rare (absent in >120 000 individuals in the gnomAD database) de novo missense variant, p.Arg480Thr in DDX3X, in the helicase C-terminal domain.

This gene was recently implicated as a common cause of female-ID in a series detecting de novo DDX3X variants ascertained from a female cohort of intellectual disability. This series of 38 females manifests a 35% prevalence of ACC (a core but not constant TCS feature) with 37% having skin pigmentary abnormalities. Males were only identified as offspring to unaffected carrier females bearing missense variants. Interestingly, individual 15 (female) of this series harbored a missense variant in this same residue, p.Arg480Ser. Subsequently, prior to the return of exome results Ute Moog and colleagues identified DDX3X variants in 2 females with a TCS-assignment, although lacking skin features. Our case with an emergent skin sign adds to the putative identification of DDX3X as a causative gene for TCS, which may well yet emerge to be a predominately female disorder.

SCHIMKE IMMUNOOSSEOUS DYSPLASIA DUE TO SMARCAL1 HELICASE: DISTINCTIVE FEATURES AND MANAGEMENT OF CARDIO/NEUROVASCULAR AND RENAL RISKS OVER TIME

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Schimke Immunoosseous Dysplasia (SIOD) is characterized by spondyloepiphyseal dysplasia and T-cell deficiency. Features that are particularly important in the natural history include progressive renal failure, refractory hypertension, early atherosclerosis, and TIAs or ischemic events. Neurologic events can be fatal and are sometimes preceded by headaches for which the underlying correlation is unclear. Dyslipidemia relative to early atherosclerosis is also poorly understood. Mortality can be within the first few years of life, though an average age of death of 11 years old has been reported. A milder course could potentially be facilitated by appropriate management. Causes of death include infection (23%), stroke (13%), and pulmonary hypertension/heart failure (13%). Such phenotypic heterogeneity suggests that the long-term course of SIOD is likely affected by genetic, epigenetic, or environmental factors. We specifically investigate whether migraine-like headaches may be related to blood pressure dysregulation and describe new management ideas for neurovascular/renal care. We also characterize dyslipidemia relative to atherosclerosis risk and describe a novel pathogenic SMARCAL1 variant as well as new management strategies for optimizing organ system care. The patient remained undiagnosed until seeing genetics in school age despite earlier clinical signs and characteristic lentigines. The birth weight was 4lbs. In early childhood, she was seen by multiple specialists and had hip dysplasia (seen in 89% of reported SIOD patients) and short stature (99%). She also had diffuse lentigines on the face, neck, torso, and extremities (70%) and lumbar lordosis (74%) and was chronically lymphopenic (74%), hypothyroid (36%), and hyperlipidemic (% unclear). Renal insufficiency was also diagnosed (99%). She was a good student but had frequent migraine-like headaches. Sequencing by Sanger method revealed a pathogenic c.2291G>A variant and a novel c.1931G>A variant in SMARCAL1 that localizes to a well-conserved putative nuclear localization domain. Parental heterozygous status was confirmed. As hyperlipidemia may be a risk factor for early atherosclerosis and cardiovascular disease (CVD), cholesterol was assessed by Vertical Auto Profile (VAP) and LDL-C was optimized with atorvastatin to a target goal of less than 100 mg/dL for both renal disease and African American heritage, an independent CVD risk factor. As headaches may precede fatal neurovascular events, we investigated whether headaches may be related to blood pressure dysregulation. Brachial blood pressure was measured by Ambulatory Blood Pressure Monitor (ABPM) with benazepril and three months after adding propranolol and showed a 44% decrease in bp percentile with correlating decrease in headache frequency. Renal insufficiency was unaffected. To improve the natural history, SIOD management should include multisystem care. Our results suggest that blood pressure and cholesterol optimization may decrease symptoms, and could ultimately impact survival when combined with other interventions.

PRENATAL ULTRASOUND FINDINGS CONSISTENT WITH NAGER SYNDROME ALLOW FOR IMPROVED PREGNANCY MANAGEMENT

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Nager syndrome is an acrofacial dysostosis characterized by mandibulofacial dysostosis and preaxial limb abnormalities. Intelligence is usually normal. The diagnosis is usually made postnatally. We report two cases of Nager syndrome in which the diagnosis was suspected prenatally based on ultrasound (US) findings, which allowed for appropriate postnatal management. Case 1: Concern for a possible skeletal dysplasia was raised on prenatal US at 19 weeks gestation on the basis of recessed chin and possible shortening of the forearms. At 24 weeks and 4 days, fetal US findings included shortened long bones, abnormal fingers and toes, and micrognathia. Prenatal chromosomal microarray was normal. Fetal MRI at 30 weeks gestation revealed severe micrognathia with a high-riding and posteriorly-positioned tongue abutting the oropharyngeal airway. The extremities were not well-visualized on this study. However, US that day revealed likely absent thumbs with only four digits visualized and all limbs measuring small for gestational age. Based on these findings, the diagnosis of Nager syndrome was raised. Because of the micrognathia, an ex utero intrapartum treatment (EXIT) procedure was performed for management of the airway. Postnatally, the patient was found to have severe micrognathia, dysplastic ears, downslanting palpebral fissures, absent thumbs, camptodactyly of remaining fingers bilaterally, and shortened forearms with radio-ulnar synostosis. Genetic testing was sent confirming the diagnosis of Nager syndrome: heterozygous for a presumed pathogenic variant in SF3B4, c.345dupG, p. Leu116Alafs*3. Case 2: Limb abnormalities and severe micrognathia were noted on prenatal US at 20 weeks and 3 days gestation. A follow-up US at 22

weeks gestation revealed severe micrognathia, shortened upper extremities, bilaterally absent radii, severe clubbing of the hands, and syndactyly of the second and third toes bilaterally. The thumbs were not visualized in this study. Again, concern for Nager syndrome was raised and an EXIT procedure was performed. Postnatally, the following findings were noted: downslanting palpebral fissures, telecanthus, severe micrognathia, cleft palate, malar hypoplasia, and bilateral limb abnormalities (absent thumbs, camptodactyly of third-fifth fingers, and sandal gap with overlapping digits and 2nd toe macrodactyly). Genetic testing for Nager syndrome was performed: 5.5kb deletion resulting in whole gene deletion of SF3B4. We believe that Nager syndrome should be strongly considered when fetal ultrasound findings of micro-retrognathia and upper limb defects, particularly radial ray defects, are noted. Detecting these prenatal features can allow care providers to plan careful delivery strategies, including the use of the EXIT procedure, in order to ensure the best possible outcome for these patients.

EXOME SEQUENCINGIN OF 103 PAEDIATRIC PATIENTS IN HONG KONG – IMPORTANCE OF CLINICAL GENETICIST IN THE INTERPRETATION

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Large published reports on the clinical application of whole exome sequencing (WES) are predominately based on Caucasian families. Interpretation of WES results with the relative lack of ethnic-specific information on phenotypic and genotypic variations in East Asia poses a unique challenge to clinical geneticists and genetic counsellors working in the region. In the Medical Genetic Clinic of the University of Hong Kong, patients with suspected undiagnosed genetic condition are offered WES in clinical laboratories. This is followed by a systematic evaluation of each report by the medical genetic team, including variant/gene level interpretation, segregation analysis, clinical correlation, recommendation in management and in some cases expert consultation and functional studies.

To this date, we have offered singleton-based WES to 103 patients. The median age at the time of enrollment was 4 years and 1 month and the patients were predominantly Chinese (94%). Overall, 93 of 103 reports (90%) required further action from the clinician before a conclusion can be made. A molecular diagnosis that explained the clinical phenotype was achieved in 42 patients (41%). Among them, clinician's interpretation changed the diagnostic category of the variants in 18 (35%) reports according to ACMG classification. The diagnoses include very rare genetic conditions e.g. Lenz-Majewski syndrome (PTDSS1), X-linked glycosylation disorder (SLC35A), recently described genetic syndromes e.g. Schuurs- Hoeijmakers syndrome (PACS1), Bainbridge-Ropers syndrome (ASXL3), Noonan-like syndrome due to PPP1CB mutation, KMT2B-related dystonia syndrome, or new syndromes e.g. ATP6V1A-related autosomal recessive cutis laxa syndrome (AJHG 2017; 100(2):216–227). Overall, WES aids clinical management in 80% patients which were based on evidence from disease-specific management guidelines (n = 13, 31%) or knowledge from case series/reports or known function of genes (n = 20, 48%).

Clinician's interpretation of WES results is a time consuming, nonetheless essential step to maximize the clinical utility of the test. Our findings confirm the usefulness of singleton-based WES and the importance of the medical geneticist in the interpretation of the WES findings and in the post-WES diagnostic work-up and management recommendation for these previously undiagnosed patients.

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GENOMIC ANALYSIS OF CONGENITAL DISORDERS IN VIETNAMESE PATIENTS

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The importance of establishing correct diagnoses in patients with suspected genetic disorders includes the ability to provide appropriate care, surveillance and recurrence risk for the patient and family. There are significant challenges diagnosing patients in a tertiary care center in a developed country; these challenges are amplified when similar patients present in developing countries with limitations of trained professionals, access to technology, and financial resources.

Our nearly 10 year partnership with medical geneticists in Hanoi and Hue, Viet Nam, has recently been enhanced by capacity whole exome and/or whole genome sequencing of patients from Viet Nam with suspected genetic conditions and their informative relatives. The present series comprises 23 patients (from 22 families) who were evaluated by medical geneticists from the U.S. and from Viet Nam. Of the 17 families for whom sampling and sequencing are complete, 13 families have genetic results that are likely the explanation for the observed phenotypic findings; a different gene is implicated in each of these 13 families. Three families have candidate variants that require additional functional analysis to determine if novel genes are involved. One patient with normal exome sequence and positive titers for CMV has clinical findings consistent with congenital CMV.

Of the critical genotypes identified in the 13 resolved cases, 5 involve *de novo* mutations, 2 are compound heterozygous, 1 is heterozygous and inherited from an affected parent, 1 is X- linked, and 4 are homozygous. Only one family was aware of even distant relationship between the parents. A particularly interesting patient has Crouzon syndrome with an identified *FGFR2* missense mutation, but more severe cognitive disabilities than described in his father and sister who share the Crouzon syndrome diagnosis. It may be that mutations in a second gene contribute to this patient's phenotype. Another interesting patient has both Robinow syndrome and G6PD deficiency, resolved by a *de novo* deletion in *DVL1* and a point mutation in G6PD.

Among the many benefits from this collaboration are the ability to provide diagnoses for patients and families, including the possibility of

pre-gestational diagnosis for future pregnancies, extending the phenotypes of recognized genetic conditions, identification of genes not previously known to be responsible for human genetic disorders, and most importantly, supporting the development of clinical and molecular diagnostic capacity in a rapidly developing country.

CORNELIA DE LANGE SYNDROME IN UNDERREPRESENTED MINORITY POPULATIONS

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Global and ethnic diversity have been poorly recorded in the study of dysmorphology, as recently described [Genet Med 2016;18,1069-74]. Specific genetic syndromes are now being catalogued in many populations in order to expand available resources. Cornelia de Lange syndrome (CdLS) has been described in only a handful of non-European/USA reports worldwide, including China, Korea, Japan, India, Senegal and Brazil, with no specific mention of ethnic diversity. We hypothesized that in our clinical cohort, there would be a higher percentage of underrepresented minority populations in the more severe subgroup than in the more mildly affected individuals, and that there would be specific features that would be distinctive in some of the populations. We reviewed 136 patients seen in our biannual multidisciplinary CdLS aging clinic, and 30 outpatients with CdLS seen in our pediatric genetics clinic, for a total of 166. Of these, 32 (19%) were found to be either Black (13, or 8%), Latino (11, or 7%), Asian (4, or 2.4%), or Pacific Islander (4, 2.4%). Some were mixed racial (7, or 5%), and no patients were Native American. The numbers of underrepresented minorities in the severity subgroups are as follow: 22 (29%) in the 77 most severe patients, 7 (22%) in the 36 moderately involved patients, 2 (6%) of the 34 mildly involved patients, and 1 (5.3%) of the 19 very mildly involved patients. When assessing patients from each minority population, including additional children evaluated at multiple national and international meetings, specific facial characteristics can be identified. In the Black population, a longer face, smaller chin and more prominent forehead are noted, as well as a broader nasal root, and a prominent rounded shape to the philtrum. In the Latino population, there is a more oval face with squarer chin, brows are more finely arched, and a more bulbous nasal tip. The East Asian population has a rounder face with a flatter midfacial region and smaller mouth. The South Asian population has a squarer face, prominent cheeks and slightly straighter brows. The Pacific Islander population, including Filipino, has marked synophrys, a flatter nasal bridge, slightly down-slanting eyes with hooding and longer philtrum. Hirsutism and limb findings are noted across all populations. All of these groups are at a lower percentage than seen in the underrepresented minority populations in the United States, other than Pacific Islander. According to the 2016 census, there are 38% individuals of minority populations, double our incidence. A higher detection rate of these populations in more severely affected individuals implies that features are more easily detected when more severe, especially when accompanied by smaller stature and a greater degree of intellectual disability. Using these statistics, there are clearly many individuals who have been missed in the diagnosis of CdLS or have not come to our medical attention. Only some of the minority patients in our cohort underwent genetic testing. In terms of mechanism, there are likely many as yet undetected background loci which modify the phenotype on top of the initial mutation.

A NOVEL HOMOZYGOUS PATHOGENIC *BBS7* VARIANT IN A PATIENT OF HMONG ANCESTRY: CHARACTERISTICS OF BARDET-BIEDL SYNDROME IN PATIENTS FROM CHINA AND SOUTHEAST ASIA

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The Hmong are an ancient people originally from Southwestern China, with migration of some over time into Southeast Asia (Myanmar, Laos, Thailand, and Vietnam). Migration to the US started around 1975, and ~270,000 Hmong currently live in the US. The largest US communities are found in California, Minnesota, Wisconsin, North Carolina, and Michigan.

We report here on a 6-year-old Hmong male born at full term to parents with no reported consanguinity. His clinical features include global developmental delay, autism spectrum disorder, truncal obesity, round face, small penis, and postaxial polydactyly of the hands and feet (including 7 digits of the left hand). A chromosomal microarray performed previously showed three regions of homozygosity (17.5 Mb total), one of which involved chromosome 4q27 that included *BBS7*. Sequencing of this gene identified a novel homozygous pathogenic variant, c.389_390delAC (p.Asn130Thrfs*4), confirming a diagnosis of Bardet-Biedl syndrome (BBS). An eye exam and renal ultrasound are currently pending.

BBS is an autosomal recessive ciliopathy currently associated with 19 genes. Common features include retinal dystrophy, renal dysfunction, postaxial polydactyly, obesity, cognitive deficit (DD/ID), and hypogenitalism. BBS was first described in China in 1954. A review of available published literature from China and Southeast Asia (C/SA) describes variable clinical information of 82 patients. No Hmong patient was reported, but BBS in China has been seen in over 17 provinces, including the Southwest region (origin of the Hmong). Overall, retinal dystrophy is seen in 77% of C/SA patients, renal dysfunction in 19%, polydactyly in 77%, obesity in 90%, DD/ID in 92%, and hypogenitalism in 76%. Male-to-female ratio is 2.1:1. Mutations in *BBS2*, *ARL6* (BBS3), *BBS4*, *MKKS* (BBS6), *BBS7*, and *MKS1* (BBS13) were identified in 8 total patients, with 75% of the patients possessing homozygous variants.

C/SA patients with BBS appear to have less retinal dystrophy (77% vs 93%) and renal dysfunction (19% vs 53%), and higher DD/ID (92% vs 61%), respectively, when compared to a previous BBS study by Forsythe and Beales (2013). Polydactyly, obesity, and hypogenitalism rates were similar between the two groups. It also appears that, when described in detail, more patients in the C/SA cohort have polydactyly involving the upper and lower limbs (70% vs 21%). Phenotypic differences between these cohorts could be related to genetic

modifiers, mutation type (eg, homozygous vs compound heterozygous), or a complex (triallelic) mode of inheritance.

Our patient appears to be the first report of BBS in the Hmong, adding to the few reports of genetic abnormalities in these patients, including pulmonary hypoplasia-diaphragmatic hernia- anophthalmia-cardiac defect (PDAC), short/branched-chain acyl-CoA dehydrogenase (SBCAD) deficiency, GM2-gangliosidosis, and osteogenesis imperfecta with cerebellar hypoplasia. Only homozygous variants of the genes associated with these conditions have been identified, consistent with sanctioned consanguinity present in their culture. Homozygosity analysis should therefore be considered when performing genetic testing in an affected Hmong patient.

POLR1A MUTATION ASSOCIATED WITH A NOVEL PHENOTYPE OF METOPIC CRANIOSYNOSTOSIS AND OROFACIAL CLEFT IN A PACIFIC NORTHWEST NATIVE AMERICAN POPULATION

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Mutations in the distal portion of *POLR1A* have been reported to cause Cincinnati type mandibulofacial dysostosis. Three individuals have been previously reported including 1) a severely affected 2-year old with ablepharon, absent zygoma, bilateral anotia, cleft palate, underdeveloped maxilla and severe micrognathia 2) a 6-year old with bilateral choanal atresia, upper and lower eyelid clefts and microcephaly; 3) a mildly affected 52-year old man with down-slanting palpebral fissures, malar flattening, unilateral microtia and mild micrognathia. Studies in zebrafish reveal loss of *polr1a* expression causes a severe craniofacial phenotype due to disturbed ribosome biogenesis with subsequent deficiency of neural-crest-derived skeletal precursor cells. To our knowledge, no patients have been described with mutations in the proximal portion of *POLR1A*.

We report two brothers with heterozygous missense mutation in exon 2 of *POLR1A* identified on whole exome sequencing. Patient 1 was born with bilateral cleft lip and palate, metopic craniosynostosis and bilateral hydroceles. Echocardiogram and newborn screening results were normal. His cleft lip was repaired at 3 months of age. His hospital course was uncomplicated with no vital sign instability. On the day of discharge from the hospital, he was found dead in his carseat. Autopsy did not reveal an obvious cause of death, and morphine levels in his blood at the time of death were therapeutic. Patient 2, brother of patient 1, was born with unilateral cleft lip and palate and metopic craniosynostosis. Because of his brother's sudden death, he had increased monitoring in the neonatal period. He was found to have mixed obstructive and central apneas and CO2 retention, which resolved after a few months. He later required gastrostomy tube because of dysphagia and aspiration.

Exome sequencing of brothers and parents revealed a paternally inherited missense variant c.176A>T (p.Asp59Val, D59V) in exon 2 of *POLR1A*. This variant has not been previously reported, but was predicted to be pathogenic with in silico analysis. The variant occurs at a site conserved across species and is predicted to impact secondary structure as the residues differ in polarity, charge and size. The father was mildly affected, with bitemporal narrowing and a prominent metopic ridge. The family reports at least 3 other members of the Swinomish tribe born with craniosynostosis and orofacial clefts.

We propose that proximal mutations in *POLR1A* are responsible for metopic craniosynostosis and cleft lip and palate seen in our patients, thus expanding the phenotype of *POLRA1* mutations. Due to the mixed sleep apnea and dysphagia with aspiration in patient 2 and sudden perioperative death in patient 1, patients with proximal mutations should undergo careful monitoring during neonatal and perioperative periods. Further studies will investigate whether other members of the Swinomish tribe with similar presentations have *POLR1A* mutations.

INTERPRETATION OF WHOLE EXOME SEQUENCING RESULTS AND RACE/ETHNIC BACKGROUND

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Allele frequencies can demonstrate significant differences in diverse ethnic groups, thus determination of ethnicity-specific, allele frequencies is critical for accurate interpretation of whole exome sequencing (WES) results. Minority populations are often less well represented in control reference databases, which may impact the interpretation of their WES testing results.

There is little data about ethnicity-specific rates of variant reporting in patients undergoing WES. We hypothesize that the frequencies of diagnostic variants and variants of unknown significance (VUS) would differ between patients of different ethnicities in whom WES is performed.

We conducted a retrospective chart review of 199 patients who underwent WES and were seen at the Personalized Genomics clinic at UCSF between 2013 and 2017. Patients were sequenced as part of a trio with both biological parents or as proband only, using several academic and commercial laboratories. We categorized the results as interpreted by the testing laboratories into three groups: (A) patients with diagnostic sequence variants: pathogenic or likely pathogenic; (B) patients with VUS that were non-diagnostic; and (C) patients with no reported variants. To determine race/ethnic backgrounds, we used parental or family report and categorized them according to the National Institute of Health (NIH) major race and ethnic groups: Non-Hispanic whites (51%), African American (1%), Hispanic/Latino (11%), American Indian/Alaskan Native (0%), Native Hawaiian/Pacific Islander (0%), Middle Eastern (3.5%), East Asian (8%), South Asian (5.5%), and more than one race (21%). We compared the proportions in the three different results categories A-C with the race/ethnic backgrounds and calculated the Odds Ratio (OR), along with the significance level (p-value) for combinations of ethnicity and results categories.

A total of 69/199 (34.7%) patients received a diagnosis from the laboratory report; this figure might be an underestimate of the true diagnostic rate in our patients, as clinicians could interpret a VUS as

causative for the patient's phenotype. 118/199 (59.3%) of patients had at least one VUS, regardless of the overall WES test result. 38% of NHW had a diagnostic test result, and this was significantly higher than those of Middle Eastern ethnicity (14%) OR = 3.7 (95%CI 1.9, 7.4; $p = .0002$), and higher than that of South Asian ethnicity (27%) OR = 1.68 (95% CI 0.9, 3.0; $p = .09$). However, the numbers of patients undergoing WES were small for those of Middle Eastern and South Asian ethnicities. In addition, non-Hispanic whites had 2.1 times the odds of having no VUS compared to Hispanics ($p = .42$). Notable in our study was a high proportion of patients of more than one race (21%), illustrating the diverse clinic population that we serve.

A potential bias is the variation in the proportions of patients undergoing sequencing at different laboratories, at which variant reporting thresholds may be discrepant. A potential confounder is the biological parental availability for trio sequencing. Our results suggest that reference databases may lack information relevant to specific ethnic groups. We conclude that further studies of allelic frequency in different ethnic groups are needed for accurate interpretation of WES results.

PALLISTER-KILLIAN SYNDROME ACROSS DIFFERENT ANCESTRY GROUPS

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Pallister-Killian Syndrome (PKS, MIM 601803) is a multisystemic genetic disorder characterized by typical craniofacial findings including skin pigmentary anomalies, neurodevelopmental delay, intellectual disability, congenital diaphragmatic hernia, congenital heart defects, gastrointestinal malformations, genitourinary malformations, rhizomelic limb shortening, ophthalmologic involvement, and hearing loss. A hallmark of this syndrome is the tissue limited mosaicism for isochromosome 12p. Given the historic difficulty of cytogenetic diagnosis in conjunction with mosaicism, it remains an underdiagnosed condition (prevalence ~1:20000). This highlights the importance of clinical recognition as a diagnostic tool for accurate genetic counseling and an appropriate preventive care plan for these patients. However, the vast majority of illustrative examples of PKS in the literature only include images of individuals of Caucasian background, and do not sufficiently reflect ancestral diversity. This study is focused on delineating the clinical features of PKS in underrepresented minority (URM) populations.

We reviewed our PKS patient database for individuals with confirmed diagnoses who had photos and clinical information available for analysis. We collected clinical features on 14 patients with PKS from different ancestral backgrounds including 4 Asians (AS), 5 Hispanic/Latinos (H/L), and 5 African Americans (AA). We also investigated the ability of facial recognition technology to identify characteristic facial landmarks in our 14 URM patients versus matched Caucasian individuals with PKS. All subjects are enrolled in an IRB-protocol of informed consent.

In terms of differences noted amongst structural anomalies: the AS cohort showed a higher frequency of cerebral findings (most often characterized by enlargement of lateral ventricles) than the other cohorts. Cardiac differences were seen less frequently in both AS and H/L patients than in individuals from other ancestral backgrounds with PKS. Functional gastrointestinal manifestations (including feeding problems, gastroesophageal reflux and constipation) were reported in nearly all URM and Caucasian patients, while structural gastrointestinal malformations occurred more often in H/L and AA patients. Genitourinary involvement was less common in the URMs than in the Caucasian cohort. Musculoskeletal differences were rarely reported in URMs, while ophthalmologic and auditory involvement closely mirrored existing data from Caucasian population with PKS. Dermatologic findings were commonly noted in both Caucasian and URM populations. However, in the URM cohort these were described mainly as hypopigmentation in AS and H/L individuals, and as hypo/hyperpigmentation, eczema, and dry skin in AA patients. Physical findings reported by trained dysmorphologists demonstrated features typical of PKS, although some variation in facial features existed even within the same ancestry group. We propose that genetic background and epigenetic variation likely influence individual DNA methylation and expression profiles seen within the context of one's genomic background, leading to the phenotypic variability noted in our cohorts.

In conclusion, a small catalog of minority PKS patients have been identified and characterized. While the numbers in our study limit statistical significance in this rare syndrome, the differences and similarities across several ancestries will be presented.

FGFR2 MUTATION IN MULTIPLEX FAMILY WITH CUP-SHAPED EARS

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Multiplex families with non-syndromic microtia have been clinically described, but causative mutations have been only identified for two families with HOXA2 mutations. We performed whole exome sequencing (WES) on a family from Colombia, presenting with isolated cup-shaped microtia in an autosomal dominant mode of inheritance with incomplete penetrance, where 5 individuals were affected and two individuals were unaffected, but transmitted the mutation (carriers). WES revealed that affected individuals and mutation carriers shared 7 conserved and rare variants (GERP>4, frequency < 0.0001), of those 2 were expressed in the mouse 2nd branchial arch (E10.5 and E11.5) and in the human embryo developing ear (at 54 days). One of these two genes is FGFR2, an heterozygous TTC insertion was identified in this gene resulting in an insertion of a serine. This mutation has not been

described in ExAC and in 40 trios with non-syndromic microtia from the same region. It is predicted to be a frameshift disruptive mutation, localized in exon 8 of FGFR2 of variants 2, 3 and 9. Variant 2 results in isoform IIIb, for which FGF3, 7 and 10 are activating ligands. FGFR2 IIIb has an important role in epithelial-mesenchymal signaling during organogenesis. The mutation occurred in the loop of the third immunoglobulin (Ig)-like domain of FGFR, a conserved domain, where FGF10 binds to FGFR2. Mutations in FGFR2 have been extensively described in syndromic craniosynostosis in different regions of the gene. Heterozygous mutations in the tyrosine kinase domains of FGFR2 have also been described in four multiplex families with lacrimo-auriculo-dento-digital (LADD) syndrome. LADD syndrome is a rare autosomal dominant syndrome characterized by defects in tear and saliva production, accompanied by microtia (cup-shaped type), microdontia, and thumb anomalies. A wide range of clinical symptoms with variable expression, even within a family is usually observed. Mutations in fibroblast growth factor 10 (FGF10) and FGFR3 genes have also been reported. Structural modelling and biochemical studies have attributed the human defects to impaired FGF10–FGFR2-IIIb interaction, or production of unstable proteins. The patients did not present craniosynostosis or any additional features of LADD syndrome such as tear or saliva production issues or limb abnormalities. The highly clinical variability in LADD syndrome might indicate that this family presents with a very mild presentation of this condition. In-depth clinical history and exam of all the individuals from the family is currently underway to identify any subtle symptoms not previously diagnosed. In the other hand this might represent one of the genes causing non-syndromic microtia in this family and, potentially, in a proportion of other non-syndromic cases.

WIEDEMANN-STEINER SYNDROME IN AFRICAN AMERICAN PATIENTS

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WIEDEMANN-STEINER SYNDROME IN AFRICAN AMERICAN PATIENTS J. Kaplan, MD, E. Boothe, MS, CGC, A.T. Gunter, MS, CGC, O. Abdul-Rahman, MD University of Mississippi Medical Center, Jackson, MS Wiedemann-Steiner syndrome (WSS) is a rare autosomal dominant disorder classically characterized by hypertrichosis cubiti (hairy elbows), short stature, intellectual disability, and distinct facial features including downslanting, narrowed palpebral fissures, hypertelorism, thick eyebrows, long eyelashes, a broad nose, and a thin upper lip. In 2012, mutations in KMT2A were identified as a cause for WSS. Since that time, there have been fewer than 30 WSS patients reported in the literature with confirmed mutations in KMT2A. These patients have been of Caucasian, Hispanic, Middle Eastern, and Asian ancestry. We report three African American patients with clinical WSS and changes in the KMT2A gene; to our knowledge, these would be the first African American individuals reported in the literature. Patient 1 is a 3 year-old African American female initially seen by our genetics team in the newborn period for short stature, microcephaly, and dysmorphic features including epicanthal folds, hypertelorism and telecanthus, narrowed palpebral fissures, and a broad nasal tip. At her most recent visit, she was found to be hirsute with synophrys; she had ptosis, left optic nerve hypoplasia, sensorineural hearing loss, oral dysphagia, tapered fingers, and partial fusion of C2-C3 vertebral bodies. Whole exome sequencing (WES) showed a change in KMT2A, classified as a VUS. Patient 2 is an 11 year-old African American female initially seen at age 6 due to short stature, congenital hip dislocation, and learning disability. In addition, she had upslanting, narrowed palpebral fissures, a bulbous nasal tip with broad midportion, bilateral ptosis, arched eyebrows, obesity, macrocephaly, bilateral metatarsus adductus, and fusion of the posterior elements of C2-C3 vertebral bodies. WES showed a pathogenic variant in KMT2A that had not been previously reported. Patient 3 is an 8 month-old African American male who initially presented in the prenatal period with cystic hygroma, clenched hands, and single umbilical artery. Postnatally, he was found to have normal growth, hypertelorism, narrowed palpebral fissures, arched eyebrows, PDA, PFO, mild dilatation of the aortic annulus, mixed hearing loss, overlapping fingers, congenital fusion of C2-C3, segmentation/fusion anomalies of the thoracic spine with associated rib deformities, sacral dimple, and mild oropharyngeal dysphagia. WES showed a de novo, mosaic, likely pathogenic change in KMT2A. To our knowledge, these three patients are the first reported African American individuals with WSS. Only Patient 1 was particularly hirsute, but did not have hypertrichosis cubiti. All three patients had narrowed palpebral fissures and C2-C3 fusion. We present the similarities and differences of our African American patients compared to individuals of other ethnicities with Wiedemann- Steiner syndrome.

DISCOVERY AND CHALLENGES IN THE APPLICATION OF WHOLE GENOME SEQUENCING IN A RESOURCE LIMITED DYSMORPHOLOGY CLINIC IN TIJUANA, MEXICO

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Despite rapid advancements in the diagnosis and the discovery of genetic disorders provided by whole exome or genome sequencing (WES/WGS), access to genetic testing remains limited for many underserved communities. The Clinica de Dismorfologia at the Hospital Infantil de las Californias is an established dysmorphology clinic staffed by American and Mexican volunteer geneticists, a genetic counselor, and fellows that serves Baja California, Mexico. Limited genetic testing is available to families who can afford such studies. Most patients who are suspected of having a genetic disorder that cannot be diagnosed by clinical exam alone will not undergo any cytogenetic or molecular genetic studies. This population represents a unique and underserved pediatric population in which clinical WGS testing can show a diagnostic advantage when resources are limited.

WGS was performed in 23 children (15 males) with suspected genetic disorders. The mean age of the probands is 4.8 ± 3.9 years. The primary phenotype was classified into one of three categories:

patterns of malformation (69.6%, n = 16), non-dysmorphic children with a neurologic phenotype (21.7%, n = 5), and myopathies (8.7%, n = 2). The cohort is comprised of 14 trios, 6 duos, and 3 quad WGS. Participating families attended "Genome Days" where they underwent pre-test counseling, informed consent, blood draw, review of family and medical history, and examination. Clinical WGS was performed by Illumina Clinical Services Laboratory through a philanthropic partnership with the Foundation for the Children, a US-based 501(c)3 that supports Hospital Infantil de Las Californias. Secondary findings and a pharmacogenomics screen were reported for all participating individuals.

WGS yielded a diagnostic result in 60.9% including 3 copy number variants (CNVs), 2 structural anomalies (1 uniparental disomy and 1 unbalanced translocation), and 9 single gene disorders. Children with patterns of malformation had the highest diagnostic yield (68.8%, n = 11). Suspected genetic diagnoses were confirmed in 2 children (Angelman syndrome, maternally inherited nemaline myopathy). A novel congenital disorder of glycosylation was identified in two siblings with a homozygous indel in *PIGS*, a GPI-biosynthesis gene. One child had a variant in a candidate gene (*USP7*) and he is participating in additional functional studies. One child had a secondary finding of a multi-exon deletion in *PMS2* concerning for Lynch syndrome.

These data highlight the diagnostic potential of clinical WGS, with included reporting of CNVs and structural variants, as a first-tier testing option for patients with suspected genetic disorders, particularly in resource limited communities. Securing a diagnosis provides crucial information to the family for prognosis and recurrence risk and empowers families to allocate resources for treatment rather than diagnostic studies.

TURNER SYNDROME ASSOCIATED WITH A CO-OCCURRING DISORDER: THE TIP OF A DIAGNOSTIC ICEBERG

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Turner syndrome (TS) is a well-recognized sex chromosome abnormality syndrome due to a single or structurally deficient X chromosome. Despite the "classic" TS appearance, the spectrum is broader, and atypical features often lead to additional testing. At the 2016 DWS Workshop, we described a girl with TS and neuroblastoma who also had Li-Fraumeni (LFS) and Noonan syndromes (mutations in *TP53* p. R282W and *PTPN11* p.N308D). We have become aware of additional TS patients (pts) with a co-occurring genetic disorder in our clinics and in the literature. With the advent of whole exome sequencing (WES), there has been increasing interest in pts with more than one syndrome (Kurolap et al., 2016). In a recent study of pts undergoing WES, almost 5% of pts with a molecular diagnosis had at least two diagnoses (Posey et al., 2017). We hypothesize that the increased incidence of X-linked conditions may be due to the unmasking of recessive alleles and aneuploidies because of meiotic/mitotic defects due to the abnormal complement of sex chromosomes. Additional conditions may occur as a chance occurrence because of their frequency in the population. We reviewed TS pts at MGH and U of Mississippi and the literature seeking genetic syndromes which have co-occurred in pts with TS. We performed a PubMed search using key words of "Turner syndrome" and over 100 other genetic syndromes. Among TS clinic pts, we found two more pts who had another genetic disorder: a TS woman (45,X) with LFS and a girl with mosaic TS with clinically diagnosed blepharophimosis- ptosis-epicanthanus inversus. Including our pts, there were at least 104 pts with TS with another genetic syndrome with a total of 24 disorders. The most common were chromosomal disorders/aneuploidies with Down syndrome being the most common (50 pts). Other groups included autosomal dominant, mitochondrial, imprinted and X-linked disorders. Only 19 pts had an X-linked disorder with a total of 8 different X-linked disorders reported. The most common X-linked disorder reported was Duchenne muscular dystrophy. Only 17% of the pts had 45, X karyotype while 83% pts had mosaicism for TS. Most of these reports were isolated case reports. Given that TS is associated with a single or structurally abnormal second sex chromosome, we anticipated that there would be an increase in X-linked disorders, but we did not observe this, possibly because of reporting bias. Some X-linked disorders may not display characteristic dysmorphic features and may have been presumed to be part of the TS phenotype. Another surprising aspect to the literature is that only 17% of patients with a 2nd genetic syndrome had the classic 45, X karyotype, which is less than the expected prevalence among all patients with TS (35–50%). This may be because the mosaicism may tolerant of another genetic abnormality or that the phenotype in the patients is more severe than what the karyotype would predict prompting further evaluations from providers. We anticipate that with improved diagnostic technology including WES, more pts with TS will be found with co-occurring genetic syndromes.

DIFFERENCES OF SEX DEVELOPMENT SECONDARY TO TRANSCRIPTION FACTOR DOSAGE DYSREGULATION: A POTENTIAL NEW MECHANISM

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The sex development pathway predominantly consists of genes that encode transcription factors, and alterations in these genes can cause Differences of Sex Development (DSD). The bipotential gonad gives rise to the ovaries, testes, or in cases of maldevelopment, non-dimorphic gonads. Typical testes and ovaries differ substantially in morphology and physiology, despite sharing common origin and reproductive/endocrine functions. The divergence of the bipotential gonad, i.e. sex determination, is driven primarily by the regulated cascade of sex-determining transcription factor regulation. Later sexually- divergent embryonal patterning of both internal and external reproductive organs and genitalia, i.e. sex differentiation, is subsequently driven by the sex-committed gonad. Disturbance of the finely tuned and mutually anatagonistic testes and ovarian developmental pathways results in non-dimorphic gonads and, secondarily, non- dimorphism of the genitals and internal reproductive organs. i.e. ambiguous genitalia.

Here we present a novel individual with classic ambiguous genitalia, found to have dose alterations of *Zinc Finger Protein, Multitype* 2 (*ZFPM2*) (previously *FOG2*) [MIM 603693]. The full term neonate was delivered after an unremarkable pregnancy, and noted to have ambiguous genitalia with no other dysmorphic findings. Congenital adrenal hyperplasia testing was negative, testosterone was elevated, and *SRY* was absent by FISH. Genome-wide array showed 46,XX with ~35% mosaic duplication of 76.5Mb at chromosome 8q13.2-q24.3, containing over 50 OMIM genes including *ZFPM2* and *CYP11B1*. The infant was noted with a uterus and two gonads on imaging. Subsequent surgical and pathologic evaluation revealed one grossly streak gonad with 5% mosaicism of the 8q duplication, and ovarian parenchyma. The other gonad had testicular parenchyma with occasional germ cells, and 75% mosaicism of the 8q duplication.

ZPFM2 hypomorphic mutations have recently been described to cause 46,XY male to female sex reversal. Of note, ZPFM2 is known to bind the transcription factor GATA4, and together drive *SRY* expression in the male-determining sex development pathway. In contrast to 46, XY sex reversal, we hypothesize that increased dosage of the *ZPFM2* caused 46,XX sex reversal, similarly driving away from bimodal female sex development toward a sexually non-dimorphic phenotype. This case would add to several examples of increased transcription factor activity in the 46,XX context resulting in DSD with non- dimorphic genitalia or sex reversal. In addition to the classic example of ectopic *SRY*, the primary male sex-determining transcription factor, a similar phenomenon is known for *NR5A1*, with increased activity causing 46, XX non-dimorphic sex development and differentiation. This mechanism of increased *ZPFM2* dosage provides a novel example of how perturbations of transcription factors results in altered sex development and DSD.

UNIPARENTAL DISOMY FOR CHROMOSOME 6 AND AMBIGUOUS MALE GENITALIA: TWO CASES OF THIS UNUSUAL ASSOCIATION

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Uniparental disomy (UPD) is a rare condition and reports of UPD of chromosome 6 (UPD6) have mainly focused on paternal UPD 6, associated with transient neonatal diabetes and intrauterine growth restriction (IUGR). Maternal UPD6 is more rare, and has only been previously reported fewer than twenty times. The main associated phenotype is IUGR. Ambiguous genitalia in females with UPD6 has been reported due to congenital adrenal hypoplasia. We recently reported the first case of a male with partial maternal UPD6 who presented with a 46,XY karyotype, and both ambiguous external and internal genitalia. We now present one further case of a patient with isodisomy of chromosome 6 resulting in severe IUGR and ambiguous genitalia.

The patient presented failure to thrive, along with ambiguous genitalia. He had been adopted from China, so birth parents, family history, and birth history are not known. Genital findings included bilateral cryptorchidism, a small penis, bifid scrotum, and perineal hypospadias with chordee. Testing showed a 46,XY karyotype and UPD for the chromosome 6.

To our best knowledge, these are the only cases of XY patients with UPD6 and ambiguous genitalia. There is a known association between male hypovirilization and IUGR, which may be contributing in these cases. Given the involvement of both internal and external genitalia in the first case, we had postulated that the pathway linking IUGR to hypogonadism could also interfere with AMH function. The recurrence here, and the fact that this presentation only involved external genitalia, highlights that this is not always the case. This broadens the possible genital presentations in UPD6, suggesting there may be other more mildly affected patients. The fact that the genital anomalies may be only secondary to the IUGR also reinforces the importance of screening patients with syndrome-related growth restriction for other long term consequences more commonly associated with maternal or placental IUGR. This case adds to the growing literature linking maternal UPD6 with IUGR, and supports the link between IUGR and both under-virilization and abnormalities in AMH functioning during male gonadal development.

QARS ASSOCIATED AUTOSOMAL RECESSIVE PROGRESSIVE MICROCEPHALY - REPORT ON FOUR NEW PATIENTS

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Compound heterozygous variants in the *QARS* gene (OMIM 603727) have been identified in only four patients from two families with autosomal recessive progressive microcephaly with seizures and cerebral and cerebellar atrophy (MSCCA), to date. These patients showed severe developmental delay, progressive primary microcephaly, intractable seizures, hypomyelination or delayed myelination, thin corpus callosum, and small cerebellar vermis on brain imaging. *QARS* encodes for Glutaminyl-tRNA Synthetase important for mRNA translation, and seems to play an essential role during human brain development.

We were able to collect data on four new patients from three families with progressive primary microcephaly, profound developmental delay, severe hypotonia, early onset and severe forms of epilepsy, brain anomalies, and short stature in some patients. NGS analysis using trio exome or panel analysis in each of the families revealed different combinations of compound heterozygous variants in the *QARS* gene. All identified variants are not reported in any database yet. The first

patient was born to non-consanguineous German parents. Her similarly affected brother died in infancy. At birth, she was too short (-2.8 *SD*) and mildly microcephalic (-2.3 *SD*). She developed intractable seizures within the first hour of life. Her growth continued to be mildly retarded (-2.8 *SD* at the age of 9 years) and microcephaly was progressive (-6.5 *SD* at the age of 9 years). She did not achieve any of the motor or cognitive developmental milestones, she did not have eye contact, and the only interaction with her surrounding was a mild reaction of being touched. The third patient was initially evaluated at 11 days of age when she exhibited myoclonic seizures, intrauterine growth retardation, microcephaly, and elevated lactic acid. At birth, she was microcephalic (-2.9 *SD*) and microcephaly was progressive (-5.4 *SD* at the age of 19 months). Later she was found to have nystagmus, but this appeared to be secondary to therapy with valproic acid. She has required a gastrostomy feeding tube. The fourth patient was born to non-consanguineous German parents. At birth, she was also too short (-2.7 *SD*) and microcephalic (-3.9 *SD*). At 2 years of age, she still showed short stature (-4.7 *SD*) and progressive microcephaly (-9.7 *SD*). She developed pharmacoresistant epilepsy, beginning 3 hr after birth. Initially, she showed hypotonia, and presented with severe spasticity at follow up. She shows profound delay with no visual reactions. We performed detailed evaluation of cranial MRI in the patients. Characteristic neuroimaging features include delayed myelination, hypoplasia or aplasia of corpus callosum, cortical anomalies and enlarged cerebral ventricles.

QARS variants may likely be diagnosed more frequently in the future due to the wide and increasing application of NGS technologies. NGS panels of genes associated with microcephaly, epilepsy and brain anomalies should include *QARS*. These new patients and additional cases will allow for further delineation of the phenotype of this rare syndrome.

THE GENETIC LANDSCAPE OF FAMILIAL CONGENITAL HYDROCEPHALUS

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Congenital hydrocephalus is an important birth defect the genetics of which remains incompletely understood. To date, only four genes are known to cause Mendelian diseases in which congenital hydrocephalus is the main or sole clinical feature, two X-linked (L1CAM and AP1S2) and two autosomal recessive (CCDC88C and MPDZ). In this study, we aimed to determine the genetic etiology of familial congenital hydrocephalus with the assumption that these cases represent Mendelian forms of the disease. Exome sequencing combined, where applicable, with positional mapping identified a likely causal mutation in the majority of these families (22/27, 81%), spanning 16 genes, none of which is X-linked. Ciliopathies and dystroglyconpathies were the most common etiologies of congenital hydrocephalus in our cohort (19% and 30%, respectively). In one family with four affected members, we identified a homozygous truncating variant in EML1, which we propose as a novel disease gene in congenital hydrocephalus in addition to its suggested role in cortical malformation. Similarly, we show that recessive mutations in WDR81, previously linked to cerebellar ataxia, mental retardation, and dysequilibrium syndrome 2, cause severe congenital hydrocephalus. Furthermore, we confirm the previously reported candidacy of MPDZ by presenting a phenotypic spectrum of congenital hydrocephalus associated with five recessive alleles. Our study highlights the importance of recessive mutations in familial congenital hydrocephalus and expands the locus heterogeneity of this condition.

DE NOVO COPY NUMBER VARIANTS AND PARENTAL AGE

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It is known that older parents are at increased risk of having children with genetic disorders, however most of this relates to a maternal age effect on nondisjunction. Recent studies suggest advancing paternal age may increase the risk of de novo single nucleotide mutations. The aim of our study was to investigate whether increased parental age is associated with an increased risk for de novo copy number variation (CNV) formation in offspring. CNV calls from 2323 individuals referred to Signature Genomic Laboratories for clinical microarray-based comparative genomic hybridization were investigated. Overall 17% (388, 127 cases and 261 controls) were prenatal and 83% (1935, 664 cases and 1271 controls) were postnatal. Controls included the samples submitted for clinical indications with inherited CNVs and cases included samples with de novo CNVs. Prenatal samples were collected from pregnant women who underwent an invasive procedure for prenatal diagnosis, and postnatal samples were collected from cases with clinical indications. The de novo CNV data were further split into de novo CNVs bound by low copy repeats (LCRs) and de novo CNVs not bound by LCRs. All statistical measures were calculated by SAS software (SAS

9.4 X64_7PRO platform. Student's t-test and one-way ANOVA test were used where indicated. We did not find any association between de novo CNV occurrence and paternal age in either the prenatal ($p = .6795$) or postnatal ($p = .1741$) cohorts. Advanced maternal age was associated with an increased de novo CNV occurrence in the postnatal cohort ($p = .0126$). Advanced maternal age was associated with higher rate of de novo CNVs that are bound by LCRs ($p = .0027$). Conclusion: Advanced maternal age was associated with an increased rate of de novo CNVs. Advanced paternal age was not associated with the occurrence of de novo CNVs.

A UNIQUE MICRODELETION SYNDROME OF CHROMOSOME 17q11.2: A STUDY OF THREE GENERATIONS

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Microdeletions of 17q11.2 have long been described with respect to neurofibromatosis, the larger and more common type 1 deletion and smaller, type 2 deletion causing a more severe form of neurofibromatosis with overgrowth, macrocephaly, and hypertelorism. In 2007, Douglas et al. described a phenotype associated with the deletion or mutation of the gene *RNF135*, within this region, associated with tall stature, macrocephaly, and a distinct facial phenotype with hypertelorism and downslanting palpebral fissures. These authors proposed that it was the *RNF135* gene itself that caused the overgrowth. More recently, Xie et al. in 2016, describe a novel microdeletion adjacent to this causing a distinct phenotype with short stature and microcephaly.

I present a family with a 464 kb deletion at 17.2 that does not encompass the *RNF135* gene nor does it include any part of the *NF1* gene, but is associated with overgrowth without macrocephaly and includes long fingers, large feet, and a facial phenotype without hypertelorism. The proband was a girl seen in the neurology clinic at the age of 2 for developmental delay. On physical examination, she was noted to have tall stature, prominent eyes, a somewhat bulbous nose, and a head circumference at the 5th % ile. She was also far-sighted and wore glasses at the age of 2 years. Her family history was positive for learning problems in both parents. A subsequent microarray revealed a 464 kb deletion at 17q11.2 that was felt to be of unknown significance. The family was referred to genetics when a subsequent maternal sample identified the same deletion.

This began a long relationship in the genetics clinic involving subsequent children and the previous generation. The proband's mother, maternal grandmother, and maternal half-sister were found to have the microdeletion and share the characteristics of tall stature, normal head circumference, long fingers and large feet and facial features that are more recognizable as younger children, but include prominent eyes, a pointed chin and a triangular face. All affected individuals have developmental delay or intellectual disability and some degree of vision and hearing problems. The proband also has a full brother and a maternal half-sister who do not have the deletion, nor do they have the same phenotype.

The deleted interval involves six known genes. The original report included two pseudogenes in the deletion as well. The six genes do not include the gene *RNF135* suggesting that the tall stature in this microdeletion is due to a different gene. The microdeletion in this family does include *SUZ12*, a zinc finger gene that is at the breakpoint of the recurrent NF1 microdeletion. I will present the phenotype in this family and its expression over three generations. I propose that there is a recognizable phenotype associated with this phenotype that is distinct from the phenotypes described in the other microdeletions at 17q11.2.

IS SOME MATERNAL MOSAICISM REALLY AN ARTIFACT OF FETAL MICROCHIMERISM?

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Parental somatic mosaicism for genetic problems in the offspring is well documented and can involve either parent. Fetal cell microchimerism is the persistence of fetal cells in maternal blood. Fetal cells can persist for decades, though this has not been considered a complicating factor for genetic testing in adult women. Here we report a case of apparent 22q13.2 deletion mosaicism in a post partum mother, most consistent with microchimerism.

This was a natural dizygotic twin pregnancy of a 30 year old G2P1 > 3 mother. It was complicated by maternal hypothyroidism and peripartum cardiomyopathy due to previous Hodgkin lymphoma treatment at age 18. The twins delivered at 36 + 6 weeks. Our patient was twin A. Birth weight was 2840 grams (5th centile), birth length 47 centimeters (35th centile), birth head circumference 35 centimeters (80th centile). He underwent genetic evaluation because of feeding problems and dysmorphic features. There were no major malformations.

Evaluation of the baby by conventional cytogenetics, fluorescence *in situ* hybridization (FISH) and cytogenomic microarray analysis (CMA) showed the following karyotype: mos 46,XY,add(22)(q13.2)[12]/46,XY[8].ish add(22)(D22S163-)/add(22)(MS607-).nuc ish(D18Z1x2).arr[hg19]6q26(162,553,626-162,710,973)x3,22q13.2q13.33(43,194,382-51,178,150)x1. The 7.98 Mb 22q13.2q13.33 deletion was consistent with Phelan-McDermott syndrome. The 0.157 Mb duplication of 6q26 was deemed noncontributory. We interpreted these results as the baby being mosaic for 22q13 deletion due to an unbalanced translocation. Prospectively, it was presumed to be a post-zygotic event.

The parents were interested in further testing. Maternal testing also showed a mosaic karyotype: mos 46,XX[30].ish 22qtel(MS607x2)[10].nuc ish 22qtel(MS607x1)[15/100]. Therefore this interphase FISH evaluation showed evidence of a 15% population of cells with the same 22q deletion present in the baby. This testing was performed at 8 weeks after delivery and exceeded the cut-off established by a concurrent normal control.

The possible mechanisms to explain mosaicism in both generations include: 1) the baby inherited the maternal translocation/deletion with postzygotic establishment of a normal cell line;

2) the baby inherited the translocation then became a chimera with his normal twin brother; 3) the baby inherited a normal karyotype

then became a chimera with an affected lost triplet, or; 4) the abnormal interphase FISH nuclei in the maternal blood were persistent fetal cells resulting in a fetal microchimerism. The last of these seems most likely: that the mother's germline is normal and the interphase FISH result is acquired. This interpretation is also consistent with the normal female karyotype present in 30 cells since a 30 cell evaluation excludes a 10% level of mosaicism at a 95% confidence interval.

In a research setting, women with previous pregnancies have been blood tested for the presence of fetal chromosome markers, most commonly *SRY*. This work has shown that fetal cells can persist in the maternal circulation for at least 27 years. It is reasonable to presume that fetal genetic and cytogenetic abnormalities would also persist as microchimerism. This should be taken into consideration when women test positive for a low level of mosaicism indicating that they share the variant with an affected child. Microchimerism would confer a lower recurrence risk.

MITOCHONDRIAL DYSMORPHOLOGY: *DNM1L* AND MITOCHONDRIAL FISSION DISORDERS.

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Mitochondria exist as part of a dynamic tubular network that undergoes continuous fission (division) and fusion, processes critical for the maintenance of cellular health. Dynamin-related protein 1 (DRP1), encoded by *DNM1L*, belongs to a GTP-hydrolyzing superfamily and is the central protein that promotes mitochondrial fission through constriction cleavage of the outer mitochondrial membrane (OMM). Although peroxisomes are evolutionarily unrelated organelles, DRP1 also plays a role in promoting peroxisomal proliferation through a similar cleavage mechanism. DRP1 is produced in the cytosol and contains GTPase, middle and effector domains. The middle domain is critical for the oligomerization of DRP1, which is necessary for its recruitment to the OMM where it forms the higher order bands needed to constrict the mitochondrial tubule, leading to scission.

In 2007, the first patient with a *DNM1L*-related disorder was reported. She had a heterozygous de novo dominant-negative missense mutation in the middle domain and died as a neonate. She presented with a severe neurologic disorder, little spontaneous movement and brain MRI patterning abnormalities. Skin fibroblasts demonstrated abnormally elongated mitochondria and morphologically abnormal peroxisomes due to impaired fission. In 2016, ten additional severe patients were reported. These included six children who survived the neonatal period, but with profound neurologic impairment and who each had de novo heterozygous missense mutations in the middle domain. Also reported were two siblings who died as infants with compound heterozygous null mutations. Notably, the mildest reported individuals to date were two brothers who presented with psychomotor delay at 12 months and spastic ataxia at 3 years. They were compound heterozygous for a null allele and a hypomorphic p.Ser36Gly variant in the GTPase domain of DRP1.

We have identified two sisters from Newfoundland who are homozygous for the hypomorphic p.Ser36Gly mutation. They are remarkably milder, as they achieved independent ambulation, and at ages 28 and 25 years, are intellectually normal. They developed ataxia, dysarthria and nystagmus at 5 years, spasticity in their mid teens and lost the ability to ambulate in their early 20's. Both have normal brain MRIs and MRS. Significantly, as with some previously reported cases, routine mitochondrial and peroxisomal testing was normal. However, immunofluorescence imaging of patient fibroblasts showed significantly elongated mitochondria and morphologically abnormal peroxisomes.

Since the sisters' homozygous variant is located in the GTPase domain, leaving an intact middle domain, this suggests that they have a kinetic defect, and less impaired DRP1-mediated mitochondrial fission than previously reported cases. Mitochondrial fission allows damaged sections of the mitochondrial network to be excised and is particularly important for the maintenance of neuronal axons. These sisters demonstrate that this novel disorder, that affects both mitochondrial and peroxisomal function, can occur in a wide spectrum of severity. In addition, their phenotype suggests that sensitivity to impaired fission varies between types of neurons, with the cerebellum and cortical spinal tracts being more susceptible than the cerebral cortex. Since small molecules have been identified that alter DRP1 function, these patients are excellent candidates for novel therapies aimed at restoring disrupted mitochondrial fission.

METABOLIC PERTURBATIONS IN SECOND-TRIMESTER ARE AMNIOTIC FLUID ASSOCIATED WITH LOW-LEVEL MATERNAL EXPOSURE TO NICOTINE

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Decades of public health research have documented that active smoking in pregnancy poses significant health risks to both mother and child. More recent studies have shown that even passive maternal exposure to secondhand smoke is associated with negative birth outcomes (1,2). However, the mechanisms linking exposure to outcomes have remained obscure. As a first step toward defining the metabolic consequence of passive exposure on fetal development, we conducted an untargeted metabolomic analysis of 81 paired samples of maternal serum and amniotic fluid collected from karyotypically normal pregnancies in the second trimester. We used calculated cotinine concentration, a derivative of nicotine, to classify our maternal serum samples into exposure groups. We found that cotinine levels consistent with low-level maternal exposures to nicotine were associated with distinct metabolic perturbations, particularly in amniotic fluid. In fact, the

metabolic effects in amniotic fluid from mothers with low-level nicotine exposure showed greater overlap with perturbations previously observed in the sera of adult smokers than did the perturbations observed in the corresponding maternal sera (3).

Dysregulated fetal pathways included aspartate and asparagine metabolism, pyrimidine metabolism, and metabolism of other amino acids. We also observed a strong negative association between level of maternal serum cotinine and acetylated polyamines in the amniotic fluid. Combined, these results confirm that maternal exposure to low-level nicotine is associated with striking metabolic alterations in the fetal compartment and that the affected pathways overlap those perturbed in the serum of actively smoking adults. Although the data may be limited, we are in the process of collecting pregnancy outcomes and postnatal development for the fetuses included in this study.

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VARIABLE INTELLECTUAL DISABILITY AND A FAMILIAL PATHOGENIC MUTATION IN THE ARX GENE

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The ARX gene is known to be associated with 1)early infantile epileptic encephalopathy [MIM308350], 2)hydranencephaly with abnormal genitalia [MIM300215], 3)lissencephaly [MIM300215], 4)mental retardation [MIM300419], 5)Partington [MIM309510] and 6)Proud [MIM300004] syndromes in an X linked recessive fashion. I present a family of multiple affected individuals with a pathogenic mutation in the ARX gene and variable phenotype. A nearly two year-old boy with developmental delay and a microarray variant of unknown significance was referred to genetics for evaluation and counseling. The family history was notable intellectual disability for all three young boys of the couple. The microarray variant was paternally inherited and likely benign. The comprehensive intellectual disability panel, revealed a 24 base pair duplication in exon 2 of the ARX gene, c.441–464dup. The mother and the other two affected brothers were positive as well. The maternal aunt and two of her adult sons also tested positive. Similar duplications in this region have been reported to be pathogenic. All the affected males share the feature of cognitive impairment, but to varying degrees. Of the two adults, age 21 and 27, the 27 year-old attended university and has been gainfully employed, where the 21 years old has had difficulties keeping employment. Where he was employed he had been tricked out of his money by fellow workers. Of the three younger boys, age 2, 8 and 11, the youngest suffered infantile spasms starting at 3 months of age, and global developmental delay. The 11 year-old had significant speech delay, though his motor skills were within normal limits. Socially and intellectually he functioned at 4–5 year-old level. The 8 year-old had mild speech delay and could get by in school with minimal help. Four of the affected had brain MRI, and no structural malformation was noted in any. On physical exam, they were all of normal stature (50- 75th%) and head circumference (50–98th%) and not overtly dysmorphic. The younger boys had normal male genitalia. The adult males had normal sexual development and secondary sexual features (exam declined). None of the affected has movement disorders or spasticity. The 21 year-old has significant issues with social sexual boundaries and the mother is very worried that he might be arrested for sexually inappropriate behaviours. The ARX gene belongs to homeodomain transcript factor family that is involved in cerebral genesis and patterning. ARX-related phenotype ranges from mild delay to lethal brain malformation. While truncating and null mutations tend to give rise to severe phenotype, missense and duplications are often associated with variable expressivity. In the latter case, intracellular mislocalization of the transcription factor may be the underlying mechanism for the proteins dysfunction and the resultant clinical phenotype.

17q12 DELETION: A COMMON CAUSE OF KIDNEY AND URINARY TRACT ABNORMALITIES IDENTIFIED PRENATALLY?

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Congenital abnormalities of the kidney and urinary tract (CAKUT) include a wide range of anomalies from hyperechogenic kidneys on prenatal ultrasound (US) to cystic kidneys to renal agenesis to abnormalities involving the collecting system. One recently recognized cause of these abnormalities is a recurrent deletion involving 17q12 that encompasses the *HNF1B* gene. We report two cases of 17q12 with widely variable prenatal manifestations suggesting that this microdeletion may be a common cause of renal and urinary tract abnormalities detected *in utero*.

Case 1: Prenatal US at 13 weeks gestation identified findings consistent with lower urinary tract obstruction (LUTO): markedly distended fetal bladder, bilateral hydronephrotic kidneys with dilated calyces and thinning of the cortex. Termination of pregnancy was performed and fetal tissue was sent for aCGH which revealed a 1.39 Mb loss at 17q12 encompassing 15 genes including *HNF1B*.

Case 2: Prenatal US at 19 weeks gestation revealed echogenic kidneys with bilateral pelviectasis. At 22 weeks, enlarged echogenic fetal kidneys with unilateral parenchymal cysts were noted. The renal pyramids appeared well preserved, inconsistent with ARPKD. MRI revealed bilateral renal enlargement with numerous peripheral and intraparenchymal cysts in both kidneys. Termination of pregnancy was performed and aCGH on fetal tissue revealed a 1.57 Mb deletion involving 17q12 encompassing 24 genes including *HNF1B*.

Deletion of 17q12 including *HNF1B* has been identified in 1/14,500 in an Icelandic population and was the second most common microdeletion reported in a large group of prenatal patients who had aCGH (Wapner et al., 2012). Major findings include structural renal

anomalies in 80–85%, mature onset diabetes of the young, type 5 (MODY5) in ~40%, and some degree of developmental delay/learning disability in ~50%. Because major malformations involving other organ systems are less common and occur later in life, apparently isolated structural anomalies involving the kidneys and urinary tract identified prenatally should warrant aCGH to identify this relatively common recurrent microdeletion.

SEVERE DISPROPORTIONATE SHORT STATURE DUE TO A 16P13.11 DELETION ACCOMPANIED BY HYPERMETHYLATION OF *XYLT1* ON THE NON-DELETED ALLELE

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Desbuquois dysplasia (DBQD) is characterized by large joint dislocations, severe short stature, and joint laxity due to biallelic mutations in *CANT1* (type I) or *XYLT1* (type II). Radiographic features include short long bones, advanced carpal and tarsal ossification, and a monkey wrench appearance of the proximal femurs. We report two individuals who have severe short stature with shortened long bones, no history of dislocated joints, and developmental delay due to an unexpected molecular mechanism affecting both copies of *XYLT1*.

Patient 1 is a 5 year old female born with right clubfoot. She has limited speech. She has had significant pulmonary issues that have improved over time. Her height is 76.5 cm (-9 standard deviations) with a head circumference is 44 cm (-2.05 standard deviations). She has a rounded face with a small upturned nose, evidence of rhizomelia and mesomelia, significant lordosis and abnormal gait.

Patient 2 is 9-9/12 year old male with severe short stature and developmental delay. He walked at 2-1/2 years of age and is currently able to speak in short sentences but he is not yet toilet trained. He has no history of joint dislocations. Height is 93.2 cm (-7.7 standard deviations) and head circumference is 50.3 cm (9^{th} centile). He has rhizomelic and mesomelic shortening of all extremities. A skeletal survey demonstrated failure of abnormal widening of the interpediculate distances, bilateral coxa vara, and shortening of the limbs, with no other distinctive features.

Both patients underwent chromosomal microarray that detected a 2.6 Megabase (Mb) deletion of 16p13.11, which includes *XYLT1*. A custom array with high density probes over the 16p13 region found no additional copy number variants. Targeted sequencing of the exons and intron/exon boundaries of *XYLT1* was normal. Low pass genomes to evaluate for chromosomal rearrangements was also normal. DNA methylation by bisulfite treatment and direct Sanger sequencing revealed hypermethylation of exon 1 in the remaining *XYLT1* allele of both patients. For patient 1, familial testing revealed the 16p13 heterozygous deletion was shared with the father and 3 siblings, with no evidence of hypermethylation on the remaining *XYLT1* allele. The mother is not a carrier for the 16p13 deletion, but did show heterozygous hypermethylation over *XYLT1* exon 1. All family members have normal stature. Familial segregation analysis for patient 2 is underway.

Xylosyltransferase 1 (the protein product of *XYLT1*) catalyzes the first step in proteoglycan biosynthesis, which is an important component of the extracellular matrix of connective tissue. Loss of function of both alleles through mutation and/or deletion of *XYLT1* can lead to a severe skeletal phenotype. There are no reports of hypermethylation of *XYLT1* leading to loss of function published in the literature. Interestingly, neither patient had joint dislocations or radiographic features that suggested a diagnosis of DBQD. Therefore, DBQD may be one end of a phenotypic spectrum, with severe long bone shortening and nonspecific radiographic findings at the other end.

HIRSCHSPRUNG DISEASE IN AURICULOCONDYLAR SYNDROME: THE FIRST REPORT

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Auriculocondylar syndrome (ACS, MIM 614669) is a rare first and second pharyngeal arch disorder, segregating as an autosomal dominant (rarely autosomal recessive) trait with mild to severe micrognathia, dysplastic ears with cleft lobules ("question mark ear"), prominent cheeks and a small mouth. Some affected individuals have had central apnea. We propose that Hirschsprung disease may be an extra-craniofacial manifestation of ACS. We report an AGA term female with suspected bowel obstruction due to nonbilious emesis on the second day of life. Hirschsprung disease was diagnosed by rectal suction biopsy and treated with a rectal pull-through procedure. At 8 weeks, she was referred to our Craniofacial Clinic for bilateral clefts at the lobule/helix junction and a small chin, which was clinically diagnosed as Auriculocondylar syndrome. The family history was negative.

Genetic testing (sequencing and del/dup) revealed a novel missense variant of unknown significance in the gene that encodes phospholipase C beta 4, *PLCB4* (exon 17, c.1345C>T; p.Pro449Ser; Fulgent) and no variants in *GNAI3* or *EDN1*. The asymptomatic parents have not been tested for this variant. The wild type amino acid, Pro449, is completely conserved in all vertebrates examined. Computational tools predict that this variant is deleterious (Align GVGD, SIFT). This variant occurs in the X-Y catalytic domain of the protein where other recurrent missense mutations cluster (Asp360 and Arg621). Structural protein modeling predicts that pathogenic missense mutations act as dominant negatives that interfere with the function of the wild type allele.

Although Hirschsprung disease has not previously been reported in ACS, poor GI motility has been described in autosomal recessive ACS. In two brothers with autosomal recessive ACS, caused by compound heterozygous splice site mutations in *PLCB4*, severe constipation required daily enemas but rectal biopsies were not performed (Kido et al., 2013). Another affected male with a homozygous frameshift mutation had antral dyskinesia, delayed gastric emptying and constipation (Leoni et al., 2016).

The three ACS genes act within the EDN1-EDNRA signaling pathway, which inhibits apoptosis and controls cell specification early in the

development of the cranial neural crest. However, mutations in other endothelin genes, *EDN3* and *EDNRB*, which interact with this pathway and other phospholipase C beta isoforms, are known to cause Hirschsprung disease. Mammals have thirteen different isoforms of phospholipase C (PLC), each with its own tissue-specific expression. Another PLC isoform, PLCB2 interacts with the EDN3-EDNRB pathway. One possible mechanism for the presence of Hirschsprung disease in this craniofacial condition is that some mutations in *PLCB4* may decrease the specificity of this isoform. A mutation that alters an isoform's unique binding properties could make it more promiscuous, causing a dominant negative effect in a related signaling network. In such a scenario, *mutPLCB4* could compete with wild type *PLCB2* affecting the EDN3-EDNRB pathway and interfering with the development of the enteric nervous system.

ANALYSIS OF POPULATION-BASED DATA FOR THE STUDY OF THE ETIOLOGY AND PATHOGENESIS OF FOREGUT MALFORMATIONS

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The embryonic foregut is derived from the anterior portion of the endoderm-lined cavity. It is bound anteriorly by the pharyngeal gut and caudally by the midgut and goes on to form the esophagus, respiratory tree, stomach, duodenum, pancreas, liver, and biliary tree. Major malformations of the foregut are relatively common and generally present prenatally or neonatally. These malformations can occur as part of a genetic syndrome but are often seen in isolation or as part of a recognized association without known etiology. Specific types of foregut malformations have often been studied individually, but a more inclusive epidemiologic study is needed to examine relationships among the various malformations and thus provide insight into etiology and pathogenesis.

The Utah Birth Defects Network (UBDN), established in 1994, has since 1999 been a repository for surveillance of structural defects. Etiological classification in the UBDN is based on that used by the Spanish Collaborative Project and applied to all congenital malformations. We reviewed the UBDN for all infants born from 2000 to 2011 and reported to have at least one foregut malformation. 617,690 cases were tracked in our catchment area in that time period; this included live births as well as stillborn, miscarried, and aborted fetuses.

1145 cases (0.2%) were reported to have foregut malformations. In 70 cases (6.1%) etiology was known, and 41 cases (3.6%) were familial. A chromosome abnormality was causative in 57 cases; additional causative conditions included CHARGE, Alagille, Fanconi anemia, Beckwith Wiedemann, and Feingold syndrome. Cardiac, skeletal, renal, and brain anomalies were frequently associated. The five most commonly reported foregut malformations were pyloric stenosis (incidence ~1/700, as compared to reported incidence of 1/250 −1/500); esophageal atresia with or without tracheoesophageal fistula (incidence ~1/4500, as compared to reported incidence of 1/3500); duodenal atresia (incidence ~1/7000, as compared to reported incidence of 1/6000); biliary atresia (incidence ~1/13,000, as compared to reported incidence of 1/18,000); and annular pancreas (incidence ~1/50,000, as compared to reported incidence of 1/20,000 to 1/7000).

Differences between incidence of birth defects in our cohort as compared to in the literature are likely multifactorial. Inclusion of stillborn infants and terminated pregnancies in our group would affect incidence of certain malformations; method of ascertainment, which depended on reporting of the anomalies to the UBDN, could also have led to omission of later presentations of defects such as annular pancreas. Additionally, some of this discrepancy may be related to over- or under-representation of certain ethnic groups in the region. Overall, however, reviewing the foregut anomalies reported in this database provides a useful overview of associated syndromes and malformations.

EXPANDING THE PHENOTYPIC SPECTRUM OF *TP63*-RELATED DISORDERS: A PLOT TWIST IN THE TALE OF A NAIL

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Individuals with *Tumor Protein P63-* (*TP63)*-related disorders are known to present with a range of phenotypic features, encompassing several established syndromes, including Ectrodactyly Ectodermal Dysplasia Clefting, Rapp-Hodgkin, Hay-Wells, and Limb-Mammary syndromes.

We present eight individuals from three families who expand the phenotypic spectrum of *TP63*- related disorders, including the first reported set of molecularly confirmed monozygotic twins. The "tale of a nail sign", which includes a hypoplastic fifth distal phalanx with a hooked or volar nail, was reported by Vogt and colleages in 2006 as pathognomonic for 4q34 deletion syndrome. The critical region for the "tale of the nail sign" was further narrowed in 2014 to a smaller region within 4q34. The presence of the "tale of a nail sign" in three of our present patients with *TP63* mutation caused initial diagnostic confusion because of normal microarrays. Based on other clinical features, they were subsequently found to have *TP63*-related disorders.

Photographs of the fifth finger of the patients in the original publications are indistinguishable from the patients in our cohort, suggesting *TP63*-related disorders should be considered in children with the "tale of a nail sign" and normal microarrays. Two of our present patients also failed the newborn screen for Severe Combined Immunodeficiency because of T-cell lymphopenia. TREC-based newborn screening for SCID has only recently been introduced, and there is one previous case of a child with *TP63*-related disorder who failed TREC-based newborn screening for SCID. Previous descriptions of *TP63*-related disorders suggested that their increased rates of infections were due to disruptions of the skin barrier from ectodermal dysplasia and genitourinary anomalies predisposing to urinary tract infections. Our two patients

and the previously reported patient suggest the mechanism for increased infections may be T- cell lymphopenia rather than as a secondary cause. Also in our cohort are the first molecularly confirmed set of monozygotic twins, who are discordant for major malformations (e.g. ectrodactyly in one twin, syndactyly in the other; cleft lip and palate in one twin only) but concordant in minor malformations, fair complexion, coarse hair and poor dentition. Finally, in a larger family with multiple affected individuals with the same *TP63* mutation, most individuals have a classic appearance, but one child had a laryngeal web and hydrocele without other classic features. Another individual was phenotypically normal, but had a brother and a child who had classic features. Together, these cases illustrate that 1) mutations in *TP63* should be considered in individuals with the "tale of a nail sign", which was previously thought to be pathognomonic for 4q34 deletion syndrome; 2) Failed SCID newborn screening due to T-cell lymphopenia may be part of the phenotypic spectrum of *TP63*-related disorders, and may be a primary cause of susceptibility to infections in the syndrome; 3) There is significant intra-familial variability including discordant major but concordant minor anomalies in the first reported set of molecularly confirmed monozygotic twins with *TP63* mutations and individuals without classic features (e.g. laryngeal web and hydrocele alone and a phenotypically normal individual) interspersed with classically-affected individuals within the same kindred.

PUBLICALLY AVAILABLE FACEBASE CONSORTIA DATA CAN HELP CLINICAL INVESTIGATORS IDENTIFY POTENTIAL CANDIDATE GENES IMPORTANT FOR CRANIOFACIAL DEVELOPMENT.

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In this article, we suggest that the cartilage and extracellular matrix gene ACAN is a candidate gene for palatogenesis. Using publically available data, we highlight the benefit of NIDCR supported multi-spoke FaceBase data sharing and its integration. We present a 2-year-old female referred to our craniofacial team with cleft palate, macrocephaly and short stature of unclear etiology. Family history was positive for short stature and macrocephaly in the probands 4-year- old sister, her father, and her father's sister and niece. Whole exome sequencing identified a single heterozygous mutation in the ACAN gene (c.7202G>A; p.W2401X) that also segregated in both the father and affected sibling. There have been no reports in the medical literature linking the ACAN gene with a human cleft palate. Defects in ACAN have been found to cause one of three defined genetic syndromes: Osteochondritis dissecans (OD), spondyloepiphyseal dysplasia type Kimberly (SEDK) and Spondyloepimetaphyseal dysplasia aggrecan type (SEMD- ACAN) none of which have cleft palate as an associated phenotype. The ACAN gene (chondroitin sulphate proteoglycan core protein), is a major cartilage component mediating interactions between chondrocytes and matrix. Although there are no other human case reports of individuals with ACAN mutations and a cleft palate, there are several lines of evidence showing that this gene may be important in palatogenesis. There are several animal models with both an Acan mutation and cleft palate. The spontaneous mouse mutation Acancmd/NKruJ published in 1978 and the recent 2017 paper by Metzger et al. on Shetland ponies with Acan mutations both have a cleft palate phenotype. Using publically available FaseBase gene expression data, we find that ACAN gene expression is present during the critical window of palatogenesis (E13.5 & E14.5). Using the The Human Genomics Analysis Interface website, we were able to review meta-analysis of existing European cleft palate GWAS data in and around the ACAN gene and found several SNPs with p-values fluctuating around 0.1 and 0.01. Analysis of enhancers in this region are being pursued. Future Cohort studies may be able to recruit families with ACAN mutations to define the penetrance of a cleft palate phenotype. We suggest that the ACAN gene should be studied further as a potential candidate gene important in craniofacial development. 1. Brinkley JF, Fisher S, Harris MP, Holmes G, Hooper JE, Jabs EW, Jones KL, Kesselman C, Klein OD, Maas RL, Marazita ML, Selleri L, Spritz RA, van Bakel H, Visel A, Williams TJ, Wysocka J, Chai Y. The FaceBase Consortium: a comprehensive resource for craniofacial researchers. Developmen. 2016;143(14):2677-88.

TRPV6 HOMOZYGOTE/COMPOUND HETEROZYGOTE GENE MUTATIONS ASSOCIATED WITH NEONATAL TRANSIENT HYPERPARATHYROIDISM – AN AUTOSOMAL RECESSIVE PLACENTAL DISORDER

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Neonatal transient hyperparathyroidism [NTHP] with prenatal onset of skeletal changes is most probably an etiologically heterogeneous condition with one of the aetiologies being an insufficient maternal-fetal Ca^{2+} transport through the placenta. The molecular mechanism responsible for this transplacental transfer has not been delineated. We report five cases with homozygous/compound heterozygous mutations in the gene encoding the transient receptor potential cation channel, subfamily V, member 6 (*TRPV6*) [OMIM # 606680], an epithelial Ca^{2+}-selective channel associated with this condition. Exome sequencing followed by Sanger sequencing identified homozygous nonsense mutation before the first transmembrane domain in a patient of Pakistani descent born to first cousins parents. Targeted mutation analysis of the TRPV6 gene, performed on 4 other cases of Japanese origin identified compound heterozygote mutations. Patch-clamp recordings, intracellular Ca^{2+} imaging, and plasma membrane biotinylation analysis identified 3 types of mutations: 1) mutations at the outer edges of the second and third transmembrane domains (R425Q, G428R and

R483W) affecting the localization of the TRPV6 proteins to the plasma membrane 2) G451E impaired $[Ca^{2+}]_i$- dependent inactivation leading to calcium overload and cell death, 3) C212Y and I223T in the forth ankyrin repeat domain affecting the TRPV6 protein stability. These results suggest that the NTHP with prenatal onset of skeletal changes is a novel autosomal recessive placental TRP channel disease caused by TRPV6 mutations that affects the maternal-fetal Ca^{2+} transport.

Further follow-up is conducted to find if these patients will have Ca malabsorption and thus osteopenia later in life.

NOVEL INFANTILE PRESENTATION OF HOMOZYGOUS VARIANTS IN *CSF1R* WITH MACROCEPHALY, STRUCTURAL BRAIN ABNORMALITIES, DIFFUSE PERIVENTRICULAR CALCIFICATIONS, INCREASED BONE DENSITY, AND DYSMORPHIC FEATURES

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Hereditary diffuse leukoencephalopathy with spheroids (HDLS) is a rare autosomal dominant white matter disease caused by heterozygous mutations in *CSF1R*. The clinical presentation of HDLS includes rapidly progressive cognitive and motor impairment, seizures, and variable behavioral abnormalities typically with onset in the fourth to fifth decades of life. *CSF1R* encodes colony stimulating factor 1 receptor, a tyrosine kinase required for survival, proliferation, and differentiation of mononuclear phagocytic cells, which include microglia in the CNS, and osteoclasts in bone. *CSF1R* knockout mice show complete absence of microglia and severe osteoclast deficiency, but the phenotype of homozygous loss-of-function *CSF1R* mutations in humans has never been reported.

We report the first description of a patient with homozygous splice acceptor (c.1754-1G>C) in *CSF1R*, which destroys the canonical splice acceptor site and is predicted to result in exon skipping and in-frame replacement of 34 amino acids within the CSF1R protein kinase domain (p.G585_K619delinsA). The patient was a term male infant who presented with macrocephaly, structural brain anomalies, generalized increased bone density, hypocalcemia, and dysmorphic features. Neuroimaging showed agenesis of the corpus callosum, diffuse periventricular white matter calcifications, and severe cerebellar hypoplasia. He died at 8 months of age. Autopsy showed diffuse intracranial calcifications and structural brain abnormalities were confirmed. Histopathology revealed numerous spheroids and scarce microglia on CD68 staining.

Our findings suggest a novel severe, infantile-onset phenotype in patients that lack CSF1R function. The phenotype can be attributed to a lack of proper monocyte/macrophage lineage differentiation, resulting in reduction of microglia and osteoclasts.

RARE PRESENTING FEATURES OF OVERGROWTH SYNDROMES: DON'T FORGET TO LOOK AT GROWTH CURVE!

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Children with overgrowth syndromes typically present with large size and acceleration of growth of neonatal onset. Clinical diagnosis can be achieved by exam, supported by advanced bone age, and confirmed by molecular testing. We describe two patients who presented with findings not typically observed – multisuture craniosynostosis and cervical spine stenosis – in Sotos and Weaver syndrome, respectively. Syndrome diagnosis was delayed until review of growth curves at follow-up visits prompted testing for overgrowth syndromes. Review of literature supports expansion of clinical findings in Sotos syndrome to include multisuture craniosynostosis and cervical spine stenosis in Weaver syndrome.

The first patient presented to craniofacial center as infant with tongue-based upper airway obstruction due to micrognathia, hypospadias, and wide cranial sutures. At age 22 months he was incidentally found to have cervical spine kyphosis with critical stenosis during video- fluoroscopic swallow study requiring urgent surgical management. Reassessment of the child at age 3 showed acceleration of growth from 90% to greater than 98%, advanced bone age, and pathogenic EZH2 variant consistent with Weaver syndrome.

The second patient presented to craniofacial center in infancy with abnormal head shape. Imaging confirmed diagnosis of bilateral lambdoid and sagittal craniosynostosis (BLSS) and cranial vault expansion was pursued at 11 months. Examination at age 2 years during genetics assessment for hypotonia and developmental delays showed acceleration of linear growth from 80% to 98% and macrocephaly. Bone age was advanced and testing for Sotos syndrome revealed pathogenic NSD1 gene variant.

Review of Weaver syndrome literature revealed two case reports of cervical spine kyphosis/stenosis prior to available molecular testing in 1990 and 2000 although risk for cervical spine stenosis is not discussed in review articles or 2015 GeneReview for EZH2-related disorders. As infants with Weaver syndrome often present with micrognathia and associated tongue-based airway obstruction, we recommend review of growth parameters and rate of growth. If growth is accelerated and bone age advanced, testing for Weaver syndrome should be considered, and if diagnosis achieved, cervical spine studies should be pursued.

Although individuals with Sotos syndrome present with macrocephaly, craniosynostosis (described as scaphocephaly in large series of NSD1-positive patients) is said to occur rarely and multisuture synostosis has not be reported. BLSS is a rare multisuture craniosynostosis that results in brachycephalic head shape with increased risk for chiari I malformation. When isolated, the etiology of BLSS is not known. For children with BLSS, if macrocephaly, tall stature, and accelerated rate of growth is found, testing for Sotos syndrome should be considered.

BIALLELIC LOSS OF FUNCTION *WNT5A* MUTATIONS IN AN INFANT WITH SEVERE AND ATYPICAL MANIFESTATIONS OF ROBINOW SYNDROME AND UNAFFECTED PARENTS – A NEW LOCUS FOR AUTOSOMAL RECESSIVE DISEASE

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We have previously reported an infant born to consanguineous parents with a severe 46,XY DSD featuring dysmorphic facies, congenital diaphragmatic hernia, truncus arteriosus, bifid thymus, adactyly and intestinal malrotation. These distinctive clinical features were not strongly suggestive of a single specific Mendelian disorder. We considered the possibility of an atypically severe presentation of a known disorder, a novel disorder or the concurrent presentation of multiple disorders. Trio whole exome sequencing identified a novel homozygous frameshift mutation in *WNT5A*, c.561delC. Missense mutations in *WNT5A* encoding a ligand of ROR2, have previously been associated with dominant Robinow syndrome (RS). The proband's carrier parents show no findings of RS on history or exam.

Our findings support the conclusion that biallelic *WNT5A* loss of function mutations can result in a severe, autosomal recessive presentation of RS including the atypical features of congenital diaphragmatic hernia, malrotation and bifid thymus. Further substantiating this finding, we have recently become aware of a second, unrelated case of severe, apparently autosomal recessive *WNT5A* related RS. Additionally, a *WNT5A* knockout mouse strain displays features reminiscent of those observed in the proband including absent digits, shortened body length, hypoplastic genitalia as well as small snout and jaw. The apparent unaffected status of the proband's parents suggests that dominant *WNT5A* related RS may not result from simple haploinsufficiency as has been proposed and may rather be the result of dominant negative or other effects – all presently reported dominant mutations have been missense. However, loss of function variants in *WNT5A* are significantly underrepresented in the gnomAD database raising the possibility of a more complicated mechanism.

In summary, we present the clinical and genetic characterization of a first family affected by a severe, *WNT5A* associated, recessive form of RS and are aware of a second similarly affected family. These findings expand understanding of both the clinical and molecular spectra of the condition and provide data relevant to the mechanisms responsible for dominant versus recessive *WNT5A* related disease.

SEVERE PHENOTYPE OF INTELLECTUAL DISABILITY, SEIZURES AND DYSMORPHIC FEATURES CAUSED BY HOMOZYGOUS MUTATION IN *PIGS*: ADDING TO THE FAMILY OF DISORDERS ASSOCIATED WITH MUTATIONS IN GLYCOSYLPHOSPHATIDYLINOSITOL (GPI) ANCHOR PROTEINS

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Inherited GPI deficiencies are an expanding subtype of congenital disorders of glycosylation due to defects in synthesis and/or transport of GPI anchored proteins (GPI- APs). This class of cell-surface expressing proteins performs diverse cellular functions including adhesion, complement regulation, and hydrolysis. Several GPI-APs play a critical role in embryo- and neurogenesis. To date roughly 200 mammalian GPI-APs have been identified. Nearly 30 proteins are known to be involved in the biosynthesis of GPI-APs. The PIG (phosphatidylinositol glycan) genes encode a subset of these proteins. Mutations in *PIGA*, *PIGL*, *PIGW*, *PIGV*, *PIGY*, *PIGO*, *PIGN*, *PIGT*, *and PIGG* have been associated with a variety of phenotypes that share features of intellectual disability (ID), hypotonia and seizures. To date no phenotype has been associated with mutation in PIGS. We report two brothers with homozygous mutations in PIGS with a pattern of malformation including ID, seizures, hypotonia and coarse features resembling storage disorders.

The brothers are the only children born to healthy, non-consanguineous parents who come from a small pueblo in Vera Cruz, Mexico. The older boy was born by C- section for fetal distress weighing 3.7 kg. He had recurrent aspiration and seizures. He was evaluated on various occasions between 23 months and 7 years. His phenotype was not progressive; however, he made no developmental progress and had poorly controlled seizures. He had distinctly coarse facies, wrinkled forehead, macroglossia with a secondary alveolar ridge, pectus carinatum, hepatomegaly, inguinal and umbilical hernias, short broad stubby fingers. The younger boy was born by C-section weighing 4.06 kg. He was recognized to be similarly affected at birth. He also has made no developmental progress with poorly controlled seizures

Whole genome sequencing on the whole family documented a novel homozygous mutation *PIGS* in both boys with heterozygous mutations in parents: c.1316_1352delCCACCACCCTTACCTCCCT GGCGCAGCTTCTGGGCAAinsGGTTG CT (p.Thr439_Lys451delinsArgLeuLeu) in a region of homozygosity. Prediction programs suggested this variant was deleterious.

PIGS is involved in the biosynthesis of GPI-APs in the transamindase complex. *PIGS* and *PIGT* are essential for the formation of carboxyl intermediates during the transfer of GPI group to the protein. Biallelic mutations in PIGT have been documented in patients with a pattern of malformation quite similar to that observed in these brothers. Functional studies are in progress.

MAKING A DIAGNOSIS FROM A DELIVERY ROOM SURPRISE: DISCORDANT NON-INVASIVE PRENATAL SCREENING AND PHENOTYPIC SEX

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Since its clinical introduction in 2011, non-invasive prenatal screening (NIPS) utilizing circulating cell-free DNA (cfDNA) has revolutionized prenatal screening for fetal chromosome disorders. Prior to NIPS, fetal

sex was optionally assessed at the mid-gestation anatomic ultrasound or through invasive diagnostic testing, typically because of an alternative indication (i.e. maternal age or fetal anomalies). In contrast, NIPS routinely assesses a sex chromosome complement, providing a highly sensitive and specific assessment of genetic sex. Herein, we present a case series of diagnoses to consider when cfDNA and phenotypic sex are discordant.

Patient 1 was the second child of healthy parents. NIPS was at 10 weeks gestation; results were reported "male". However, the patient had normal female external genitalia at birth. Given this discrepancy, the pediatrician referred the patient to the Differences of Sexual Development (DSD). Specialized evaluation by the DSD team revealed a palpable gonad in the left inguinal canal. Karyotype was normal 46, XY. She was diagnosed with a non-syndromic 46,XY DSD.

Patient 2 was a term infant born after an uncomplicated delivery. NIPS done at 11 weeks gestation (and repeated at 22 weeks) was consistent with "male". However, mid-gestation ultrasound examination suggested female external genitalia and a single amniotic sac. Postnatal chromosome analysis was normal 46, XX [60/60] at a 500-band level. By diagnosis of exclusion, the most likely etiology was considered a co-twin demise of a male fetus.

Patient 3 was a demise at 21 weeks due to fetal hydrops. cfDNA results were "male". However, at autopsy, normal internal and external female genitalia were noted. Postnatal karyotype was 45,X[18]/46,X,i(Y)(q10)[2], consistent with mosaic Turner syndrome with an iso- dicentric Y-chromosome. Placental circulating cfDNA is a small fraction of cfDNA in maternal circulation and sex chromosome mosaicism cannot be accurately detected.

Patient 4 was a 26-year-old primigravida with history of a renal transplant from a male donor. cfDNA screening results were reported as "male" though mid-gestation ultrasound and postnatal examination were consistent with female external genitalia. Diagnostic chromosome analysis was declined for the neonate; circulating cfDNA from the male donor kidney was considered the most likely source of cfDNA screening result and phenotypic discordance.

NIPS is the first non-invasive prenatal screening test to routinely evaluate for sex chromosome complement. This technology has been widely adopted in prenatal clinics and introduces both challenges and opportunities postnatally. A basic understanding of NIPS and the etiologies of discordant results provide an opportunity to diagnosis conditions that may not otherwise be appreciated in the neonatal period as well as to guide medical management.

PARASPINAL AND INTRA-ABDOMINAL NEUROFIBROMAS ASSOCIATED WITH *SOS2* GENE MUTATION IN A PATIENT WITH NOONAN SYNDROME

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Mutations in the son of sevenless, Drosophila, homolog 2 (*SOS2*) gene have been recently described to cause Noonan syndrome, characterized by marked ectodermal involvement and minimally affected to preserved intellect, in 12 reported individuals. Meanwhile, Neurofibromatosis-Noonan syndrome has been a recognized clinical entity for decades; the majority of individuals harbour a *NF1* gene mutation. Mutations in other rat sarcoma (RAS)- mitogen-activated protein kinase (MAPK) pathway genes have infrequently been implicated in neurogenic tumours, with none of the individuals with *SOS2* mutations having neurofibromas. We report the rare occurrence of extensive paraspinal, abdominal, and pelvic neurofibromas in a 58-year-old woman with Noonan syndrome and a likely pathogenic variant in the *SOS2* gene.

The patient was born to healthy 27-year-old mother and 33-year-old father of Ashkenazi Jewish (AJ) descent. She had reportedly normal development, required bilateral ptosis surgery at 10 years of age and developed glaucoma at 22 years of age. Following the birth of her daughter with pulmonary stenosis, both the patient and her daughter were diagnosed with Noonan syndrome. Her features included curly hair, high anterior hairline, sparse eyebrows, ptosis, downslanting palpebral fissures, thick lips, short and webbed neck, pectus deformity, hyperkeratosis pilaris, ulerythema ophryogenes, and short stature. At 51 years of age, she developed significant lower limb lymphedema and subsequent abdominal computed tomography (CT) revealed cystic abdominal masses. Abdominal and pelvic magnetic resonance imaging (MRI) confirmed innumerable bilateral thoracic, abdominal, and pelvic masses consistent with neurofibromas. Spine MRI revealed sacral nerve root expansion with mixed solid and cystic components, suggestive of a plexiform neurofibroma.

Genetic testing of the *PTPN11* gene found a variant, p.Ile309Val, which was initially reported as pathogenic but retracted ten years later as a variant of uncertain significance due to high allele frequency in the AJ population. *NF1* gene sequencing and dosage were normal. Expansion of an initially normal RASopathy panel at The Hospital for Sick Children, Toronto, to include the *SOS2* gene revealed a likely pathogenic *SOS2* variant, p.Met267Arg. This variant has been described in two affected individuals and is predicted to activate the SOS2 protein.

Neurofibromas are a rare and potentially overlooked manifestation of Noonan syndrome. Our report suggests that their presence may be explained by the shared RAS-MAPK pathway of *NF1* and other RASopathy genes, including *SOS2*.

A NEW FLNA SYNDROME IN TWO BROTHERS WITH INTELLECTUAL DISABILITY, MITRAL VALVE PROLAPSE AND RADIOULNAR SYNOSTOSIS

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Filamin A (FLNA) is one member of a family of high molecular weight cytoskeletal proteins with cross link the actin cytoskeleton into

networks and modulate the response of cells to their chemical and mechanical environment by regulating changes in shape and motility. Mutation in the X-linked FLNA gene are associated with a large number of diverse disorders with a wide range of phenotypes, including otopalatodigital syndrome types I and II, Melnick-Needles syndrome, frontometaphyseal dysplasia, cardiac valvular dysplasia, FG syndrome 2, and nodular periventricular heterotopia. We have seen two brothers who presented with intellectual disability, radioulnar synostosis and mitral valve prolapse who have a mutation in FLNA. The proband is a 17 year old male initially seen in the Genetics Clinic at age 6 years for velopharyngeal dysfunction. He had a history of congenital radioulnar synostosis, mild speech disorder. He was seen again at 16 years for developmental disability. Previously he had an abnormal EEG (epileptiform) with no overt seizures. Treatment anticonvulsants was unsuccessful and discontinued. He was also treated for ADHD. The patient was receiving special education for most school subjects. Brain MRI showed persistent hypomyelination in frontal and temporal lobes. Neuropsychological evaluation at 6 years showed a full scale IQ of 68 and at 16 years 75 (WISC IV). He was diagnosed with mitral valve prolapse with mitral regurgitation. Physical examination showed a thin young man in no distress. Weight 49.7 kg (2nd centile), height 172.9 cm (35th centile) and head circumference 54.5 cm (15th centile). Face was oval and narrow, palate was narrow and arched, the sternum was flat, cardiovascular examination demonstrated a mid-systolic click with late systolic murmur. He had bilateral radio-ulnar synostosis.

Neurological examination was significant for a mild tremor. His brother was suspected to have developmental delay at 4 years. He was later diagnosed with ADHD and neuropsychological testing showed a full scale IQ of 73. At 11 years weight was 36.7 kg (37th centile), height 147 cm (50th centile) and head circumference 53 cm (25 centile). He had a long oval face, narrow and arched palate, mild pectus carinatum, radioulnar synostosis of the right upper extremity, and cardiovascular examination showed a mid-systolic click with late systolic murmur. He did not have a brain MRI. Examinations of parents were normal except the mother had a narrow arched palate. NextGen sequencing for intellectual disability (Claritas) demonstrated a missense mutation, c.7811G>A, p.Arg>Gln in both brothers and their mother. This mutation has not been has not been associated with any other of the known FLNA disorders and has not been reported in HGMD or ClinVar. FLNA pathogenic variants cause a large number of different disorders, including skeletal dysplasia, valvular dysplasia, brain heterotopia with seizures and craniofacial disorders. These brothers have an apparently new FLNA mutation syndrome with intellectual disability, skeletal anomalies, and valvular dysplasia. Functional studies are being planned.

NOONAN-LIKE SYNDROME WITH LOOSE ANAGEN HAIR – EXPANDING THE PHENOTYPE

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Noonan-like syndrome with loose anagen hair (NS/LAH) is a condition characterized by Noonan-like facies, congenital heart defects, darkly-pigmented skin, brittle nails, short stature, intellectual disability and sparse, thin, slow-growing hair that is easily plucked. The clinical phenotype is caused by a missense mutation in the *SHOC2* gene, c.4A>G, p.Ser2Gly, which is the only mutation reported to cause this condition. We report on a patient with NS/LAH who has a number of vascular abnormalities, including skin, hepatic, and extrahepatic hemangiomas, as well as vascular changes of the subdural membrane. She also developed seizures. These findings appear to expand the phenotype of this condition.

The patient is a 4-year-old female with an identified c.4A>G mutation in the *SHOC2* gene, with a phenotype consistent NS/LAH. Her mutation was identified on whole exome sequencing. She presented initially at 2 months of age with a 6-week history of non-bilious, non-bloody vomiting. An abdominal CT scan and an abdominal MRI both demonstrated hepatomegaly, findings consistent with multiple hepatic hemangiomas, and an extrahepatic, extra-adrenal mass suggestive of a hemangioma. She also had several cutaneous hemangiomas. At 6 months of age, she was hospitalized for increased intracranial pressure with findings of papilledema and a bulging anterior fontanel. Because of the increased intracranial pressure, she first had a decompression followed by placement of an extraventricular drain (EVD), and she later had a ventriculoperitoneal (VP) shunt placement. During her EVD placement, her subdural membrane was described as thick, white, and riddled with distended veins. Further, during the abdominal laparoscopy for the VP shunt placement, there was an increased vascular pattern noted on the surface of her liver. She subsequently developed epilepsy, and the burr hole used for the initial decompression never closed.

There have been two patients with SHOC2 p.Ser2Gly mutation reported with hemangiomas (Gripp et al., 2013). Ekvall et al. (2011) reported a patient with a familial *PTPN11* mutation (p.Gly409Ala) as well as the NS/LAH mutation mutation in *SHOC2* (p.Ser2Gly), who had characteristic features of NS/LAH and a hepatic hemangioma; the authors proposed that the atypical phenotype in this patient was the result of an additive effect, with PTPN11 p.Gly409Ala acting as a modifier. Additionally, there have been three cases of moyamoya disease reported in this condition (Cho et al., 2015; Lo et al., 2015). Our patient's abnormal subdural membrane venous finding appears to be another intracranial vascular anomaly in this condition. She has not had intracranial vascular studies that could elucidate whether she has moyamoya disease.

MUTATIONS IN FIBRONECTIN CAUSE A SUBTYPE OF SPONDYLOMETAPHYSEAL DYSPLASIA WITH "CORNER FRACTURES"

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Fibronectin is a master organizer of extracellular matrices (ECM), promoting assembly of collagens, fibrillin-1, and other proteins. It is also known to play roles in skeletal tissues through its expression in osteoblasts, chondrocytes and mesenchymal cells. Spondylometaphyseal dysplasias (SMD) comprise a diverse group of skeletal dysplasias often presenting with short stature, growth plate irregularities, and vertebral anomalies, such as scoliosis. By comparing the exomes of individuals with SMD with the radiographic appearance of "corner fractures" at metaphyses, we identified three individuals with novel variants in the fibronectin gene (FN1) affecting highly conserved residues. Furthermore, using matching tools and the SkelDys emailing list, we identified other individuals with de novo FN1 variants and a similar phenotye. The severe scoliosis in most individuals and rare developmental coxa vara distinguishes individuals with FN1 mutations from those with classical Sutcliffe type SMD. To study functional consequences of these FN1 mutations, we introduced three disease-associated missense mutations (p.Cys87Phe, p.Tyr240Asp, p.Cys260Gly) in a recombinant secreted N- terminal 70 kDa fragment (rF70K) as well as in the full length fibronectin (rFN). The wild-type rF70K fragment and rFN were secreted into the culture medium, whereas all mutant proteins were either not secreted or secreted at significantly lower amounts. Immunofluorescence analysis demonstrated increased intracellular retention of the mutant proteins. In summary, mutations in FN1 that cause defective fibronectin secretion are found in SMD, and we thus provide additional evidence for a critical function of fibronectin in cartilage and bone.

ANORECTAL ANOMALIES IN NOONAN SYNDROME

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Noonan syndrome is an autosomal dominant disorder caused by germline mutations in genes critical to the Ras/MAPK pathway, a signal transduction pathway important for cell growth and differentiation.

Noonan syndrome is characterized by short stature, cardiac defects, cryptorchidism, chest wall anomalies, and characteristic facial features. With respect to gastrointestinal (GI) complications, the neonatal period can be marked by feeding difficulties and failure to thrive. Other anomalies of the GI tract are less commonly reported and there has only been one prior report of an anorectal malformation and Noonan syndrome (rectal atresia).

We report three additional patients with Noonan syndrome and anorectal anomalies. The first was evaluated at 3 months of age and found to have a patent, but significantly anteriorly displaced anus. She had a de novo c.770C>T (p.Ser257Leu) variant in *RAF1*. Our second patient was a 12-day old male with anteriorly displaced anus versus anal stricture. He was found to have the same c.770C>T (p.Ser257Leu) variant in *RAF1* that was identified in our first patient. Both patients required anal dilation. Our third patient was evaluated at 4 days of age. He was found to have a prolapsed rectum. He had a c.236A>G (p.Gln79Arg) variant in *PTPN11*.

Based on the anorectal findings of these cases, we retrospectively reviewed 53 cases of Noonan syndrome from a research cohort to further explore the relative frequency of associated GI concerns. Of the 53 individuals with Noonan syndrome, 29 had known mutations in causative genes, (79% were in *PTPN11* and 7% were in *RAF1*). A GI complication was identified in 19 individuals (36%) within this research cohort. These complications ranged from feeding issues (i.e. swallowing difficulties, oral aversion, NG tube feeds) in 5 individuals (9%), to structural anomalies (i.e. malrotation) in 2 individuals (4%). A gastrostomy tube was present in 7 individuals (13%) within this cohort, though the indication was not specified. Anorectal anomalies were not reported for any of these patients.

The embryogenesis of anorectal malformations is complex. *PTPN11* and *RAF1* are highly expressed in the GI tract. The Ras/MAPK pathway is critical for cellular differentiation and proliferation and we postulate that alterations of this pathway could contribute to the development of anorectal anomalies.

Our data suggest that there are variable GI complications in Noonan syndrome. Our report adds three cases of anorectal anomalies to the one case previously reported. Two of these patients were identified to have the same variant in *RAF1* and raises the question of a genotype- phenotype correlation. However, anorectal anomalies in Noonan syndrome are likely rare, which is supported by review of our research cohort. It is possible that these cases may represent a chance association given the relative frequency of Noonan syndrome (1 in 1000–2500). This report helps to potentially expand the phenotype of Noonan syndrome and suggests that further studies are needed to better establish whether there exists an association with anorectal anomalies with Noonan syndrome, and the role of the Ras/MAPK pathway in GI development.

CONGENITAL HEART DISEASE AND AORTIC ARCH VARIANTS ASSOCIATED WITH MUTATION IN PHOX2B

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Congenital Central Hypoventilation Syndrome (CCHS, OMIM 209880) is a rare autosomal dominant disorder caused by mutation in *PHOX2B*. It is believed to manifest as a consequence of abnormal neural crest cell migration during embryogenesis. Characteristic features include decreased sensitivity to hypercapnia, Hirschsprung disease, risk for tumors of neural crest cell origin, and risk for arrhythmias. The prevalence of congenital heart disease (CHD) in this population, however, has not been previously assessed. To determine the prevalence of CHD in patients with CCHS as compared to the estimated prevalence among live births within the United States. A single institution retrospective review of patients with CCHS was performed. Patient dataset was identified using the search terms congenital central hypoventilation, central hypoventilation syndrome, and central alveolar hypoventilation. Data was queried and manually extracted from the electronic health record system. Echocardiogram reports were reviewed and if findings were ambiguous, manual review of images was performed. Chi

square analysis was utilized to compare prevalence of CHD in the sample population as compared to the general population. A total of 27 patients with CCHS were identified. Five patients had clinically significant CHD including left anomalous coronary artery, complete vascular ring, moderate secundum ASD with deficient retroaortic rim, and patent ductus arteriosus requiring surgical closure. A rare aortic arch variant, separate origin of the left vertebral artery off the aortic arch was also identified. The prevalence of CHD in this study population was 18.5% ($p<0.001$) with a relative risk of 26.6 (CI 9.02–78.61, $p<0.001$) as compared to current estimates of CHD among live births in the United States. This is first report of association between congenital heart disease and mutation in *PHOX2B*. Six patients (22.2%) had abnormalities or variants that involved the proximal aortic arch, a region predominantly derived from neural crest cell lineages. There was a significant increase in both the prevalence and relative risk for CHD in patients with mutation in *PHOX2B*. This study emphasizes the need for careful cardiac anatomical screening for patients with CCHS. If the aortic arch cannot be adequately visualized, cross sectional imaging should be considered.

A MALE INFANT WITH XQ22.2àQ22.3 DUPLICATION CONTAINING *PLP1* AND *MID2* GENES

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A one week old male infant with a history of IUGR and maternal diabetes was referred to genetics because of direct hypebilirubinemia and elevated liver function studies. His physical examination was significant for pendular nystagmus and micrognathia. Eye spacing and ear sizes were within normal limits. Liver biopsy revealed neonatal hepatitis. Over the three months that he was hospitalized he developed head titubation and spasticity. He had normal EEG and MRI. He was subsequently placed in a chronic care facility. The whole chromosome SNP microarray (reveal) analysis revealed a 6.7 Mb interstitial duplication of Xq22.2àq22.3. Among 20 OMIM genes in this interval, the copy number change of *PLP1* gene (OMIM# 300401) and MID2 genes (OMIM# 300294) are noteworthy with respect to potential contribution to the patients' clinical features. Mutations and duplications of *PLP1* are associated with X-linked recessive Pelizaeus Merzbacher disease (PMD, OMIM# 312080). Duplications involving *PLP1* account for 60–70% of reported PMD cases. Patients with *PLP1* duplications typically display PMD features including nystagmus, spasticity, ataxia, and intellectual disability, however, the course and degree of clinical severity may vary among patients (Inoue et al. (1996); *Am J Hum Genet* 59: 32–39). Our patient's clinical features are largely in consistent with PMD which relates his microarray finding of a *PLP1* gene duplication to the PMD diagnosis. Our patient's mother and maternal uncle both have intellectual disability. The Fluorescence In Situ Hybridization (FISH) study showed that the patient's mother possesses the duplication in the region of Xq22.3->q22.3, as seen in this patient, thus indicating that the duplication found in the patient might be familial and maternal in origin.

FG syndrome is an X-linked multiple congenital anomalies (MCA) syndrome. It has been mapped to four distinct loci *FGS1–4* on Xq13.1 (FGS1), Xq28 (FGS2), Xp22.3 (FGS3) and Xp11.4 (FGS4). Jehee et al. (*Am J Med Genet* 2005; 139A: 221–225) detected an inherited duplication at Xq22.3 by comparative genomic hybridization microarray in a FG syndrome patient, and identified a new locus responsible for FG syndrome, named FG syndrome 5 (OMIM#300581). The gene in this region, *MID2*, is highly homologous to *MID1*, a gene is known to be mutated in Opitz G/BBB syndrome, was proposed as a candidate gene for FG syndrome at locus Xq22.3. In the report by Jehee et al. (2005), a male child with FG syndrome presented with dysmorphic features including trigonocephaly, upslanting palpebral fissures, depressed nasal bridge, anteverted nares, long philtrum, diastema of upper central incisors, strabismus and hypospadias. He had hypotonia and developmental delay and died at 4 years of age due to generalized infection and multiple organ failure. The patient reported herein displays clinical features influenced by the *PLP1* gene duplication. The effect of the *MID2* duplication on the patient's overall phenotype appears less evident but may become manifest over time.

WHAT IS LEFT? AN ANALYSIS OF THE REMAINING UNSOLVED MENDELIAN MULTIPLE MALFORMATION DISEASES IN OMIM

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Since 1966, Online Mendelian Inheritance in Man (OMIM) has provided continuous curation and classification of Mendelian disease for the Genetics community. OMIM mines the biomedical literature and according to expert review, curates gene and phenotype entries and links them once a gene-phenotype association is identified; not a small undertaking given the recent implementation of next generation sequencing methods for gene discovery. Of the 6500+ Mendelian phenotypes within OMIM, focus is typically given to the 5000 phenotypes with an established molecular basis ($n = 4988$ on April 25th, 2017). We, however, set out to dissect the lesser known and likely rarely accessed group in OMIM; the confirmed Mendelian phenotypes or phenotypic loci that remained without a confirmed etiology, with a particular interest in those characterized by multiple malformations.

The full morbid map from OMIM was queried in April 2017 for such entries ($n = 1607$, denoted by a percent [%] sign). Thus far, a random selection of 161 entries has been manually reviewed to better understand the constitution of this group. Of the 161 entries, 91 (56.5%) are descriptions of what we consider true Mendelian phenotypes. These include rare clinical presentations that have been described in <10 kindreds ($n = 47$, 29.2%, e.g., Wahab syndrome), more recognizable syndromes that remain without a confirmed etiology ($n = 1$, 0.6%, e.g., Dubowitz syndrome), mapped loci for a disease known to be genetically heterogeneous ($n = 33$, 20.5%, e.g. retinitis

pigmentosa), relatively common multifactorial entities (n = 1, 0.6%, e.g., orofacial cleft) and descriptions of minor traits (n = 9, 5.6%, e.g., Darwinian tubercle of the ear, musical perfect pitch). The remaining 70 entries (43.5%) describe loci associated with either quantitative traits (e.g., ALT levels) or susceptibility to common disorders (e.g., glaucoma) or traits (e.g., myopia, nocturnal enuresis).

We also considered how these phenotypic entries were being reclassified from one year to the next. Twenty-two entries denoted by a % sign in 2016 (May 2016, n = 1622) had been reclassified by 2017. Of these, 17 phenotypes were now associated with pathogenic variants in a specific gene (e.g., uncombable hair syndrome: *PADI3*). The remaining 5 were incorporated into other OMIM entries. For example, Miles-Carpenter syndrome (previously 309605), which was suspected to be an X-linked mental retardation syndrome, has now been incorporated into Wieacker-Wolff syndrome (314580), with the gene identified to be *ZC4H2* on chromosome X. Interestingly, of the 7 new % phenotypes in 2017, some (2/7) appear to be reclassified due to downgraded genetic evidence for causality based on new control databases.

We will present our review of the 1600 unsolved clinical presentations with a focus on those associated with multiple malformations and will discuss the contribution of these unsolved syndromes to current practices of dysmorphology.

NANCE-HORAN SYNDROME IN A FAMILY OF THREE GENERATION

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Nance-Horan syndrome (NHS: OMIM 302350) is a rare X-linked disorder characterized by congenital cataract, specific dental anomalies, and dysmorphic features. Mental retardation is present in about 30% of the cases. It is caused by mutations in the NHS action remodelling regulator gene on the chromosome Xp22.13. The encoded protein functions in eye, tooth, craniofacial and brain development. NHS syndrome is also known as cataract-oto-dental syndrome.

Ophthalmological findings in affected males are severe bilateral congenital dense nuclear cataracts which lead to profound vision loss and usually require surgery at an early age. Microcornea, microphthalmia and nystagmus have also been reported. Characteristic facial features such as large anteverted and simple pinnaes, long narrow face, prominent nose and nasal bridge, and distinctive dental anomalies such as supernumerary incisors (mesiodens), diastema between the teeth and screwdriver blade-shaped incisors are seen in affected males. Also, several families have been reported to have lateral brachymetacarpalia. The disorder appears to be inherited in a semi-dominant fashion, with heterozygous females often manifesting similar but less severe features.

To date the number of cases reported is very small and the spectrum of phenotypic features especially in females, is not well defined. Here we report the clinical and molecular findings in a three generation Finnish family due to a novel mutation in NHS gene. Method used for the first patient was next generation sequencing (NGS) panel testing and for the rest of the family Sanger sequencing was used to verify mutation. Clinical and ophthalmological examinations were performed on all mutation positive family members.

Testing result revealed a mutation c.3808C>T p.(Gln1270Ter) in exon 6 of the NHS gene, co-segregating with the disease in the family. The mutation led to the truncation of the NHS protein. Multiple sequence alignments showed that codon, where the mutation occurred, was located within a phylogenetically conserved region. The clinical features in affected male and all female carriers are described in detail.

In conclusion, we report a novel nonsense mutation in NHS gene. The spectrum of clinical features in Nance-Horan syndrome is variable. In particular, for female patients, it can be very subdued. Our findings broaden the spectrum of NHS mutations causing Nance-Horan syndrome and describe detailed clinical findings of phenotypic spectrum in all three generations.

BEHAVIORAL AND PHYSICAL FEATURES ASSOCIATED WITH VARIANTS OF SHANK2

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SHANK2 (11q13.2) encodes SH3 and multiple ankyrin repeat domains protein 2. It is the only gene attributed to OMIM #613436, Autism Susceptibility Type 17. The SHANK family of proteins serve as scaffolding at the level of neuronal dendritic spine and has a critical role in synaptic plasticity (Moteiro & Fang, 2017). There is an association between copy number variants (CNVs) that include SHANK2 and autism spectrum disorder (ASD). Multiple cohort studies show that these CNVs may be responsible for up to 0.5% of cases of ASD. Likewise, this association is further supported by the Shank2 knockout mouse model which demonstrates; decreased communication, increased grooming, hyperactivity, and repetitive jumping. This report proposes a more specific phenotype beyond 'autism susceptibility' and includes patient photos and cohort data compiled from services like Gene Matcher and MyGene2. Patients with loss of function or haploinsuficiency of SHANK2 appear to have speech apraxia without the corresponding narrow range of interest or impaired social skills diagnostic for autism. Likewise in our patient affected by a de novo loss of function SHANK2 variant there are distinctive facial features including; telocanthus, downslanting palpebral fissures, wide columella, thin ala nasi, smooth philtrum, wide mouth and thin upper vermillion. These facial features are similar to those found in Phalen-McDermid syndrome which is due to deletions of SHANK3. Therefore it is not surprising that individuals heterozygous for loss of function variants in SHANK2 may have a recognizable syndrome.

SOPH SYNDROME: MULTISYSTEM DISORDER LEADING TO INFANTILE LIVER FAILURE, PELGER-HUET ANOMALY, DYSMORPHIC FEATURES AND SKELETAL DYSPLASIA

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SOPH syndrome (OMIM #614800) is an autosomal recessive condition characterized by skeletal dysplasia, brachydactyly, Pelger-Huet anomaly and episodic liver failure triggered by fever due to biallelic mutations in NBAS.

The NBAS gene at 2p24.3 was first identified in Neuroblastoma cell lines. NBAS protein (NAG) is a subunit in the syntaxin-18 complex which is implicated in ER/Golgi transport. It has been proposed that missense mutations in NBAS gene renders syntaxin-18 complex thermolabile. Febrile illnesses cause increased catabolism with subsequent disordered ER/Golgi transport.

Our proband initially presented at age 16 years during her 4th episode of acute liver failure. This episode was associated with acute renal failure requiring hemodialysis. She had three prior episodes between ages 3 and 6 years. She was born at 26 weeks to a non-consanguineous Caucasian couple. She had failure to thrive requiring G-tube. Additionally, she had precocious puberty, hypothyroidism and insulin resistance requiring medical management. She has intellectual disability with an IQ of 72. She is dysmorphic (including triangular face, prominent forehead, pointed chin) with long fingers, prominent PIP joints and has short stature (Z-score −3). NK cell function was substantially decreased. Interestingly, her IgG level was low during the episode and normalized upon recovery. IgA level was low during the recovery period. Skeletal imaging revealed relatively long phalanges, thin long bones, dynamic anterior subluxation of C2 on C3, rightwards thoracic curvature, gracile ribs and narrow upper chest. Novel compound heterozygous missense mutations in the NBAS gene were found by whole exome sequencing. Our patient supports the theory of multisystem involvement in biallelic NBAS mutations. In addition, she has unique features that raise questions for further functional studies.

SOPH syndrome is a rare, autosomal recessive disorder first described in 2010 by Maksimova et al. (*J Med Genet* 2010; 47: 538–548) in an isolated population of Yakuts in East Russia. Clinical features in their cohort of 33 patients included short stature, facial dysmorphism, senile appearance, brachydactyly, optic atrophy and Pelger-Huet anomaly. Homozygosity mapping revealed homozygous missense mutations in Neuroblastoma amplified sequence (NBAS) gene. Biallelic mutations in NBAS gene were found causative in infantile onset recurrent acute liver failure (OMIM #616483) in a separate cohort of 11 German individuals by Haack et al. (*AJHG* 2015; 97:163–169). The affected individuals had episodes of acute liver failure triggered by fever, with recovery of liver function in between crises. These individuals reportedly did not have any features of SOPH syndrome. This was closely followed by a clinical report by Segarra et al. (*AJMG Part A* 2015; 167A: 2902–2912) of two unrelated individuals with biallelic NBAS mutations presenting with a multisystem disease that included progeroid appearance, recurrent acute liver failure, immune dysfunction, hypogammaglobulinemia and skeletal dysplasia with C1-C2 instability. Our patient supports the idea that SOPH syndrome and recurrent episodes of liver failure may be part of the same spectrum and can coexist in some patients.

NOVEL PAATHOGENIC VARIANT IN *OFD1* RESULTS IN MALE LETHAL ORAL FACIAL DIGITAL SYNDROME TYPE 1 WITH PITUITARY APLASIA

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We report live birth of a male patient with OFD Type I secondary to a novel mutation in *OFD1*, c.515T>C, p.Leu172Pro, and complete pituitary aplasia with severe hypoplasia of peripheral endocrine glands. The infant was first identified with severe brain malformations and multiple congenital anomalies at 22 weeks gestation on routine ultrasound. Subsequent fetal MRI demonstrated polymicrogyria, gray matter heterotopia, ventriculomegaly, corpus callosum agenesis, and abnormalities of the posterior fossa and brainstem concerning for Dandy-Walker malformation and molar tooth sign. The infant was delivered at 36 weeks gestation via c-section with immediate intubation. Recurrent episodes of hypotension and hypoglycemia prompted evaluation of the adrenal glands which could not be visualized on ultrasound. Postnatal brain MRI confirmed fetal findings and was unable to identify the pituitary. Extensive endocrine work up was consistent with panhypopituitarism. Given severity of brain malformations and poor prognosis, family withdrew care on day of life 10. Autopsy confirmed absence of pituitary tissue. Careful analysis identified two areas of tissue near the kidneys. Microscopic analysis identified hypoplastic islands of adrenal cortex consistent with severe adrenal hypoplasia. The thyroid and testes demonstrated similar degrees of hypoplasia and only one parathyroid gland was identified. The pancreas was normal in size with normal histology.

OFD type 1 is an X-linked dominant condition with near universal lethality in early gestation in male infants. Live birth is exceedingly rare and has been reported in only 5 patients to date.

Although a spectrum of brain malformations have been associated with mutations in *OFD1*, this is the first report of pituitary aplasia. It is likely that most if not all of the other abnormalities in endocrine organs were secondary to the lack of pituitary stimulation. The severity of adrenal insufficiency made clinical stabilization difficult with recurrent hypotension and bradycardia despite steroid and fluid administration. Temperature and glucose regulation was also problematic. Maternal testing was performed and confirmed carrier status. Extended family history identified a maternal male sibling that died at approximately 30 hr of life from severe brain malformations. At this time, confirmatory testing in the maternal grandmother is ongoing and the family has elected to pursue preimplantation diagnosis for future pregnancies.

The exact function of OFD1 gene is not fully understood, but it is been shown that OFD1 gene encodes a protein that localizes to the centromere and basal body of primary cilia, which plays a role in cilia formation and left-right axis specification. It is also been noted that there's an inverse correlation between OFD1 mutant protein length and the severity of phenotype. It has been suggested that OFD1 might be involved in the SHH signaling pathway via interaction with other genes such as Hox, thus affecting pituitary gland development.

OCCASIONALLY, WILLIAM OCKHAM GOT IT WRONG.

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The law of parsimony or "Occam's razor" is interpreted in the medical field as a preference for simplicity and the search for one diagnosis to account for all medical complications is often sought. Here, we describe an 18 year old female with both Kleefstra Syndrome and α-N-acetylgalactosaminidase deficiency or Schindler/Kanzaki Disease. She presented with mild intellectual disability, learning delays, stable bilateral sensorineural hearing loss, visual impairment, behavioral concerns and psychomotor retardation. She is dysmorphic with coarse facial features. Karyotype, microarray and a brain MRI at age 10 were normal. A pathogenic variant in *EHMT1* and homozygous pathogenic variants in *NAGA* were identified using whole exome sequencing.

Kleefstra Syndrome is characterized by intellectual disability, seizures, hypotonia and craniofacial anomalies. Both deletions in the long arm of chromosome 9 and loss of function mutations in euchromatic histone methyltransferase-1 (*EHMT1*) result in the haploinsufficiency of histone methyltransferase, which is specific for lysine 9 at histone H3 and is involved in transcriptional repression of genes. The exact mechanism for how this disruption leads to the phenotypic characteristics of Kleefstra Syndrome is not well understood. Recently, Benevento suggested that repression in neuronal dimethylated H3 at lysine 9 (H3K9me2) results in an imbalance in homeostatic plasticity and disrupts the fine balance between excitation and inhibition in the developing and mature neuronal system (Benevento et al., 2016).

Deficiencies in lysosomal α-N-acetylgalactose-aminidase have been reported in less than 20 people to date. Resulting in a broad clinical spectrum that includes early onset neuroaxonal dystrophy (Schindler disease) and late onset angiokeratoma corporis diffusum (Kanzaki disease) the clinical heterogeneity of the spectrum and the actual cause of disease in these patients is still heavily debated. The homozygous E325K was initially reported by van Diggelen et al. in 1987, when they presented two brothers with early-onset severe progressive neurological disorder. Three additional patients were later reported with varying degrees of symptoms, including a 7- year-old male who was asymptomatic at the time of publication (Bakker et al., 2001). Kanzaki disease is associated with late onset angiokeratomas, psychomotor retardation, sensori-motor polyneuropathy, intellectual disability and sensori-neural hearing loss.

Our patient's phenotype overlaps Kleefstra and Kanzaki syndromes. The relative stability of her features at 18 years is surprising with homozygous E325K mutation. She does not have any of the classically described skin findings associated with later onset NAGA mutations; however, her slow jerky and purposeful movements are classic for this syndrome. Genetic mastery of yet another theory – Chaos theory is now applicable. The study of the apparent randomness of chaotic complex systems allows understanding of patterns and how the final point is infinitely dependent on the initial condition. Theorist Edward Lorenz described chaos as this: when the present determines the future, but the approximate present does not approximately determine the future. The field of genetics must now pursue the understanding of these present complexities and how they contribute to the longitudinal history of these diseases.

Workshop Session 4
SYNDROMES & ISOLATED BIRTH DEFECTS INVOLVING MALFORMATIONS OF THE DEVELOPING FOREGUT I

NEW INSIGHTS INTO LUNG EPITHELIAL ONTOGENY AND ITS IMPACT ON POSTNATAL DEVELOPMENT AND REGENERATION

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The development of the lung has been extensively studied over the course of several decades and has resulted in a deep understanding of the processes that define the basic structure of the respiratory system. Many of these studies have focused on the process of branching morphogenesis, which is responsible for generating the basic arborized airway structure in the lungs. Less is understood about the cellular and molecular processes that drive later stages of lung development including alveologenesis, which is critical for refining and tuning the epithelial-vascular interface essential for gas exchange through generation of the alveolus. Formation of the alveolus entails appropriate specification of lung epithelial progenitor cells into a type 1 (AT1) or type 2 (AT2) alveolar epithelial cells and maturation of a juxtaposed capillary plexus and mesenchymal niche. Our lab has recently focused on the generation and regeneration of the lung alveolus. These studies have revealed novel origins of lung alveolar epithelial cell fates and identified important molecular pathways that are critical for alveologenesis. Importantly, some of these cellular and molecular processes are also essential or proper alveolar regeneration after acute lung injury. These ongoing studies provide critical insight into reinitiating lung growth in developmental lung disease and regenerating lung epithelium following injury.

ACINAR DYSPLASIA, *TBX4* AND LUNG BRANCHING MORPHOGENESIS

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Congenital acinar dysplasia is a rare form of primary interstitial lung disease characterized by impaired development of the lower respiratory tract leading to deficiency of the respiratory bronchioles, alveolar ducts, and alveoli. The condition typically results in neonatal lethality due to respiratory insufficiency. While likely under-recognized, there are approximately10 confirmed cases in the medical literature including

reports of affected siblings. A recent report noted acinar dysplasia in a single patient due to a *de novo* missense mutation in *TBX4* (Szafranski et al., 2016; c.256G>C, p.E86Q). Nearly concurrently, another report noted an infant with acinar dysplasia and ectrodactyly due to homozygous missense mutation in *FGFR2* (Barnett et al., 2016; c.764G>A, p.R255Q), suggesting locus heterogeneity. Here we report the second case of congenital acinar dysplasia due to a *de novo* mutation in *TBX4*.

The proband was the product of a natural conception to a 26 yo G1P0->1 mother. Pregnancy was uncomplicated. She was delivered at 41 1/7 weeks gestation via c-section due to failure to progress. Labor and delivery were complicated by presumed meconium aspiration requiring intubation, chest compressions, epinephrine, nitric oxide, chest tubes for pneumothoraces, and active cooling. Despite these efforts, she remained hypoxemic and was placed on ECMO. Her course was complicated by continued respiratory failure, coagulopathy, seizures, and hypertension. Brain imaging also noted evolving hypoxic ischemic injury. A lung biopsy revealed absence of normal alveolar development, consistent with congenital acinar dysplasia. Her physical exam revealed an otherwise non-dysmorphic infant with appropriate growth parameters. Given the poor prognosis, care was withdrawn. Autopsy revealed widespread bilateral pulmonary dysplasia and confirmed the lung biopsy finding of congenital acinar dysplasia. Exome sequencing revealed a *de novo* missense variant in *TBX4* (c.688T>C, p.Y230H) in the T-box DNA binding domain.

TBX4 is a member of the T-box transcription factor family and is expressed in multiple tissues during embryonic development including the lung mesenchyme. The fetal lungs, which form from the primitive foregut, require *TBX4* and *FGFR2* for initial lung bud formation and branching of the respiratory tract (Cebra-Thomas et al., 2003; Sakiyama et al., 2003). In further support of the role of *TBX4* in lung development, Arora et al. (2012) showed that homozygous knockout mice had hypoplastic lungs with decreased branching. Heterozygous mutations in *TBX4* have previously been reported in patients with Ischiocoxopodopatellar syndrome as well as childhood-onset pulmonary arterial hypertension (Kerstjens-Frederikse et al., 2013). These two patients with *de novo* missense mutations in the T-box DNA-binding domain suggest a dominant negative effect in these individuals with a more severe clinical presentation. These findings reinforce the role of *TBX4* in the developing fetal lung and as an etiology of congenital acinar dysplasia.

SEGMENTAL TRACHEAL ATRESIA: IT'S NOT ALL CHAOS

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Segmental tracheal atresia is a rare and usually fatal malformation. Controversy exists concerning development of the trachea and its separation from the esophagus. Recent investigations question the existence of a tracheoesophageal septum, but show budding of bilateral lung primordia into a region of splanchnic mesenchyme; elongation of this pulmonary- bronchial offshoot later forms the trachea. Complete tracheal agenesis, where the bronchi arise directly from the esophagus, supports this outgrowth model, but makes segmental tracheal atresia more difficult to explain. Recent delivery of an affected infant led to review of the literature as well as our local experience with this anomaly.

Our recent case was a stillborn male born at 22w. Ultrasonography at 20w had revealed massive lung hyperplasia, hydrops and ascites typical of Congenital High Airway Obstruction Sequence (CHAOS). Autopsy identified segmental tracheal atresia with blind ending segments, ankyloglossia, and single right palmar crease. The second case was a female born at 31w after a pregnancy complicated by polyhydramnios and hydrops. The infant could not be resuscitated. Autopsy revealed hyperplastic lungs, a 1cm long segment of tracheal atresia and a small sacral dimple. The third local case was a female with omphalocele born at 36w. She could not be resuscitated. Polyhydramnios was noted prenatally. Autopsy revealed multiple defects including VSD, SUA and malrotation. The trachea was occluded by a 0.8cm block of cartilage. The slightly enlarged lungs showed bilateral lobation defects and the right was much smaller than the left.

In these cases, as in most with CHAOS, there was no tracheoesophageal connection to drain pulmonary secretions *in utero*. However, segmental tracheal atresia more often occurs with such a fistula. Our last local case was a male born at 33w with duplicated R thumb who could not be resuscitated. Ultrasonography had shown polyhydramnios. At autopsy, the proximal esophagus was found to be a 2.5cm blind-ending tube. The proximal trachea was occluded, but the distal trachea was patent with a fistula to the distal esophagus. The lungs were hypoplastic. Similar literature cases also had small or normal size lungs, presumably due to drainage of lung secretions into the lower gut.

Our review highlights several issues regarding tracheoesophageal development. Since segmental tracheal atresia presumably occurs during or after separation of trachea and esophagus, it may be less influenced by the complex molecular mechanisms that set up the ventral-dorsal patterning of the undivided foregut. The high frequency of tracheoesophageal fistulas and other anomalies, frequently those seen in VACTERL, indicate a more widespread dysmorphogenetic process in many cases. Other pathogenetic mechanisms could include faulty maintenance of the tracheal lumen, aberrant cartilage formation, or atresia secondary to vascular compromise. Segmental atresia with blind ending upper and lower portions is still hard to explain, but could involve a breakdown in epithelial continuity during tracheal elongation or other epithelial-mesenchymal interactions involved in foregut development.

LOSS-OF-FUNCTION VARIANTS IN *MED12* ARE A CAUSE OF HARDIKAR SYNDROME

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Hardikar syndrome [MIM 612726] is a rare multiple congenital anomaly syndrome characterized by biliary foregut malformations, genitourinary malformations, distinctive pigmentary retinopathy, cleft lip and palate,

and grossly normal development. Here, we present all of the known affected individuals in the world, as well as the first molecular diagnoses.

It was originally described in 1992, and four affected individuals (Individuals 1–4) have been published to date. Individual 1 was a female with biliary malformation, cleft lip and palate, retinal pigmentation, aortic coarctation, low ureteric obstruction, and intestinal malrotation. Individual 2 was a female with biliary malformation, cleft palate, retinal pigmentation, urinary tract dilation, and intestinal malrotation. Individual 3 was a female with cleft lip and palate, preauricular pits, "cat's paw" retinal pigmentation, and ventricular septal defect. She died at 21 years due to massive intracerebral hemorrhage. Individual 4 was a female with biliary abnormalities, cleft lip and palate, intestinal malrotation, "cat's paw" retinal pigmentation, lacrimal duct stenosis, and umbilical hernia. She required liver transplant for severe cholestatic disease.

Individuals 5–9 are previously unpublished cases: Individual 5 is a female with biliary atresia, cleft lip and palate, and urogenital anomalies. She is now 7 years old, with normal intelligence and portal hypertension. Individual 6 is a 21-month-old female with biliary malformation, duodenal stenosis, cleft lip and palate, hydronephrosis, and abnormal retinal pigmentation. She is developing normally, but has poor intestinal motility and strabismus. Individual 7 has a choledochal cyst with absent gall bladder, cleft palate, aortic coarctation, cloacal anomaly, preauricular pits, scalp hemangioma, and retinal findings thought to be consistent with "cat's paw" pigmentation. She was hypotonic, but now at age 2 years has normal development. Individual 8 has biliary atresia, cleft lip and palate, and normal development. Individual 9 has developmental delay, cleft lip and palate, and biliary atresia requiring liver transplant.

Biliary anomalies are a rare form of foregut malformation, and may be seen infrequently in Lambert, Mitchell-Riley, Cat-eye, Martinez-Frias, COACH, Kabuki, Meckel, and Zimmermann- Laband syndromes. None of these patients had features consistent with these syndromes. Previous analyses have failed to reveal a molecular etiology for Hardikar syndrome. However, since all four reported patients had been female, it was suggested to be an X-linked dominant syndrome with male embryonic lethality. We have performed whole exome sequencing on four Hardikar patients: Individuals 3, 4, 5, and 7. Of these, we have identified nonsense mutations and frameshift indels (loss-of-function variants) in *MED12*, located on the X chromosome, in Individuals 3, 4, and 7. Missense mutations in *MED12* cause FG syndrome, Lujan syndrome, and Ohdo syndrome. Carrier females are frequently unaffected. These loss-of-function *MED12* mutations detected in individuals with Hardikar syndrome lend credence to the hypothesized male-embryonic-lethal mechanism of inheritance. Individual 9 underwent chromosomal microarray, which revealed a 1.25Mb deletion within chromosome 7q31.1 q31.2, which may suggest genetic heterogeneity; no further genetic testing was pursued. We hope to perform whole exome sequencing on Individuals 1, 2, 6, 8, and 9 in the future, to further investigate *MED12*'s role in Hardikar syndrome.

Workshop Session 5
SYNDROMES & ISOLATED BIRTH DEFECTS INVOLVING MALFORMATIONS OF THE DEVELOPING FOREGUT II

INTESTINAL MALROTATION: A REVIEW AND REPORT OF A FAMILY WITH CRANIOSYNOSTOSIS AND MALROTATION

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Intestinal malrotation has a prevalence of 0.3%-1% making it one of the most common malformations, yet it gets little attention when compared with less common but more dramatic intestinal anomalies. Approximately half the cases are isolated (with or without volvulus), but the remaining cases include other intestinal defects, abdominal wall defects, and/or multiple congenital anomalies (MAC). An increasing number of MCA syndromes are being recognized to be associated with intestinal malrotation, many of which have identified gene causation, which may help localize the gene or genes controlling for intestinal malrotation at 10–12 fetal weeks.

We review a series of cases plus an MCA family with recurrent malrotation. Additionally, a tabulation of literature MCA syndromes associated with malrotation will be presented.

A classification study done by the first author (BDH) identified 54 cases of malrotation. Malrotation alone (with or without Ladd's bands) occurred in 37%, malrotation with volvulus was noted in 20.4%, malrotation with additional defects of the intestinal tract was present in 20.4%, and the remainder (22.2%) consisted of multiple non-intestinal defects or MCA syndromes. A family of the second author (CJC) with craniosynostosis and malrotation will also be presented. Statistics regarding MCA syndromes (i.e., Curry-Jones, Fryns, etc.) associated with malrotation will be given and possible genes of interest will be discussed.

THE ROLE OF THE KRUPPEL-LIKE TRANSCRIPTION FACTOR KLF5 IN FOREGUT DEVELOPMENT

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The foregut is the precursor of the esophagus, stomach, intestines, liver, gall bladder and pancreas. All of their anatomic formation is achieved in the fourth fetal week through cell proliferation, growth and morphogenesis. On a cellular basis, these mechanisms are regulated by homeobox genes, cell to cell interactions, epithelial-mesenchymal interactions and transcription factors. Interruption of any of these mechanisms can alter the normal development of the foregut which may result in various GI tract malformations. We present patients with foregut malformations and 13q21q22 microdeletion including the KLF5 gene, discuss the role of the Kruppel-like transcription factor genes (KLFs) in foregut development, and propose a mechanism for GI tract

malformations in these KLF5-deleted individuals. Case 1: M.P. was born at 27 weeks gestation and noted to have a "double bubble" sign on radiograph. He was diagnosed at surgery with duodenal atresia type 1 plus malrotation of the duodenum. Microarray revealed a 13q21.33q22.1 deletion of approximately 1.65Mb, containing 6 OMIM genes, including KLF5. Case 2: D.P., the mother of M.P., was born at 33 weeks gestation and noted to have esophageal atresia, distal tracheoesophageal fistula and annular pancreas. She has the same microdeletion involving KLF5 as her child. Case 3: E.D. was born at 35 weeks gestation and noted to have polyhydramnios and duodenal atresia on prenatal ultrasound. Postnatal findings included cervical ribs and right-sided aortic arch. Microarray revealed a 13q22.1 deletion of approximately 1.2Mb, containing 3 OMIM genes, including KLF5. At 2 years of age, she has normal growth and mild motor delay. Case 4: Brother of E.D. Fetal demise at 35 weeks gestation and noted to have polyhydramnios and duodenal atresia on prenatal ultrasound. DNA was not available on patient. Case 5: Patient reported in DECIPHER database (case# 289511) was noted to have duodenal atresia and renal abnormality. Microarray revealed a maternally inherited 13q21.33q22.11 deletion of approximately 1Mb, containing 6 OMIM genes, including KLF5. Phenotype of the mother was not provided. The Kruppel-like zinc finger transcription factors are key regulators of multiple mammalian biological processes including cell growth, differentiation, embryogenesis and tumorigenesis. KLF5 (formerly BTEB2/IKLF) is primarily expressed in the gastrointestinal tract, with concentration at the base of the crypt epithelium where active cell division occurs. We theorize that deletion of KLF5 in our patients disrupts the migration of epithelial cells and thus normal epithelial homeostasis in the gut, resulting in abnormalities of foregut development. In conclusion, the KLFs play an important role in foregut development. We present patients with 13q21q22 microdeletion, including the KLF5 gene, and propose a mechanism for their foregut malformations based upon disruption of gastrointestinal epithelial homeostasis.

THE ROLE OF *FOXF1* IN FOREGUT DEVELOPMENT

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FOXF1 (Forkhead Box F1) is regulated by the Sonic Hedgehog (SHH) pathway and is critical in epithelial-mesenchyme signaling. Patients with mutations or deletions in this gene suffer from the lethal lung developmental disorder, Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACDMPV). Patients with this condition frequently have cardiovascular, urogenital, and foregut anomalies. FOXF1 is expressed in splanchnic and septum transversum mesenchyme during organogenesis. As such, it is critical for the development of all derivatives of the foregut. There have been reported abnormalities in all derivatives of the foregut (esophagus, respiratory tract, stomach, duodenum, liver, gallbladder, pancreas and spleen) in patients with ACDMPV. We present a female patient born to a 24 yo G1P0 white female with hypertension and obesity, who was noted at birth to have a small omphalocele. She quickly went into respiratory distress requiring intubation with 100% FIO2 and nitrous oxide. Echocardiogram revealed pulmonary hypertension, and a hypoplastic left heart variant with ASD, VSD, PDAs, bilateral SVCs, and coarctation of the aorta. Pulmonary hypertension continued to worsen, and the patient was placed on ECMO. A lung biopsy was performed which showed evidence of alveolar capillary dysplasia with misalignment of pulmonary veins. Given worsening cardiopulmonary status and biopsy results, the family decided to withdraw ECMO and the patient died at 14 days of age. A chromosomal microarray was negative. Sequence analysis of the *FOXF1* gene found a pathogenic deletion of two nucleotides (c.236_237delAG, p.Ser80ProfsX293). This is a previously unreported *de novo* mutation. This is only the second known case of ACDMPV with omphalocele. We will review the clinical and histopathologic findings of ACDMPV. We will also review all of the reported foregut derivative abnormalities seen in patients with *FOXF1* mutations and deletions. Finally, we will review the interaction between mesenchymal, endodermal and endothelial cells in foregut development, and the role of FOXF1 in this development.

ESOPHAGEAL ATRESIA/TRACHEOESOPHAGEAL FISTULA: RADY CHILDREN'S HOSPITAL SAN DIEGO EXPERIENCE

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Esophageal atresia (EA) and tracheoesophageal fistula (TEF) are major congenital malformations estimated to affect 1 in 3500 live births. These defects usually occur together and are classified into 5 anatomic subtypes (Gross classification), with the most common being proximal EA with distal TEF (Gross Type C). EA/TEF can either present in isolation or be associated with other birth defects. The co-occurrence of congenital anomalies as part of the VATER association is present in 10 to 30% of EA/TEF patients. In 6–10% of all EA/TEF cases, a defined genetic syndrome can be diagnosed, but in the remainder, the etiology is unknown. Rady Children's Hospital San Diego (RCHSD) is the largest surgical pediatric referral center in San Diego county. We performed a retrospective chart review of all the EA/TEF patients seen at this center from 2012–2016. The great majority of patients were managed at RCHSD neonatal critical care unit, but patients seen at the outpatient clinics or emergency department were also included if adequate records were available. A query to the electronic record system resulted in 67 cases. Eight were excluded because there was no EA or TEF, and nine were excluded because of incomplete records. This left us with 50 patients to review. As expected, the most common subtype was EA Gross Type C (78%), followed by EA without Fistula (Gross Type A, 10%) and type H TEF without EA (Gross Type E, 6%). About half (54%) of all the EA/TEF cases were evaluated by a geneticist. In 78% of the cases, there were other congenital anomalies present, most commonly cardiac (38%), vertebral (24%) and renal (22%). VATER association was diagnosed in 26% of cases. There were no cases clearly associated with teratogenic exposure. Gestational diabetes was present in two mothers (4%) but both cases were detected in the third trimester and were well controlled with diet. There was one case of maternal

hyperthyroidism with third trimester methimazole exposure. Eleven of the 50 cases (22%) had an identified syndrome (See Table), most confirmed with laboratory testing. This number is higher than reported in the literature, which may reflect the increased likelihood of referral of complex cases.

Diagnosis	Number of cases
Trisomy 21	3
CHARGE syndrome	2
Goldenhar syndrome	2
Trisomy 18	1
Cri-du-chat syndrome	1
Suspected 22q11.2 deletion syndrome	1
Feingold syndrome	1
Total	11

The pathologic mechanisms leading to EA/TEF remain unknown. The trachea, esophagus and lungs are foregut derived structures. A variety of genetic and environmental factors likely play a role in the development of EA/TEF. Large cohorts of patients will be required to unravel this heterogeneous etiology. We have conducted a preliminary retrospective study and plan to expand the time period to include the last 20 years with the aim of comprehensively describing the human syndromes and associations involving EA/TEF.

GENETIC DIAGNOSES AND ASSOCIATED MALFORMATIONS IN FETUSES PRENATALLY DIAGNOSED WITH ESOPHAGEAL ATRESIA

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Esophageal atresia (EA) is a major congenital malformation affecting 1 in 3500 livebirths. In about half the cases, other malformations exist as part of either a genetic syndrome or an association such as VATER/VACTERL (**V**ertebral defects, **A**nal atresia, **C**ardiac malformations, **T**racheoesophageal fistula with **E**sophageal atresia, **R**enal dysplasia, and **L**imb anomalies).

Advances in prenatal care have allowed for EA to be diagnosed prenatally, but the data are scarce on the genetic diagnoses, associated malformations, and postnatal outcome in these prenatally diagnosed cases. To address this knowledge gap, we reviewed the charts of all mothers who were referred to the Advanced Fetal Care Center at Boston Children's Hospital from January 2002 to March 2017 with a diagnosis of possible EA in the fetus.

We identified 49 cases of intrauterine EA. Prenatal genetic consult was performed in 8/49 (16%) of the cases and prenatal genetic testing was done in 27/49 (55%), including karyotype in 24/27 (89%), FISH for specific chromosomes in 5/27 (19%), and chromosomal microarray in 6/27 (22%).

Associated anomalies identified after birth included: airway/pulmonary (77%), cardiac (75%), spine (58%), genitourinary (42%), other gastrointestinal (38%), neurological (34%), limb (29%), and ophthalmic (27%) malformations. Three (6%) had isolated EA with or without tracheoesophageal fistula. Twenty-one (45%) of these cases were born prematurely before 37 weeks' gestation. More than half of (21/37 (57%)) children did not have any documented developmental delay.

Three cases were born in an outside hospital and were excluded from further analysis due to no access to their postnatal information. Twenty-one out of 46 (45%) of the patients were evaluated by a clinical geneticist after birth, and genetic testing was done in 23/46 (50%) of patients postnatally, including some who have had prenatal testing. Postnatal genetic tests included karyotype in 9/46 (19%), chromosomal microarray in 13/46 (28%), and a variety of single genes in 6/46 (13%). Out of those patients seen by a geneticist prenatally, 59% were seen in follow up by a geneticist postnatally.

Twenty-six of 46 patients (57%) were given a genetic diagnosis. VACTERL association was the most common clinical diagnosis (21/26 (80%)), followed by Down syndrome in 3/26 (11%), Feingold syndrome in one patient and CHARGE syndrome in one patient. For 20/46 of patients (43%), no genetic diagnosis was made. While many of the diagnoses were made by clinical geneticists (9/21 (42%)), nine VACTERL patients and two Down syndrome patients were diagnosed by surgeons and neonatologists. Patients were followed up for an average of 6.7 years (median = 6.2, range = 0 – 14.9). Based on our current data, 75% of the patients were still alive, 23% were deceased, and 2% were lost to follow-up.

Our data suggest that many patients with EA have a unifying genetic diagnosis and would benefit from a clinical genetics consultation, which the majority of patients have not had. These data will help improve our counseling of women who are carrying a fetus with EA and provide the first insights into the natural history of EA.

BIALLELIC MUTATIONS IN WAARDENBURG SYNDROME GENES CAUSE RECOGNIZABLE ARTHROGRYPOSIS SYNDROMES

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Random mating in the general population tends to limit the occurrence of homozygous and compound heterozygous forms of dominant hereditary disorders. Certain phenotypes, the most recognized being dwarfism/short stature, lead to cultural interaction and assortative mating. To this well-known example, we add deafness which brings together individuals with a variety of deafness genotypes, some being dominant. Waardenburg syndrome is one such autosomal dominant disorder in which affected individuals may interact culturally because of deafness.

Three pregnancies to a young African American couple, both of whom have Waardenburg syndrome, resulted in the birth of one boy with Waardenburg syndrome and two fetuses who died in the early third trimester with multiple congenital anomalies.

The first pregnancy resulted in a male infant with iris heterochromia and the presumed diagnosis of Waardenburg syndrome. The second pregnancy resulted in a stillborn female infant at 31 weeks gestation. The infant had white hair, telecanthus, low set small ears, micrognathia, contractures of the upper and lower limbs, rocker bottom feet, muscle atrophy, and absence of palmar and plantar creases. Internal examination showed pulmonary hypoplasia and a VSD with overriding aorta.

The third pregnancy resulted in a stillbirth at 32 weeks gestation. The fetus had increased cranial-length ratio, striking white hair, small upslanted palpebral fissures, small ears, small nose, cleft palate, unused scrotum, micropenis, pterygium at all large joints, long and overlapping fingers with diminished flexion creases, overhanging heels, varus left foot, and flat right foot.

Genomic microarray and gene sequencing on DNA from the second fetus documented a 397 kb deletion of 22q including *SOX10* and a nested 7 kb deletion of *SOX10* on the other allele. The mother carried the 397 kb deletion and the father carried the 7 kb deletion.

This family is the first in which biallelic mutations of *SOX10* cause a distinctive arthrogryposis syndrome but joins others in which documented or presumed homozygous or compound heterozygous mutations of *PAX3* cause a similar arthrogryposis phenotype.

Historical Note: A (posthumous) feud of sorts has arisen in the Waardenburg world. Four years after Petrus Waardenburg's death (1886–1979), David Klein (1908–1983) published in AJMG an article in which he claims to have been slighted by Waardenburg. Klein presented a 10-year-old girl, now recognized a WSIII, in August 1947 in Geneva. Waardenburg presented his first case in December 1947 in Utrecht. Klein subsequently presented his case to Waardenburg and was disappointed to read in Waardenburg's first paper (1951) that it was Waardenburg's research alone that led to discovery of a "hitherto unknown human disorder". WSIII, also called Klein-Waardenburg is now known to be caused by compound heterozygosity of *PAX3* (Bottani et al., 1999), one of the 2 possibilities posed by Waardenburg in 1951 but about which he was silent in 1970.

Workshop Session 6
TERATOGENS AND MALFORMATIONS

MECHANISMS OF GENE-ENVIRONMENT INTERACTION IN HOLOPROSENCEPHALY

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Holoprosencephaly (HPE) is a common developmental defect in which bilateral symmetry of the forebrain and/or midface fails to form. HPE is associated with heterozygous mutations in the Nodal and Sonic hedgehog (SHH) pathways, but clinical presentation is highly variable, and many mutation carriers are unaffected. This scenario appears to be explained by a "mutation- plus-modifier" model. Complex gene-environment interactions are thought to underlie many developmental defects, and potential teratogens may be HPE modifiers. CDON is SHH coreceptor. *CDON* mutations are found in HPE patients. *Cdon* mutant mice develop HPE in a strain-dependent manner. 129S6 *Cdon*$^{-/-}$ mice have a sub-threshold defect of *Shh* signaling and are sensitized to HPE-modifying factors. Previously we found that, while individual loss of *Cdon* or in utero exposure to the teratogen ethanol did not cause HPE in 129S6 mice, the two together produced defects in early midline patterning, inhibition of *Shh* signaling in the developing forebrain, and a broad spectrum of HPE phenotypes later in development. We have pursued how mutation of *Cdon* and in utero ethanol synergize to interfere with developmental signaling pathways and how ethanol acts as a teratogen. We report here that the window of sensitivity to ethanol-induced HPE in *Cdon* mutants is very narrow and over by E7.5, prior to initial expression of *Shh*. The Nodal signaling pathway lies upstream of the Shh pathway developmentally and is active during the window of sensitivity to ethanol. Expression of the Nodal pathway target genes *FoxA2*, *Gsc*, and *Lefty2* were decreased specifically in ethanol-treated *Cdon*$^{-/-}$ embryos. Additionally, CDON binds to Cripto, an essential Nodal coreceptor, and to Chordin, a soluble BMP inhibitor required for optimal Nodal signaling strength during gastrulation. We hypothesize that transient disruption of Nodal signaling during gastrulation by loss of *Cdon* and ethanol exposure results in subsequent defective *Shh* signaling and HPE. Much of ethanol's toxicity is ascribed to its oxidative metabolism by alcohol and aldehyde dehydrogenase. We find that treatment of *Cdon* mutant mice with *t*-butyl alcohol, which is not subject to such oxidative metabolism, induces HPE and defects in Shh signaling in *Cdon*$^{-/-}$ embryos. We propose a model wherein ethanol itself, not a consequence of its metabolism, is an HPE-inducing teratogen and that it acts to modify Nodal signaling in *Cdon*$^{-/-}$ embryos.

UPDATE ON A TIMELINE OF CRITICAL DEVELOPMENTAL STAGES FOR THE TERATOGENIC CAUSATION OF BIRTH DEFECTS

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Several years ago at this meeting we presented preliminary results of a project to collate data regarding critical periods during gestation when interference with normal development causes specific malformations. The purpose of this presentation is to update progress on this project to identify and catalogue windows of teratogenic exposure for common birth defects using published data from human observations as well as animal studies (mouse, rat, golden hamster, and chick), translating non-human developmental stages to the corresponding human

developmental days. Such an integrated timeline may be useful for epidemiologic studies involving multiple birth defects, in understanding gene-environment interactions, and for teaching normal embryologic development.

Human epidemiologic studies of birth defects may include multiple phenotypes and exposures to maximize resources. Statistical analyses of small groups can lead to spurious, misleading conclusions with low power and inflated false positive rates. Grouping defects can increase statistical power, but may cause a loss of biologic significance unless the groupings are based on common causal and/or pathogenetic factors. Our strategy is to consider clustering birth defects caused at the same stage in development. We propose that such a grouping can be combined with advanced statistical methods to find a compromise between lumping and splitting in studies designed to investigate numerous birth defects and potential risk factors.

In our previous, preliminary report at this meeting, we noted that a group of malformations appear to be caused early during gastrulation and neurogenesis, while there seemed to be another cluster during subsequent formation of the heart, sensory placodes, and pharyngeal arches. In addition, we found that some malformations appear to result from teratogenic exposures at more than one specific developmental stage, suggesting multiple mechanisms of causation. This presentation will provide an update of progress on this timeline, with the annotation of more than 30 clinically important structural anomalies.

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DISCOVERY AND CHALLENGES IN THE APPLICATION OF WHOLE GENOME SEQUENCING IN A RESOURCE LIMITED DYSMORPHOLOGY CLINIC IN TIJUANA, MEXICO

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Despite rapid advancements in the diagnosis and the discovery of genetic disorders provided by whole exome or genome sequencing (WES/WGS), access to genetic testing remains limited for many underserved communities. The Clinica de Dismorfologia at the Hospital Infantil de las Californias is an established dysmorphology clinic staffed by American and Mexican volunteer geneticists, a genetic counselor, and fellows that serves Baja California, Mexico. Limited genetic testing is available to families who can afford such studies. Most patients who are suspected of having a genetic disorder that cannot be diagnosed by clinical exam alone will not undergo any cytogenetic or molecular genetic studies. This population represents a unique and underserved pediatric population in which clinical WGS testing can show a diagnostic advantage when resources are limited. WGS was performed in 23 children (15 males) with suspected genetic disorders. The mean age of the probands is 4.8 + 3.9 years. The primary phenotype was classified into one of three categories: patterns of malformation (69.6%, n = 16), non-dysmorphic children with a neurologic phenotype (21.7%, n = 5), and myopathies (8.7%, n = 2). The cohort is comprised of 14 trios, 6 duos, and 3 quad WGS. Participating families attended "Genome Days" where they underwent pre-test counseling, informed consent, blood draw, review of family and medical history, and examination. Clinical WGS was performed by Illumina Clinical Services Laboratory through a philanthropic partnership with the Foundation for the Children, a US-based 501(c)3 that supports Hospital Infantil de Las Californias. Secondary findings and a pharmacogenomics screen were reported for all participating individuals. WGS yielded a diagnostic result in 60.9% including 3 copy number variants (CNVs), 2 structural anomalies (1 uniparental disomy and 1 unbalanced translocation), and 9 single gene disorders. Children with patterns of malformation had the highest diagnostic yield (68.8%, n = 11). Suspected genetic diagnoses were confirmed in 2 children (Angelman syndrome, maternally inherited nemaline myopathy). A novel congenital disorder of glycosylation was identified in two siblings with a homozygous indel in PIGS, a GPI-biosynthesis gene. One child had a variant in a candidate gene (USP7) and he is participating in additional functional studies. One child had a secondary finding of a multi-exon deletion in PMS2 concerning for Lynch syndrome. These data highlight the diagnostic potential of clinical WGS, with included reporting of CNVs and structural variants, as a first-tier testing option for patients with suspected genetic disorders, particularly in resource limited communities. Securing a diagnosis provides crucial information to the family for prognosis and recurrence risk and empowers families to allocate resources for treatment rather than diagnostic studies.

UNILATERAL ABDOMINAL WALL HYPOPLASIA - A FEATURE OF DIABETIC EMBRYOPATHY?

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Approximately 1–2% of pregnancies are complicated by pre-gestational diabetes, either type 1 or type 2. The risk of congenital anomalies in infants born to diabetic mothers is 2–8 times higher than the background risk. These anomalies most frequently involve the craniofacial region, CNS, heart, GI tract, urogenital tract, and skeleton. This report describes an infant born to a diabetic mother with unilateral abdominal wall hypoplasia, preaxial hallucal polydactyly, and other anomalies and reviews the evidence that abdominal wall hypoplasia may be due to diabetic embryopathy.

A female infant was born to an insulin-dependent diabetic mother at 37 weeks gestation; HgbA1C levels were significantly elevated in early pregnancy. At birth she had unilateral preaxial hallucal polydactyly, absent left kidney, multiple thoracic, lumbar, and sacral segmentation anomalies, sacral dysgenesis, hip dysplasia, and PDA requiring ligation. Each of these findings occurs more commonly in infants born to diabetic mothers. She also had the very uncommon finding of left abdominal wall hypoplasia. Microarray analysis and head ultrasound were normal. At 6 months of age she had mild short stature, normal facies, unilateral abdominal wall hypoplasia, unilateral preaxial hallucal

polydactyly, normal hip ultrasound and echocardiogram, and normal development.

An abstract by Shapiro et al. (1999) described 3 infants of mothers with insulin-dependent diabetes who had 3 or more VATER anomalies who also had left lateral abdominal wall hernias and bilateral preaxial polydactyly of the feet. Donnelly and Johnson (1995) reported an infant girl born to a mother with gestational diabetes who had left abdominal wall hypoplasia, bifid first toes, multiple vertebral and rib anomalies, absent left kidney, VSD, and bilateral hip dislocation. Smolkin et al. (2008) reported a male infant born to a mother with uncontrolled gestational diabetes requiring insulin who had bilateral abdominal wall protrusion, multiple thoracic hemivertebrae, rib anomalies, absent left kidney, and hip dislocation.

Prune belly syndrome and unilateral abdominal wall hypoplasia have not been previously noted to occur more frequently in infants born to diabetic mothers, although malformations of the urogenital tract are increased. However preaxial hallucal polydactyly has been reported in several infants of diabetic mothers. Each of the anomalies in the patient described in this report has been noted to occur more commonly in infants of diabetic mothers except the abdominal wall hypoplasia. It is difficult to determine whether this pattern of anomalies is due to a unique syndrome or whether the anomalies represent diabetic embryopathy. This report and the others in the medical literature suggest that unilateral hypoplasia of the abdominal wall musculature may be due to the teratogenic influence of maternal diabetes mellitus although the mechanism is unknown. However, since this is a very uncommon finding additional clinical reports and epidemiologic studies will be necessary to determine if this hypothesis is valid.

CONGENITAL ZIKA VIRUS INFECTION WITH ARTHROGRYPOSIS AND PARALYSIS OF THE DIAPHRAGM

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Since the first reports of newborns with microcephaly in Brazil in September 2015, much has been learned about the causative agent, Zika virus (ZIKV), and the variability of the clinical phenotype in infants who are affected *in utero*. Joint contractures have been documented in infants with confirmed or suspected ZIKV infection with almost 15% of infants affected in one case series by Sarno, Aquino and colleagues in 2016. Among seven infants with arthrogryposis in a retrospective case series reported by van der Linden, Filho and colleagues in 2016, thinning of the cord and reduction in the ventral roots was documented on MRI of the spine in four. Among these seven infants, electromyography (EMG) findings suggested long-term involvement of peripheral and central motor neurons.

Diaphragmatic paralysis and laboratory-confirmed congenital ZIKV infection has been briefly described in one infant each in reports by Melo and colleagues and Souza and colleagues in 2016 and in three infants by Meneses and colleagues in 2017. The infant reported by Souza had upper and lower limb contractures with unilateral diaphragmatic paralysis. All three infants reported by Meneses had arthrogryposis with unilateral diaphragmatic hernia. No information on the mechanism of the diaphragmatic paralysis was provided in any of these reports.

We report three infants from northeastern Brazil with congenital Zika syndrome, arthrogryposis, and unilateral diaphragmatic paralysis; two had Zika-specific IgM detected in CSF, and IgM results were negative at 7 months in the third. Pneumonia was a presenting feature in two infants. Contractures involved the upper and lower limbs in two infants and the lower limbs in one. Unilateral diaphragmatic paralysis was documented by ultrasound in all infants.

EMG findings in all three suggested moderate chronic involvement of peripheral motor neurons; compound muscle action potential of the phrenic nerve was reduced unilaterally in two infants and bilaterally in one. The spinal cord in one infant was thinner than normal in the thoracic area, predominantly in the ventral aspect reducing the ventral roots.

All mammals have movement of the diaphragm *in utero* preparing the newborn to breathe effectively upon delivery. In experimental animals, control of this process has been shown to be the result of medullary centers that generate the rhythmic bursts and interaction between the phrenic motor neuron and the muscles of the diaphragm. The phrenic nerve arises from the neck at C3-C5 and receives innervation from both the cervical and brachial plexus. One hypothesis suggests that the muscle of the diaphragm may arise from forelimb muscle cells.

We postulate that one clinical phenotype associated with congenital ZIKV infection includes arthrogryposis and paralysis of the diaphragm. Interestingly, diaphragmatic dysfunction has been reported with congenital CMV infection; however, it is unclear whether there was eventration or paralysis of the diaphragm in these infants. In infants with this phenotype, significant morbidity and mortality can be expected; infants with bilateral diaphragmatic paralysis might not survive after birth and could remain undetected.

GENETIC SENSITIVITY TO DEPAKOATE-INDUCED BIRTH DEFECTS: EFFORTS TO IDENTIFY SUSCEPTIBILITY GENES

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Since the original publication of an association between *in utero* Depakote (Valproic Acid, VPA) exposure and an increased risk for neural tube defects (NTDs) was described by Elisabeth Robert in 1982, efforts have been underway to identify susceptibility genes that place selected embryos at increased risk for congenital malformations. In 1987, Edward Lammer published a Teratogen Update to better delineate the spectrum of malformations associate with this significant human teratogen. He continued publishing on VPA teratogenicity and at the time of his passing, we were collaborating on a final effort to demonstrate how low dose VPA exposure can disrupt normal morphogenesis in highly susceptible embryos.

In parallel to Ed's efforts to understand how VPA induced NTDs and congenital heart defects in humans, our laboratory performed genetic studies seeking to identify the gene- environment interactions in our mouse model of VPA teratogenicity. It is critically important to understand the underlying teratogenic mechanisms by which VPA increases the NTDs risk in order to prevent VPA exposure to high-risk mother-child pairs.Identifying which genes were responsible for sensitivity to VPA induced NTDs would have immediate translational impact on patient care.

In mice, VPA increases the risk of exencephaly, an anterior NTD, and the degree of susceptibility depends on the genetic background of the mouse strain exposed. Since the early 1980s, we have developed two mice strains who respond differently to maternal VPA treatment. One strain (C57BL/6J) is resistant to VPA, with only a modest portion (10%) of the exposed embryos present with NTDs. While the SWV/Fnn inbred mouse strain is highly sensitive to *in utero* VPA exposure, with no less than 80% of the embryos having NTDs after the dam exposed to VPA at E8. Using these two strains in a series of backcrossing linkage studies, we were able to map purported sensitivity genes to a 6Mb chromosomal region located on chromosome 7, which are responsible for VPA interactions in the SWV/Fnn embryos. To further this initial investigation, we performed whole genome sequencing (WGS) in VPA-exposed embryos from these two strains, and called genetic variants including both single nucleotide variants (SNVs) and copy number variants (CNVs) that differed between these two strains. Using SNVs identified from WGS, we fine mapped a SNP in the *SOX6* which is we believe to be associated with increasing the risk for VPA induced NTDs. We demonstrated that SOX6 is downregulated in embryos 4 hr post-VPA injection. Allelic expression imbalance demonstrated that expression of the two alleles (C/T) for the SNP in question was significantly different.

This represents the first significant candidate sensitivity gene for VPA teratogenicity after some 30 years of experimentation. As *in utero* VPA exposure not only increases the risk for NTDs, but it also increases risk for other structural malformations including congenital heart defects, limb defects, craniofacial defects and even for neurobehavioral disorders such as autism. Identifying genes and variants that are responsible for the VPA-induced birth defects represents a significant step towards preventing these preventable birth defects.

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Workshop Session 7
NEW SYNDROMES AND NEW INSIGHTS INTO OLD SYNDROMES

OF MICE AND MEN: FIRST EXAMPLE OF HOMOZYGOUS VARIANT IN EDNRA CAUSING LETHAL CRANIOFACIAL ANOMALIES SIMILAR TO THOSE IN KNOCKOUT MICE

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The importance of the Endothelin signaling system (including Endothelin-1 (EDN1) ligand and Endothelin receptor type A (EDNRA)) is well known for normal craniofacial development. During embryonic development, neural crest cells migrate from the dorsal neural tube to populate the pharyngeal arches, where they give rise to the cartilage, bone and connective tissue of the face. *Edn1*$^{-/-}$ and *Ednra*$^{-/-}$ mice are born with severe craniofacial defects resulting in neonatal lethality. In these mice, most lower jaw structures undergo a transformation into maxillary-like structures. Since EDN1/EDNRA signaling is restricted to the mandibular portion of arch one (in addition to more caudal arches), these findings indicate that EDN1-EDNRA signaling is crucial for specifying mandibular identity.

Mutations in *EDN1* have been reported in individuals with recessive auriculo-condylar syndrome (ACS) and in dominant isolated question mark ears. Recently *de novo* heterozygous variants in *EDNRA* have been identified in patients with mandibulofacial dysostosis with alopecia. Functional studies demonstrated that a p.x129T substitution in three of the individuals altered the affinity of *EDNRA* for Endothelin ligands, resulting in aberrant signaling in the maxillary first arch. While mouse and zebrafish loss-of-function studies illustrate that mutations in *EDNRA* mechanism can result in multiple defects in craniofacial and cardiovascular structures, isolating these mutations is difficult, due in part to the fact that most *EDNRA* mutations would not be well-tolerated and likely result in neonatal lethality.

Here we report the first human with a homozygous variant p. Glu381Pro in exon 7 of the *EDNRA* gene (for which each parent is heterozygous) which was identified by whole exome sequencing. Notably, a region of homozygosity of 7.51 Mb, which includes *EDNRA*, was found on prenatal microarray. Data from general population variant databases reveal that there are fewer missense alterations in the *EDNRA* gene than expected for a gene of its size. The variant results in the non-conservative substitution of an amino acid that is highly evolutionarily conserved.The male infant was born at 27 3/7 week gestation to a 31-year-old G3P0 conceived by IVF secondary to left salpingectomy. Prenatal imaging revealed micrognathia, microtia, a large VSD, and aortic arch anomaly. The infant could not be orally intubated and had an event with bradycardia and hypoxemia, which was unresponsive to resuscitation. On physical and autopsy exam, there was marked micrognathia and microstomia with an inability to fully open the mouth. Lower jaw structures had a maxillary-like appearance. There was aglossia and severe posterior oropharyngeal stenosis. The ears were replaced with nub-like tissue bilaterally. There was a VSD with posterior malalignment, a bicuspid aortic valve, and hypoplastic aortic arch.

The infant's abnormalities, compared to ACS patients with EDN1-EDNRA signaling defects, were much more severe, resembling more closely the phenotype observed in *Edn1* and *Ednra* knockout mice. Preliminary results of in vitro functional studies indicate that the mutation results in EDN1 acting as an inverse agonist. While the biophysical basis for this change is not yet clear, these findings indicate that the p. Glu381Pro likely leads to diminished EDNRA activity during early neural crest patterning and craniofacial dysmorphology.

A RECOGNIZABLE PHENOTYPE INCLUDING MACROCEPHALY, LIGAMENTOUS LAXITY AND DEVELOPMENTAL DELAY IS ASSOCIATED WITH GERMLINE DE NOVO *TAOK1* VARIANTS

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Trio based exome analysis identifies de novo variants in genes without known disease association. We report a male patient evaluated for developmental and physical abnormalities in whom a de novo nonsense variant in *TAOK1* was identified. Six additional individuals with de novo *TAOK1* variants shared similar findings, suggesting a gene disease association.

The patient was the product of a pregnancy complicated by polyhydramnios. Prenatally identified macrocephaly persisted. Developmental delay included walking at 15.5 months; hyperactivity; immaturity and a preference for interaction with younger peers. Generalized hypotonia and ligamentous laxity of small joints were present. Cognitive testing (WISC-IV at 10.5 y) showed a full scale IQ of 82. At 14 years, his height was 25–50th%; OFC +3SD. He had distinctive facial features with mildly downslanted palpebral fissures, prominent/thick alae nasi and prominent ears. Exome analysis showed a de novo *TAOK1* c.1819C>T; p.Q607X VUS.

Six additional unrelated patients (4M; 2F) ranging in age from 5 to 14 years, identified through clinical exome analysis, carried a de novo *TAOK1* variant (2 splice site; 2 missense; 1 nonsense; 1 frameshift). Developmental delay was present in all, with intellectual disability present or suspected in 5/7. Learning disabilities in the absence of intellectual disability were reported in 2 with missense variants. Behavior problems occurred in 5/7, with ADHD related issues in 3. Relative or absolute macrocephaly was present in 4/6.

TAOK1 (MIM 610266) encodes a ubiquitously expressed 1005 amino acid product, thousand and one amino acid kinase 1, also referred to as Ste20-related mitogen-activated protein kinase kinase kinase (MAP3Ks). *TAOK1* expression levels increase during the G2 phase/mitosis and decrease as cells progress through the metaphase-anaphase transition and exit mitosis. Reduced *TAOK1* expression results in the cell's inability to engage the DNA damage-induced G2/M checkpoint. TAO1 kinase maintains mitotic microtubule dynamics, establishes proper chromosome-microtubule attachment, and ensures the accurate segregation of the chromosomes in mitosis and interphase human cells.

The nature of the identified *TAOK1* variants suggests a loss of function mechanism, however, functional studies are necessary to elucidate the disease mechanism. Deletions encompassing *TAOK1* on 17q11.2 often affect *NF1*. Few individuals with small deletions excluding *NF1* have been reported in DECIPHER, these were not reported to have a distinctive phenotype. Identification of additional individuals with de novo germline variants in *TAOK1* will support the ongoing phenotypic delineation.

BIALLELIC MUTATIONS IN *PISD* IDENTIFIED IN SIBLINGS WITH CONGENITAL CATARACTS AND EXTREME SHORT STATURE ADDS A NOVEL DISORDER TO THE EMERGING FAMILY OF MITOCHONDRIAL CHAPERONOPATHIES

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Whole exome sequencing (WES) studies have contributed to the identification of hundreds of novel diseases causing rare diseases. As a result classes of genes causing related disorders have emerged, such as the recent discoveries that multiple different spliceosomal genes cause distinct yet overlapping craniofacial disorders. While this does not replace the need for other functional experiments, recognizing such classes of disorders allows for the identification of plausible novel candidates based even on observations from few families.

We report two adult sisters followed since birth. Both share a remarkably similar phenotype with congenital cataracts, severe (-6 *SD*) postnatal short stature, hypomyelination on MRI, distinctive facial features and mild neurological involvement. After negative results on conventional tests, WES on the siblings identified rare biallelic variants in *PISD*, which encodes the enzyme that converts phospatidylserine (PS) to phosphatidylethanolamine (PE) – an abundant phospholipid essential for mitochondrial structure and function- in the inner mitochondrial membrane. PISD has not previously been linked to human disease, and *PISD* knockout is embryonic lethal in mice. Interestingly gain of function mutations in an enzyme catalyzing a reverse reaction (*PTDSS1*) cause Lenz-Majewski hyperostic dwarfism.

Studies in fibroblasts from one sister confirmed a decrease in cellular PE levels. These cells also died in the presence of the glycolysis inhibitor 2-deoxyglucose, which forces cells to rely on mitochondrial function. Consistent with previous reports in cells depleted of *PISD*, patient fibroblasts exhibited a more fragmented mitochondrial network, a feature often associated with mitochondrial dysfunction. We also demonstrate that treatment with lyso-PE, which can replenish mitochondrial PE, restores mitochondrial morphology in patient fibroblasts. Taken together these results support that decreased function of PISD causes a novel mitochondrial disorder. The patients clinical features with short stature, cataracts and neurological impairment overlaps several other emerging disorders (collectively recognized as 'mitochondrial chaperonopathies') including CODAS (*LONP1*), Even-Plus syndrome (*HSPA9*), CAGSSS syndrome (*IARS2*) and X-linked SEMD-MR (*AIFM1*). We will review this family of conditions and discuss our theories regarding common pathogenic mechanisms.

SONIC HEDGEHOG SIGNALING TARGETS FOXF2 DURING UPPER LIP MORPHOGENESIS AND CLEFT LIP PATHOGENESIS

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Cleft lip is one of the most common human structural birth defects, yet our understanding of the mechanisms that regulate upper lip development and cleft pathogenesis is extremely limited. Using a clinically-relevant mouse model of cleft lip, we show that Sonic Hedgehog (Shh)- induced proliferation of cranial neural crest cell (cNCC) mesenchyme is required for upper lip closure. Gene expression profiling revealed a subset of Forkhead box (Fox) genes regulated by Shh signaling during upper lip morphogenesis. During cleft pathogenesis, reduced cell proliferation paralleled expression of *Foxf2* and canonical pathway target Gli1 in the medial nasal process mesenchyme. SHH ligand induction of *Foxf2* expression was dependent upon Shh pathway effectors in cNCCs, while a functional GLI binding site was identified downstream of *Foxf2*, demonstrating that it is a direct target of canonical Shh signaling. Consistent with the cellular mechanism demonstrated for cleft lip pathogenesis, we found that either SHH ligand addition or *FOXF2* overexpression is sufficient to induce proliferation of cNCCs. Finally, analysis of a multiethnic human population with cleft lip identified clusters of single-nucleotide polymorphisms in *FOXF2*. These data suggest that Shh targeting of *Foxf2* drives cNCC mesenchyme proliferation during upper lip morphogenesis and that disruption of this sequence results in cleft lip. This study also presents additional candidate human clefting genes and an integrated approach to define their role in upper lip development and birth defect etiology.

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Workshop Session 8 DYSMORPHOLOGY (SYNDROMES AND MALFORMATIONS) IN MINORITY AND UNIQUE POPULATIONS I

GENETIC SYNDROMES IN DIVERSE POPULATIONS

Maximilian Muenke, MD[1]; Paul Kruszka, MD[1]; Adebowale A. Adeyemo, M.D.[2], Marius George Linguraru, Ph.D.[3], and Clinicians around the World

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As a trainee in pediatrics and clinical genetics I (MM) was fortunate to have had as mentors Prof. Hans-Rudolf Wiedemann in Kiel, Germany and Dr. Elaine Zackai in Philadelphia. My textbooks included Wiedemann's *Atlas Klinischer Syndrome* (1976; now Kunze, 2010) and Smith's *Recognizable Patterns of Human Malformations* (1970; now Jones et al., 2013). As virtually all genetic syndromes have been first described in individuals of European descent, most medical genetics journals, textbooks and atlases depict photos of patients from this background. For example, Down syndrome was not even recognized in Africans until 1950. Compounding the paucity of patients from diverse populations in the medical literature are reports of difficulties with diagnosing even common genetic syndromes in Africans and Asians. More recently, textbooks have been published with patient photos from Asia (Kajii et al., 1998: *New Atlas of Congenital Malformation Syndromes* (in Japanese); Shotelersuk, 2004: *Clinical Genetics in Children*. (in Thai)), Latin America (Zighelboim et al., 2012: *Atlas de Dismorfología Pediátrica* (in Spanish)), and patients with specific syndromes from Africa (Christianson (1996); *JMG* 33: 89–92; De Decker R et al. (2016); *South Afr Med J 106*: S82–S86; Tekendo-Ngongang et al. (2014); *Mol Syndromol* 5: 287–292).

As part of an international collaboration (see *Atlas* website: www.genome.gov/atlas, Koretzky M et al. (2016); *Genet Med 18*: 1069–1074, 2016; Muenke M et al. (2016); *Genet Med 18*: 1085–1087) we have begun to compare individuals who have a variety of genetic syndromes including Down syndrome (Kruszka P et al. (2017); *AJMG A 173*: 42–53), deletion 22q11.2 syndrome (Kruszka P et al. (2017); *AJMG A 173*: 879–888), Noonan syndrome (Kruszka et al. (in press); *AJMG A 173*, Williams-Beuren syndrome, Turner syndrome, Fragile X syndrome, and Alagille syndrome. These individuals originate from Europe, Africa, Asia, and Latin America. Both subjective clinical findings and objective facial recognition technology has found differences between various populations groups in selected syndromes. As example, brachycephaly, ear anomalies, and clinodactyly were significant less frequent in individuals of African descent with Down syndrome compared to other ethnic groups. Using a facial analysis algorithm, we showed that when applied to individuals with Down syndrome from around the world, we found the sensitivity to be 96.1% and specificity to be 92.4%. Importantly, the accuracy of the technology increased significantly when applied to distinct population groups (African, Asian, and Caucasian). When using facial analysis technology in 22q11.2 deletion syndrome, we also found differences between population groups. Interestingly, all four groups (Caucasian, African, Asian, and Latin American) only shared two common geographic facial analysis features: telecanthus and narrow palpebral fissures.

In summary, genetic syndromes in diverse populations are underrepresented in the medical literature and underdiagnosed in the clinics. We have shown that both subjective clinical examination and objective facial analysis technology have found differences in diverse population groups with selected genetic syndrome. And most importantly, we know that facial analysis technology is both sensitive and specific. We believe in both developed countries and in parts of the world where molecular testing is not available, facial analysis technology will be a valuable tool that will assist in earlier diagnoses and earlier treatment of patients with genetic syndromes.

UNUSUAL PHYSICAL FINDINGS IN KNOWN GENETIC SYNDROMES AFFECTING THE AFRICAN AMERICAN AND HISPANIC POPULATIONS: REPORT FROM THE LARGEST GENETIC CENTER IN THE DMV AREA

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The Genetics and Metabolism clinic at Children's National Health System (CNHS) receives over 8,500 patient visits per year. The families it serves come from a wide range of ethnic and socioeconomic backgrounds from the greater DC-Maryland-Virginia (DMV) metro area in the United States. The African American and Hispanic populations represent the majority of the patients seen in our center.

We report more than 10 African American and Hispanic patients affected with different genetic syndromes with positive molecular testing that presented with unusual physical findings that have not been described in the medical literature. At least half of them were initially detected by DNA testing and after clinical reevaluation the genetic diagnosis was then confirmed. We have photographic evidence of all of the findings.

One of our patients was initially believed to have a primary overgrowth syndrome after presenting with macrocephaly and very tall stature along with morbid obesity and intellectual disability. He was diagnosed many years later with Kleefstra syndrome via whole exome sequencing. Another case belongs to a Hispanic toddler with normal development and no history of seizures presenting with very large and symmetric dental "cavities" in central incisors along with hypopigmented skin macules. She was found to have a known mutation in one of the analyzed Tuberous Sclerosis genes. An adult African American with high arched palate, aortic root dilation, polyvalvular heart disease and hearing loss, found to have an in-frame deletion in the *FLNA* gene on connective tissue gene panel; his mother declined carrier testing. A Hispanic boy with clinical diagnosis of Carpenter syndrome due to his mild but typical facial configuration was found to have Primrose syndrome many years later after whole exome sequencing analysis. These cases are just part of the multiple unusual presentations seen in our cohort.

We conclude that initial genetics evaluation, of patients considered being part of the "minority" populations, needs to be carefully performed due to their atypical presentations and numerous limitations to receive a comprehensive genetic evaluation. We believe that some of these unusual findings can be part of a different presentation of the clinical spectrum of the genetic syndromes and some may be due the ethnic related physical traits. It is also possible that there are other unknown confounding genetic factors or modifiers that need to be studied further.

EMBRYONIC LETHAL MENDELIAN PHENOTYPES: A LARGE COHORT FROM A CONSANGUINEOUS POPULATION

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Embryonic lethality is a well-studied phenomenon in many model organisms, and the proportion of genes that are indispensable to embryonic development is remarkable. Building on the successful approach of using Mendelian genetics of birth defects to understand normal morphogenesis of individual organs, we reckoned that Mendelian forms of embryonic lethality offer a window into the essential genetic components of early organismal development in humans. In this study, we report our genomic analysis of multiplex consanguineous pedigrees in which embryonic lethality appears to follow a Mendelian recessive pattern. A total of 38 families were recruited, typically with a second affected pregnancy, and these were analyzed by exome sequencing and autozygome analysis of the affected fetus. We also recruited consanguineous couples with history of two or more affected pregnancies even when no access to the affected fetus was possible, in which case we resorted to haplotype analysis and duo exome sequencing of the couples. The phenotypes ranged from nonspecific nonimmune hydrops fetalis, to highly specific syndromes some of which are novel. A likely causal variant was identified in the majority of our cohort (90%). These variants fall in three categories: 1) established disease genes that are known to have severe fetal presentations, 2) established disease genes for which no prenatal lethality has been described, and 3) novel candidate genes. The candidacy of the novel genes is typically supported by data from model organisms. Our study expands the list of genes that can result in non-viable Mendelian phenotypes, defines novel lethal syndromes, and demonstrates the power of consanguineous populations in the study of these phenotypes.

CORNELIA DE LANGE SYNDROME IN DIVERSE POPULATIONS

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Cornelia de Lange syndrome (CdLS, MIM 122470) is a dominant multisystemic malformation syndrome typically caused by de novo mutations in all ancestry groups with no racial or ethnic predilection. The characteristic facial dysmorphisms, critical in establishing a clinical diagnosis, include arched eyebrows, synophrys, short nose, anteverted nares, long philtrum, thin lips, maxillary prognathism, micrognathia, hirsutism, and a low anterior hairline. While wide phenotypic variability exists within the CdLS spectrum, ranging from mild to severe, most patients have growth deficiency, intellectual disability, and oftentimes can have upper limb anomalies. There are currently 5 identified genes known to cause CdLS when mutated – *NIPBL*, *SMC1A*, *HDAC8*, *SMC3*, and *RAD21*.

Genetic, environmental and epigenetic factors contribute to gene expression differences during development, and these same factors likely play a role in modifying facial features as significantly as a strong Mendelian mutation. It is also clear that these modifiers play a key role

in phenotypic variability between different ancestry groups. Of note, there are higher proportions of Caucasians in the databases used for this analysis. However, our approach to use the objectivity of computer software facial anthropometric analysis will allow us to accurately identify differences across underrepresented minorities (URM), and further assess its utility as a tool to help the clinician in the diagnosis of CdLS across diverse ancestral groups.

We have evaluated the dysmorphologic features in a large cohort of individuals who were self- or family-identified as a URM with CdLS. All subjects had previously been enrolled in an IRB-approved protocol of informed consent. Our CdLS subject database was reviewed for all individuals with a clinical diagnosis. This identified 3078 overall subjects of whom 147 were identified as a URM belonging to one of the following ancestry cohorts – African American, Asian, Native American, Middle Eastern, and Hispanic/Latino. Of these clinically diagnosed individuals, we refined our analysis to patients with clinical images available, and identified 40 URM individuals and 180 matched Caucasians who also had confirmed molecular diagnoses. We restricted subsequent analyses to these 220 subjects.

Using facial recognition technology, we compared frontal photos between the URM and Caucasian cohorts. We assessed average scores between individuals grouped by mutated gene. We subsequently compared the technology's ability to recognize facial landmarks in the URM versus Caucasian cohorts. Our preliminary assessment indicates that comparative analysis of the two cohorts does not have a lower diagnostic yield in URMs compared to Caucasians.

Workshop Session 9
DYSMORPHOLOGY (SYNDROMES AND MALFORMATIONS) IN MINORITY AND UNIQUE POPULATIONS II

CONGENITAL HEART MALFORMATIONS IN SUB-SAHARAN AFRICA AND ASIA

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Congenital heart disease is the most common birth defect, affecting approximately 1% of all newborns. There have been multiple large studies genotyping humans with structural CHD in resource rich countries and very little study in developing nations. In this study, we focus on the genomic analysis of individuals with CHD in resource poor countries in Sub-Saharan African and Asia. Genomic evaluation of non-European individuals offers the opportunity to investigate genetic causes in different genetic backgrounds and different environmental backgrounds such as vitamin A deficiency which is endemic in Sub-Saharan Africa and parts of Asia and lower obesity rates. Clinical examination and echocardiography were used to diagnose patients with both syndromic and isolated CHD. Exome sequencing, assembly, genotyping, and annotation were performed on probands and their parents (trios) by the National Intramural Sequencing Center (NISC). DNA variant list manipulation was performed using perl scripts developed by our group and significant findings were confirmed with Sanger sequencing. Copy number variations were evaluated using both the Illumina HumanExome BeadChip-12v1_A (Illumina Inc. San Diego, CA) and the XHMM (eXome-Hidden Markov Model) software, which recovers information on CNVs from targeted exome sequence data. Selected variants are being evaluated functionally in the mouse model and zebrafish model. 124 probands have completed the pipeline including 111 parent-offspring trios and 13 probands with one parent. Currently, 60 more trios are in analysis pipeline. Probands included 83 (67%) from Sub-Saharan Africa and 41 (33%) from Asia. Tetralogy of fallot (TOF) was the most common CHD in our cohort occurring in 29 probands (23%), followed by ventricular septal defects in 27 (22%), and then pulmonary stenosis in 13 (10%). Large copy number variations were found in 19 probands (15%) with 22q11.2 deletion syndrome being most common (6%). Syndromic single gene disorders were found 11 probands (9%) with RASopathy variants found in 8 patients (6%). Three probands had pathogenic variants in genes known to cause cardiomyopathies (*ACTC1*, *ACTN2*, *DSP*). Novel candidate genes were chosen using a strict criterion to minimize false positive: present in two unrelated families (including families found in Genematcher and other databases), genes not known to be associated with CHD, variants not present in the ExAC database, and CADD scores greater than 15. Sixteen genes met this criteria and zebrafish evaluation is currently underway to validate pathogenicity. We have initiated the largest CHD study in a non-European cohort using next generation sequencing project to search for novel genetic associations with CHD. With 2/3 of next generation sequencing complete on our cohort, we are now finding novel genes that explain the CHD phenotype. Similar to European populations, we found that CHD in diverse populations is enriched with genes associated with genetic syndromes in which CHD comprises a major part of the phenotype. More interesting is our new gene discovery that will increase our understanding of the genetic basis of CHD. Additionally, we are conducting mouse model experiments on unique vitamin A pathway variants from our cohort that will contribute to our und rstanding of gene-environmental interactions.

RITSCHER-SCHINZEL/3-C SYNDROME – FURTHER DELINEATION OF A FIRST NATIONS COHORT AND IMPLICATIONS IN CHOLESTEROL HOMEOSTASIS

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Background: A presumed autosomal recessive multi-system disorder was first described by Ritscher et al. (*Am J Med Gen, 1987*) in two sisters with distinctive craniofacial findings, cerebellar anomalies, congenital heart defects and intellectual disability (ID). The diagnosis of "Ritscher-Schinzel syndrome" (RSS); also referred to as "3-C syndrome" (cranio-cerebello- cardiac; OMIM 220210) has subsequently been

proposed in numerous patients with findings involving these three systems. The genetic and phenotypic heterogeneity of RSS was recently addressed by Dr. Ian Krantz at these meetings in 2014. Leonardi et al. (*Am J Med Gen 2001*) attempted to define diagnostic criteria and discussed the importance of the craniofacial phenotype, which has been emphasized by others (*Craft* et al., *Am J Med Gen, 2011*). Marles et al. (*Am J Med Gen 1995*) described a series of First Nations (FN) patients from Northern Manitoba with findings consistent with RSS. The underlying genetic disorder was subsequently elucidated; affected individuals were homozygous for a novel splice site mutation in *KIA00196* and showed a 60% reduction in the resulting protein, strumpellin. Study of newborn blood spots revealed a carrier frequency of 1 in 9 in this population (*Elliott* et al., *J Med Gen* 2013).

Materials and Methods: A detailed literature review of patients with presumed 3-C/RSS was performed. Phenotypic findings and underlying mechanisms were summarized.

Results: Craniofacial findings among the FN cohort included: macrocephaly, prominent forehead, hypertelorism, wide and downslanting palpebral fissures and low set ears; consistent with the original reported family and is reported in other patients. Hands showed brachydactyly with a characteristic wavy pattern, seemingly specific to this cohort, although brachydactyly and camptodactyly have also been reported. As with other patients, not all three systems were always involved, although ID was consistent and the craniofacial features were an important indicator. Hypercholesterolemia was reported in two cohorts including those with *KIAA0196* variants.

Discussion: Strumpellin is a component of the WASH complex that is involved in endosomal trafficking and sorting. The report of an X-linked disorder of affected male siblings with ID, growth deficiency, Dandy-Walker malformation, septal defects, relative macrocephaly, hypertelorism and distal limb findings revealed a shared a missense variant in exon 15 in *CCDC22* (Kolanczyk et al., *Eur J Hum Genet* 2015); a subunit of the CCC complex, which interacts with the WASH complex. The authors proposed the term "washopathies" for this group of disorders. The CCC complex regulates circulating low density lipoprotein cholesterol by mediating the endosomal trafficking of the low density lipoprotein receptor (LDLR). Recent studies have found patients with variants in *CCDC22* and *KIAA0196* to be hypercholesterolemic, demonstrating involvement of the CCC and WASH in cholesterol homeostasis (Bartuzi et al., *Nat Comm* 2016). Further characterization of WASH subunits and related proteins may help to elucidate the underlying mechanism in this heterogeneous group of disorders.

CLINICAL FEATURES OF BECKWITH-WIEDEMANN SYNDROME IN DIVERSE POPULATIONS

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Beckwith-Wiedemann Syndrome (BWS) is the most common epigenetic disorder to affect children. A recent international consensus for diagnosis and management of BWS has proposed the term "Beckwith-Wiedemann Spectrum" (BWSp) to encompass the wide range of features caused by the underlying genetic and epigenetic changes on chromosome 11p15. To carefully assess the range of clinical features that fall under this spectrum, we utilized the new international BWS criteria and the most current molecular diagnostic strategies to investigate whether clinical features recognized as part of the diagnosis of BWSp vary within different racial and ethnic backgrounds. The aim of this study was to compare the incidence of common BWS features between Caucasian and multiracial/non-Caucasian subjects within the BWS patient registry that has been established at CHOP.

BWS registry subjects with molecularly confirmed BWS in one or more tissues, available medical records, and demographic information available were included for analysis. Within the cohort, 70% were Caucasian and 30% were multiracial/non-Caucasian. We noted an equal distribution of molecular subtypes between the two groups. Pearson chi- square analysis was performed to compare the incidences of common BWS features using SPSS Statistics with a significance of 0.05.

The majority of BWS features such as macrosomia, nevus flammeus, ear creases, hemihypertrophy (lateralized overgrowth), and tumor incidence did not vary between the groups. However, several observations were notable. We found that although the incidence of macroglossia was similar between the groups, Caucasian subjects were found to have a significantly higher incidence of tongue reduction surgery ($p = .026$) than multiracial/non-Caucasian subjects. This suggests that macroglossia was either more severe in the Caucasian patients, or these families were more likely to opt for tongue reduction surgery. We also noted that abnormal ultrasound findings outside of the renal system ($p = .045$) were more often noted in Caucasian subjects. Additionally, multiracial/non-Caucasian subjects had a significantly higher incidence of hyperinsulinism (severe persistent hypoglycemia) compared to Caucasian subjects ($p = .013$) while Caucasian subjects tended to have a slightly increased incidence of transient hypoglycemia.

Overall, this analysis demonstrates that most BWS features did not significantly differ across diverse populations. We are extending this work to investigate the variation in the nature and management of macroglossia and hyperinsulinism to determine if there are additional factors that vary between populations, to include more detailed facial feature analyses, and will work to broaden these analyses to larger BWSp population cohorts.

A NEW ASHKENAZI JEWISH SYNDROME? NUP188 AND ITS ROLE IN A NEWLY DESCRIBED OCULO-FACIAL-NEURO SYNDROME

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Nuclear pore complexes are composed of approximately 30 distinct proteins termed nucleoporins (NUP). Alterations in NUP genes are

linked to several human diseases including primary biliary cirrhosis, triple A syndrome (achalasia, Addison disease, alacrima), atrial fibrillation, and neoplastic disease. NUP188, with other nucleoporins, constitutes the NUP93 complex, the second largest nuclear pore complex structural unit. NUP188 and NUP93 form a barrier preventing membrane proteins from passing from the outer to inner nuclear membrane (Nofrini, 2016). Although NUP188 has never been directly implicated in human disease, NUP188 duplication has been suggested to contribute to heterotaxy. Here we present three patients from two unrelated Ashkenazi families with the same compound heterozygous truncating variants and a distinct phenotype, representing a new Ashkenazi Jewish syndrome. Patient 1 is a 4-month-old patient born prematurely who presented to our institution with multiple congenital anomalies found at birth. Family history was significant for maternal and paternal Ashkenazi Jewish descent and maternal history of Tetralogy of Fallot. The patient was born in breech presentation at 36 1/7 weeks to a 23-year-old G1P1 mother and presented with severe microcephaly, bitemporal narrowing, bilateral congenital cataracts, epicanthal folds, small palpebral fissures, wide nasal bridge and wide nose, micrognathia, cleft palate, generalized hypotonia, overlapping toes, and transitional palmar creases. Brain MRI findings showed marked volume loss of the cerebral and cerebellar white matter, ventriculomegaly, and deficient appearance of the corpus callosum, brainstem, and inferior cerebellar vermis. Echocardiogram showed trivial mitral regurgitation and a large PDA that closed by age 3 months. Renal, bladder, abdominal and pelvic ultrasounds were normal and EEG showed hypsarrhythmia starting at 3 months old. Patient was also noted to have pancytopenia early in life requiring filgrastim, platelet and RBC transfusions. Exome sequencing identified biallelic truncating variants in NUP188: c.904_907delATTT (p.I302VfsX7) and c.3144 C>G (p.Y1048X). Using GeneMatcher, we identified a collaborator with two affected Ashkenazi siblings. Both affected siblings have the same compound heterozygous truncating variants as patient 1. The phenotypes were also strikingly similar, presenting with thin corpus callosum, progressive microcephaly, severely delayed brain myelination, prenatal onset ventriculomegaly, gliosis at autopsy, congenital cataracts, partial anomalous pulmonary venous return, bicuspid aortic valve, large great toe, long gracile fingers, preaxial polydactyly, and streak ovaries. NUP188 has many described functions, including cilia formation and regulation of chromosome segregation. Del Viso (Cell, 2016) showed that knockdown of Nup188 or its binding partner Nup93 leads to a loss of cilia during embryonic development. This new syndrome shares phenotypes with other syndromes of cilia dysfunction, especially Lowe oculocerebrorenal syndrome. Additionally, Foerster (Development, 2017) presents data to suggest that primary cilia regulate ventricle morphogenesis by acting as a brake on the mTORC1 pathway; this may contribute to the ventriculomegaly in these patients. In the gnomAD database, the p.I302VfsX7 is present at an allele frequency of 0.077% and p.Y1048X at 0.0406% in the Ashkenazi population; neither variant is present in other populations. Therefore, although there appears to be a low carrier frequency, this syndrome may be considered for inclusion on Ashkenazi carrier panels.

HOMOZYGOUS BORICUA *TBCK* MUTATION CAUSES NEURODEGENERATION AND ABERRANT AUTOPHAGY

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Autosomal recessive mutations in *TBCK* cause intellectual disability of variable severity. Children of Puerto Rican (Boricua) descent sharing the homozygous *TBCK* p.R126X exhibit characteristic coarse facial features and progressive neurologic decline. Although the physiologic function of TBCK remains unclear, loss-of-function mutations are associated with inhibition of mTORC1 signaling. As mTORC1 signaling is known to regulate autophagy, we tested whether Puerto Rican patients with TBCK-encephalopathy have defects in autophagic-lysosomal dysfunction. We also performed extensive neurophysiological phenotyping to better establish the distinct clinical syndrome in children of Puerto Rican descent affected with homozygous *TBCK* p.R126X mutations (n = 8). We found that the neurologic phenotype of children with *TBCK* p.R126X mutations, which we call TBCK-encephaloneuronopathy (TBCKE), include congenital hypotonia, progressive motor neuronopathy, leukoencephalopathy and epilepsy. Systemic features consist of coarse facies, dyslipidemia, and osteoporosis. $TBCK^{-/-}$ fibroblasts exhibit increased numbers of LC3+ autophagosomes *in vitro*, and upregulation of autophagic markers LC3b and beclin-1. Aberrant free oligosaccharide profiles were identified in fibroblasts and urine of TBCKE patients, which was ameliorated by treatment with the mTORC1 activator leucine. We conclude that TBCKE in patients with the p.R126X mutation is a clinically distinguishable syndrome with progressive central and peripheral nervous system dysfunction. We provide evidence that inappropriate autophagy in the absence of cellular stressors may play a role in this disorder, and that mTORC1 activation may ameliorate the autophagic-lysosomal system dysfunction. Free oligosaccharide profiles could serve as a novel biomarker for this disorder as well as a tool to evaluate potential therapeutic interventions.

CATALOGUING INHERITED DISORDERS AMONGST THE IRISH TRAVELLER POPULATION

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Irish Travellers are an endogamous, ethnically Irish population numbering ~40,000 on the island of Ireland. Consanguinity is common. Knowledge of Traveller disorders exists but mainly in specialised Irish centres. Most Traveller disorders are published but ethnicity is not explicit, hampering diagnoses, particularly if the patient lives overseas where knowledge about this population is poor. To catalogue inherited Irish Traveller disorders through identifying the disorders, detailing mutations, use of coding, (OMIM, Orphacodes & ICD10), publications, and move towards database development to facilitate diagnoses. A literature review was undertaken. Key national and international Clinician/scientists were contacted to identify relevant disorders and publications. Laboratory and clinical databases were searched to retrieve disorders & mutations. Annotations were updated. An Excel database was established listing each disorder, its appropriate code, associated mutation and relevant publication. 86 distinct rare genetic disorders were identified which resulted in 76 phenotypes. 78/86 were autosomal recessive; 4 of these were dominant disorders but presented only in the recessive state. Seven dominant disorders with no recessive phenotype were included as > one affected individual existed. One common 17q12 duplication was included, presenting in two unrelated families. Homozygous mutations were found in all recessive disorders bar one. The genetic basis of 78/86 was established. A further 2/76 have common haplotypes; the genetic basis of six disorders remains unclear. Linkage disequilibrium was observed in 4 families with co- existing McArdles disease and microcephaly & 11 individuals have co-existing Friedreich's ataxia & galactosemia. We present for the first time, a comprehensive catalogue of the currently known Traveller disorders, their causative mutations (where known), including several novel or previously unreported mutations, relevant publications, disease codes (Orphacodes, OMIM and ICD10 coding). Future challenges include development of an online mutation database; development of a NGS Traveller panel with a view to population screening.

Workshop Session 10
NATURAL HISTORY OF SYNDROMES I

THE NATURAL HISTORY OF GENETIC DISORDERS: THE CENTERPIECE OF THE "CENTRAL DOGMA" OF CLINICAL GENETICS

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The concept, phenotype, was proposed as a term by the botanist, Wilhelm Johannsen in the early 1900s. Phenotype has become a widely recognized term in recent years, even to those outside of the genetics community, because of the remarkable identification of human disease genes. The notion of **disease** phenotype comprises a number of important elements: diagnostic criteria/definition of a condition; the component manifestations of the condition; and the **natural history.** The importance of natural history in the study of genetic disorders and multiple congenital anomaly syndromes was initially emphasized by Judith Hall in her seminal paper in the *Journal of Medical Genetics* in 1988. She defined natural history of a disorder as "an account of all of the consequences of that disorder .... as the study of the disease process with emphasis on the sequences of events and the effects of time." Thus, natural history is the evolution of the features of the condition over time.

I would propose that the characterization and analysis of the natural history of a condition is the centerpiece of what is de facto the "central dogma" of clinical genetics: disease phenotype -> natural history -> management (health supervision and treatment). There have been a number of strategies that investigators in the field have used over the years to characterize natural history. These include: (1) the listing all of the component manifestations of the condition and the frequency of the finding; (2) the development of severity scores that characterize the seriousness within the variability of the condition; (3) population investigations of mortality in individuals in a region with the disorder. The typical listing of manifestations with frequencies within a disorder is very familiar to this audience (e.g., NF1, del22q11). The challenge, however, is that these frequency figures are laden with publication and selection biases. It is very difficult to obtain a real sense of the frequency because of the limitations in methodology and the difficulties in studying rare diseases longitudinally. Recently, many genetic support groups (e.g., Unique in the UK, CTF) and the NIH (patient-reported outcomes) have developed registries which provide a different ascertainment of conditions and may lead to better representations of the actual natural history of the condition.

Health supervision guidelines have been developed over the last 3 decades for many syndromes including those published by the AAP (e.g., Down syndrome), GeneReviews, and leading experts in the 3 editions of Cassidy & Allanson's text, Management of Genetic Syndromes. A number of disorders well known to this audience provide prototypes for the study of natural history; these include NF1, achondroplasia, and WHS/4p-. Future studies of natural history need to include the evolution of findings in adults with syndromes, standardized efforts for transition of care, analysis of the patient-reported outcomes model, and development of evidence-based guidelines for management.

PHENOTYPE AND NATURAL HISTORY IN 49 INDIVIDUALS WITH *SATB2*- ASSOCIATED SYNDROME

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SATB2-associated syndrome (SAS, Glass syndrome) is a multi-system disorder characterized by significant neurodevelopmental compromise with limited or absent speech, behavioral issues, and craniofacial anomalies. Since the description of the initial cases of SAS, additional reports over the last few years have validated the most common features.

Since its inception, 56 families (37 unpublished) with SAS have enrolled in the SAS clinical registry. Of these, 49 families have completed additional phenotypic questionnaires and their medical records have been reviewed. 53% of the participating SAS individuals were male. The mean age was 7.4 years (median age 7, range 0–18 years) with a mean age at diagnosis 5.1 years (median age 4, range 0–18 years). The predominant molecular mechanism was heterozygous intragenic pathogenic variants of *SATB2* (63%).

Mean anthropometric measurements at birth were 3.3 ± 0.6 kg for weight, 50.4 ± 2.9cm for length, and 34.7 ± 1.22cm head circumference. Micrognathia, cleft palate, or high arched palates were noted in 45%, 41%, and 35% of individuals, respectively. Feeding difficulties (80%), sialorrhea (81%), and hypotonia (66%) were common during infancy. During childhood, abnormal dental shape/size was reported in 90% of individuals. Other relevant medical issues found during this time period included strabismus (36%), seizures (17%), and osteopenia (10%). Gross motor developmental milestones were overall achieved at later ages with mean age for walking at 25.6 months (median 21, range 11–144). Speech was universally delayed with mean age at first word of 33 months (median 24, range 10–144); 80% of individuals currently have absent or very limited (less than 10 words) total vocabulary. Formal cognitive evaluations had been conducted in some individuals (n = 8) with an average IQ score of 45 (range 32–52). Where performed (n = 34), brain MRI was abnormal in 53% of individuals (at mean age 2.7 years) most commonly with reported delayed myelination and/or brain signal abnormalities (83%).

Combined with previously reported cases (total = 102), this data allows the outline of the common features of this syndrome: universal developmental delay and speech delay, dental anomalies (77%), behavioral abnormalities (66%), and cleft palate (47%). Understanding the timing, presentation, and age-specific phenotype of SAS will allow earlier recognition of the syndrome and the development of adequate surveillance guidelines for this rare syndrome.

NATURAL HISTORY OF SPINE DISEASE IN THE MUCOPOLYSACCHARIDOSES: INTEGRATING HUMAN AND ANIMAL MODEL DATA

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The mucopolysaccharidoses (MPS) are a family of lysosomal storage disorders characterized by the deficient degradation of glycosaminoglycans (GAGs) due to different enzymatic deficiencies. Many subtypes, including MPS IVA (Morquio Syndrome, Type A) and MPS VII (Sly Syndrome), exhibit skeletal abnormalities that range from mild to severe. Spine manifestations are common and include both developmental and degenerative abnormalities, such as progressive kyphoscoliosis, odontoid hypoplasia, cervical stenosis, and can lead to spinal cord compression (Peck et al., 2016). Spine disease is an important feature of MPS as it is often one of the first signs (e.g. gibbus), leads to significant morbidity, and current treatments have little efficacy on improving disease. Due to the variability in presentation and the progression of disease, diagnosis of MPS is often delayed. Here we describe three siblings, 3, 9, and 14 years of age, that presented to our clinic at different ages. The two older brothers, were followed for years until the diagnosis of Morquio Syndrome, Type A (MPS IVA), an autosomal recessive MPS, was made via whole exome sequencing. Over the years, their progression of spine disease can be noted through physical exam and imaging, beginning first with anterior involvement of the vertebrae, progressing to platyspondyly, spinal stenosis, and thoracolumbar scoliosis. In addition to spinal disease progression, facial coarsening, digital involvement, and dental anomalies have become more evident over time. The youngest brother was diagnosed via enzyme screening at 3 years of age. At this time, he has a wide based gait and lumbar lordosis without facial dysmorphia or dental anomalies. Heterogeneous mineralization of the vertebral bodies with subtle anterior beaking is noted on imaging. The mechanism of the variability in spine disease and of GAG accumulation leading to spine disease across MPS subtypes is poorly understood.

Using a naturally occurring MPS VII canine model that closely parallels human skeletal disease, our group identified the developmental window in which failed vertebral bone formation first occurs and that it is due to the failure of chondrocytes to undergo hypertrophic differentiation (Peck et al., 2015). Using whole transcriptome sequencing, we found that failed chondrocyte hypertrophy is associated with impaired activation of the Wnt/ß-catenin signaling pathway (Peck et al., 2016). We hypothesize that GAG accumulation contributes to impaired Wnt signaling activation. Extracellular GAGs are important in the distribution and activity of Wnt ligands and abnormal accumulation may prevent Wnts from reaching target cells. Intracellular GAGs may increase cell stress, negatively affecting Wnt synthesis and signaling. Thus, therapeutic targeting of the Wnt/ß-catenin signaling pathway represents a potential treatment strategy for spine disease. Lithium is used in multiple other diseases and is a GSK3ß inhibitor, which can activate Wnt signaling. We have shown with in vitro studies that MPS VII chondrocytes respond to lithium and undergo hypertrophy (Peck et al., 2016). Ongoing studies using lithium in the MPS VII canine model are underway and will establish if lithium can be used for bone formation during postnatal growth in MPS VII and other subtypes.

THE NATURAL HISTORY OF CRANIOSYNOSTOSIS AND TUMOR RISK

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Certain pediatric syndromes are associated with increased risk of cancer. Juvenile myelomonocytic leukemia is a well-established risk in Noonan syndrome, and other tumors may occur in Rasopathies (Bertola et al., 2017; McWilliams et al., 2016). A number of syndromes harbor increased tumor risk (DW Smith workshop discussion on mTOR, PIK3CA, sex chromosome conditions) (Ripperger et al., 2017), yet precise determinants of tumor risk and tumor patterns in many pediatric syndromes are not clear.

Here we seek further evidence of germline predisposition and somatic tumor occurrence. Interestingly, many genes that are somatically mutated in human tumors (in individuals without syndromes) are the same genes that can cause germline genetic conditions. This leads to an interesting question of whether these germline genetic conditions (or syndromes) harbor an increased risk of tumor. This may be most important for patients with existing mutations in cancer-predisposing genes.

We present a study of the 127 most-commonly-mutated somatic cancer genes from The Cancer Genome Atlas project. We examine the corresponding germline genetic syndromes associated with each gene and examine all reports of cancer in the syndromes. We hypothesized that the most common somatically-mutated genes in cancer identify pediatric germline genetic conditions that have unrecognized tumor risk. After excluding 34 known cancer-predisposition conditions and 55 genes without corresponding germline conditions, we found that of 38 syndromes, 13(34.2%), had tumor case reports suggesting increased tumor risk. As an example, we investigated FGFR2-associated craniosynostosis further, after noting unusual tumors in these patients. We analyzed over 13 million U.S.- based hospitalizations to find 1,893 with Apert craniosynostosis. Interestingly, we found examples of tumors in these patients at higher rates than expected, including tumor types unlikely to be due to chance. We also investigated potential candidates for FGFR2 co- occurring gene mutations by analyzing somatic tumor exome sequencing data. We present tumor suppressors and oncogenes that could act in concert with FGFR2 in tumors. Over the years of a patient's life, such additional gene mutations may be important in tumor predisposition. In conclusion, we developed a new means for identifying tumor association in germline genetic conditions. Further efforts examining more tumors in both the unselected population and in individuals with syndromes will aid future counseling, tumor prediction or screening methods.

NATURAL HISTORY OF NEVOID BASAL CELL CARCINOMA (GORLIN) SYNDROME

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Nevoid basal cell carcinoma syndrome (NBCCS), also known as Gorlin syndrome, causes a range of congenital anomalies and later-onset complications. NBCCS is associated with loss-of- function mutations in *PTCH1*, *PTCH2*, and *SUFU* resulting in upregulation of the hedgehog signaling pathway, but 15–27% of individuals have no known molecular diagnosis. Many of the clinical diagnostic criteria do not manifest until late childhood. As such, early identification of NBCCS remains challenging. A better understanding of the disorder's natural history may facilitate the diagnosis of NBCCS before complications develop.

We reviewed electronic medical records at Boston Children's Hospital from 2000 to 2017 and identified 14 patients with a diagnosis of NBCCS. All 11 individuals with a molecular diagnosis had mutations in *PTCH1*. Three additional patients met clinical diagnostic criteria for NBCCS. For each of these 14 patients, we performed a longitudinal review of their charts to identify various clinical manifestations and their ages of onset. All individuals were alive at the time of chart review. Current ages range from 2.5 months to 23 years (mean = 9.3 years). Two patients were known to have an affected father and received prenatal diagnoses. The remaining patients received a diagnosis at a mean age of 6.6 years.

Prenatal findings of NBCCS in our cohort were ventriculomegaly (n = 6), including two cases of unilateral ventriculomegaly, and cleft lip and palate (n = 1). Features identified in the neonatal period were macrocephaly (n = 4), cardiac fibroma (n = 2), and metopic craniosynostosis (n = 2). Rhabdomyoma, facial myofibroma, lingual cyst, and natal tooth were each observed in one individual. Manifestations in the three years after the neonatal period were medulloblastoma (n = 3), nasal dermoid cyst (n = 1), and cardiac fibroma (n = 1). No individual developed jaw keratocysts or basal cell carcinomas (BCC) prior to three years of age.

The most common manifestation in middle childhood and adolescence was jaw keratocysts (n = 7), which developed at a mean age of 9 years. Only 3 patients had BCC. One developed thousands of BCC at age 3, following radiation therapy at age 7 months for medulloblastoma; the other two individuals had BCC by 6 and 13 years of age. One patient developed an osteochondroma of the tibia at 17 years.

Almost all patients had either neurologic or ophthalmologic manifestations. By 12 years old, all individuals had macrocephaly. Most had developmental delay (n = 9). Many had ophthalmologic abnormalities (n = 5). Aside from non-specific findings, such as strabismus, myopia, and delayed visual maturation, 2 patients had pupillary defects and 1 had myelinated nerve fiber, first noted at 4 years.

Two widely recognized features of NBCCS, bifid ribs and palmar pits, were identified in a small number of patients. Only 2 of 6 patients who were evaluated for the presence of rib anomalies had positive findings. Of the 10 patients whose hands were carefully examined, 4 had palmar pits.

Our findings suggest that many of the diagnostic features of NBCCS do not emerge until after 3 years of age. Young children, however, do manifest several early findings, underscoring the need for revised diagnostic criteria for NBCCS in neonates and toddlers.

THEY'RE NOT ALL GIVING US THE (FIFTH) FINGER: NATURAL HISTORY OF 82 PATIENTS FROM THE COFFIN-SIRIS SYNDROME/BAF PATHWAY REGISTRY

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Coffin-Siris syndrome (CSS, MIM 135900) is a now well-described, congenital anomaly syndrome, caused by mutations in the BAF-complex pathway (including *ARID1A*, *ARID1B*, *ARID2*, *SMARCE1*, *SMARCB1*, *SMARCA4*, *SOX11*) (Hempel et al., 2016; Kosho et al., 2013; Tsurusaki et al., 2012, 2014). It is classically characterized by coarse facial features, sparse scalp hair, hypertrichosis, and other organ system anomalies. One of the most historically notable features is the presence of hypo- or aplasia of the distal fifth digit phalanges or nails, hence the original name of 'fifth digit syndrome' (Coffin and Siris, 1970). In 2011, prior to the discovery of the BAF pathway genes in CSS, Schrier et al. proposed that the fifth-digit abnormalities are the hallmark of this disorder, and a prerequisite for a clinical diagnosis.

Since that time, as technology has advanced, and as more patients are being diagnosed through next-generation panels and whole-exome sequencing, clinicians are discovering the breadth the variability of the phenotype in this disorder. In 2015, the CSS-BAF pathway disorders registry was created, and currently has 82 individuals enrolled. In examining these probands, it is becoming clear that some of the 'classic' features of this syndrome are less consistent. Of the individuals in the registry, only 42/82 (51%) are reported to have fifth-digit abnormalities.

Probands with mutations in the *ARID1B* gene, the most commonly mutated in CSS, tend to have the most variability with the fifth digits, with only 46% reporting abnormalities to the nails or digits. Only 20% of individuals with *SMARCB1* mutations had fifth digit or nail abnormalities, and probands with *SMARCE1* or *SMARCA4* mutations had a slightly higher proportion of fifth digit anomalies, at 75% and 67%, respectively. Although still common, fifth-digit anomalies no longer appear to be the *sine qua non* of this syndrome.

While examining individuals enrolled in the registry, we have also looked for the presence or absence of some of the other key features of CSS, including sparse scalp hair, hirsutism/hypertrichosis, feeding difficulties, developmental milestones, and immune-related issues. These, too, are certainly not present across the board in individuals with the syndrome, reflecting the broad variability particularly among the various genes. Abnormal dentition was reported in 65% of individuals, hypertrichosis 57%, and sparse scalp hair only 35%.

Gastrointestinal abnormalities, including reflux, feeding difficulties, and gastrostomy-tube use, were reported in about 65% of individuals, with the highest prevalence among *ARID1B* and *SMARCA4* mutation-positive probands.

We discuss these findings here to reflect the broadening phenotype of CSS, in order to encourage clinicians to consider the diagnosis even when previously reported "key features" may be absent.

REVIEW & NATURAL HISTORY OF THE *NAA10*-ASSOCIATED DISORDERS

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The *NAA10*-related disorders are X-linked conditions with a broad spectrum of findings, ranging from a very distinctive, lethal phenotype in males, originally described as Ogden syndrome (OMIM 300855) [Rope et al.], to non-specific, intellectual disability syndrome in both males and females [Rauch et al., Popp et al., Saunier et al.]. Although developmental delay and intellectual disability may be presenting features (and in some cases the only finding), many individuals also have cardiac findings, distinctive physical features, and disorders of growth, but are largely indistinguishable from other intellectual disability syndromes.

Ogden syndrome is associated with a (c.109T>C) missense mutation, ascertained in eight affected males from two, unrelated families sharing a remarkably consistent phenotype. The average life span of the affected boys was 10.2 months, with the oldest only surviving until 16 months. In the absence of significant medical interventions, one of the affected infants survived for only 4.5 months. The cause of death in all cases, was congestive heart failure stemming from underlying cardiac arrhythmias.

Although not found in the majority of those fitting the description of Lenz microphthalmia syndrome, four related males were identified as having a splice site mutation (c.471 + 2T>A) [Esmailour et al.] in *NAA10*. Their clinical features included anophthalmia, hypotonia, moderate to severe intellectual disability and anomalies of skeletal, cardiac and renal development. Heterozygote females demonstrated 2–3 toe syndactyly as their only apparent anomaly.

Seven additional *NAA10* mutations have been identified in nine males and sixteen manifesting females (the vast majority of which, were proven to be *de novo*). As one might expect, females generally had a milder and less characteristic clinical presentation than the males, but commonalities included moderate to severe neurocognitive impairments, postnatal growth failure commonly occurred, also resulting in severe microcephaly. Skeletal, brain, and organ anomalies were frequent, but without any apparent specific pattern.

Mutations in *NAA10* are associated with reduced enzyme activity of N-Acetyltransferase (NatA). As much as 40% of the human proteome is a substrate for this enzyme complex. Specific mutations affect enzymatic activity of NatA differently. However, the phenotypic severity does not correlate with activity of the enzyme, suggesting that there is more to the underlying mechanism, than what was originally hypothesized.

CANTU SYNDROME NATURAL HISTORY STUDIES: CLINICAL INVESTIGATIONS ON 18 PATIENTS AND REPORT ON INTERNATIONAL REDCAP REGISTRY DATA ON 58 PATIENTS

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Cantu syndrome (CS) is an autosomal dominant condition associated with congenital hypertrichosis, neonatal macrosomia, macrocephaly, craniofacial dysmorphic features, osteochondrodysplasia, peripheral edema/lymphedema and cardiovascular abnormalities including PDA, cardiomegaly with high cardiac output and low peripheral vascular resistance, pericardial effusion and tortuous and dilated vasculature. CS is caused by heterozygous activating mutations in either *ABCC9* or *KCJN8* that encode the regulatory SUR2 and Kir6.1 subunits, respectively, of the ATP-sensitive potassium channel (K_{ATP}). K_{ATP} channels formed from SUR2 and Kir6.1 subunits are prominent in cardiovascular tissues, and K_{ATP} activation in vascular smooth muscle results in decreased vascular contractility.

Through establishment of a Cantu Syndrome Research Clinic in 2013 at Washington University, we have performed extensive clinical evaluations on 18 patients over the past 4 years, including cardiac and brain imaging and functional studies. We have identified several novel features of CS. We have characterized a distinct cardiovascular phenotype in patients with CS that includes low blood pressure in combination with decreased pulse wave velocity (increased elasticity) in the vasculature, enlarged cardiac ventricles (with high cardiac output state) along with evidence of sympathetic dysregulation and cardiac repolarization abnormalities. CS patients have persistent fetal circulation manifested by PDA and pulmonary hypertension, as well as persistence of fetal circulation in the form of persistent thoracic arterial collaterals and trigeminal arteries. CS patients have lymphatic circulation abnormalities with lymphedema. We hypothesize that CS patients have contractile dysfunction of the lymphatic system due to decreased lymphatic smooth muscle excitability. Pericardial effusions occur in 25% of CS patients, and polyhydramnios is present in 60% of pregnancies of mothers carrying a CS fetus; these complications might also be related to lymphatic abnormalities. In all CS patients who have had brain imaging thus far, we have observed diffusely dilated and tortuous intracranial arteries. Many patients have migraine headaches, as well as neurobehavioral abnormalities, including developmental delays, attention deficit disorder and autism spectrum disorder.

We have developed a REDCap registry and have gathered clinical information on 58 patients with CS from the USA, UK, Netherlands, and Australia. Analysis of *ABCC9* in our cohort indicates that mutations cluster in the second transmembrane domain in 80% of cases, and two recurrent mutations account for 40% of cases. Mutations in *KCNJ8* are a rare cause of Cantu, with only two known cases. With regard to cardiac involvement, 65% of patients had cardiac enlargement, 86% had PDA, 17% had pulmonary hypertension, 28% had pericardial effusion and 13% had a dilated aortic root. 53% of patients report edema or lymphedema. Additional clinical data from the registry will be presented.

Activating mutations in the pancreatic form of K_{ATP} cause neonatal diabetes, which can be treated with sulfonylureas. Therefore, we hypothesize that some of the phenotypic features of CS may be ameliorated by these agents. As an international consortium, we have devised strategies to address this question by further clinical investigation of the natural history CS and the effects of *ABCC9/KCNJ8* mutations. Phenotypic data derived from patient investigations will be utilized to direct ongoing animal studies, in addition to providing the necessary metrics for future drug safety and efficacy testing.

AN INVESTIGATION INTO THE NATURAL HISTORY OF BARDET-BIEDL SYNDROME

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Bardet-Biedl Syndrome (BBS) is an autosomal recessive ciliopathy caused by biallelic loss-of-function mutations in a family of genes associated with ciliary structure or function.

BBS is characterized by retinal degeneration, obesity, polydactyly, cognitive impairment, renal anomalies, and hypogenitalism. The clinical features and natural history of BBS can be extremely variable. Diagnosis can often be delayed in early childhood since malformations are inconsistently present, and the clinical presentation may not prompt diagnostic evaluation for BBS until the vision symptoms develop in an obese child. Even after the diagnosis has been clinically or molecularly confirmed, predicting the natural history can be challenging considering the genetic and clinical heterogeneity with at least 20 genes that have now been associated with BBS, as well as inter- and intra-familial variability. Moreover, the natural history, including the ages of onset of the different manifestations, has not been well studied, which makes it difficult for clinicians to offer accurate information on prognosis.

We reviewed the electronic medical records at Boston Children's Hospital from 2004 to 2017 and identified 91 patients in 74 different families who were given a clinical diagnosis of BBS. Among the 91 patients, 47 have a confirmed molecular diagnosis of BBS in the genes *BBS1* [n = 26], *BBS2* [n = 5], *BBS7* [n = 3], *BBS9* [n = 1], *BBS10* [n = 7], *BBS 12* [n = 2], and *BBS13* [n = 3]. One of the individuals with biallelic mutations in *BBS13* also carried a heterozygous pathogenic mutation in *BBS9*. These 47 patients ranged from ages 2 years to 50 years old (mean age 18 years), and included 28 males and 19 females. We reviewed the records of the patients with a confirmed molecular diagnosis in a retrospective longitudinal study evaluating the age of onset and severity of each of the clinical manifestations associated with BBS.

Among the first 20 patients we reviewed in detail, all of them had some degree of retinal degeneration, visual field constriction, and night blindness starting between ages 3 to 8 years of age. Seventy five percent had obesity (BMI greater than 30 kg/m^2 in adults or $\geq 95^{th}$ percentile for ages 2–20 years), with onset between ages 5 to 22 years

old. Fifty percent of the patients had intellectual disability or developmental delay. Postaxial polydactyly was present in sixty percent. Renal function was normal for all 20, but renal ultrasound abnormalities were present in 5 patients (calyceal blunting, pelviectasis, hydronephrosis, ureteropelvic junction obstruction, and renal hypoplasia). Hypogenitalism including small penile shaft, reduced volume of the testes, and cryptorchidism was present in 5 patients. We will present data on the prevalence and age of onset of all primary and secondary findings of BBS in all 47 molecularly confirmed cases evaluated at our center.

The presence of both obesity and retinal disease are the most consistent findings in BBS in childhood, and these findings should prompt diagnostic workup for BBS even without other clinical findings to allow appropriate anticipatory management. Better phenotypic and natural history information will be critical for better management of this complex multisystem condition.

MEDICALLY ACTIONABLE COMORBIDITIES IN ADULTS WITH COSTELLO SYNDROME

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Costello syndrome (CS) is a rare, autosomal-dominant condition caused by activating missense mutations in *HRAS*. There is very little published literature describing health concerns specific to adults with CS. Parents of individuals with CS are eager to gain a better understanding of what to anticipate as their children age. We surveyed a group of 16 individuals with CS, 9 women and 7 men (range 16 to 38y, mean 24.75y), regarding their medical concerns and lifestyle characteristics. All but 2 had the most common *HRAS* mutation G12S. Surveys were completed either at the 9th International CS Family Conference (July 2015) or via email. Five individuals had a current diagnosis of hypertrophic cardiomyopathy (HCM) and 2 had a history of HCM. Six individuals had Chiari I malformations; 4 were treated surgically. The oldest age of Chiari I diagnosis was 15.7 years and the oldest age of surgical treatment was 19 years. Seven individuals reported gastroesophageal reflux (GER) and an additional 2 have cyclic vomiting. Eight of the women reported delayed or absent menarche, and most were on hormone replacement.

Decreased bone density was reported by seven individuals (4 women, 3 men) although no fragility fractures were reported. Seven individuals reported use of a wheelchair for long distances. An additional two patients (aged 26 and 30) reported worsening distal contractures, one of whom became non-ambulatory at age 19 after progressive balance problems with falls. Five individuals reported needing some assistance with walking on uneven surface or stairs. Ten individuals reported anxiety and two reported aggressive behavior. Four individuals were on medication for anxiety and 1 did not tolerate medication due to side effects. Only one individual in the group had a previous diagnosis of rhabdomyosarcoma; no other cancers or tumors were reported.

Previous studies have identified cardiomyopathy, bladder carcinoma, benign tumors, Chiari malformations, gastroesophageal reflux (GER), delayed puberty, and abnormal bone density (Hopkins et al., 2009; White et al., 2005) as the most prevalent medical concerns for adults with CS. A quality of life (QOL) study demonstrated decreasing QOL as the number of medical issues increased in individuals with CS (Hopkins et al., 2009). The current study identifies several previously undescribed actionable medical concerns in adults with CS. First, the high prevalence of anxiety in this cohort indicates that screening for anxiety is warranted since this is a treatable condition that can have a significant impact quality of life. Second, adults with CS should be monitored for progressive contractures or other problems that could decrease mobility. This is especially important in a population that seems to have increased risk for osteopenia. Also of note is the lack of cancer diagnoses in adulthood in this group, although the cohort is too small to draw any definitive conclusions about cancer risk in adults with CS. We plan ongoing follow-up of the current cohort of adults with CS in order to better understand progressive medical and physical problems, which is essential for providing targeted management recommendations and anticipatory guidance to families.

RASA1-RELATED DISORDERS

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Germline variations in *RASA1* have been shown to cause vascular malformations with a wide range of phenotypic variability. The hallmark features are capillary malformations (CM) and arteriovenous malformations (AVMs). Lymphatic malformations have also been reported in some cases. Based on the initial observation of characteristic capillary malformations and arteriovenous malformations, CM-AVM syndrome was coined. Some individuals with a clinical diagnosis of Parkes Weber syndrome were also found to have germline mutations in *RASA1*.

In order to investigate the natural history and pathophysiology of *RASA1*-related disorders, we obtained the clinical and molecular findings of 70 unrelated cases that tested positive for a germline *RASA1* variant at a single reference laboratory. Standardized patient history forms were used. Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) were mainly used. Several atypical cases were evaluated using next generation sequencing (NGS), array-comparative genomic hybridization, and/or microarray.

Sixty-one individuals had a pathogenic *RASA1* mutation of which 30 were novel and 6 were large *RASA1* deletions. The remaining 9 individuals had a variant of uncertain significance. Of those with family history information, a family history of similar findings was present in 56%. Localized hypertrophy was reported in 11 individuals. AVMs were reported in the brain, colon, lung, leg, arm, back, lip, and face, and 32 individuals were reported to have an AVM/AVF. The large majority had multifocal CMs and only eight were reported to have a solitary CM. Two individuals had macrocephaly. Several individuals had a history of epistaxis and/or telangiectasia and had testing for genes

associated with HHT which was negative. Further clinical details will be presented.

Our cohort confirms that the phenotype of individuals with germline *RASA1* variants has significant variable expressivity. Although the majority of individuals had multifocal CMs, approximately 10% had a solitary CM. In addition, the AVMs were located in multiple different body regions beyond just the brain. Based on the relatively random locations and variation in the number of vascular malformations between individuals, we hypothesized that stochastic events were key drivers of the phenotypic expression. We previously documented a somatic second hit in one capillary malformation of an individual with CM-AVM syndrome (MacMurdo et al., 2016), and to further support this hypothesis, we obtained blood and tissue from the hypertrophic affected limb of an individual with the clinical phenotype of Parkes Weber syndrome. A *RASA1* germline (c.1981_1985del, p.Thr662Glufs*6) and somatic (c.463G>T, p.Glu155*) mutation (6%) were identified, further supporting the role of "second hits" in the etiology of the vascular malformations in *RASA1*-related disorders. Although a somatic *RASA1* mutation was documented in the biopsied tissue, the low level of mosacism suggests a mixed cellular origin. We hypothesize that the cells of origin are the endothelial cells and future studies of specific cell types are warranted. Our findings also show that *RASA1*-related disorders have a high degree of variable expressivity, which is likely secondary to stochastic events such as a" second hit".

MALADAPTIVE BEHAVIORS IN CHILDREN WITH ANGELMAN SYNDROME

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Angelman syndrome (AS) is a neurodevelopmental disorder in which disruptive or maladaptive behaviors are displayed by the majority of patients. Most often, these behaviors result from excitability, desire for attention, poor control over movements, and inability to verbally communicate needs/desires. Noncompliance, tantrums, and repetitive/stereotyped behaviors have also been described.

302 individuals with AS were enrolled in a Natural History study and completed an average of 3.3 visits (range 1–9). 76% had at least two visits. Behavior was assessed at each visit through caregiver report using questionnaires, including the Aberrant Behavior Checklist (ABC), a 56-item survey with 5 subscales (Irritability, Hyperactivity, Lethargy, Stereotypy and Inappropriate Speech – the last omitted due to absent speech in AS). Results of one-way ANOVAs indicated that for each of the 4 subscales there were significant differences between the three molecular types (deletion, UPD/ID and *UBE3A* mutation). Results are tabulated below.

	ABC Score for each subscale (mean [*SD*]) at Baseline			
Molecular class of AS	Irritability (Max score 45)	Lethargy (Max score 48)	Stereotypy (Max score 21)	Hyperactivity (Max score 48)
Deletion	4.5 [5.51]	4.2 [4.02]	4.2 [3.90]	13.9 [9.85]
UPD/ID	7.4 [6.51]	2.7 [3.40]	2.4 [3.30]	17.8 [11.17]
UBE3A mutation	8.6 [9.57]	3.0 [3.79]	3.0 [3.91]	17.1 [14.06]
p value	<.001	.026	.006	.035

The following observations emerged:

- At baseline, there were statistically significant differences in the domains of irritability, lethargy, stereotypy and hyperactivity based on molecular subtype and level of developmental functioning.
- At baseline, AS females were more lethargic than males
- Stereotypy did not change over time
- As individuals with AS mature, those with *UBE3A* mutations had greater increases in irritability, lethargy and stereotypy than those with Deletion or UPD/ID.
- Hyperactivity increases with age for all molecular subclasses.

Correlations between severity of behavior problems with caregiver stress and family quality of life will be discussed.

NEONATAL MARFAN SYNDROME – A COMPREHENSIVE REVIEW AND ASSESSMENT OF PROGNOSIS

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Neonatal Marfan syndrome (nMFS) is the most severe form of Marfan syndrome and typically is associated with a rapidly deteriorating course and death within a few years of life. The majority of cases results from mutations in exons 24–32 of the fibrillin 1 (*FBN1*) gene. In addition, the physical findings are slightly different from those found in classic Marfan syndrome. Here we present three new cases of nMFS and report on a comprehensive literature review of the condition.

At birth Patient 1 had constricted pupils, enophthalmos, iridodonesis, dolichostenomelia with arachnodactyly and a murmur. At 2 months of age she had mitral valve prolapse, moderate aortic root dilation with a Z-score of 3.8 and bilateral ectopia lentis. At age 2.5 years she had her mitral valve replaced. At age 3 her aorta had enlarged to a Z-score of 11.6. She died at age 4.5 years following kyphoscoliosis surgery. She had a c.3218G>A mutation in exon 26 of the *FBN1*. Patient 2 presented at 1 month with mitral valve prolapse and regurgitation, a long face and trunk, deep-set eyes, arachnodactyly and long feet. Her aorta had become enlarged by age 18 months and at ages 5 and 9 years, her aortic Z-scores were 4.1 and 5.8, respectively. She has not had aorta replacement. As an infant, she also had a dislocated right lens, which was removed and replaced at age 18 months. At age 2 years she had

rod-placement back surgery. At age 11 she was stable. She has an IVS30 + 5G>A mutation in *FBN1*. Patient 3 had cardiovascular involvement as an infant and at 10 years had her aortic valve and aortic root replaced. At the age of 20 she had aorta replacement, and at age 25, a mitral valve replacement. On physical exam at age 32 she had down slanting palpebral fissures, enophthalmos, abnormal ears, pectus excavatum, severe scoliosis, dolichostenomelia, arachnodactyly and decreased muscle mass. She has a c.3130T>G mutation in exon 25 of *FBN1*.

We also report on 105 patients from the literature who we believe have had nMFS. Fifty-six patients had *FBN1* testing of which 44 had mutations in exons 24–32. The most common exon involved was exon 25 with 17 mutations. The most common abnormalities reported in these 56 patients included arachnodactyly (53), ear abnormalities (42), aortic root aneurysm (40), mitral valve prolapse with regurgitation (39), joint contractures (38), tricuspid valve prolapse with regurgitation (32), cardiomegaly (28), enophthalmos (23), redundant skin (23) and ectopia lentis (19). Thirty seven of these patients had died with death ranging from before birth to 17 years with a mean of death of 5 months. Of the 14 individuals who were living, their ages ranging from 2 months to 32 years (our third patient) with the mean age being 7.4 years. The other 59 patients had had no *FBN1* testing. The spectrum of abnormalities in this group was similar to the first group. Twenty nine of these patients had died between birth to 3 years with a mean of 7.2 months. Twenty children were alive at the time of being reported and their ages ranged from 12 days to 11 years with a mean age of 2.7 years. For both groups, there was also a large variety of other defects reported occurring at lesser frequencies.

At the present time there is no agreed on criteria for nMFS. We propose that the criteria include the presence of one or more systemic features of classic Marfan syndrome, aortic root dilation and major involvement of the mitral and/or tricuspid valves all before age 1 year, and an *FBN1* mutation within exons 24–32. While we are not completely satisfied with the name nMFS, since not all children are diagnosed with the condition in the neonatal period, we do not have a better term and recommend continuous use of this name. A genotype-phenotype correlation has not been made for mutations in exons 24–32. Such a correlation could account for the longer survival in some children.

CARDIOVASCULAR MANIFESTATIONS AND EVALUATION OF HIGH BLOOD PRESSURE IN WILLIAMS-BEUREN SYNDROME

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Williams-Beuren syndrome (WBS, OMIM 194050) is a neurocognitive disorder with multisystemic manifestations caused by a 1.55–1.83 Mb hemizygous deletion at chromosome band 7q11.23. Cardiovascular disease is present in approximately 75% of patients. Supravalvar aortic stenosis is the most common symptomatic structural lesion. Stenosis of many other medium and large sized arteries can also occur. High blood pressure (HBP) affects approximately 50% of patients, but is often under-recognized and not treated, when severe complications such as stroke and coronary artery disease can occur.

We collected and analyzed the clinical information on cardiovascular disease and HBP in 136 patients with WBS, with a mean age of 14.46 (range: 0.4–47 years). In a smaller cohort of 47 patients we recorded 24h Ambulatory Blood Pressure Monitoring (ABPM) with a Holter device. We measured serum levels of the renin-angiotensin-aldosterone axis and several markers of oxidative stress damage. We analyzed the findings of echocardiography, abdominal Doppler ultrasound (n = 47) and CT angiogram (n = 14). We treated hypertensive patients with the angiotensin II receptor blocker Losartan, and evaluated its efficacy after 12-months. Finally, we investigated the association of candidate genetic variants with HBP in order to identify possible modifiers of the cardiovascular phenotype.

Approximately 79% presented cardiovascular anomalies visible on echocardiogram, SVAS, peripheral pulmonary stenosis, coarctation of the aorta being the most frequent and more frequent and severe in males. Mitral and aortic valvular dysfunctions were more frequent than previously identified. Left ventricular hypertrophy and cardiac wall remodeling were frequent in cases of HBP. Stenoses of the abdominal branches of the aorta –celiac trunk, mesenteric or renal arteries, were present in 43% of patients and were best identified by CT.

Based on office BP measurements, 52.2% (71/136) had systolic HTN and 42.6% (58/136) had diastolic HTN, but only 30% were treated. When measured on a 24h ABPM, 33% (14/42) had a MBP above the 95th percentile, 42.9% for SBP (18/42), 31% for DBP (13/42) and 57.1% (24/42) for nocturnal BP, with loss of the nocturnal DIP in most patients and normal BP during daytime in 28.6%. Vascular stenoses were seen in all patients with HBP on CT. Office and ambulatory hypertension were significantly associated with an increased BMI. Plasma Renin activity and Aldosterone levels were normal in most patients, and not significantly different in those with HBP. Among measures of oxidative stress, only higher levels of MDA were observed in the hypertensive group. We identified genetic associations to HBP. Hypertension was less frequent with *NCF* deletion only in females. Higher frequency of HBP was associated with specific genotypes of the *PLCE1* gene, structural cardiovascular lesions were more frequent with variants in *PLCE1* and the *ELN* gene and the presence of valvular disease was associated with variants in *FBLN2*.

Finally, hypertensive patients treated with Losartan had a significant decrease of all blood pressure indexes without adverse effects. Early identification of HBP and vascular stenoses and understanding of the natural history of HBP may lead to improved management and a reduction in morbidity and mortality later in life in WBS.

CHARACTERIZATION AND NATURAL HISTORY OF GENITAL TRACT ANOMALIES AND TUMORS IN PROTEUS SYNDROME

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Proteus syndrome (PS) is a progressive disorder with asymmetric, disproportionate overgrowth of the body, connective tissue nevi, epidermal nevi, dysregulated adipose tissue, and vascular malformations caused by a somatic activating mutation in AKT1 [Lindhurst et al., 2011]. Certain tumors occurring before the second decade, specifically bilateral ovarian cystadenomas and parotid monomorphic adenomas, are part of the clinical diagnostic criteria for PS. We have observed that tumors of the testes and adjacent structures of both the testes are as prevalent as ovarian cystadenomas. While mostly benign, these tumors have been diagnosed as serous, borderline and low-grade carcinomas. Serous borderline cystadenomas of the ovary/paraovarian structures and testis/paratestis are rare in the general population in adults and especially children. Management of these borderline tumors, including staging and treatment is controversial, especially since the mechanisms of tumorigenesis are still being studied. The purposes of this study are to: 1) describe the prevalence, types, and management of genital tract tumors and anomalies in 65 patients seen at the NIH, and in 16 reported patients with PS, and to review the mechanisms, in addition to *AKT1* mutation, which may lead to development of malignancy in these tumors.

Of 65 patients with PS evaluated at the NIH (1997-present), 43 had evidence of genital tract anomalies or tumors on physical exam, surgical or autopsy pathology, and/or pelvic or testicular imaging. Age range at diagnosis was 1.5 – 52 yrs with a median age of 12 yrs for females and 15 years for males. Of 15 females, 4 (27%) had enlarged external genitalia; 4(27%) had infantile uterus; 7(47%) had enlarged/cystic ovaries; 4(27%) had uterine masses; 6(40%) had ovarian serous cystadenomas with 3 diagnosed $<$ 6 years, and the remainder at 14, 28 and 44 yrs (1- bilateral fallopian tube, 2 bilateral, 3 right-sided) and two of the children had borderline tumors with peritoneal implants. Of 28 males, 14(50%) had hydroceles; 7(25%) testicular enlargement/cysts; 11(39%) epididymal cysts/mass; 6(21%) testicular serous cystadenomas (one was borderline) with age of diagnosis at 1, 4, 13, 19, and two at 22 years, and one had yolk sac tumor at 5 years. Overall, 3/43 (7%) had borderline serous cystadenomas with one being described as low-grade serous carcinoma of the ovary. All were surgically removed, and one was treated with 3 courses of chemotherapy, followed by pelvic imaging and tumor markers.

Benign ovarian/paraovarian cystadenomas occur in 5–10% of postmenopausal woman, while borderline or malignant tumors are extremely rare in the general population. Similarly, these tumors of the testes/paratestes are rare with less than 50 reported, only 2 in children. These tumors are frequent in PS, occurring in 28% of our cohort and reported in 16 individuals with PS in the literature, with the majority developing during childhood. This increase in occurrence in PS suggests that somatic *AKT1* mutations play a significant causative role in cystadenoma development. Clonal expansion of mutant spermatogonial stem cells leading to formation of testicular tumors has been linked to "selfish" pathogenic mutations in the RTK/RAS pathway, which interacts with AKT [Goriely et al., 2009]. Understanding the genetic and molecular mechanisms that interact with the *AKT1* mutation may provide insight into natural history of these tumors and help direct management.

Workshop Session 13 MISCELLANEOUS TOPICS

CARDIAC ANOMALIES IN MONOZYGOTIC TWINS

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Monozygotic twinning is in itself an abnormality of early embryonic development. In addition to the obstetric risks associated with twinning, monozygotic twins face unique risks for birth defects related to unequal perfusion, disturbed laterality or asymmetric division of the inner cell mass. Cardiovascular anomalies are more common in MZ twins than in singletons or DZ twins and are frequently discordant, implying an epigenetic mechanism.

To further investigate the occurrence of cardiac malformations in twins, we studied all 174 pairs of twins (95 MZ including 73 pairs with twin-twin transfusion (TTT) or twin-twin disruption (TTD), 23 DZ, and 56 unknown), in the cohort of $>$3100 late miscarriages and stillbirths identified through the Wisconsin Stillbirth Service Program. These were compared to 337 twin pairs selected from Marshfield Clinic Twin Cohort (MCTC), who had been identified based on last name, date-of-birth, home address, billing accounts, and/or NLP from electronic health records (EHR). The MCTC twins included 39 pairs diagnosed with TTT from EHR data, 153 like sex and 145 opposite sex control pairs. Chart review of these 337 pairs identified an additional 6 TTT cases from the 153 like sex pairs ffor a total of 45 live born TTT pairs.

Among the stillborn MZ twins with TTT/TTD, 11/146 (7.5%) had structural cardiac defects including 8 with acardia, 1 with ectopia cordis, 1 with MCA including TOF, and 1 with ASD. Except one pair with an unknown MCA syndrome in both twins including TOF in one twin and acardia in the other, all were discordant. Cardiomegaly without structural heart disease. was reported in 12/146 (8.2%), all recipients of TTT. Among like sex twins of unknown zygosity, 3/112 had congenital heart defects, all of which were syndromic. None of the 44 MZ twins without TTT/TTD or the 46 DZ twins had cardiac anomalies.

Among the liveborn twins with TTT, 8/90 (8.9%) had structural heart defects including 7 with VSD (6 donors). All were discordant. 9/90 (all recipients) had cardiomegaly or cardiomyopathy at birth. 3 of these also had structural heart defects, 2 had a co-twin with VSD, and 1 had a co- twin with renal failure. Among like sex twin controls, excluding those diagnosed with TTT, 14/294(3.7%) had congenital heart disease including 11/294 with VSD. 3 pairs were concordant (1 with VSD alone, 1 with VSD/ASD, and 1 pair with valvular disease affecting different valves). Among opposite sex twin controls, only 5/

290 (1.7%) had congenital heart disease including 3/290 VSD (two syndromic).

We postulate that in addition to the risk for CHF and other complications of circulatory overload in the recipient, monozygotic twins are at increased risk for a spectrum of structural cardiac malformations which reflect a smaller number of cells affecting not only the cardiac primordium, but also overall fetal and placental growth. In severe cases the affected twin has a poorly developed heart and inadequate circulation, eventually becoming acardiac, while in less severe cases, the smaller infant has not only deficient septal growth sometimes resulting in VSD, but also less angiogenesis compared to the co-twin and becomes the "donor". Our observations of low concordance for cardiac defects and greater weight differential for pairs with structural heart disease among both liveborn and stillborn pairs with TTT relative to like sex controls without TTT support this hypothesis.

LANDSCAPE OF PLEIOTROPIC PROTEINS CAUSING HUMAN MALFORMATION SYNDROMES

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Pleiotropy is the phenomenon by which the same gene can result in multiple phenotypes. Although this occurrence has been known for over 100 years, its contribution to rare genetic diseases has only really been appreciated since the boom in genetic discoveries precipitated by the advent of next-generation sequencing strategies. In the past year alone, the percentage of genes with phenotype-causing mutations associated with >1 phenotype in OMIM has increased from 25% to 32%. Pleiotropic proteins are therefore emerging as an important contributor to both rare and common disorders, but little is known of the mechanisms underlying pleiotropy, particularly with how they relate to congenital malformation syndromes.

To examine the contribution of this phenomenon to congenital malformation syndromes we referred to the 7th edition of Smith's Recognizable Patterns of Human Malformations and compared it to OMIM data accessed in April 2017. Each listed syndrome was queried within OMIM and PubMed to identify the currently understood molecular etiologies (all genes in which pathogenic variants have been found to cause the syndrome). Syndromes associated with chromosomal abnormalities and disorders caused by contiguous gene deletions were excluded. In total, 231 syndromes were examined. Of these, 10 remained 'unsolved' (defined as molecular cause unknown).

In total, 402 unique genes had been implicated in Smith malformation syndromes; 72 of the 'solved' 221 syndromes (32%) were associated with pathogenic variants in more than one gene (genetic heterogeneity). Of the 402 genes, 271 (67%) were associated with more than one phenotype within OMIM; a rate that is twice as high as observed for all genes listed in the OMIM dataset (32%). Forty-eight of the 270 genes (18%) were associated with more than one distinct malformation syndrome within Smith's. Not surprisingly, the rates differed amongst categories of syndrome; being highest in neurological phenotypes (75%) and lowest amongst storage disorders (40%). The mechanisms of pleiotropy also varied tremendously at the protein level and could be sorted into four different categories: (1) distinct location of the mutations in different domains of the protein (e.g., *FGFR3*), (2) quantitative differences in the level of protein (e.g., *POMT1*) (3) qualitative effects on the protein (e.g., GOF vs LOF; *FLNA*), and (4) other influences on the protein (i.e., same mutation but different phenotypes due to unknown contributions; *LMNA*).

These results provide a better understanding of the pleiotropic proteins related to congenital malformations. They further support evidence that pleiotropic proteins are more likely to be 'essential' in human development and will be used to guide future discovery efforts for unsolved malformation syndromes.

MOUSE KNOCK-IN OF A PREMATURE STOP ALLELE OF *FRIZZLED-2* RECAPITULATES HUMAN AUTOSOMAL DOMINANT OMODYSPLASIA PHENOTYPES

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Autosomal dominant omodysplasia is a rare skeletal dysplasia characterized by short humeri, radial dislocations, short stature, frontal bossing, small nose with broad nasal tip, and long philtrum. We have previously reported on a mother (proband) and daughter with omodysplasia. Whole exome sequencing was performed on both affected individuals and the parents of the proband (Saal et al., 2015). A nonsense mutation in *FZD2* was identified (c.1644G>A) which causes a coding change in the FRIZZLED2 protein. This missense mutation codes for a premature stop codon at position 458, rather than a tryptophan residue (p.Trp548*). This is predicted to create a truncated protein with the loss of 17 amino acids including a portion of the consensus DISHEVELLED (DVL) binding sequence (KTxxxW) required for Wnt signal transduction. Frizzled proteins act as receptors for Wnt proteins which play a critical role in cell polarity. Wnt pathway genes have an important role in skeletal and craniofacial development. We have previous shown the truncated FZD2 protein is stable and can negatively affect canonical Wnt signaling. We have now gone on to test these models *in vivo* to formally address pathogenicity of this variant.

While the $FZD2^{W548^*}$ patients exhibited autosomal dominant omodysplasia, a previously published deletion of the *Fzd2* gene in mouse exhibited incompletely penetrant autosomal recessive cleft palate. Here, we have utilized CRISPR-mediated transgenesis to generate an allelic series of mouse mutations near the human variant. We generated two germline mouse alleles with small deletions, $Fzd2^{p.W552fs^*60}$ and $Fzd2^{p.W552del}$. Homozygotes for each allele survive embryonic development at normal ratios but exhibit a >90% penetrant recessive phenotype of cleft palate, a wide/short snout and perinatal lethality. Heterozygotes have fused palates, but $Fzd2^{p.W552fs^*60/wt}$ mice exhibit an incompletely penetrant dominant phenotype of a wider snout and

failure to thrive, with only 50% surviving to weaning. We also generated embryos mosaic for humanized *Fzd2*^{W553*} knock-in; a "F0-CRISPR" approach of harvesting injected embryos at E17.5 revealed five edited embryos with the *Fzd2*^{W553*} mutation. All F0 embryos exhibited cleft palate, smaller weights, and shortened limbs, consistent with *FZD2*^{W548*} patients. This *in-vivo* replication of clinical features seen in patients represents an approach which may be used to further investigate the mechanism of the autosomal dominant omodysplasia phenotypes and validates the utility of CRISPR knock-in mice as a tool for demonstrating pathogenicity of human genetic variants. Ongoing studies are focused on clarifying the mechanism of dominant inheritance via the role of DVL-FZD2 binding mediating canonical Wnt signaling in the face, as well as the effects of genetic background on the observed phenotypes.

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