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Supplemental Data

The Genomics of Arthrogryposis, a Complex Trait: Candidate Genes and Further Evidence for Oligogenic Inheritance

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SUPPLEMENTARY INFORMATION

CLINICAL AND MOLECULAR INFORMATION FOR CANDIDATE, OLIGOGENIC, AND PHENOTYPIC EXPANSION FAMILIES

PROBABLY CAUSATIVE ARTHROGRYPOSIS GENES

RYR3

BAB7845 (Figure 2A-D)

BAB7845 is a 6 month old female who was diagnosed with arthrogryposis multiplex congenita. She had global hypotonia, facial dysmorphism including long eyelashes, right ptosis, left strabismus, thin lips, diffuse hyporeflexia and left hip dislocation. Musculoskeletal system evaluation showed contractures that were more severe in the fingers and toes and less severe in the elbows, knees and ankles (Figure 2B). Her parents were first degree cousins and she had one unaffected male sibling. Exome on the proband revealed a compound heterozygous mutation in *MYO18B* (NM_032608:c.[2879C>T];[3397C>T]:p.[Ala960Val];[Arg1133Trp]) and a homozygous variant in *RYR3* (NM_001243996:c.2486G>A:p.Arg829His). All three variants were within highly conserved regions and prediction scores were pathogenic for all three variants. The *RYR3* variant has been reported as heterozygous in 312 individuals and as homozygous in 1 individual in gnomAD. The *MYO18B* c.2879C>T variant was reported as heterozygous in 122 individuals and as homozygous in 1 individual in gnomAD and the c.3397C>T variant was reported as heterozygous in 15 individuals. Echocardiogram and spine X-ray were performed to evaluate for possible cardiomyopathy and Klippel-Feil deformity findings which were reported in *MYO18B* mutation carrier individuals.[1; 2] Echocardiogram

revealed a patent foramen ovale but no findings of cardiomyopathy, and spine X-ray showed kyphoscoliosis without Klippel-Feil anomaly.

PAED187 (Figure 2E-F)

This female proband is the second child of her unrelated healthy parents. Her sister is healthy as well. An older maternal half-sister has spastic paraplegia of the lower limbs attributed to a preterm birth and hypoxemia in the perinatal period. The remainder of the family history is unremarkable. The pregnancy was complicated by an abnormal ultrasound with bilateral club foot and echogenic intracardiac focus. She was born at term with normal anthropometric measurements. The diagnosis of bilateral club foot was confirmed; in addition she had contractures in all fingers of both hands. Conservative treatment of club feet, including tenotomy of Achilles tendon, did not resolve the severe bilateral club foot and equinus deformation. Eventually she underwent surgical intervention of equinus deformity at the age of 16 months. The finger contractures resolved well under physiotherapy. After the age of 10 months, global developmental delay became evident: the proband began crawling at 12 months, sitting at 16 months and walking unsupported at 25 months. She spoke her first words with 32 months. At the age of 3 years and one month she spoke 30 words with slurred speech. Intellectual development was also not age-appropriate. At the age of 2 years, she had repeated episodes resembling seizures over a period of four months. Multiple EEGs during this period were normal, and these episodes disappeared without treatment. Also starting at age of two years she developed an enteropathy with diarrhea up to ten times a day, which led to poor weight gain.

At the most recent examination at the age of 3 years and one month, evaluation was notable for a friendly girl with normal measurements (length -1.6 SD; weight -1.0 SD; OFC -0.4 SD). She was

able to walk short distances, but she fell frequently. Muscle weakness or other signs of muscle disease were not present, but she demonstrated muscular atrophy of the lower legs and limited movements of the feet that were caused by the surgical treatment of club feet. The fingers and all other joints did not show contractures, but fine motor skills were not age appropriate. She had dysmorphic features, such as a broad forehead, epicanthus, a broad and depressed nasal bridge, and small and abnormally spaced teeth. The fingers were small and short, as were the toes. The toe nails were dysplastic, and in addition she had 2-3 toe partial syndactyly. Extensive clinical, metabolic and genetic investigations were normal, except CGH-array analysis, which revealed a 144kb microdeletion in 6q22.31 of unknown significance. Trio exome identified compound heterozygosity for *RYR3* missense (NM_001036.3:c.2000A>G:p.Asp667Gly) and splice site donor (intron 83, c.11164+1G>A) alterations consistent with an autosomal recessive mode of inheritance [minor allele frequency (MAF) < 0.01]. Segregation analyses showed parents are heterozygous for each variant and the unaffected sister is wild type for both variants.

BAB8988 (Figure 2G-I)

BAB8988 is a 15 year old female, who was born to first degree cousin parents. She has four elder healthy siblings. At birth, she was found to have contractures in the right elbow and left wrist, and left hip dislocation which was surgically corrected at 2 years of age. On exam, she had a prominent forehead, blue sclera, high arched palate, bilateral ulnar deviation in both hands, flexion contractures in the elbows, fingers and toes, and scoliosis (Figure 2H). Diagnostic work up including a karyotype was normal. Trio exome revealed a deleterious, homozygous variant, NM_001243996:c.8939G>T:p.Arg2980Leu, in *RYR3*. This variant was reported in 19 individuals in gnomAD and 7 individuals in the Center for Mendelian Genomics (CMG) cohort as heterozygous.

MYOM2
BAB8905 (Figure 3A-C)

BAB8905 was born to a 27 year old G2P1 mother at term via standard vaginal delivery. She stayed in the newborn intensive care unit (NICU) for 10 days due to feeding problems. Birth weight was 3090 g (26^{th} percentile). She achieved head control at 2 months of life. She was not sitting unsupported by age 18 months. Parents did not report consanguinity; however they were from the same small village. The mother's first pregnancy was a spontaneous miscarriage at the 10^{th} week of gestation. Physical exam was performed at 18 months old. Growth parameters showed weight: 5.4 kg (-5.5 SD), height: 55 cm (-7.7 SD) and OFC: 41 cm (-4.1 SD). The proband had mild dysmorphic facial features including a depressed nasal root, small mouth and micrognathia. Musculoskeletal exam showed contractures in bilateral fingers and knees, bilateral hip dislocation and rocker bottom feet (Figure 3B). Cranial ultrasound at birth, ophthalmologic evaluation and hearing tests were unremarkable. Exome sequencing revealed a homozygous nonsynonymous variant in Myomesin-2, *MYOM2*, (NM_003970:c.621C>G:p.Ser207Arg). This amino acid is highly conserved throughout species and the amino acid change was predicted to be deleterious. This variant was reported 15 times in a heterozygous state in gnomAD and is not present in other public databases.

IN076 (Figure 3D-G)

We ascertained a consanguineous couple. The first pregnancy resulted in intrauterine fetal demise at four months of gestation. The second pregnancy was a preterm delivery at 28 weeks of gestation with intrauterine growth restriction (IUGR); the child succumbed to respiratory distress syndrome. The third pregnancy was a spontaneous abortion at six weeks of gestation. No fetal anomalies were documented in the first three pregnancies. The following fourth pregnancy, the

couple gave birth to a male baby weighing 1.96 kg and 43 cm in length. Antenatally, the fetus was noted to have echogenic bowel, IUGR, and dilatation of right atrium and right ventricle. Karyotype of the baby was normal. Postnatal echocardiography revealed a patent ductus arteriosus (4 mm). The baby had global developmental delay and did not achieve any developmental milestones. Eventually the baby died at six months of life. The fifth pregnancy was aborted at 24 weeks of gestation due to multiple congenital anomalies: antenatal ultrasonography at 21 weeks of gestation revealed cardiomegaly, ventricular septal defect, pulmonary valve reflux, hydrops fetalis, severe biventricular dysfunction, poor contractility, atrioventricular valve reflux and mild pericardial effusion. Following medical termination, postnatal examination was carried out in another center. The fetus weighed 575 g (normal), measured 23 cm (normal) in crown rump length, and had a head circumference of 22 cm (normal). Pleural and pericardial effusions were noted. Bilateral hypoplasia of the lungs was observed. Echocardiogram showed dilated atrium and hypertrophic ventricles. Ascites and hepatosplenomegaly were noted. Microscopic examination of fetal organs revealed calcification of the liver, brain and placenta.

The proband was the product of the sixth pregnancy. Antenatal ultrasonography at 19 weeks of gestation revealed hydrops fetalis, global myocardial hypokinesia, ascites, pleural and pericardial effusion, fetal akinesia and echogenic kidneys in the fetus. Following termination of pregnancy at 20 weeks of gestation, fetal autopsy was carried out. Fetal karyotype as well as parental karyotypes revealed a pericentric inversion of chromosome 9. A detailed postnatal evaluation including perinatal autopsy, radiographic evaluation, MRI of the brain and histopathological examination was performed. The index (male) weighed 380 g (normal), measured 23 cm (normal) in length, and had a head circumference of 18 cm (normal). The fetus had dysmorphic

facies with telecanthus, a depressed nasal bridge, microstomia and retrognathia. Generalized subcutaneous edema was noted. A short neck with protuberant abdomen was seen. Bilateral multiple joint contractures were noted across the shoulder, elbow, hip and knee joints. Bilateral pterygia were observed across the axillae (Figure 3E). Muscle mass was grossly reduced in all four limbs. On internal examination, lung hypoplasia was noted. Heart and major vessels were grossly normal. Abdominal visceral organs and intestines were normal. External and internal genitalia were normal. Brain examination revealed partial agenesis of the corpus callosum. Radiographs of the fetus showed a normal skeleton. Magnetic resonance imaging of the fetal brain was suggestive of corpus callosum agenesis which was confirmed by autopsy. Skeletal muscle (triceps), cardiac muscle (right ventricle), liver tissue, blood vessel (brachial artery), somatic nerve (radial nerve) and different parts of the brain (cerebrum, thalamus and cerebellum) were taken for histopathology examination. Hematoxylin and eosin (H&E) of all tissues and Periodic acid–Schiff (PAS) staining of liver and skeletal muscle was performed. Skeletal muscle showed perivascular and perimysial lymphocytic infiltrates on H&E stain. Lymphocytic infiltrates were also noted in the cardiac muscle and nerve as well. PAS stain was negative for both liver and skeletal muscle. No significant histopathological abnormality was noted in brain tissues. Exome sequencing of the proband revealed a stopgain variant, NM_003970:c.2797C>T:p.Gln933*, in *MYOM2* in homozygous state. This variant is seen in gnomAD in 27 individuals as heterozygotes. Another nonsynonymous homozygous missense variant NM_016381.5:c.553G>A:p.Asp185Asn in *TREX1* was noted in proband. This variant is not present in publically available databases like 1000 Genome project, gnomAD database and Exome Variant Server in homozygous or in heterozygous state. The variant c.553G>A occurs at amino acids conserved across various species. *In-silico* tools are consistent in predicting the

variant to be damaging the TREX1 protein function. However, segregation analysis was not performed for the variant. Pathogenic variants in *TREX1* are known to cause Aicardi-Goutieres syndrome 1, dominant and recessive (MIM: 225750); Chilblain lupus, autosomal dominant (MIM: 610448), Vasculopathy, retinal, with cerebral leukodystrophy, autosomal dominant (MIM: 192315) and Systemic lupus erythematosus, susceptibility to, autosomal dominant (MIM: 152700). Inflammatory myopathy is a known feature in Aicardi-Goutieres syndrome 1 and histopathological examination of multiple tissues including muscles of the proband showed inflammatory infiltrates.[3] However, inflammatory myopathy has been reported with other causes of arthrogryposis multiplex congenita.[4; 5] Calcification of basal ganglia, a characteristic feature of Aicardi-Goutieres syndrome 1, was not observed in the proband.[6] However, it was documented in the previous (5^{th}) pregnancy loss. Hence, there is possibility of a blended phenotype due to two recessive disorders in this family.

ABCA7
BAB6807-BAB6808 (Figure 4A-B)

BAB6807 and BAB6808 are two affected siblings born to a consanguineous union with a clinical diagnosis of distal SMA. Both siblings had distal contractures and DTRs were unobtainable. The older male sibling, BAB6808, additionally had DD/ID. Diagnostic work up including CPK level, *SMN1-2* gene testing and brain MRI were normal. We performed exome on both affected siblings and found three variants of interest. The female affected sibling, BAB6807, was found to have a homozygous stop gain mutation (NM_057166:c.619C>T:p.Gln207*) in the known *COL6A3* gene (Figure 4A). This variant has not been reported in the literature previously. Segregation studies showed that the remaining members of the family including the affected sibling, BAB6808, are heterozygous for the variant. Exome analysis additional showed

(NM_019112:c.5092C>T:p.Arg1698Trp) in *ABCA7*. This variant was reported in 8 individuals in gnomAD as heterozygous. BAB6808 who has additional DD/ID phenotype, was found to carry a deleterious homozygous variant (NM_015339:c.775A>C:p.Asn259His) in *ADNP*. Functional prediction scores for shared homozygous *ABCA7* and *ADNP* variant were deleterious and the affected amino acid residue was highly conserved between species. Sanger segregation showed that the parents were heterozygous and unaffected female sibling was wild type for the variant. Sanger segregation study for *ADNP* revealed that only the proband carries this variant in the homozygous state and the rest of the available relatives are heterozygous.

BAB10708 (Figure 4C-D)

BAB10708 is a 4 year old male born to healthy unrelated parents. He had an older unaffected female sibling. Anthropometric measurements at 4 years old revealed weight: 11.5 kg (-3SD), height: 91 cm (-2.7SD) and head circumference: 48 cm (5^{th} percentile). Physical examination revealed age appropriate speech and cooperation. He had down slanting palpebral fissures, high arched palate, 4x4 cm hyperpigmentation in left chest and pectus excavatum. Restrictions in bilateral wrists, knees and ankles and 2-5^{th} camptodactyly were noticed. He had right cryptorchidism which was confirmed by testicular ultrasound. Other diagnostic work up including echocardiogram, ophthalmologic evaluation, urinary ultrasound and karyotype were unremarkable. Trio exome sequencing revealed *de novo* variants in two known genes (*TPM2* and *SPEG*) and a compound heterozygous variant in *ABCA7* (Figure 4C). Both c.3076C>T and c.4045C>T residues were highly conserved through species and prediction scores were highly deleterious for both amino acid changes. *De novo* c.620_631dup non-frameshift insertion in *TPM2* is not reported in public databases. Monoallelic variants in *TPM2* cause broad spectrum of neuromuscular disorders including arthrogryposis multiplex congenita type 1, distal

arthrogryposis type 2B, nemaline myopathy type 4 and CAP myopathy type 2 (MIM: 108120, 601680, 609285 and 609285, respectively). c.9575C>A in *SPEG* is not reported in public databases however c.9575C>T was reported in two individuals in gnomAD. The region is partly conserved and the amino acid change is predicted to be deleterious by multiple prediction tools. Biallelic variants in *SPEG* are known to cause centronuclear myopathy 5 (MIM: 615959) however, we believe *de novo* variant in *SPEG* in this proband is also contributing to neuromuscular phenotype.

ERGIC1 (Figure 5)

BAB8802 is a 1 month old female who was born to first degree cousin parents. She is the first child of the parents and there are no children with similar findings in the family. The pregnancy was uncomplicated, with normal fetal movements and a normal ultrasound evaluation. She was born at term via C-section with a birth weight of 2660 g (7^{th} percentile). She was found to have *pes equinovarus* (PEV) and ulnar deviation of both hands at birth (Figure 5B). Anthropometric measurements were age appropriate: weight 3340 g (9^{th} percentile), height 51 cm (17^{th} percentile), and OFC 35 cm (9^{th} percentile). She had retromicrognathia and a capillary hemangioma covering the forehead and nose. She had normal tone and had social smile, consistent with age-appropriate development. Laboratory studies including a complete blood count and creatine phosphokinase (CPK) levels were normal. Imaging work-up including brain MRI, hip ultrasound, abdominal ultrasound, hearing screening, and spinal ultrasound were unremarkable. Echocardiogram showed a secundum type atrial septal defect (ASD) and patent foramen ovale (PFO). Trio exome showed a homozygous change in *ERGIC1* (NM_001031711:c.782G>A:p.Gly261Asp). This variant was predicted to be deleterious by all mutation prediction algorithms and the amino acid was in a highly conserved region. This variant

was reported neither in public nor in internal databases. Segregation study showed that the parents were heterozygous and the proband was homozygous as expected.

SPTBN4 **(Figure 6)**

BAB8691 was first evaluated at 13 months of age. Her parents were first cousins, once removed. Family history was notable for two deceased older siblings. The first child was male and died at 1 week of life due to respiratory problems. He had facial dysmorphic features and contractures in his hands and feet. The second child was a male individual and died at 7 months. He had lung malformation but parents do not report any contractures in his extremities. The proband had abnormal prenatal screening: she was found to have hydrocephaly on ultrasound and amniocentesis was recommended, but parents declined genetic testing. She was born at term via C-section with a birth weight of 2850 g (14^{th} percentile). She was intubated soon after birth due to respiratory distress and hospitalized for 3 months in NICU. She required a gastric tube for feeding. She developed seizures and was started on antiepileptic treatment. Her development was severely delayed and she did not achieve head control. She weighed 9340 g (33^{rd} percentile), with a length of 63 cm (-3.9SD) and OFC of 37 cm (-6.6 SD). She was hypotonic. She had dysmorphic facial features including a short neck, short nose, anteverted nares, bilateral epicanthus and microphthalmia, sparse eyelashes and a low posterior hairline. She had distal joint contractures, kyphoscoliosis and bilateral rocker bottom foot deformity. Brain MRI showed enlarged lateral ventricles. Abdominal ultrasound and echocardiogram were unremarkable. Ophthalmological evaluation revealed pale optic discs. CPK level was normal. She failed two hearing evaluations. The subject died at 2 years old due to respiratory failure. Trio exome revealed that she has a homozygous missense variant in *SPTBN4* causing NM_020971:c.6433G>A:p.Ala2145Thr in a well conserved region of the protein (Figure 6D). This variant was reported neither in the public databases nor internal databases. Prediction scores

are mostly deleterious for this variant. Sanger sequencing showed that parents are heterozygous and proband is homozygous as expected for an autosomal recessive disease trait.

MULTILOCUS PATHOGENIC VARIATION FAMILIES

FBN2-COL6A3 (Figure S6)

BAB8600 is a 15 month-old female who was born to an affected 33 year old mother (BAB8601). The parents of the proband were not consanguineous by report; the mother had 32 Mb of AOH, and the father had 28.3 Mb total AOH by BafCalculator, whereas the index did not have AOH blocks larger than 3 Mb. The index, BAB8600, had a Marfanoid habitus including a high arched palate and arachnodactyly, and mild restriction at the elbows and knees and bilateral PEV deformity. The mother was more severely affected and had additional neuromuscular findings of scoliosis and 2-5th finger camptodactyly (Figure S6). We performed trio exome and focused on shared variants between the mother and index. Novel variants in two known genes, *FBN2* and *COL6A3*, were found in the family. The variant in *FBN2* was a *de novo* missense change (NM_001999:c.4094G>C:p.Cys1365Ser) in the mother that was transmitted to the affected child. The *COL6A3* variant (NM_057167:c.367G>A:p.Val123Met) was a homozygous missense change in the mother and heterozygous in the affected child and maternal grandparents. Mutations in *FBN2* are known to cause autosomal dominant contractural arachnodactyly (MIM: 121050). Various mutations in *COL6A3* are known to cause several neuromuscular phenotypes including dominantly and recessively inherited Bethlem Myopathy 1 (MIM: 158810). Additional features in the mother, scoliosis and camptodactyly, can potentially be parsimoniously explained by her homozygosity for the identified variant in *COL6A3*.

NEB-MID1IP1 (Figure S7)

BAB8397 is an 11 year-old male born to a consanguineous marriage with multilocus pathogenic variation; rare variants were identified in one known disease gene and one candidate gene. He

had contractures in bilateral wrists, elbows, knees and 2-5th fingers with bilateral PEV. Trio exome showed a homozygous splice site variant (c.19101+5G>A) in *NEB* and a hemizygous nonsynonymous variant (NM_001098791:c.297C>G:p.Asn99Lys) in *MID1IP1* (Figure S7). Bohm *et al.* reported an infant with neonatal hypotonia, severe arthrogryposis and respiratory insufficiency who died at 10 day of life. The proband was found to have our variant, c.19101+5G>A, and nonsense variant p.Tyr1858* change. Muscle biopsy showed nemaline bodies, fiber size variability and type I fiber predominance.[7] Lethality in the subject can be explained by nonsense variant in the second allele. Segregation studies showed that parents are heterozygous for *NEB* variant and mother is heterozygous for the *MID1IP1* variant as expected with AR and X-linked Mendelian disease traits.

DRG1-TANC1-BRWD3

BAB8807 presented at age 4 years, having been born to a first degree cousin union with features of arthrogryposis including 5th finger camptodactyly and bilateral PEV, and developmental delay. He was found to have a dysplastic corpus callosum and abnormal visual evoked potentials (VEP) and electroretinogram (ERG). He had a normal echocardiogram, hearing test, and karyotype. We found three potential candidate genes: a homozygous nonsense mutation (NM_004147:c.118C>T:p.Arg40*) in *DRG1*, a homozygous missense variant in *TANC1* (NM_033394:c.2830C>T:p.His944Tyr), and a hemizygous splice site mutation in *BRWD3* (NM_153252:c.592-3T>C) which is known to cause developmental delay/intellectual disability (DD/ID) by deleterious variants (MIM: 300553). The *DRG1* variant was in a highly conserved region and not reported in public databases. The *TANC1* variant was in a highly conserved region as well, and was reported in the ExAC database 12 times in a heterozygous state, but no homozygous variation was reported. Prediction scores were damaging/disease causing by all algorithms. Segregation studies revealed that the parents were heterozygous and the proband

homozygous for both *DRG1* and *TANC1* variants, as expected for autosomal recessive disease traits; the mother was heterozygous and the proband hemizygous for the *BRWD3* variant. *DRG1*, Developmentally Regulated GTP binding protein 1, had not been shown to cause any disease phenotype previously. *DRG1* has been shown to have increased expression with feeding in Salmon fish and during myogenesis as well.[8] Expression studies showed that *DRG1* has a higher expression in fast muscle and eye which may explain the neuromuscular and eye findings in our subject.[8] Dysferlin was shown to bind alpha-tubulin and interacts with microtubules which are required for myogenesis and myotube elongation.[9] Additionally, Schellhaus *et al.* recently showed that DRG1 binds to microtubules, promotes microtubule polymerization and bundling and stabilizes them.[10] *TANC1* is not linked to any human phenotype previously. Granot-Hershkovitz *et al.* reported a female with psychomotor delay who was found to have a chromosome 2 inversion, inv(2)(p15;q24.2) disrupting the *TANC1* and *RBMS1* genes.[11] Avirneni-Vadlamudi *et al.* showed that *TANC1* is biologically relevant for myoblast fusion/development and has a critical role in rhabdomyosarcoma development as a downstream effector in the setting of the PAX-FOXO1 fusion oncoprotein.[12] Thus, we propose that homozygous missense variant in *TANC1* may explain the developmental delay and/or neuromuscular phenotype in our proband. *BRWD3* is known to cause Mental retardation, X-linked 93 (MIM: 300659) and the variant is likely contributing to proband's phenotype.

SPEG-MYOM3-CIT

BAB8532 is a 3 months old girl who presented with contractures involving the elbows, fingers and knees, as well as hip dislocations and contractures at birth. She also had DD/ID and ophthalmoplegia. The parents were not related but they were from the same small village. Trio exome sequencing revealed three candidate genes: two candidate genes and one known myopathy gene. She had a homozygous missense change,

NM_005876:c.6971T>A:p.Ile2324Asn, in *SPEG*. This region is highly conserved within orthologs and the variant was reported in 14 individuals in CMG cohort and 159 individuals in gnomAD. The *SPEG* gene was linked to centronuclear myopathy-5 (MIM: 615959). Prediction scores were deleterious by all algorithms. She was also found to have compound heterozygous variants in the Myomesin 3 gene (*MYOM3*) (NM_152372:c.1684G>A:p.Val562Ile and intronic [NM_152372.3:c.3534+56C>T]). The intronic region was highly conserved but the exonic variant, c.1684G>A, was not in a conserved region. The intronic variant was quite common and reported in 103 individuals as homozygous in gnomAD. Segregation studies for both genes revealed that the parents were heterozygous for the *SPEG* variant and heterozygous for each variant of the *MYOM3* gene. *MYOM3* has not been previously linked to any disorders. Only two studies involving 5 families have been reported for *SPEG*.[13; 14] Individuals with *SPEG* biallelic variants typically have myopathy, weakness, cardiomyopathy, and ophthalmoplegia. Our subject has muscle weakness and ophthalmoplegia. Myomesins (MYOM1, MYOM2 and MYOM3) are the principal components of the M-band that cross-link filamin-C and titin filaments in the middle of the sarcomere.[15; 16] Similar to titin, myomesin is a molecular spring whose elasticity guards the stability of the sarcomere.[17; 18] *MYOM3* is mainly expressed in intermediate fibers (type IIa) of skeletal muscle, *MYOM2* is mainly expressed in fast fibers, whereas *MYOM1* is expressed in all muscle fibers.[16] Interestingly, contractures were not a common feature in subjects with *SPEG* mutations, with the exception of a single person who was reported to have hip contractures. Thus, we believe *MYOM3* is contributing to the contracture phenotype in our subject. Our proband additionally has DD/ID which is not a feature in individuals with centronuclear myopathy-5, thus we searched for a molecular etiology for the observed DD/ID. We found a *de novo* deleterious heterozygous variant, NM_007174:c.2651A>C:p.Gln884Pro, in

CIT causing a nonsynonymous change. Biallelic variants in *CIT* have been shown to cause microcephaly and DD/ID (MIM: 617090).[19-21] We propose that the identified monoallelic *de novo* variant is likely explaining the DD/ID and microcephaly in our proband. We have previously demonstrated evidence for monoallelic mutations in disease genes associated with phenotypes segregating in a recessive mode of inheritance.

KLHL7-HOXA11-TNRC6C

BAB8688 presented at 7 months old with contractures of multiple joints. She was born at 35 weeks gestation via C-section with a birth weight of 2350 g. Pregnancy was complicated by polyhydramnios and IUGR with normal fetal movements. Anthropometric measurements at 7 months revealed height: 57 cm (-3.7 SD), weight: 4160 g (-4.3 SD) and OFC: 38.5 cm (-3.7 SD)]. Physical examination revealed hypotonia, microcephaly, failure to thrive, global developmental delay, arthrogryposis multiplex congenita, bilateral kidney stones. Parents were second degree cousins, and the proband had one healthy elder brother. Cranial ultrasound revealed hydrocephalus, echocardiogram showed a patent foramen ovale, and abdominal ultrasound displayed multiple kidney stones bilaterally. Her CPK level and routine bloodwork were unremarkable. She died at 14 months of life due to respiratory infection. Trio exome showed homozygous missense variants in *KLHL7* (NM_001031710:c.1258C>T:p.Arg420Cys) and *HOXA11* (NM_005523:c.304G>A:p.Val102Met) which are within the same AOH block. Both variants were predicted to be disease causing and embedded within highly conserved regions. The *KLHL7* variant has been previously reported in a Turkish individual.[22] Both variants were reported as heterozygous in two individuals in gnomAD. Additionally, Bruel *et al.* reported *KLHL7* mutations in Bohring-Opitz syndrome (BOS) subjects and highlighted the phenotypic similarity between cold-induced sweating syndrome (CISS3) and BOS.[23] The constellation of observed features in our proband was consistent with the BOS phenotype. This subject had an

additional homozygous missense variant in a highly conserved region in Trinucleotide repeat-containing gene 6 (*TNRC6C*) which was not previously linked to any disease (NM_018996:c.1022G>A:p.Gly341Glu). This variant was reported as disease causing by all mutation prediction algorithms. This gene is globally expressed with highest expression in brain per Genotype-Tissue Expression (GTEx) portal. Segregation studies for all three variants showed that the parents are heterozygous and the index is homozygous for the variants. TNRC6C plays a role in RNA-mediated gene silencing by micro-RNAs. This gene was not associated with any disease previously. Based on the mutation prediction scores, conservation and expression pattern, we propose that this gene may be responsible for the hydrocephalus phenotype in our proband that cannot be explained by *KLHL7*. Our subject additionally has a homozygous missense variant in *HOXA11*. Heterozygous frameshift mutation in this gene was initially linked to radioulnar synostosis with amegakaryocytic thrombocytopenia 1 in two families with AD inheritance pattern (MIM: 605432).[24] Radioulnar synostosis was a persistent feature in all mutation carriers but thrombocytopenia was not present in all affected individuals. Our subject has limited elbow movements but X-ray during the infantile period did not confirm the radioulnar synostosis. The family later declined repeat X-rays. *KLHL7* and *HOXA11* are embedded within the same region of AOH, and we propose that *KLHL7*, and potentially also *HOXA11*, are contributing to the observed phenotype.

ECEL1-FLII

BAB7710 is a 5 years old male, born at 28 weeks' gestation via C-section due to preterm labor with a birth weight of 2750 g. His parents were first degree cousins, and he is their only child; family history is notable for maternal cousins described as having a similar condition. The proband has down-slanting palpebral fissures, right congenital ptosis, contractures in all joints, severe kyphoscoliosis and cryptorchidism. He is tracheostomy-dependent. Echocardiogram and

abdominal ultrasound were normal. EMG/NCS showed myopathic changes and fibrosis. He was initially diagnosed with Escobar syndrome and *CHRNG* mutation screening was inconclusive. The index underwent exome sequencing which revealed two potential candidates. He had a homozygous frameshift variant in exon 2 of the *ECEL1* gene (NM_004826.3: c.505_529del;p.Gly169Serfs*26) and a homozygous nonsynonymous change (NM_001256265:c.2590C>T:p.Arg864Trp) in Flightless I Drosophila Homolog (*FLII*). This amino acid was highly conserved throughout orthologs. The *ECEL1* frameshift variant has been reported in only one individual as heterozygous in gnomAD, and the *FLII* variant has not been reported in any of the publicly available and internal databases. All computational tools predicted the *FLII* variant to be disease causing. Sanger PCR confirmed that both parents are heterozygous, and proband is homozygous for both variants. Deleterious changes in *ECEL1* are known to cause distal arthrogryposis type 5D (MIM: 615065), and it is the second most common mutated gene within our cohort. Our variant was not previously reported in subjects with *ECEL1* mutation. *FLII* is not linked to any human phenotype. It is globally expressed but highest expression is in the muscle tissue. *FLII* encodes a protein with a gelsolin-like actin binding domain and an N-terminal leucine-rich repeat-protein protein interaction domain which is similar to a Drosophila protein involved in early embryogenesis and the structural organization of indirect flight muscle. *TRPV4* which is a known gene for distal nonprogressive SMA, and present in one person within our cohort, mediates Ca^{+2} influx through interaction with fliI and non-muscle myosin IIA in fibroblast cell culture.[25] Naganawa and Hirata studied zebrafish mutants and showed that Zebrafish flii mutants show normal behavior at 24 hours but slow swimming at 48 hour through a mechanism involving actin disorganization in fast muscle fibers.[26] Campbell *et al.* constructed Flii -/- mice and demonstrated early embryonic lethality for the knockout; heterozygous carriers

did not have a detectable phenotype.[27] Since mutations in *ECEL1* are known to cause distal arthrogryposis and our proband has much more severe phenotype than would be expected, we propose that *FLII* is likely contributing to the disease phenotype.

GBE1- AP4M1-TAF9B

We found 3 potential candidate variants in BAB8400 that may explain the proband's phenotype. The proband was evaluated at age 3 years 8 months of age due to hypotonia, global developmental delay, muscle atrophy, arthrogryposis multiplex and cryptorchidism. Muscle biopsy revealed severe atrophy of type 2 fibers. Exome of the proband showed several potential disease causing candidates including homozygous nonframeshift deletion (NM_000158.3:c.1864_1866del:p.Leu622del) in the *GBE1* gene, a homozygous change (NM_004722:c.136C>G:p.Pro46Ala) in *AP4M1* and a hemizygous missense variant in *TAF9B* (NM_015975:c.133C>A:p.Arg45Ser). None of the homozygous variants in *GBE1*, *AP4M1* and *TAF9B* were reported as homozygous/hemizygous in internal and public databases. Functional prediction scores were deleterious for all variants. Segregation studies were consistent with the observed disease trait, homozygous for recessive conditions (*GBE1* and *AP4M1*) and hemizygous for X-linked recessive condition (*TAF9*). *GBE1* is known to cause glycogen storage disease, type IV which can present with neuromuscular features (MIM: 232500). Our mutation was not previously reported in the literature. Individuals with *GBE1* mutations frequently have other features of glycogen storage disorder such as cardiac and liver involvement. However, there is a recent report of a 3 years old male who presented with arthrogryposis, motor developmental delay and rigid spine who was found to have compound heterozygous mutations in *GBE1*.[28] *TAF9B* has not been linked to any human disease, however it interacts closely with *TAF6* in which deleterious variants are known to cause Alazami-Yuan syndrome, characterized by DD/ID (MIM: 617126).[29; 30] Moreover, it has been shown to regulate neuronal gene

expression.[31] Thus, we here propose that variant in *TAF9B* is contributing to the DD/ID phenotype in our proband. Mutations in *AP4M1* are known to cause Spastic Paraplegia type 50 (MIM: 612936). Although our subject has overlapping features such as DD/ID and club feet, he did not exhibit the classical hyperreflexia and spasticity phenotypes observed in spastic paraplegia. Thus it is difficult to determine the contribution of the *AP4M1* variant to the disease phenotype.

COG6-MED27

BAB8606 is a 17 day old female with had medically complicated family history. Parents were first degree cousins and she had three female siblings who died ages between 3 days of life to 3 months of life with similar features including polyhydramnios, contractures and cataract. Another female sibling with pontocerebellar hypoplasia and cataract died at 9 years old. She has a 21 year old living, male sibling, BAB8609, who has developmental delay, ataxia, dysmetria, mild contracture in knees and in wrists and bilateral cataract. Brain MRI of this sibling, BAB8609, also showed cerebellar hypoplasia. Index, BAB8606, was born at 38th gestational week with a birth weight of 2200 g (-2.3 SD) after a pregnancy complicated by polyhydramnios. She developed respiratory distress after birth. She was found to have contractures in both hands and feet and bilateral cataract. Head ultrasound showed hydrocephaly. We performed quadruple exome in this family and found a homozygous frameshift variant (NM_001145079.1:c.726del:p.Cys242Trpfs*7) in *COG6* which is linked to congenital disorder of glycosylation (CDG), type III (MIM: 614576) and Shaheen syndrome (MIM: 615328). Segregation studies showed that parents and living sibling carries this variant in heterozygous state and proband is homozygous. The gross features of this syndrome are microcephaly, DD/ID, liver involvement, recurrent infections, early lethality and hypohydrosis.[32] Cerebellar hypoplasia, early lethality and contractures are well-known clinical features of other CDG phenotypes. Our

family has multiple individuals who died early in the life and cerebellar hypoplasia in some individuals. Since our proband was evaluated at 17 day of life, we could not evaluate the typical features of CDG. Since both siblings carry additional similar clinical features including bilateral cataract, and DD/ID phenotype, we searched for shared variants in both siblings. A deleterious homozygous missense (NM_001253881:c.770C>T:p.Pro257Leu) variant in Mediator Complex Subunit 27 (*MED27*) is found in both siblings. c.770C>T variant is called to be disease causing by all mutation prediction tools, embedded in highly conserved region and not reported in any of the public databases or our internal database except within the family. Mediator Complex Subunit genes are well known to cause DD/ID phenotype, cerebellar anomalies and various eye findings.[33] *MED17* mutations are known to cause microcephaly, DD/ID and cerebellar atrophy (MIM: 613668), *MED25* mutations are known to cause Basel-Vanagait-Smirin-Yosef syndrome (MIM: 616449) which is characterized by DD/ID, various brain malformations and eye malformations including cataract. Thus, we here propose *MED27* variant is likely contributing DD/ID, cerebellar and eye findings in our siblings and more severe DD/ID and multiple early deaths among siblings can be explained by *COG6* homozygous frameshift mutation and other potential variants in other genes.

POSSIBLY CAUSATIVE ARTHROGRYPOSIS GENES

***CACUL1* (Figure S5)**

BAB9729 is a 3.5 months old male who was found to have bilateral hydronephrosis during the prenatal period. He was found to have contractures in both hands and feet at birth and diagnosed with amyoplasia congenita. Neonatal testing confirmed the prenatal diagnosis of hydronephrosis and demonstrated bilateral vesicoureteral reflux and end stage renal disease. His parents were first degree cousins, and he has two healthy siblings. He had dysmorphic facial features including a prominent forehead, hypotelorism, a long philtrum, thin lips, retromicrognathia and

low set ears. Growth parameters revealed he is small in all parameters: weight 3 kg (-4.1 SD), height 55.5 cm (-2.3 SD), and head circumference 36 cm (-3.2 SD). Head CT, karyotype and array-CGH were normal. Trio exome revealed a homozygous 2 bp deletion in a candidate gene, *CACUL1*, causing (NM_153810:c.910_911del:p.Leu304Ilefs*3) (Figure S5). This gene was not previously linked to any disease. Segregation studies showed that the parents are heterozygous, and the proband is homozygous for the deletion.

FGFRL1 **(Figure S3)**

BAB3944 is a 3 year old female, born to consanguineous parents, who first presented at the age of 6 months. She had myopathic facies, dolicocephaly, and contractures in the fingers and knees (Figure S3). She had a normal karyotype and echocardiogram. Exome sequencing initially did not reveal any candidate gene but re-analysis showed a homozygous nonsynonymous change in the *FGFRL1* gene (NM_001004356:c.124C>T:p.Arg42Trp). This amino acid is conserved in orthologs, and the variant was evaluated as disease causing in all functional prediction algorithms. This variant was present in only one individual in our internal BHCMG database and one individual in gnomAD in the heterozygous state. Segregation studies revealed that both parents and the unaffected brother are heterozygous for the variant, consistent with an autosomal recessive disease trait.

TMEM214 **(Figure S4)**

BAB5192 was first evaluated at age 2 years 4 months old with chief complaints of restricted joint movements, motor developmental delay and respiratory distress. She was born at 34 weeks and 1 day gestation by C-section. Physical examination revealed myopathic facies, scoliosis, and contractures in the shoulders, elbows, hips and knees with absent deep tendon reflexes. She was born to a first degree cousin marriage. The mother reported three miscarriages, one affected male child who died at the age of 2 with similar clinical features, one female child who died 4 day of

life of unknown clear etiology and one healthy female sibling. Diagnostic work up including blood chemistry and metabolic profile, CPK level, cranial and abdominal ultrasound, cranial MRI, congenital disorder of glycosylation testing, proband and parental karyotypes, and SMA gene testing were unremarkable. EMG/NCS was consistent with diffuse anterior horn cell disease. The proband died at 2 years 6 months of age due to respiratory complications. Exome sequencing of the proband revealed a homozygous missense change (NM_001083590:c.764G>A:p.Arg255Gln) in the *TMEM214* gene. This variant was reported in only one person in our internal database and three individuals in gnomAD as a heterozygous variant. The amino acid was present in a highly conserved region and prediction scores reported it as a disease-causing variant (Figure S4). Sanger sequencing from the available relatives showed that the father is heterozygous and the proband is homozygous, consistent with a recessive disease trait.

NR2C1

BAB8086 and BAB8087 are two affected siblings born to a first degree cousin marriage. BAB8086 is a 14 years old male and BAB8087 is a 9 years old female. Both siblings had normal growth parameters and intellectual development. They developed hirsutism and arthrogryposis after infancy. Diagnostic work up including CPK level, karyotype, EMG/NCS and brain MRI were normal. Exome sequencing was performed on two affected siblings. Exome analysis revealed a homozygous splicing variant in a highly conserved region in the *NR2C1* gene (NM_003297:c.544+1G>C). This variant was not reported in public databases. *NR2C1*, nuclear receptor subfamily 2 group C member 1, is the only convincing gene that is homozygous in both affected individuals. *NR2C1* is an orphan nuclear receptor and belongs to the nuclear hormone receptor family. It is globally expressed and together with NR2C2, forms the core of the DRED (direct repeat erythroid-definitive) complex that represses embryonic and fetal globin

transcription. Lee *et al.* recently showed its role in erythroid cell proliferation and maturation.[34] Interestingly, a homozygous knock-out mice model did not survive beyond embryonic day 7, however heterozygous animals showed increased expression of cyclin dependent kinase inhibitor (*Cdkn1c*). *CDKN1C* deleterious variants are known to cause developmental disorders including Beckwith Wiedemann syndrome and IMAGE syndrome (MIM: 130650 and 614732, respectively). Our probands have unique clinical features such as hirsutism and contractures that developed after infancy and which are atypical for classical arthrogryposis phenotypes. Given the pathogenicity of the variant, conservation and segregation studies, we propose *NR2C1* as disease causing in this family.

PRDM2 **(Figure 7)**

BAB9309 was born to a 29 year old G4P3A1 mother via normal spontaneous vaginal delivery at term. Parents were first degree consanguineous and she has two healthy siblings. Birth weight was 2390 g (-2 SD) and head circumference 30 cm (-3.3 SD). Intrauterine fetal movements and ultrasounds were unremarkable. She was consulted due to small for gestational age, microcephaly, contractures and dysmorphic features. Evaluation at 7 day of life revealed she has weak cry, bulbous nose, low set ears, micrognathia, contractures in fingers and rocker-bottom feet. Anthropometric measurements showed weight 2350 g (-2.3 SD), height 44 cm (-2.9 SD) and head circumference 30.5 cm (-3.3 SD). She failed with the initial hearing screening test. Trio exome revealed a *de novo* frameshift deletion (NM_012231:c.4283_4295del:p.Leu1428Glnfs*15) in *PRDM2* in a highly conserved region (Figure 7). Based on variant read/total read ratio (12/150=~8%), mosaicism is suspected. Sanger PCR did not show the variant thus, we conveyed ddPCR which showed mosaic *de novo* indel in the proband.

PHENOTYPIC EXPANSION

SYT2 (Figure S8)

BAB7308 is an 8 month old male born at term with via C-section with a birth weight 3600 g (53rd percentile). Mother reports decreased fetal movements during pregnancy. He was born to unrelated parents and has two healthy female siblings. He had delayed motor milestones. Physical examination showed contractures in fingers, bilateral PEV deformity and undescended testicles. Diagnostic work up including EEG and abdominal ultrasound were unremarkable however brain MRI showed partial corpus callosum agenesis and EMG/NCS was consistent with sensorimotor polyneuropathy. Ophthalmologic evaluation showed megalocornea (Figure S8). Proband exome was inconclusive thus expanding to trio exome revealed a *de novo* missense change (NM_177402:c.1081G>C:p.Asp361His) in Synaptotagmin 2 (*SYT2*) through our DNM-finder tool.[35] *SYT2* mutations have been described in two families with congenital presynaptic Myasthenia (MIM: 616040).[36] Based on functional studies[37; 38] and strong association between presynaptic Myasthenia syndrome genes and arthrogryposis (*e.g. CHRNA1* and *CHRND* mutations are known to cause both phenotypes), we propose that this family represents an expansion of the known phenotype of *SYT2* to include arthrogryposis. We could not identify any other gene/s that can explain the DD/ID, partial corpus callosum agenesis and megalocornea phenotype.

FAT1 (Figure S9)

BAB8356 is a 5 month old male born to a first degree cousin marriage. He was born term after an uncomplicated pregnancy. He was found to have a webbed neck and contractures involving both hands. Additionally he had a large fontanel, thick lips and cleft palate. Eye exam and genitourinary ultrasound were unremarkable. Skeletal survey revealed elongated metaphysis and flat vertebral bodies. Echo showed mild pulmonary stenosis. Exome sequencing of the proband

revealed a homozygous nonsynonymous change, NM_005245:c.6026A>G:p.Asn2009Ser in *FAT1* (Figure S9). This variant was reported in heterozygous state in the heterozygous state in 15 individuals from the gnomAD database and not reported in other public databases nor our internal cohort. Sanger segregation showed that the parents are heterozygous for the variant, which is consistent with an autosomal recessive Mendelian trait.

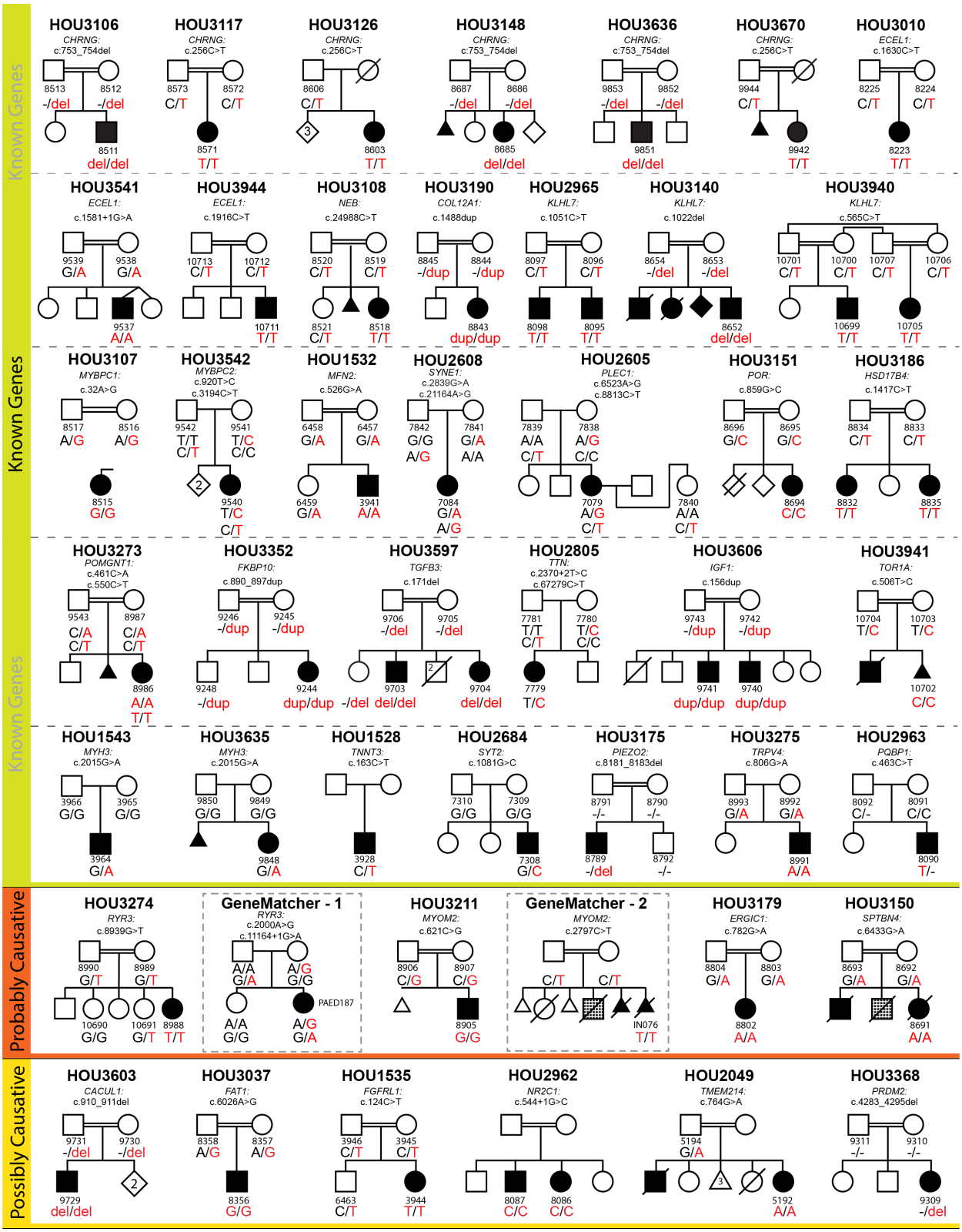


Figure S1: Pedigrees and Sanger confirmed genotypes of the known and proposed candidate gene families.

Figure S1: Pedigrees and Sanger confirmed genotypes of the known and proposed candidate gene families. Family IDs (HOU#) are written above each pedigree; filled circles (females) and squares (males) with clinical diagnosis of arthrogryposis. Genotypes are shown below each individual. Mutations are written in red, whereas wild type alleles are written in black. Subject numbers (BAB) are written under each individual whose samples were available. GeneMatcher families in probably causative genes are framed with a dashed line.

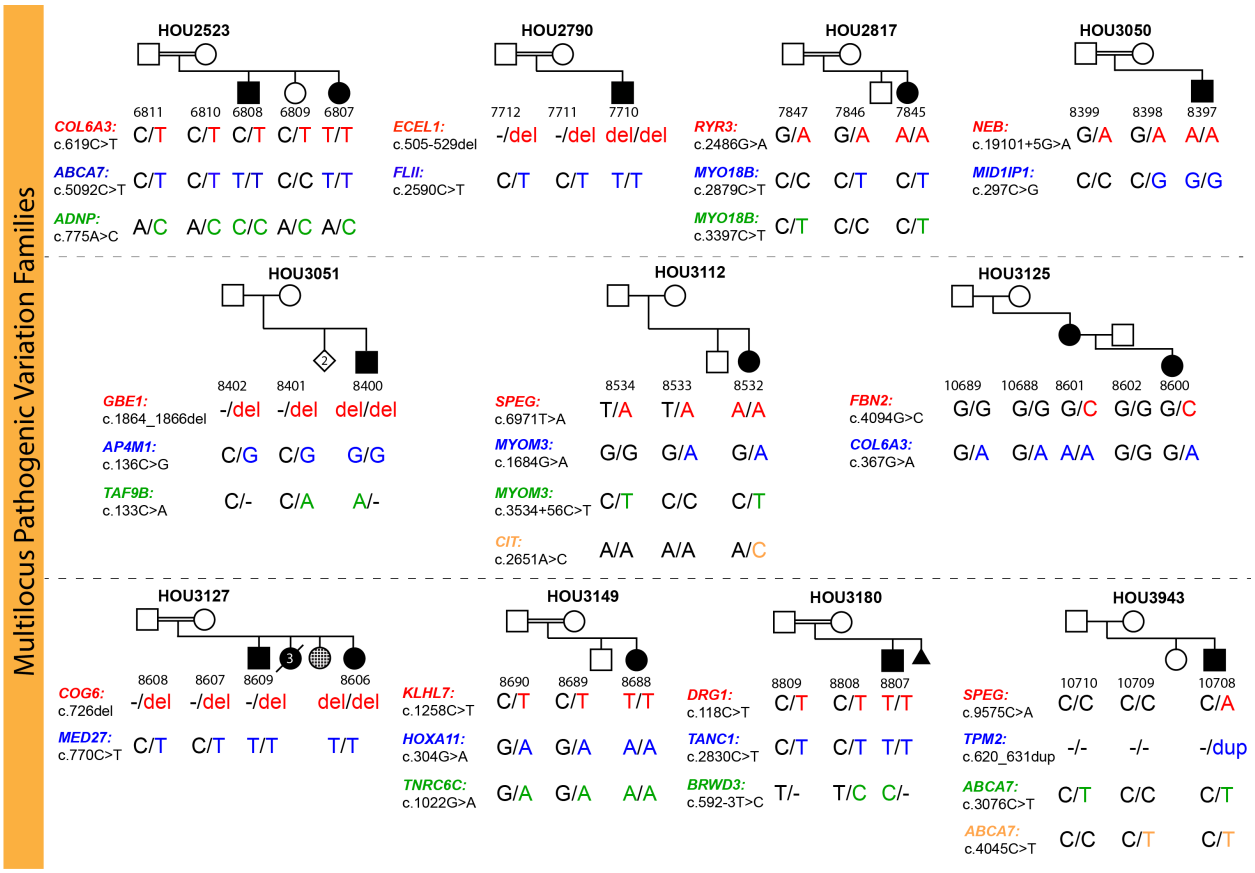


Figure S2: Segregation studies of the families with multilocus pathogenic variation. Family identification numbers are displayed on top of each pedigree. Genotypes for each variant are written in columns with each row for the given gene locus specified. BAB numbers indicating the studied subject are displayed under each shape.

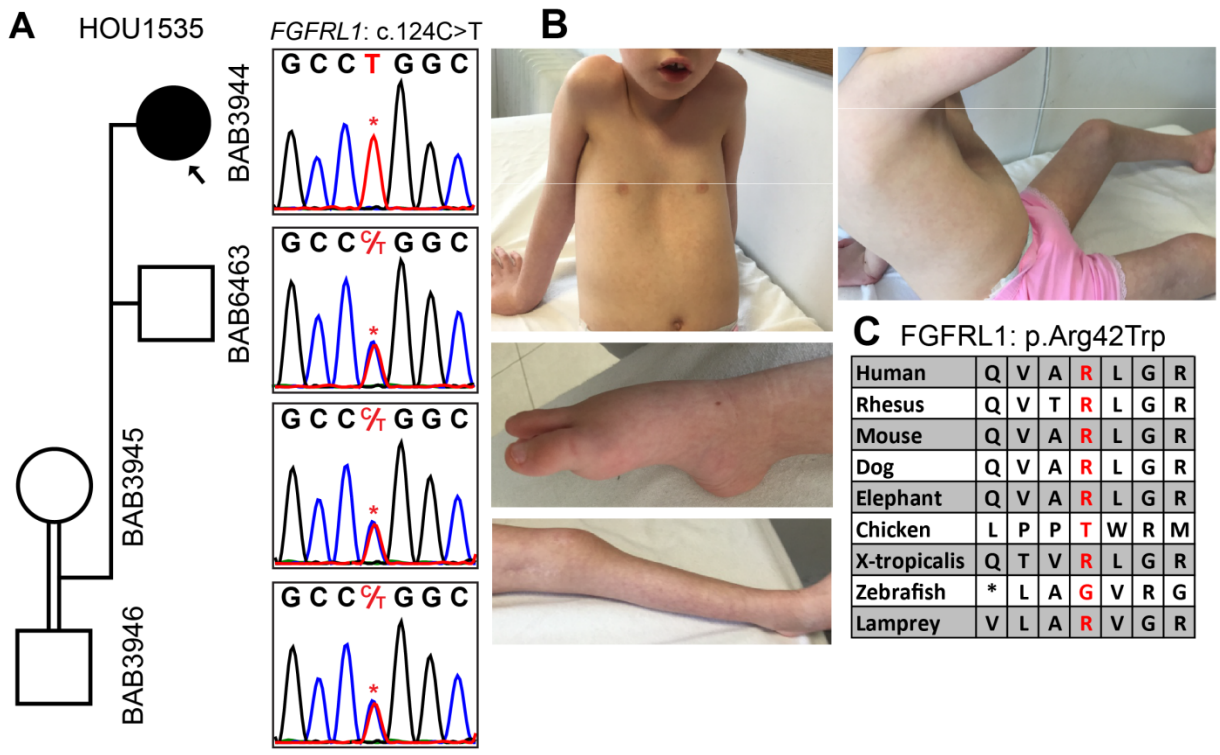


Figure S3: Pedigrees and Sanger validation of the identified variant in BAB3944. (A): Homozygous c.124C>T variant in *FGFRL1* is segregating within the family consistent with recessive Mendelian traits, *i.e.* parents and unaffected brother is heterozygous and the proband is homozygous. (B) Photographs showing contractures in hands and knees, and *pes equinovarus* deformity. (C) Highly conserved amino acid residue across different species at position 42.

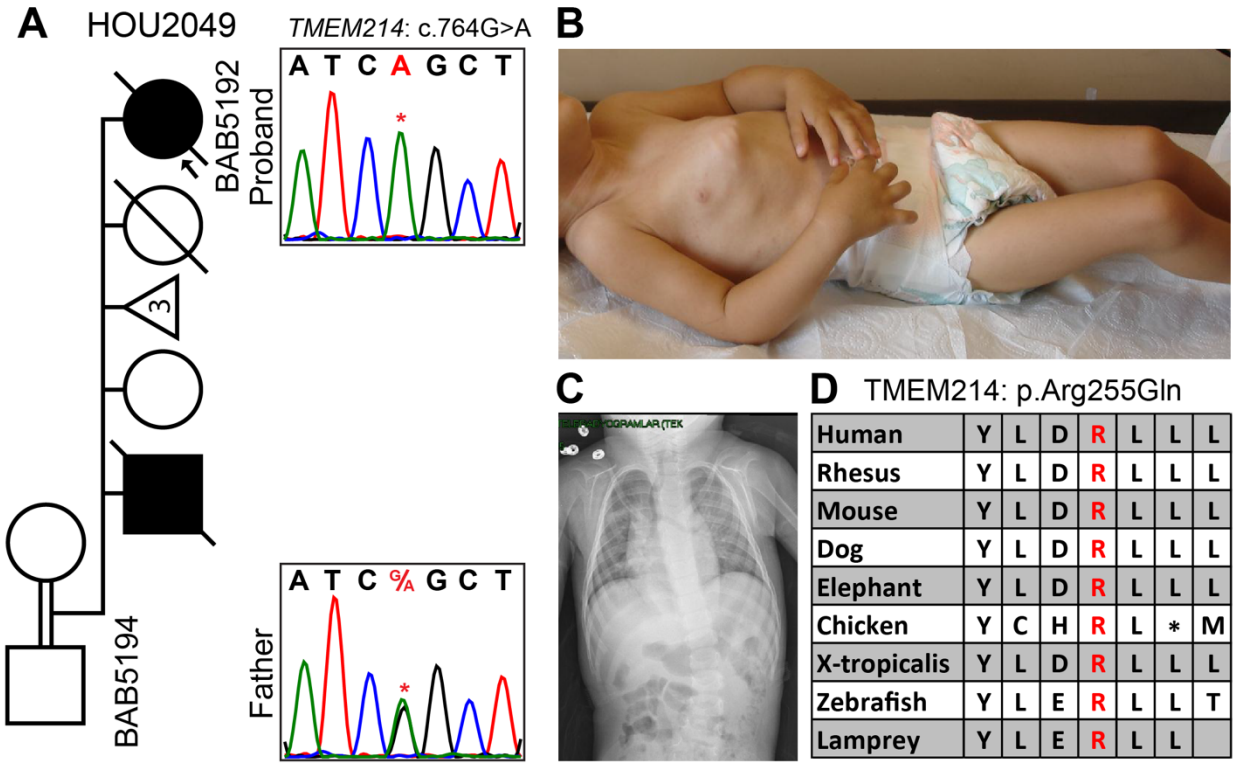


Figure S4: Details of homozygous *TMEM214* variant in BAB5192. (A) Pedigree and Sanger chromatographs of the family for segregation analyses. Affected individual has homozygous mutation whereas unaffected father is heterozygous. Mother was not available for segregation studies. (B) Picture showing contractures in shoulders, elbows, hip and knees. (C) Spinal X-ray showing scoliosis in the proband. (D) Conserved Arg (R) residue at position 255 with red font color.

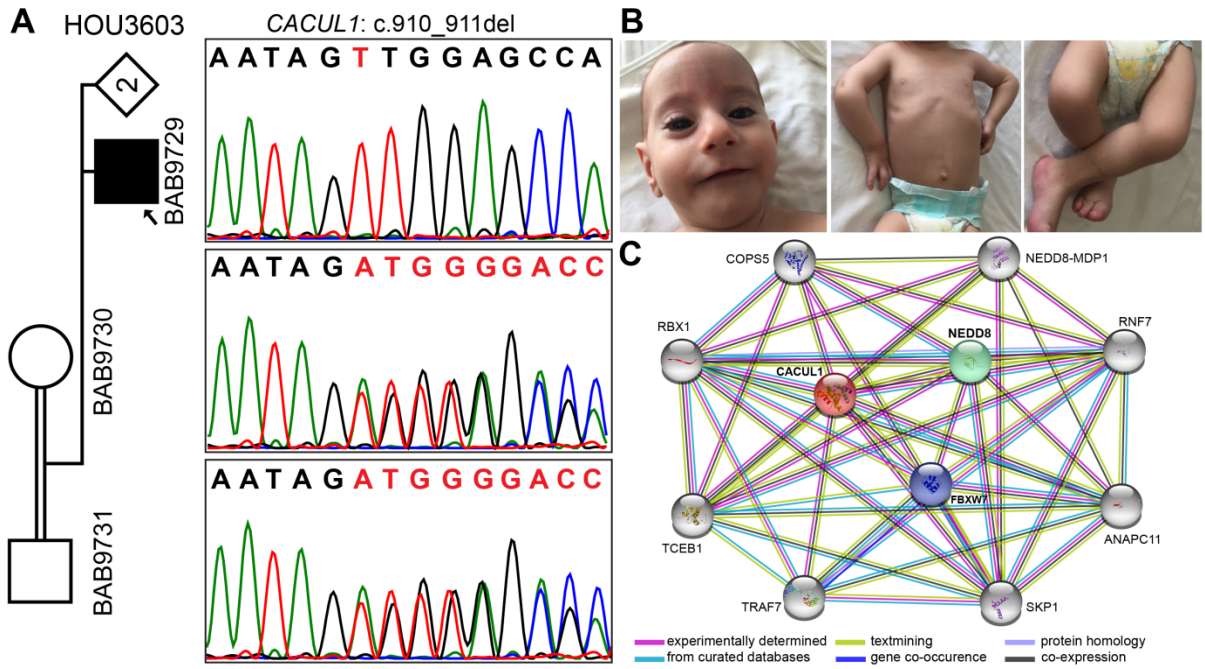


Figure S5: Clinical and molecular findings in subject with *CACUL1* variant. A) Segregation analyses of the homozygous *CACUL1* homozygous deletion in the family HOU3603. Note the peak on peak appearance on the heterozygous parents. (B) Proband's pictures showing contracture in the elbows and fingers. (C) The interaction network of *CACUL1* (green circle) with genes which have role in muscle function (blue circles). Gray circles indicate the proteins in the same network with no known neuromuscular function/phenotype. Interactome diagram is obtained through the STRING database, version 11.0. Each colored line between proteins indicates the source of the association and is indicated directly under the panel.

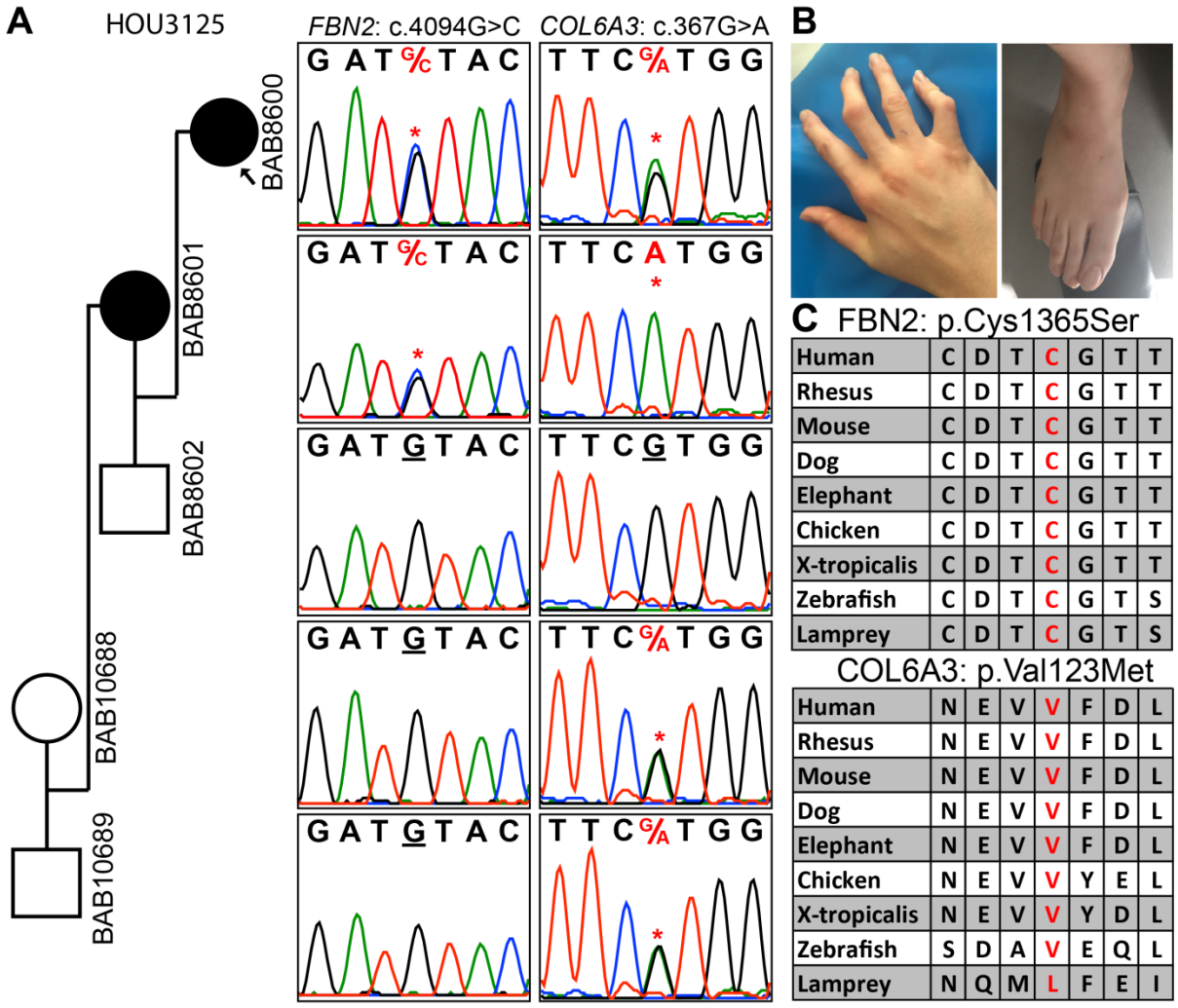


Figure S6: Known - known oligogenic model: heterozygous *FBN2* variant identified in BAB8600 and BAB8601 and a homozygous *COL6A3 variant* in the mother. (A) Sanger sequencing studies showing *de novo* heterozygous *FBN2* variant (c.4094G>C) in mother which was shared with affected daughter and a homozygous *COL6A3* variant (c.367G>A) in affected mother who has additional contractural features. Mother's affected daughter and grandparents are heterozygous for the *COL6A3* variant. (B) Photographs of the contractural arachnodactyly in fingers and toes in mother. (C) High conservation of the mutation amino acid residue for both *FBN2* and *COL6A3* variants

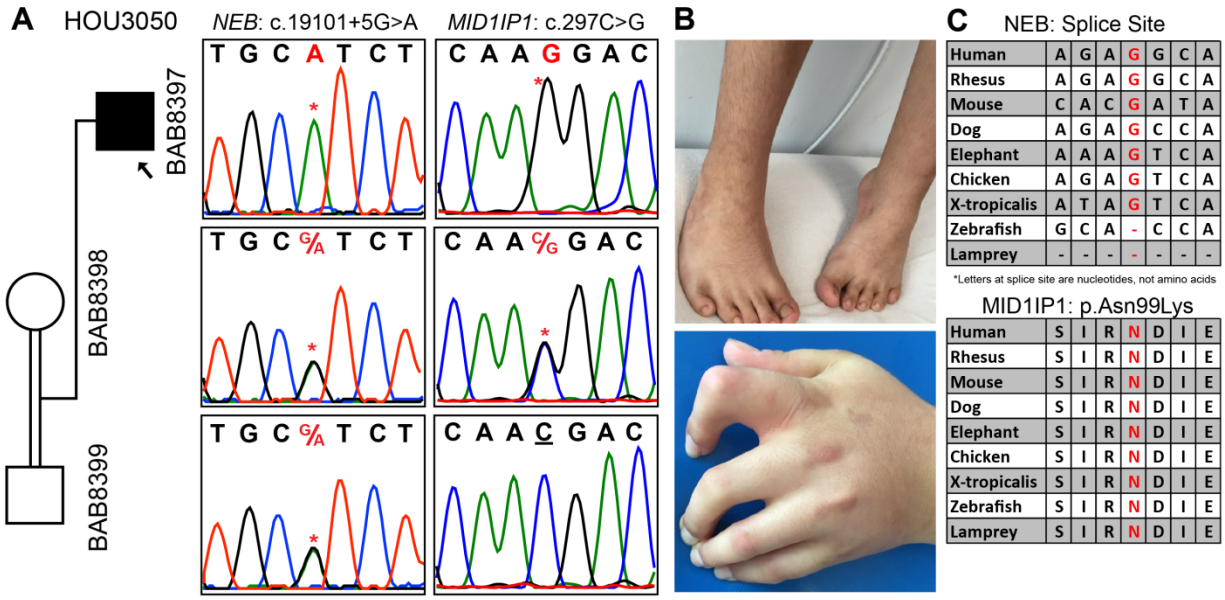
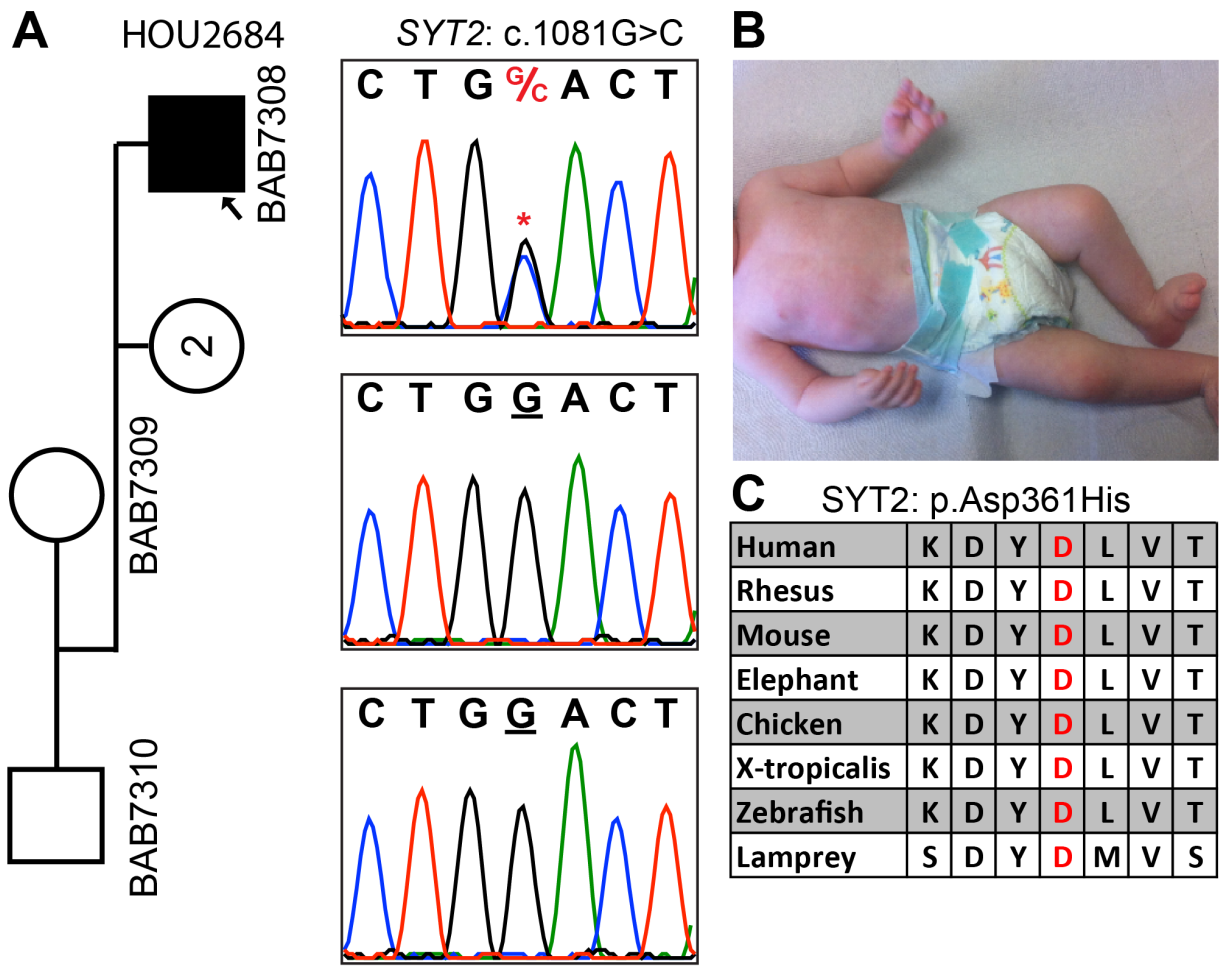


Figure S7: Known – candidate oligogenic model: Reported splice site mutation in the known *NEB* gene and missense mutation in the candidate *MID1IP1* gene. (A) Segregation analyses showing *NEB* variant (c.19101+5G>A) and a homozygous *MID1IP1* variant (c.297C>G) in the proband. Both parents are heterozygous as expected with consanguineous marriages. (B) Pictures of the proband showing contractures in fingers and *pes equinovarus* deformity in feet. (C) Conservation table for the peptide residues around *NEB* and *MID1IP1* variants.



Human	K	D	Y	D	L	V	T
Rhesus	K	D	Y	D	L	V	T
Mouse	K	D	Y	D	L	V	T
Elephant	K	D	Y	D	L	V	T
Chicken	K	D	Y	D	L	V	T
X-tropicalis	K	D	Y	D	L	V	T
Zebrafish	K	D	Y	D	L	V	T
Lamprey	S	D	Y	D	M	V	S

Figure S8: *De novo* heterozygous *SYT2* variant in BAB7308. (A) Pedigree and segregation analyses of the c.1081G>C variant. (B) Proband's photographs showing the contractures in the hands and feet. (C) High conservation of Asp361His peptide in other species. Asp (D) amino acid is written in red font.

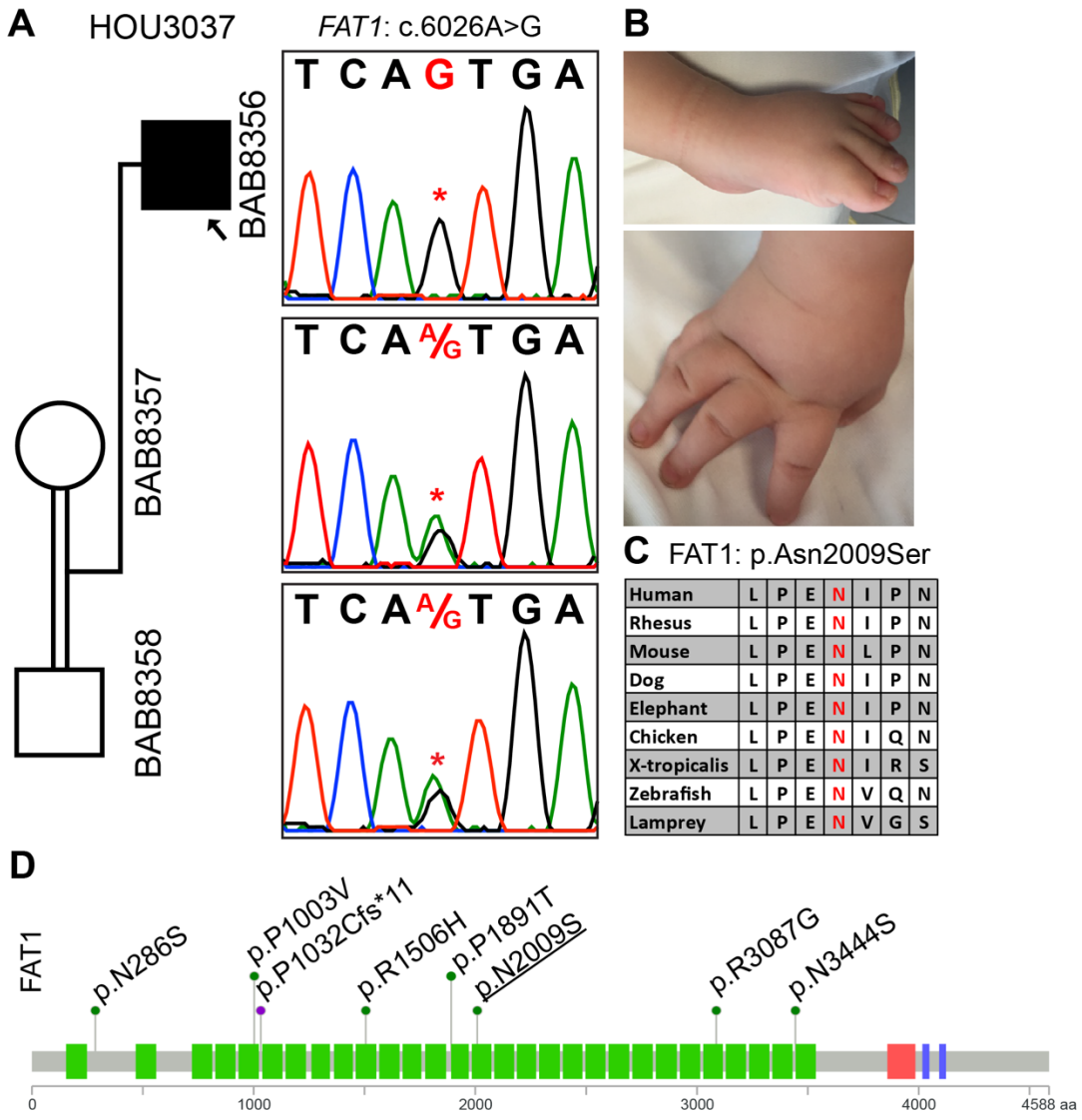


Figure S9: Clinical and genomic studies for homozygous *FAT1* variant in BAB8356. (A) Family HOU3037 pedigree and segregation of the variant c.6026A>G, consistent with autosomal recessive inheritance pattern. (B) Photographs of the hand and feet showing the contractures in fingers and toes. (C) Sequence alignment of human FAT1 in other species. N2009 is a conserved residue across all vertebrates. (D) Protein domains of FAT1 and localization of the identified mutations marked with small circles in Facioscapulohumeral Muscular Dystrophy and glomerulotubular nephropathy. Purple filled circle indicates the frameshift mutation and green filled circles point missense mutations. The variant identified in our case is underlined to distinguish.

Table S2: Molecular summary of the putative pathogenic Copy Number Variants (CNVs) cohort			
Proband	**CNV coordinates**	**CNV type**	**Size**
BAB7128	chr5:129673070-136365890	Deletion	6.6 Mb
BAB8145	chr10:58374373-67805420	Deletion	9.4 Mb
	chr11:90798107-91499657	Deletion	0.7 Mb
	chr11:92950818-100534639	Deletion	7.5 Mb
BAB9312	chr18:21901302-43716565	Duplication	21.8 Mb
	chr18:66350129-78002264	Deletion	11.6 Mb

Table S4: Patients diagnosed with Reanalysis of ES data and Expansion to additional family members				
Proband	**Gene**	**Nucleotide;Protein**	**Zygosity**	**Contributing factors to diagnosis**
BAB3928	*TNNT3*	c.163C>T;p.Arg55Cys	Htz	DNMfinder and literature update
BAB3941	*MFN2*	c.526G>A;p.Gly176Ser	Hmz	Literature update
BAB3944	*FGFRL1*	c.124C>T;p.Arg42Trp	Hmz	Literature update
BAB5192	*TMEM214*	c.764G>A;p.Arg255Gln	Hmz	Literature update
BAB6807 / BAB6808	*COL6A3*	c.619C>T;p.Gln207*	Hmz	Literature update
	ADNP	c.775A>C;p.Asn259His	Hmz	
	ABCA7	c.5092C>T;p.Arg1698Trp	Hmz	
BAB7079	*PLEC*	c.8813C>T;p.Thr2938Met c.6523A>G;p.Lys2175Glu	Comp Htz	Trio expansion
BAB7084	*SYNE1*	c.2839G>A;p.Glu947Lys c.21164A>G;p.Lys7055Arg	Comp Htz	Trio expansion and Literature update
BAB7128	Copy Number Variant			Trio expansion and HMZDelFinder
BAB7308	*SYT2*	c.1081G>C;p.Asp361His	Htz	Trio expansion and DNMfinder

Hmz: Homozygous, Htz: heterozygous, Comp Htz: Compound Heterozygous.
Headings and column titles are bolded, gene names are italicized.

References

1. Alazami, A.M., Kentab, A.Y., Faqeih, E., Mohamed, J.Y., Alkhalidi, H., Hijazi, H., and Alkuraya, F.S. (2015). A novel syndrome of Klippel-Feil anomaly, myopathy, and characteristic facies is linked to a null mutation in *MYO18B*. J Med Genet 52, 400-404.

2. Malfatti, E., Bohm, J., Lacene, E., Beuvin, M., Romero, N.B., and Laporte, J. (2015). A Premature Stop Codon in *MYO18B* is Associated with Severe Nemaline Myopathy with Cardiomyopathy. J Neuromuscul Dis 2, 219-227.

3. Tumiene, B., Voisin, N., Preiksaitiene, E., Petroska, D., Grikiniene, J., Samaitiene, R., Utkus, A., Reymond, A., and Kucinskas, V. (2017). Inflammatory myopathy in a patient with Aicardi-Goutieres syndrome. European journal of medical genetics 60, 154-158.

4. Dlamini, N., Josifova, D.J., Paine, S.M., Wraige, E., Pitt, M., Murphy, A.J., King, A., Buk, S., Smith, F., Abbs, S., et al. (2013). Clinical and neuropathological features of X-linked spinal muscular atrophy (SMAX2) associated with a novel mutation in the *UBA1* gene. Neuromuscular disorders : NMD 23, 391-398.

5. North, K.N., and Ryan, M.M. (1993). Nemaline Myopathy. In GeneReviews((R)), M.P. Adam, H.H. Ardinger, R.A. Pagon, S.E. Wallace, L.J.H. Bean, K. Stephens, andA. Amemiya, eds. Seattle (WA), University of Washington, Seattle

6. Tolmie, J.L., Shillito, P., Hughes-Benzie, R., and Stephenson, J.B. (1995). The Aicardi-Goutieres syndrome (familial, early onset encephalopathy with calcifications of the basal ganglia and chronic cerebrospinal fluid lymphocytosis). Journal of medical genetics 32, 881-884.

7. Bohm, J., Vasli, N., Malfatti, E., Le Gras, S., Feger, C., Jost, B., Monnier, N., Brocard, J., Karasoy, H., Gerard, M., et al. (2013). An integrated diagnosis strategy for congenital myopathies. PLoS One 8, e67527.

8. Bower, N.I., and Johnston, I.A. (2010). Discovery and characterization of nutritionally regulated genes associated with muscle growth in Atlantic salmon. Physiol Genomics 42A, 114-130.

9. Azakir, B.A., Di Fulvio, S., Therrien, C., and Sinnreich, M. (2010). Dysferlin interacts with tubulin and microtubules in mouse skeletal muscle. PLoS One 5, e10122.

10. Schellhaus, A.K., Moreno-Andres, D., Chugh, M., Yokoyama, H., Moschopoulou, A., De, S., Bono, F., Hipp, K., Schaffer, E., and Antonin, W. (2017). Developmentally Regulated GTP binding protein 1 (DRG1) controls microtubule dynamics. Sci Rep 7, 9996.

11. Granot-Hershkovitz, E., Raas-Rothschild, A., Frumkin, A., Granot, D., Silverstein, S., and Abeliovich, D. (2011). Complex chromosomal rearrangement in a girl with psychomotor-retardation and a de novo inversion: inv(2)(p15;q24.2). Am J Med Genet A 155A, 1825-1832.

12. Avirneni-Vadlamudi, U., Galindo, K.A., Endicott, T.R., Paulson, V., Cameron, S., and Galindo, R.L. (2012). Drosophila and mammalian models uncover a role for the myoblast fusion gene *TANC1* in rhabdomyosarcoma. J Clin Invest 122, 403-407.

13. Agrawal, P.B., Pierson, C.R., Joshi, M., Liu, X., Ravenscroft, G., Moghadaszadeh, B., Talabere, T., Viola, M., Swanson, L.C., Haliloglu, G., et al. (2014). SPEG interacts with myotubularin, and its deficiency causes centronuclear myopathy with dilated cardiomyopathy. Am J Hum Genet 95, 218-226.

14. Wang, H., Castiglioni, C., Kacar Bayram, A., Fattori, F., Pekuz, S., Araneda, D., Per, H., Erazo, R., Gumus, H., Zorludemir, S., et al. (2017). Insights from genotype-phenotype correlations by novel *SPEG* mutations causing centronuclear myopathy. Neuromuscul Disord 27, 836-842.

15. Agarkova, I., and Perriard, J.C. (2005). The M-band: an elastic web that crosslinks thick filaments in the center of the sarcomere. Trends Cell Biol 15, 477-485.

16. Schoenauer, R., Lange, S., Hirschy, A., Ehler, E., Perriard, J.C., and Agarkova, I. (2008). Myomesin 3, a novel structural component of the M-band in striated muscle. J Mol Biol 376, 338-351.

17. Lange, S., Agarkova, I., Perriard, J.C., and Ehler, E. (2005). The sarcomeric M-band during development and in disease. J Muscle Res Cell Motil 26, 375-379.

18. Schoenauer, R., Bertoncini, P., Machaidze, G., Aebi, U., Perriard, J.C., Hegner, M., and Agarkova, I. (2005). Myomesin is a molecular spring with adaptable elasticity. J Mol Biol 349, 367-379.

19. Li, H., Bielas, S.L., Zaki, M.S., Ismail, S., Farfara, D., Um, K., Rosti, R.O., Scott, E.C., Tu, S., Chi, N.C., et al. (2016). Biallelic Mutations in Citron Kinase Link Mitotic Cytokinesis to Human Primary Microcephaly. Am J Hum Genet 99, 501-510.

20. Shaheen, R., Hashem, A., Abdel-Salam, G.M., Al-Fadhli, F., Ewida, N., and Alkuraya, F.S. (2016). Mutations in *CIT*, encoding citron rho-interacting serine/threonine kinase, cause severe primary microcephaly in humans. Hum Genet 135, 1191-1197.

21. Basit, S., Al-Harbi, K.M., Alhijji, S.A., Albalawi, A.M., Alharby, E., Eldardear, A., and Samman, M.I. (2016). *CIT*, a gene involved in neurogenic cytokinesis, is mutated in human primary microcephaly. Hum Genet 135, 1199-1207.

22. Angius, A., Uva, P., Buers, I., Oppo, M., Puddu, A., Onano, S., Persico, I., Loi, A., Marcia, L., Hohne, W., et al. (2016). Bi-allelic Mutations in *KLHL7* Cause a Crisponi/CISS1-like Phenotype Associated with Early-Onset Retinitis Pigmentosa. Am J Hum Genet 99, 236-245.

23. Bruel, A.L., Bigoni, S., Kennedy, J., Whiteford, M., Buxton, C., Parmeggiani, G., Wherlock, M., Woodward, G., Greenslade, M., Williams, M., et al. (2017). Expanding the clinical spectrum of recessive truncating mutations of *KLHL7* to a Bohring-Opitz-like phenotype. J Med Genet 54, 830-835.

24. Thompson, A.A., and Nguyen, L.T. (2000). Amegakaryocytic thrombocytopenia and radio-ulnar synostosis are associated with *HOXA11* mutation. Nat Genet 26, 397-398.

25. Arora, P.D., Di Gregorio, M., He, P., and McCulloch, C.A. (2017). TRPV4 mediates the Ca(2+) influx required for the interaction between flightless-1 and non-muscle myosin, and collagen remodeling. J Cell Sci 130, 2196-2208.

26. Naganawa, Y., and Hirata, H. (2011). Developmental transition of touch response from slow muscle-mediated coilings to fast muscle-mediated burst swimming in zebrafish. Dev Biol 355, 194-204.

27. Campbell, H.D., Fountain, S., McLennan, I.S., Berven, L.A., Crouch, M.F., Davy, D.A., Hooper, J.A., Waterford, K., Chen, K.S., Lupski, J.R., et al. (2002). Fliih, a gelsolin-related cytoskeletal regulator essential for early mammalian embryonic development. Mol Cell Biol 22, 3518-3526.

28. Malfatti, E., Barnerias, C., Hedberg-Oldfors, C., Gitiaux, C., Benezit, A., Oldfors, A., Carlier, R.Y., Quijano-Roy, S., and Romero, N.B. (2016). A novel neuromuscular form

of glycogen storage disease type IV with arthrogryposis, spinal stiffness and rare polyglucosan bodies in muscle. Neuromuscul Disord 26, 681-687.

29. Yuan, B., Pehlivan, D., Karaca, E., Patel, N., Charng, W.L., Gambin, T., Gonzaga-Jauregui, C., Sutton, V.R., Yesil, G., Bozdogan, S.T., et al. (2015). Global transcriptional disturbances underlie Cornelia de Lange syndrome and related phenotypes. J Clin Invest 125, 636-651.

30. Alazami, A.M., Patel, N., Shamseldin, H.E., Anazi, S., Al-Dosari, M.S., Alzahrani, F., Hijazi, H., Alshammari, M., Aldahmesh, M.A., Salih, M.A., et al. (2015). Accelerating novel candidate gene discovery in neurogenetic disorders via whole-exome sequencing of prescreened multiplex consanguineous families. Cell Rep 10, 148-161.

31. Herrera, F.J., Yamaguchi, T., Roelink, H., and Tjian, R. (2014). Core promoter factor TAF9B regulates neuronal gene expression. Elife 3, e02559.

32. Rymen, D., Winter, J., Van Hasselt, P.M., Jaeken, J., Kasapkara, C., Gokcay, G., Haijes, H., Goyens, P., Tokatli, A., Thiel, C., et al. (2015). Key features and clinical variability of *COG6*-CDG. Mol Genet Metab 116, 163-170.

33. Soutourina, J. (2018). Transcription regulation by the Mediator complex. Nat Rev Mol Cell Biol 19, 262-274.

34. Lee, M.P., Tanabe, O., Shi, L., Jearawiriyapaisarn, N., Lucas, D., and Engel, J.D. (2017). The orphan nuclear receptor TR4 regulates erythroid cell proliferation and maturation. Blood 130, 2537-2547.

35. Eldomery, M.K., Coban-Akdemir, Z., Harel, T., Rosenfeld, J.A., Gambin, T., Stray-Pedersen, A., Kury, S., Mercier, S., Lessel, D., Denecke, J., et al. (2017). Lessons learned from additional research analyses of unsolved clinical exome cases. Genome Med 9, 26.

36. Herrmann, D.N., Horvath, R., Sowden, J.E., Gonzalez, M., Sanchez-Mejias, A., Guan, Z., Whittaker, R.G., Almodovar, J.L., Lane, M., Bansagi, B., et al. (2014). Synaptotagmin 2 mutations cause an autosomal-dominant form of lambert-eaton myasthenic syndrome and nonprogressive motor neuropathy. Am J Hum Genet 95, 332-339.

37. Pang, Z.P., Melicoff, E., Padgett, D., Liu, Y., Teich, A.F., Dickey, B.F., Lin, W., Adachi, R., and Sudhof, T.C. (2006). Synaptotagmin-2 is essential for survival and contributes to Ca2+ triggering of neurotransmitter release in central and neuromuscular synapses. J Neurosci 26, 13493-13504.

38. Tejero, R., Lopez-Manzaneda, M., Arumugam, S., and Tabares, L. (2016). Synaptotagmin-2, and -1, linked to neurotransmission impairment and vulnerability in Spinal Muscular Atrophy. Hum Mol Genet 25, 4703-4716.