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Title:

Neurogenetic fetal akinesia and arthrogryposis: genetics, expanding genotype-phenotypes and functional genomics.

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ABSTRACT (250 words)

Background: Fetal akinesia and arthrogryposis are clinically and genetically heterogeneous and have traditionally been refractive to genetic diagnosis. The widespread availability of affordable genome-wide sequencing has facilitated accurate genetic diagnosis and gene discovery in these conditions.

Methods: We performed next generation sequencing (NGS) in 190 probands with a diagnosis of arthrogryposis multiplex congenita, distal arthrogryposis, fetal akinesia deformation sequence or multiple pterygium syndrome. This sequencing was a combination of bespoke neurogenetic disease gene panels and whole exome sequencing. Only Class 4 and 5 variants were reported, except for two cases where the identified variants of unknown significance (VUS) are most likely to be causative for the observed phenotype. Co-segregation studies and confirmation of variants identified by NGS were performed where possible. Functional genomics was performed as required.

Results: Of the 190 probands, 81 received an accurate genetic diagnosis. All except two of these cases harboured Class 4 and/or 5 variants based on ACMG guidelines. We identified phenotypic expansions associated with *CACNA1S, CHRNB1, GMPPB* and *STAC3*. We describe a total of 50 novel variants, including a novel missense variant in the recently identified gene for arthrogryposis with brain malformations – *SMPD4*.

Conclusions: Comprehensive gene panels give a diagnosis for a substantial proportion (42%) of fetal akinesia and arthrogryposis cases, even in an unselected cohort. Recently identified genes account for a relatively large proportion, 32%, of the diagnoses. Diagnostic-research collaboration was critical to the diagnosis and variant interpretation in many cases, facilitated genotype-phenotype expansions and reclassified VUS through functional genomics.

Keywords: fetal akinesia, arthrogryposis, next generation sequencing, genotype-phenotype, functional genomics, *SMPD4, STAC3, CHRNB1, GLDN, GMPPB*

INTRODUCTION

Fetal akinesia or hypokinesia can result in a range of clinical presentations, including; fetal akinesia deformation sequence (FADS),[1] arthrogryposis multiplex *congenita* (AMC), distal arthrogryposis (DA),[2] lethal congenital contracture syndromes (LCCS), and multiple pterygium syndrome (MPS). Many of these conditions have overlapping features including: joint contractures, pterygia, fetal hydrops, lung hypoplasia and dysmorphic features. While fetal akinesia is frequently diagnosed *in utero*, following abnormal indications on routine ultrasounds and/or maternal reporting of reduced fetal movements, many cases are not detected until birth, when they present with contractures and associated complications.[3] For the purposes of this manuscript, the term fetal akinesia is used as an umbrella description for all entities described above.

Fetal akinesia can arise from gene defects or maternal/external factors. In recent years the contribution of variants in genes that encode critical neuromuscular system proteins has become increasingly recognised as a cause of fetal akinesia. Variants in >70 of these genes are now known to cause fetal akinesia.[4-7]

As was foreshadowed for rare diseases,[8] many novel phenotypes have been attributed to variants in known disease genes – this is also true for the fetal akinesias. In particular, bi-allelic null variants in genes previously associated with autosomal dominant neuromuscular disease have now been associated with fetal akinesia, e.g. recessive *CACNA1S*,[9] *DNM2*,[10] *RYR1*,[11] *SCN4A*,[12] *TOR1A*,[13] and *TTN*[14] disease.

In this study we present our findings from massively parallel sequencing in a cohort of 190 probands presenting with fetal akinesia. We achieved an accurate genetic diagnosis in 81 of these cases, identified two then novel disease genes,[15,16] described 50 novel variants and extended the phenotypes associated with known neuromuscular disease genes.

METHODS

Cohort details

This cohort includes 190 probands with a clinical diagnosis of arthrogryposis multiplex *congenita*, distal arthrogryposis, fetal akinesia deformation sequence, multiple pterygium syndrome (Escobar-variant or lethal) or Schwartz Jampel Syndrome [n=2]. Additional features included CNS involvement (lissencephaly [n=3], polymicrogyria [n=5]), neuropathy (n=2), and congenital myopathy (n=5).

Recruitment into this project was via two arms: (1) cases submitted for diagnostic testing via the PathWest neurogenetic gene panels (some of which were then enrolled in research testing, if no genetic diagnosis was provided in the diagnostic setting), and (2) cases consented for research that underwent testing via the PathWest neurogenetic gene panels and/or whole exome sequencing (WES). Of the 190 patients, 146 underwent diagnostic testing, 16 were studied in both the diagnostic and research setting, and 28 were analysed in the research setting only. Of the 190 probands, all except 15 were from Australia/NZ. These 15 cases were part of the research recruitment arm of this study.

Testing was performed via the PathWest neurogenetic gene panel alone for 150 probands. Combined panel and WES was performed in 29 probands whilst in the remaining 11 probands only WES was performed. The number of probands sequenced on each version of the panel were: 33 (v1), 37 (v2) and 109 (v3).

NGS: Neurogenetic disease gene panel sequencing, mapping and analysis

Next generation sequencing was performed on custom-designed neurogenetic disease gene panels (versions 1-3). Details of versions 1 and 2 of the panel are outlined in Beecroft *et al*.[17] For design of the third iteration of the panel, the genes were split into two panels: a muscle disease gene panel (v3 muscle) and a neurogenic disease gene panel (v3 neuro), with some overlap as appropriate. Sequencing for v3 was performed using Illumina Nextera Rapid Capture Custom Enrichment Kit and sequenced on an Illumina Benchtop Sequencer. Average coverage was 309 fold for versions 1 and 2 of the panel and 350-fold for v3. The minimum accepted coverage cutoffs were as follows; average coverage> 150-fold (v1-3), and 92% (v1-2) or 95% (v3) of te target regions covered to >20x. Details of the genes included in each iteration of the panel are included in

Supplementary Table 1. During the iterative process, panels were spiked with probes to known low-coverage regions to facilitate deeper sequencing.[17] Base calling, mapping, and variant calling were performed using Torrent Suite 3.6.2 or 4.2 with germline high stringency settings (GRCh37), with default values as outlined at: https://github.com/iontorrent/TS/blob/master/plugin/variantCaller/pluginMedia/parameter_sets/4_4/ampliseqexome_germline_highstringency_p1_parameters.json. For v3 of the panel all mapping and calling was done by the BWA Enrichment App v2.1.2 on the Illumina Basespace Sequence Hub using our custom bed files.

In the diagnostic setting, Cartagenia BenchLab/Alissa software (Agilent Technologies) was used for annotation and analysis. Bioinformatic filters were used as a first pass to restrict analysis to a sub-panel of genes associated with each clinical phenotype. If no pathogenic variant was identified, rare coding variants across all genes on the panel were analysed. Variants with a minor allele frequency of >2% were filtered from the analysis. The diagnostic laboratory adopted the American College of Medical Genetics (ACMG) guidelines to classify variants when they became available in 2015.[18]

The research team was able to interpret variants on a research-only basis in Cartagenia/Alissa software, without analysis restricted to sub-panels of genes. If a likely reportable variant was found (Class 4 or 5), interpretation was referred to PathWest for validation in the Australian National Association of Testing Authorities (NATA) accredited laboratory or, in the case of overseas samples, the research findings were reported to the consenting clinician for confirmation by their corresponding diagnostic genomics laboratory. Class 3 variants were followed up with appropriate literature searchs, consultation with international experts, and functional genomics where possible.

This project was approved by the Human Research Ethics Committee of the University of Western Australia (approval number RA/4/20/1008) and participants provided informed consent.

NGS - Whole exome sequencing, mapping and analysis

Ampliseq whole exome sequencing was performed by the Lotterywest State Biomedical Facility Genomics (LSBFG) as previously described.[5,16] Illumina whole exome sequencing

(WES) was performed at either the Australian Genomics Research Facility (AGRF) or the Center for Mendelian Genomics (CMG; The Broad). For AGRF and LSBFG exomes, processing from FASTQ to VCF was performed as per the GATK best practice guidelines version 3.6 (https://software.broadinstitute.org/gatk). VCFs and pedigree information were compiled in a GEMINI database for variant annotation and querying.[19] Variant annotation was performed as described previously[20] with additional filtering: variants with a minor allele frequency of >1%, or which were seen in the homozygous state in ExAC and gnomAD were excluded from downstream analysis. Variants observed >10 times within the GEMINI database were also removed, which removed many sequencing artefacts. Scripts were used to extract variants compatible with the presumed modes of inheritance (autosomal recessive and *de novo* dominant).

CMG exomes were analysed in seqr (https://seqr.broadinstitute.org). CNV detection was performed using The Broad's GATK gCNV calling pipeline, as outlined (https://github.com/broadinstitute/gatk-protected/blob/master/docs/CNVs/CNV-methods.pdf).

Variant confirmation and interpretation

Bi-directional Sanger sequencing was used to confirm missense and indel variants and to determine co-segregation with disease where parental and sibling DNAs were available. CNVs were confirmed using multiplex ligation-dependent probe amplification (MLPA).

In the case of apparent *de novo* variants, samples from the parents and affected individual were sequenced across a panel of microsatellites to check that the samples were compatible with the provided family structure (i.e. to confirm paternity and rule out the possibility of sample mix-ups).

For missense variants, likely pathogenicity was explored using *in silico* tools including: CADD,[21], MuPro,[22] MutationAssessor, MutationTaster, PolyPhen-2[23] PROVEAN[24] and SIFT.[25]

cDNA studies

For muscle cDNA studies, 10 µm frozen sections were cut on a Leica ultramicrotome and collected into a pre-chilled microfuge tube. RNA extraction was performed using the RNeasy (fibrous tissue) kit (QIAGEN). cDNA was generated using Superscript III reverse transcriptase (ThermoFisher Scientific). Sanger sequencing of regions of interest was performed using standard protocols. Primer details are available upon request.

Minigene assays were performed as previously described,[26,27] with slight modifications. This assay relies on the use of a minigene vector which contains a fragment of the *SERPING1* gene, with two exonic regions separated by an intron, cloned into the mammalian expression vector pcDNA 3.1(-), downstream of the cytomegalovirus (CMV) promoter. Following transient transfection into human cells, chimeric transcripts can be analysed by reverse transcriptase PCR (RT-PCR) and sequencing. In brief, the exon of interest and approximately 150 bp of flanking intronic sequence either side, were PCR amplified from patient and control DNA using 2X SuperFi (ThermoFisher Scientific) and cloned into the pCas2.1 minigene construct (a kind gift from Dr. Alexandra Martins) using *Bam*HI/*Mlu*I (ThermoFisher Scientific) to generate pCas2.1-STAC3_e3 (https://benchling.com/s/seq-MpmgmQUFtsZtWsXCHHS9). Wild-type and variant-containing plasmids were purified using a QIAprep miniprep kit (QIAGEN) and sequences verified by Sanger sequencing. Expression constructs were then transfected into human HEK293FT cells using Lipofectamine 3000 (ThermoFisher Scientific), and RNA harvested after 24 hours using an RNeasy mini kit (QIAGEN). cDNA was generated using SuperScript III reverse transcriptase (ThermoFisher Scientific) and chimeric cDNA amplified by RT-PCR using 2X GoTaq G2 (Promega) using forward primer KO1F (5'-TGACGTCGCCGCCCATCAC) and reverse primer pCAS2R (5'-ATTGGTTGTTGAGTTGGTTGTC) and 30 cycles of amplification. RT-PCR products were analysed on a 2% agarose gel stained with ethidium bromide (0.5 µg/mL), and gel extracted and purified using a QIAquick gel extraction kit (QIAGEN) for Sanger sequencing. Splicing differences were assessed by comparing RT-PCR and sequencing results for the wild-type and variant (c.312T>G) constructs. Sequence alignments were performed in benchling (benchling.com) using the MAFFT algorithm.

GLDN cell assay

The plasmid expressing human GLDN (hGLDN) with a myc-tag epitope at the extracellular C-terminus was obtained from Dr Jerome Devaux.[28] Site-directed mutagenesis was performed by Genscript to generate a construct containing the p.Leu20Pro substitution. HEK cells were grown in tissue culture plates as per standard conditions and transfected with either the WT or mutant hGLDN constructs using Lipofectamine 3000. After 48 hours the live cells were incubated for 2 hours with a c-myc (dylight 550) monoclonal antibody (Myc.A7, ThermoFisher Scientific) to label GLDN at the cell surface. Cells were washed 3 times for 5 minutes with PBS to remove unbound antibody and then fixed with 2% paraformaldehyde for 5 minutes. Cells were washed again 3x for 5 minutes in PBS and then blocked and permeabilised with 10% FCS and 0.1% saponin in PBS for 30 minutes. Cells were then incubated with a c-myc FITC-labeled monoclonal antibody (FITCR953-25, ThermoFisher Scientific) to label total GLDN and then washed in PBS containing Hoechst to label nuclei. Imaging was performed on a Nikon inverted microscope.

RESULTS AND DISCUSSION

Diagnostic yield

Through diagnostic and/or research testing (including bespoke targeted neurogenetic disease gene panels and whole exome sequencing) we investigated the genetics of a cohort of 190 Australasian cases with a primary diagnosis of arthrogryposis, fetal akinesia or multiple pterygium syndrome. A genetic diagnosis was obtained in 81 cases (42.6% diagnostic yield, Table 1, Supplementary Table 2). Fifty variants not previously associated with disease were identified (Supplementary Table 2).

TTN was the most frequently identified causative gene (*n*=8). Of the eight *TTN* cases, two showed striking amyoplasia – a feature which has previously been described in congenital titinopathy.[14] Five patients harboured variants within the triplicate repeat region of *TTN*, which is frequently poorly sequenced on exome sequencing.[29] The most frequently observed pathogenic variant within the cohort is the common 1 bp duplication in *CHRNG* (rs774279192)[30] which was present on five alleles (allele frequency 0.0132). The allele frequency of this variant in gnomAD is 0.000343 (97 of 282,834 alleles).

Recently described genes represent a substantial proportion of diagnoses

Of the 81 cases, 26 harboured variants in genes which were added in v2 and v3 iterations of the targeted gene panel (*BICD2*, *ECEL1*, *MAGEL2*, *MYO18B*, *NALCN*, *PIEZO2*, *STAC3*, *ZC4H2*), or within genes not then or only recently associated with disease (*GLDN, MYL1, NUP88, SMPD4*), i.e. none were on v3 gene panel. Thus, within our cohort, the more recently identified genes for arthrogryposis and fetal akinesia made up a substantial proportion of the genetic diagnoses (32.1%, Table 1). To illustrate this point, the diagnostic yield has increased with each iteration of the comprehensive panel (v1 = 30.3%, v2 = 37.8% and v3 = 44.0%). The improved diagnostic yield in v3 of the panel is also in part due to improved coverage and uniformity of coverage thus facilitating calling of variants within the repetitive regions of *NEB* and *TTN* and the improved ability to detect CNVs due to greater and more even coverage. Three variants in *NEB* and four in *TTN* occurred within the repetitive regions which are typically refractory to mapping and sequencing with exome sequencing.

Genotype-phenotype expansions

BICD2: Interestingly, six cases harboured *de novo* variants in *BICD2*. This includes three novel variants not previously reported in the literature or in ClinVar (c.628C>A, p.His201Asn; c.1559T>C, p.Leu520Pro; c.2113G>A, pGlu705Lys). One patient with a *de novo* p.His210Asn substitution presented with arthrogryposis multiplex *congenita*, as did cases with the recurrent *de novo* p.Arg694Cys substitution.[31,32] A series of papers originally described heterozygous *BICD2* variants in families with an SMA-LED phenotype;[33-35] *BICD2*-opathies now account for cases with lethal arthrogryposis through to asymptomatic individuals with mild subclinical features (i.e. myopathic MRI).[32] Over a similar period, the PathWest Diagnostic Genomics service has performed sequencing using v2 of the panel (which includes *BICD2*) on >1,100 neuromuscular disease patients. *BICD2* variants were not identified by the PathWest Diagnostic Genomics service in any other neuromuscular disease groups. Thus, at least in the Australasian setting *BICD2* variants seem to cause arthrogryposis more frequently than the SMA-LED phenotype for which *BICD2* variants were first described.

CACNA1S: In a Caucasian family (Figure 1A) that presented with recurrent fetal akinesia, panel sequencing identified two missense VUS in the *CACNA1S* gene (c.665T>A, p.Met222Lys and c.2365C>T, p.Arg789Cys) in the proband (II:4). Sanger sequencing

confirmed the variants and showed that the c.665T>A variant was paternally inherited and the c.2365C>T variant was maternally inherited. The affected sibling (II:1) was compound heterozygous for these variants, while both healthy siblings carried only the maternal variant. Both variants were predicted to be disease-causing by MutationAssessor, MutationTaster, PolyPhen-2, PROVEAN, and SIFT. MuPro predicted that both variants reduce protein stability. Both substitutions alter highly conserved amino acids (p.Met222 to *D. rerio* and p.Arg789 to *C. elegans,* Figure 1B) and return high CADD scores (p.Met222Lys = 27.1, p.Arg789Cys = 28.9). The p.Met222Lys and p.Arg789Cys substitutions occur within an ion transport domain and an intracellular loop domain of CACNA1S, respectively. The c.665T>A variant is absent from gnomAD and c.2365C>T is present on only 2 of 157,372 alleles. Heterozygous dominantly acting variants in the *CACNA1S* gene cause malignant hyperthermia, hypokalemic periodic paralysis and thyrotoxic periodic paralysis (OMIM 114208). More recently, Schartner *et al.* identified dominant and recessive *CACNA1S* variants associated with congenital myopathy.[9] Ophthalmoplegia, ptosis and high-arched palate were common within this cohort. On muscle biopsy, central nuclei, cores and myofibre size variation were observed. Antenatal onset was detected in three of the seven families based on decreased fetal movements on ultrasound.[9] Affected individuals in two of these families had bi-allelic null alleles, and the third had a single *de novo* missense variant.[(9)] Hunter *et al*. described an isolated case with bi-allelic *CACNA1S* variants and a congenital myopathy with ophthalmoplegia.[36] The proband (II:4) in our family was a fetus in which polyhydramnios, scalp oedema, wrist contractures and talipes were detected on ultrasound. Fetal movements were reported to be reduced by the mother and the pregnancy was terminated at 26 weeks gestation (wg). Mild facial dysmorphic features were noted on autopsy, including low anterior hairline, mild hypertelorism, and moderate retrognathia. Muscle biopsy did not detect atrophy or myofibre disorganisation. A previously affected sibling (II:1) was delivered by emergency Caesarean section at week 32/40 due to placental abruption and died at 10 days of age. The pregnancy was complicated by unexplained polyhydramnios. In retrospect, reduced fetal movements (II:1) were noted compared subsequent pregnancies (II:2/II:3). Subjectively, this baby had ptosis (based on photographic review) and a broad nasal tip (II:1). Unfortunately, cell lines and/or muscle biopsy material was not available for either case for follow-up of the identified class 3 *CACNA1S* variants.

CHRNB1: In a non-consanguineous family of mixed European ancestry (Figure 2A) with recurrent lethal multiple pterygium syndrome, a novel homozygous deletion of *CHRNB1* exon 8 was identified by WES and gCNV at the Broad Center for Mendelian Genomics (Figure 2B). Both parents were shown to be carriers. This deletion was also present in the NSES data; however it was not called due to variable coverage across neighbouring exons. The deletion was confirmed by MLPA. *CHRNB1* variants are a rare cause of autosomal dominant congenital myasthenic syndrome (OMIM 616313) and have been identified to cause recessive CMS in one family (OMIM 616314). Affected individuals in this published recessive family harboured compound heterozygous variants: a 9bp in-frame deletion and skipping of exon 8 on the other allele.[37] Skipping of exon 8 resulted in almost complete loss of pentameric AChR expression. The in-frame deletion resulted in ~70% reduced acetylcholine receptor (AChR) expression.[37] In 2016, a Danish group published the identification of a homozygous 1 bp deletion in exon 1 of *Chrnb1* in Red dairy cattle with arthrogryposis multiplex *congenita*.[38] Three related stillborn calves showed severe generalised contractures of the joints of the spine and limbs. In patients with *CHRNB1*-related CMS there is some residual AChR expression, however in the calves, complete loss of *CHRNB1* resulted in a lethal presentation. These data support the severe phenotype in our family with homozygous exon 8 deletion.

FLNC: A proband (Figure 3) presented at birth with hip dislocation, clenched hands, adducted thumbs, small mouth and high palate, and posteriorly rotated ears. On examination, she had mild arthrogryposis, reduced shoulder movement, elbow dimples and scoliosis. She remained undiagnosed after testing on a commercial arthrogryposis gene panel (143 genes). Trio whole exome sequencing identified a missense variant in *FLNC* (c.3557C>T, p. Ala1186Val). *FLNC* variants are associated with myofibrillar myopathy and distal myopathy[39] and increasingly with cardiomyopathies. Recently, Kiselev *et al.* described a series of four cases with early-onset restrictive cardiomyopathy (RCM) and congenital myopathy.[40] Three of these cases harboured the same *de novo* variant identified in our family and two of these also presented with arthrogryposis at birth. The RCM presented between 6 months and 15 years of age with the median onset of ~2 years of age.[40] Follow-up echocardiogram at 5 years of age in our proband showed a mildly dilatated left atrium, with otherwise normal heart structure and function.

Cardiac surveillance in these cases is important and this work highlights the importance of an accurate genetic diagnosis for optimal clinical care.

GMPPB: In a case from the NICU, that was born after a history of reduced fetal movements, known biallelic *GMPPB* variants were identified (c.220C>T, p.Arg74* and c.1081G>A, p.Asp361Asn). In another family, 18 week gestation ultrasound detected hydrops, pterygia and talipes; the pregnancy was terminated at 20 wg. Post mortem reported facial dysmorphism with wide-set eyes, bilaterally low-set ears, retrognathia and a wide mouth. There was a cleft of the soft and hard palate. Multiple pterygia were noted, including the elbows, shoulders, knees and hips; there was also severe bilateral talipes. Palmar and finger creases were absent. There was thin muscle in all limbs and the psoas appeared similarly. CNS examination showed an overall small brain with a small hypoplastic cerebellum and suggestion of delayed sulcation. There was ambiguous genitalia. Babygram was normal. CGH array revealed a normal female karyotype. Bi-allelic missense variants were identified in *GMPPB* (c.95C>T, p.Pro32Leu and c.1069G>A, p.Val357Ile). Recessive variants in *GMPPB* were initially described in patients with congenital and limb-girdle muscular dystrophies.[41] Bi-allelic *GMPPB* variants have since been described in patients with diverse phenotypes including congenital myasthenic syndrome and isolated episodic rhabdomyolysis.[20,42] Our cases represent a substantial phenotypic expansion of *GMPPB* disease.

SCN4A: Two families within this cohort harboured bi-allelic variants in *SCN4A*. We previously described one of these families, that presented with recurrent lethal amyoplasia, in an autosomal recessive *SCN4A* cohort paper.[12] In the second family, bi-allelic novel missense variants were identified (c.2266C>T, p.Arg756Cys and c.4433C>T, p.Ser1478Leu) in a fetus with distal arthrogryposis. This case had contractures of all limbs and polyhydramnios. The pregnancy was terminated at 22 wg. Both substitutions alter highly conserved amino acids (conserved to *D. melanogaster*, Figure 1C) and are predicted to be deleterious by SIFT and probably-damaging by PolyPhen-2; CADD scores of 32 (p.R756C) and 27.4 (p.S1478L). The p.Ser1478 residue lies within the S4-5 loop of the fourth domain which is involved in channel inactivation. Previous work showed that substitution of p.Ser1478 to cysteine enhanced channel in-activation, i.e. partial loss of function.[43] It would be anticipated that the p.Ser1478Leu substitution would also

generate a channel with partial loss-of-function. Based on this, these variants are highly suspicious but remain Class 3 variants under ACMG guidelines; functional validation of these variants is required to substantiate causality. More recently, *SCN4A* variants were postulated to cause sudden infant death syndrome.[44]

STAC3: In a consanguineous family (Figure 4A) with a history of recurrent fetal akinesia and limb contractures detected on first-trimester ultrasound, we identified a homozygous missense variant in *STAC3* (exon 3, c.312T>G, p.Asp104Glu) in the proband (II:3). Both parents were found to be carriers, and sequencing of DNA from formalin-fixed paraffin-embedded tissue of the first affected fetus (II:1) showed this case was also homozygous for the *STAC3* variant. This variant is absent from gnomAD and affects a highly conserved amino acid. Splicing predictors in Alamut suggested the variant introduced a cryptic donor splice-site. To investigate this possibility, we generated minigene constructs containing the normal exon 3 or the variant exon 3. Studies of RNA (produced in HEK cells) showed that the variant resulted in skipping of the last 22 nucleotides of exon 3 from the mRNA (Figure 4B-C). The consequence of this variant is then (c.313_334del, p.Asp104Glu*fs**73) which is likely to lead to a loss of function. A homozygous missense variant (p.Trp284Ser) in *STAC3* was originally identified as the cause of Native American myopathy.[45] This variant has since been identified in other populations. Zaharieva *et al.* identified 18 patients from 12 families presenting with a congenital myopathy with dysmorphic features and susceptibility to malignant hyperthermia.[46] Seventeen cases were homozygous for the p.Trp284Ser variant, and another proband was compound heterozygous for this variant and an essential splice site variant (c.997-1G>T). Functional analysis has shown reduced sarcoplasmic reticulum Ca^{2+} release in response to KCl depolarisation in patient myotubes.[(46)] Affected cases from our family are likely *STAC3* nulls and thus the very early and severe presentation in our cases fit with the genotype. In support of this, *Stac3* null (*Stac3*$^{-/-}$) mice are born at near Mendelian ratios but are all found dead at birth, and show curved bodies and dropped forelimbs.[47] When dissected from the uterus, *Stac3*$^{-/-}$ fetuses did not move or respond to touch but did have a heartbeat. Histologically, muscle from *Stac3*$^{-/-}$ mice showed central nuclei, reduced myofibril number and size and deranged sarcomeres.[47] STAC3 appears to be critical to normal muscle development and function. Thus, bi-allelic loss-of-function

variants in genes critical to excitation-contraction coupling (*CACNA1S, SCN4A, STAC3, RYR1*)[5,9,11,12,48] cause lethal early-onset disease.

Identification of variants in novel human disease genes

Four cases harboured variants in disease genes that had not been described at the time of whole exome sequencing (*GLDN*,[28] *MYL1*,[16] *NUP88*[15] and *SMPD4*[49]).

The identification of a proband within this cohort with a homozygous *MYL1* missense variant, and another with compound heterozygous variants in *NUP88,* were recently described in separate publications, which also combined additional cases from other research centres with bi-allelic variants in these genes.[15,16]

GLDN: In a family from Hong Kong (Figure 5A) with a history of recurrent fetal akinesia, we identified two VUS in the gliomedin gene (*GLDN*). The first affected baby was born at 36/40 and died at 1 day of age. She had hypoplastic heart and lungs and a high-arched palate. In the second pregnancy, the fetus presented at 18/40 with decreased fetal movements and the pregnancy was terminated at 22 wg. In the third pregnancy, reduced fetal movements were reported at 20/40 and the pregnancy was terminated at 22 wg. This case had fetal hydrops, multiple joint contractures, pterygia and increased muscle bulk. All three affected cases harboured bi-allelic *GLDN* variants: a maternally inherited missense variant, c.59C>T [p.Leu20Pro], and a paternally-inherited essential splice site (ESS) change, c.363+1G>A (Figure5A). Analysis of cDNA derived from muscle from the second case indicated that the missense variant was homozygous at the transcript level, suggesting loss of expression from the allele harbouring the ESS change (Figure 5B). Using the myc-GLDN reporter assay defined in Maluenda *et al*.[28] we showed that, unlike WT human GLDN (hGLDN), gliomedin containing the p.Leu20Pro substitution fails to localise at the surface membrane of HEK cells (Figure 5C). In comparison, Maluenda *et al*. found that gliomedin mutants failed to localise at the cell surface and also did not bind its axonal partner neurofascin-186. Thus, the *GLDN* variants identified in our family are likely functional nulls.

SMPD4: A consanguineous family, from Melbourne, presented with recurrent arthrogryposis multiplex *congenita* and complex brain malformations (Figure 6A). All

cases presented with AMC, were small for gestation age and displayed hypoplasia of the corpus callosum (Figure 6B). Additional features present in two of the three cases included: congenital encephalopathy and microcephaly, cerebellar malformation and hypoplasia (Figure 6B) and hypomyelination (Figure 6C). We performed panel sequencing on the proband (II:3) with the v3 muscle and v3 neuro panels but did not identify any causative variants. We subsequently performed trio whole exome sequencing and identified a novel homozygous missense variant in the *SMPD4* gene (c.575C>T, p.Pro192Leu) in the proband. Each parent was a carrier. This variant was confirmed by bi-directional Sanger sequencing and we also showed that an affected sibling (II:1) was homozygous for this variant. The *SMPD4* variant was also homozygous in a clinical exome (performed elsewhere) on the most recently affected baby (II:4). The variant is predicted to be damaging by MutationTaster, SIFT and PolyPhen-2 and has a CADD score of 22.8. The variant is present on three alleles in gnomAD and alters a highly conserved amino acid (up to *D. rerio*, Figure 6D). *SMPD4* was recently described as a likely disease gene for a syndrome presenting as a skeletal dysplasia with cortical malformations and epilepsy.[50] In a follow-up study, 12 families with bi-allelic *SMPD4* variants and a phenotype encompassing microcephaly, hypomyelination, cerebellar atrophy, congenital arthrogryposis and fetal/postnatal demise were described.[49] *SMPD4* encodes the neutral sphingomyelinase-3; sphingomyelinases are important for the properties of cell membranes and the regulation of transmembrane and peripheral membrane proteins, they are also highly enriched in the nervous system. Magini *et al.*[49] showed via over-expression studies that SMPD4 localises to the ER and nuclear envelope. Immunoprecipitation assays revealed that SMPD4 interacts with several nuclear pore proteins; highlighting a role for the nuclear pore in the disease pathogenesis. In support of this, the role of the nuclear pore in human disease is becoming increasingly recognised.[51,52]

Conclusions

The utility of next generation sequencing in arthrogryposis and fetal akinesia is undeniable, yielding a genetic diagnosis in 42% of cases. In many instances, couples have gone on to have IVF and preimplantation genetic diagnosis or prenatal genetic diagnosis of subsequent pregnancies.

Of note is the substantial contribution of relatively recently identified disease genes and the large muscle genes (*NEB, RYR1* and *TTN*) to disease burden. Previously, most cases did not receive a genetic diagnosis. The clinical and genetic heterogeneity, along with the sporadic nature of the disease or small families, meant that genetic testing was difficult or unattainable beyond a handful of known genes or gene hotspots. The number of genes known to cause these diseases and the phenotypic expansions associated with known genes has been quite remarkable. In this report we show that homozygous null variants in *CHRNB1* or *STAC3* and known *GMPPB* variants cause lethal fetal akinesia, expanding the phenotypes associated with each of these genes.

Moving forward, it is critically important that interdisciplinary teams discuss interpretation of VUS in candidate genes and perform functional genomics to reclassify VUS as likely pathogenic or benign. Alternative splicing appears to be a particularly important mechanism underlying protein regulation in skeletal muscle.[53] Thus, splicing defects represent a substantial contribution to muscle disease burden.[29,54]

Submission of variants into well-curated gene databases (e.g. the LOVD) is also critical to the reclassification of VUS and mapping of known phenotype-genotype associations. Identification of the same rare VUS in two unrelated patients with a similar clinical presentation will facilitate reclassification as likely-pathogenic. By way of illustration, we identified in the research laboratory a then novel, *de novo* missense variant in *BICD2* in a patient with arthrogryposis multiplex *congenita*. The diagnostic laboratory within PathWest had a similar case with the same *BICD2* variant. Follow-up revealed that the variant in this diagnostic case had also arisen *de novo*.[31] In the words of Johan den Dunnen: many VUS may be "*variants of under sharing*" (International Congress of the World Muscle Society 2019, Copenhagen).

As the affordability of and availability of massively parallel sequencing has improved, variant interpretation has become the new bottleneck in accurate genetic diagnosis. Scalable, relatively robust and affordable assays (e.g. the cell-surface localisation assay for gliomedin) that can be utilised to assay VUS in multiple genes should be an ideal that the rare diseases community work towards. Saturation mutagenesis together with appropriate functional read-outs[55] have already been performed for some common

disease genes including *BRCA1* and *PTEN*.[56,57] A historical mutagenesis study of a small region of the SCN4A protein to cysteines informed our interpretation of the likely pathogenicity of a Class 3 variant in this gene,[43] and highlights the clinical utility of such experimental work.

Based on studies in model organisms,[58,59] it is likely that many more fetal akinesia and arthrogryposis genes await discovery. Exome and genome sequencing, along with RNA-seq in genetically unresolved cases, will likely identify additional novel disease genes.

ADDITIONAL RESOURCES

Benchling: https://www.benchling.com/
CADD: https://bio.tools/CADD_Phredh
GEMINI: https://github.com/arq5x/gemini
gnomAD: https://gnomad.broadinstitute.org/
MutationAssessor: http://mutationassessor.org/r3/
MuPro: https://omictools.com/mupro-tool
MutationTaster: http://mutationtaster.org/
PolyPhen-2: http://genetics.bwh.harvard.edu/pph2/
PROVEAN: http://provean.jcvi.org/index.php
Seqr: https://seqr.broadinstitute.org
SIFT: https://sift.bii.a-star.edu.sg/

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COMPETING INTERESTS

The authors do not have any competing interests to declare.

AUTHOR CONTRIBUTIONS

GR, NGL, MRD conceived the study. JSC, FF and PS performed experiments. TZ, KC, AO-L, GR, MRD, JSC, FF and PS analysed data. DM, RC, PM, BK, ME, MD, PJL, SHSC, AM, AC, FC, LH, GG and SG contributed clinical data. GR wrote the manuscript. All authors approved the final manuscript.

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TABLE

***Table 1**: Causative genes identified within the 81 genetically diagnosed probands*

Gene	**Cases (n)**	***Gene***	**Cases (n)**
TTN	8	*CACNA1S*	1
TNNI2	7	*CHANB1*	1
BICD2	**6**	*FBN2*	1
NEB	5	*FLNC1*	1
CHRNG	4	*GBE1*	1
COL6A1	4	*GLDN**	1
MYH3	4	*KLHL40*	1
ECEL1	**3**	*MYL1**	1
MAGEL2	**3**	***MYO18B***	**1**
PIEZO2	**3**	*NUP88**	1
TPM2	3	*RAPSN*	1
TRPV4	3	*RYR1*	1
ZC4H2	**3**	*SMPD4**	1
ACTA1	2	***STAC3***	**1**
DYNC1H1	2	*TNNT3*	1
GMPPB	2		
NALCN	**2**		
SCN4A	2		

Genes in bold denote genes that were added into v2 or v3 of the panel. Genes tagged with an "" represent then novel human disease genes identified by WES (i.e. not on v1-3 of the panel)*

FIGURE LEGENDS

Figure 1: Bi-allelic class 3 variants in CACNA1S and SCN4A associated with fetal akinesia. (A) A pedigree co-segregating bi-allelic missense variants in *CACNA1S* with fetal akinesia and (B) conservation across species at the observed substitutions: p.Met222 and p.Arg789. (C) Evolutionarily conservation at two SCN4A residues substituted, bi-allelically, in another case of fetal akinesia. Importantly, previous work substituting p.Ser1478 to cysteine showed enhanced inactivation of the Na^+ channel. Abbreviations: d. 10 d = died at 10 days of age, TOP 26wg = termination of pregnancy at 26 weeks gestation.

Figure 2: Homozygous deletion within CHRNB1 causes lethal multiple pterygia syndrome. (A) A nonconsanguineous family with recurrent lethal multiple pterygia syndrome; IUFD: *in utero* fetal demise and TOP: termination of pregnancy . (B) Visualisation of the *CHRNB1* exon 8 deletion in the exome sequencing data from the proband (blue trace) and both parents (purple traces). The grey lines represent other samples in the same batch of CNV calling and show the average amount of noise across the region. The y-axis denotes the copy number as inferred by gCNV, and the x-axis shows the position along the gene in kilobases. The dots on the plot represent a probe for exon capture, which roughly represent exons. The copy number estimation is expected to hover around 2.0 for autosomes. For the X chromosome, the copy number estimation should hover around 2.0 for females and 1.0 for males.

Figure 3: Clinical presentation in a case with mild distal arthrogryposis due to a de novo missense variant in FLNC. Images A and B were taken at age 8 months and image C was taken at age 2 months. The images demonstrate reduced elbow extension with dimples, excessive ankle hypermobility, and subtle facial findings including plagiocephaly and micrognathia.

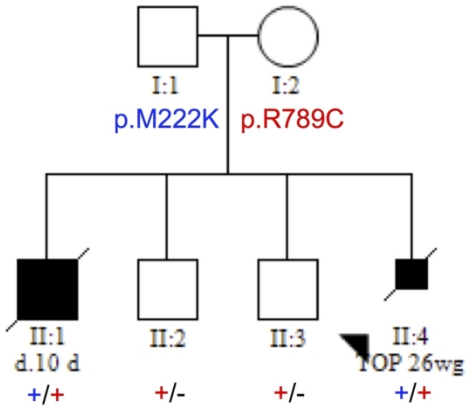
Figure 4: Homozygous variant in STAC3. (A) Pedigree showing segregation of a homozygous variant in *STAC3*. (B) 2% agarose gel of RT-PCR products from the *STAC3* minigene assay. WT – normal exon 3 of *STAC3*, MUT – exon 3 containing the c.312T>G variant. +P or -P indicates the cells were grown in the presence (+) or absence (-) of puromycin (to inhibit potential nonsense-mediated decay). NTC = no template control.

The red arrowhead indicates the smaller product in the samples containing the variant. Sanger sequencing of the RT-PCR product shows that this smaller product corresponds to loss of the 22 nucleotides following the variant in the cDNA (C).

Figure 5: Novel GLDN variants identified in a family with recurrent fetal akinesia. (A) Pedigree showing segregation of bi-allelic VUS in *GLDN*. (B) Sanger sequencing showed that the heterozygous c.59T>C on gDNA (in A) appeared homozygous in muscle cDNA, suggesting loss of expression from the allele containing the essential-splice site change. (C) Detection of hGLDN and mutant hGLDN fused to an extracellular myc-tag in HEK cells in culture (red) and post-fixation and permeabilization (green). Nuclei are stained with Hoechst.

Figure 6: A family with recurrent arthrogryposis and central involvement due to a homozygous missense variant in *SMPD4*. (A) Pedigree, (B) T1 midline sagittal image (Individual II:2, neonatal MRI brain scan) showing absence of the genu and rostrum, thinning and elongation of the callosal body. Microcephaly, hypoplasia of the inferior cerebellar vermis and prominent venous sinuses are also evident. (C) T2 axial image (Individual II:2) showing simplified gyration, compensatory ventriculomegaly and absent myelination. (D) Alignment showing evolutionary conservation of the p.Pro192 residue.

A



B

CACNA1S: p.Met222Lys		CACNA1S: p.Arg789Cys	
Human	LFKGKMHKTCY	Human	RNQKIRVLCHR
D16-0750	LFKGKKHKTCY	D16-0750	RNQKICVLCHR
Mouse	LFKGKMHKTCY	Mouse	RNQKIRVLCHR
Chicken	LFKGKMHKTCY	Chicken	RNQKFRMLCHR
Frog	LFKGKMHKTCY	Frog	RNQKFRKLCYK
Zebrafish	LFKCKMHKTCY	Zebrafish	RNKKFRKLCHR
Fruitfly	LFSGKLHKACR	Fruitfly	LNKRFRVFCHW
Worm	LFCGKLHSTCI	Worm	RNMSFRVFCNM

C

SCN4A: p.Ser1478Leu		SCN4A: p.Arg756Cys	
Human	FALMMSLPALF	Human	FLIVFRILCGE
D16-1073	FALMMLLPALF	D16-1073	FLIVFCILCGE
Mouse	FALMMSLPALF	Mouse	FLIVFRILCGE
Chicken	FALMMSLPALF	Chicken	FLIVFRILCGE
Frog	FALMMSLPALF	Frog	FLIVFRILCGE
Zebrafish	FALMMSLPALF	Zebrafish	FLIIFRVLCGE
Fruitfly	FALAMSLPALF	Fruitfly	FMIVFRVLCGE

Figure 1

124x191mm (300 x 300 DPI)

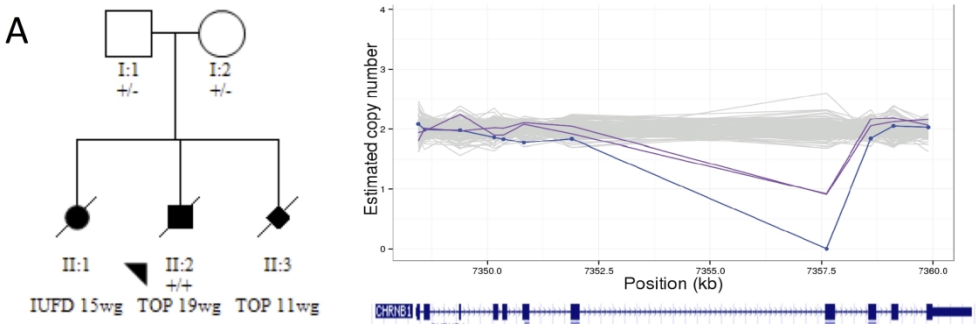
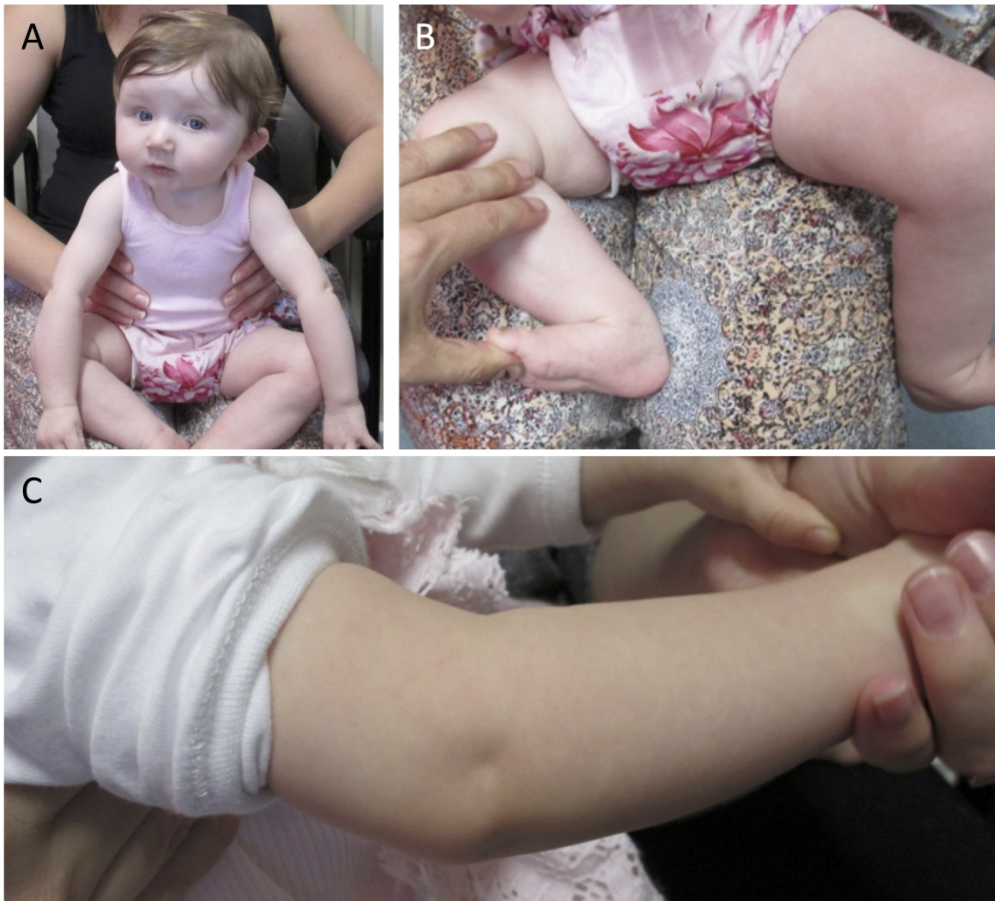


Figure 2

155x51mm (600 x 600 DPI)



158x142mm (600 x 600 DPI)

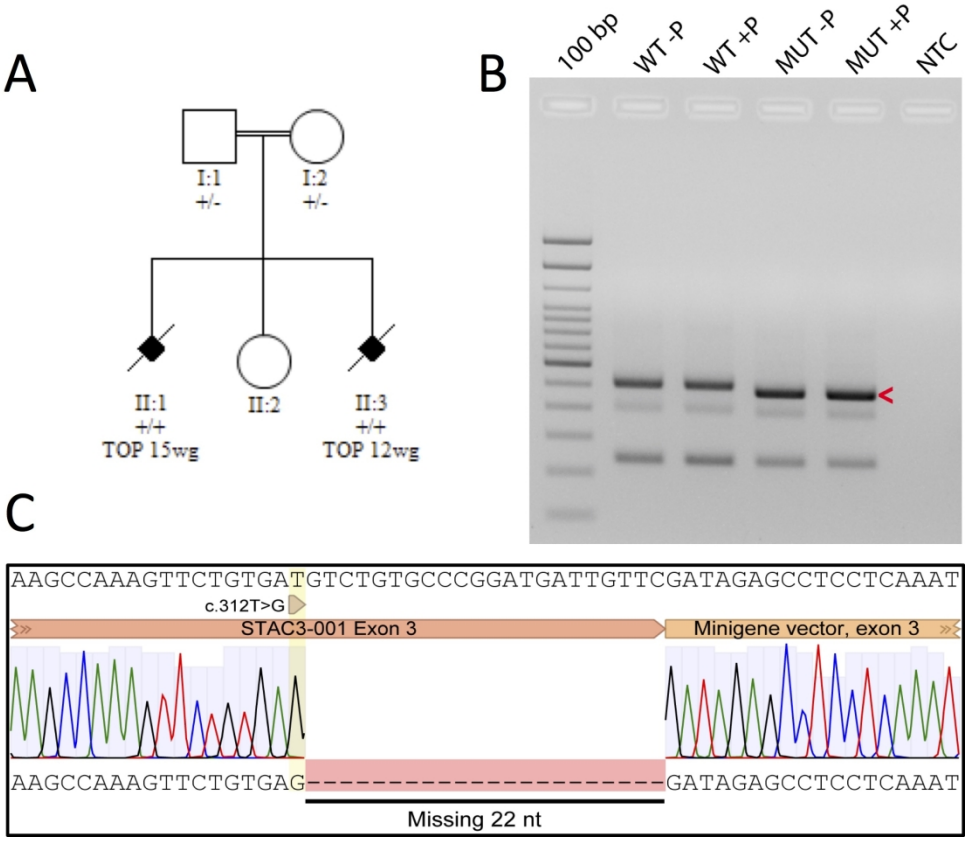


Figure 4

108x91mm (600 x 600 DPI)

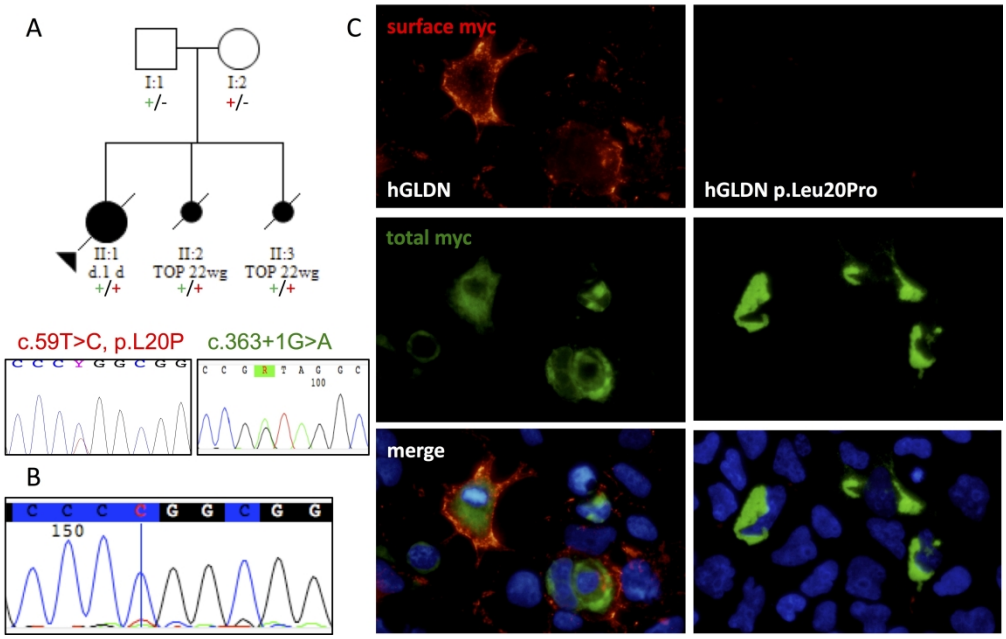


Figure 5

222x141mm (600 x 600 DPI)

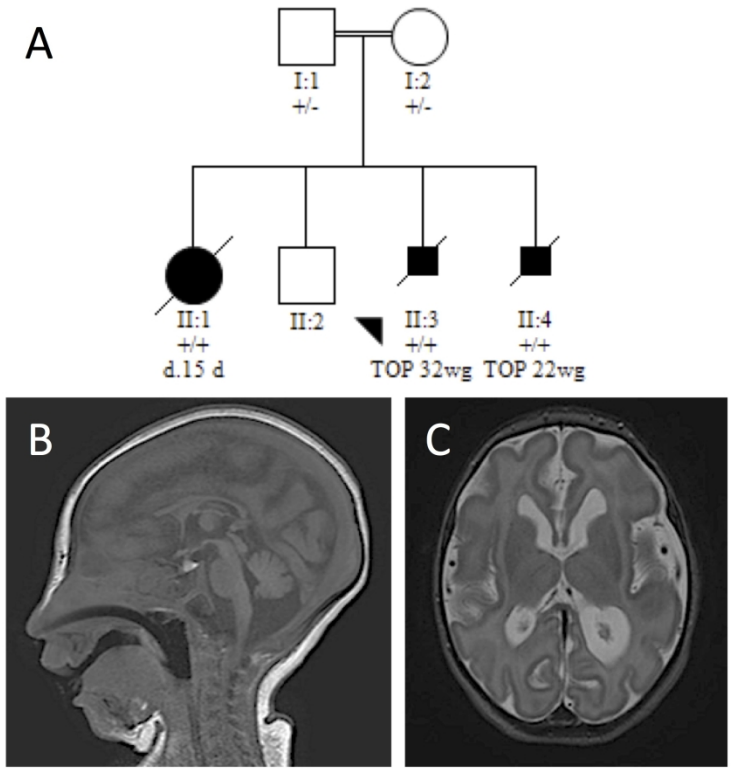


Figure 6

113x154mm (600 x 600 DPI)

Supplementary Table 1: Genes included on each iteration of the PathWest gene panel

v1	***v2***	***v3 neuro***	***v3 muscle***
AARS	AARS	AARS	AARS2
ABCC9	AARS2	ABCB7	ABHD5
ABCD1	ABCD1	ABCD1	ACAD9
ABHD12	ABHD12	ABHD12	ACADVL
ABHD5	ABHD5	ACTB	ACTA1
ACADVL	ACAD9	ACTG1	ACTC1
ACADVL	ACADVL	ADAR	ACTN2
ACTA1	ACTA1	ADCK3	ADCY6
ACTC1	ACTB	ADGRG1	ADGRG6
ACTN2	ADCK3	AFG3L2	ADSSL1
ACVR1	ADCY6	AIFM1	AGK
ADCK3	AFG3L2	AIMP1	AGL
AFG3L2	AGK	AKT1	AGRN
AGL	AGL	AKT3	AIFM1
AGRN	AGRN	ALDH18A1	ALDOA
AHNAK	AIFM1	ALDH3A2	ALG14
AIFM1	AIMP1	ALS2	ALG2
ALDH3A2	ALDH3A2	AMPD2	AMPD1
ALS2	ALG14	ANG	ANKRD1
ANG	ALG2	ANO10	ANKRD2
ANK2	ALS2	ANO3	ANO5
ANKRD1	AMPD1	AP4B1	APOD
ANO10	AMPD2	AP4E1	ASCC1
ANO5	ANG	AP4M1	ATP1A2
AP5Z1	ANO10	AP4S1	ATP2A1
APOA1	ANO3	AP5Z1	ATP5A1
APTX	ANO5	APTX	B3GALNT2
AR	AP4B1	ARFGEF2	B3GALT6
ARHGEF10	AP4E1	ARHGEF10	B4GAT1
ARSA	AP4M1	ARL6IP1	BAG3
ARX	AP4S1	ARSA	BIN1
ASAH1	AP5Z1	ARSI	BVES
ATL1	APTX	ARX	C10orf2
ATM	ARHGEF10	ASAH1	C12orf65
ATP2A1	ARL6IP1	ASCC1	C8orf22
ATP2B3	ARSA	ASPA	CA3
ATP7A	ARSI	ASPM	CACNA1S
B3GALNT2	ASAH1	ATCAY	CAPN3
BAG3	ASPA	ATL1	CASQ1
BEAN1	ATL1	ATL3	CAV3
BIN1	ATL3	ATM	CCDC78
BSCL2	ATM	ATP1A3	CD99
C10orf2	ATP1A3	ATP2B3	CEP89
CACNA1A	ATP2A1	ATP2B4	CFL2
CACNA1C	ATP2B3	ATP6AP2	CHAT
CACNA1S	ATP2B4	ATP7A	CHCHD10
CACNB2	ATP5A1	ATR	CHD7
CACNB4	ATP6AP2	B3GALNT2	CHKA
CAPN3	ATP7A	B4GALNT1	CHKB

CASQ2	B3GALNT2	BCAP31	CHRNA1
CAV3	B3GALT6	BEAN1	CHRNB1
CCT5	B3GNT1	BICD2	CHRND
CFL2	B4GALNT1	BSCL2	CHRNE
CHAT	BAG3	C12orf65	CHRNG
CHRNA1	BCAP31	C19orf12	CHST14
CHRNB1	BEAN1	CACNA1A	CKM
CHRND	BICD2	CACNA1B	CKMT2
CHRNE	BIN1	CACNA1G	CLCN1
CHRNG	BSCL2	CACNB4	CLDN11
CLCN1	C10orf2	CCT5	CMYA5
CNBP	C12orf65	CDK5RAP2	CNTN1
CNTN1	C19orf12	CENPJ	CNTNAP1
COL6A1	CACNA1A	CEP152	COL12A1
COL6A2	CACNA1S	CHCHD10	COL13A1
COL6A3	CACNB4	CHMP2B	COL1A1
COLQ	CAPN3	CLCN2	COL3A1
COX15	CASQ1	CLPP	COL6A1
CPT1B	CAV3	COASY	COL6A2
CPT2	CCDC78	COL4A1	COL6A3
CRYAB	CCT5	COL4A2	COLQ
CSRP3	CEP89	COX6A1	COX14
CTDP1	CFL2	CPT1C	COX6A2
CYP7B1	CHAT	CSF1R	COX6B1
DAG1	CHCHD10	CTDP1	CPT2
DCTN1	CHD7	CUL3	CRYAB
DCX	CHKB	CWF19L1	CYC1
DES	CHMP2B	CYP2U1	DAG1
DHKTD1	CHRNA1	CYP7B1	DCST2
DMD	CHRNB1	DARS2	DES
DMPK	CHRND	DCAF8	DLK1
DNAJB2	CHRNE	DCTN1	DMD
DNAJB6	CHRNG	DCX	DNA2
DNM2	CLCN1	DDHD1	DNAJB6
DNMT1	CLCN2	DDHD2	DNM2
DOCK3	CLPP	DENR	DOK7
DOK7	CNTN1	DHTKD1	DPAGT1
DPAGT1	CNTNAP1	DNAJB2	DPM1
DPM2	COASY	DNAJB5	DPM2
DSC2	COL12A1	DNM2	DPM3
DSG2	COL1A1	DNMT1	DYSF
DSP	COL6A1	DRD2	ECEL1
DTNA	COL6A2	DRP2	EDN3
DYNC1H1	COL6A3	DST	EGR2
DYSF	COLQ	DYNC1H1	EMD
EGR2	COX14	EDN3	ENO3
EMD	COX6B1	EGR2	ERBB3
ENO3	CPT2	EIF2B1	ETFDH
ERBB3	CRYAB	EIF2B2	FARS2
ETFA	CSF1R	EIF2B3	FBN2
ETFB	CTDP1	EIF2B4	FBXL4
ETFDH	CYC1	EIF2B5	FDX1L

EYA4	CYP2U1	ELOVL4	FGF7
FA2H	CYP7B1	ELOVL5	FHL1
FAM134B	DAG1	ENTPD1	FHL3
FBLN5	DARS2	EOMES	FKBP14
FGD4	DCAF8	ERLIN1	FKRP
FGF14	DCST2	ERLIN2	FKTN
FGFR2	DCTN1	EXOSC3	FLNA
FHL1	DDHD1	EXOSC8	FLNC
FIG4	DDHD2	FA2H	GAA
FKRP	DES	FAM126A	GAPDH
FKTN	DHTKD1	FAM134B	GBE1
FLNA	DMD	FARS2	GFPT1
FLNC	DNA2	FAT3	GGPS1
FUS	DNAJB2	FAT4	GLE1
FXN	DNAJB6	FBLN5	GMPPB
GAA	DNM2	FBXO38	GNE
GAN	DNMT1	FGD4	GOLGA2
GARS	DOK7	FGF14	GREM1
GATAD1	DPAGT1	FIG4	GYG1
GBE1	DPM2	FKRP	GYS1
GDAP1	DPM3	FKTN	HACD1
GFPT1	DRD2	FLNA	HADHA
GJA5	DST	FLRT1	HADHB
GJB1	DYNC1H1	FLVCR1	HBB
GJB3	DYSF	FTL	HIST1H3H
GLE1	ECEL1	FUS	HNRNPA1
GMPPB	EGR2	FXN	HNRNPA2B1
GNE	EIF2B1	GAD1	HNRNPDL
GPD1L	EIF2B2	GALC	HRAS
GTDC2	EIF2B3	GAN	HSPB6
GYG1	EIF2B4	GARS	HSPB7
GYS1	EIF2B5	GBA2	HSPB8
HARS	ELOVL4	GCH1	HSPG2
HCN4	ELOVL5	GDAP1	IGF2
HINT1	EMD	GFAP	ISCU
HK1	ENO3	GJB1	ISPD
HOXD10	ENTPD1	GJB3	ITGA7
HSPB1	ERBB3	GJC2	ITGA9
HSPB3	ERLIN1	GNAL	KBTBD13
HSPB8	ERLIN2	GNB4	KCNA1
HSPD1	ETFDH	GOSR2	KCNJ2
HSPG2	EXOSC3	GRID2	KIF21A
IFRD1	EXOSC8	GRM1	KLHL31
IGHMBP2	FA2H	HACE1	KLHL40
IKBKAP	FAM126A	HARS	KLHL41
ILK	FAM134B	HEPACAM	KLHL9
INF2	FBLN5	HINT1	KY
ISCU	FBXL4	HK1	L1CAM
ISPD	FBXO38	HNRNPA1	LAMA2
ITGA7	FDX1L	HNRNPA2B1	LAMB2
ITPR1	FGD4	HNRNPUL1	LAMP2
JPH2	FGF14	HNRNPUL2	LARGE1

JUP
KARS
KBTBD10
KBTBD13
KBTBD5
KCNA1
KCNA5
KCNC3
KCNE1
KCNE2
KCNE3
KCNH2
KCNJ12
KCNJ18
KCNJ2
KCNQ1
KIAA0196
KIF1A
KIF1B
KIF21A
KIF5A
KLHL9
L1CAM
LAMA2
LAMA4
LAMB2
LAMP2
LARGE
LDB3
LDHA
LITAF
LMNA
LMOD3
LPIN1
LRSAM1
MATR3
MED25
MEGF10
MFN2
MPZ
MRE11A
MRPL3
MSTN
MTM1
MTMR2
MTTP
MURC
MUSK
MYBPC1
MYBPC3
MYH2
MYH3
FHL1
FIG4
FKRP
FKTN
FLNA
FLNC
FLRT1
FLVCR1
FTL
FUS
FXN
GAA
GAD1
GALC
GAN
GARS
GBA2
GBE1
GCH1
GDAP1
GFAP
GFPT1
GJB1
GJB3
GJC2
GLE1
GMPPB
GNAL
GNB4
GNE
GOSR2
GRID2
GYG1
GYS1
HADHB
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HEPACAM
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HK1
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HSPB8
HSPD1
HSPG2
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ISCU
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IBA57
IFIH1
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IKBKAP
INF2
ISPD
ITPR1
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KIF1C
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KIF2A
KIF5A
KIF5C
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KLC4
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LARGE1
LAS1L
LITAF
LMNA
LMNB1
LRSAM1
LYST
MAG
MARS
MARS2
MATR3
MCPH1
MED25
MFN2
MLC1
MMP28
MORC2
MPZ
MR1
MRE11A
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LDB3
LDHA
LIMS2
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LMOD2
LMOD3
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LRP4
LYZ
MAGEL2
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MGME1
MICU1
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MUSK
MUSTN1
MYBPC1
MYBPC3
MYF6
MYH13
MYH14
MYH2
MYH3
MYH7
MYH8
MYL1
MYL12A
MYL2
MYL3
MYL4
MYLPF
MYO18B
MYOD1
MYOT
MYOZ1
NALCN
NDUFAF1
NDUFAF2
NEB
NEFL
NFU1
NNAT
NRAP
NUP88
OBSCN

MYH6	ISPD	MYH14	OPA1
MYH7	ITGA7	MYH7	ORAI1
MYH8	ITPR1	NAGLU	P4HA1
MYL2	KARS	NDE1	PABPN1
MYL3	KBTBD13	NDRG1	PBLD
MYLK2	KCNA1	NEFL	PDE4DIP
MYOT	KCNC3	NGF	PDK4
MYOZ2	KCNJ2	NIPA1	PDLIM3
MYPN	KIAA0196	NOP56	PFKM
NDRG1	KIF1A	NOTCH3	PGAM2
NDUFAF1	KIF1B	NT5C2	PGK1
NEB	KIF1C	NTRK1	PGM1
NEFL	KIF21A	OPA1	PHKA1
NEXN	KIF5A	OPTN	PHKB
NGF	KLHL40	OTUD4	PHOX2A
NIPA1	KLHL41	PAFAH1B1	PIEZO2
NOTCH3	KLHL9	PANK2	PIGY
NPPA	L1CAM	PARK2	PIP5K1C
NTRK1	LAMA2	PAX6	PLEC
OPA1	LAMB2	PBLD	PLOD1
PABPN1	LAMP2	PCNT	PMP22
PAFAH1B1	LARGE	PDK3	PNPLA2
PDK3	LAS1L	PDYN	POGLUT1
PEX7	LDB3	PEX10	POLG
PFKM	LDHA	PFN1	POLG2
PFN1	LIMS2	PGAP1	POMGNT1
PGAM2	LITAF	PIK3R5	POMGNT2
PGK1	LMNA	PLA2G6	POMK
PGM1	LMNB1	PLEKHG5	POMT1
PHKA1	LMOD3	PLOD1	POMT2
PHOX2A	LPIN1	PLP1	POSTN
PHYH	LRP4	PMP2	PPP1R27
PIP5K1C	LRSAM1	PMP22	PRELP
PKP2	LYST	PMPCA	PREPL
PLEC	MAG	PNKP	PRX
PLEKHG5	MAGEL2	PNPLA6	PTRF
PLN	MARS	POLG	PYGM
PLP1	MARS2	POLR3A	PYROXD1
PMP22	MATR3	POLR3B	RAPSN
PNPLA2	MED25	POMGNT1	RBCK1
PNPLA6	MEGF10	POMGNT2	RMND1
POLG	MFN2	POMK	RRM2B
POLG2	MGME1	POMT1	RYR1
POMGNT1	MICU1	POMT2	SCN4A
POMT1	MLC1	PPP2R2B	SDHA
POMT2	MPZ	PRDM12	SEPN1
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PRKCG	MRE11A	PRKRA	SGCB
PRPS1	MRPL3	PRNP	SGCD
PRRT2	MSTN	PRPH	SGCG
PRX	MTM1	PRPS1	SLC18A3
PSEN1	MTMR2	PRRT2	SLC19A3

PSEN2
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RELN
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RYR1
RYR2
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SBF2
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SCN5A
SDHA
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SETX
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SGCB
SGCD
SGCE
SGCG
SH3TC2
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SLC22A5
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SLC33A1
SMCHD1
SOD1
SOX10
SPAST
SPG11
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STIM1
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SYNE1
SYNE2
TARDBP
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PGM1
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PLEKHG5
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PNPLA6
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POLR3B
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PSAP
PTEN
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RAB7A
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REEP2
RELN
RNASEH2B
RNASET2
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RUBCN
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SCN1A
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SCYL1
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SETX
SGCE
SH3TC2
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SLC6A3
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SRPK3
STAC3
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STMN2
SUCLA2
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SYNE1
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SYNPO2
SYT2
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UNC79

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TNNT3
TNPO3
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TUBB3
UBA1
UTRN
VAPB
VCL
VCP
VMA21
VRK1
WNK1
YARS
ZFYVE26
ZFYVE27

POMGNT1
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PRKRA
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PSAP
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REEP2
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SCN4A
SCN9A
SDHA
SEPN1
SEPT9
SETX
SGCA
SGCB
SGCD
SGCE
SGCG
SH3TC2
SIGMAR1
SIL1
SLC12A6
SLC16A2
SLC17A5

SPG7
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SPTLC3
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STUB1
SYNE1
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THAP1
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TSEN54
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TUBB
TUBB2B
TUBB3
TUBB4A
TUBG1
UBA1
UBA5
UBQLN2
UNC79
UNC80
USP8
VAMP1
VAPB
VCP
VLDLR

UNC80
UQCRC2
VCP
VMA21
XIRP1
XIRP2
ZBTB42
ZC4H2

SLC19A3
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SLC25A20
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SLC5A7
SLC6A3
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SOD1
SOX10
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SPG7
SPR
SPTBN2
SPTLC1
SPTLC2
STAC3
STIM1
STUB1
SUCLA2
SURF1
SYNE1
SYNE2
TAF1
TARDBP
TAZ
TBP
TCAP
TDP1
TECPR2
TFG
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TH
THAP1
TIA1
TK2
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TMEM5
TNNI2
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VPS37A
VRK1
WDR45
WDR48
WDR62
WNK1
WWOX
YARS
YWHAE
ZBTB18
ZFR
ZFYVE26
ZFYVE27

TNNT1
TNNT3
TNPO3
TNXB
TOR1A
TOR1AIP1
TPM2
TPM3
TPP1
TRAPPC11
TREX1
TRIM2
TRIM32
TRPV4
TTBK2
TTN
TTPA
TTR
TUBB4A
UBA1
UBQLN2
UQCRC2
USP8
VAMP1
VAPB
VCP
VMA21
VPS13A
VPS37A
VRK1
WDR45
WDR48
WNK1
YARS
ZC4H2
ZFR
ZFYVE26
ZFYVE27

***Supplementary Table 2**: Genetic diagnoses made in each of the genetically-resolved 81 cases.*

Clinical Diagnosis	D-number	Setting	*Gene*	Variant/s	Inheritance mode	HPO terms
DA	D18-0172	D	*ACTA1*	c.220G>A (p.Glu74Lys)	*de novo*	Abnormality of prenatal development or birth Arthrogryposis multiplex congenita Amyoplasia
FADS	D16-1596	D	*ACTA1*	**c.440C>A (p.Ser147Tyr)**	*de novo*	Antenatal onset Fetal akinesia sequence Neonatal hypotonia Macrocephaly at birth Frontal bossing Cryptorchidism Congenital finger flexion contractures Contractures of the joints of the upper limbs Contractures of the joints of the lower limbs Aplasia/Hypoplasia of the palmar creases
AMC	D14-1209	D	*BICD2*	**c.628C>A (p.His210Asn)**	*de novo*	Arthrogryposis multiplex congenita Muscle weakness
DA	D16-0307	D	*BICD2*	**c.1559T>C (p.Leu520Pro)**	*de novo*	Arthrogryposis multiplex congenita Bulbar signs Restrictive ventilatory defect Kyphoscoliosis
LMPS	D16-0032	D	*BICD2*	c.1604C>T (p.Ala535Val)	*de novo*	Bilateral congenital talipes Congenital hip dislocation Knee flexion contracture Cryptorchidism Decreased muscle mass
AMC	D11-949[1]	D	*BICD2*	c.2080C>T (p.Arg694Cys)	*de novo*	Arthrogryposis multiplex congenita Neonatal hypotonia Respiratory distress Bilateral perisylvian polymicrogyria Hand clenching Talipes Micrognathia
AMC	D13-094[1]	R	*BICD2*	c.2080C>T (p.Arg694Cys)	*de novo*	Reduced fetal movements Arthrogryposis multiplex congenita Bilateral perisylvian polymicrogyria Cerebellar hypoplasia Hypoplasia of the corpus callosum Femur fracture Central apnea Talipes Ulnar deviation of finger Single transverse palmian crease
DA	D13-090	R	*BICD2*	**c.2113G>A (p.Glu705Lys)**	*de novo*	Hydrops fetalis Arthrogryposis multiplex congenita Pterygium

						Rocker bottom foot Hand clenching Talipes
FADS	D16-0750	D/R	*CACNA1S*	**c.665T>A (p.Met222Lys)** **c.2365C>T (p.Arg789Cys)**	AR	Antenatal onset Talipes Polyhydramnios
LMPS	D15-1251	D/R	*CHRNB1*	**c.[(820+1_821-1)_(1044+1_1045-1)del] - hmz**	AR	Multiple pterygia
DA	D16-0492	D	*CHRNG*	c.136C>T (p.Arg46*) - hmz	AR	Distal arthrogryposis Autosomal recessive inheritance
FADS	D13-1032	D	*CHRNG*	c.459dupA (p.Val154Ser*fs**24) - hmz	AR	Arthrogryposis multiplex congenita Pretibial dimple
DA	D16- 0928	R	*CHRNG*	c.459dupA (p.Val154Ser*fs**24) - hmz	AR	Distal arthrogryposis
FADS	D18-0294	D	*CHRNG*	**c.56-1G>A** c.459dupA (p.Val154Ser*fs**24)	AR	Antenatal onset Distal arthrogryposis
DA	D14-0923	D	*COL6A1*	c.850G>A (p.Gly284Arg)	*de novo*	Hip dislocation Peripheral neuropathy Arthrogryposis multiplex congenita
DA	D17-0991	D	*COL6A1*	**c.906_920dup (p.Glu303_Arg307dup)**	NI	Hip dislocation Bilateral congenital talipes Knee flexion contracture Elbow flexion contracture
DA	D19-1254	D	*COL6A1*	**c.987_995del (p.Asp331_Val333del)**	*de novo*	Elbow flexion contracture Knee flexion contracture Hyperextensibility at wrists Long fingers Long toe
DA	D19-1391	D	*COL6A1*	**c.6248G>A (p.Gly2083Asp)**	NI	Distal arthrogryposis Myopathy Abnormal facial shape Short stature Bilateral congenital talipes Limb-girdle muscle weakness (proximal)
DA	D08-745[2]	D	*DYNC1H1*	c.1792C>T (p.Arg598Cys)	NI	Distal arthrogryposis
DA	D14-1028[2]	D	*DYNC1H1*	c.2327C>T (p.Pro776Leu)	*de novo*	Distal arthrogryposis
DA	D16-0968	D	*ECEL1*	**c.1339C>T (p.Arg447Cys) - hmz**	AR	Antenatal onset Arthrogryposis multiplex congenita Unilateral ptosis Nonprogressive Muscle weakness
DA	D16-1653	D	*ECEL1*	**c.110_155del (p.Phe37Cys*fs**151)** **c.589G>A (p.Gly197Ser)**	AR	Flexion contracture (multiple) Aortic root aneurysm Progressive sensorineural hearing impairment Midface retrusion Micrognathia

DA	D18-0102	D	*ECEL1*	**c.1408-1G>T - hmz**	AR	Progressive distal muscle weakness Lower limb muscle weakness Onset (assymetric L>R) Elevated serum creatine kinase Myopathy
DA	D17-1980	D	*FBN2*	**c.3968G>C (p.Cys1323Ser)**	AD	Distal arthrogryposis
DA	D15-1576	R	*FLNC*	c.3557C>T (p.Ala1186Val)	*de novo*	Hand clenching Hip dislocation Hitchhiker thumb Distal arthrogryposis Scoliosis High palate Posteriorly rotated ears
DA	D18-0978	D	*GBE1*	**c.708G>C (p.Gln236His)** 691+2T>C	AR	Arthrogryposis multiplex congenita
FADS	D12-950	R	*GLDN*	**c.59T>C (p.Leu20Pro)** **c.363+1G>A**	AR	Fetal akinesia sequence Decreased fetal movement Arthrogryposis multiplex congenita Hydrops fetalis Pterygium Hypoplastic heart Pulmonary hypoplasia Skeletal muscle hypertrophy High palate
DA	D19-0998	D	*GMPPB*	c.220C>T (p.Arg74*) c.1081G>A (p.Asp361Asn)	AR	Arthrogryposis multiplex congenita Knee flexion contracture Hip contracture Flexion contracture of finger Wrist flexion contracture Stiff shoulders Overlapping fingers Talipes Limited elbow extension Amyoplasia (not typical) Decreased fetal movement (antenatal) Diaphragmatic paralysis Elevated serum creatine kinase Hirsutism
DA	D18-0741	D	*GMPPB*	c.95C>T (p.Pro32Leu) c.1069G>A (p.Val357Ile)	AR	Antenatal onset Hydrops fetalis Abnormal facial shape Small anterior fontanelle Sagittal craniosynostosis Hypertelorism Wide mouth Retrognathia Cleft palate

						Webbed neck Low-set ears Multiple pterygia Bilateral talipes equinovarus (severe) Abnormality of the musculature of the limbs Abnormality of brain morphology
DA	D16-1428	D	*KLHL40*	c.602G>A (p.Trp201*) - hmz	AR	Antenatal onset Arthrogryposis multiplex congenita
DA	D18-2007	D	*MAGEL2*	**c.1869dup (p.Thr624His*fs**89)**	NI	Elbow flexion contracture Flexion contracture of fingers and thumbs Hand clenching Torticollis
DA	D16-1250	D	*MAGEL2*	**c.1880G>A (p.Trp627*)**	*de novo*	Antenatal onset Arthrogryposis multiplex congenita Flexion contracture (all major joints) Hypertonia Elevated serum creatine kinase
DA	D18-1694	D	*MAGEL2*	**c.2895G>A p.(Trp965*)**	paternal (imprinted gene)	Distal arthrogryposis Obstructive sleep apnea Bulbar signs Feeding difficulties
DA	D16-1217	D	*MYH3*	**c.725C>G (p.Ser242Cys)**	*de novo*	Distal arthrogryposis
DA	D19-0178	D	*MYH3*^	**c.1539C>G (p.Asp513Glu)** **c.2036C>T (p.Thr679Ile)** **c.2113A>C (p.Ile705Leu)**	AD	Distal arthrogryposis Micrognathia Chin with H-shaped crease
DA	D14-0774	D	*MYH3*	c.2014C>T (p.Arg672Cys)	NI	Antenatal onset Abnormal facial shape
DA	D19-0489	D	*MYH3*	**c.2474T>G (p.Val825Gly)**	NI	Distal arthrogryposis Adducted thumb Bilateral talipes equinovarus Micrognathia Respiratory infections in early life
DA/CM	D14-1355[3]	R	*MYL1*	c.488T>G (p.Met163Arg) - hmz	AR	Decreased fetal movement Apnea Respiratory insufficiency due to muscle weakness Generalized hypotonia Myopathic facies Axial muscle weakness High palate
FADS	D17-0045	D	*MYO18B*	**c.193C>T (p.Arg65*)** **c.1537G>C (p.Ala513Pro)**	AR	Antenatal onset Hypoplasia of the musculature Elbow flexion contracture Knee flexion contracture Hip contracture Pterygium

						Cystic hygroma Dextrotransposition of the great arteries Ventricular septal defect Hypoplastic left heart Abnormal cerebral morphology
DA/CM	D18-0198	D	*NALCN*	**c.1745A>G (p.Tyr582Cys)**	*de novo*	Distal arthrogryposis
DA	D16-1913	D	*NALCN*	c.3542G>A (p.Arg1181Gln)	*de novo*	Antenatal onset Hand clenching
FADS	D17-1855	D	*NEB*	**c.739del (p.Ala247Leu*fs**16)** c.13476+1G>A~	AR	Stillbirth Multiple pterygia Fetal akinesia sequence Aplasia/Hypoplasia involving the skeletal musculature Talipes equinovarus Flexion contracture Cerebral palsy Abnormal lung lobation
LMPS	D14-1786[4]	R	*NEB*	c.10872+1G>T - hmz	AR	Multiple pterygia Fetal akinesia sequence Arthrogryposis multiplex congenita Cystic hygroma Hydrops fetalis Polyhydramnios Intaruterine growth restriction Camptodactyly Ulnar deviation of finger Long philtrum Downslanted palpebral fissures Hypertelorism Low-set ears
LMPS	D15-1283	D	*NEB*	**c.11610C>A (p.Tyr3870*)** **c.18024_18027del (p.Val6009Trp*fs**67)**	AR	Antenatal onset Fetal akinesia sequence
DA	D17-0450	D	*NEB*	**c.12361del (p.Arg4121Val*fs**13)~** **c.13788+2dup~**	AR	Generalized hypotonia Distal arthrogryposis Myopathy
FADS	D14-0067	R	*NEB*	c.3987+1_3987+2delinsTG **c.24543dup (p.Thr8182His*fs**15)**	AR	Reduced fetal movements Polyhydramnios Distal arthrogryposis Talipes Generalized hypotonia Myopathic facies Short chin Cleft palate
FADS	D15-1176[5]	R	*NUP88*	c.1525C>T (p.Arg509*) c.1889_1901del (p.Glu634del)	AR	Decreased fetal movements Polyhydramnios Arthrogryposis multiplex congenita Hypoplasia of the musculature

						Posteriorly rotated ears Microretrognathia
DA	D15-1255	D	*PIEZO2*	c.7067C>T (p.Thr2356Met)	AD	Distal arthrogryposis
DA	D16-0479	D	*PIEZO2*	c.8057G>A (p.Arg2686His)	NI	Talipes (severe) Joint contracture of the hand Down-sloping shoulders Limited elbow movement Short hallux Distal arthrogryposis
DA	D15-1298	D	*PIEZO2*	c.8057G>A (p.Arg2686His)	NI	Skeletal muscle atrophy Shoulder flexion contracture Elbow flexion contracture Knee flexion contracture Hip contracture Bilateral talipes equinovarus Absent palmar crease Stiff fingers Interphalangeal joint contracture of finger
DA	D17-1198	D	*RAPSN*	c.264C>A (p.Asn88Lys) **c.712C>T (p.Gln238*)**	AR	Arthrogryposis multiplex congenita Bilateral talipes equinovarus Contractures of the joints of the lower limbs (knee, hip) Elbow flexion contracture (mild) Respiratory insufficiency Sleep disturbance
DA	D17-1678	D	*RYR1*	**c.2255C>T (p.Pro752Leu)** c.14804-1G>T	AR	Neonatal respiratory distress Shoulder flexion contracture Aplasia/Hypoplasia of the palmar creases Hip contracture Knee flexion contracture Bilateral talipes equinovarus (mild) Severe intrauterine growth retardation
FADS	D16-1073	D	*SCN4A*	**c.2266C>T (p.Arg756Cys)** **c.4433C>T (p.Ser1478Leu)**	AR	Distal arthrogryposis Polyhydramnios
DA	D14-0912[6]	D/R	*SCN4A*	c.608T>A (p.Met203Lys) c.4779C>A (p.Tyr1593*)	AR	Amyoplasia (lethal) Arthrogryposis multiplex congenita
DA	D18-0398	D/R	*SMPD4*	**c.575C>T (p.Pro192Leu) - hmz**	AR	Arthrogryposis multiplex congenita Small for gestational age Lissencephaly Hypoplasia of the corpus callosum Congenital encephalopathy Cerebellar hypoplasia
LMPS	D17-0476	D/R	*STAC3*	**c.312T>G (p.Asp104Glu) - hmz**	AR	Multiple pterygia Fetal akinesia sequence Arthrogryposis multiplex congenita

DA	D16-0627	D	*TNNI2*	c.521G>A (p.Arg174Gln)	*de novo*	Antenatal onset Wrist flexion contracture Abnormality of the ankles Decreased fetal movement (Knees/elbows)
DA	D16-0481	D	*TNNI2*	c.466C>T (p.Arg156*)	AD	Distal arthrogryposis Autosomal dominant inheritance
DA	D18-2142	D	*TNNI2*	c.499_501delGAG (p.Glu167del)	NI	Distal arthrogryposis
DA	D19-0904	D	*TNNI2*	c.521G>A (p.Arg174Gln)	AD	Distal arthrogryposis Hydronephrosis (left) Feeding difficulties
DA	D19-1111	D	*TNNI2*	c.521G>A (p.Arg174Gln)	NI	Distal arthrogryposis Shoulder flexion contracture Limb joint contracture
DA	D18-1716	D	*TNNI2*	c.527_529delAGA (p.Lys176del)	NI	Distal arthrogryposis
DA	D19-0754	D	*TNNI2*	c.527_529delAGA (p.Lys176del)	NI	Congenital onset Distal arthrogryposis Laryngomalacia Micrognathia
DA	D14-1680[7]	D	*TNNT3*	c.681+1G>A - hmz	AR	Distal arthrogryposis Abnormal facial shape Scoliosis Respiratory insufficiency Contractures of the joints of the upper limbs Contractures of the joints of the lower limbs
DA	D13-1137	D	*TPM2*	c.20-22del (p.Lys7del)	AD	Arthrogryposis multiplex congenita Autosomal dominant inheritance
DA	D17-2016	D	*TPM2*	c.463G>A (p.Ala155Thr)	AD	Congenital onset Wrist flexion contracture Elbow flexion contracture Knee flexion contracture Rocker bottom foot
DA	D17-1613	D	*TPM2*	c.541G>A (p.Glu181Lys)	NI	Distal arthrogryposis
DA	D17-1807	D	*TRPV4*	**c.1058G>A (p.Cys353Tyr)**	de novo	Arthrogryposis multiplex congenita Limb muscle weakness Myopathic facies Bulbar palsy Hypoventilation Skeletal dysplasia
DA	D16-1146	D	*TRPV4*	c.557G>A (p.Arg186Gln)	AD	Distal arthrogryposis Bilateral talipes equinovarus Micrognathia Rocker bottom foot Ventricular septal defect Knee flexion contracture
DA	D15-1019	D	*TRPV4*	c.806G>A (p.Arg269His)	NI	Arthrogryposis multiplex congenita

DA	D16-1830	D	*TTN*	c.10303+2T>C **c.37193del (p.Pro12398Leu*fs**549)~**	AR	Distal arthrogryposis
DA	D12-502[8]	R	*TTN*	c.16621+1G>T c.28561C>T (p.Gln9521*)	AR	Distal arthrogryposis Pterygium Webbed neck Cleft palate Bilateral ptosis
DA	D17-1146	R	*TTN*	**c.31208-2A>C** c.38660delA (p.Lys12887Arg*fs**60) ~	AR	Amyoplasia Arthrogryposis multiplex congenita Scoliosis Nevus flammeus of the forehead
DA	D15-0954	D	*TTN*	c.35756del (p.Pro11919Leu*fs**51) c.93110G>A (p.Trp31037*)	AR	Distal arthrogryposis
DA	D19-0564	D	*TTN*	**c.37154del (p.Lys12385Arg*fs**562)~** **c.58034_58035del p.(Thr19345Ser*fs**2)**	AR	Distal arthrogryposis Horseshoe kidney
EV-MPS	D17-0237[9]	D	*TTN*	c.37228delC (p.Glu12411Lys*fs**536)~ c.39974-11T>G	AR	Distal arthrogryposis Multiple pterygia Low-set ears Narrow mouth Short nose Depressed nasal ridge Anteverted nares
AMC	D09-079	R	*TTN*	c.38660delA (p.Lys12887Arg*fs**60)~ **c.56329A>G (p.Met18777Val) - alters splicing**	AR	Decreased fetal movement Polyhydramnios Amyoplasia
FADS	D18-0091	D	*TTN*	c.39974-11T>G **c.44815+1G>A**	AR	Arthrogryposis multiplex congenita Central hypotonia Feeding difficulties Bilateral cryptorchidism
DA	D15-0320	D	*ZC4H2*	**c.399-1G>A**	*de novo*	Distal arthrogryposis Rocker bottom foot Hip contracture Amyoplasia
DA	D17-0639	D	*ZC4H2*	**c.53+1G>A**	NI	Distal arthrogryposis Weakness of facial musculature Ptosis Keratosis pilaris Sleep apnea Atrial septal defect Dilatation of the renal pelvis
DA	D17-0141	D	*ZC4H2*	**c.601C>A (p.Pro201Thr)**	NI	Distal arthrogryposis Abnormality of the extraocular muscles

Numbers in superscript denote reference pertaining to the case where cases were published previously as case reports, as part of gene-specific cohort studies or novel disease gene discoveries. Setting describes whether the case was sequenced and analysed in a diagnostic (D) or research (R) laboratory or both (D/R). Variants in bold represent variants, that to the best of our knowledge have not been previously described as disease-causing. ~denotes variants within the triplicate repeat region of NEB and TTN. AMC = arthrogryposis multiplex congenita, CM = congenital myopathy, DA = distal arthrogryposis, EV = Escobar variant, FADS = fetal akinesia deformation sequence, hmz = homozygous, LMPS = lethal multiple pterygium syndrome. MYH3^ represents a variant allele present in the proband, affected father and affected paternal uncle. NI = no information.

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