



# Recognizable Pattern of Arthrogryposis and Congenital Myopathy Caused by the Recurrent *TTN* Metatranscript-only c.39974-11T > G Splice Variant

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## Abstract

**Introduction** Arthrogryposis is characterized by the presence of multiple contractures at birth and can be caused by pathogenic variants in *TTN* (*Titin*). Exons and variants that are not expressed in one of the three major isoforms of titin are referred to as “metatranscript-only” and have been considered to be only expressed during fetal development. Recently, the metatranscript-only variant (c.39974–11T > G) in *TTN* with a second truncating *TTN* variant has been linked to arthrogryposis multiplex congenita and myopathy.

**Methods** Via exome sequencing we identified the *TTN* c.39974–11T > G splice variant *in trans* with one of three truncating variants (p.Arg8922\*, p.Lys32998Asnfs\*63, p.Tyr10345\*) in five individuals from three families. Clinical presentation and muscle ultrasound as well as MRI images were analyzed.

**Results** All five patients presented with generalized muscular hypotonia, reduced muscle bulk, and congenital contractures most prominently affecting the upper limbs and distal joints. Muscular hypotonia persisted and contractures improved over time. One individual, the recipient twin in the setting of twin-to-twin transfusion syndrome, died from severe cardiac hypertrophy 1 day after birth. Ultrasound and MRI imaging studies revealed a recognizable pattern of muscle involvement with striking fibrofatty involvement of the hamstrings and calves, and relative sparing of the femoral adductors and anterior segment of the thighs.

## Keywords

- ▶ congenital titinopathy
- ▶ myopathy
- ▶ titin
- ▶ cardiomyopathy
- ▶ case series
- ▶ arthrogryposis

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**Conclusion** The recurrent *TTN* c.39974–11T > G variant consistently causes congenital arthrogryposis and persisting myopathy providing evidence that the metatranscript-only 213 to 217 exons impact muscle elasticity during early development and beyond. There is a recognizable pattern of muscle involvement, which is distinct from other myopathies and provides valuable clues for diagnostic work-up.

## Introduction

Arthrogryposis is characterized by congenital non-progressive contractures involving more than one joint. Arthrogryposis is attributed to variable conditions, which all cause decreased fetal movements.<sup>1</sup> The disease spectrum includes disorders of the peripheral (i.e., neuropathies), or central nervous system, congenital myopathies, myasthenic syndromes, metabolic diseases, and inherited disorders of the connective tissue, mirroring the broad clinical spectrum.<sup>2</sup>

Pathogenic variants in *TTN*, encoding the sarcomeric protein titin, account for a heterogeneous group of muscular diseases including cardiomyopathy, congenital myopathies, and other skeletal myopathies such as autosomal dominant tibial muscular dystrophy and limb-girdle muscular dystrophy.<sup>3</sup> *TTN* is the most common gene causing autosomal dominant dilated cardiomyopathy (DCM) in adulthood.<sup>4,5,6</sup>

Titin, along with myosin and actin, is one of the three major structural proteins in the sarcomere. Titin elastically connects myosin to the Z-disk of the sarcomere, thereby holding myosin in position, mediating passive elasticity, and preventing overstretching of the sarcomere. Titin is an extraordinarily large (approximately 3 megadalton) protein spanning over a distance of 1 to 2 μm (Z- to M-line) and consisting of up to 34,000 amino acids.<sup>7</sup> *TTN* undergoes extensive differential splicing and many alternatively spliced and differentially expressed isoforms have been identified, making *TTN* a very complex gene and a challenge for genetic diagnostics.<sup>8,9</sup> The N2B *TTN* isoform containing the N2B element is cardiac specific, while the N2A isoform containing N2A elements is primarily expressed in skeletal muscle. The larger N2BA isoforms include both the N2B and N2A elements and are expressed in the heart, with a maximal expression during fetal development.<sup>10</sup> The theoretical, inferred isoform that includes all exons is referred to as metatranscript (NM\_001267550.1). Many of the metatranscript-only exons were formerly thought to be only expressed during fetal development.<sup>9</sup>

First, recessive *TTN* splice variants predicted to affect all three major isoforms N2A, N2B, N2BA and were reported to cause congenital arthrogryposis with cardiac involvement including left ventricular non-compaction and DCM.<sup>11</sup> Later, metatranscript-only variants were reported in association with congenital arthrogryposis without cardiac involvement.<sup>12,13</sup>

Recently, a metatranscript-only intronic variant (c.39974–11T > G) in *TTN* inherited *in trans* with a second truncating *TTN* variant was identified to cause arthrogryposis multiplex congenita and myopathy.<sup>14</sup> Individuals presented with variable congenital contractures and muscular

hypotonia, but none of the patients was noted to have cardiac involvement.

Here, we report five additional patients from three families with congenital arthrogryposis and myopathy harboring the recurrent intronic variant c.39974–11T > G (NM\_001267550.1: c.39974–11T > G) in *TTN* (designated “*TTN-11*”) and a second truncating variant *in trans*. One individual, the recipient twin in the setting of twin-to-twin transfusion syndrome, died from severe cardiac hypertrophy 1 day after birth suggesting that *TTN-11*-arthrogryposis might be associated with cardiomyopathy under specific circumstances. This series confirms the distinct presentation of *TTN-11*-associated myopathy and reveals a recognizable pattern of muscle involvement on MRI and ultrasound images.

## Methods

### Study Design and Patients Recruitment

The study is designed as an observational case series. From March 2020 to March 2021, five patients with arthrogryposis and congenital myopathies were identified newly in-hospital and were enrolled for further analyses. The legal guardians gave informed consent. Ethical approval was obtained from the institutional ethical review boards in Düsseldorf and the NIH (Bethesda, Maryland).

### Exome Sequencing, Clinical Investigation, Radiographic Analyses

Exome sequencing was performed at two centers: Tübingen (Family A + B) and Genomics Platform at the Broad Institute of MIT and Harvard (Family C) (► **Supplementary Material**, available in the online version). Clinical information, including natural histories of clinical symptoms, evaluation of cardiac and pulmonary function, serum creatine levels were reviewed. Muscle biopsies and autopsy samples were prepared for sectioning and staining using standard protocols and evaluated by pathologists (A.S.J.). Ultrasound scans were assessed according to standard protocol (► **Supplementary Material**, available in the online version). Axial and coronal muscle MR images of the legs were acquired in conventional T1-weighted and short T1 inversion recovery sequences. Fatty infiltration and edema were evaluated by radiologists and neurologists (S.S.).

## Results

### Clinical History of Patients AII-1, AII-2, BII-1, CII-1, and CII-2

BII-1 is a 3-year-old girl, and CII-1 and CII-2 are 11-year-old and 4-year-old boys, respectively. AII-1 and AII-2 are

monozygotic monochorionic-diamniotic twins and their pregnancy was complicated by twin-to-twin transfusion syndrome. The recipient twin AII-1 died of cardiac failure at day 1 after birth.

### Perinatal and Neonatal Period

During pregnancy slightly reduced fetal movements were noted in all patients.

All reported patients were born by C-section (causes are listed in ►Table 1). Except for the twins (AII-1 and AII-2), who had twin-to-twin transfusion syndrome, the auxological data were within normal range. All patients (5/5) presented with congenital onset muscular hypotonia with frog-leg position and weakness with abnormal positioning of extremities and fixed contractures (►Fig. 1B-i + ii; ►Supplementary Table S1, available in the online version). Four patients (4/5) had fractures of the upper extremities. All patients had a weak cry and suck and some had feeding difficulties. AII-1 required nasogastric tube feeding for 2 weeks. CII-1 had severe dysphagia and aspiration requiring G-tube placement and intensive care during the first 3 months of life. AII-1 and BII-1 required respiratory support (continuous positive airway pressure [CPAP]) during their first weeks of life.

### Neuromuscular and Motor Development

In all patients, the muscular weakness presented at birth. Contractures showed a consistent interindividual pattern and were most evident in the upper extremities. Elbows were most severely affected. All patients had a recognizable ulnar abduction and extension of wrist (►Fig. 1B). In all patients, flexion contractures improved over time. For example, the degree of reduced elbow extension improved from 110 degrees (1 month) to 40 degrees (12 months) (►Fig. 1C). The degree of knee flexion contracture was only mild (5–10 degrees). Most patients had adducted thumbs (3/5), flexed fingers including distal interphalangeal joints (5/5), clubfeet (3/5), and second toes overlapping first toes (3/4). Torticollis (3/5) or scoliosis (2/5) was mild (►Fig. 1B-vi + vii) (details are provided in ►Supplementary Table S1, available in the online version).

Muscular weakness was proximal more pronounced than distal in both upper and lower extremities and was stable, non-progressive. Pulmonary function tests in CII only showed mildly decreased forced vital capacity. While language and cognitive development were unremarkable, all patients had motor delay. At 10 months of age, AII-1 was not able to lift the upper arms against gravity, and elevation of legs was incomplete. BII-1 and CII-1 learned walking (at 3 years and 5 years of age, respectively), but required assist devices for ambulation and walking. C-II required a wheelchair for mobility. There was generalized hyporeflexia in all patients. Serum creatinine kinase was within normal range (5/5). The neuromuscular phenotype significantly overlaps with the clinical description of the nine patients with the recurrent *TTN-11* splice variant reported by Bryen et al (►Table 1).<sup>14</sup>

### Facial Features

Facial features included high arched palates (►Fig. 1B-vii) (5/5) and retrognathia (3/5) (►Fig. 1B-iii) and three patients had positional plagiocephaly.

### Muscle Ultrasound

Muscle ultrasound was performed in all patients who survived (4/5) (AII-1: 6 months; BII-1: 1.5 years, CII-2: 4 years, CII-1: 11 years). In all patients, ultrasound imaging revealed moderate to severe involvement of hamstrings muscles with most severe fatty fibrotic changes in semitendinosus (“semitendinosus sign,” ►Fig. 2D, F). There was a consistent sparing of the adductor muscles and the tibialis anterior and peroneus muscles when compared with the other muscles of the legs (►Fig. 2B, D, F). In all patients, there was mild to moderate involvement of upper extremity muscles, including M. biceps brachii (►Fig. 2D, F, grading of muscle involvement see ►Supplementary Tables S2 and S3, available in the online version).

### Muscle Magnetic Resonance Imaging (MRI)

Muscle MRI was performed in AII-1 (6 months), CII-2 (4 years), and CII-1 (11 years). MRI of the lower extremity showed generalized reduced muscle bulk and fatty-fibrotic changes throughout (see ►Fig. 2A, C, E) and severe involvement of quadriceps and all hamstring muscles. The semitendinosus exhibited the most severe fatty-fibrotic changes, while the adductor muscles (M. sartorius and M. gracilis) were relatively spared. In the lower leg, there was prominent involvement of posterior compartment (M. gastrocnemius) and relative sparing of anterior compartment (►Fig. 2C).

### Cardiac Involvement

Echocardiography of BII-1 showed a small, apical ventricular septal defect, which was not present anymore at the age of 6 months. Echocardiography of patient BII-1 (3 weeks; 6 months), CII-2 (4 years), and CII-1 (11 years) was unremarkable and there was no dilated or hypertrophic cardiomyopathy.

The twins of family A were subject to twin-to-twin transfusion syndrome and were born preterm (29 weeks of gestation): AII-1 was the donor twin with oligohydramnios (birth weight 730 g; –2.08 SD) and AII-2 was the recipient twin (1,550 g; +0.47 SD) with polyhydramnios requiring repeated amniotic fluid punctures. Echocardiography of the smaller, donor twin showed mildly restricted ejection function on the day of birth and a normal cardiac function on the follow-up examination (►Fig. 3A). In contrast, echocardiography of the recipient twin (AII-2) revealed severe non-obstructive, global myocardial hypertrophy, moderate pulmonary hypertension (two-thirds of systemic pressure). Chest X-ray depicted pulmonary edema, and invasive inhalation was necessary (►Fig. 3E). While cardiac function was initially hyperdynamic, pump function rapidly decreased (►Fig. 3B–D, ►Videos 1–3, ►Supplementary Tables S4 available in the online version). Fifteen hours after birth, severe bradycardia occurred. Despite cardiac resuscitation and maximal care, the neonate died of low-output cardiac failure.

**Table 1** Clinical characteristics and genetic variants of all five patients with the recurrent metatranscript-only variant c.39974-11T > G (TTN-11) and arthrogryposis

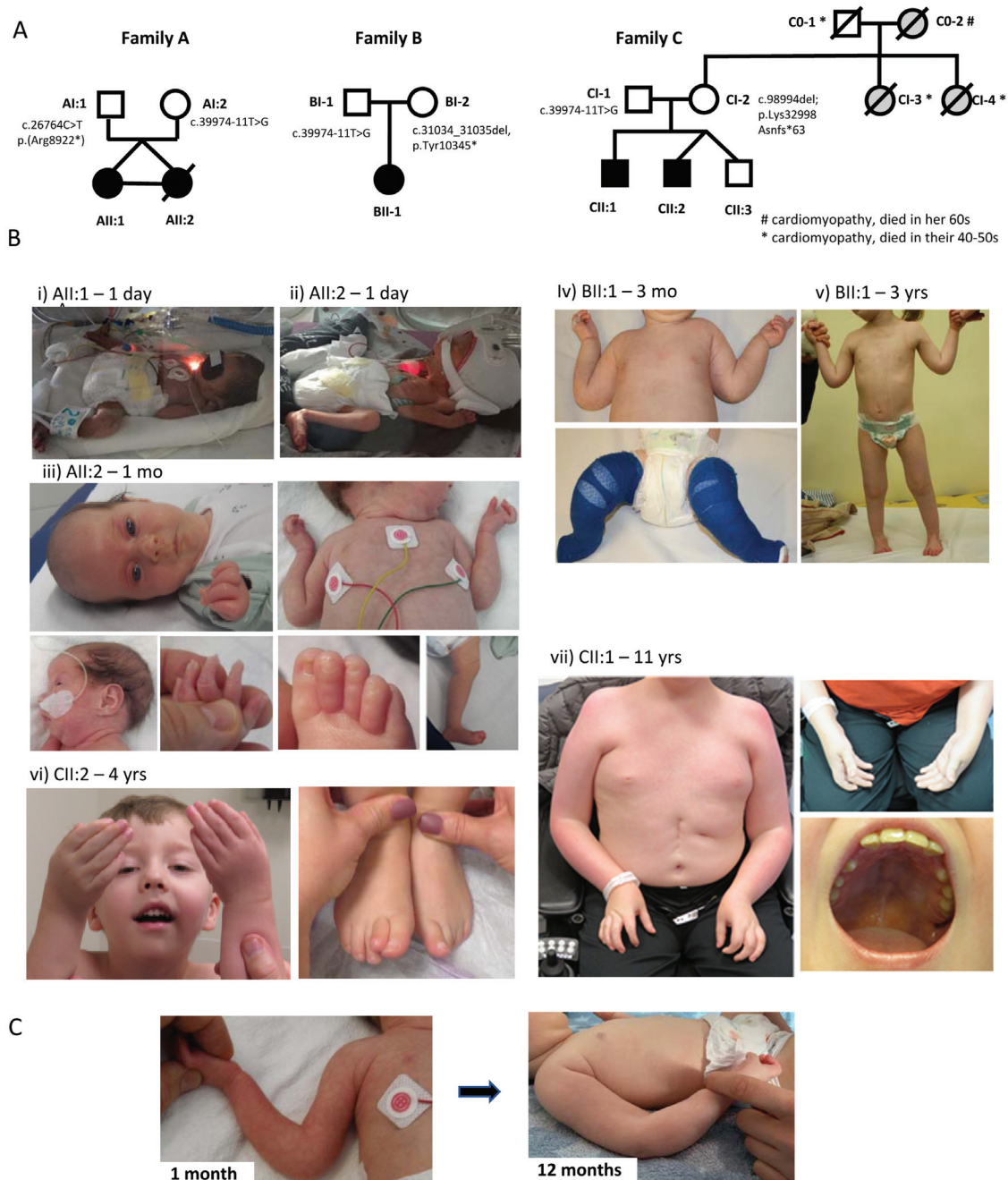
	All-1	All-2 (deceased)	Bill-1	CII-1	CII-2	Bryen et al
Allele 1	c.39974-11T > G					
Allele 2	c.26764C > T,					
	p.Arg8922*					
Sex	F	F	F	M	M	3F/6M/1 n/r
	Monozygotic monochorionic-diamniotic twins					
Origin	Germany		Germany	Germany/Ireland		Most Caucasian
Consanguineous parents	No		No	No		-
Gestation age at birth	29		38	40	37	-
Anomalies and complications during pregnancy	Oligo-hydramnios TTTS, donor, IUGR	Poly-hydramnios TTTS, recipient	Breech presentation	Reportedly normal	Oligo-hydramnios	4/10 oligo-hydramnios
Delivery complications	23 weeks: shortening of cervix, prolapsing arm, emergency C-section		C-section	Non-reassuring fetal heart tones (NRFTH), C-section	Failure to progress NRFTH, C-section	-
Reduced fetal movements	Yes	Yes	Yes + delayed (28w)	Yes	Yes	6/9
Problems at birth and in the neonatal period						
Congenital contractures	Yes	Yes	Yes	Yes	Yes	Yes
Of upper + lower extremities including: ulnar deviation of wrist, flexion contracture of fingers, elbows, shoulder, knee, abduction of hips, talipes						
Muscular hypotonia	Yes	Yes	Yes	Yes	Yes	10/10
Fractures	Green-stick fracture at humerus (4 months)	No	Yes, humerus, congenital	Yes, ulna + radial bone, congenital	Yes, arm, right ulna, congenital	3/10
Feeding difficulties	Yes, nasogastric tube feeding 2 weeks	-	Weak suck and weak cry in neonatal period	Weak suck and cry, dysphagia requiring G-tube for 2 mo	Weak suck and weak cry, some feeding difficulties	10/10
Respiratory difficulties	CPAP for 2 weeks		Yes, CPAP for 2 d	Aspiration in early infancy	-	6/9
Motor findings						
	10 months	1 day	3 years	11 years	4 years	

**Table 1** (Continued)

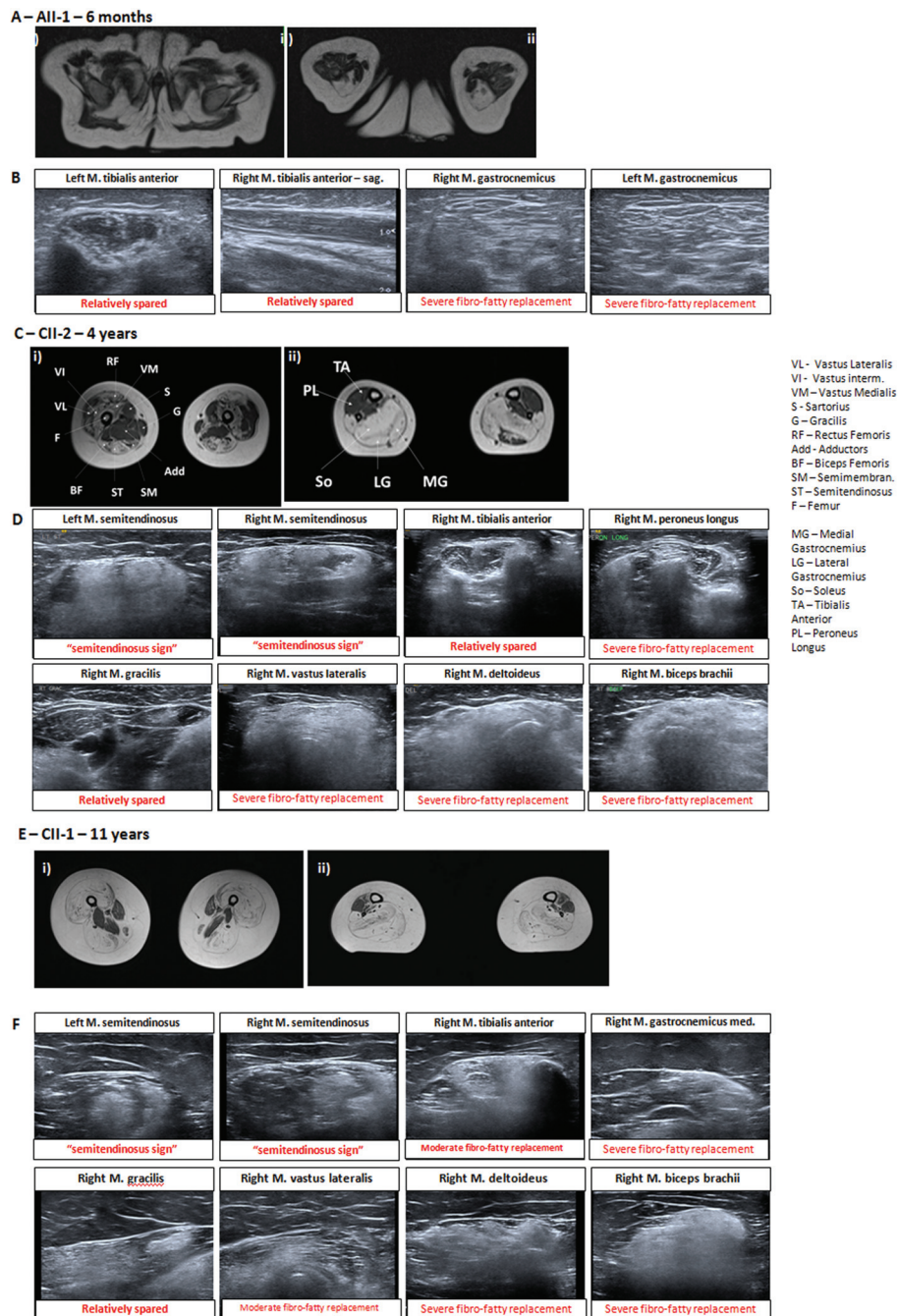
	All-1	All-2 (deceased)	Bill-1	Cil-1	Cil-2	Bryen et al
Age of last clinical review						
Contractures improved in course of time?	Yes	-	Yes	Yes	Yes	4/9
Axial weakness	Yes	Yes	Yes	Yes (head lag)	Yes (Severe)	5/9 severe
Facial hypotonia	Mild	-	Mild	No	No	7/9
Deep tendon reflexes	Reduced (10 months)	-	Reduced	Generally reduced	Generally reduced	6/9 reduced
Joint hypermobility	No	-	No	Mild distal hyperlaxity	Shoulders, elbows, hands	7/9
Reduced muscle bulk	Yes	-	Yes	Distal legs	Yes	
Motor delay	Yes	-	Yes	Yes	Yes	9/9
Head control	Not yet	-	2 years	n/r	n/r	
Sitting	Not yet	-	n/r	n/r	No (4 years)	n/r
Walking	Not yet	-	3 years: assist devices to walk independently	11 years: shorter distances without support	No, requires wheelchair for mobility	8/9
Cognitive development	Normal	-	Normal	Normal	Normal	Normal
<b>Cardiac involvement</b>						
Echocardiography (age)	1 day: Mildly reduced pump function, 6 months: normal	1 day: Severe hypertrophy, cardiac failure	1 month: Apical VSD, 6 months: spontaneous closure	11 years: normal	4 years: normal	1/10 cardiopulmonary resuscitation

Abbreviations: D, day; Mos, months; w; weeks; yr(s), year(s).





**Fig. 1** Three families presenting with arthrogryposis multiplex congenita and myopathy. **(A)** Family pedigrees and segregation of the recurrent metatranscript only *TTN* variant c.39974-11T > G (NM\_001267550) (*TTN*-11) and the truncating *TTN* variant in trans. While the family history was unremarkable in families A and B, three of the relatives on the maternal site had cardiomyopathy. Circles, squares, and scratched symbols designate women, men, and deceased family members, respectively. The filling of the symbols indicates whether the individuals were clinically unaffected (white) or affected either by arthrogryposis (black) or cardiomyopathy (gray). **(B)** Clinical photographs of the five affected patients (i) shows the recipient twin All:2 (1,550 g) and (ii) shows the donor twin All-1 (780 g) born at 29 weeks' gestation on day 1 after birth. All:1 required CPAP ventilation and All:2 required invasive ventilation and died 1 day after birth due to severe hypertrophic cardiomyopathy and cardiac failure. Note the muscular hypotonia with flexed legs resting in the abducted "frog leg position." (iii) All-2 at the age of 1 month showing flexion contractures of the fingers and the spontaneous posture with adducted and externally rotated upper arms, flexed elbow joints, dorsally extended and ulnar deviated wrists, flexed knees, feet in dorsiflexion. Knee extension (max. 5 degrees) and plantar flexion of feet (max. 20 degrees) were mildly reduced. The images of the head show retrognathia and asymmetric skull (plagiocephaly). (iv) BII-1 at age of 3 months with the typical flexion contractures of the elbows and fingers and, ulnar deviation. The lower images show BII-1 at the same age undergoing redresson therapy of clubfeet. Note. (v) BII-1 at the age of 3 years standing with assistance. (vi) CII-2 at age of 4 years with ulnar deviation of wrist and thumbs holds in opposing position; a typical feature is the second toe overlapping the great toe. (vii) CII-1 at age of 11 years sitting in the wheelchair. Contractures are still evident. Oral cavity of CII-1 showing a high palate. **(C)** Images show the arms of All-1 with flexion contractures of elbows improving from age of 1 month (110 degrees) until the age of 12 months (40 degrees); mo, month(s); yrs, years.



**Fig. 2** (A) MRI-T2-weighted of AII-1 at age of 6 months (i) coronal section of upper body shows global muscle atrophy and increased proportion of intramuscular fat. (ii) Axial section of gluteal muscles shows muscle atrophy most prominent in the gluteus medius and maximus; axial sections of proximal calf show a predominant involvement of the hamstring muscles. (B) Muscle ultrasound (US) images of AII-1 (6 months) of the calf muscles show extensive fibrofatty replacement of gastrocnemii and relative sparing of the anterior compartment (grade 1). (C) MRI-T1 weighted axial sections of CII-2 (4 years) at approximately mid-thigh (i) show slightly asymmetric involvement of rectus femoris, vastus medialis, intermedius and lateralis. Of the hamstring muscles semitendinosus is more involved than semimembranosus and biceps femoris. There is relative sparing of adductors, sartorius, and gracilis. Axial sections of mid-calf (ii) show predominant involvement of posterior compartment muscles. There is relative sparing of anterior compartment muscles including tibialis anterior and peroneus longus. (MRI for the younger sibling – 4 year old). (D) Cross sectional muscle ultrasound (US) images of CII-2 (4 years) show extensive fibrofatty replacement of hamstrings, most specifically in the M. semitendinosus (increased granular echogenicity indicating and semitendinosus sign) and only mild involvement of, with relative sparing of tibialis anterior and peroneus (grade 0–1). M. vastus lateralis and, in upper extremities, M. deltoideus and M. biceps brachii are moderately involved (grade 2–3). (E) MRI-T1 weighted axial sections of CII-1 (11 years) at mid-thigh show extensive fibrofatty replacement of rectus femoris, vastus lateralis, medialis, and intermedius. All hamstring muscles are involved. There is relative sparing of adductors, sartorius, and gracilis. Axial sections of mid-leg show predominant involvement of posterior compartment muscles of the leg including gastrocnemius and soleus. There is relative sparing of anterior compartment. (F) Muscle US of CII-1 (11 years) with predominant involvement of the quadriceps and posterior compartment muscles (hamstrings, especially M. semitendinosus, medial and lateral M. gastrocnemius), with relative sparing of the anterior compartment (M. tibialis anterior, M. peroneus) and of the adductors (M. gracilis, M. sartorius).

Post-mortem autopsy showed severe myocardial hypertrophy (heart weight, 15 g, reference:  $7.2 \pm 2.7$  g) (► **Fig. 3F**, ► **Supplementary Table S4**, available in the online version). Histology of the myocardium showed variable muscle fiber size, cardiomyocyte degeneration, and mild immune cell infiltration (► **Fig. 3G**).

#### Video 1

Echocardiography of AII-2 at the first day after birth (short axis). Online content including video sequences viewable at: <https://www.thieme-connect.com/products/ejournals/html/10.1055/a-1859-0800>.

#### Video 2

Echocardiography of AII-2 at the first day after birth (four chambers view). Online content including video sequences viewable at: <https://www.thieme-connect.com/products/ejournals/html/10.1055/a-1859-0800>.

#### Video 3

Echocardiography of AII-2 at the first day after birth (five chambers view). Online content including video sequences viewable at: <https://www.thieme-connect.com/products/ejournals/html/10.1055/a-1859-0800>.

Family histories of family A and B were unremarkable with respect to cardiac conditions. In family C, two sisters of the maternal grandmother died of cardiomyopathy at the age of 40 to 50 years and the maternal grandmother's mother died of cardiomyopathy in her 60s.

#### Variants and Isoforms

Via exome sequencing, we identified the c.39974–11T > G (*TTN*-11) extended splice site variant in *TTN* (reference transcript: NM\_001267550.1, ENST00000589042) in the four tested affected patients in compound heterozygosity with a truncating *TTN* variant. In the monozygotic twin affected by twin-to-twin transfusion syndrome who deceased 1 day after birth (AII-2), no specimen was available for genetic testing; however, given monozygosity and consistent phenotype the same genotype is assumed.

The variant *TTN*-11 is located in the intron 213 (► **Fig. 4A**). It was shown that *TTN*-11 either causes abnormal exon 214 skipping by removing exon 214 (28 amino-acids) or abnormal use of cryptic 3' splice site resulting in a frameshift and premature termination.<sup>14</sup> *TTN*-11 is predicted not to impact any of the major skeletal or cardiac isoforms and thus is referred to as a metatranscript-only variant (► **Fig. 4B**).

In the previous report, the *TTN*-11 splice variant was discussed as a potential founder mutation, since it co-segregated with the c.23177C > T, c.45328G > A, c.70969G > C polymorphisms (► **Supplementary Table S5**, available in the online version).<sup>14</sup> In the three families presented here, the three polymorphisms were present with the *TTN*-11 variant. Thus, our data confirm a shared haplotype and a common founder pathogenic variant.

All affected patients harbored a second pathogenic or likely pathogenic *TTN* truncating or splicing variant on the other allele. Parental testing confirmed the inheritance *in trans* (► **Fig. 1A**). The variants were either absent from the population database gnomAD (family A + B) or reported once (family C).

All variants but *TTN*-11 are predicted to impact the long skeletal N2A isoform (► **Fig. 4B**, ► **Supplementary Table S6**, available in the online version). While the truncating variants of families A and B were also predicted to impact the longer cardiac isoform N2BA, the variant of family C was predicted to impact, both, the longer cardiac isoform N2BA as well as the short cardiac isoform N2B (► **Fig. 4B**, ► **Supplementary Table S6**, available in the online version). This is important to notice as in family C, two aunts and one grandmother have died of cardiomyopathy in their forties to sixties.

#### Discussion

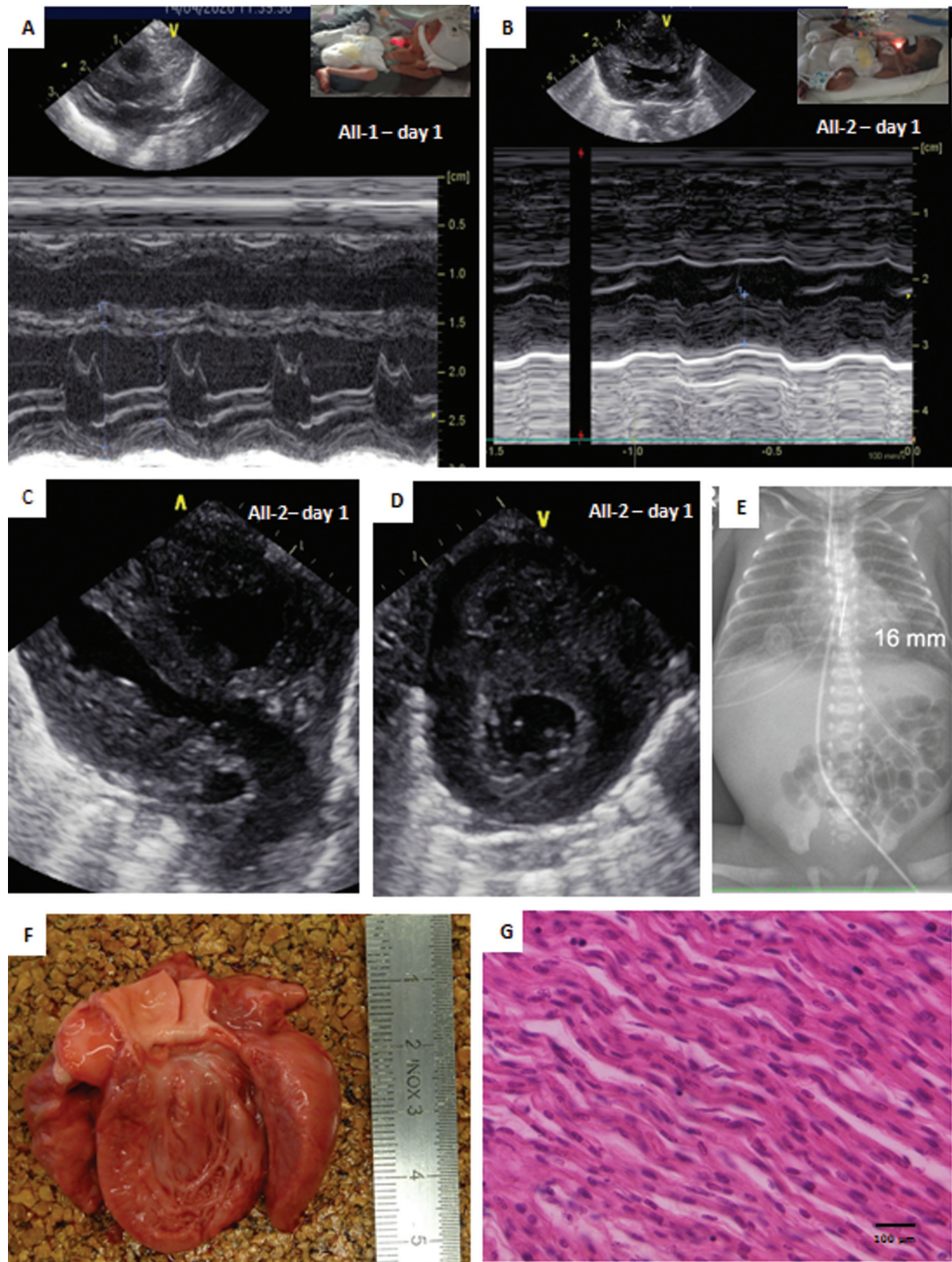
This study reports on five additional patients with the recurrent intronic pathogenic variant c.39974–11T > G in *TTN* (*TTN*-11) presenting with congenital myopathy and confirms the severe, consistent, and recognizable manifestation of this variant.<sup>14</sup>

This variant impacts the near-splice acceptor site of intron 213 and is assumed to alter exclusively the splicing or expression of the metatranscript-only isoform (NM\_001267550.1). The metatranscript-only isoform is a hypothetical isoform including all known exons and was formerly thought to be only expressed during fetal development.<sup>9</sup> Based on this, during adulthood one would not expect a clinical significance of genetic alterations in exons 213 or 214 and only minor significance during embryonic development.

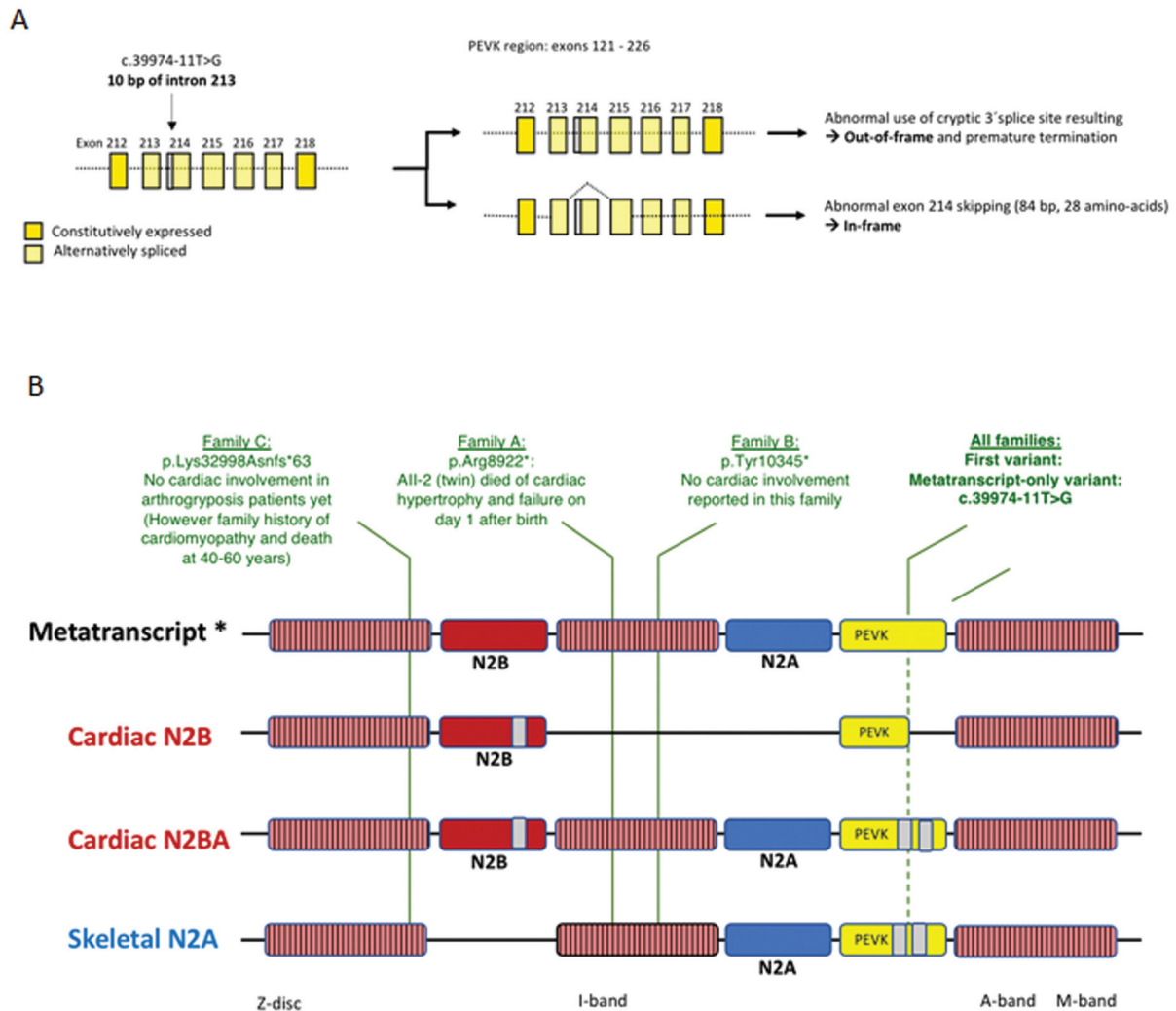
However, recent transcript analyses of skeletal muscle in healthy patients revealed that *TTN* exons 213 to 217 are not only expressed in the fetal muscle, but are also expressed to a lower extent (60%) in adult muscle.<sup>9,14,15</sup> In studies dealing with recessive titinopathies, almost all patients with pathogenic metatranscript-only variants clinically presented with arthrogryposis, supporting the notion, that isoforms bearing metatranscript-only exons might be of importance during early prenatal and postnatal development.<sup>13</sup>

Depending on differential splicing, *TTN*-11 can cause either abnormal in-frame exon 214 skipping by removing exon 214 (28 amino-acids), or abnormal use of a cryptic 3' splice site resulting in the inclusion of intronic bases into the mature messenger RNA, causing a frameshift and premature termination.<sup>14</sup> Transcript analyses of three muscle biopsies from patients with *TTN*-11 variants via RT-PCR confirmed the presence of both transcripts lacking exon 214 or transcript





**Fig. 3** Cardiac findings of both twins All-1 and All-2 with arthrogyriposis congenita and myopathy and twin-to-twin transfusions syndrome (TTTS). (A) Echocardiography of the donor TTTS twin All-1 showing normal ventricular pump function and only mild cardiac hypertrophy. (B–D) Echocardiography of the recipient TTTS twin All-2 showing severe biventricular hypertrophic non-obstructive cardiomyopathy, including hypertrophy of septum and severely reduced cardiac function (see also ► **Supplementary Video** materials, available in the online version). (E) Chest X-ray depicted diffuse, reduced lung opacity consistent with mild pulmonary edema (fluid lung), most likely on the ground of reduced cardiac function. (F) Macroscopic photos of heart (autopsy) showing increased ventricular hypertrophy (left ventricle 8 mm, right ventricle 4 mm diameter; 15 g; reference:  $7.2 \pm 2.7$  g). (G) Hematoxylin and eosin (H&E) staining of postmortem of FFPE (formalin fixed paraffin embedded) sections of cardiac muscle. Cardiac muscle shows hypertrophic muscle fibers, cardiomyocyte cell death, and lymphocytes.



**Fig. 4** (A) Schematic representation of the molecular consequences of the variant c.39974-11T>G. The recurrent intronic metatranscript-only variant c.39974-11T>G (*TTN-11*) is located in the intron 213. It was shown that c.39974-11T>G either causes abnormal exon 214 skipping by removing exon 214 (28 amino-acids) or abnormal use of cryptic 3'splice site resulting in a frameshift and premature termination.<sup>14</sup> Exon 214 contains PEVK elements which are crucial for muscle elasticity and prevention of contractures. (B) Illustration of major titin isoforms and location of patients' pathogenic variants (elements not drawn to scale). There are three major transcriptional isoforms of *Titin*: The N2B isoforms contain N2B elements and are cardiac specific, while the N2A isoforms contain N2A elements and are primarily expressed in the skeletal muscle. The larger N2BA isoform include both, the N2B and N2A elements, and is expressed in the heart with a maximal expression during fetal development.<sup>4</sup> The proline-glutamine-valine-lysine (PEVK)-element is one of the spring elements that mediate the passive, elastic forces of titin in the I-band region. The metatranscript isoform (\*) is a hypothetical isoform including all known exons (formerly thought to be only expressed during fetal development). All affected patients harbor a second, truncating variant in trans: While the truncating variants of family A and B only affect the skeletal N2A and cardiac N2BA (maximal expression during fetal development!), the variant of family C also affects the cardiac isoform N2B. Of note, in family C there is a family history of cardiomyopathy after age of 40 years, while the family history is unremarkable in family A and B.

harboring intronic bases and a frameshift transcript.<sup>14</sup> While a damaging effect can be assumed for the frameshift variants, the interpretation and functional implication of the loss of exon 214 is unclear.

There are three spring elements ([1] proline-glutamine-valine-lysine [PEVK]-, [2] N2B- and the [3] tandem Ig-spring segments that mediate the passive, elastic forces of titin in the I-band region.<sup>15</sup> The PEVK elements account for the majority of the passive tension response of titin. Skeletal muscle and cardiac specific isoforms differ in the number of Ig and PEVK domains.<sup>16</sup> Exon-skipping events of PEVK region were shown to mediate myogenic differentiation resulting in muscle types with unique titin-based elastic properties, e.g.,

soas fibers have a higher degree of passive tension than soleus fibers.<sup>16</sup> Exon 214 includes such PEVK repeat units.<sup>9,16,17</sup> Based on the function of PEVK repeat region in tension regulation, it can be speculated that loss of exon 214 might reduce muscle elasticity eventually resulting in contractures during early embryonic development. Fetal and neonatal skeletal muscle in mice and rabbits expresses large titin isoforms and additional exons in the PEVK regions, which is accompanied by a lower titin-based passive stiffness of the fetal and neonatal muscle.<sup>17</sup>

During the first year of life the large isoforms are gradually replaced by smaller isoforms.<sup>17</sup> In line with a declining functional importance of the large isoforms during infancy,



the contractures of the patients were most pronounced at birth, and showed improvement over time.

None of the previously published individuals with arthrogryposis and the *TTN*-11 variant were explicitly reported to have cardiac involvement. However, one patient required cardiopulmonary resuscitation in the neonatal period, and one individual died at 26 years of age from unknown cause. Of note, the twins we report here had twin-to-twin transfusion syndrome. Due to intrauterine volume overload, the recipient twin is at increased risk of myocardial hypertrophy.<sup>18,19</sup>

*TTN*-11 can cause exon 214 skipping and shortening of PEVK.<sup>14</sup> In a mouse model PEVK knockout resulted in cardiac hypertrophy in line with cardiac hypertrophy being present in AII-2.<sup>20</sup> Former transcript studies showed a relatively low fractional expression of exon 213 and exon 217 in the heart when compared with skeletal muscle (exon 213: fetal skeletal muscle: >95% vs. fetal heart approx. 23%; adult skeletal muscle: approx. 60% vs. adult heart approx. 20%).<sup>9</sup> However, the low level of transcription containing exons 213, 217, and presumably exon 214 in fetal and neonatal heart does not essentially rule out a role in cardiac function and adaptation. Similarly, heterozygous truncating variants in *TTN* are associated with dilated and hypertrophic cardiomyopathy which might unmask during cardiac stress by volume overload.<sup>21</sup> Whether the recurrent intronic splice variant *TTN*-11 or the truncating variant in *trans* contributed to postnatal heart failure is difficult to prove.

Larger sequencing studies of cardiomyopathy cohorts might give further information of functional impact of exons 213, 214, and 217 and *TTN*-11 on cardiac function.

The variant p.Tyr10345\* of family B only affects the long cardiac N2BA isoform. Patient BII-1 had an apical ventricular septal defect at birth which spontaneously resolved. Recessive *TTN* pathogenic variants have been associated with septal defect, however, the evidence is still sparse.<sup>11</sup>

The variant p.Lys32998Asnfs\*63 of family C affects both, the short N2B and the long cardiac N2BA isoforms, and three first degree relatives of CII-1 of the truncating *TTN* variant (CII-1) passed away from cardiomyopathy before the age of 60 years. This is consistent with studies reporting variants affecting both, the long N2BA and the short N2B cardiac isoforms, but not variants that affect only either of both, are significantly associated with cardiomyopathy.<sup>12,13</sup>

Three of the ten previous published *TTN*-11 cases and four of our five patients had fractures affecting the upper extremities. Ten to 25% of individuals with arthrogryposis congenita are reported to have congenital fractures which are suggested to be caused by decreased fetal movement and inactivity-induced osteoporosis of long bones making bones more prone to fractures.<sup>22,23</sup>

The evaluation of muscle involvement via ultrasound and MRI imaging in our patients with a “metatranscript-only” titinopathy revealed a consistent and recognizable pattern with severe involvement of quadriceps and hamstring muscles with relative sparing of adductors, sartorius, and gracilis in upper thigh. In the lower legs imaging reveals prominent involvement of the posterior compartment muscles and relative

sparing of the anterior compartment muscles.<sup>24</sup> Similar findings were recently observed in three patients from two families all harboring biallelic *TTN* pathogenic variants including one metatranscript-only variant in *trans* (exon 163, p.Glu11932\*; exon 201; p.Leu12974Trpfs\*104).<sup>12,24</sup> These patients also had a marked calf involvement, clear adductor sparing, and sparing of the anterior compartment of the lower legs.

Taking together, these findings suggest that the distribution of the *TTN*-11-associated muscle involvement is specific and distinct from other forms of congenital myopathies.<sup>25,26,27</sup> The identified pattern enables imaging-based clinical phenotyping, which is especially important for variant interpretation in the current “era of reverse phenotyping after exome or genome sequencing.”

## Conclusion

The assessment of the functional relevance of different *TTN* transcripts and variants is a challenge for clinical diagnostics. Our findings confirm that the metatranscript-only isoforms including exons 213 to 217 are of importance during early fetal development and beyond, possibly related to its role in mediating higher elasticity and preventing contractures. The lethal cardiac involvement of one patient might point to a potential functional relevance of the variants in the developing heart. There is a distinct pattern of muscle involvement in “metatranscript-only” congenital titinopathy, providing valuable clues for genetic diagnostic work-up and suggesting specific genotype–phenotype correlations in *TTN* myopathies.

### Full Data Access Statement

The principal author and the senior author take full responsibility for the data, analyses, and interpretation, and the conduct of the research; they have full access to all of the data; and that they have the right to publish any and all data, separate and apart from the guidance of any sponsor.

### Data Availability Statement

Data is not provided in the article because of space limitations but may be shared (anonymized) at the request of any qualified investigator for the purpose of replicating procedures and results.

### Author Contributions

L.A., F.D., and S.D. contributed toward conceptualization. L.A., S.D., D.H., S.W., S.S., U.L., A.S.J., T.H., D.G., A.R., K.R.C., and S.B.N. contributed toward data acquisition. L.A., S.W., and S.S. did the illustrations. F.D., E.M., and C.G.B. reviewed the manuscript. L.A. and F.D. drafted the manuscript.

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#### Conflict of Interest

None declared.

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