ARTHROGRYPOSIS

Causes, Consequences and Clinical Course in Amyoplasia and Distal Arthrogryposis

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Göteborg 2009

Picture on cover page:
Ribera, Jusepe de (lo Spagnoletto) (c.1590-1652) The Club Foot, 1642 (oil on canvas) Italian C17th oil on canvas Bridgeman Art Library /IBL Bildbyrå

Abstract

Background. Arthrogryposis Multiplex Congenita, AMC, is a heterogeneous condition defined as multiple congenital joint contractures in two or more body areas. The pathogenesis is impaired fetal movements. Amyoplasia, the most frequent form, is a sporadically occurring condition with hypoplastic muscles and joint contractures. Distal arthrogryposis (DA) syndromes are often hereditary, and joint involvement is predominantly in hands and feet. Arhrogryposis with CNS involvement includes chromosomal and other syndromes.

Aims. The purpose of this study was to investigate patients with arthrogryposis, to classify the different occurring forms, and to investigate causes, muscle and joint involvement, motor function, treatment and outcome.

Methods. Patients were identified via pediatric rehabilitation centers. Family and case history including perinatal findings were recorded. Physical investigation included joint range of motion, muscle strength and motor function. In patients with DA molecular genetic and, in selected cases, muscle morphologic investigations were carried out.

Results. 131 patients with arthrogryposis were investigated. The most frequent diagnoses were amyoplasia and DA. In amyoplasia, community ambulators had the best muscle strength, household ambulators had severe contractures in legs but good muscle strength in arms, and non-ambulators had the most severe contractures and muscle weakness. Muscle strength was found to be more important than joint range of motion for motor function.
In DA, muscle weakness was present in 44% of investigated patients. Mutations in sarcomeric muscle protein genes were found in seven families with autosomal dominant and in one child with sporadic DA. In one family with a mutation in *TNNI2* there were mild myopathic findings, in one family with mutation in *TPM2* no obvious myopathy, and in patients from three families with *MYH3* mutations mild myopathic findings. Clinical findings were found to be highly variable between families and also within families with DA.

Conclusions. Different forms of arthrogryposis were identified. In amyoplasia, attention should be directed at development of muscle strength with early stimulation of active movements. Immobilisation should be minimized. DA syndromes are clinically and genetically heterogeneous conditions. Fetal myopathy due to sarcomeric protein dysfunction can cause DA. An early multidisciplinary team evaluation for specific diagnosis and planning of treatment is recommended.

Key words. Arthrogryposis, amyoplasia, distal arthrogryposis, muscle involvement, motor function, contractures, muscle morphology, sarcomeric protein dysfunction.

ISBN 978-91-628-7928-0 Göteborg 2009

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Neurology 2006;67:597-601

III. **Tajsharghi H, Kimber E, Holmgren D, Tulinius M, Oldfors A**
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Neurology 2007;68:772-5

IV. **Tajsharghi H, Kimber E, Kroksmark AK, Jerre R, Tulinius M, Oldfors A**
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Arch Neurol 2008;65:1083-90

V. **Kimber E, Tajsharghi H, Kroksmark AK, Oldfors A, Tulinius M**
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Abbreviations

ADHD	Attention deficit hyperactivity disorder
AMC	Arthrogryposis Multiplex Congenita
ATP	Adenosin triphosphate
BAC	Bacterial artificial chromosome
CA	Community Ambulator
CGH	Comparative genomic hybridisation
CHRNG	Cholinergic receptor nicotinic gamma gene
CNS	Central Nervous System
CK	Creatine kinase
COFS	Cerebro-oculo-facio-skeletal
CT	Computed tomography
DA	Distal arthrogryposis
DNA	Deoxyribonucleic acid
EMG	Electromyography
FBN	Fibrillin gene
HA	Household ambulator
MCP	Metacarpophalangeal
MR	Mental retardation
MRI	Magnetic resonance imaging
MYH	Myosin heavy chain gene
MyHC	Myosin heavy chain
NA	Non ambulator
NADH	Nicotinamide adenine dinucleotide
NCS	Nerve conduction studies
NFA	Non functional ambulator
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
ROM	Range of motion
SD	Standard deviation
SMA	Spinal muscular atrophy
TM	Tropomyosin
Tn	Troponin
TNN	Troponin gene
TPM	Tropomyosin gene

INTRODUCTION

In the field of neuromuscular disorders in children and adolescents, arthrogryposis is found to be a diverse and confusing diagnosis. Parents and professionals are often in doubt regarding specific diagnosis, treatment and prognosis in the child with arthrogryposis. This study was initiated by professionals treating arthrogryposis and by arthrogryposis patient organisations in Scandinavia. The study was conducted as a multicenter study with the investigators travelling to the local child rehabilitation centers in all health care regions of Sweden for personal interviews and investigation of included patients. The overall aims of the study were to clarify causes, clinical consequences and clinical course in arthrogryposis.

Definition

Arthrogryposis Multiplex Congenita (AMC) is defined as congenital, non-progressive contractures in more than two joints and in multiple body areas. The term arthrogryposis derives from the Greek words *arthron* – joint and *grypos* – curved. The term multiple congenital contractures can be used synonymously with arthrogryposis. The diagnosis is purely descriptive, and arthrogryposis can be part of a large number of different syndromes, at least 200 [1].

Compromised fetal mobility is the main background factor, common to all different types of arthrogryposis. The cause can be pathology in the peripheral or central nervous system (CNS), in muscles or in connective tissue, defects in neuromuscular transmission, compromised space *in utero*, maternal disease, external factors like medication or drugs, or compromised vascular supply to the fetus [2].

Arthrogryposis refers to a large and heterogeneous group of conditions, both sporadically occurring and hereditary. The literature is confusing regarding types of arthrogryposis described, as different diagnoses are often lumped together and regarded as one entity. The most frequently occurring form of arthrogryposis is amyoplasia [3], a sporadically occurring condition sometimes referred to as "classical arthrogryposis". The second most common form is probably distal arthrogryposis, (DA) [4, 5]. DA is not one single entity, but a group of syndromes with mainly distal joint contractures [6, 7].

Background

Historically, a patient with AMC is described by Thomas of Monmouth in "The life and Miracles of St William of Norwich" in 1156, as reported by Gordon [8]. A boy with arthrogryposis is depicted in a painting by Jusepe de Ribera ("The club foot") from 1642 [9]. The first description of arthrogryposis in the medical literature is thought to be by AW Otto, professor of anatomy in Breslau, in a textbook from 1841 [10]. The term Arthrogryposis was probably first used by Rosencranz in 1905 [11], and the term Arthrogryposis Multiplex Congenita by Stern in 1923 [12]. The term Amyoplasia Congenita is used by Sheldon in 1932 [13].

AMC is discussed and described in orthopedic reviews from 1930 to 1950, and numerous specific syndromes with AMC are described in the medical literature from 1950 to 1960. Further research regarding the pathogenesis was published in the following decades [12, 14-16]. Hall described clinical and genetic evaluation and diagnosis in patients with AMC [17, 18]. There are a great number of publications on genetic diagnostics and further descriptions of mechanisms leading to reduced fetal mobility.

Recently, advances in molecular genetics have made it possible to understand pathogenic mechanisms, especially in distal arthrogryposis (DA) syndromes, where mutations in genes that encode for contractile muscle proteins have been found [19-22].

Pathogenesis

Animal studies demonstrate that congenital joint contractures can be caused by fetal immobilization. A study from 1962 by Drachman and Coloumbre showed that congenital joint ankylosis can be produced by relatively short periods of immobilization in chick embryos [16]. In 1983, Moessinger (1983) [14] published an animal study where rat fetuses paralysed during part of gestation showed the same anomalies as those described in Pena Shokeir I syndrome: multiple joint contractures, pulmonary hypoplasia, micrognathia, fetal growth retardation, short umbilical cord, and polyhydramnios. These features were previously described by Pena and Shokeir in an early lethal disorder with autosomal recessive inheritance, Pena Shokeir I syndrome [23].

Polyhydramnios is thought to be due to lack of swallowing during fetal life. Oligohydramnios deformation sequence includes similar features, also demonstrated in animal studies, possibly caused by fetal immobilization from external factors. However, renal defects are also often present in oligohydramnios [15].

Swinyard [12] discusses the etiology of multiple congenital contractures in animals and in humans and suggests that the joint fixations are caused by a proliferation of capsular connective tissue, a compensatory connective tissue response to lack of movement *in utero*.

Hall (1986) suggested that the Pena Shokeir syndrome represents a phenotype, fetal akinesia deformation sequence, caused by severely decreased or absent fetal movements [24], including joint contractures, short gut, pulmonary hypoplasia, short umbilical cord, intrauterine growth retardation, and craniofacial abnormalities. At least 20 familial types of Pena-Shokeir phenotype are now recognized, in addition to sporadic cases [25].

Pathologic changes in 96 children with AMC were described in a study by Banker in 1986 [15]. Abnormalities in the neuromuscular system were found in all cases, with the primary alterations in anterior horn cells, roots, peripheral nerves, motor end-plates or muscles. All had onset during fetal development. The vast majority of patients in this study were considered to have a neurogenic cause. Dys-

genesis or degeneration of the spinal cord, with abnormalities in anterior horn cells, were found in a majority of cases with CNS involvement, while the spinal cord and anterior roots were normal in myopathic cases (*e.g.* central core disease, congenital muscular dystrophy, nemaline myopathy, myotonic dystrophy). Fiber type predominance or disproportion was also found in some patients, in whom involvement of the spinal cord was not seen. Presumably secondary changes of muscle included fiber type predominance and disproportion, aplasia of muscle (amyoplasia) signifying a primary defect in the anterior horn cells early in the fetal development leading to hypoplasia of muscle and progressive denervation of muscle. CNS involvement included dysgenesis or degeneration of the brain and/or spinal cord in association with cases of trisomy 18, trisomy 21, Möbius syndrome, Zellweger syndrome, spinal muscular atrophy, and others.

In a further pathology study of 83 cases of lethal arthrogryposis in Finland, the majority of cases were also found to have a neurogenic cause, a few myopathic, while a non-neuromuscular cause was present in 10 cases [26].

Investigation of the spinal cord in infants with neurogenic arthrogryposis has demonstrated abnormal histology and unequal distribution of alpha motor neurons in anterior horn cells, the latter also predictive of involved muscle groups [27]. Several studies report that neurogenic arthrogryposis (of the amyoplasia type) can be caused by vascular compromise in early fetal development, with ischemia of anterior horn cells leading to fetal akinesia and poor or absent muscle development, but also in some cases to co-existing anomalies with presumed vascular genesis, *i.e.* gastroschisis, bowel atresia, Möbius syndrome, and focal muscle defects [28-31].

Arthrogryposis can be present in several congenital myopathic disorders, *e.g.* nemaline myopathy [32], centronuclear myopathy [33], central core disease and others, in congenital muscular dystrophies (Fukuyama, Ullrich) [1], in severe SMA1 with fetal onset [34], and in severe congenital polyneuropathies [35-37].

Several DA syndromes have recently been discovered to be caused by mutations in sarcomeric muscle proteins [19-22]. A number of collagen disorders can also appear with arthrogryposis, for example Ehlers-Danlos syndrome [38], Marfan syndrome [39] and Larsen syndrome. DA9, Beal syndrome, has also been found to be a collagen disorder with a mutation in the *FBN2* gene [40].

Disease affecting the neuromuscular junction with resulting weakness can cause arthrogryposis. Congenital myasthenic syndrome with arthrogryposis has been reported [41], congenital myotonic dystrophy [42], and maternal antibodies to fetal neurotransmittors [43] can all cause arthrogryposis.

Movement restriction *in utero* caused by *e.g.* oligohydramniosis, myoma/fibroma of the uterus and bicornate uterus can be associated with arthrogryposis. Arthrogryposis of the amyoplasia type occurs with increased frequency in one of monozygotic twins, but the cause in these cases is thought to be more related to vascular compromise than to actual crowding [28, 44].

A separate group of arthrogryposis syndromes are those caused by maternal disease during pregnancy, such as maternal multiple sclerosis, maternal myasthenia gravis [1], maternal diabetes mellitus, and maternal hyperthermia [1]. Metabolic disease such as phosphofructokinase deficiency can cause arthrogryposis and drugs taken during pregnancy can also be associated with arthrogryposis (*e.g.* muscle relaxants, misoprostol, cocaine, alcohol)[1].

Contractures caused by compromised space in utero have a relatively late gestational onset and are relatively mild. These types of congenital contractures regress more easily compared to contractures caused by early immobility of the fetus [45].

Epidemiology

Several epidemiological surveys of arthrogryposis have been published. The occurrence has previously been estimated to 1 in 3,300 live births in a Finnish study from 1966 [46], 1 in 56,000 live births in a Scottish study of 66 sporadic cases from 1976 [47], and 1 in 12,000 live births in a study from Western Australia from 1976 [48].

In a retrospective epidemiologic study in western Sweden by Darin *et.al.*, all children born with multiple congenital contractures between 1979 and 1994 were identified through screening of registers, reviews of medical records and re-examination of children. 68 cases were identified, and the birth prevalence was found to be 1 in 5,100 live births [49]. In this study, 39 patients had cerebral or spinal involvement, three patients had mechanical restriction in utero, 12 neuromuscular and nine connective tissue involvement.

Clinical classification

Since there is a very large number of disorders with arthrogryposis, differential diagnosis can be difficult. It is, however, important to make as specific a diagnosis as possible for several reasons:

Treatment can vary depending on the underlying cause in the individual child. For example, stretching should be minimized in diastrophic dysplasia, where too intensive mobilisation may damage joint cartilage. The joint contractures can also be more resistant to treatment in certain types of AMC, especially amyoplasia [50] and surgery and splinting may need to be planned accordingly.
Risk of re-occurrence varies greatly: amyoplasia occurs sporadically, while several forms of DA have autosomal dominant inheritance with a recurrence rate of 50%.

The prognosis is also very much dependent on diagnosis, where conditions with CNS involvement may have a poor prognosis, sometimes with early death, while other conditions have a normal lifespan but may need extensive orthopaedic treatment and rehabilitation.

An approach to clinical evaluation that has been found to be very useful has been suggested and further developed by Hall [1, 17, 18]. According to this, patients can be divided into three main groups of disorders: 1 Primarily musculoskeletal involvement; 2 Musculoskeletal involvement plus other system anomalies and, 3 Musculoskeletal involvement plus central nervous system dysfunction and/or mental retardation (MR).

Amyoplasia or "classic arthrogryposis" belongs to the first group, as do several camptodactyly syndromes, distal arthrogryposis type 1, popliteal pterygium syndrome, several symphalangism syndromes, and others.

In the second group, *i.e.* involvement of limbs and other body areas, several other camptodactyly syndromes, several DA syndromes, myotonic dystrophy, congenital myopathies, myasthenia gravis, connective tissue disorders such as Larsen syndrome and Marfan syndrome with congenital contractures can be included.

The third group includes a great number of genetic syndromes and chromosomal anomalies. Examples from this group are COFS (cerebro-oculo-facio-skeletal) syndrome, congenital muscular dystrophy, Miller-Dieker (lissencephaly), lethal multiple pterygium syndrome, Pena-Shokeir phenotype, Potter syndrome, Zellweger syndrome, trisomy 8/mosaicism, trisomy 18, and many others. This group includes lethal syndromes and syndromes with severe disabilities due to central nervous system malfunction. Mental retardation/CNS involvement is found in approximately 25% of individuals with AMC [2, 51, 52].

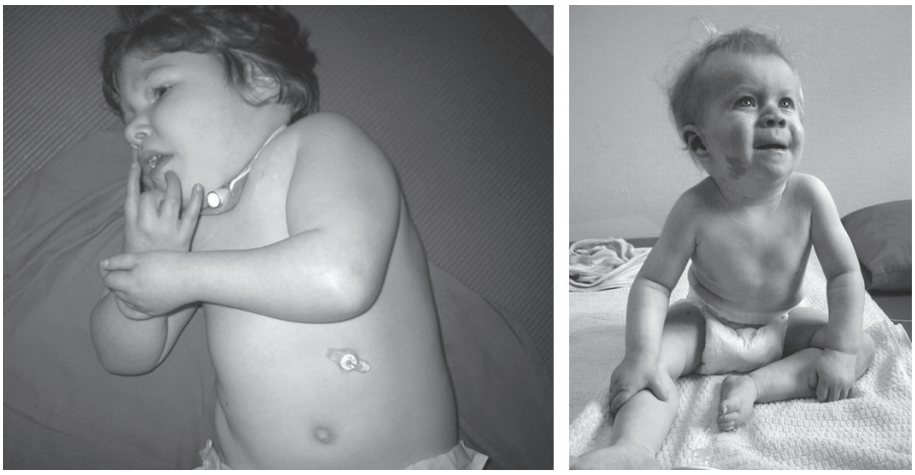

Figure 1. *Two children with arthrogryposis and CNS involvement at age six years (left) and one year (right). Note tracheostomy and gastrostomy in the girl to the left in the picture. Photographs are published by permission of the parents.*

Investigation

To clarify the specific diagnosis in a child with arthrogryposis, a careful clinical evaluation including joint mobility, muscle strength, and associated anomalies is important. Family history, pregnancy history including infections, trauma, bleeding, drugs and medication, delivery history, developmental milestones in an older child, and associated problems should all be recorded. Evaluation of the mother of a newborn child with arthrogryposis should not be overlooked, regarding *e.g.* maternal myasthenia or myotonic dystrophy.

Possible CNS involvement must be evaluated. Ultrasound/CT scan or MRI of the brain should be performed if there are signs of CNS involvement, and also MRI of the spinal cord if there are signs of spinal involvement. Muscle ultrasound [53], CT-scanning of skeletal muscle [54], and MRI of muscle [55] can all be helpful in evaluating affected muscle involvement in planning muscle biopsy or surgery, and also to follow muscle development over time.

Dysmorphic features, which could indicate chromosomal anomalies, should be looked for and, if present, chromosomal analysis should be performed. Microarray based Comparative Genomic hybridisation (arrayCGH), is a method which in recent years has proved to be a reliable diagnostic tool for genome-wide detection of small chromosomal abnormalities, copy number changes, in patients with MR. Studies have demonstrated arrayCGH to detect copy number variations in 10-20% of patients with mental retardation with and without dysmorphic features and/or multiple congenital anomalies [56-59].

If there is any suspicion of myopathic involvement serum muscle enzymes should be analyzed. Other blood or urine chemistry investigations, such as serum lactate and metabolic screening, should be carried out if indicated from clinical findings (such as suspicion of mitochondrial or metabolic disease)

Nerve conduction studies, NCS, can be helpful if there are signs of peripheral nerve involvement/polyneuropathy, and electromyography, EMG, if there are signs of myopathy.

Muscle biopsy for pathological evaluation is essential for a correct diagnosis in myopathic conditions [15], and can be of help if the diagnosis is unclear [60]. Therefore, if orthopedic surgery is planned a muscle biopsy should also be considered.

In a study of the diagnostic value of NCS/EMG and muscle biopsy in the evaluation of 38 patients with AMC, it was found that when clinical evaluation suggests a specific syndrome, developmental, or exogenous cause, NCS/EMG and muscle biopsy are not helpful, but when the diagnosis is unclear NCS/EMG and muscle biopsy together can help in the diagnostic workup [61].

In two muscle morphologic studies from patients with neurogenic AMC [62] and AMC

where known myopathic conditions were excluded [63], findings indicating neurogenic cause were found, *i.e.* variation in fiber size and abnormal fiber type distribution. With careful evaluation and investigation, a specific diagnosis can be reached in at least 30-50% of individuals with arthrogryposis [1]. Prenatal diagnosis of AMC by ultrasound is possible, assessing fetal movement [64].

Family history

Clinical investigation

- Joint involvement
  - Proximal/distal
  - Jaws
  - Spine
  - ROM, range of motion
- Muscle involvement
  - Absence/hypoplasia of muscles
  - Muscle weakness
- Other organ involvement
  - Eyes, palate, heart, lungs, gastrointestinal, genitourinary system
- Cognitive function
- Developmental history
- Associated problems/disease

Functional assessment

- Gross motor function
- Hand function
- ADL, activities of daily living

Laboratory investigations

- Genetic tests
  - Chromosomes,
  - DNA-analysis (if signs of specific genetic syndrome)
- Blood chemistry:
  - lactate, muscle enzymes,
- MRI of brain/spinal cord if signs of CNS involvement
- CT/MRI of muscles in amyoplasia
- EMG if signs of myopathy
- NCS if signs of polyneuropathy

Figure 2. *Suggested baseline investigations in arthrogryposis.*

Treatment

Physical therapy, splinting, and orthopedic surgery are the main treatment methods in arthrogryposis [45]. A correct genetic diagnosis is important, as treatment needs may vary [65-67].

Early physical therapy is important to avoid further muscle atrophy and, for the same reason, splinting combined with physical therapy is mostly preferable to casting, especially in amyoplasia [1, 5]. The first three to four months of life are especially valuable in activating and stimulating muscle function and in stretching contracted joints [66]. Daily passive stretching and serial splinting in infants has been found to increase function [68].

Children with arthrogryposis may have major feeding difficulties in infancy, *i.e.* problems with chewing, sucking, and swallowing, sometimes requiring tube feeding. These problems are in many cases related to structural abnormalities in the jaw and tongue. Secondary to this chest infections, constipation, poor growth, and also language problems can be seen [69].

Malignant hyperthermia can occur in some forms of arthrogryposis, which must

be kept in mind prior to anesthesia for orthopedic and other surgery. Maxillar and mandibular dysplasia and limited mouth opening are common problems, which can make intubation difficult [70, 71].

A multidisciplinary team management is needed, as many aspects need to be taken into account in the planning of treatment to optimize the child´s functional outcome. In planning treatment, the child´s general development and social activities must be kept in mind. A considerable part of the child´s day may be occupied by physical therapy and training [65]. The goal of treatment must be to achieve independence in adult life, and there must also be time for play and other important activities in daily life. Factors of importance to achieve independence are, in order of importance, communication skills, activities of daily living, mobility, and walking [5, 50].

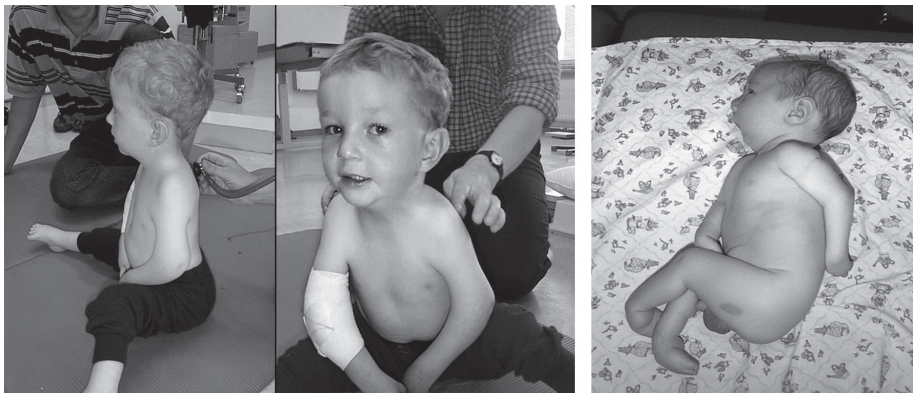

Figure 3. *Two boys with amyoplasia at ages three years (left) and 2.5 months (right). Photographs are published by permission of the parents.*

Amyoplasia

The most common form of arthrogryposis is amyoplasia, which accounts for approximately one third of all cases [3]. The word amyoplasia means no muscle formation. Amyoplasia occurs sporadically. Pathogenesis is unknown but thought to be impaired blood circulation to the fetus early in pregnancy with hypotension and hypoxia damaging the anterior horn cells and resulting in lack of or underdevelopment of muscle tissue with fatty or connective tissue replacement [3, 72]. Clinically, the common morphologic features suggest a genetic syndrome, but occurrence is sporadic and individuals with amyoplasia have unaffected children [1]. Amyoplasia occurs in increased frequency in one of monozygotic twins [73].

The diagnostic criteria for amyoplasia are highly specific with decreased muscle mass, typical joint contractures and limb positioning at birth, mostly symmetrical in all four limbs. There may be involvement only of the lower limbs or, less commonly, only of the upper limbs and asymmetric limb involvement [3, 74]. Typically, the shoulders are adducted and internally rotated, and the elbows are extended with the forearms pronated and wrists and fingers flexed. The hips are either in abduction and external rotation with flexed knees, or flexed with extended or flexed knees. Hips

and knees can be dislocated. The feet are most often in an equinovarus adductus position, although other types of foot deformities occur. Involvement of the spine is also described [3, 74]. The contractures can be fixed or flexible.

There is usually dimpling of the skin over affected joints. Common associated findings are midline facial hemangiomas and a round facial appearance. Muscle defects in the abdominal wall and inguinal hernias occur in about 10% of children born with amyoplasia, and gastroschisis and bowel atresia have also been recorded [28]. Mental development is normal [74], unless there has been a concomitant birth asphyxia.

Contractures in children with amyoplasia are at their maximum at birth. To increase the joint range of motion (ROM) and to obtain a functional position of the joint, a combination of stretching, splinting, and orthopedic surgery is often necessary [65, 66, 68]. Early physical therapy is very important, both to mobilize joints and to stimulate muscle growth and to prevent further muscular atrophy [50]. In amyoplasia, the joint contractures can be severe and have a tendency to recur after correction [65].

Figure 4. *Eight months old girl with familial DA and a pathogenic mutation in TNNI2 (left) and hands of a one year old boy with familial DA and no identified gene mutation (right). Photographs are published by permission of the parents.*

Distal arthrogryposis

Clinical classification

Distal arthrogryposis (DA) syndromes are characterized by mainly distal congenital joint contractures, *i.e.* in the hands and feet. The exact incidence of DA is not known. In a large study of over 350 patients with arthrogryposis, 44 (12,6%) patients with DA were identified [6]. In another review, 35% of 155 patients with artrogryposis were diagnosed with DA [5]. In an epidemiological survey from western Sweden, 5 (7%) patients with DA syndromes were identified in a total of 68 patients with multiple congenital contractures [49].

Table 1. *Classification of DA syndromes*

Distinguishing features	Classification Hall	Classification Bamshad	Other name
Overlapping fingers neonatally, ulnar deviation	DA type I	DA1	Digitotalar dysmorphism
Facial contractures, small pursed mouth		DA2A	Freeman Sheldon syndrome, FSS
Intermediate DA1/2A		DA2B	Sheldon Hall syndrome
Cleft palate, short stature	DA type IIA	DA3	Gordon syndrome
Scoliosis	DA type IID	DA4	Arthrogryposis with severe scoliosis
Cleft lip	DA type IIC		
Ptosis, limited ocular mobility	DA type IIB	DA5	Arthrogryposis with oculomotor limitation and electroretinal abnormalities
Sensorineural hearing loss		DA6	Arthrogryposis-like hand anomaly and s ensorineural hearing loss
Trismus, facultative finger contractures	DA type IIE	DA7	Trismus-pseudocamptodactyly syndrome, Hecht syndrome
Multiple pterygium		DA8	AD multiple pterygium syndrome
Ear deformity, long fingers		DA9	Beal syndrome, congenital contractural arachnodactyly
Plantar flexion contractures		DA10	Short tendo calcaneus

DA has been defined as arthrogryposis with congenital hand and foot involvement by Hall *et. al.* in 1982 [6]: type I with only distal joint involvement and characteristic hands at birth with flexed and overlapping fingers, and type IIA-IIE with distal limb contractures and additional characteristic manifestations.

A revised and extended classification was made by Bamshad *et.al.* in 1996 [7]. In this classification, DA is defined as an inherited primary limb malformation disorder characterized by congenital contractures of two or more different body areas and without primary neurological and/or muscle disease that affects limb function. Included disorders are characterized by distal joint involvement, limited proximal joint involvement, autosomal dominant inheritance, reduced penetrance, and variable expressivity. In this classification, nine different clinical forms are originally described, with a 10th syndrome defined in 2006 [7, 75].

The prevalence of DA syndromes is not known. The most commonly described forms of DA are DA1 and DA2B. DA1 is characterized by clenched fists at birth, ulnar deviation, medially overlapping fingers and club feet or other foot malpositions.

The hips may be affected, calves small and opening of the mouth mildly limited [76]. DA2B, Sheldon Hall syndrome, is similar to but milder than DA2A [4, 77]. DA2A, Freeman Sheldon syndrome, is characterized by a small mouth, facial contractures, scoliosis, mainly distal joint contractures and short stature [77]. Typical findings in DA2B are vertical talus, ulnar deviation, severe camptodactyly, triangularly shaped face, prominent nasolabial folds, downslanting palpebral fissures, small mouth and prominent chin [4]. Foot deformities may be asymmetric [78].

The sarcomer is the functional unit of striated muscle contraction. Mutations in sarcomeric proteins are found in at least 20 different skeletal muscle diseases [79] and can result in DA or in congenital myopathy [33]. Mutations in genes encoding sarcomeric muscle proteins are found in several DA syndromes: Sung *et.al.* described mutations in β tropomyosin (*TPM2*) in DA1, and mutations in fast troponin I (*TNNI2)* and fast troponin T (*TNNT3*) in DA2B [19]. Toydemir *et. al.* describes mutations in embryonic myosin heavy chain (*MYH3*) in DA2A, Freeman Sheldon syndrome, and in DA2B, Sheldon-Hall syndrome [21]. Further, a mutation in fetal myosin heavy chain (*MYH8*) has been described in DA7, trismus-pseudocamptodactyly syndrome [22]. A defective function of contractile muscle proteins during fetal life influencing fetal mobility seems to be the common cause of congenital joint contractures in these syndromes [21, 22].

Gene mutations and sarcomeric muscle proteins in DA

Two different mutations in the gene encoding an isoform of troponin I (TnI) specific for fast-twitch muscle fibers (*TNNI2*) have been associated with DA2B. DA2B has also been associated with a mutation in the gene encoding an isoform of troponin T specific for fast twitch contractile proteins (*TNNT3*) [20]. DA1 has, in one affected family, been associated with a mutation in the gene encoding β-tropomyosin (*TPM2*), [80] which is expressed mainly in slow twitch muscle fibers [81].

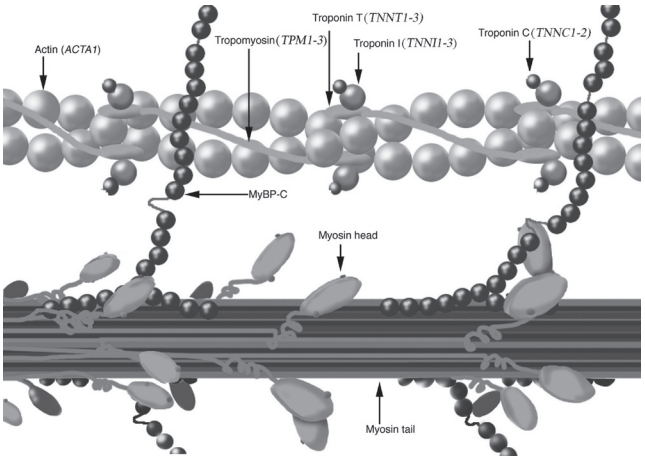

Figure 5a. *Sarcomeric proteins in the thin (actin) filament(top) and thick (myosin) filament (bottom) in striated skeletal muscle. The thick and thin filaments slide past each other in muscle contraction. Figure by Homa Tajsharghi, reproduced by permission.*

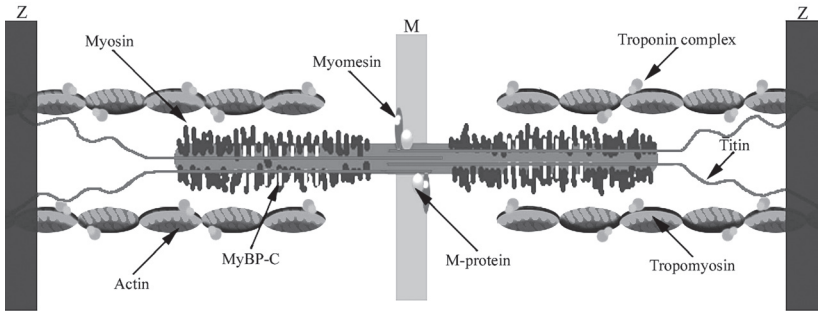

Figure 5b. *The sarcomere is divided into four major components: the Z-disc, I band, A band and M-line, with one sarcomere stretching from one Z-disc to the next. Figure by Homa Tajsharghi, reproduced by permission.*

The thin filament in striated muscle contains actin, tropomyosin, nebulin, and the troponin complex (TnI, TnT, and TnC). TM is composed of two α-helical chains, forming a rod-shaped coiled-coil dimer. It is localized head to tail along the length of the actin filament, providing stability, and is essential for myosin-actin interaction [81, 82].

There are four TM genes: *TPM1*, *TPM2*, *TPM3*, and *TPM4*. By alternative splicing, the use of alternative promoters and differential RNA processing, various transcripts are produced, which are specific for striated muscle, smooth muscle, and nonmuscle tissues [81].

There are three primary striated muscle TM isoforms, α-TM, β–TM, and γ–TM, which are highly homologous but are thought to exhibit unique physiologic properties [81]. In human striated muscle, α-TM is a product of *TPM1*, β-TM is encoded from *TPM2*, and γ-TM is encoded from *TPM3*. These striated muscle-specific isoforms are expressed in developmental and fiber-type-specific patterns in skeletal muscle and heart [81, 83]. In humans, the muscle isoform encoded by *TPM1* is predominantly expressed in cardiac muscle and in fast type 2 muscle fibers. *TPM2* is mainly expressed in slow type 1, and, to some extent, in fast muscle fibers and cardiac muscle. *TPM3* is predominantly expressed in slow muscle fibers and is also expressed in the heart [81]. In one family, DA1 was caused by the substitution of a highly conserved amino acid residue (R91G) in *TPM2* [19].

Myosin is the main component of skeletal muscle sarcomeric thick filaments. It consists of two globular heads attached to a long-helical-coiled coil rod domain. It is a hexamer composed of one pair of myosin heavy chains (MyHCs) and two pairs of myosin light chains. The myosin globular head domain of the myosin motor (myosin subfragment 1 [S1]) contains actin and adenosine triphosphate (ATP)-binding regions and is responsible for the force transduction properties of myosin [84].

Several striated muscle MyHC genes have been described [85]. The expression of myosin isoforms is developmentally regulated [86-88]. Myosin myopathies have evolved as a new group of muscle diseases caused by mutations in skeletal muscle myosin heavy-chain (MyHC) genes. The phenotypes of these diseases vary, ranging from prenatal nonprogressive arthrogryposis syndromes to adult-onset progressive muscle weakness. Mutations have been reported in two of three MyHC isoforms expressed in adult limb skeletal muscle. In addition to familial hypertrophic or dilated cardiomyopathy 11 mutations in the slow or cardiac MyHC gene (*MYH7*) cause skeletal myopathies such as myosin storage myopathy [89-93] and Laing early-onset distal myopathy [94, 95]. A mutation in the MyHC IIa gene (*MYH2*) is associated with dominant myopathy characterized by ophthalmoplegia, congenital joint contractures, and rimmed vacuoles in muscle fibers [96-98].

DA2A, Freeman-Sheldon syndrome, and DA2B, Sheldon-Hall syndrome, have been reported as the first disorders associated with mutations in embryonic MyHC (*MYH3*)[21]. DA syndromes are associated with missense mutations in various genes coding for sarcomeric proteins. The genes thus far demonstrated to be involved in DA syndromes are *TNNI2* (troponin I), *TPM2* (β-tropomyosin) [19], *TNNT3* (troponinT) [20], *MYH8* (perinatal MyHC), and *MYH3* (embryonic MyHC)[21].

These findings indicate that DA syndromes are caused by myopathies with onset during fetal development, but few studies have involved analysis of muscle tissue in these diseases.

In a recent study aiming to investigate the mechanisms of impaired muscle function in two patients with DA2B and a β-tropomyosin mutation (R133W), significant differences in regulation of muscle contraction was demonstrated in type 1 fibers. The found mutation appears to induce alteration in myosin-actin kinetics causing a reduced number of myosin molecules in the strong actin-binding state, resulting in muscle weakness in the absence of muscle wasting [9].

AIMS OF THE STUDY

The aims of the study were:

- To investigate children and adolescents with arthrogryposis in Sweden and to classify the different occurring forms.

- To describe a group of individuals with amyoplasia, to investigate how muscle strength and joint contractures affect their motor function, and to relate their current functional status to joint position at birth.

- To investigate the clinical, muscle morphologic and genetic findings in families with DA and pathogenic mutations in the sarcomeric muscle protein genes TNNI2, TPM2 and MYH3.

- To describe the clinical findings, clinical course, additional problems and disabilities in 40 individuals with DA and to evaluate genotype-phenotype correlation.

- To study and evaluate given treatment in patients with amyoplasia and DA.

MATERIAL AND METHODS

Patients

In a national survey in Sweden, 127 children, adolescents and young adults with arthrogryposis were investigated. The index patients were identified through pediatric rehabilitation centers or, in a few cases, through the orthopaedic surgeon or the Swedish AMC-association. A further four children with familial DA were added to the study after the initial survey was completed, making the total number 131.

The inclusion criteria were contractures present at birth in more than two joints and in multiple body areas. Children born with myelomeningocele, isolated congenital hip dislocation or pes equinovarus adductus were excluded.

Written informed consent was obtained from adult participating individuals and for participating children, from the parents.

The study was approved by the Ethical Board at the Universities of Gothenburg, Uppsala, Stockholm, Umeå, Örebro, Malmö, Lund and Linköping, and by the heads of Paediatric clinics in the Swedish health-care regions.

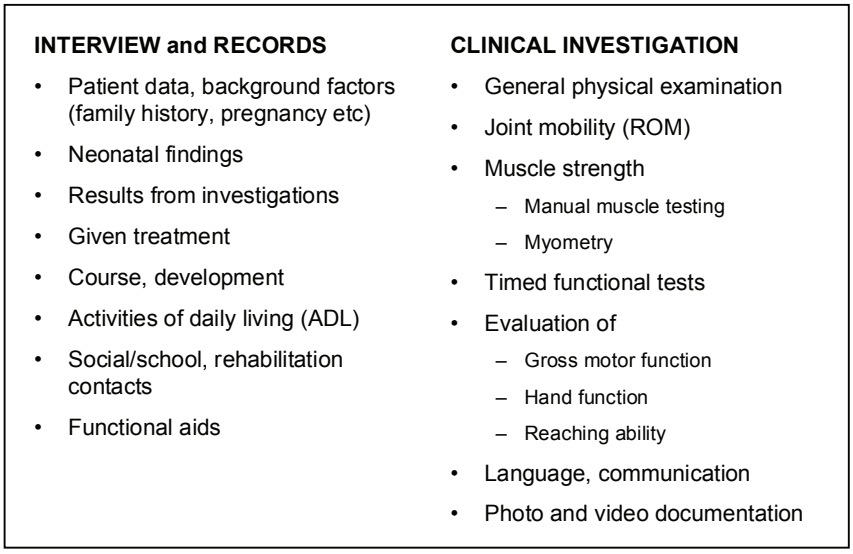

Figure 6. *Summary of performed investigations in the survey of children and adolescents with arthrogryposis in Sweden.*

Clinical investigation

All patients were investigated by the same pediatric neurologist (EK) and physiotherapist (AKK). Interview with the families, examination of the patients, and review of medical records were carried out at the local child rehabilitation centers.

A structured interview including family history, prenatal and perinatal history, neonatal findings including joint involvement, developmental milestones, associated medical problems, treatment, and outcome was carried out for all index cases.

A detailed clinical examination was carried out. Physical examination included evaluation of facial involvement, other associated signs and symptoms and a neurological examination.

For each patient, medical records were studied and results from previous investigations, including orthopedic procedures and muscle biopsies, were recorded. In patients with amyoplasia (paper I), an orthopedic surgeon (RJ) also reviewed the orthopaedic treatment records.

Affected adult family members were also examined by the same physician and physiotherapist, and information on family history was obtained by personal interviews. Extended family members who were found to be asymptomatic carriers of the pathogenic gene mutation in the family were seen and interviewed personally to exclude the presence of previously undiagnosed distal joint involvement.

Diagnosis was determined based on clinical findings and medical records, using known diagnostic criteria. For all patients with dysmorphic signs and/or suspected genetic syndrome, clinical findings and features of the patients were discussed with a clinical geneticist and in some cases a search in a genetic data base, Possum, was performed to aid in the diagnosis. Short stature was defined as below 3 SD. Cardiac investigation was performed in two patients with DA and TPM2 mutation.

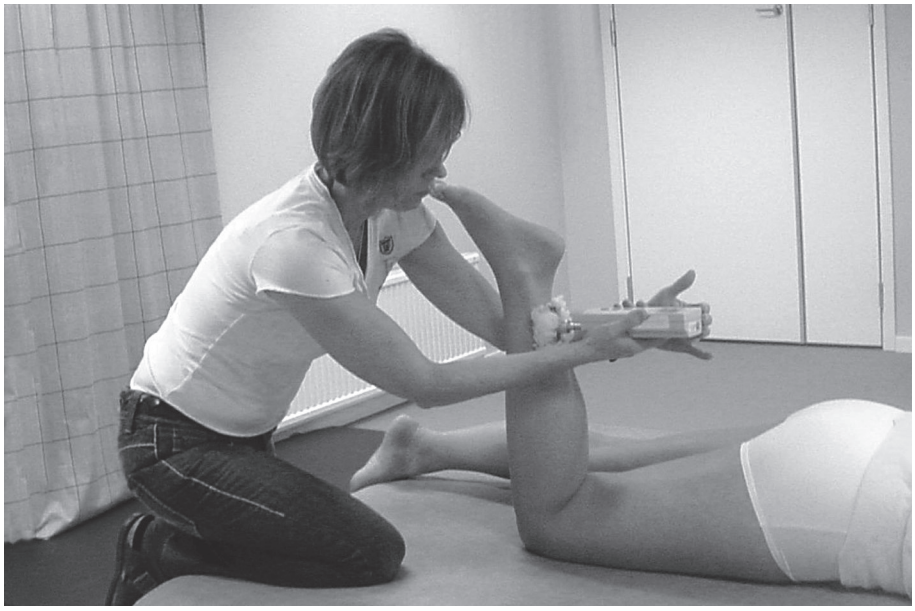

Figure 7. *Measuring muscle strength by a hand-held dynamometer.*

Muscle strength

Isometric muscle strength was measured with an electronic hand-held dynamometer (adapted Chatillon, Axel Ericson Medical AB, Göteborg, Sweden) with a method standardized by Eek *et.al*. [100] or, in children too young to participate, by clinical evaluation. Nine muscle groups were measured. An isometric contraction of at least five sec was required and the peak force value in Newton was recorded. The best of three values obtained was compared with the mean value from weight-related reference values for healthy children and adolescents [100]. To be able to compare isometric muscle strength independent of age and gender, a percentage of normal muscle strength was calculated.

Range of motion, ROM

Passive ROM was measured with an ordinary goniometer. Information on hip dislocation was collected from medical records.

Motor function

Motor function was assessed using a scale designed by Scott *et.al*. [101]. Twenty movements were assessed, including head-lifting in the supine position, rolling, sitting up from lying down, sitting, getting off a chair, standing up from lying, standing, standing on heels, standing on toes, standing on one leg, jumping, and climbing up and down stairs. The performance is scored according to a three-point scale: 0 (unable), 1 (needs self-reinforcement), and 2 (succeeds). Maximum score is 40.

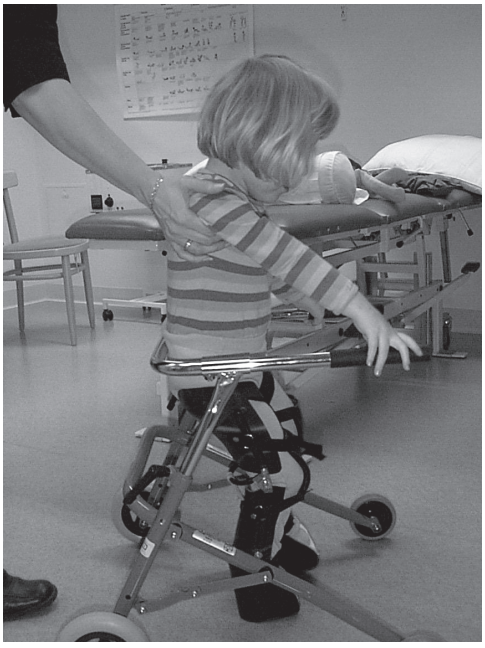

Figure 8. *A three-year-old girl with amyoplasia using orthoses and walker. Level of ambulation was household ambulator. The photograph is published by permission of the parents.*

According to their level of ambulation the patients were divided into functional groups according to the classification of Hoffer *et al* [102]:

1. Community ambulators (CA) walk with aids in the community and do not need a wheelchair;
2. Household ambulators (HA) walk with aids in the household and use a wheel chair in the community;
3. Non-functional ambulators (NFA) use parallel bars or walkers with support;
4. Non-ambulators (NA) do not walk.

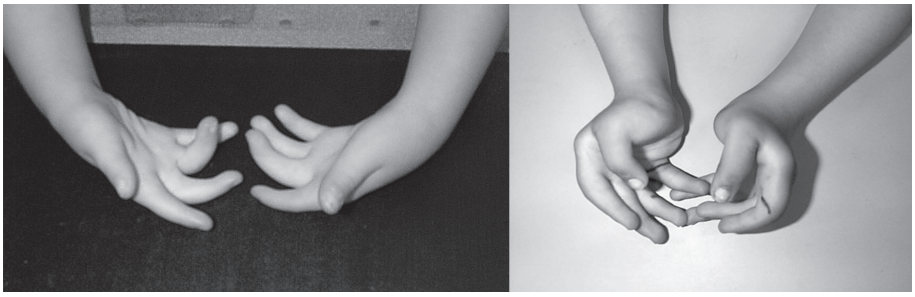

Figure 9. *Illustration of hand involvement in a child with amyoplasia (left) and a child with distal arthrogryposis (right).*

Hand function

Hand function was evaluated as follows: The ability to grasp a piece of paper, grasp a pencil, draw/write, build with duplo and lego bricks, thread beads on a string and screw a nut and bolt was evaluated and videotaped in all investigated children from the age of four years. In younger children hand function was observed and videotaped during play.

Four categories of hand function were used:

1. Normal function;
2. Able to perform all tasks completely but with compensatory strategies;
3. Able to perform all tasks but incompletely and with compensatory strategies;
4. Able to perform tasks incompletely, very limited hand function.

Blood chemistry and neurophysiology

Serum creatine kinase (S-CK) concentrations were measured in affected individuals with DA. In one individual, urine myoglobin levels before and after exercise were measured and electromyography (EMG) was performed.

Muscle morphology

In 11 individuals muscle biopsies were obtained and analysed by the same pathologist (AO). In five patients, results from previously performed muscle biopsies were recorded from medical charts. Morphologic stainings included hematoxylin–eosin and Gomori trichrome. Histochemical analyses included myofibrillar ATPase, oxidative enzymes (NADH-tetrazolium reductase, succinate dehydrogenase, and cytochrome oxidase), glycogen, lipids, and major histocompatibility complex class 1 antigen.

To identify embryonic (*MYH3*) MyHC expression, a monoclonal antibody, F1.652 (Developmental Studies Hydridoma Bank, Department of Biologic Sciences, University of Iowa, Iowa City), at a concentration of 1:100 was used.

In muscle tissue from four patients with DA and *MYH3* mutations, investigation of expression of MYHC isoforms at the protein and transcript levels were carried out in addition to morphologic analysis.

Molecular genetic investigation

Blood samples for genetic analysis were obtained and analysed from 25 index patients with DA and from their relevant family members. Blood samples were also obtained from 11 patients with CNS involvement/MR and dysmorphic signs, with normal karyotype on previous investigation.

Distal arthrogryposis

Extraction of genomic DNA, polymerase chain reaction (PCR) and sequence analysis were performed. The entire coding sequence of *MYH3, TPM2* and *TNNI2* was sequenced in relevant patients using a previously described primer. The presence of each mutation was confirmed in each affected individual by restriction fragment length polymorphism (RFLP) analysis. The RFLP was also used to screen for the presence of each mutation in 200 control chromosomes. The entire coding region of *MYH3, TPM2*, and *TNNI2* genes was sequenced in 14 patients. In a few patients, in whom mutations in *MYH3*, T*PM2* and *TNNI2* had been excluded*, TNNT3, TNNT1, TNNC1, TNNC2* and *TNNT1* were also analysed. In one patient, the acetylcholine receptor gene gamma, *CHRNG*, was investigated in addition to *TPM2*.

CNS involvement and dysmorphic signs

In 11 cases of arthrogryposis with CNS-involvement/MR and dysmorphic signs, where routine chromosome analysis had been normal but clinical signs suggested a chromosomal background, further investigation with arrayCGH, was performed. Eight patients were analyzed by arrayCGH consisting of 32 000 BAC clones as previously described by de Ståhl *et al* and three patients by Affymetrix 250K Nsp array according to the standard Affymetrix GeneChip protocol (Affymetrix Inc, Santa Clara, California, USA).

Statistical analysis (paper I)

Stat View 4.02 for Macintosh was used to analyze the data. Nonparametric tests were used. Wilcoxon's signed rank test was used for the calculation of paired differences and the Mann-Whitney U-test was used for the calculation of unpaired differences. Spearman's rank correlation was used for the calculation of correlation coefficients between isometric muscle strength and motor function/hand function, ROM and motor function/hand function and between isometric muscle strength and ROM. The correlation values were interpreted according to guidelines adapted from Altman [103], where $r<0.20$ poor, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 good, and 0.81–1.00 very good. The level of significance was determined to be 0.05.

Results

Survey results

In a nationwide study of arthrogryposis in children and adolescents in Sweden, 131 individuals were investigated. Ages ranged from newborn to 28 years of age, with a mean age of 8.25 years; 59 females and 72 males. The majority of included individuals were under 20 years of age at the time of investigation. Diagnostic groups are presented in table 2.

Amyoplasia was diagnosed in 48 (37%) patients; 35 (27%) of these were regarded as definite and 13 (10%) as probable amyoplasia. Five had only upper limb involvement, eight only lower limb involvement, four lower limb plus discrete additional contractures (jaw/ thumbs/unilateral hand/bilateral hands). Of the remaining 31 patients with involvement of all four limbs, one had dysmelia of the feet and one had Blackfan Diamond anemia in addition to amyoplasia.

DA was found in 27 (21%) index patients. Sixteen were autosomal dominant and 11 were sporadic cases. Among the sporadic cases, five had DA1, one DA2B, Sheldon Hall syndrome, and there was one boy with DA3, Gordon syndrome, one boy with DA2A, Freeman Sheldon syndrome, and one girl and one boy each with DA8, multiple pterygeum syndrome. One young man was classified as having DA7, trismus-pseudocamptodactyly syndrome. Among familial cases 10 had DA2B, four DA1, and one DA9, Beal syndrome, with autosomal dominant inheritance.

Six children had clinical signs of myopathy, none of these with specific myopathic diagnosis. Four children had Larsen syndrome, one further child had suspected Larsen syndrome, one girl had Turner syndrome and one boy facio-audio-symphalangism syndrome. Three children had suspected syndrome without MR: one boy with suspected Sprenger syndrome, one girl with severe hearing impairment and arthrogryposis and one boy in whom signs of a not defined syndrome were present.

Twenty-two (17%) children were found to have arthrogryposis with CNS-involvement. Of these, one had a perisylvian syndrome, one agenesia of the corpus callosum, one Marden Walker syndrome, and one Schwartz-Jampel syndrome. There was one case each of triploidy mosaicism, trisomy 6q and trisomy 18q. One boy had clinical signs of Kabuki make-up syndrome. Fourteen children had non-defined syndromes with mental retardation and arthrogryposis. Four children also had increased muscle tone/spasticity.

Preliminary results from arrayCGH investigation in 11 patients with arthrogryposis, CNS-involvement and dysmorphic features indicate that microaberrations likely to be pathogenic are found in the same frequency (10-20%) as in patients with MR and dysmorphic signs without the additional finding of arthrogryposis [58, 104].
Six children had arthrogryposis in the lower extremities only, two of these with

vertebral anomalies and caudal regression syndrome. A further 12 children had arthrogryposis that we could not specify further at present.

Table 2. *Survey results.The 131 investigated individuals with arthrogryposis according to identified diagnostic groups.*

AMYOPLASIA		**N=48**
Upper and lower extremities	31	
Lower extremities	12	
Upper extremities	5	
DISTAL ARTHROGRYPOSIS		**N=27**
DA1	9	
DA2A, Freeman Sheldon syndrome	1	
DA2B, Sheldon Hall syndrome	12	
DA3, Gordon syndrome	1	
DA7, trismus-pseudocamptodactyly	1	
DA8, multiple pterygium syndrome	2	
DA9, Beal syndrome	1	
CNS/MENTAL RETARDATION		**N=22**
Spasticity	4	
Perisylvian syndrome	1	
Marden Walker syndrome	1	
Schwartz-Jampel syndrome	1	
Kabuki syndrome	1	
Mosaic triploidy	1	
Trisomy 6q	1	
Trisomy 18q	1	
OTHER SYNDROMATIC		**N=10**
Larsen syndrome	5	
Facio-audio-symphalangism syndrome	1	
Turner syndrome	1	
Not defined syndrome	3	
MYOPATHIC SIGNS		**N=6**
LOWER LIMB ARTHROGRYPOSIS		**N=6**
Vertebral anomalies	2	
Other	4	
OTHER ARTHROGRYPOSIS		**N=12**
TOTAL NUMBER		**N=131**

Paper 1
Muscle involvement and motor function in amyoplasia

Patients

Thirty-five individuals (28%) were diagnosed with definite amyoplasia. The diagnostic inclusion criteria were typical contractures at birth, muscle involvement, and associated findings such as dimpling and facial hemangiomas [3]. There were 23 males and 12 females. The age at investigation ranged between 0.2 and 22.8 years, with a median age of 7.0 years. Involvement of only the upper limbs was seen in four, only the lower limbs in six and both the upper and lower limbs in 25 patients.
Eleven cases were categorized as community ambulators, 11 cases as household ambulators and seven as non-ambulartors.

Gestational and perinatal information

There were three cases of twin pregnancies. Early spontaneous abortion of one fetus was seen in two of these, leading to birth of affected singletons. The third twin pregnancy resulted in the birth of one affected girl and one healthy boy. There were two documented cases of maternal bicornate uterus, one of oligohydramnios and one of fetal ascites. Twenty mothers described decreased or other abnormal fetal movements. Three cases of pre-eclampsia and one of placental insufficiency were described. Ten children were delivered by Caesarian section and two by vacuum extraction. Eight children were born in breech presentation, one in foot presentation and 12 in normal presentation. Information about presentation is missing in two cases. Twenty-seven children were born at term and 7 were premature (gestational age ranging from 33 weeks to 36 weeks and 5 days). In one case information about gestational age is missing. Birth weight was within normal range in 27 children, there were six children with low birth weight (<2,500 g) and one with very low birth weight (<1,500 g). One child was born with gastroschisis and one with an inguinal hernia. In one child fractures occurred during delivery.

Joint Position at Birth

Twenty-three children were born with internal rotation of the shoulders. The elbows were extended in 22 children and flexed in seven. Flexion deformities at the wrist were noted in 24 children. Seven children were born with flexion, external rotation and abduction of the hips with flexed knees. Eleven children were born with extended or hyperextended knees. The most common foot deformity was pes equinovarus adductus seen in 24 children. There were four children with isolated short Achilles tendon and one child with pes calcaneovalgus.

Orthopedic operations

Nine hip dislocations were found in seven patients. Open reduction was performed in three patients/four hips. Extending osteotomies had been performed in two patients/four knees. The original deformity recurred after two to three years in these patients. Posterior soft tissue release had been performed in seven patients/14 knees, and quadriceps lengthening was performed in six patients/9 knees.

Lengthening of the Achilles tendon was performed in nine patients. Six of these patients were subject to repeated surgery. Eleven patients underwent surgery with posterior release as the primary operation and in six of these patients repeated surgery was performed. Talectomy as the primary orthopaedic operation was performed in three children under the age of two years. The median age of two months at the first operation was lower in 13 patients requiring repeated surgery, compared to the median age of two years in 11 patients who were operated on only once.

In the upper limbs 28 operations were performed in 12 patients. Transfer of muscles (m. latissimus dorsi, m. deltoideus, m. pectoralis major) to restore active elbow flexion was performed in six patients. One or several lengthening procedures or releases of the triceps tendon to increase ROM were performed in four patients. Six patients underwent surgery of the hand. Operations included transfers, lengthening, or release of tendons, capsulotomies and, in one case, an extending osteotomy with the main purpose of treating wrist flexion and thumb-in-hand deformities.

Stretching
All children except one had received regular stretching during their first years of life. The frequency varied from three to four times per week up to three to five times daily. After the first year the frequency gradually decreased, but in most patients stretching was continued two to three times per week. Splints were frequently used to increase or maintain joint motion during the first year of life, and later to maintain range of motion after orthopaedic surgery.

Isometric muscle strength
All investigated muscle groups, except elbow extensors in the household ambulators, were significantly weakened compared with reference values [100]. The most severely reduced isometric muscle strength was found in non-ambulators. They were significantly weaker than household ambulators in elbow extensors (P=0.013), hip flexors (P=0.037), hip abductors (P=0.004), and knee extensors (P=0.001). Non-ambulators were significantly weaker than community ambulators in shoulder abductors (P=0.001), elbow extensors (P=0.006), wrist dorsiflexors (P= 0.002), hip flexors (P<0.001), hip extensors (P<0.001), hip abductors (P<0.001), knee flexors (P=0.043), knee extensors (P=0.001), and ankle dorsiflexors (P=0.012). Household ambulators were significantly weaker than community ambulators in hip flexors (P<0.001), hip extensors (P<0.001), hip abductors (P=0.001), knee flexors (P=0.004), and knee extensors (P<0.001). There was no significant difference in the muscle groups of the upper extremities between household ambulators and community ambulators.

Range of motion at time of investigation
The most severe contractures were found in non-ambulators. Non-ambulators had significantly more severe contractures than household ambulators in shoulder abduction (P=0.019), elbow flexion (P=0.002), hip extension (P=0.019), knee extension (P=0.001), and knee flexion (P=0.038). Non-ambulators had significantly more severe contractures than community ambulators in shoulder elevation (P=0.048),

elbow flexion (P=0.001), hip extension (P=0.001), hip abduction (P=0.023), hip adduction (P=0.037), hip inward rotation (P=0.003), knee extension (P<0.001), ankle dorsiflexion (P=0.002), and plantar flexion (P=0.001). Household ambulators had significantly more severe contractures than community ambulators in hip extension (P=0.034), hip internal rotation (P=0.006), knee flexion (P=0.008), knee extension (P=0.001), ankle dorsiflexion (P=0.010), and plantar flexion (P=0.001).

Motor Function
Motor function scores were related to age in all the assessed individuals. The youngest children had poor motor function scores partly due to their age. Community ambulators had the highest scores and none-ambulators the lowest. Community ambulators achieved independent walking at an earlier median age compared to household ambulators.

Hand Function
Nine patients had normal hand function, 13 were able to perform tasks completely but with compensatory strategies, four were able to perform tasks incompletely and with compensatory strategies, and nine patients were able to perform tasks incompletely with very limited hand function.

Main correlations
There were strong correlations between muscle strength and motor function. There were only moderate correlations between range of motion and motor function. Community ambulators had the best muscle strength, and none had knee flexion contractures of more than 20 degrees. Household ambulators had severe contractures in the legs, but good muscle strength in the arms. Non-ambulators had the most severe joint contractures and the most pronounced muscle weakness. The majority were born with hips in pronounced abduction, flexion and external rotation.

Paper 2
A mutation in the fast skeletal muscle troponin I gene causes myopathy and distal arthrogryposis

Patients
This family included five affected individuals in three generations at the time of investigation: the index patient who was a young girl, her father, paternal aunt and paternal uncle as well as her paternal grandfather. The clinical findings were, in summary, mainly distal joint involvement and mild facial involvement (mild micrognathia, narrow palpebral fissures). There was no evident muscle weakness.

Blood chemistry and neurophysiology
In all four affected adults, S-CK levels were mildly elevated at the time of investigation, ranging between 5.7 and 7.9 µkat/L (reference interval <3 µkat/L). In one case urine myoglobin concentration before and after exercise was measured and found to be normal. EMG was performed in one case. A small amount of denervation activity was found, but there were no convincing myopathic findings.

Muscle morphology
Similar myopathic changes were found in all four adult cases. There was an increased variability of fiber size and frequent muscle fibers with internalized nuclei. Fiber size variability was present among type 1 as well as type 2 fibers but was more pronounced among type 2 fibers, which were generally much larger than the type 1 fibers. Occasional signs of fiber splitting in type 2 fibers were present. The fibers with internalized nuclei were type 2. There was type 1 fiber predominance (which is normal for the tibialis anterior muscle) except in one case where the area occupied by type 2 fibers was equal to or larger than that of type 1 fibers. Regenerating fibers and increased interstitial connective tissue was also found.

Genetic findings
We performed mutation analysis of *TNNI2* in 16 members of the family. The entire coding sequence of *TNNI2* was investigated in the index patient. We identified a heterozygous three-base in-frame deletion, 2,918–2,920del, skipping the highly conserved lysine (K) at position 176. The K176del is located in the distal end of the TNNI2 filament, seven residues from the stop codon. The deletion was identified in all five affected family members and not in any of the 11 investigated relatives without DA. The mutated residue is highly conserved among species and corresponds to residue K206 in cardiac TnI (cTnI; *TNNI3*). A mutation of this residue, K206Q in cTnI, has been associated with hypertrophic cardiomyopathy [105].

Paper 3
Distal arthrogryposis and muscle weakness associated with a ß-tropomyosin mutation

Patients
We investigated a mother and daughter (index patient), age 65 and 28 years respectively at time of investigation. Both had predominantly distal joint contractures at birth. At the time of investigation, the mother had contractures in jaws, hands, feet, elbows and shoulders and scoliosis. The daughter had contractures in all joints, proximal and distal, proximal syndactyly in the hands, and kyphosis. Both patients had short stature, and both had muscle weakness in proximal and distal muscles, most prominent in the hands and feet. The mother described progressive muscle weakness. Results of cardiac investigations were normal. The clinical findings were consistent with DA2B. No other family members were affected.

Genetic findings
We performed mutation analysis of the the entire coding sequence of *TPM2* in the index patient. A heterozygous missense mutation in exon 4, C5396T, was identified. The mutation was also present in the mother, but it was not identified in any of the three investigated relatives without DA (father and maternal grandparents), or in 200 control chromosomes.
Muscle morphology
Muscle biopsies were obtained from the tibialis anterior muscle in both patients. Histochemical and ultrastructural investigation revealed type 1 fiber predominance

but no other major morphologic abnormalities. Immunohistochemistry of myosin isoforms also demonstrated a marked predominance of muscle fibers expressing slow myosin.

Paper 4

Embryonic myosin heavy chain mutations cause distal arthrogryposis and developmental myosin myopathy that persists postnatally

Patients

Patients 1 and 2 were a mother and daughter with DA2B, with clinical features including short stature, scoliosis, mild facial dysmorphism, joint contractures in proximal and distal joints, and muscle weakness.

Patient 3 was a man with a milder form of DA2B, father of three children who all had DA. Clinical features included contractures in the hands and mild involvement of jaws, feet, and elbows, and normal muscle strength.

Patient 4 was a boy with sporadic DA2A, Freeman Sheldon syndrome with facial contractures, small mouth, ptosis, short stature, and joint contractures in the proximal and distal joints. Muscle strength was difficult to evaluate owing to his young age, which was four years at the last assessment.

Genetic findings

The entire coding sequence of *MYH3* was investigated in the index subjects of the respective families. Three different missense mutations were identified:
In patients 1 and 2 with DA2B, a heterozygous missense mutation in exon 13, A1454G, was identified. The mutation was *de novo*, and not present in the maternal grandparents. In patient 3 with DA2B, a heterozygous missense mutation in exon 7, C769T, was identified. The mutation was also identified in all three affected children of patient 3 and also in the asymptomatic paternal grandfather. In patient 4 with sporadic DA2A, a heterozygous missense mutation in exon 5, C602T, was identified. This was also an apparent *de novo* mutation, as neither of the parents carried the mutation.

Expression of MYHC isoforms

The relative expression of the 3 major MyHC isoforms in skeletal muscle and the presence of embryonic MyHC were determined at the messenger RNA level using reverse transcription-polymerase chain reaction analysis. None of the muscle biopsy specimens from the patients demonstrated expression of embryonic MyHC.

Muscle morphology

Muscle biopsy specimens from the deltoid muscle in patients 1 and 2 exhibited slight pathological changes. There was increased variability of fiber size owing to the presence of frequent small type-1 fibers, and also a slightly abnormal type-1 fiber predominance. A muscle biopsy specimen from the deltoid muscle of patient 3 showed scattered, small type-1 fibers but no obvious pathological changes. Muscle biopsy

specimens from the tibialis anterior muscle of patient 4 at ages 15 months and five years showed slight pathological changes. The major abnormality at age 15 months was numerous fibers expressing the fetal (perinatal) isoform of MyHC (*MYH8*), and the biopsy specimen obtained at age five years showed marked type-1 fiber predominance and scattered, small type-1 fibers. No fibers expressed fetal MyHC at age five years in patient 4. Expression of embryonic MyHC (*MYH3*) was not identified in any patients.

Paper 5
Distal arthrogryposis: clinical and genetic findings

Patients

In the original study of 131 patients with arthrogryposis, 27 cases with DA from 21 families were identified. Of the 27 index cases, 11 were sporadic and 16 familial. A further 13 affected relatives were identified and investigated. Including affected parents and extended family members, 40 individuals with familial or sporadic DA were interviewed and examined.

DA classification

Fourteen patients, seven familial and seven sporadic cases, were classified as having DA1 and 17 patients, 15 familial and two sporadic cases, were classified as having DA2B. In two familial patients clinical findings were intermediate between DA1 and DA2B. Clinically, dividing lines between these two syndromes where not clear cut.

One child was classified as having DA2A, Freeman Sheldon syndrome, one child as DA3, Gordon syndrome, and one young man as DA7, Trismus-pseudocamptodactyly syndrome. These three were all sporadic cases. Multiple pterygium syndrome, DA8, was found in two sporadic cases, and Beal syndrome, DA9, in one family with affected mother and son. We did not identify any patients with DA4 (DA with scoliosis as the predominant feature), DA5 (DA with limited ocular motility), DA6 (DA with sensorineural hearing loss) or 10 (DA with plantar flexion contractures).

Perinatal data

Fourteen of 27 (52%) of the children were born by Caesarean section. Eight of these children were in breech position. Feeding problems in the neonatal period were common (60%).

At birth, hand involvement included ulnar deviation, thumbs in hand, clenched fists, contractures in fingers, contractures in wrists, and overlapping fingers. Foot involvement included pes equinovarus, pes calcaneovalgus, and other foot malpositions. Asymmetric foot involvement was found in nine familial cases. Involvement of proximal joints included contractures in hips, knees, shoulders and elbows, dislocation of hips, scoliosis/kyphosis, and torticollis.

Facial involvement
Facial involvement was found more frequently in familial than in sporadic cases. The most frequent findings, in decreasing order, were impaired mouth opening (mild contractures in jaw joints), low set ears, high arched palate, micrognathia, high nose bridge, down-slanting and/or narrow palpebral fissures, facial asymmetry, small mouth, and epicanthal folds. Prominent chin was not a frequent finding

Hand involvement
Flexion or, more seldom, extension contractures in the wrist were the most frequently found involvement of hands, followed by ulnar deviation in wrists/fingers. Smooth palms with absent flexion creases and contractures in MCP and finger joints resulting in camptodactyly were also seen frequently. Thumbs in hands and other thumb malpositioning were also found.

Foot involvement
Where foot malpositioning was present at birth, orthopaedic treatment had mostly taken place when patients were examined after the first year of life. The most common present types of foot involvement, in order of frequency, were ankle contractures, adduction/metatarsus varus, prominent heel pads (also a common finding in other types of arthrogryposis), overlapping toes, short Achilles tendons, pes planovalgus and equinus feet. Asymmetric involvement of the feet was a common finding, while asymmetric size of the feet was found in a few cases.

Involvement of proximal joints
Proximal joints were more often engaged in the upper limbs than in the lower limbs, with elbow contractures the most common finding. Impaired shoulder mobility was also relatively frequent. Contractures in the hips and knees were found in decreasing frequency. Asymmetric legs (length and/or muscle bulk, especially in calves) were also seen.

Spinal involvement
Scoliosis was found in nine cases, kyphosis in two. Stiff spine, lumbar or thoracolumbar, was found in three cases. Short neck and/or contracted neck muscles were found in 15 cases.

Short stature
Five individuals had a short stature, four familial and one sporadic case.

Gross motor function and hand function
Ambulation was affected in 4/38 patients: a mother and daughter with DA2B and *MYH3* mutation, one sporadic case with DA2B and no identified mutation, and one sporadic case of DA8.

Hand function was mildly affected in 28/40 patients (able to perform all tasks but with compensatory strategies or aberrant function), and impaired in 5/40 patients.

Muscle involvement
Muscle weakness, generalised or in hands/feet, was found in 15/36 individuals: 8/29 familial cases, five of these with an identified mutation (two *MYH3*, two *TPM2* and one *TNNI2*), and 9/11 sporadic cases, one with an identified mutation (*MYH3*). Calves were thin in 11 familial and five sporadic cases. Muscle pathology had been demonstrated in 10/16 biopsied cases.

Orthopedic surgery
The number of performed orthopedic operations varied between none and 22 in different individuals. The majority of operations were in the feet, with only a few in hands, proximal joints or spine.

Pain
Pain, either generalised muscle pain or localised pain, was present in 16/39 patients. Pain was a more frequently occurring problem in patients with more severe joint contractures, and in patients who had undergone multiple surgeries. In several of the adult patients, muscle fatigue and pain on exertion was described.

Molecular genetics
Genetic investigation for mutations in genes encoding for sarcomeric proteins identified pathogenic mutations in 21/37 affected cases, 20/29 familial and 1/8 sporadically occurring. In three families, one with mutation in *MYH3*, one *TPM2*, and one *TNNI2,* the mutation was found to be *de novo*. Four asymptomatic carriers were found. In seven patients with DA2B, five familial cases from two families and two sporadic cases, and six patients with DA1, two familial and four sporadic, no mutation was identified. Clinical findings in these cases did not differ from cases were pathogenic mutations were identified. In one patient with DA8, no pathogenic mutation was identified on investigation.

Clinical vs genetic findings
In DA1 and DA2B families, symptoms did not comply with the different mutations, or even with whether a pathogenic mutation was found or not. This would indicate firstly that dysfunction of several different sarcomeric proteins during fetal life result in similar clinical symptoms, and secondly that the presence of so far non-identified gene mutations can cause the same clinical symptoms as the identified mutations in *MYH3*, *TPM2* and *TNNI2*.

The likelihood of identifying a genetic cause of DA was found to be far greater in familial than in sporadic cases. The only sporadic case of DA with an identified pathogenic mutation in our study was a child with DA2A, Freeman Sheldon syndrome. However, in three families with familial DA a *de novo* mutation could be demonstrated in the oldest affected individual, which demonstrates that pathogenic sarcomeric gene mutations may also be present in other sporadic cases. In two extended families asymptomatic carriers were found, demonstrating varying penetrance.

DISCUSSION

Amyoplasia (Paper I)

Patients

There were twice as many boys as girls in our study of muscle involvement and motor function in 35 children with amyoplasia, and boys were also more severely affected than girls. The reason for this is unclear. In a review of AMC by Hageman *et al* [106] a sexratio of two boys to one girl is described, referring to a publication by Kite from 1955. As far as we know this relationship has not been found in other studies. However, in our background survey of arthrogryposis in 131 cases in Sweden with a total of 48 patients with definite or possible amyoplasia, the male preponderance was not as high: 28 boys/20 girls.

Joint contractures

The extent and severity of joint contractures are important predictors of ambulatory level and motor function outcome in amyoplasia. Patients who turned out to be non-ambulators or household ambulators had a larger number of joints involved at birth compared to community ambulators. The combination of lower limb joint contractures and severe muscle weakness is negative for ambulation development. In non-ambulators, the typical joint positions at birth in lower extremities were flexion, abduction, and external rotation in the hips with knee flexion contractures. However, with good muscle strength in the upper extremities some of the patients with this combination of congenital contractures became household ambulators and used walking aids for support. The importance of good hand function for the ability to use walking aids has been described previously [102], as has the importance of contractures in level of ambulatory function [65, 107]. Non-ambulators all had severe flexion contractures in the hips and knees. The negative impact on flexion contractures in the lower extremities on the ability to walk has also been demonstrated in previous studies. More than 20 degrees of flexion contractures in the knees make use of orthoses for ambulation impossible[108]. Flexion contractures in the lower extremities also seem to be more difficult to treat successfully [50].

Orthopedic treatment

The majority of performed orthopedic operations were in the feet. Achilles tendon lengthening and posterior release often had to be repeated, especially if performed at an early age (during the first four months). No community ambulators had been subject to hip surgery, and only one had knee surgery, while surgery in hips and knees was more often performed in household ambulators and non-ambulators, a consequence of their more severe contractures in the lower extremities. Surgery in the upper limbs mainly included tendon transfers and lengthenings to increase range of motion. Although increased range of motion was often achieved, functional improvement was rare.

Muscle strength

The most important finding in our study was that muscle strength is more important than joint contractures in the development of motor function. This implies that muscle mass in the infant with amyoplasia is more important than severity of joint contractures in predicting future motor abilities. Muscle mass can be difficult to evaluate on inspection, as the small infant has a relatively large amount of adipose tissue that may disguise lack of muscle bulk. Functional assessment (observation of the baby´s spontaneous motor function) and ultrasound/CT scan/MRI to evaluate muscle bulk are ways to get a better evaluation. The importance of muscle strength in development of motor function/level of ambulation in children also has implications in the planning of treatment.

Distal artrogryposis (Papers II-V)

Clinical and genetic findings

Clinical findings were found to be highly variable in individuals with DA. This was true also between families, and within families with DA. Familial cases were often found to have more severe symptoms than sporadic cases, but this was not true of all patients.

In DA1 predominantly distal joint contractures with no associated anomalies are described, whereas in DA2B, Sheldon Hall syndrome, mild facial involvement (triangular face, down-slanting palpebral fissures, prominent nasolabial folds, small mouth), sometimes asymmetric foot involvement and short stature are typical findings [76]. In DA2A, Freeman Sheldon syndrome, severe facial involvement with small and pursed mouth, joint contractures, and short stature are common findings. The majority of cases in our study were diagnosed with DA1 or DA2B. We found that the clinical division between the two syndromes was unclear, especially in patients where facial involvement was very mild. Both syndromes were found in sporadic and familial patients, although familial involvement was more common in DA2B. The only other familiar patients found were a mother and son with DA9. Other DA forms found were all sporadic cases of DA2A, DA3, DA7 and DA8. We found no cases of DA4, DA5, or DA6. DA1 and DA2B are the most commonly occurring forms of DA [6, 21], while other forms are more rare.

Mutations in genes encoding sarcomeric (contractile) muscle proteins are involved in DA syndromes. Genes initially found to be involved in DA are troponin I (*TNNI2*), troponin T (*TNNT3*), and β-tropomyosin (*TPM2*). Mutations in *TPM2* can also be found in nemaline myopathy [109] and cap disease [110]. Recently, mutations in *MYH3* have also been found in DA syndromes [21]. There are several known myosin myopathies: familial cardiomyopathy, myosin storage myopathy, Laing early onset myopathy and autosomal dominant myopathy with rimmed vacuoles, ophtalmoplegia and congenital joint contractures [111]. The pathogenic mechanisms in sarcomeric protein dysfunction leading to myopathy and DA have been unclear. Increased contractility of developing fast-twitch skeletal muscles, causing muscle contraction and secondary joint contractures has been a proposed pathogenic mechanism [112].

Earlier studies have shown that fetal immobility is the common background for all arthrogryposis syndromes. Defective muscle relaxation may lead to fetal immobility and arthrogryposis. Troponin and tropomyosin are both involved in the regulation of muscle contraction. In a study of R133W β-tropomyosin mutation on regulation of muscle contraction in single human muscle fibers, results indicate that the mutation induces alterations in myosin-actin kinetics causing a reduced number of myosin molecules in the strong actin-binding state, resulting in overall muscle weakness without simultaneous muscle wasting [99].

Myosin is an essential part of the thick filament in striated muscle (paper IV). The expression of myosin heavy-chain (MyHC) is developmentally regulated. The embryonic isoform encoded from *MYH3* is expressed during fetal life. In muscle biopsy specimens from patients with DA and *MYH3* mutations, the embryonic isoform of MyHC (MYH3) was not detected. This indicates a normal down regulation of MYH3. On morphologic analysis, mild and variable pathologic changes were found, and in one patient also pathologic upgrading of the fetal MyHC isoform (MYH8). These findings indicate that DA syndromes are caused by myopathies with onset during fetal life, and that postnatal muscle manifestations are variable.

TNNI2

Two families with a pathogenic *TNNI2* mutation were identified. In the first family a novel K176del mutation was found in all affected individuals (paper II). The clinical findings in six affected individuals (five at the time of primary investigation; one more child born during the study) included distal joint contractures but also involvement of proximal joints, short neck muscles, and mild facial involvement including mildly narrowed palpebral fissures and a high arched palate. The feet were asymmetrically involved in one individual. Clinical findings were intermediate between DA1 and DA2B. One further family with a different *TNNI2* mutation, R174Q, in three affected individuals (mother and two children) was identified in our study. This mutation has previously been reported in DA2B [4]. In this family, clinical findings were more consistent with DA2B. Muscle weakness was not apparent in the first described family, while muscle weakness was present in one investigated individual in the second family. In the first of these two families, the mutation was most likely *de novo*.

TPM2

One family with a novel *TPM2* mutation, R133W, was found (paper III). The two patients, mother and daughter, had contractures predominantly in distal joints but also proximal joint involvement, smooth palms, short stature, and spinal involvement. Both had muscle weakness in proximal and distal muscles, most prominent in the hands and feet. The mother described progressive muscle weakness. The clinical history and findings were consistent with DA2B. The mutation was found to be *de novo*.

MYH3

Three families and one sporadic case with *MYH3* mutations were identified. In the first family (patients 1 and 2 in paper IV) with a D462G mutation, the affected mother and daughter both had severe joint involvement in both distal and proximal joints, relatively mild facial involvement, spinal involvement, short stature, asymmetric foot involvement, all consistent with DA2B. Both had weak muscles. The mutation found was *de novo*.

In the second family, a A234T mutation in *MYH3* was found in the affected father (patient 3 in paper IV), in his three affected children, but also in the unaffected paternal grandfather. Clinical signs were consistent with DA2B in the father and in one child, whereas in the other two affected children there was no facial involvement and clinical findings were more like DA1. Clinical features in affected family members included contractures primarily in the hands and/or feet, mild involvement of the jaws and elbows, and normal muscle strength. In one child with predominantly hand involvement the contractures seemed to be progressive, which is an unusual finding. Incidentally, in the extended family the maternal grandfather was found to have DA1 and a different mutation in *MYH3*, A1198T. This mutation was also found in his two unaffected sons but not in his also unaffected daughter (mother of the three affected children).

In a third family, an A1752T *MYH3*-mutation was found in two brothers, father and an unaffected paternal grandmother. The older brother also had a mild mental retardation and the younger additional malformations (cleft palate, bowel atresia and VOC). Both had attention deficit hyperactivity disorder, ADHD. The father in this family had a history of contractures in his feet as a child, but was asymptomatic at the time of investigation. The mutation found in this family was considered to be a possible polymorphism.

Finally, a T178I *MYH3*-mutation was identified in a boy with DA2A, Freeman-Sheldon syndrome (patient 4 in paper IV). Symptoms included ptosis, blepharophimosis, short stature, small and contract mouth, and joint contractures in proximal and distal joints.

DA with no identified mutation

We found two families with DA2B, one with an affected mother and son, and the second family with an affected father and two sons, with typical symptoms in both families including mild facial involvement, predominantly distal joint contractures and asymmetric foot involvement in several patients, and no identified mutation in investigated genes. Symptoms in these two families with DA2B were no less severe compared to those where a pathogenic mutation in genes encoding for sarcomeric proteins were identified. No pathogenic mutation was identified in sporadic cases except the one child with DA2A.

Phenotypic variability in DA is well described previously [78, 113]. Clinical features of-

ten overlap, especially between DA1 and DA2B and between DA2B and DA2A [114]. Mutations in *MYH3*, *TNNI2*, and *TNNT3* have been described in approximately 50% of cases with DA2B, Sheldon-Hall syndrome, in previous studies [4] *TPM2* mutations in DA2B have also been found recently [114].

In a study of *MYH3*-mutations in patients with DA2A and DA2B by Toydemir [21], a mutation was found in 58% of investigated cases. According to Bamshad [114], mutations in *MYH3* have been found in approximately 90% of DA2A, and 40% of DA2B cases, and mutations in *TNNI2*, *TNNT3*, and *TPM2* have been found in DA1 and DA2B, suggesting variable expressivity of the same syndrome. Other sarcomeric protein genes involved in DA are *MYH8* in DA7, where a single missense mutation has been found in studied families [22], and *MYH2* and *MYH13* found in two families with DA5 (DA with ophtalmoplegia and ptosis). Both *MYH2* and *MYH13* are expressed in skeletal and extraocular muscles [114].

We identified mutations in *TNNI2* in nine cases with DA1 and DA2B, a *TPM2* mutation in two cases with DA2B, and *MYH3* mutations in nine DA1, one DA2A and three DA2B cases.

Muscle morphologic findings and muscle weakness
Muscle biopsy performed in four affected adults with a *TNNI2*-mutation revealed mild myopathic changes. There was an increased variability of fiber size, frequent internalized nuclei, and signs of muscle fiber regeneration and increased interstitial connective tissue in some cases. The structural changes and internalized nuclei were mainly confined to type 2 muscle fibers, which express the mutant TnI isoform. Muscle weakness was not found in these four patients, but in the oldest patient, who also had the most prominent muscle morphologic findings, muscle fatigue was described.

Histochemical and ultrastructural investigation of muscle biopsies in the two affected individuals with *TPM2*-mutation revealed type 1 fiber predominance but no other major morphologic abnormalities. Immunohistochemistry of myosin isoforms also demonstrated a marked predominance of muscle fibers expressing slow myosin. Muscle weakness was found in both patients.

Muscle biopsy specimens in affected patients with *MYH3*-mutations, patients 1 and 2 (paper IV) exhibited increased variability of fiber size owing to the presence of frequent small type-1 fibers, and slightly abnormal type-1 fiber predominance. These two patients both had muscle weakness. A muscle biopsy specimen from patient 3 (paper IV) showed scattered, small, type-1 fibers but no obvious pathologic changes. This patient had no muscle weakness. Muscle biopsy specimens from patient 4 (paper IV) showed slight pathological changes; at age 15 months there were numerous fibers (>20% of all fibers) expressing the fetal (perinatal) isoform of MyHC (MYH8), and at age five years there was type-1 fiber predominance and scattered small type-1 fibers. In this patient, muscle weakness was also found.

Clinical involvement in individuals with DA and *MYH3*-mutations is highly variable, from very mild or no joint involvement to severe contractures. DA associated with *MYH3* mutations is secondary to myosin myopathy, and postnatal muscle manifestations are variable.

Mutations in the same gene were found in different DA syndromes, suggesting varying clinical penetrance and expression rather than separate genetic syndromes and, *vice versa*, different gene mutations were found in the same clinical syndrome suggesting multiple gene background.
DA has previously been defined as an inherited primary limb malformation disorder characterized by congenital contractures of two or more different body areas and without primary neurologic and/or muscle disease that affects limb function [7].

We suggest that the present classification of DA syndromes could be modified, and DA syndromes re-defined according to pathogenic findings, *i. e.* sarcomeric protein dysfunction, connective tissue dysfunction or other pathology. DA1, DA2B,Sheldon Hall syndrome, and DA2A, Freeman Sheldon syndrome, may then be regarded as related syndromes with fetal sarcomeric myopathy of increasing severity. At least DA5 and DA7 also belong to this group of DA syndromes caused by sarcomeric protein dysfunction. In DA8, mutations both in *TPM2* and in acetylcholinreceptor genes have been described, and DA9 is known to be caused by a mutation in *FBN2*, and so should rather be regarded as a collagen dysfunction disorder.

Molecular genetic and muscle pathology investigations, in addition to careful clinical evaluation including evaluation of muscle strength and joint involvement, are of importance in patients with DA syndromes in order to further define and understand these disorders.

General discussion

Epidemiology

The birth prevalence of multiple congenital contractures was found to be 1/5100 live born children in an epidemiological study from western Sweden [49.] Based on this study, there should be approximately 1800 individuals with arthrogryposis in Sweden, 400 of which should be individuals of 18 years and younger, with a present total population of 9.3 million and 2.05 million of 18 years and under. However, there is an increased mortality in many syndromes with arthrogryposis, especially those with CNS-involvement, so the actual number of present cases can be assumed to be lower. In our study of arthrogryposis primarily in children and adolescents, we investigated 131 index cases, with a further 13 affected relatives. It can be assumed that we saw a maximum of 1/3 of all present cases in children and adolescents in Sweden. The reasons for "missed" cases are several: first, not all cases are registered at the local rehabilitation center; second, not all cases are registered under the diagnosis of arthrogryposis; third, not all families who had children with arthrogryposis were considered suitable to partake in the study at the present time by the local rehabilitation professionals and therefore not asked; and fourth, some families declined

participation in the study. We also assume that some of the cases with mild joint involvement may not have any medical contacts at all, or sporadic contact with an orthopedic/hand surgeon. The children who were not identified as having the diagnosis of arthrogryposis in spite of the presence of multiple congenital contractures were mainly those who had a diagnosis of congenital myopathy/muscle dystrophy or diagnosed genetic syndromes/malformation syndromes. Children with myelomeningocele were not included in the study. These children often have arthrogryposis in the lower extremities, but as a group they are well defined and described in other studies, and the causes are well known.

Classification

The majority of cases investigated did not have a specific diagnosis other than arthrogryposis at the time of investigation. In some cases, further medical investigations such as muscle biopsy or radiology investigations were needed to clarify the diagnosis, but in many cases careful clinical evaluation by professionals with experience in evaluation of children with arthrogryposis was sufficient. Both in amyoplasia and in DA syndromes, as in many types of arthrogryposis, diagnosis is based on clinical evaluation. An evaluation by a clinical geneticist was also invaluable in a number of cases, mainly those with specific malformation syndromes or dysmorphic features. Our conclusion is that a multidisciplinary evaluation by an experienced arthrogryposis team is needed for a correct diagnosis in the majority of cases.

The relatively high proportion of amyoplasia/probable amyoplasia (37%) and distal arthrogrypos (21%) patients in our study is probably due to the fact that these are the most typical arthrogryposis diagnoses, while other causes may not be as obvious. Intellectual abilities are generally not affected in these syndromes, but in the arthrogryposis group as a whole mental retardation is present in approximately 25% of all subjects [52]. In our study mental retardation/CNS involvement was present in at least 16% (at least two more patients had a suspected mental retardation, but were too young to be evaluated). This is a lower frequency than has previously been reported, probably due to the described bias in reported patients.

Assessment

There is a need for expert teams with clinical experience of diagnostics and treatment in arthrogryposis. There are many different syndromes, often rare disorders, the clinical picture can be complicated, and arthrogryposis often leads to severe disabilities. A correct diagnosis is important in order to give a relevant prognosis, inform about possible risk of recurrence, and to plan treatment. The first contact should be as early as possible, preferably already in the neonatal ward. Repeated functional assessments are often necessary. Early treatment should be initiated, avoiding immobilisation as far as possible. Treatment must always be planned in close collaboration with the family and the local rehabilitation team. A multidisciplinary approach is needed, with pediatric neurologist, clinical geneticist, orthopedic surgeon, hand/plastic surgeon, physical therapist, occupational therapist, and orthopedic technician. Other specialists that are often involved are neonatologist

for the newborn, dentist, nutritionist, and others. Regional referral teams may be a solution, even though the patient organisation advocates a national competence center for arthrogryposis.

Aspects of treatment

Information about given treatment was difficult to evaluate and validate. For the majority of cases, a specific diagnosis was not available prior to our investigation, so in planning treatment the underlying cause of arthrogryposis in the individual child was not known. Since joint contractures in amyoplasia have been found to be more resistant to treatment than in DA [50, 65] this information is necessary to compare and evaluate treatment results.

Given treatment was also difficult to recapitulate: The amount of actual stretching (how many times a day, which joints, for how long time, etc) was almost impossible to assess, as documentation and parents recollection could differ considerably. The same was true regarding splinting, were prescribed splints where not always used, at least not as assumed in the medical records. Records of casting and actual orthopaedic surgical interventions were easier to collect from medical charts, but even then it was sometimes difficult to find the duration of casting and also the exact surgical procedure performed. The results of treatment given was also difficult to evaluate in retrospect, as the opinion of the orthopaedic surgeon, the physiotherapist, the parents and, in cases where the child was old enough, the patient could differ considerably.

Functional outcome is the most important aspect of treatment. In our study of amyoplasia, we found that muscle strength is more important than severity of joint contractures for the ability to move/walk. In amyoplasia, muscles are absent or severely dysplastic due to anterior cell dysplasia [15]. Usually, not all muscle groups are involved, and commonly muscles in the trunk, neck and face are spared. It has been assumed that the underdeveloped muscles do not regenerate, but one recent study has shown increase of muscle mass as demonstrated with MRI studies of muscle in one child in infancy and again at five years of age [55]. This finding has important implications for treatment and underscores the importance of stimulating muscle use and activity even in very weak muscles in amyoplasia. Ultrasound or MRI investigation of muscle in the neonatal period should be useful to evaluate the possibilites for future muscle strength development and to predict results of treatment.

Early intensive treatment (stretching and splinting) has been found to be especially important in amyoplasia. Casting, especially during the first three or four months, should be minimized [5,50]. In planning surgery, it is essential to carefully assess muscle bulk and muscle function to make sure the planned intervention does not impair future motor abilities.

Problems with muscle pain and increasing muscle fatigue have been described by adults with amyoplasia and DA. The symptoms described are similar to those found

in post-polio syndrome, with increasing symptoms in older patients due to lack of reserve capacity in the muscles.

In DA, the pathogenic background is different. In the most commonly occurring DA syndromes, DA1 and DA2B with the present nomenclature, a "new" kind of myopathy with defects in sarcomeric proteins affecting the contractile function in striated skeletal muscles, expressed mainly during fetal life, has been demonstrated. The muscle weakness resulting in congenital, mainly distal joint contractures is presumably at maximum during fetal life. This fetal myopathy can result in mild myopathic findings in adult muscle, and also in muscle weakness in adult life. There are, however, no signs of progressive myopathic symptoms in our investigated patients, other than increasing muscle fatigueability with increasing age. Congenital contractures in DA have been reported to be easier to correct than in amyoplasia [5], even though, in our study, we found individuals with severe joint involvement who had been subject to multiple surgical corrections. The implications for treatment of muscle findings in DA may be that care should be taken not to "over-exercise", in order to avoid pain and fatigue in muscles with increasing age, and in DA as well as in amyoplasia care should be taken not to cause additional muscle atrophy and weakness by immobilisation or muscle damage due to the surgical procedure when treating joint contractures.

Other aspects of treatment must also be taken into consideration. Jaw involvement is a frequent finding, both in amyoplasia and in DA. Feeding difficulties in infancy are common. Anesthesiological implications are difficulties with intubation. Malign hyperthermia is described in rare cases of arthrogryposis [70]. As children with arthrogryposis are often subject to repeated surgical treatment, care must be taken with pain management and to avoid painful procedures as far as possible. Other than postoperative pain, pain found in children with arthrogryposis in our study was related to multiple surgeries, especially in the feet. The general development and wellbeing of the child must also be considered in planning treatment. The following parameters have been found to be important in preparing for an independent adult life in individuals with physical disabilities, in order of importance: 1: Communication skills. 2: Activities of daily living, 3:Mobility, and 4:Walking [50]. Consequently, time for play and interaction with peers are at least as important as treatment and training to achieve good motor function in the overall development of the child.

In summary

A correct specific diagnosis should be aimed for. Early physical therapy with stretching of joint and muscle contractures is important, and in the first 3-4 months of life especially rewarding. Active muscle use should be promoted, and immobilisation should be avoided as far as possible. Assessment of muscle strength and motor function should be done before surgery. It is important to care for pain management and avoid painful procedures, since these children are often subject to repeated surgical interventions.

CONCLUSIONS

A classification of the investigated patients with arthrogryposis into different specific diagnoses identified the three largest groups, in decreasing order, as amyoplasia, distal arthrogryposis syndromes and arthrogryposis with CNS-involvement.

In amyoplasia, muscle strength was reduced in the majority of patients. The extent of muscle involvement varied between individuals and also between muscle groups in the individual patient. Ultrasound or MRI of muscle tissue in the newborn period would be useful to evaluate prerequisites for future development of muscle strength and motor function, and thereby anticipate response to therapy.

Muscle function in amyoplasia is more important than severity of joint contractures for the prediction of walking ability and functional level. Joint positions at birth are also of importance, especially for the prediction of ambulatory function. We conclude that more attention should be paid to development of muscle strength with early stimulation of active movements. Immobilisation should be minimized.

In distal arthrogryposis, pathogenic mutations in the same gene were found in different clinical syndromes, implying that these syndromes are expressions of varying clinical penetrance and expression of the found mutation rather than separate genetic syndromes. Different gene mutations were also found in the same clinical syndrome, suggesting multiple gene background. We conclude that DA syndromes are clinically and genetically heterogenous conditions.

A pathogenic mutation is more often found in familial than in sporadically occuring cases of DA, but de novo mutations are not uncommon.

In DA1, 2A and 2B pathogenic mutations were found in TNNI2, TPM2 and MYH3. Troponin, tropomyosin and myosin are sarcomeric proteins important for muscle contraction. We conclude that these DA syndromes are caused by fetal myopathy due to sarcomeric protein dysfunction causing muscle weakness and secondary joint contractures, and that DA syndromes may be re-defined according to pathogenic background.

In studying treatment of arthrogryposis, our main conclusion is that a careful clinical evaluation by a team with experience in arthrogryposis as early as possible is important for correct diagnosis and planning of treatment, and that care should be taken to minimize immobilisation in order to avoid further muscle atrophy, especially during early life.

Acknowledgements

I would like to express my sincere gratitude and appreciation to:

All the children and families who participated in this study

Professor **Már Tulinius**, my supervisor, for his always patient and friendly supervision, scientific guidance, encouragement and support throughout this whole research project

Associate Professor **Gösta Blennow,** co-supervisor, for encouragement, support and inspiration in research and in the clinical practice of pediatric neurology, and for initiating my participation in this research project

Professor **Göran Annerén**, co-supervisor, for his enthusiasm and support, and for expert guidance in clinical genetics and dysmorphology

Anna-Karin Kroksmark, co-writer and co-investigator, for generously sharing scientific and clinical experience and for companionship in all our excursions during the clinical investigation

Homa Tajsharghi, co-writer and scientist, for expert work and friendly collaboration in the molecular genetic studies

Professor **Anders Oldfors** for constructive criticism and expert knowledge in muscle pathology

Associate Professor **Ragnar Jerre** for collaboration and expert opinions in evaluation of orthopaedic treatment

Associate Professor **Niklas Darin** for his epidemiologic work on multiple congenital contractures and for constructive criticism

Associate Professor **Daniel Holmgren** for cardiac investigations and evaluation

Associate professor **Eva Beckung** for collaboration on the work with amyoplasia

Ann-Charlotte Thuresson for collaboration in arrayCGH studies

Professionals at all the local **child rehabilitation clinics** for their assistance and hospitality

Sonja Högseth for sharing clinical experience and for practical assistance and cooperation

Ulrika Sterky and **Anette Ekberg** at ward 334 at The Queen Silvia Childrens Hospital for always efficient and cheerful assistance

Karin Marcusson and **Tora Hanssen** for proof reading and language corrections

Ann-Sofie Petersson and **Anna-Karin Larsson** for professional assistance with layout and illustrations

Barbro Westerberg at the Department of Womens and Childrens Health, Uppsala University for support and computer assistance

Associate Professor **Gunnar Ahlsten**, my present boss, for letting me have time off from clinical practice for research

Colleagues and **staff** at the department of Pediatric Neurology, the pediatric neurology ward 95B and at Uppsala University Childrens Hospital for daily clinical collaboration, friendship and support

The **Swedish AMC-association** and especially **Ulf Larsson** for cooperation and support

All my **family** and personal **friends**, none mentioned and none forgotten

Thank you!!!

This study was supported by grants from the National Board of Health and Welfare funding for small and lesser known handicap groups, the Linnéa and Josef Carlsson Foundation, The National Association for Disabled Children and Youths (RBU), the Majblomman Foundation and the Folke Bernadotte Foundation.

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