INVISIBLE FOOTPRINTS

A JOURNEY TO IMPROVE THE PRENATAL DETECTION OF LIMB ANOMALIES

ARDA ARDUÇ

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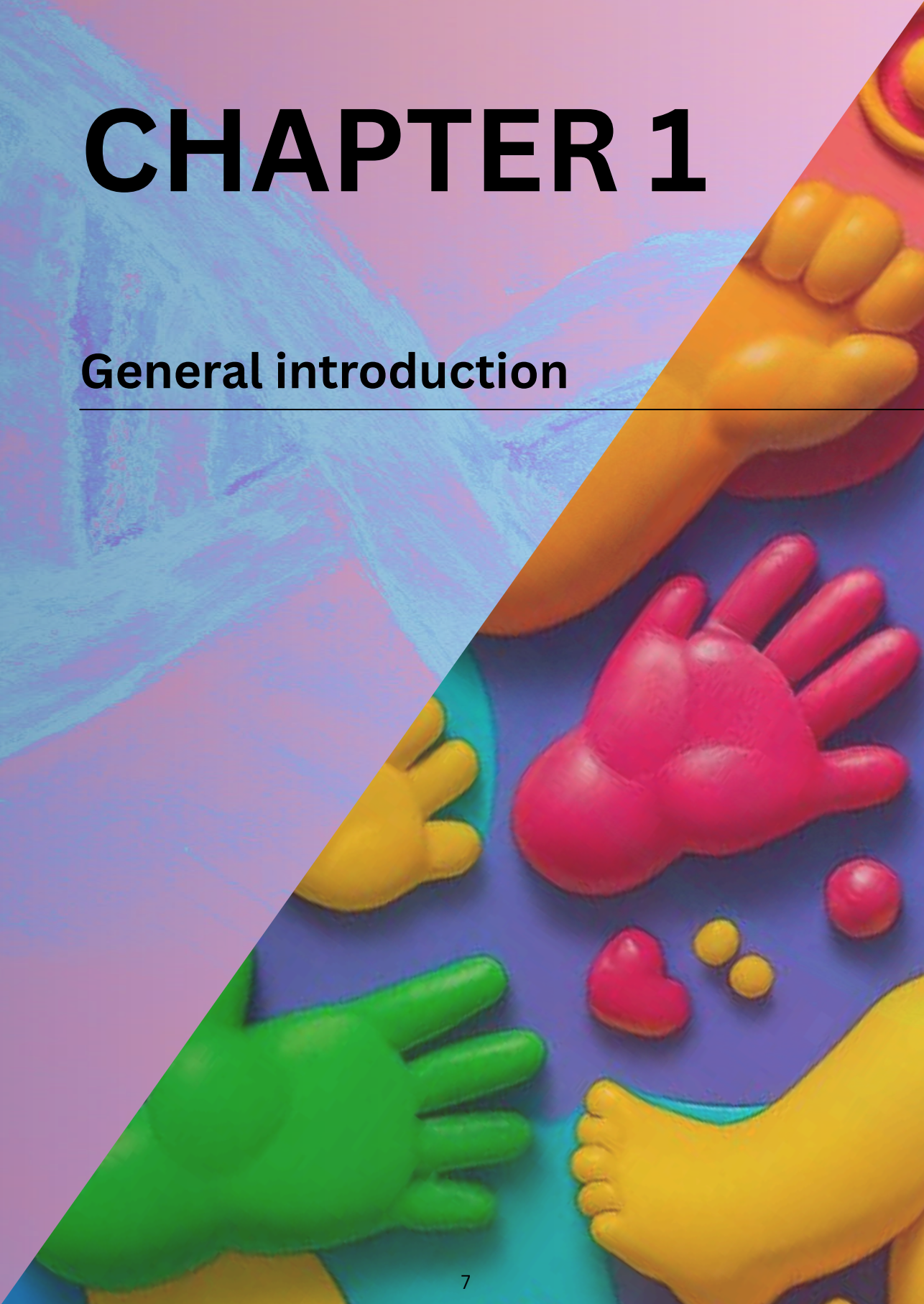
List of abbreviations

- AMC: arthrogryposis multiplex congenita
- AUMC: Amsterdam University Medical Center
- AER: Apical Ectodermal Ridge
- AI: Artificial Intelligence
- CMA: Chromosomal Microarray Analysis
- CNV: Copy Number Variant
- CNS: Central Nervous System
- DAIPT: Distal Arthrogryposis with Impaired Proprioception and Touch
- DNA: Deoxyribonucleic Acid
- ES: Exome Sequencing
- EPIC: Electronic Portfolio of International Credentials (EPD-systeem)
- EUROCAT: European Surveillance of Congenital Anomalies
- FADS: Fetal Akinesia Deformation Sequence
- FISH: Fluorescence In Situ Hybridization
- FMU: Fetal Medicine Unit
- FTAS: First-Trimester Anomaly Scan
- HPO: Human Phenotype Ontology
- ISO: Isolated
- MECA: Maternal Experience of fetal movements from a Child with AMC
- MLPA: Multiplex Ligation-dependent Probe Amplification
- MRI: Magnetic Resonance Imaging
- NISO: Non-isolated
- NGS: Next-Generation Sequencing
- NICU: Neonatal Intensive Care Unit
- NIPT: Non-Invasive Prenatal Test
- NVOG: Dutch Society of Obstetrics and Gynecology (Nederlandse Vereniging voor Obstetrie en Gynaecologie)
- OMIM: Online Mendelian Inheritance in Man
- OMT: Oberg–Manske–Tonkin (Classification for Upper Limb Anomalies)
- PRELLIM: Prenatal lower limb impairment
- PRISMA-ScR: Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews
- SPSS: Statistical Package for the Social Sciences
- QF-PCR: Quantitative Fluorescent Polymerase Chain Reaction
- SNV: Single Nucleotide Variant
- STAS: Second-Trimester Anomaly Scan
- TOP: Termination of Pregnancy
- UMC: University Medical Center
- VUmc: VU University Medical Center
- VUS: Variant of Uncertain Significance
- WES: Whole Exome Sequencing
- WGS: Whole Genome Sequencing
- 2D: Two-dimensional
- 3D: Three-dimensional

CHAPTER 1

General introduction



The unseen anomaly

At the routine 20-week ultrasound examination, my patient was reassured to hear that no structural anomalies were visible at that time. Like many expectant mothers, she hoped for a normal outcome and relied on the scan to provide reassurance. Yet, after birth, it became clear that her child was born with an unexpected abnormality: several fingers were missing from one hand, a congenital transverse upper limb reduction defect.

Following delivery, a multidisciplinary Hand Team thoroughly assessed the child, and genetic testing was initiated to investigate possible underlying causes. In the aftermath of this unexpected finding, several important questions emerged, both from the parents and from myself:

- Could this anomaly reasonably have been detected during the routine 20-week ultrasound examination, given current imaging capabilities and standards?
- What is the likelihood of identifying an underlying genetic cause or syndromic set of symptoms in a case like this, and how should further investigations be guided?
- How should prenatal counseling address the limitations and uncertainties inherent in routine anomaly screening, particularly for subtle or rare abnormalities?

These questions underscore the theme of this thesis: the challenges and opportunities in the prenatal detection of limb anomalies. While great strides have been made in fetal imaging over the past few decades, certain structural abnormalities remain difficult to identify prenatally, for example limb anomalies.

Background

Congenital anomalies are observed in approximately 2.5% of all newborns, with limb anomalies being among the commonest subtypes (1-4). According to the European Surveillance of Congenital Anomalies (EUROCAT), the overall prevalence of limb anomalies is 45 per 10,000 births with an upper-to-lower-limb ratio of 2:1 (1-5).

While the sensitivity of prenatal ultrasound is relatively high for major anomalies involving the entire upper limb, with reported detection rates ranging from 70% to 100%, the sensitivity markedly decreases for more subtle abnormalities, such as digital anomalies (i.e., anomalies involving the fingers or toes)(6). Detection rates for digital anomalies remain low, estimated between 4% and 19% according to recent studies (6).

For lower limb anomalies, detection rates have also improved considerably over time, particularly for more easily recognizable conditions. Clubfoot, a contracture of the ankle, represents the most commonly observed lower limb anomaly at birth and serves as a prime example of a condition that is now frequently detected prenatally. Offerdal and colleagues demonstrated a significant increase in the prenatal detection of isolated clubfoot, from 23% during the period 1987–1992 to 81% between 1999–2004 (7). Subsequent advances in imaging technology, increased awareness, and systematic screening approaches have further improved detection rates, with studies reporting rates approaching 90% (8).

However, the prenatal identification of more complex or generalized limb abnormalities, such as multiple joint contractures as seen in arthrogryposis multiplex congenita (AMC), remains more variable. Globally, the reported detection rates for AMC range from 26% to 53%, depending on the study and population evaluated (9-11). Nevertheless, when a pregnancy is assessed in a specialized fetal medicine center that utilize serial ultrasound examinations, detailed fetal movement assessments, and multidisciplinary team evaluations, the sensitivity for detecting AMC can approach nearly 100% (12).

Following the identification of a limb anomaly, the possibility of an underlying genetic or syndromic etiology must be systematically considered. While many cases of limb anomalies occur as isolated anomalies, a proportion is associated with syndromes, genetic abnormalities, environmental influences, or still unknown causes. The likelihood of non-isolated limb anomaly increases substantially when targeted ultrasound examination reveals additional anomalies, or when there is a positive family history on limb anomalies or history on possible external influences. Parents should receive counseling on updated genetic testing possibilities tailored to the found anomalies as part of the prenatal counseling. This will enhance the chance for the prenatal diagnosis and thus implications for prognosis, recurrence risk assessment, and multidisciplinary care planning.

A multidisciplinary team approach is crucial to integrate knowledge, and to enhance awareness of the variable timing, pathogenesis, and phenotypic variability inherent to these conditions. Such an approach aims to provide parents with the most targeted counseling possible, based on the available phenotypic and genotypic information after ultrasound and genetic examinations. A thorough understanding of normal fetal musculoskeletal and central nervous system development is necessary to differentiate physiological variants in phenotypic expressions from true pathological findings. Classification systems for anomalies affecting the upper extremities, lower extremities, or both serve to further refine the prenatal detection and inform appropriate (follow-up) investigations.

Skeleton and limb development

A comprehensive understanding of normal embryonic and fetal skeletal development is essential for evaluating the possibilities and limitations of prenatal imaging in detecting limb anomalies. In particular, insight into the timing and nature of skeletal and limb formation is crucial to assess at what stages prenatal ultrasound examination, whether in the first or second trimester, may realistically contribute to anomaly detection.

Early limb development and genetic regulation

The development of the limb buds is orchestrated by complex interactions between genetic and molecular signals. A key position in this interaction is the tuning between the Hox gene clusters, that regulate positional identity, and retinoic acid signaling, which plays a critical role in the initiation of limb bud formation (13). Limb development follows a precise chronological pattern. The upper limb buds emerge as lateral swellings on the embryonic body wall around day 26 postconceptional age, followed shortly thereafter by the lower limb buds at approximately day 28 postconceptional age (14).

Histologically, the early limb bud consists of a mesenchymal core derived from the lateral plate mesoderm, covered by ectoderm. The apical ectodermal ridge (AER), a thickened layer at the distal tip of the limb bud, forms shortly after bud emergence and is essential for proximodistal outgrowth. The interaction between the AER and the underlying mesenchyme regulates the growth and patterning of the limbs (14).

Milestones in limb differentiation and skeletal ossification

The skeletal elements of the limbs develop progressively through a series of well-defined stages (14):

- Week 5 postconceptional age: Mesenchymal condensations appear within the limb buds, marking the early organization of future skeletal structures.
- Week 6: Cartilage models form through chondrification, providing a blueprint for future bones.
- Weeks 7–8: The first ossification centers emerge, and the process of endochondral ossification (conversion of cartilage to bone) begins.

These stages have been described using a variety of techniques, including histological analysis of deceased embryos, classical anatomical studies, and more recently, high-resolution imaging technologies such as phase-contrast X-ray computed tomography (14,15).

Rotation and positioning of the limbs

By the end of the embryonic period (postconceptional week 8), the limbs undergo characteristic rotations:

- The upper limbs rotate laterally so that the extensor muscles face posteriorly.
- The lower limbs rotate medially, resulting in the extensor muscles facing anteriorly.

This coordinated rotation is essential for establishing the normal anatomical orientation of the limbs (Figure 1).

Influences on limb development

Genetic disruptions, environmental exposures, multifactorial, or unknown causes can interfere with the highly regulated processes of limb formation. The molecular signaling by retinoic acid signaling, for example, must be tightly regulated; experimental models showed that both an excess and a deficiency of retinoic acid can lead to severe limb abnormalities, including limb reduction defects and duplications (13).

Development of bones and joints

Following the initial formation of skeletal elements, joints develop by cavitation within the mesenchymal condensations (16):

- Week 7: Interzones of dense mesenchyme appear at future joint sites, initiating joint development.
- Week 8 onwards: Articular cavities form, and the first features of joint mobility are established.

By the end of the embryonic period, the fetal skeleton demonstrates a recognizable posture, and the foundations of all major skeletal structures are established. Normal posture and joint movement are critical indirect indicators of normal skeletal development.

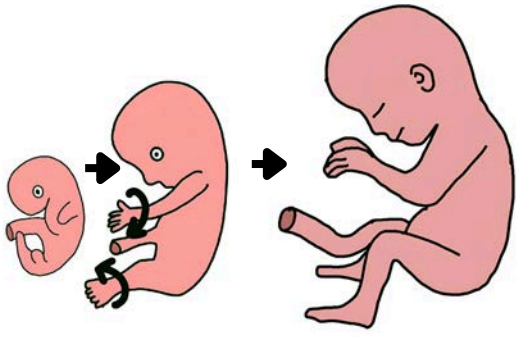


Figure 1 Key milestones in the embryonic and fetal development of the limbs. This schematic picture was designed by Arda Arduç.

Development of neuromuscular system

In addition to the skeletal framework, the development of the neuromuscular system, soft tissues (including muscles, tendons, and connective tissue), and skin plays a critical role in the formation and function of the limbs. Proper interaction among these tissues is essential not only for achieving correct anatomical structures but also for enabling fetal movements, posture, and functional integrity.

Neuromuscular development begins early in gestation and is tightly coordinated with skeletal maturation. One of the earliest indicators of neuromuscular integrity is the appearance of spontaneous embryonic and fetal movements. These movements are not merely a sign of activity but are essential for normal development of the musculoskeletal and neurological systems (17).

The first observable embryonic movements occur at 7 weeks of gestational (postmenstrual) age (17). Initially, these movements consist of regular, low-amplitude lateral flexions of the head and trunk, and later extend including limb movements, all characterized by small and slow motions in one direction(18). These early movements are believed to result from spontaneous activity from spine and brainstem before cortical control is established (18).

By 9 weeks of gestational age, general movements emerge (17). These movements are characterized by variable amplitude, speed, direction and the participation of all body parts, including the trunk, head, and all four limbs. General movements are the most complex performed movement patterns, involving a continuous fluent flow of motion with changing intensity, reflecting the maturing coordination between muscles, nerves and central nervous system including supraspinal influences.

Importantly, general movements persist throughout fetal life and are recognized as a hallmark of neurological function not only during gestation but also postnatally, up to approximately three months of age after a term birth. Abnormalities in the quality or presence of general movements in children at three months corrected for term age have been shown to correlate with later neurological impairments, emphasizing their clinical significance for early detection of developmental disorders (18).

Development of soft tissues and skin

Parallel to neuromuscular maturation, the limb buds also undergo critical development of soft tissues and skin (15). Muscle formation begins with the migration of myogenic precursor cells into the limb bud mesenchyme by the fifth week of development, where they differentiate into myoblasts and eventually form organized muscle masses. Tendons and ligaments derive from the lateral plate mesoderm and become distinguishable as separate connective tissue structures by the seventh week (13). Skin formation involves the proliferation and stratification of the ectoderm, with epidermal stratification becoming apparent by the end of the first trimester (19).

Together, the coordinated development of the skeletal, neuromuscular, connective tissue, and cutaneous systems shapes the final anatomical and functional architecture of the fetal limbs. Disturbances in any of these components can result in structural anomalies or functional impairments.

Etiology of limb anomalies

Congenital limb anomalies arise from disruptions of normal embryonic development, caused by heterogeneous factors (20). A thorough understanding of the etiological background of these anomalies is critical for accurate prenatal diagnosis, effective counseling of parents, and realistic prognostication (21). Genetic factors play a major role in the development of limb anomalies (21). For instance, Holt-Oram syndrome is characterized by upper limb defects in combination with congenital heart disease, while Fanconi anemia presents with upper limb reduction defects alongside hematologic abnormalities (21). These findings highlight the importance of considering a genetic cause even when limb anomalies occur in isolation.

Environmental factors can also disrupt normal limb development. A classic example is the amniotic band syndrome, where fibrous strands of amniotic tissue entangle fetal limbs, resulting in constrictions, amputations, or other severe deformities (22). Exposure to certain medications during pregnancy, notably thalidomide (Softenon®), led to a well-documented epidemic of limb reduction defects in the mid-20th century (23). Maternal hyperthermia during early pregnancy has also been associated with an increased risk of limb defects, likely due to its disruptive effects on critical developmental processes (24).

The timing of the disruption plays a crucial role in determining the type of anomaly observed. Disruptions occurring during the early embryonic period, between four and eight weeks post conception, when limb buds are forming and differentiating, typically result in major reduction defects, such as transverse or longitudinal reduction defects. Transverse reduction defects involve the absence of limb structures beyond a certain level, resembling an amputation, while longitudinal deficiencies affect structures along the limb's length, such as the radius, fibula, or specific digits. In contrast, anomalies that involve secondary effects due to limited joint movements, such as joint contractures seen in conditions like AMC, emerge depending on the underlying cause from first through third trimester of pregnancy (25,26).

Despite advances in prenatal imaging and genetic technologies, a significant proportion of congenital limb anomalies' etiology remains unexplained. This persistent gap in knowledge underscores the complexity of limb development and the multifactorial nature of its disruption.

Postnatal classification of limb anomalies

Accurate classification of congenital limb anomalies is fundamental for guiding diagnosis, prognosis, genetic evaluation, and parental counseling. Moreover, precise classification, especially when combined with family history and information about environmental exposures, can facilitate the discovery of new underlying causes of these anomalies, as emphasized in international surveillance programs such as EUROCAT which systematically register all major congenital anomalies in Europe and monitor trends, potential risk factors, and regional differences (27).

Most established classification systems for limb anomalies have been developed based on postnatal assessments. Among the most commonly referenced are the Swanson classification, the Frantz and O'Rahilly system, the Oberg-Manske-Tonken (OMT classification), and the classification schemes used by EUROCAT (27-30). Each system has contributed to standardizing diagnostic categories, improving clinical communication, and enabling research across different centers. However, significant differences exist in terminology, definitions, and subdivisions among these systems.

Each of these classifications highlights different aspects of congenital limb anomalies. For instance, Swanson's system incorporates embryological development, distinguishing between failures of formation, differentiation, and duplication, while Frantz and O'Rahilly's system focuses primarily on anatomical descriptions without referencing developmental pathways (28-30). The EUROCAT classification further refines limb defects by separating limb reduction defects into transverse and longitudinal types, with longitudinal defects subdivided into preaxial, postaxial, central, and intercalary types (27).

Specific conditions such as clubfoot (talipes equinovarus) and AMC have dedicated postnatal classification systems. For clubfoot, the Pirani score and the Dimeglio classification are widely used to assess severity based on anatomical deformity and flexibility, guiding treatment strategies (31,32). For AMC, Hall's system provides amongst others one type based on the phenotypic expression with extent and type of contractures and associated anomalies (33). In addition, the Bamshad classification for AMC provides a more genetically oriented framework (34).

Limitations in prenatal classification

Despite these comprehensive postnatal systems, there is currently no fully standardized classification specifically applied to prenatal ultrasound. Applying a postnatal classification directly to prenatal findings is challenging, because not all features assessed postnatally are visible or evaluable before birth.

Prenatally, detection begins through routine planned ultrasound examinations, which vary by country in timing and extend of ultrasound examination. Ultrasound assessment of fetal limbs requires extensive training and expertise to systematically evaluate limb presence and morphology (35). However, certain characteristics, such as minor skin anomalies (e.g., skin dimples or polydactyly), subtle hand deformities, or detailed neurological function, cannot always be reliably examined before birth. Furthermore, other factors can influence the prenatal detection of anomalies (36,37):

- Gestational age: earlier scans (first trimester) focus primarily on major structural integrity, while detailed limb assessments are often reserved for second-trimester scans.
- Detailed visualization of limbs or digits can be obscured by fetal positioning, multiple fetuses, maternal obesity, placental localization and amniotic fluid.
- Operator experience: the sonographer's expertise significantly affects the ability to detect and classify anomalies accurately.

Without a prenatal classification, adapted to the realistic capabilities of ultrasound imaging, clinicians may apply inconsistent terminology, leading to confusion in clinical reports, prognostic estimations, and parental counseling. Thus, while efforts are ongoing, there remains a need for standardized, prenatal-specific classification systems that align with postnatal frameworks which can be detected with imaging and genetic modalities.

1. Prenatal imaging

Ultrasound remains the cornerstone of prenatal limb anomaly detection and assessment. Advances in two-dimensional (2D) and three-dimensional (3D) ultrasound technologies, and to a lesser extent, magnetic resonance imaging (MRI), have markedly enhanced our ability to visualize fetal structures. However, significant challenges persist in distinguishing between normal developmental variations and clinically relevant anomalies.

In the Netherlands, prenatal screening is offered to all pregnant women at standardized timepoints within the prenatal care. These examinations include currently (35,39,40-42):

- Non-invasive prenatal testing (NIPT) can be performed after 10 weeks of gestation to screen not only for common chromosomal abnormalities (trisomies 13, 18, and 21) but, since 2023, also for large structural chromosomal abnormalities in which parts of a chromosome are duplicated or missing.
- First-trimester anomaly scan (12+3 to 14+3 weeks) (39,42): the introduction of first-trimester fetal anomaly scanning in the Netherlands, initially within a research setting in 2021, aimed to facilitate earlier detection of severe structural anomalies compared to the traditional 20-week scan. Severe limb anomalies, such as transverse limb reduction defects or major skeletal malformations, may already be identifiable at this stage. The protocol includes 2D visualization of both upper and lower extremities, including assessment of the presence of both hands and both feet.
- Second-trimester anomaly scan (18–21 weeks) (35,40): introduced nationally in 2007 in the Netherlands, the second-trimester fetal anomaly scan represents the standard moment for detailed structural assessment.

Assessment of the extremities includes evaluation of both arms and legs, as well as the presence and position of both hands and feet using 2D ultrasound.

International and national guidelines, notably from the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) and Dutch Society of Obstetrics and Gynecology (NVOG), provide standardized protocols for fetal limb assessment (35,39,41,42). The Dutch prenatal healthcare system facilitates universal access to fetal anomaly scans at primary or secondary care centers. Suspected anomalies lead to referral to one of the seven fetal medicine units (FMUs) for advanced targeted ultrasound evaluation, in 2D and/or 3D, and multidisciplinary counseling. Invasive genetic testing, including chorionic villus sampling (after 11 weeks) and amniocentesis (after 16 weeks), is offered where indicated and requested by the future parent(s) to rule out or confirm chromosomal or monogenic abnormalities.

Accurate prenatal assessment of fetal limbs depends heavily on the timing of the examination, given the sequential nature of limb development. Key milestones in limb visualization presented in postmenstrual age are as follows:

- By 9–10 weeks' gestation: limb buds with developing hand plates can be visualized. Discrete movement of arms and legs may also be seen on high-quality transvaginal ultrasound.
- By 11–12 weeks: the separation of individual digits can begin to be distinguished. Complete absence of a long bone can be noted or skeletal dysplasia.
- By 12 weeks: clubfoot-like positioning may be physiologic. Research by Cohen-Overbeek *et al*. using I-Space 3D imaging has demonstrated that clubfoot positioning can be a normal finding until approximately 12 weeks, emphasizing caution in early diagnosis (43).
- By 13–14 weeks: clear visualization of all limb segments (humerus, radius/ulna, femur, tibia/fibula) is generally possible. Gross postural abnormalities, persistent fixed flexion of for example the wrist, or absent limb segments can often be identified at this stage with high-quality ultrasound.
- Mid-trimester (by 18-21 weeks): detailed assessment of bone length, alignment, joint flexion/extension, and hand opening/closing movements can be systematically performed. At this stage, the distinction between normal and abnormal limb development becomes more reliable, forming the basis of most second-trimester anomaly scanning protocols.

2. Genetic testing

Advances in genetic testing have significantly enhanced our knowledge of congenital limb anomalies. The integration of molecular diagnostics into prenatal care now plays a critical role in elucidating the etiology of both isolated and syndromic limb malformations.

Historically, insights into the genetic regulation of limb development began with the discovery of the Hox gene clusters, which were recognized in the late 1970s and early 1980s as master regulators of embryonic body patterning (44). Hox genes determine the identity and positioning of limb segments.

The evolution of genetic diagnostic technologies has further improved genotype-phenotype correlations. Initially limited to karyotyping and single-gene testing, the field of genetics has rapidly evolved with the introduction of chromosomal microarray analysis (CMA), followed by next-generation sequencing (NGS) techniques such as exome sequencing (ES) (45-54). These advances have enabled the detection of both copy number variants (CNVs) and single nucleotide variants (SNVs), either through targeted panels or broader approaches, such as whole exome sequencing (WES). For both examinations trio sampling is essential, with the goal of comparing the fetal and parental genotype. Most recently, whole genome sequencing (WGS) has emerged as a comprehensive tool that captures both coding and non-coding regions of the genome (45-54).

A key advancement in clinical genetics has been the adoption of the Human Phenotype Ontology (HPO) framework (55). Careful and standardized phenotypic description using HPO terms allows for more effective matching of clinical presentations to known genetic disorders and optimizes bioinformatic analyses during exome or genome sequencing. In the prenatal setting, this concept becomes even more urgent. Serial ultrasound examinations can track evolving phenotypic features over time during gestation. The resulting phenotypic profile can ultimately be correlated with postnatal literature on phenotype-genotype associations (25,26). This dynamic phenotyping approach enables iterative refinement of the genetic differential diagnosis and guides more targeted molecular testing.

In the context of congenital limb anomalies, prenatal genetic evaluation follows a structured approach in our center:

- Initial step: quantitative fluorescent-polymerase chain reaction (QF-PCR) with or without a chromosomal microarray to exclude chromosomal aberrations (e.g., trisomy, (micro)deletion, or -duplication).

- Next steps: CNV and/or SNV analysis in exome sequencing (ES) data, either through targeted panels or WES. In selected cases, WGS may be considered to increase diagnostic yield, as it enables the detection of variants in non-coding regions and structural variants not captured by ES.

3. Counseling

The complexity of congenital limb anomalies necessitates a multidisciplinary approach to diagnosis, counseling, and management. Effective care requires collaboration among various specialists, including maternal-fetal medicine physicians, clinical geneticists, neonatologists, pediatric neurologists, pediatric orthopedic surgeons, plastic and reconstructive surgeons, and physiatrists. Each discipline provides complementary expertise, ensuring that both medical and functional aspects of the anomalies are thoroughly evaluated. Additionally, patient support organizations play an increasingly important role. These groups offer invaluable perspectives from affected individuals and contribute to research efforts through surveys, advocacy initiatives, and the sharing of lived experiences.

Parental counseling is an integral component of prenatal care for congenital anomalies. Early detection of limb anomalies has been shown to significantly impact parental preparedness, psychological adjustment, and decision-making processes(56). Parents who receive timely, structured information are better equipped to understand the prognosis, consider all available options, and prepare emotionally and practically for the birth of a child with a limb anomaly.

The outcome of congenital limb anomalies varies widely depending on the specific condition, severity, and presence of associated anomalies. For example, children born with isolated arm reduction defects generally exhibit remarkable adaptive capabilities. With early intervention and appropriate support, they are functionally capable of achieving independence in daily activities and often demonstrate excellent long-term outcomes (57).

In cases of clubfoot, the introduction of standardized care pathways, has led to consistently positive functional outcomes. The majority of cases, when treated promptly with the Ponseti method or similar approaches, result in good ambulatory function and quality of life (58).

The prognosis for AMC has also improved significantly over time. Recent research demonstrates that many individuals with AMC experience a high quality of life (59-68). Despite the physical challenges associated with joint contractures and limited range of motion, most women with AMC attain high education, engage in employment, maintain active social lives, and express the desire to start or expand their families.

These findings underscore the importance of not only addressing the functional implications of congenital anomalies but also recognizing the personal aspirations and reproductive health needs of affected individuals. Therefore, counseling must extend beyond prenatal diagnosis alone. It should also encompass guidance for women with pre-existing limb anomalies, particularly those with AMC, who seek information on pregnancy planning, maternal health risks, and fetal outcomes. These women may encounter specific challenges during pregnancy, such as reduced mobility, mechanical difficulties during labor, or associated comorbidities, necessitating careful multidisciplinary evaluation.

Scope and outline of this thesis

This thesis aims to enhance the prenatal detection of limb anomalies by improving prenatal imaging, expanding genetic diagnostics, and strengthening parental counseling and decision-making. It also seeks to optimize perinatal care for fetuses diagnosed with congenital limb anomalies. In addition, it addresses the specific needs and pregnancy-related concerns of women with AMC, thereby broadening the scope of fetal medicine to include maternal outcomes.

The work is organized into three interconnected parts (Figure 1):

- I. Improving the prenatal identification of fetal limb anomalies: exploring how the timing, technique, and interpretation of prenatal imaging can influence prenatal diagnosis.
- II. Understanding and improving the prenatal identification of contractures, in case of isolated (clubfoot) and multiple contractures (AMC).
- III. Understanding the etiology of AMC and advancing care for women with AMC, focusing on pregnancy guidance, maternal health management, and postpartum care.

To achieve these goals, this thesis utilizes a variety of methodological approaches, including retrospective and prospective cohort studies, systematic literature reviews, and questionnaire surveys. Data is drawn from both clinical research and collaboration with multidisciplinary specialist teams and patient support groups. Specific clinical teams involved in the management and counseling of patients within this thesis include:

- The Congenital Hand Team of Amsterdam UMC (multidisciplinary team for congenital hand anomalies), providing genetic, surgical, orthopedic, and rehabilitative expertise.
- The Amsterdam Clubfoot Center (Amsterdam Klompvoeten Centrum), specialized in the diagnosis, treatment, and follow-up of children with congenital clubfoot by pediatric orthopedic surgeons.
- The Amsterdam UMC Expertise Center for Fetal Akinesia Deformation Sequence (FADS) and AMC, providing interdisciplinary guidance for affected

individuals with contractures, from prenatal diagnosis to adult care. This multidisciplinary team includes fetal medicine specialists, clinical geneticists, pediatric neurologists, physiatrists, neonatologists, orthopedic surgeons, plastic surgeon, and a social worker.

Through these collaborations and approaches, this thesis seeks to provide a comprehensive view of congenital limb anomalies from the prenatal period into adulthood, bridging gaps between diagnosis, counseling, management, and long-term outcomes.

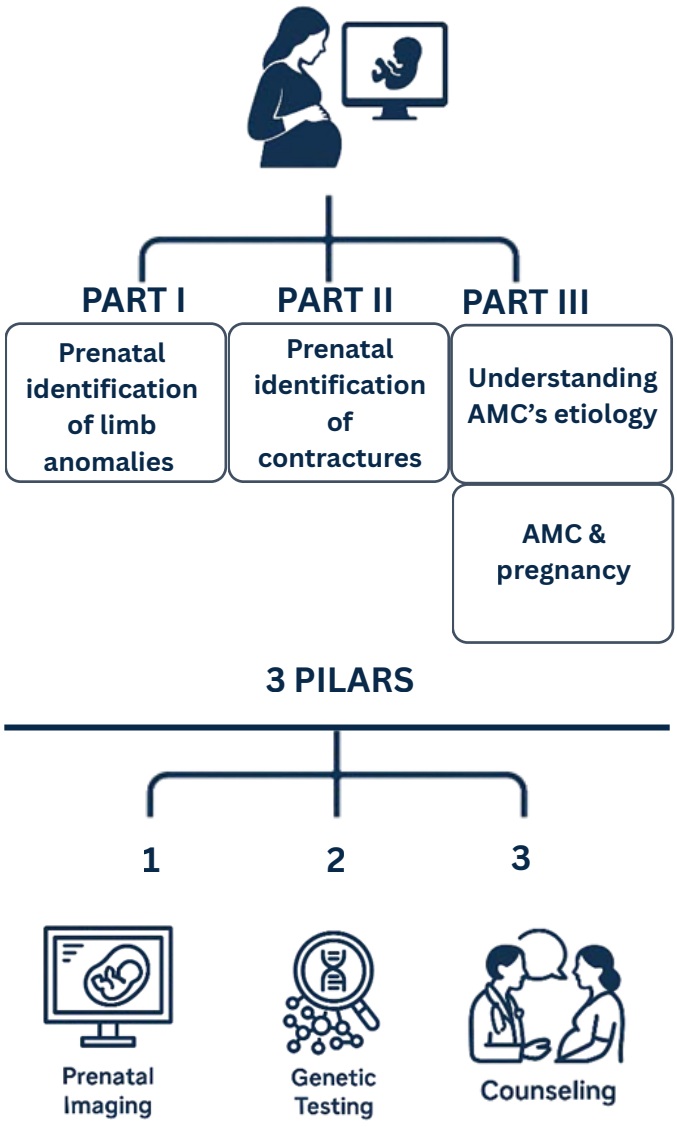


Figure 1. Three parts of this thesis and three pillars in prenatal detection of limb anomalies (prenatal imaging, genetic testing, and counseling).

Part I: Prenatal identification of limb anomalies

- Chapter 2 presents a retrospective cohort study analyzing congenital upper limb anomalies through a phenotype-to-genotype correlation, comparing prenatally and postnatally detected cases.

- Chapter 3 examines the impact of structural anomaly scans, counseling, and parental decision-making on the choice for termination of pregnancy, based on retrospective data.

- Chapter 4 introduces the PRELLIM classification, a newly developed prenatal classification system, designed to standardize the prenatal description and categorization of fetal lower limb anomalies.

Part II: Prenatal identification of contractures

- Chapter 5 focuses on the prenatal detection of clubfoot using structural anomaly scans, with a retrospective cohort study.

- Chapter 6 investigates the additional value of genetic testing in fetuses suspected of having isolated clubfoot, by a retrospective cohort study.

- Chapter 7 reports on the genetic diagnostic yield in a cohort of fetuses with AMC, analyzing the results of genetic testing over a 15-year period and providing insights into genetic testing strategies and evolving diagnostic practices.

Part III: arthrogryposis multiplex congenita & pregnancy

- Chapter 8 presents a scoping review of the literature on pregnancy outcomes in women with AMC, summarizing maternal, fetal, and delivery-related possibilities and challenges.

- Chapter 9 explores with an online questionnaire study the pregnancy-related experiences, complications, and needs among women with AMC.

- Chapter 10 evaluates in a questionnaire-based study the maternal perception of fetal movements in pregnancies affected by AMC.

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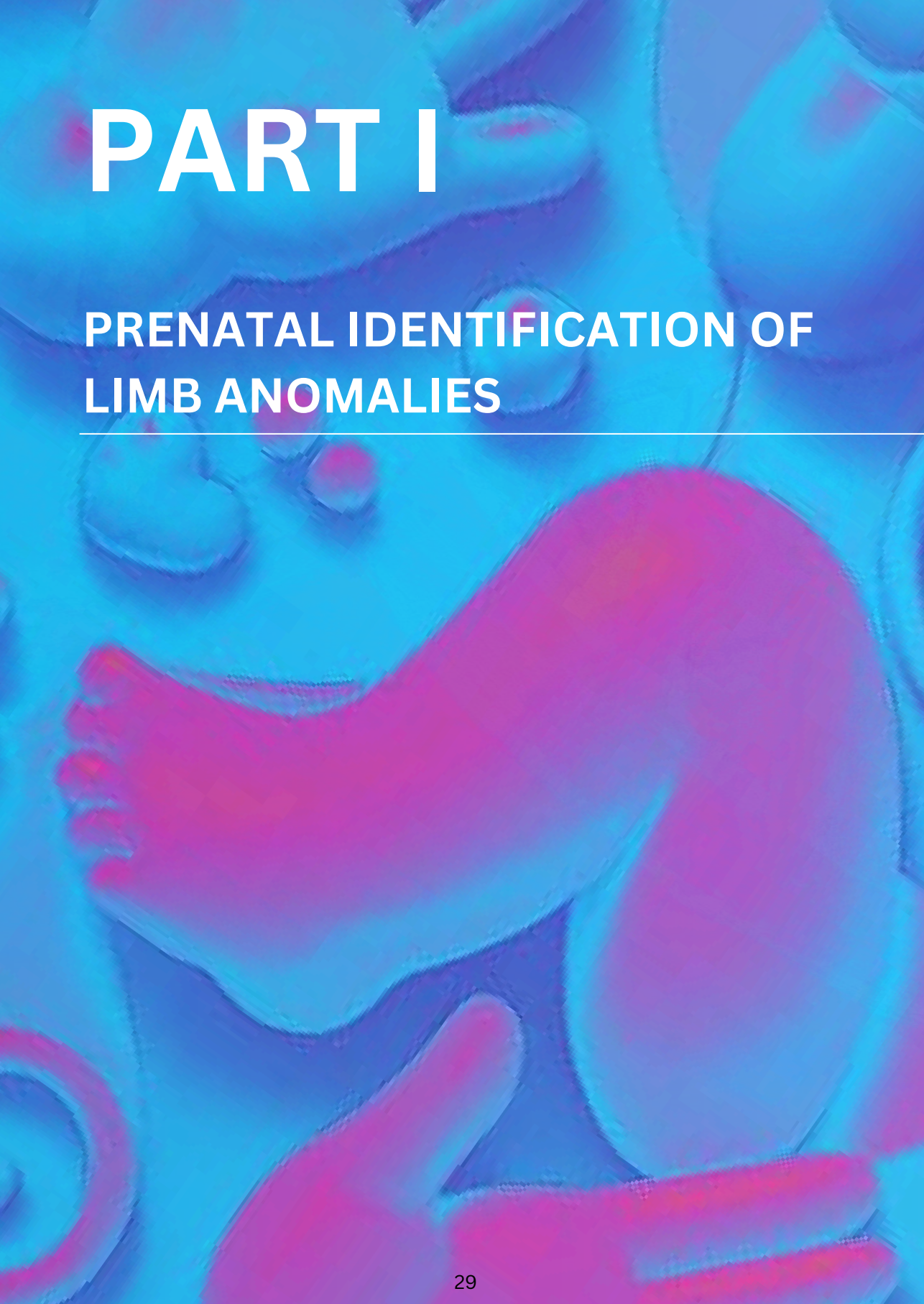
PART I

PRENATAL IDENTIFICATION OF LIMB ANOMALIES



CHAPTER 2

Phenotype-to-genotype description of prenatal suspected and postnatal discovered upper limb anomalies: a retrospective cohort study.

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Abstract

Objective To evaluate phenotype and genotype characteristics of fetuses and children with upper limb anomalies.

Method Retrospective cohort study of a prenatal and postnatal cohort with upper limb anomalies from January 2007 to December 2021 in a Fetal Medicine Unit. Prenatally on ultrasound suspected upper limb anomalies, such as transverse and longitudinal reduction defects, polydactyly, and syndactyly, and postnatally identified children referred to the Congenital Hand Team were evaluated separately.

Results The prenatal group included 199 pregnancies: 64 transverse and 19 longitudinal reduction defects, 103 polydactylies, and 13 cases with syndactyly. The majority of cases with longitudinal reduction defects (n=10, 52.6%), polydactyly (n=62, 60.2%), and syndactyly (n=10, 76.9%) were non-isolated, as opposed to transverse reduction defects which were generally isolated (n=41, 64.1%). The postnatal cohort included 362 children with upper limb anomalies with 49 transverse and 22 longitudinal reduction defects, 226 polydactylies, and 65 syndactylies. Chromosomal or monogenic abnormalities were identified in 76/199 (38.2%) cases of the prenatal cohort and in 31/362 (8.6%) cases of the postnatal cohort.

Conclusion Prenatal identification of minor defects of the digits holds a challenge, with more postnatal than prenatal cases. The majority of cases with isolated anomalies in both groups had no underlying chromosomal or monogenic cause, nor a were they associated with a syndrome, as compared to the non-isolated cases. Conducting structural anomaly scans and genetic counseling are crucial to assess the risk of genetic abnormalities.

Introduction

Congenital anomalies affect approximately 2.5% of all newborns (1). Limb anomalies of the upper and lower limbs are frequently observed, with an estimated prevalence of 39 per 10.000 pregnancies in the Netherlands (1). Polydactyly is the most frequently observed upper limb anomaly followed by reduction defects, and syndactyly (1). Polydactyly is the occurrence of complete or partial extra digit(s), with or without a bony content on the thumb side or on the side of the little digit (2). A reduction defect is the absence, aplasia or hypoplasia of skeletal structures of the limb: transverse when the limb (distal or proximal arm, forearm and/or hand) is absent, and longitudinal when the long axis of a limb is affected (2,3). A syndactyly is the (partial or complete) fusion of two or more digits (2).

Considering the significant role that upper limbs play in a child's psychosocial and motor development, it is crucial to provide information, support, and guidance by a multidisciplinary team to parents and their child when an upper limb anomaly is identified in the prenatal or postnatal period. Although the majority of children appear to adjust well to their condition, this is by no means the case for all children (4). Although surgeons and parents may be good judges of functional outcomes, quality of life can only really be accurately measured if self-reported (4). Reconstructive operations could have a positive impact on the function of the limbs, a child's self-confidence and self-image (4). Prenatal or postnatal genetic testing can be offered to define the underlying genetic etiology and the recurrence risk in a future pregnancy. Additionally, a prenatal diagnosis empowers parents with informed reproductive choices and enables planning of optimal neonatal and surgical care (5).

The aim of this study was to evaluate the phenotype and genotype characteristics of a prenatal group of fetuses with suspected upper limb anomalies, including transverse and longitudinal reduction defects, polydactyly, and syndactyly. Additionally, a postnatal group of children with the same upper limb anomalies, not identified prenatally, was also described. The comparison in the phenotype-to-genotype characterization between the prenatal and postnatal group will support healthcare providers in advising and informing parents during parental genetic counseling.

Methods

Dutch prenatal healthcare system

In the Netherlands, prenatal screening occurs majorly in primary and secondary healthcare centers as part of a government-led national screening program(6-8). All pregnant women are offered a first trimester viability and dating scan around 10 weeks of pregnancy.

Additionally, women can opt for two structural anomaly scans, that is a second trimester anomaly scan since 2007 and in research setting a first trimester anomaly scan since 2021 (6,7). Both structural anomaly scans have a strict protocol and are performed by trained sonographers. The second trimester protocol includes the visualization of the following structures of the upper arm, using only 2D ultrasound: upper arm (including humerus), forearm (including ulna and radius), position of the wrist, and hand. Evaluation of the digits is not mandatory. Moreover, all women are offered prenatal screening for fetal aneuploidy by the first trimester combined test (until 2021) or cell free fetal DNA testing (since 2017). Ultrasound examination in the third trimester is only performed for obstetric indications such as suspected growth restriction or abnormal presentation of the fetus. In contrast to low risk women, those with a high risk for fetal anomalies due to an obstetric history or underlying medical conditions, can opt for medical ultrasounds in the first and second trimester in a Fetal Medicine Unit. Invasive prenatal testing (chorionic villus sampling or amniocentesis) is offered in cases of parental chromosome rearrangement, familial pathogenic variants or detected fetal abnormality.

If a fetal anomaly is suspected, the pregnant women are referred for an advanced ultrasound to a Fetal Medicine Unit(8). In the North-West region of the Netherlands, this is conducted at Amsterdam University Medical Center (AUMC). Here the length of the fetal limb bones are assessed by using the charts of Chitty *et al*., 2002 (9). If an upper limb anomaly is suspected, the possibility of genetic testing is discussed, depending on the findings. Moreover, referral to the Congenital Hand Team for additional counseling is also provided. This multidisciplinary team, consisting of clinical geneticists, plastic surgeons, rehabilitation doctors, and occupational therapists specialized in congenital anomalies of the upper limbs, offers prenatal and/or postnatal counseling and treatment until adulthood. Both the Fetal Medicine Unit and the Congenital Hand Team serve as referral centers for the same geographical area. Termination of pregnancy is permissible up to 24 weeks of gestation in the Netherlands.

Prenatal group
This is a retrospective cohort study of pregnant women who underwent prenatal ultrasound examinations at the Fetal Medicine Unit of AUMC from January 2007 to December 2021. We included all fetuses with the following fetal upper limb anomalies: transverse and longitudinal reduction defects, polydactyly, and syndactyly.

After approval from the Medical Ethical Committee of AUMC (reference number W21_361 # 21.401), we extracted data on the medical and obstetric history of the mothers, prenatal ultrasound findings, results of invasive genetic tests, and postnatal follow-up of the newborns. Additional information regarding postnatal findings was evaluated using the children's electronic patient files. When data were missing, we contacted other healthcare providers (e.g. midwife or gynecologist) of the mothers for information on the outcome. We excluded pregnancies with other upper limb anomalies, such as dysplasia (e.g. contractures and lymphangioma).

In case of a termination of pregnancy, postnatal confirmation of the diagnosis occurred by external physical examination, autopsy, and/or X-ray. If no autopsy was performed, all fetuses were structurally examined externally by our medical professionals to see if the suspected anomalies were present in case of a medical termination. In cases without documentation of the postnatal examination, it was assumed that the postnatal findings were in accordance with prenatal findings.

Postnatal group
The postnatal group consisted of all live born children, who were seen by the Congenital Hand Team of AUMC between January 2007 and December 2021 with a transverse and longitudinal reduction defect, polydactyly, and syndactyly. All mothers had received their second trimester anomaly scan in the North-West region of the Netherlands. Duplicate cases were removed when the case was already included in the prenatal group.

Classification
Cases were grouped per affected axis (proximodistal, radioulnar or unspecified) according to the Oberg-Manske-Tonkin (OMT) classification for upper limb anomalies(2). The anomalies were classified as isolated (ISO) if no other fetal abnormality was observed during prenatal sonography (prenatal group) or during postnatal physical examination (postnatal group), and as non-isolated (NISO) when other structural anomalies were identified.

In case of multiple anomalies of the upper limb, the case was classified as non-isolated and scored according to the most severe upper limb anomaly. Pregnancy outcomes were classified as termination of pregnancy, stillbirth, neonatal death in the first 28 days of life, or live birth. Outcomes of genetic testing were reported and classified as chromosomal or as monogenic. In cases with multiple anomalies without a genetic diagnosis, the case was classified as syndromic.

The performed genetic tests per case were dependent on the parents' request and year of diagnosis. In the last decades, advancements in genetic evaluation have shifted from karyotyping to exome sequencing (ES) (9-12). In case of suspected anomalies, rapid aneuploidy testing is the first tier test to examine aneuploidies, followed by chromosomal microarray analysis (CMA). Targeted molecular testing was mainly performed in families with known genetic pathogenic variants. Prenatal ES has been offered since 2019 in our unit, as opposed to a postnatal ES, which is available since 2012 (12). The approach to genetic testing differs between the prenatal and postnatal groups. During pregnancy, genetic tests such as rapid aneuploidy testing, microarray, or WES are routinely offered. In the postnatal group, the choice of genetic testing is guided by the physical examination of the newborn. In the prenatal period, only class 4 and 5 genetic variants (likely pathogenic or pathogenic variants) are reported in ES diagnostics. In contrast, variants of uncertain significance (VUS) are also reported in postnatal WES diagnostics.

Descriptive analysis

For the descriptive analysis of both groups, the findings of prenatal and postnatal genetic testing were reported in numbers and percentages. Furthermore, we estimated the prevalence (per 10,000 pregnancies) and prenatal detection rates of the specific limb anomalies.

Results

Prevalence and detection rate

In total, there were 561 cases with anomalies, of which 199 were identified in the prenatal and 362 in the postnatal period. The estimated prevalence for the North-West region was 2.3 per 10,000 pregnancies for transverse reduction defects, 0.8 for longitudinal reduction defects, 6.7 for polydactyly, and 1.6 for syndactyly, respectively. The estimated prenatal detection rates were 57% (64 of the 113) for transverse reduction defect, 46% (10 of the 41) for longitudinal reduction defect, 31% (103 of the 329) for polydactyly, and 17% (13 of the 78) for syndactyly.

Prenatal group

Between 2007 and 2021, 485.000 women received a structural anomaly scan within the North-West region of the Netherlands (source unpublished data from the yearly ultrasound unit audit files of the Fetal Medicine Unit of AUMC, including the region's primary care facilities). A total of 225 women was referred for ultrasound examinations due to suspected upper limb anomalies of which 26 (11.5%) could not be confirmed. A transverse reduction defect was finally identified in 66 cases, longitudinal reduction defect in 19, polydactyly in 124, and syndactyly in 16, of which the majority was non-isolated (Figure 1). Of the 85 cases with a termination of pregnancy, 28 (32.9%) agreed for autopsy (Figure 2). There was one lost to follow-up in the polydactyly group. All cases with genetic abnormalities are summarized in Tables 1 and 2, including the phenotypes. Genetic testing was performed in 121 of the 199 cases (60%). A genetic abnormality was identified in 76 of the 199 (38.2%) cases. The most commonly observed monogenic syndromes were Cornelia de Lange for reduction defects (4x), Greig cephalopolysyndactyly (3x) and Bardet-Biedl (3x) for polydactyly, and Apert (2x) for syndactyly (Table 2). Among the 94 isolated cases, 90 (96%) had no underlying chromosomal or monogenic cause, nor were they associated with a syndrome, while 95 of the 105 (90%) non-isolated cases had a chromosomal or monogenic cause, or a suspected syndrome.

Postnatal group

The postnatal group consisted of 362 children. These cases included 49 transverse and 22 longitudinal reduction defects, 226 polydactylies, and 65 syndactylies, of which the majority was non-isolated (Figure 3). A total of 42 out of 49 (86%) children presented with a transverse reduction defect of the digits, 5 (10%) in the forearm (radius or ulna), and/or in 2 (4%) the whole hand was affected. In 14 out of 22 (64%) children with a longitudinal reduction defect, the anomaly occurred in the digit(s) only, in 5 (22%) in the forearm (radius or ulna) and in 3 (14%) children the whole hand was affected. Reduction defects involving the humerus were not observed. In cases with a polydactyly, we observed that 57 out of 226 (25%) children had other family members with also a polydactyly, indicating a familial pattern. All cases with genetic abnormalities are summarized in Tables 1 and 3, including the phenotypes.

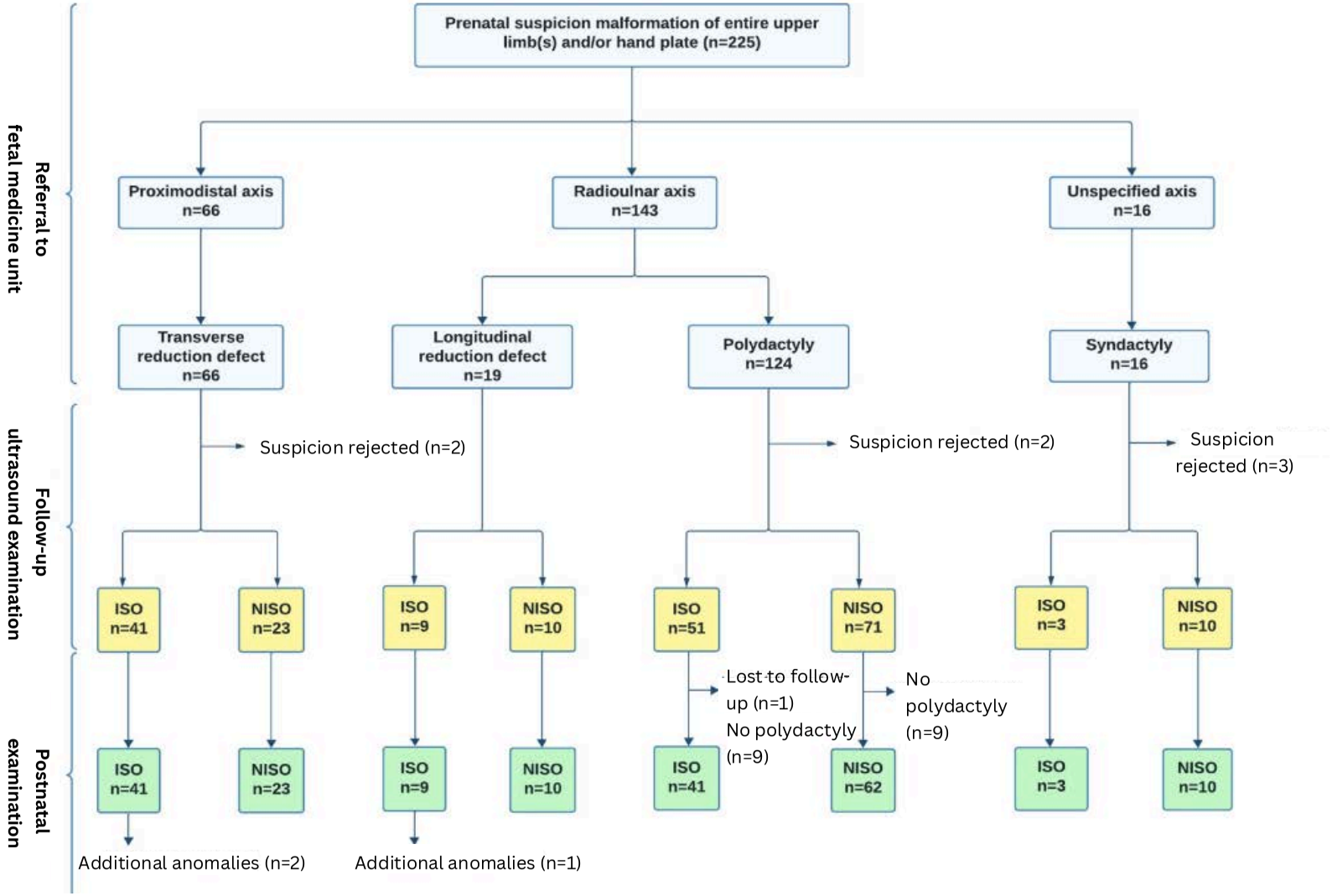

Figure 1. Prenatal group: sonographic and postnatal findings. ISO= isolated, NISO= non-isolated.

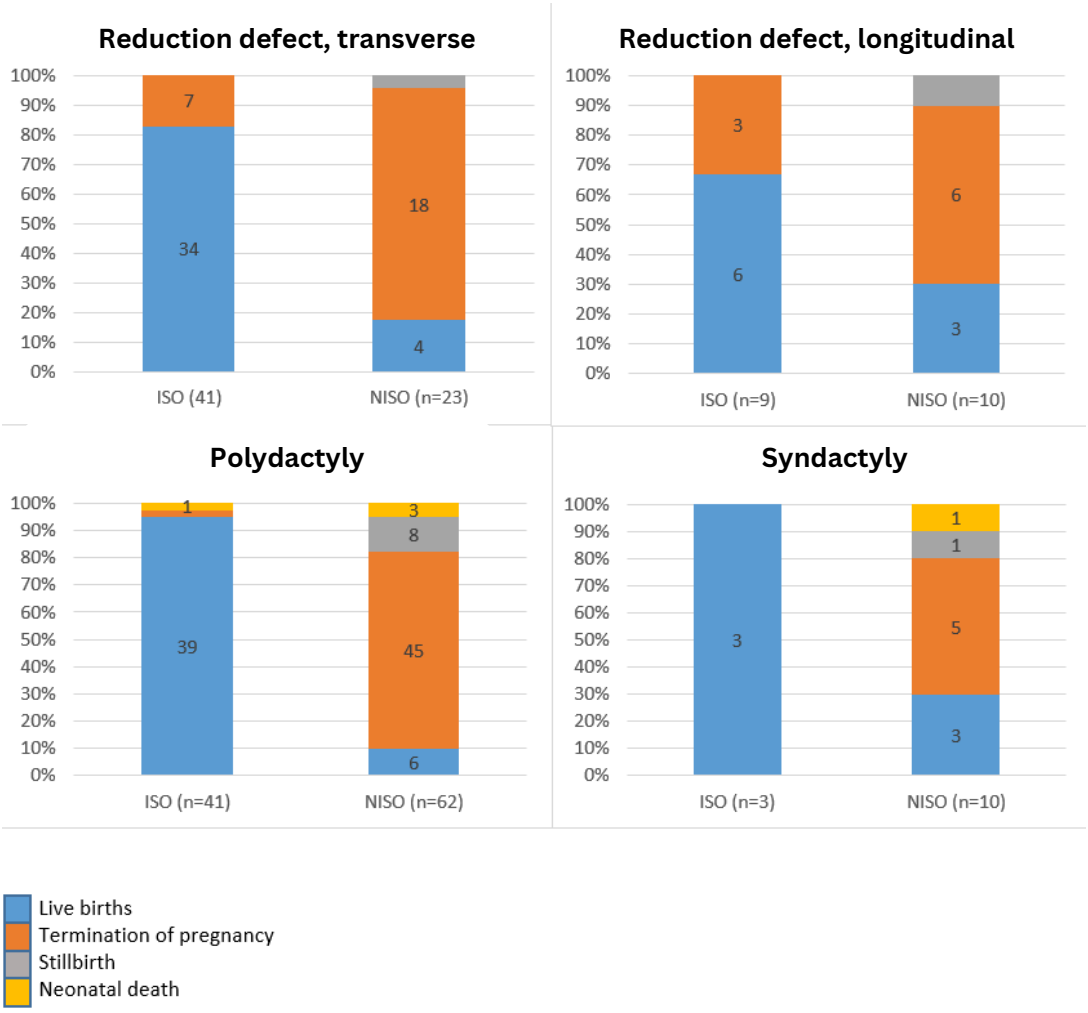

Figure 2. Percentages (y-axis) and numbers (in the colored bars) of terminations of pregnancy, stillbirths, neonatal deaths (death in the first 28 days of life), and live births for each prenatal suspected and postnatal confirmed isolated and non-isolated anomaly. ISO = isolated, NISO = non-isolated.

A genetic abnormality was identified in 31 of the 362 (8.2%) cases. The most observed monogenic syndromes were brachydactyly type C (3x) for reduction defects, Bardet-Biedl (2x) for polydactyly, and oculo-dento-digital syndrome (3x) and Apert (2x) for syndactyly (Table 3). Among the 313 isolated cases, 298 (95%) had no underlying chromosomal or monogenic cause, nor were they associated with any syndrome, while 37 of the 49 (76%) non-isolated cases had a chromosomal or monogenic cause, or a suspected syndrome.

Table 1. Genetic testing and results in n (%), divided into prenatal identified (only cases that were prenatally identified on sonography and confirmed after birth) and postnatal discovered (prenatally not identified during ultrasound examination, but discovered after birth). All the cases were categorized into three groups with abnormalities: chromosomal, monogenic, or syndromic. Chromosomal cases involve abnormalities in the chromosomes, monogenic cases are linked to single gene mutations, and syndromic cases exhibit multiple abnormalities without a known chromosomal or monogenic disorder causing them. It is expected that cases without any suspected chromosomal, monogenic, or syndromic causes are generally in good health.

	Prenatally identified *n* (%)					Postnatally discovered *n* (%)				
		Genetic		Non-genetic			Genetic		Non-genetic	
Sonographic characteristics	Tested/ total (*n*)	Chromosomal	Monogenic	Syndromic	No additional diagnosis	Tested/ total (*n*)	Chromosomal	Monogenic	Syndromic	No additional diagnosis
Reduction defect, transverse	64					49				
Isolated, unilateral	14/40	—	—	—	40 (100%)	5/34	—	—	—	34 (100%)
Isolated, bilateral	1/1	—	—	—	1 (100%)	5/8	1 (12.5%)	3 (37.5%)	—	4 (50%)
Non-isolated	19/23	7 (30.4%)	4 (17.4%)	9 (39.1%)	3 (13.1%)	3/7	1 (14.3%)	—	5 (71.4%)	1 (14.3%)
Reduction defect, longitudinal	19					22				
Isolated, unilateral	2/6	—	—	—	6 (100%)	2/11	—	—	—	11 (100%)
Isolated, bilateral	3/3	2 (66.7%)	1 (33.3%)	—	—	2/2	1 (50%)	—	—	1 (50%)
Non-isolated	9/10	3 (30%)	3 (30%)	2 (20%)	2 (20%)	6/9	—	1 (11.1%)	7 (77.8%)	1 (11.1%)
Polydactyly	103					226				
Isolated, unilateral	2/12	—	—	—	12 (100%)	7/110	1 (0.9%)	1 (0.9%)	—	108 (98.2%)
Isolated, bilateral	6/29	—	1 (3.4%)	—	28 (76.6%)	10/94	—	2 (2.1%)	—	92 (97.9%)
Non-isolated	55/62	39 (62.9%)	7 (11.3)	11 (18.1%)	2 (3.2%)	16/22	2 (9.1%)	7 (31.8%)	6 (27.3%)	7 (31.8%)
Syndactyly	13					65				
Isolated, unilateral	0/2	—	—	—	2 (100%)	2/32	—	1 (3.1%)	—	31 (96.9%)
Isolated, bilateral	0/1	—	—	—	1 (100%)	9/22	—	5 (22.7%)	—	17 (77.3%)
Non-isolated	10/10	1 (10%)	5 (50%)	4 (40%)	—	9/11	1 (9.1%)	4 (36.3%)	3 (27.3%)	3 (27.3%)

n=number

Table 2. Phenotype, genotype and postnatal findings of the prenatal group.

Number	Prenatal phenotype	Genotype	Diagnosis (by which test)	Sonographic characteristics and additional postnatal findings
1-2	Reduction defect, isolated, bilateral	TAR syndrome (microdeletion 1q21.1)	TAR syndrome (micro array)	Radial ray defect.
3	Reduction defect, isolated, bilateral	Pathogenic variant in FGFR2 gene	FGFR2 related syndrome (WES)	Radial ray defect. Postnatal additional findings: bilateral renal agenesis and syndactyly
4-5	Reduction defect, non-isolated, unilateral	Trisomy 21	Down syndrome (QF PCR)	
6-11	Reduction defect, non-isolated	Trisomy 18	Edwards syndrome (Karyotyping or QF PCR)	
12	Reduction defect, non-isolated	PIK3CA gene mutation	PIK3CA related syndrome (gene panel)	Transversal reduction defect or the right hand, oligodactyly, lymphangioma from head to thorax
13	Reduction defect, non-isolated	FANCB gene mutation	Fanconi anemia type B (WES)	Fetal growth restriction, ventriculomegaly, small cerebellum, bilateral short humerus and ulna with radial ray defects. Left hand with rudimental thumb, contractures of both legs
14	Reduction defect, non-isolated	NIPBL gene mutation	Cornelia de Lange syndrome (targeted molecular testing)	Brachycephaly, hydrops, Dandy walker malformation, dextrocardia, hypoplastic left heart, bilateral radial ray defects, oligodactyly and rocker bottom feet
15	Reduction defect, non-isolated	Tetrasomy 9p	(micro array)	Mild ventriculomegaly, vermis hypoplasia, abnormal corpus callosum, retrognathia, AVSD, empty stomach, single umbilical artery, talipes equinovarus, and syndactyly
16	Reduction defect, non-isolated	DYNC2H1-gene mutations	Jeune syndrome (gene panel)	Bilateral short humerus and femur with an abnormal stature, short ribs and bilateral polydactyly
17	Reduction defect, non-isolated	NIPBL gene mutation	Cornelia de Lange syndrome (WES)	Micrognathia, clubfeet, reduction defect lower legs, polydactyly unilateral, radial ray defect unilateral, oligodactyly hand
18	Reduction defect, non-isolated	NIPBL gene mutation	Cornelia de Lange syndrome (targeted molecular testing)	Split hand, radius aplasia, rudimentary fingers, , hypoplastic nasal bone, ventricular septal defect
19	Reduction defect, non-isolated	NIPBL gene mutation,	Cornelia de Lange syndrome (targeted molecular testing)	Thick nuchal translucency, diaphragmatic hernia, micrognathia, absent nasal bone, absence of 3 fingers unilateral
20	Reduction defect, non-isolated	Triploidy (diandric)	Triploidy (QF PCR)	Holoprosencephaly, cardiomegaly, (A)VSD, oligodactyly unilateral, omphalocele, echogenic kidneys and bowels, growth restriction

Table 2 (continued)

Number	Prenatal phenotype	Genotype	Diagnosis (by which test)	Sonographic characteristics and additional postnatal findings
21	Polydactyly, isolated, bilateral	Trisomy 13	Patau syndrome (QF PCR)	
22	Polydactyly, isolated, bilateral	GLI3 gene mutation	Greig cephalopolysyndactyly syndrome (targeted molecular testing)	Polydactyly both hands. Sibling of number case 70.
23-52	Polydactyly, non-isolated	Trisomy 13	Patau syndrome (Karyotyping or QF PCR)	
52-58	Polydactyly, non-isolated	Trisomy 18	Edwards syndrome (Karyotyping or QF PCR)	
59	Polydactyly, non-isolated	1q21.1 duplication	1q21.1 duplication syndrome (micro array)	Diaphragmatic hernia, postaxial polydactyly both hands and feet, echogenic kidneys
60	Polydactyly, non-isolated	TMEM218 gene mutations	Meckel Gruber syndrome (WES)	Encephalocele, polydactyly, polycystic dysplastic kidneys
61	Polydactyly, non-isolated	GLI3 gene mutation	Greig cephalopolysyndactyly syndrome (WES)	Polydactyly (preaxial) hands and feet, mild ventriculomegaly.
62	Polydactyly, non-isolated	BBS4 gene mutations	Bardet-Biedl syndrome type 4 (WES)	Polydactyly bilateral hands and feet, echogenic kidneys
63	Polydactyly, non-isolated	MKKS gene mutations	Bardet-Biedl syndrome type 6 (WES)	Ulnar polydactyly hands and feet, echogenic kidneys
64	Polydactyly, non-isolated	TMEM218 gene mutations	Meckel Gruber syndrome (WES)	Encephalocele, bilateral polycystic kidneys, polydactyly bilateral hands and legs, single ventricle heart, talipes equinovarus
65	Polydactyly, non-isolated	EVC gene mutations	Ellis van Crefeld syndrome (WES)	Ulnar polydactyly, short bones
66	Polydactyly, non-isolated	DHCR7 gene mutations	Smith Lemli Opitz syndrome (targeted molecular testing)	Polydactyly, overlapping fingers,
67	Polydactyly, non-isolated	Trisomy 21	Down syndrome (QF PCR)	Hydrops and bilateral polydactyly (pre- or postaxial unknown), miscarriage at a gestational age of 11 weeks.
68	Polydactyly, non-isolated	BBS5 gene mutations	Bardet-Biedl syndrome type 5 (WES)	Polycystic kidneys, hypospadias, postaxial polydactyly, oligohydramnios
69	Polydactyly, non-isolated	Trisomy 13	Patau syndrome (NIPT only)	
70	Polydactyly, non-isolated	GLI3 gene mutation	Greig cephalopolysyndactyly syndrome (targeted molecular testing)	Sibling of case 22. Prenatal suspicion of CCAM and polyhydramnios. CCAM was not confirmed after birth.

Table 2 (continued)

Number	Prenatal phenotype	Genotype	Diagnosis (by which test)	Sonographic characteristics and additional postnatal findings
71	Syndactyly, non-isolated	Triploidy (maternal)	Triploidy (QF PCR)	Large head, small abdomen, horseshoe kidney, syndactyly unilateral, growth restriction
72	Syndactyly, non-isolated	FGFR2 gene mutation	Apert syndrome (targeted molecular testing)	Dolichocephaly, syndactyly bilateral, thick nuchal translucency
73	Syndactyly, non-isolated	TP63 gene mutation	Ectrodactyly - ectodermal dysplasia - cleft syndrome (targeted molecular testing)	Bilateral cleft lip, VSD, abnormal position of the toes and fingers, syndactyly dig 3-4 bilateral hands and also feet
74	Syndactyly, non-isolated	FGFR2 gene mutation	Apert syndrome (targeted molecular testing)	Abnormal profile (frontal bossing), large cerebellum, bilateral syndactyly
75	Syndactyly, non-isolated	Triploidy (maternal)	Triploidy (QF PCR)	Syndactyly hands, small thorax, AC and FL<p3, micrognathia, VSD, empty stomach
76	Syndactyly, non-isolated	GLI3 gene mutation	Greig cephalopolysyndactyly syndrome (targeted molecular testing)	Ventriculomegaly, poly- and syndactyly, VSD

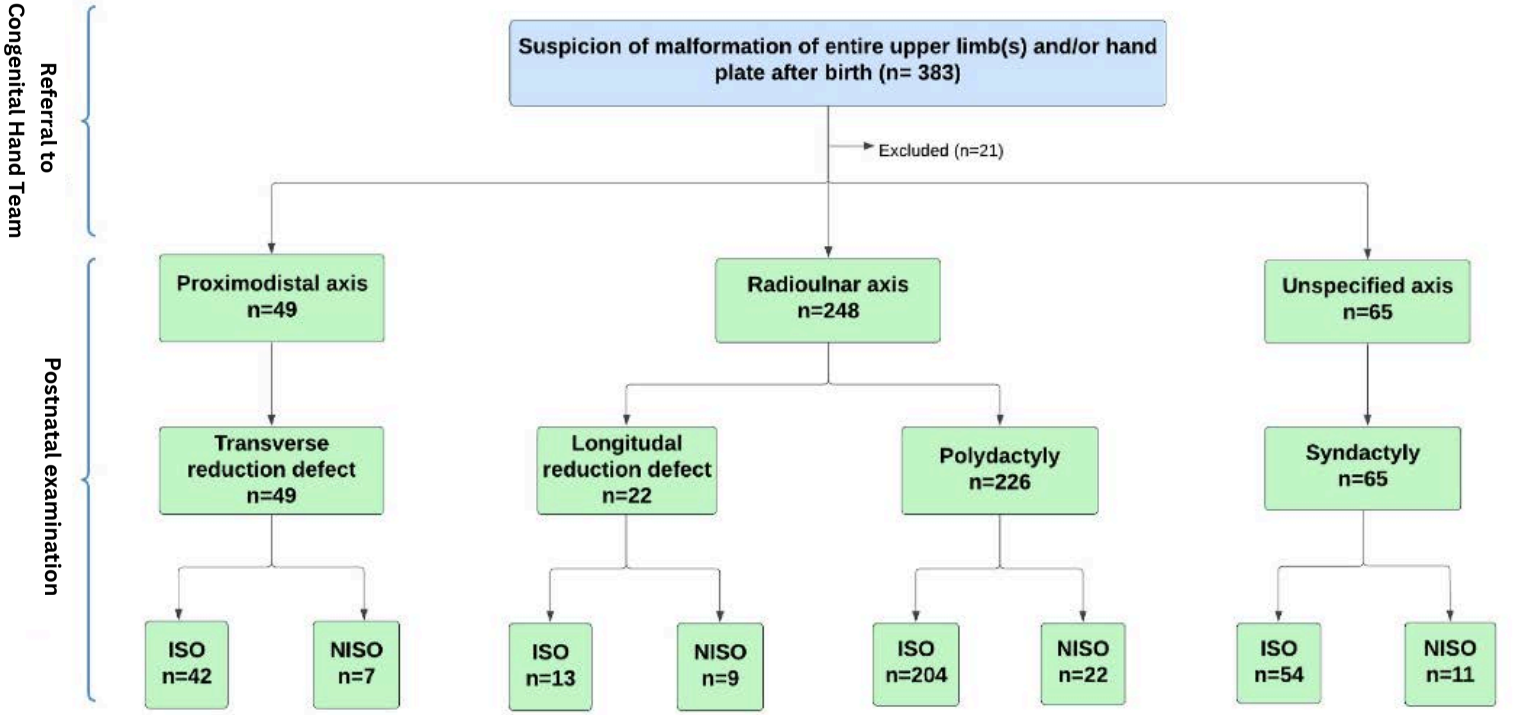

Figure 3. Postnatal group: findings of physical examination. All cases were not identified during pregnancy but discovered after birth. ISO = isolated, NISO = non-isolated.

Table 3. Phenotype, genotype and postnatal findings of the postnatal group.

Number	Postnatal phenotype	Genotype	Diagnosis (by which test)	Characteristics on postnatal physical examination and other comments
1	Reduction defect, isolated, bilateral	Duplication 20q11.22	(micro array)	Hypoplastic thumb/brachydactyly
2	Reduction defect, isolated, bilateral	Duplication 10q24	Split-Hand-Split-Foot Malformation (micro array)	Cleft hand and foot syndrome
3-5	Reduction defect, isolated, bilateral	GDF5 gene mutation	Brachydactyly type C (WES)	Reduction defect dig 2-5
6	Reduction defect, non-isolated	Mosaic trisomy 18	(FISH/micro array)	Longitudinal defect of both lower arms with hypoplastic thumbs, proximal radio-ulnar synostosis, clinodactyly dig 5 bilateral, syndactyly toes
7	Reduction defect, non-isolated	TBX5 gene mutation	Holt-Oram syndrome (targeted molecular testing)	Radius hypoplasia, partial syndactyly dig 2 unilateral, VSD
8	Polydactyly, isolated, unilateral	Deletion 17p12-17p11.2	(micro array)	Postaxial polydactyly
9	Polydactyly, isolated, unilateral	FANCA gene mutations	Fanconi anemia (Mitomycine-C test)	Radial polydactyly, fanconi anemia
10	Polydactyly, isolated, bilateral	ERF gene mutation	Chitayat syndrome (WES)	Central polydactyly bilateral (dig 2)
11	Polydactyly, isolated, bilateral	GLI3 gene mutation	Greig cephalopolysyndactyly syndrome (unknown)	Polydactyly all limbs, syndactyly foot
12	Polydactyly, non-isolated	Deletion 1q21,1-q21.2	(micro array)	Bilateral preaxial polydactyly, microcephaly
13	Polydactyly, non-isolated	TBX3 gene mutation	Ulnar-mammary syndrome (WES)	Bilateral polydactyly, VSD, tracheomalacia, mamma aplasia
14	Polydactyly, non-isolated	TFAP2A gene mutation	Branchio-oculo-facial syndrome (targeted molecular testing)	Cleft palate, polydactyly
15	Polydactyly, non-isolated	HNRNPU gene mutation	Early infantile epileptic encephalopathy type 31 (unknown)	Scoliosis, syndactyly feet, preaxial polydactyly hands and feet , epilepsy, psychomotor retardation
16	Polydactyly, non-isolated	PUF60 gene mutation, BTD gene mutations	Verheij syndrome (WES)	Cleft lip, polydactyly unilateral hand, coloboma. BTD gene mutation is a VUS.

Table 3. Phenotype, genotype and postnatal findings of the postnatal group.

Number	Postnatal phenotype	Genotype	Diagnosis (by which test)	Characteristics on postnatal physical examination and other comments
17	Polydactyly, non-isolated	CHD4 gene mutation	Sifrim-Hitz-Weiss syndrome (unknown)	Postaxial polydactyly hand (not typical for mutation, VUS), pes planovalgus deformity, cryptorchidism.
18	Polydactyly, non-isolated	Trisomy 21	Down Syndrome (QF PCR, karyotyping)	AVSD, radial polydactyly unilateral
19	Polydactyly, non-isolated	BBS10 gene mutations	Bardet-Biedl syndrome (WES)	Large kidneys, postaxial polydactyly unilateral
20	Polydactyly, non-isolated	BBS12 gene mutations	Bardet-Biedl syndrome (gene panel)	Polydactyly unilateral hand and both feet, dysplastic kidneys
21-23	Syndactyly, isolated, bilateral	GJA1 gene mutation	Oculo-dento-digital syndrome (targeted molecular testing)	Syndactyly dig 4 and 5 hands
24	Syndactyly, isolated, bilateral	BBS7 gene mutations	Bardet-Biedl syndrome (gene panel)	Syndactyly and polydactyly
25	Syndactyly, isolated, unilateral	RAF1 gene mutation	Noonan syndrome (targeted molecular testing)	Syndactyly hand dig 3-4, learning difficulties, clinodactyly
26	Syndactyly, isolated, unilateral	PIK3CA gene mutation	Megalencephaly-capillary malformation (gene panel)	Syndactyly dig 3-4, syndactyly feet, Cutis Marmorata Telangiectatica Congenita
27	Syndactyly, non-isolated, bilateral	FGFR2 gene mutation	Apert syndrome (targeted molecular testing)	Syndactyly and craniosynostosis
28	Syndactyly, non-isolated, bilateral	FGFR2 gene mutation	Apert syndrome (targeted molecular testing)	Frontal bossing, syndactyly both hands and feet, craniosynostosis
29	Syndactyly, non-isolated	BPTF gene mutation	Neurodevelopmental disorder with dysmorphic facies and distal limb anomalies (WES)	VSD, bilateral syndactyly
30	Syndactyly, non-isolated	Distal deletion of 22q11	22q11 deletion syndrome (micro array)	Unilateral syndactyly dig3 and 4, ASD, retrognathia
31	Syndactyly, isolated, bilateral	GJA1 gene mutation	Oculo-dento-digital syndrome (targeted molecular testing)	Syndactyly dig 4 and 5 both hands

Discussion

This study demonstrated that the majority of cases (90/94, 96%) with apparently isolated anomalies in the prenatal group had no underlying chromosomal or monogenic cause and were not associated with a syndrome. A similarity was observed in the postnatal group, where 298 of the 313 (95%) had no genetic cause or a syndrome (Tables 1-3). In contrast, the majority of the non-isolated cases in both groups (132/154, 86%) had an underlying genetic cause or a suspected syndrome. A higher percentage of genetic abnormalities was seen in the prenatal group in comparison with the postnatal group. The larger size of the postnatal group suggests that identifying mild anomalies of the digits during the prenatal period remains challenging.

The available literature on the prenatal detection of upper limb anomalies is primarily based on data gathered decades ago, while improvement in the prenatal anomaly identification has been observed on other fetal anomalies over the last few years (13-21). Reported prenatal detection rates of upper limb anomalies range between 22.8-42% (period 1990-2010) (13-21). Anomalies of the entire upper limb have higher sensitivities (70-100%), whereas anomalies affecting only the digits had the lowest sensitivities (4-19%) (20,21). This is in line with our finding that in 56 of the 71 (78.9%) children with postnatally discovered reduction defect, the anomaly occurred in the digit(s). Prenatal detection rates in our study varied from 17% to 57%, depending on the anomaly.

Since digit evaluation is not included in current (inter)national guidelines for second-trimester anomaly scans, it is likely that these specific anomalies are often missed during routine structural anomaly scans (6).

The high occurrence of chromosomal abnormalities in the non-isolated prenatal group of our study (26%) was similar to the findings of Paladini and colleagues (28%) (22). In contrast, the postnatal group exhibited a lower percentage of chromosomal abnormalities (7/365, 1.9%). Additionally, the occurrence of monogenic abnormalities was higher in the prenatal group (21/199, 10.5%) than in the postnatal group (24/362, 6.6%). The expected structural anomalies are mainly in the urogenital, heart and nervous system (21,22). Table 1 suggests that genetic abnormalities are more frequently associated with bilateral cases than with unilateral cases, which is in line with the findings of Pajkrt *et al*. (18). Their study also found that cases with bilateral lesions have a significantly higher association with aneuploidy and genetic abnormalities, whereas those with sonographically isolated unilateral forearm defects had a very low incidence of other underlying pathology.

The findings of this study are useful for healthcare providers who want to inform parents about the potential prenatal and postnatal outcomes. Notably, this study has one of the largest study populations with upper limb anomalies that has been described, with a total of 561 included cases.

The retrospective nature of this study is one of the study's limitations. In the prenatal group, findings about postnatal examination were not always well documented after a termination of pregnancy and we assumed that all these cases were correctly identified when no additional specific classification was made postnatally. Moreover, since not all other fetuses have been systematically evaluated externally after a termination of pregnancy, some upper limb anomalies may have been missed leading to an underestimation. Another limitation is that not all cases underwent autopsy, likely resulting in some anomalies not being confirmed postnatally. In the postnatal group, we expect that all children with clinically relevant upper limb anomalies will be referred to the Congenital Hand Team. However, we expect that simple forms of polydactyly may also have been treated outside our tertiary hospital. Regional guidelines allow plastic surgeons in secondary hospitals to treat simple forms of polydactyly, which may also have contributed to an underestimation of the postnatal group. Therefore, we think that the actual prenatal detection rate for polydactyly is lower.

An early prenatal identification of abnormalities allows parents more time to consider whether they wish to have prenatal invasive testing. A genetic consultation should be offered as a standard workup to define the underlying genetic causes if a upper limb anomaly is suspected (21,22). Evaluation of the prenatal phenotype can help to determine if it is part of a genetic syndrome (23). For example, radial polydactylies are more observed in non-isolated cases compared to ulnar polydactylies (24). Furthermore, it is important to enquire about family history for upper limb anomalies, maternal medication use and intoxication to identify factors related to the anomaly (18,25).

If invasive diagnostics is requested by parents, rapid aneuploidy testing and microarray analysis are highly recommended as a first step, particularly in non-isolated cases (18,26). In the prenatal group, 53 of the 76 (70%) abnormalities were detectable by these tests (Table 2). Finally, exome sequencing can be used to detect other genetic disorders. In case of isolated upper limb anomalies, particularly in unilateral anomalies, parents can be informed that chromosomal, monogenic and syndromic underlying causes are rare.

In conclusion, this study showed that the majority of cases with isolated limb anomalies in both the prenatal and postnatal group had no underlying chromosomal or monogenic cause, and were not associated with syndromes, while in the majority of the non-isolated cases an underlying genetic cause was found. For isolated unilateral anomalies, parents should be informed that most cases do not have an underlying genetic cause. Identification of minor defects of the hand and digits poses a challenge, given the larger size of the postnatal group compared to the prenatal group.

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CHAPTER 3

The influence of the introduction of fetal anomaly scans on pregnancy terminations in cases of upper limb anomalies: a retrospective cohort study from 2000 to 2023.

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Abstract

Objective Examining the association between the introduction of the fetal anomaly scans in the Netherlands and termination of pregnancy (TOP) in cases of prenatally detected upper limb anomalies.

Methods We conducted a retrospective study among prenatally detected upper limb anomalies between 2000 and 2023. Anomalies were categorized as reduction defects, syndactyly, or polydactyly, and classified as isolated or non-isolated. We analyzed TOP rates across three periods (2000–2006, 2007–Aug 2021, Sept 2021–2023), including an interrupted time series (ITS) analysis to assess the impact of introducing second- and first-trimester anomaly scans (STAS, FTAS).

Results We included 300 pregnancies, of which 133 (44.3%) were isolated. Overall TOP rates did not differ significantly between periods, except for isolated reduction defects, where a significant increase was observed (p=0.032). TOP rates over time did not increase for syndactyly and polydactyly. Median gestational age at diagnosis decreased across the three periods: from 20.4 weeks to 19.4 weeks and then to 14.9 weeks. Similarly, the timing of termination of pregnancy decreased from 20.5 to 16.8 and then to 15.0 weeks.

Conclusion Earlier prenatal detection followed the introduction of STAS and FTAS. Despite this shift in timing, no consistent changes in termination rates were observed across the study periods. While overall TOP rates remained stable, a trend towards higher termination rates was observed for isolated reduction defects.

Introduction

This study investigates the impact of the fetal anomaly scans on pregnancy outcomes for prenatally detected congenital upper limb anomalies in the Netherlands. The second trimester structural anomaly scan (STAS) was implemented in 2007 as part of a national prenatal screening program and is offered to all pregnant women (1,2). It is performed between 18 and 21 weeks' gestation by qualified sonographers, and costs are covered by the Dutch government (1,3). One of the components of the fetal anatomy assessment includes evaluation of the limbs and for the upper limb it includes the humerus, radius, ulna and the position of the wrist and hand (3). Digit evaluation is not mandatory (3). In September 2021, the first trimester structural anomaly scan (FTAS) was introduced in the Netherlands within the nationwide IMITAS implementation study (4). Between 2021 and 2022, the scan was offered to all pregnant women, with a reported uptake of 75%. Before 2007, no standardized national protocol existed for fetal anomalies scans in both first and mid trimester (5). Detailed ultrasounds were offered only incidentally, most often to women with an increased risk for fetal anomalies based on family or obstetric history.

Upper and lower limb defects – including reduction defects, syndactyly, and polydactyly – can be diagnosed by fetal ultrasound (6,7). A reduction defect is the aplasia or hypoplasia of the limb: transverse when the limb is absent or shortened, and longitudinal when the long axis is affected (8,9). Syndactyly is the partial or complete fusion of digits (8). Polydactyly is the occurrence of extra digit(s), with or without bony content (8). Severity varies, and some anomalies may significantly impact a child's functionality and quality of life (10).

Prenatal differentiation between isolated and non-isolated anomalies – including those with associated genetic conditions – can influence parental decisions regarding pregnancy continuation. In the Netherlands, termination of pregnancy (TOP) is permitted until 24 weeks' gestation (11). Since 2007, TOP rates for conditions such as open spina bifida and severe congenital heart defects have been evaluated and stated to have increased, whereas termination rates for facial clefts have remained unchanged (12-15). However, the impact of the introduction of the fetal anomaly scans on parental decisions in case of upper limb anomalies has not been evaluated yet. Therefore, this study aims to evaluate whether the introduction of the STAS and additionally the FTAS changed the timing of diagnosis and TOP in cases of prenatally detected upper limb anomalies. Additionally, we evaluated if TOP rates differ between type of anomaly, isolated versus non-isolated, and the presence of underlying genetic conditions.

Methods

Study Design

This retrospective cohort study was conducted at the Fetal Medicine Unit (FMU) of Amsterdam University Medical Centre (AUMC) in the Netherlands. Pregnancies with a prenatally detected upper limb anomaly observed during fetal anomaly scans between January 1, 2000, and December 31, 2023, were included. Ethical approval for this study was obtained from the Medical Ethical Committee of AUMC (reference number W21_361 # 21.401).

Inclusion and exclusion criteria

This study included three prenatally detected anomalies of the upper limbs: reduction defects (including both longitudinal and transverse defects), syndactyly, and polydactyly. Cases with these anomalies were included from an already existing database for another study with upper limb anomalies detected between 2007 and 2021 (16). In addition, data on cases that were seen between 2000 and 2006 and between 2021 and 2023 were collected retrospectively by searching the center's prenatal database for a reduction defect, syndactyly, or polydactyly.

Prenatal screening workflow

Most FTAS and STAS in the Netherlands are performed in primary and secondary healthcare settings by sonographers, midwives, and obstetricians. All ultrasound clinics conducting fetal anomaly scans must be affiliated with a tertiary center for fetal medicine. Women at increased risk for anomalies and women with a detected fetal anomaly in the North-West region of the Netherlands are referred to our FMU for targeted ultrasound assessment. If the anomaly is confirmed, invasive testing is offered, that is chorion villus sampling from 11 weeks' gestation and amniocentesis from 16 weeks' gestation. If desired, parents are offered counseling from clinical geneticists, and/or plastic surgeons and/or rehabilitation doctors to inform them on the postnatal treatment possibilities and to aid decision making on whether to continue or terminate pregnancy.

Classification of cases

According to the prenatal ultrasound diagnosis, cases were classified into one of the following groups: reduction defects (longitudinal and transverse), syndactyly, or polydactyly. In addition, cases were further categorized as either sonographically isolated or non-isolated. Isolated cases were defined as those in which no other fetal abnormalities were identified during ultrasound examinations, whereas non-isolated cases were characterized by the presence of additional structural anomalies in other organ systems.

When multiple (upper) limb anomalies were present (e.g. reduction defect and syndactyly) that are part of the same presumed etiology as the upper limb anomaly, the case remained classified as isolated and was categorized based on the functionally most severe anomaly (most severe: reduction defect, and least severe: polydactyly). As a final step, we categorized all cases based on genetic outcomes as normal, abnormal when a genetic abnormality was identified, or unknown if genetic testing was not performed.

The genetic test results of the cohort between 2007 and 2021 were discussed in a separate cohort study (16). The tests performed were based on counseling by the clinical geneticist, parental preference and year of diagnosis. Available tests included karyotyping, rapid aneuploidy testing (Fluorescence In Situ Hybridization, FISH; Quantitative Fluorescent Polymerase Chain Reaction, QF-PCR, and Multiplex Ligation-dependent Probe Amplification, MLPA), chromosomal microarray, single gene tests, targeted panels, and exome sequencing (ES). If the result of the non-invasive prenatal test (NIPT) was abnormal and ultrasound findings were in line with the NIPT result, but no invasive testing was performed, the genetic outcome was considered as abnormal.

Statistical analysis

Descriptive statistics were used to summarize the baseline characteristics. Categorical variables were reported as numbers/proportions and percentages, while continuous variables were expressed as medians and their corresponding ranges. We conducted three separate statistical tests to answer our research question.

Firstly, the proportions of TOP of all pregnancy outcomes across the three time periods (2000–2006, 2007–2021, and 2021–2023) were compared using a chi-squared test for independence in IBM SPSS Statistics 28. Secondly, trends in the 3-year moving average of TOP rates over the study period were assessed using the Mann-Kendall trend test, performed separately for each anomaly type and stratified by isolated and non-isolated cases. This non-parametric trend test examines whether there are significant increases or decreases in the outcome over time, although it does not assess whether the trend changes before versus after and event. These analyses were conducted using XLSTAT 2023. As a final step, an interrupted time series (ITS) analysis was performed to investigate the effect of an ‘event’ – the introduction of the STAS and FTAS – on the 3-year moving average of TOP rates separately for each anomaly type. To improve the distributional properties of the outcome variable, a logarithmic transformation was applied to the 3-year moving average of the percentage of TOP prior to regression analysis.

A linear regression analysis was conducted to investigate the association between GA at diagnosis, year of examination and anomaly type. Additionally, a separate linear regression analysis was performed to examine the association between GA at termination and the year of examination. Corresponding scatter plots with trend lines were generated to visualize these relationships.

Results

Study population

Upper limb anomalies were detected in 300 pregnancies between 2000 and 2023. Of these, 133 (44.3%) were isolated and 167 (55.7%) were non-isolated. Among the anomaly groups, polydactyly was the most frequently observed anomaly (n=169, 56.3%), then reduction defect (n=108, 36%), and finally syndactyly (n=23, 7.7%). Table 1 summarizes the characteristics of the study cohort and the proportion of isolated and non-isolated cases per anomaly type. A positive family history of congenital anomalies was present in 106 of the 300 cases (35.3%). Of the 108 fetuses diagnosed with a reduction defect, 66 cases (61.1%) were isolated and 42 cases (38.9%) were non-isolated. Among the 23 cases with syndactyly, 4 (17.4%) were isolated and 19 (82.6%) were non-isolated. In the polydactyly group (n=169), 63 cases (37.3%) were isolated and 106 (62.7%) were non-isolated.

Genetic testing was performed in 243 of the 300 (81%) cases with sonographically isolated and non-isolated anomalies, revealing a genetic abnormality in 107 (44%) cases (Additional File 1). Cases with an abnormal genetic outcome were eight times more likely to result in TOP compared to those with a normal outcome (OR = 8.2, 95% CI 4.767 –13.961, p <.001).

TOP rates for all isolated and non-isolated groups, further subdivided by genetic outcome for the isolated groups, are presented in Figure 1. TOP was rare in isolated syndactyly (0%, 0/4) and polydactyly (1.6%, 1/63), but occurred in 22.7% (15/66) of isolated reduction defects. In contrast, for non-isolated cases, pregnancy was terminated in 30 of 42 (71.4%) reduction defects, 9 of 19 (47.4%) syndactyly cases, and 79 of 106 (74.5%) polydactyly cases.

Pregnancy was terminated in 45 of the 108 cases (41.7%) with a **reduction defect**. Among the 66 isolated reduction defects, genetic testing was performed in 58 cases (88%). Termination of pregnancy occurred in 24.1% (13/54) of cases with a normal genetic result, 25% (1/4) of cases with an abnormal result, and 12.5% (1/8) of cases with unknown genetic outcomes. A genetic abnormality was identified in one of the 15 terminated pregnancies with a sonographically isolated reduction defect (6.7%), involving a pathogenic variant in *FGFR2*. In the group of 42 non-isolated reduction defects, parents opted for termination in 30 cases (71.4%).

Table 1. Characteristics of the study population.

***n*(%)**	**Total cases (*n* = 300)**
Isolated	133 (44.3)
Non-isolated	167 (55.7)
Anomaly type	
Reduction defects	108 (36)
Isolated	66 (61.1)
Non-isolated	42 (38.9)
Longitudinal	31 (10.3)
Transverse	77 (25.7)
Syndactyly	23 (7.7)
Isolated	4 (17.4)
Non-isolated	19 (82.6)
Polydactyly	169 (56.3)
Isolated	63 (37.3)
Non-isolated	106 (62.7)
Pregnancy outcome	
TOP	130 (43.3)
Live birth	141 (47)
IUFD	20 (6.7)
NND	9(3)

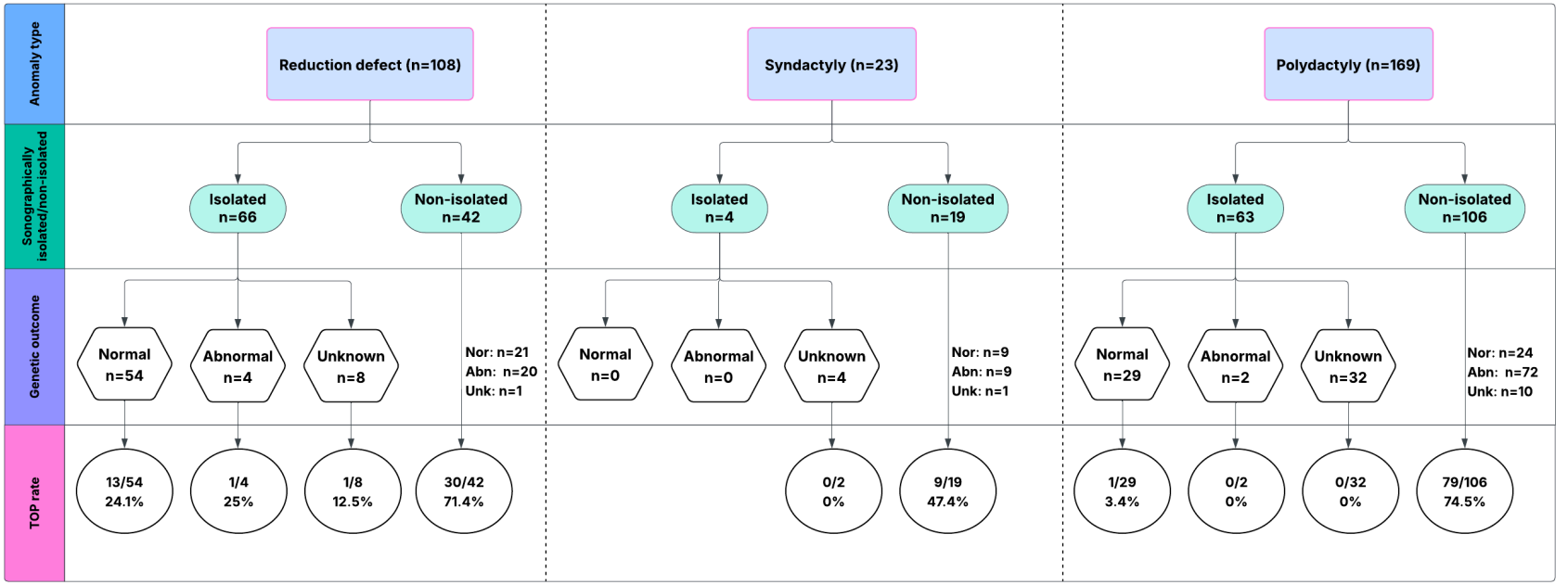
Abbreviations: FTAS = first trimester anomaly scan, IUFD = intrauterine fetal death, MA = maternal age, NND = neonatal death, STAS = second trimester anomaly scan, TOP = termination of pregnancy.

In the **syndactyly** subgroup, TOP was performed in 9 of the 23 cases (39.1%), all of which were sonographically non-isolated. No genetic testing or terminations were performed in the four cases of isolated syndactyly. Within the **polydactyly** group, TOP was chosen in 80 cases (47.3%). Among isolated polydactyly cases (n=63), 31 cases received genetic testing, and one pregnancy was terminated – after normal results of genetic testing. Details of all abnormal genetic results are provided in Additional File 1. Of the 89 isolated cases who underwent genetic testing, 6 (6.7%) had an abnormal result. In contrast, among the 152 tested non-isolated cases, 101 (66.4%) had an abnormal genetic finding.

Trends in termination of pregnancy rates

The study population was divided into three time periods: 2000–2006 (n = 37), 2007–August 2021 (n = 216), and September 2021–2023 (n = 47). TOP rates were compared across these three periods, as shown in Table 2. No statistically significant differences were observed between the periods for any of the anomaly subtypes.

Figure 1. Termination of pregnancy (TOP) rates for fetuses with reduction defects, syndactyly, and polydactyly, stratified by whether the anomaly was sonographically isolated or non-isolated, and further subdivided by genetic test outcome (normal, abnormal, or unknown). Proportions are shown as n/N (%), and visualized below each group.



TOP= termination of pregnancy, nor=normal, abn=abnormal

Figure 2. 3-year average termination of pregnancy (TOP) rates for reduction defect, syndactyly, and polydactyly, separated by whether the anomaly was isolated or non-isolated. Detailed information on these are included in Additional File 2.

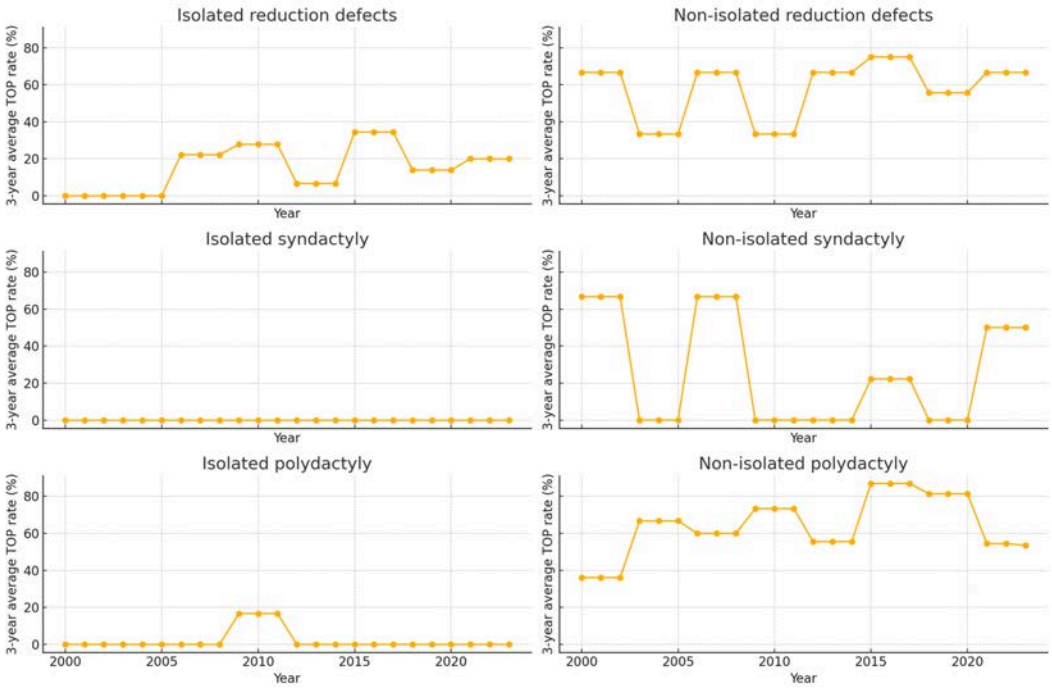


Figure 2 and Additional File 2 illustrate the trend in TOP rates during the study period for each anomaly type, stratified by sonographically isolated and non-isolated cases. In addition, stratified analyses with the Mann-Kendall trend test and ITS regression are shown in Tables 3 and 4, respectively.

Trend analyses revealed a significant upward trend in the 3-year moving average of TOP rates over the study period for **isolated reduction defects**. ITS regression indicated a significant change in trend following the introduction of both the STAS and FTAS. For **non-isolated reduction defects,** there was no difference in 3-year moving average of TOP rates over time and the ITS regression did not suggest any effect of the STAS or FTAS on this pregnancy outcome. No significant trend was seen for non-isolated syndactyly. **Non-isolated polydactyly** demonstrated no significant trend in 3-year moving average of TOP rates over time. However, ITS regression revealed a significant change in trend following the introduction of both STAS and FTAS. In addition, there were 4 TOPs after a GA of 24 weeks. All were due to a Trisomy 13 or 18.

Table 2. Termination of pregnancy (TOP) rates per period for each type of limb anomaly.

n/N (%)	Before introduction of STAS, TOP rate 2000–2006 n = 37	After STAS and before FTAS, TOP rate 2007–August 2021 n = 216	After FTAS and before FTAS, TOP rate September 2021–2023 n = 47	p value
Reduction defects isolated	2/8 (25)	10/48 (20)	3/10 (30)	0.809
Reduction defects non-isolated	4/7 (57.1)	23/32 (71.9)	3/3 (100)	0.3861
Syndactyly isolated	0/1 (0)	0/3 (0)	—	
Syndactyly non-isolated	3/3 (100)	4/13 (30.8)	2/3 (66.7)	0.0736
Polydactyly isolated	0/4 (0)	1/50 (2)	0/9 (0)	0.8763
Polydactyly non-isolated	11/14 (78.6)	52/70 (74.3)	13/22 (59.1)	0.3198

Proportions are shown as n/N (%). Differences across the three periods (2000–2006, 2007–2021, and 2021–2023) were evaluated using a chi-squared test for independence.

Table 3. Results of the Mann-Kendall trend test and Sen's slope estimation for 3-year moving average of TOP over time, stratified by anomaly type and whether the condition was isolated or non-isolated.

Group (n)	Kendall's tau	Sen's slope	95% CI (slope)	p value
Isolated reduction defects (66)	0.339	0.926	0.000 to 1.250	**0.032**
Non-isolated reduction defects (42)	0.241	0.0	0.000 to 1.489	0.154
Isolated syndactyly (4)	—	—	—	—
Non-isolated syndactyly (19)	−0.043	0.0	0.000 to 0.000	0.817
Isolated polydactyly (63)	—	—	—	—
Non-isolated polydactyly (106)	0.128	0.833	−0.371 to 1.665	0.424

Bold values indicate statistical significance at p < 0.05.

Table 4 Summary of the ITS regression results for each limb anomaly group as STAS or FTAS as intervention.

Anomaly type (n)	Intervention	Unstardized coefficient	95% CI	p value
Isolated reduction defects (66)	STAS	−935.226	−1522.98 to −327.471	**< 0.001**
Isolated reduction defects (66)	FTAS	−492.852	−685.166 to −300.539	**< 0.001**
Non-isolated reduction defects (42)	STAS	66.312	−35.626 to 168.250	0.190
Non-isolated reduction defects (42)	FTAS	−9.297	−30.048 to 11.455	0.361
Isolated syndactyly (4)	—	—	—	—
Non-isolated syndactyly (19)	STAS	1,035,008	−886.857 to 2956.873	0.275
Non-isolated syndactyly (19)	FTAS	248.083	−90.703 to 586.868	0.142
Isolated polydactyly (63)	—	—	—	
Non-isolated polydactyly (106)	STAS	−102.773	−171.351 to −34.195	**0.005**
Non-isolated polydactyly (106)	FTAS	−27.679	−39.864 to −15.495	**< 0.001**

P values for intervention effects are shown. A base-10 logarithmic transformation (log10) was applied to the 3-year moving average of TOP percentages to normalize the distribution prior to interrupted time series regression. Bold values indicate statistical significance at p < 0.05.

Gestational age at of diagnosis

Table 5 shows the GA at diagnosis across the study period. The median overall gestational age for all anomalies together was 20.4 weeks (range: 12.7–29.1) for the period before the introduction of STAS, 19.4 (range: 8.3–36.0) after the introduction of STAS and before FTAS, and 14.9 (range: 11.0–23.1) after the introduction of the FTAS. The median GA at diagnosis was 19.7 weeks (range 11.8–32.4) for isolated cases and 16.3 weeks (range 8.3-36.0) for non-isolated cases.

Gestational age at termination of pregnancy

The median GA at termination was 20.5 weeks (range: 13.4–27.4) for the period before the introduction of STAS, 16.8 (range: 11.4–30.6) after the introduction of STAS and before FTAS, and 15.0 (range: 13.0–23.7) after the introduction of the FTAS. Among terminated pregnancies, the median GA at TOP was 18.4 weeks (range 13.4-26.0) for isolated cases and 16.6 weeks (range 11.4–30.6) for non-isolated cases.

Table 5. Gestational age (GA) at suspicion, categorized into five groups.

GA at suspicion in weeks, *n* (%)	Reduction defects (*n* = 108)	Syndactyly (*n* = 23)	Polydactyly (*n* = 169)
< 12	5 (4.7)	1 (4.3)	11 (6.6)
12–15	25 (23.4)	2 (8.7)	53 (31.7)
15–18	7 (6.5)	2 (8.7)	16 (9.6)
18–22	57 (53.7)	13 (56.5)	71 (42.5)
≥ 22	13 (12.1)	5 (21.7)	16 (9.6)
Unknown	1 (0.9)	—	2 (1.1)

This table highlights differences in the timing of prenatal suspicion for each anomaly type.

Discussion

This study reveals no differences in TOP rates across the three time periods (2000–2006, 2007– Augustus 2021, and September 2021–2023) for any type of upper limb anomaly. However, time trend analyses suggest that there may be an upward significant trend in TOP for isolated reduction defects since 2000, with an increase after the introduction of STAS and FTAS. In contrast, TOP was very rare for isolated syndactyly (n=0) and polydactyly (n=1). Termination of pregnancy was more frequent in non-isolated cases than in isolated cases, underscoring the influence of associated anomalies on pregnancy outcome. The introduction of the STAS in the Netherlands in 2007, and more recently the FTAS in 2021 in research setting, may therefore have led to earlier detection.

Several factors may explain why a significant increase in 3-year moving average of TOP rates was observed in our study for isolated reduction defects. Reduction defects may be perceived by parents and healthcare providers as carrying a higher burden of functional impairment and psychosocial consequences compared to other upper limb anomalies, potentially influencing decisions towards a termination. A large study analyzing 53,000 pregnancies found that anomaly severity was one of the strongest predictors of TOP (17). A study conducted in Bangkok found that parental decisions were influenced most strongly by severity and the presence of genetic abnormalities (18).

The availability and expansion of the prenatal detection after NIPT and invasive genetic testing, such as next-generation sequencing, may also have influenced decisions over time(19,20). Previous research has already shown an increased diagnostic yield over time in fetuses with limb anomalies (21,22). In our cohort, genetic testing was performed in 58 of the 66 (87%) cases with an isolated reduction defect, and genetic abnormalities were detected in 4 (7%). One of these was a pathogenic variant in *FGFR2*, and the parents opted for a TOP. This finding indicates that increased TOP in this group is likely not only driven by genetic findings, but rather by severity and functional implications. Moreover, TOP rates were similar among isolated cases of reduction defect with a normal (24.1%) and abnormal (25%) genetic outcome.

Societal and cultural factors may further shape decision-making. A study performed in Isreal found that religious affiliation significantly impacts TOP decisions, with non-religious parents being 10 times more likely to opt for TOP after a prenatal diagnosis of congenital heart disease (23). Societal attitudes with less tolerance for imperfection and parental perceptions regarding quality of life with limb deficiencies might have shifted over the study period, leading to less tolerance for reduction defects, even though many upper limb anomalies allow for correction with prosthesis and for a good quality of life (10,24,25,26).

Furthermore, parents undergoing the decision-making process often experience significant emotional distress and require tailored support. Studies have emphasized the importance of sensitive, timely, and multidisciplinary counseling in supporting parents during this process (23,27). In addition, a positive family history may influence decision-making. Ding *et al*. (2022) found that couples with a family history of congenital anomalies were approximately 15 times more likely to opt for termination of pregnancy in case of fetal facial deformities (28).

The prenatal detection of upper limb anomalies remains challenging. Visualization is highly dependent on maternal and fetal factors (e.g., BMI, fetal movement, amniotic fluid volume) (29). Moreover, anomalies without skeletal involvement – such as soft-tissue syndactyly or non-skeletal polydactyly – can be especially difficult to detect. This is consistent with findings from a large study in Washington, where detection rates were highest for major upper limb anomalies (70–100%), and lower for isolated digital anomalies (4–19%) (30). This is also in line with the findings of our previous cohort study, where prenatally missed upper limb anomalies were isolated and mostly minor defects of the hand and digits (15).

Despite the high coverage of prenatal screening in the Netherlands, there remains room for improvement in the prenatal detection of upper limb anomalies. The FTAS, performed between 12-14 weeks' gestation, was implemented in research setting in the Netherlands in September 2021 (4). Although visualization of the digits is also not mandatory for this first trimester anomaly scan, incidental findings of upper limb anomalies may become more frequent and earlier in the pregnancy. The period between the late first trimester and the early second trimester is considered optimal for evaluating the upper limbs, particularly the fetal digits (31). At this stage, the fetal hands are more often opened, providing a more accurate assessment of the digits.

Given that a notable number of upper limb anomalies in this study were visualized between 12-15 weeks, incorporating a detailed assessment of the fetal upper limbs in the protocol of the FTAS seems to be beneficial for an earlier prenatal detection. This is in line with our findings that the median GA at diagnosis has been further decreased to 14.9 weeks since its introduction. In line, an earlier diagnosis may have contributed to the observed decrease in gestational age at termination in the period after the introduction of the FTAS. The timing of diagnosis can play a critical role in parental decision-making. Earlier identification of structural anomalies allows more time for multidisciplinary counseling, additional genetic testing, and careful deliberation prior to reaching the legal gestational limit for termination. Previous studies have suggested that earlier termination is associated with lower levels of grief, anxiety, and psychological distress in parents (5,32,33).

Providing counseling early in pregnancy ensures that parents receive timely support and information, enabling them to make a well-informed and autonomous decision. Clelland *et al*. reported that 73% of parents who received a prenatal diagnosis of an upper limb anomaly felt inadequately supported after a diagnosis that was unexpected (34).

A multidisciplinary approach is essential, ensuring the parents receive comprehensive information regarding the possible underlying cause, e.g. by a clinical geneticist, the impact of an upper limb anomaly on a child's quality of life, and the potential postnatal treatment options, e.g. by a rehabilitation doctor and plastic surgeon (34,35).

The strength of this study is the use of a large, regionally representative database, providing a reliable overview of upper limb anomalies in the North-West Netherlands. The long study period, spanning over more than two decades, enables meaningful time trend analyses. Despite these notable strengths, the study has limitations that should be acknowledged. One of the primary weaknesses is the relatively small sample size of cases diagnosed before the introduction of the STAS (n=37) and after the FTAS (n=47). Furthermore, some anomaly groups were underrepresented despite the inclusion of cases over two decades, particularly there were small numbers for syndactyly (n=23). The small sample size for syndactyly may account for the contrasting trends observed in this group. The limited sample size reduces the reliability and statistical power also for isolated polydactyly, with one TOP. In addition, this study did not focus on postnatal outcome, limiting our ability to evaluate the efficacy of prenatal ultrasound in diagnosing upper limb anomalies.

Conclusion

This study offers new insights into the potential impact of the introduction of fetal anomaly scans on TOP rates for upper limb anomalies in the North-West region of the Netherlands. The implementation of STAS and FTAS was associated with earlier prenatal detection of these anomalies. However, no consistent or marked changes in overall termination rates were observed across the three time periods. Exploratory analyses suggest a possible upward trend in TOP for isolated reduction defects, although this finding should be interpreted with caution and warrants further investigation.

Additional File 1. Abnormal genetic findings per anomaly group. Genetic test outcomes of cases seen between 2007-2021 are described in: Arduç A, van Dijk SJB, Ten Cate FJ, van Doesburg MHM, Linskens IH, van Leeuwen E, van Maarle MC, Pajkrt E. Phenotype-to-Genotype Description of Prenatal Suspected and Postnatal Discovered Upper Limb Anomalies: A Retrospective Cohort Study. Prenat Diagn. 2025 Jan;45(1):3-14. doi: 10.1002/pd.6714. Epub 2024 Nov 29. PMID: 39613947; PMCID: PMC11717735

Anomaly type	Abnormal results (n)	Outcome	Number
Reduction defect	24	Trisomy 18 Cornelia de Lange syndrome Trisomy 21 TAR syndrome (i) Jeune syndrome Tetrasomy chromosome 9p Fanconi anemia Triploidy (maternal) PIK3CA-Related Overgrowth Spectrum Klinefelter syndrome (i) Holt- Oram syndrome FGFR2 (i)	9 3 2 2 1 1 1 1 1 1 1 1
Syndactyly	9	FGFR2 Apert syndrome Triploidy (paternal) Greig syndrome Trisomy 13 Triple X syndrome Triploidy (maternal) Coffin-Siris syndrome	2 1 1 1 1 1 1 1
Polydactyly	74	Trisomy 13 Trisomy 18 Trisomy 21 Meckel-Gruber syndrome Ellis-van Creveld syndrome Greig syndrome Bardet-Biedl syndrome Turner syndrome Paternal triploidy Neurofibromatose type 1 Copy number variant - Deletion 1.2 Mb from a part of the q-arm of chromosome 1 duplication of 11.7 Mb from 8p23.3p23.1 - Deletion of 8.2 Mb from 15q26.1q26.3	49 6 1 3 2 2 3 1 1 1 2

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CHAPTER 4

A practical prenatal ultrasound classification system for lower limb anomalies –PRELLIM classification.

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Abstract

Objective To address the current lack of a prenatal classification system for fetal lower limb anomalies, we developed and evaluated the PRELLIM (PREnatal Lower Limb IMpairment) classification.

Method A systematic literature review was conducted to identify existing classifications. Based on sonographic features, we developed the PRELLIM classification and applied it to a retrospective cohort of fetuses with isolated lower limb anomalies assessed between 2007 and 2024 at Amsterdam UMC's fetal medicine unit.

Results No standardized prenatal classification system for lower limb anomalies was found. PRELLIM distinguishes isolated and non-isolated anomalies and categorizes them into clinically relevant subgroups (absent/short, duplication, fusion, contracture, bowing and other). It was applied to 643 fetuses with isolated lower limb anomalies. Contractures were most common (n = 599; 93.2%), followed by poly(syn)dactyly (n = 26; 4.0%), reduction defects (n = 9; 1.5%), bowing (n = 5; 0.8%), and a case of sirenomelia (0.1%). Three additional cases (0.4%) were classified as "other": two lymphangiomas and one amniotic band with lower leg constriction.

Conclusion PRELLIM is the first prenatal classification tailored to sonographically detectable lower limb anomalies. It hopes to enhance diagnostic consistency, improve interdisciplinary communication, and support prenatal counseling and decision-making.

Introduction

Congenital anomalies affect approximately 2.5% of newborns, with limb anomalies frequently observed (1-3). Upper limb anomalies exhibit a higher birth prevalence compared to lower limb anomalies, with rates of approximately 3.5 per 10,000 births versus 1.4 per 10,000 births, respectively (4-5). Lower limb anomalies are often unilateral and affect the right side more often in comparison to the left side (5-6). They can appear in isolation or together with other structural anomalies (6).

Most countries offer a mid-trimester scan as part of the routine prenatal care for identifying congenital anomalies (7-8). The International Society of Ultrasound in Obstetrics & Gynecology (ISUOG) Practice guidelines and the Dutch Society of Obstetrics and Gynaecology (NVOG) guidelines include evaluation of the presence and appearance of the arms, hands, legs, and feet, including the joint position (7,8). While upper limb anomalies have been studied – albeit with challenges in detecting minor hand defects – research on lower limb anomalies, apart from clubfoot, is scarce (9-12).

Postnatal classification systems for upper limb anomalies, such as those by Swanson, Frantz and O'Rahilly, and the Oberg-Manske-Tonkin (OMT), have improved diagnosis, communication between healthcare providers, and prognostic counseling (13-16). Some postnatal classification systems can be applied partially prenatally, though visualization remains challenging for certain anomalies (e.g. syndactyly) (9,17-19). However, a standardized prenatal classification does not exist for prenatally suspected lower limb anomalies, leading to inconsistencies in terminology, parental counseling and research.

The main aim of this study was to design a simple prenatal classification for lower limb anomalies, which will aid to improve data collection for research and the prenatal care for affected fetuses and their parents. The consistent terminology used in this classification will enhance parental counseling and the understanding and communication among healthcare professionals. We performed a literature search to confirm the absence of an existing prenatal classification for lower limb anomalies. Furthermore, we propose a new prenatal classification system, the PRELLIM (PREnatal Lower Limb IMpairment) classification, to standardize the diagnosis of lower limb anomalies. To illustrate its clinical utility, we applied the PRELLIM classification retrospectively to a cohort of fetuses with prenatally suspected lower limb anomalies from our fetal medicine unit (FMU).

Methods

Literature review

A literature review on prenatal classification systems of (both upper and lower) limb anomalies was conducted. Pubmed, Medline and Embase databases were searched in June 2024. The Rayyan website, which supports storage, multi-person selection, and grouping of the manuscripts, was used for the selection of manuscripts following a de-duplication process. Two investigators (AA and ILI) completed the screening process on the titles and abstracts, and full texts independently in a blinded fashion. Only English manuscripts with available prenatal sonographic data on limb anomalies were included. Manuscripts that were not selected by both investigators were excluded for this review. Discrepancies between authors were resolved through discussion until a consensus was reached. It was not necessary to consult a third reviewer. The residual manuscripts were assessed again on relevance by both investigators together. The quality of the included studies was not appraised. Data extraction was done by the two investigators.

Prenatal classification

A classification is proposed for prenatally suspected lower limb anomalies in consultation with a clinical geneticist, pediatric orthopedic surgeon, plastic surgeon, and perinatologists (Figure 1). We named it the PRELLIM (PREnatal Lower Limb IMpairment) classification. This system categorizes anomalies into two main groups, based on the ultrasound examination: isolated and non-isolated. Isolated anomalies are defined as anomalies confined to the lower extremities that share a common presumed etiology, regardless of laterality (e.g., bilateral polydactyly). They may also involve the same anomaly in the upper extremities (e.g., polydactyly of all extremities). Anomalies are classified as non-isolated when additional limb anomalies with a different presumed etiology are present (e.g., short extremities and clubfeet), when accompanied by intrauterine growth restriction, or when associated with structural anomalies in other organ systems (e.g., cardiac or cerebral malformations). The isolated anomalies are further divided in absent/short, duplications, fusion, contractures, bowing, and other. Only anomalies that can be visualized by prenatal ultrasound are added into this classification. Each aspect was labeled with a number or letter as goal to describe the anomaly in an easy manner and as short as possible.

Cohort study

In addition to the literature review, we conducted a retrospective cohort study of all fetuses with prenatally suspected isolated lower limb anomalies at the initial targeted anomaly scan, evaluated at the FMU of Amsterdam university medical center (AUMC) between January 2007 and December 2024.

After obtaining approval from the Medical Ethical Committee of AUMC (reference number W21_361 # 21.401), data were extracted from clinical ultrasound reports. Anomalies were classified using the PRELLIM system.

All women were referred to our FMU for a targeted anomaly scan and additional counseling. The following parts of the limbs were assessed during a targeted anomaly scan: the presence and appearance of the arms, hands, legs, and feet, including evaluation of all long bones and the position of all limbs. Additionally, the digits were assessed. The length of the fetal limb bones was assessed by using the charts of Chitty *et al*., 2002 (20).

Results

Literature review

We retrieved 3490 publications with our literature search for prenatal classification for (both upper and lower) limb anomalies. We excluded 3076 studies after screening for duplicates and irrelevant publications based on abstracts. After additional evaluation of the full text, we found no articles on prenatal classification of lower limb anomalies.
However, several postnatal classification systems were identified for limb anomalies. An overview of the most widely used postnatal classification systems for limb anomalies is provided in Table 1 (13-17,21).

Table 1. Overview of major postnatal classification systems for limb anomalies.

Classification system	Year	Authors/ Origin	Basis of classification	Special features	Context
Swanson classification	1976	Swanson	Embryological failures (developmental field defects)	First systematic embryological classification framework	USA
Frantz and O'Rahilly system	1961	Frantzand O'Rahilly	Descriptive anatomical classification	Purely anatomical, lacks developmental/pathogenetic insight	USA
OMT classification	2010, updated in 2020	Oberg, Maske and Tonkin	Embryological and dysmorphological classification	Modern update of the Swanson classification	USA
EUROCAT limb anomaly classification	1986, updated in 2004	European network	Registry-based epidemiological classification	Standardized surveillance across european countries; epidemiological emphasis	Europe

PRELLIM classification

Figure 1 displays the PRELLIM classification. This classification is based on the sonographic aspects which are listed below, as I. Isolated or as II. Non-isolated. Description of the anomaly should always include if the anomaly is unilateral or bilateral and which side is affected(right/left).

In case of multiple affected body parts, for example a lower leg and foot, both numbers of the affected parts should be noted. In addition, in case of multiple different limb anomalies, each anomaly should be described separately (e.g., polydactyly and reduction defect).

I. Isolated

In case of isolated anomalies, no additional structural anomalies are suspected by ultrasound. The isolated anomalies of the lower limbs are divided in the following anomalies:

1) Absent/short: in case of aplasia or hypoplasia of skeletal structures of the limb, which is also called a reduction defect. Prenatally, it is challenging to distinguish the malformations (e.g., caused by a genetic disorder) from the deformations (e.g. caused by excentric problems such as amniotic bands). Therefore, it should be described in the same manner.

A. A reduction defect is longitudinal when the long axis of a limb is affected. This could affect the lower leg (a) or the foot (b), and it can be further divided into:

- i) Tibial/preaxial when the side of the tibia is affected (in case of the foot with or without involvement of second toe), for example a tibial hemimelia.
- ii) Fibular/postaxial when the side of the fifth toe is affected (in case of the foot with or without the fourth toe involved), for example a fibular hemimelia.
- iii) Central when the central ray is affected (split foot malformation).

A longitudinal reduction of the femur does not occur, as any reduction defect in the upper leg always involves either the proximal or distal end of the femur, rather than affecting its long axis.

B. A reduction defect is transverse when the limb (distal or proximal leg and/or foot) is absent, when the terminal part of the limb is completely missing. A transverse defect can affect the upper leg (a), lower leg (b) or the foot (c). When some part of the limb is missing but the terminal part is present, it is called intercalary. For example, a proximal femoral focal deficiency (PFFD) is classified as an intercalary reduction defect of the upper leg, characterized by absence or hypoplasia of the proximal femur with preservation of distal structures.

In case of an intercalary defect such as this, the part of the affected limb should be mentioned separately according to the PRELLIM classification (I.1.B.d.femur.uni).

2) Duplication: in case of a duplication of the upper leg (a), lower leg (b), or foot (c). The occurrence of complete or partial extra digit(s), with or without a bony content, can further be divided into:

i. Polysyndactyly refers to the combined presence of polydactyly as well as syndactyly.
ii. Fibular/postaxial polydactyly when another digit is on the fibular side, after the fifth toe.
iii. Central polydactyly in case of a duplication of one or more toes in the central rays of the foot, typically involving the second, third, or fourth toe.

3) Fusion: a (partial or complete) fusion could occur of the upper leg (a), lower leg (b), and foot (c). Fusions of the upper leg and lower leg are rare (e.g., sirenomelia), but fusion of the toes, a syndactyly, is a frequently observed anomaly in newborns. It is an anomaly which is challenging to identify sonographically in the prenatal period.

4) Contracture: refers to the permanent tightening or shortening of muscles, tendons, or other soft tissues, leading to an abnormal position of the affected joint, stiffness, and restricted movement in the joint. Contractures can be a key prenatal finding, particularly in conditions such as arthrogryposis multiplex congenita (AMC), that are classified as non-isolated in our classification.

A contracture could involve the hip (a), knee (b), ankle (c), and foot (d). A clubfoot, also talipes equinovarus, is a contracture in the ankle (c) and it is one of the most common observed congenital birth defects in newborn. It is characterized by a foot that is fixed in a position of adduction, supination, and varus. Consequently, the foot typically turns inward, resulting in a club-like appearance. This condition involves subluxation of the talocalcaneonavicular joint and is often accompanied by underdevelopment of the soft tissues on the medial side of the foot, as well as hypoplasia of the calf and peroneal muscles.

Clubfeet have a wide spectrum from mild to severe (22). A calcaneovalgus foot deformity is another deformity of the ankle, which can be seen in fetuses with a posteromedial tibial bowing.

5) Bowing: refers to an abnormal curvature of the long bones. The upper leg (a) or the lower leg (b) can be involved. Bowing is a nonspecific indicator linked to various conditions with differing prognoses. In case of a fibular a/hypoplasia, it could be observed together with an anteromedial bowing of the tibia and a leg length discrepancy. Posteromedial Tibial Bowing is another congenital condition thought to be a result of intrauterine positioning that typically presents with a calcaneovalgus foot deformity and with a leg length discrepancy. Bowing of the foot does not exist since it is not a singular long bone.

6) Other: there are plenty other types of isolated lower limb anomalies. Other rare conditions, soft tissue deformities as lymphangioma, and other anomalies as macrodactyly (=overgrowth of the bone and soft tissue) could be classified in this group. These anomalies should be described by specifying the affected part, side, and appearance.

II. Non-isolated

Multiple sonographic detectable anomalies are listed as non-isolated. In case of multiple lower limb anomalies, it is important to describe all anomalies separately.

Cohort study

During the study period, 1,573 fetuses were suspected of having lower limb anomalies; 643 (40.9%) were classified as isolated and 930 (59.1%) as non-isolated.

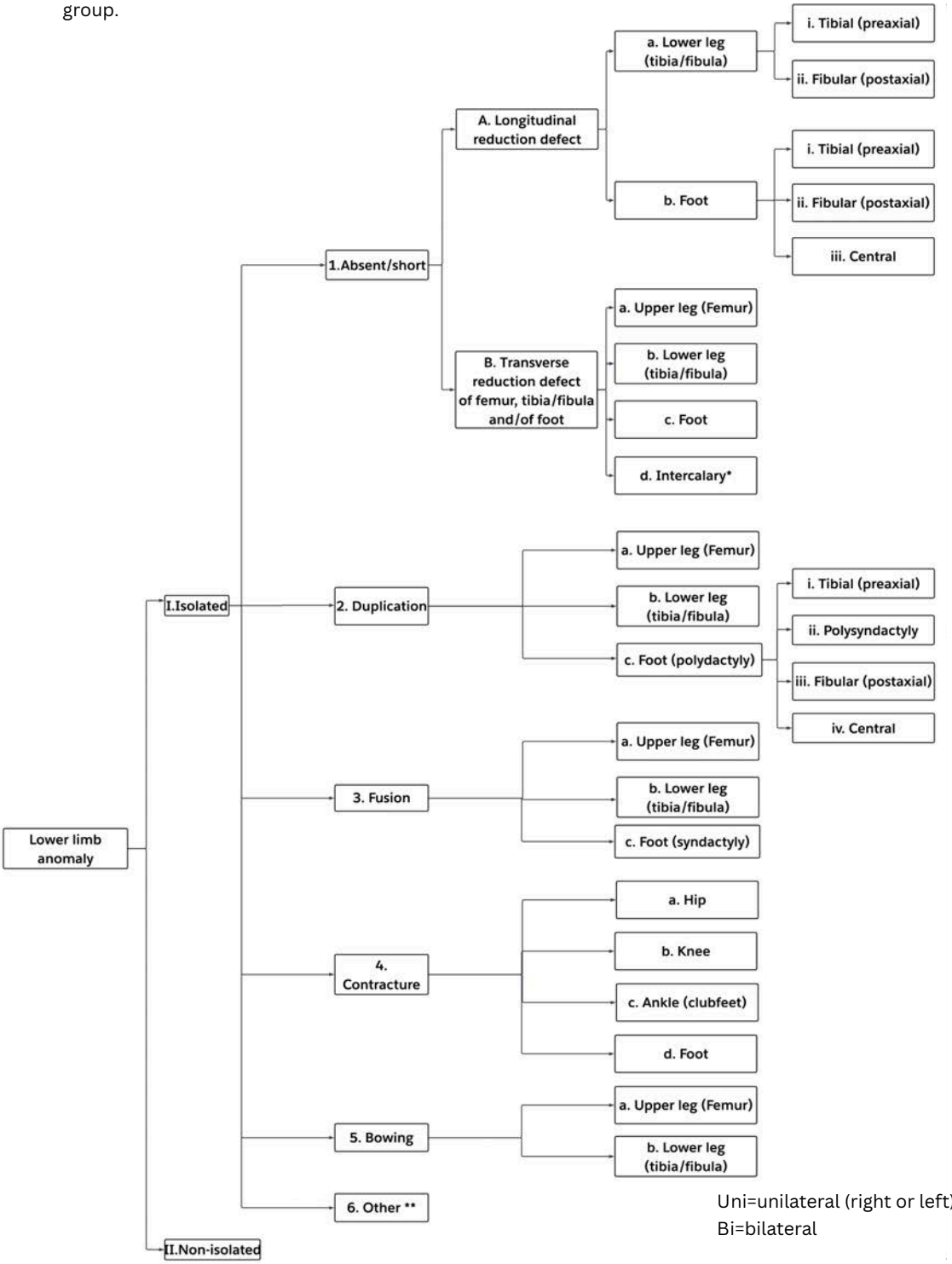
Among isolated cases, contractures were most common (n = 599; 93.2%), nearly all diagnosed as clubfoot (n = 597; 99.7%), including 266 unilateral and 331 bilateral cases, and two as extended knee contractures (0.3%). Other isolated anomalies included duplication (n = 26; 4.0%), absent/short limbs (n = 9; 1.5%), bowing (n = 5; 0.8%), fusion (n = 1; 0.1%), and three cases (0.4%) categorized as "other" (two lymphangiomas and one amniotic band with lower leg constriction). All duplication cases involved polydactyly: 22 fibular (postaxial; 84.6%), two tibial (preaxial; 7.7%), and two polysyndactyly (7.7%). The nine reduction defects were divided into longitudinal (n = 5; all fibular) and transverse types (n = 4), including two transverse cases showing intercalary defects of the lower leg. Bowing was suspected in five cases: one involving the femur and four affecting the lower limbs. The single case of fusion was consistent with sirenomelia. Detailed sonographic findings are provided in Additional File 1.

Discussion

The PRELLIM classification has been developed specifically for lower limb anomalies detectable in the prenatal period. It will facilitate a clearer communication in both a clinical and research setting, and a more effective parental counseling in case of lower limb anomalies.

This study underscores the importance of the targeted anomaly scans in detecting lower limb anomalies, distinguishing between isolated and non-isolated cases. During the study period, lower limb anomalies were suspected in 1,573 fetuses, of which 643 (40.9%) were classified as isolated. The majority of these isolated cases (93.2%) consisted of contractures, mainly diagnosed as clubfoot. The distribution of clubfoot cases was evenly split between unilateral and bilateral presentations.

Figure 1 Suggested PRELLIM classification. In case of bilateral, each limb should be described separately.
* In case of an intercalary reduction defect, the part of the affected limb should be mentioned separately.
** There is a long list of other rare and non-rare limb anomalies that could be included in the other group.



Uni=unilateral (right or left)
Bi=bilateral

Clubfoot is among the few lower limb anomalies with a relatively high prenatal detection rate, due to its sonographic features, such as persistent inward rotation of the foot and abnormal angulation of the ankle joint (23,24). Two postnatal classification systems which are widely used in the assessment of clubfoot severity are the Dimeglio classification and the Pirani scoring system (25,26). The PRELLIM classification complements these systems by providing a structured and standardized approach for describing clubfoot in the prenatal setting. While it does not quantify severity, PRELLIM helps to differentiate between for example the wide spectrum of clubfoot and conditions with similar sonographic appearances, for example posteromedial tibial bowing. In line, limb reduction defects need to be distinguished from other limb anomalies such as symmetrical shortening of the long bones in case of e.g. achondroplasia, an intra-uterine growth restriction, or a short femur length biometry in fetuses with trisomy 21.

Posteromedial tibial bowing is a rare but distinct congenital deformity characterized by curvature of the tibia with the apex directed posteriorly and medially (27). It can also result in abnormal positioning of the lower limb besides the limb-length discrepancy, which can mimic the sonographic appearance of clubfoot. Differentiating these entities is crucial, as postnatal management differs: posteromedial bowing often improves spontaneously and is managed conservatively, while clubfoot requires Ponseti treatment with serial casting (28,29).

Another prominent subgroup of lower limb anomalies in our cohort consisted of duplications (n = 26), all of which were classified as poly(syn)dactylies. Most cases (n = 22; 84.6%) involved fibular (postaxial) polydactyly, which is typically not associated with underlying genetic disorders (30). In contrast, tibial (preaxial) polydactyly (n = 2; 7.7%) and polysyndactyly (n = 2; 7.7%) were less common but carry greater clinical relevance due to their stronger association with genetic conditions (30). When tibial polydactyly is detected prenatally, a thorough examination for additional anomalies is strongly recommended, for example to search for specific craniofacial features which could be seen in fetuses with Greig syndrome (30). However, for isolated cases with tibial polydactyly, the detection rate of causative pathogenic variants remains limited with genetic testing (30).

The other anomaly groups in our cohort were relatively small. Only nine cases (1.5%) were classified as reduction defects, representing a lower proportion compared to our previous cohort on upper limb anomalies, with 50 of the 104 (48%) isolated upper limb anomalies were reduction defects (9). This finding is consistent with prior studies reporting a higher ratio in the prevalence of upper versus lower limb anomalies, with an upper: lower limb ratio of 2:1 (31).

The quality of ultrasound diagnostics can be limited by factors such as fetal position and maternal abdominal wall thickness and the limited visibility of soft tissue structures (e.g. vascular malformations), which can hinder the accurate detection of anomalies (32,33). For example, syndactyly of the upper limb remains challenging to detect in the prenatal period and therefore the majority will be not seen during the routine mid-second trimester scans (9). It is likely that syndactyly of the lower limb is even more challenging to detect due to its less distinct anatomical presentation and limited clinical attention. Postnatally, physical examination and tests such as X-rays and magnetic resonance imaging (MRI) are available to confirm anomalies (9).

A prenatal diagnosis of lower limb anomalies knows many advantages. Firstly, it allows parents to receive detailed information about their unborn child's condition. Parents can receive prenatal counseling from relevant pediatric specialists, such as orthopedic and plastic surgeons. This includes understanding the nature of the anomaly, potential outcomes, and available treatment options. A previous study showed that parents prefer to learn about their child’s limb anomaly before birth (34). Invasive tests can be offered to determine the etiology and assess the risk of recurrence in future pregnancies. Secondly, a prenatal detection enables parents to make informed decisions about continuing or terminating the pregnancy within the local legal time frame. Thirdly, prenatal diagnosis can help in planning the place of delivery and the necessary (direct) postnatal treatment. For example, in the Dutch system, a home birth is optional in case of an uncomplicated pregnancy.

PRELLIM simplifies and standardizes prenatal terminology. This classification framework minimizes the risk of errors, making it more practical and reliable for clinical application. However, it is based only on sonographic features, unlike embryology-based systems like OMT. Furthermore, the main focus of this study was on isolated cases. Sonographers may tend to be more vigilant and thorough in their assessment when a non-isolated anomaly is already detected, prompting a more detailed evaluation for other abnormalities. The spectrum of non-isolated is wide and knowns many causes (35-41). Therefore, detailed ultrasound examination of the head, thorax, spine, and limbs is needed (36).

A limitation of this study is the absence of systematic postnatal follow-up data for all included cases, which precludes confirmation of the prenatal diagnoses and assessment of the classification’s true diagnostic accuracy. The classification should be evaluated in the future concerning anomalies that may not be adequately represented in the current version. It remains essential to compare postnatal findings with the prenatally suspected anomalies.

To improve this comparison, standardized documentation, including detailed imaging and intra- and inter-rater reliability assessments should be encouraged. In conclusion, the PRELLIM classification for lower limb anomalies is designed to simplify a prenatal diagnosis, management, and parental counseling. This approach will facilitate also a clearer interdisciplinary communication and will facilitate the comparison of outcomes in both a clinical and research setting.

Additional File 1. Sonographic Findings Classified by PRELLIM Code

Main Category	Description	PRELLIM Code
Absent/short 1	Shortened right lower leg with a single visible bone, presumed to be the tibia. The tibia is hypoplastic. The foot displays only three rays (hallux and two toes), with absence of the fibular-side digits. The foot is abnormally positioned and shows plantar flexion.	I.1.A.a.ii.uni
Absent/short 2	The right fibula appears absent. The right tibia is shortened and demonstrates an abnormal, angulated contour.	I.1.A.a.ii.uni
Absent/short 3	The left foot shows a reduction defect characterized by partial absence of the forefoot, predominantly affecting the toes. The hallux may still be present.	I.1.B.c.uni
Absent/short 4	Bilateral fibular agenesis with shortened tibiae. The right femur is bowed, and the tibia is abnormally positioned outside the leg's normal contour. Both feet are present.	I.1.A.a.ii.bi
Absent/short 5	Intercalary reduction defect involving the left lower leg.	I.1.B.d.tibia/fibula.uni
Absent/short 6	Marked shortening of the left tibia and fibula, with a posteriorly located structure suggestive of a severely hypoplastic foot. No ossified elements are identifiable within the foot structure.	I.1.B.d.tibia/fibula.uni
Absent/short 7	Right-sided pes equinovarus with concurrent abnormalities of the left lower leg and foot. Only a single bone is visible in the left lower leg (<5th percentile), and the foot is abnormally positioned.	I.1.A.a.ii.uni
Absent/short 8	Mild shortening of the right femur compared to the left. The right tibia is dysmorphic, possibly bowed or fractured, and the fibula is absent. The right foot has three rays and is in inversion. The other limbs and long bones appear normal in echogenicity and shape.	I.1.A.a.ii.uni
Absent/short 9	Both the tibia and fibula on the left side are below the 5th percentile in length. The left foot shows a severe positional deformity, with an extremely flexed configuration.	I.1.B.d.tibia/fibula.uni
Duplication 1-22	Fibular polydactyly observed in 22 cases: 7 unilateral (5x left, 2x right), 15 bilateral.	I.2.c.iii.uni I.2.c.iii.bi

Additional File 1. (continued)

Duplication 23-24	Tibial polydactyly observed in 2 cases, both right sided.	I.2.c.i.uni
Duplication 25-26	Polysyndactyly observed in 2 cases, both bilateral.	I.2.c.ii.bi
Fusion 1	Fusion of both lower limbs, consistent with sirenomelia.	I.3.a,b,c.bi
Contracture 1-597	Clubfoot spectrum observed in 597 cases: 266 unilateral (116x left, 150x right), 331 bilateral.	I.4.c.uni I.4.c.bi
Contracture 598	Both knees are hyperextended. Hallux valgus is noted bilaterally. All ten toes are present. Both hands have normal positioning with five fingers each. Bone echogenicity and thoracic shape are	I.4.b.bi
Contracture 599	Both knees are extended without observed flexion, possibly indicating contractures. Other joints appear normal.	I.4.b.bi
Bowing 1	Unilateral hypoplasia of the right tibia and fibula, associated with a clubfoot deformity and bowing of the affected limb.	I.5.b.uni
Bowing 2	Abnormal positioning of the right ankle and foot, with visible bowing deformity. Postnatal evaluation confirmed posteromedial bowing.	I.5.b.uni
Bowing 3	Posteromedial tibial bowing on the left foot.	I.5.b.uni
Bowing 4	Right-sided reduction defect with shortened tibia and fibula (6–13th percentile), compared to normal length contralaterally (35–60th percentile), and inverted foot position. The foot itself	I.5.b.uni
Bowing 5	The left femur appears bowed. No evidence of fracture is observed.	I.5.a.uni
Other 1	Lymphangioma involving the right axilla and neck, extending into the mediastinum and upper right thorax. Similar lesions are present in the left upper and lower leg. The trachea is not	I.6.uni
Other 2	The left foot appears abnormal with anterior soft tissue edema. Findings suggest strangulation by the umbilical cord around the foot.	I.6.uni
Other 3	Cystic lesion in the right lateral thigh, measuring initially 15 × 29 × 26 mm with a septation and no vascular flow. No connection to the femur; lesion appears subcutaneous. No skeletal anomalies	I.6.uni

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PART II

PRENATAL IDENTIFICATION OF CONTRACTURES

CHAPTER 5

Can prenatal ultrasound and genetic testing reliably exclude non-isolated clubfoot?

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Abstract

Objective To evaluate the efficacy of prenatal second trimester ultrasound in diagnosing isolated congenital clubfoot and to assess the role of prenatal genetic testing.

Method We conducted a retrospective cohort study in the North-West region of the Netherlands with prenatally suspected clubfoot between 16 and 24 weeks of gestation from 2007 to 2021. We included isolated cases, defined as no additional structural anomalies on the initial targeted ultrasound. Rapid aneuploidy testing, chromosomal microarray analysis and/or exome sequencing was performed via invasive testing following on parental request.

Results We identified 423 cases of isolated clubfoot. The diagnosis changed to prenatal non-isolated clubfoot during prenatal follow-up in 20 cases (5%); in 10 cases during a follow-up ultrasound and in 10 cases an underlying genetic condition was found. In 11 cases, the initial suspicion of clubfoot was not confirmed at follow-up ultrasound. There were 387 ongoing pregnancies with a prenatal diagnosis of isolated clubfoot. In 47 children (12%) diagnosis changed postnatally to non-isolated. These postnatal findings were classified as major in 36 children (9%). In 40 cases (10%), the prenatal diagnosis of clubfoot was not confirmed postnatally.

Conclusion Prenatal ultrasound combined with genetic testing are components in the work-up of clubfoot, enabling the identification of associated structural anomalies and underlying genetic disorders. Despite advances in prenatal ultrasound and genetic testing, distinguishing isolated clubfoot from cases with additional structural or genetic anomalies remains challenging. Moreover, prenatal genetic testing does not exclude the absence of structural or neurodevelopmental issues diagnosed after birth.

Introduction

Congenital clubfoot (talipes equinovarus) is a deformity of the foot and ankle resulting in adduction, supination, varus, and equinus position. The severity of this deformity ranges from positional clubfoot that resolves spontaneously postnatally, to those requiring multiple interventions with disability persisting into later life (1). Clubfoot can equally manifest unilaterally or bilaterally. In most cases, it occurs as an isolated condition but may also be associated with additional structural anomalies or chromosomal and genetic syndromes.

Despite clubfoot being one of the most common congenital anomalies (1.13 per 1,000 births), its aetiology is still largely unknown (2). Isolated clubfoot is thought to be a combination of genetic and environmental factors. Polygenic inheritance is likely, given the elevated prevalence of clubfoot in some populations and the male-to-female ratio of 2:1 (3, 4). The causes of clubfoot in combination with other anomalies include a broad range of syndromes, such as arthrogryposis multiplex congenita (AMC), or structural anomalies, such as neural tube defects (1).

Prenatal screening by ultrasound for fetal anomalies has become routine prenatal care in many countries (5). Standardized use of ultrasonography, along with improving ultrasound technology, has resulted in increased prenatal detection of clubfoot, from around 10% in the 1990's to 30-77% at present (2, 6-8). Prenatal diagnosis enables parents to opt for diagnostic evaluations before birth and supports informed reproductive choices. It also helps parents and caregivers prepare for postnatal management, which consists of manipulation, serial casting, and Achilles tendon tenotomy, followed by bracing (9).

The prognosis of prenatally detected clubfoot is mainly dependent on the presence or absence of other structural anomalies and underlying chromosomal or genetic syndromes, as children with isolated clubfoot generally have a good prognosis (10, 11). Previous research showed that additional structural anomalies were identified at targeted prenatal anomaly scans in around half of the cases (8, 12). However, it is known that prenatally classified isolated clubfoot can have additional structural anomalies or syndromes detected at birth in up to 13% of cases (1, 13, 14). These can be mild deformities of the digits, but also serious (genetic) conditions (15). Offering invasive prenatal genetic tests can help to detect chromosomal and monogenic conditions both in isolated- and non-isolated clubfoot. Recent studies have reported a yield for prenatal chromosomal microarray testing between 1.9% and 3.7%, showing an increased risk for chromosomal abnormalities compared to cases without structural anomalies (16, 17).

Nevertheless, it is important to note that not all genetic tests are universally available, and international consensus is lacking on which should be offered (11, 17, 18).

In this study, we have evaluated the efficacy of prenatal ultrasound examination in case of congenital clubfoot in the North-West Region of the Netherlands and present our experience with prenatal genetic testing in case of prenatally detected isolated clubfoot. Our main outcome is to evaluate the accuracy of prenatal targeted ultrasound in distinguishing isolated and non-isolated clubfoot and the predictive value of prenatal targeted ultrasound for isolated clubfoot. Secondary objectives included determining the yield of prenatal genetic testing, assessing the impact of laterality, describing the postnatally identified non-isolated cases, and pregnancy outcomes.

Methods

A retrospective analysis of medical records of all fetuses with prenatally suspected clubfoot, at the two Fetal Medicine Units (FMU) of Amsterdam University Medical Centers (Amsterdam UMC) between January 2007 and December 2021 was performed. The two FMUs, Amsterdam Medical Center (AMC) and the VU Medical Center (VUMC), assessed all prenatally detected fetal anomalies in the North-West region of the Netherlands.

Dutch prenatal care system
The Dutch government introduced the second-trimester anomaly scan in 2007 for all low-risk women as part of a national screening program. Trained sonographers perform the scan according to a strict scanning protocol (19). Sonographers in the North-West of the Netherlands refer all pregnant women with a prenatally suspected fetal anomaly, including clubfoot, to the FMU of the Amsterdam UMC for advanced targeted ultrasound examination.

Prenatal diagnosis of clubfoot
The ultrasound diagnosis of clubfoot is made if both the long bones of the lower leg (tibia and fibula) are seen in the same plane as the sole of the foot throughout the whole examination. Clubfoot is considered isolated in case no other structural anomalies are present during the initial targeted ultrasound examination, with the exception of prenatal soft markers (e.g. single umbilical artery) (19).

At the time of clubfoot diagnosis, invasive genetic testing is offered to all parents, usually following consultation with a clinical geneticist. After initial ultrasound diagnosis parents are offered a follow-up ultrasound, in the second and/or third trimester of pregnancy, depending on the time period.

Additionally, parents receive information on postnatal prognosis and treatment from a pediatric orthopaedic surgeon. Decision-making regarding continuation or termination of the pregnancy is up to 24 weeks in the Netherlands, as regulated by law.

Inclusion and exclusion criteria
All fetuses in the second trimester (between 16 and 24 weeks of gestation) diagnosed with isolated clubfoot were included in the study. We excluded cases with non-isolated clubfoot, diagnosed at the initial targeted ultrasound examination at our FMU. We excluded cases diagnosed at earlier gestational ages because clubfoot cases are then often either transient and disappear during early development, or coincide with severe multiple congenital and chromosomal anomalies (20). We excluded cases diagnosed after 24 weeks gestation, as these cases were not diagnosed during the routine care window for fetal anomaly screening in second trimester, and have a different diagnosis and management. In addition, we excluded cases with a clubfoot diagnosis in the presence of anhydramnios due to premature rupture of membranes or oligohydramnios due to placental insufficiency. Forty cases of bilateral clubfoot included in this analysis have been previously described elsewhere (15).

Study groups
Clubfoot classification was as follow:

- **Prenatal isolated and non-isolated clubfoot:** primary classification of *prenatal isolated clubfoot* was based on the absence of additional findings during the initial targeted ultrasound. Reclassification to *prenatal non-isolated clubfoot* was made if additional findings were detected during follow-up ultrasounds in the second or third trimester, or if abnormal genetic results were obtained.

A further subclassification into *minor* and *potential major anomalies* was applied.
– *Minor anomalies* were defined as those with a low impact on quality of life and neurodevelopment in cases of multiple structural anomalies.
– *Major anomalies* were defined as those with a potentially high impact, such as requiring (major) surgical intervention or being associated with neurodevelopmental delay. Including al genetic and syndromal anomalies.

- **Postnatal isolated and non-isolated clubfoot:** classification of postnatal isolated and non-isolated clubfoot was based on physical examination, genetic test results, and/or developmental delay. Postnatal non-isolated clubfoot was further subclassified as:

– chromosomal,
– monogenic,
– multiple structural anomalies, or
– syndromic.

A subclassification into *minor* and *potential major anomalies* was again applied. Children were classified as *syndromic* if they presented with multiple structural anomalies—often in combination with neurodevelopmental delay—without a conclusive genetic result (unsuccessful, inconclusive, or normal based on the knowledge available at the time) or if genetic testing was not performed.

For subgroup analyses, *true isolated clubfoot* was defined as cases with a prenatal diagnosis of isolated clubfoot in which (1) no additional structural anomalies were identified on follow-up ultrasound or immediately after birth or known genetic diagnosis, and (2) the diagnosis of clubfoot was confirmed postnatally.

Genetic analysis

Depending on the time period genetic testing could have included fetal karyotyping (until 2013), rapid aneuploidy testing, chromosomal microarray analysis (CMA; since 2012), various genetic tests for specific genetic conditions (e.g. for Myotonic Dystrophy), and/or exome sequencing (ES; since 2021).

Currently, all parents are offered rapid aneuploidy testing, CMA and ES including single nucleotide variant (SNV) analysis in case of isolated clubfoot at initial targeted ultrasound. This was either counseled by the perinatologist and/or clinical geneticist. Genetic test results were reported according to the guidelines of the American College of Medical Genetics and Genomics (ACMG), including pathogenic and likely pathogenic (LP) variants (21). Variants of uncertain significance (VUS) were not reported in prenatal setting unless there was a strong association with the clinical presentation (ultrasound findings or phenotype) or the variant was considered clinically relevant.

The yield of prenatal genetic testing in our study, was determined based on the initial targeted ultrasound assessment of prenatal isolated clubfoot, including all cases that were later reclassified as non-isolated or in which clubfoot was no longer present. A subgroup analysis was performed for true isolated clubfoot.

Data collection

Endpoints were rate of correctly diagnosed isolated clubfoot at the initial targeted second trimester ultrasound including false-positive rates and rate of prenatally unidentified non-isolated clubfoot. Other endpoints were pregnancy outcome and rate of prenatal genetic anomalies. Data regarding the endpoints were collected from electronic patient records. Pregnancy outcome was classified as elective termination of pregnancy, stillbirth or live birth (which includes neonatal deaths within 28 days of birth). Long-term follow-up of children born with clubfoot could only be obtained when children received medical care at Amsterdam UMC. If the electronic patient records were incomplete, we contacted the general practitioners and parents by telephone to obtain information.

Cases in which medical records or telephone questionnaires were not available were marked as loss to follow-up. The study was approved by the local ethics committee (ref. number: W21_361 # 21.401). As data on false-negative prenatal screening for congenital clubfoot were unavailable, this endpoint could not be reported in our study.

Statistics

Poisson regression was used to compute crude prevalence ratios (PR) and 95% confidence intervals (CI), with PRs expressed per 10,000 pregnancies. The total number of pregnancies was determined based on the number of second-trimester anomaly scans conducted within our region from 2007 until 2021. Descriptive statistics, such as percentages and ratios, were employed to characterize the data, with differences assessed using chi-square or t-tests as appropriate. A significance level of $p < 0.05$ was applied for statistical significance. Statistical analyses were conducted using SPSS version 28 (IBM, Armonk, NY, USA).

Results

Study population and prevalence

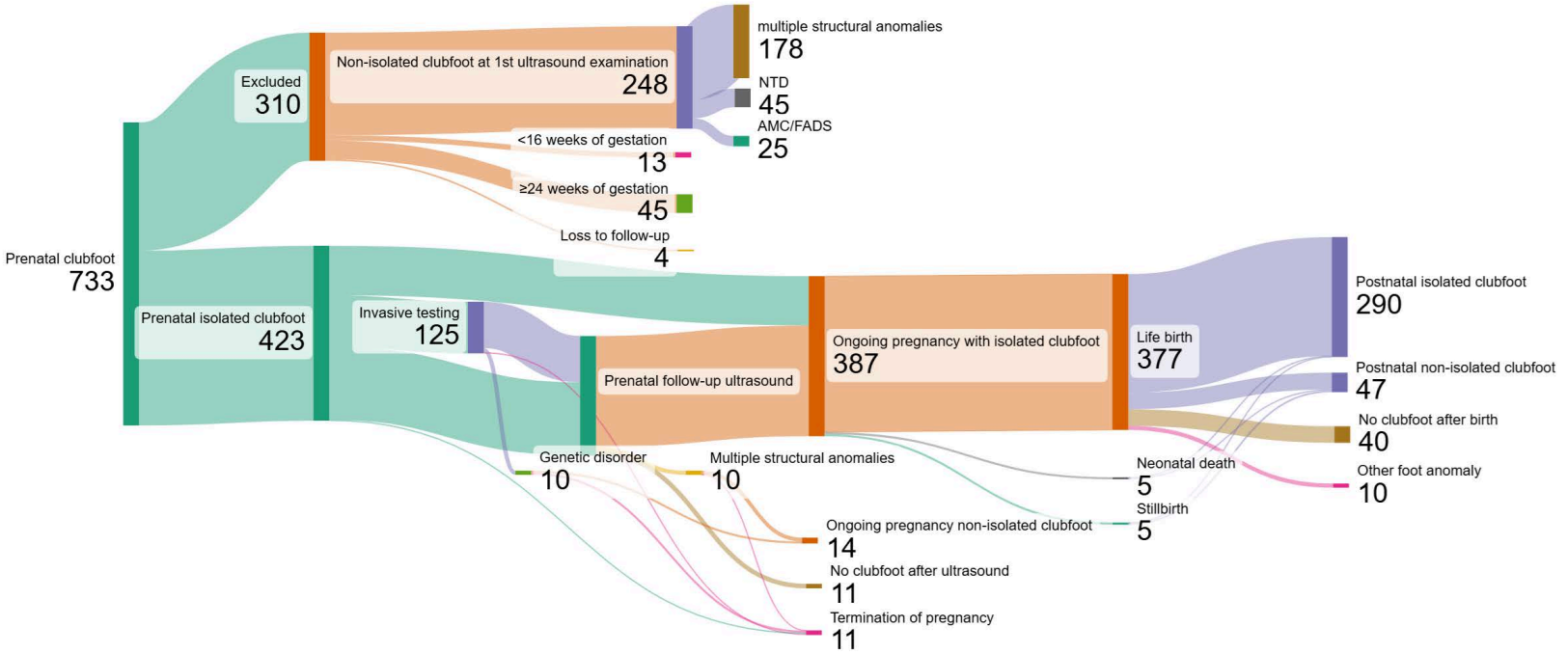
During the study period, approximately 485,000 second-trimester screening ultrasound examinations were performed In the North-West region of the Netherlands. We evaluated 733 cases referred because of a prenatally suspected clubfoot between January 2007 and December 2021 (figure 1). Of the 733 cases, 423 cases (58%) were classified as isolated clubfoot after the initial targeted ultrasound, of which 198 cases (47%) were unilateral and 225 cases (53%) bilateral.

A total of 248 cases (34%) were excluded due to non-isolated clubfoot on initial targeted ultrasound. The remaining cases were excluded because of early or late diagnosis (<16 weeks of gestation, n = 13; >24 weeks of gestation, n = 45). Four cases (1%) were excluded because of loss to follow-up. Based on these data, the estimated prevalence of isolated clubfoot, prenatally diagnosed in the second trimester in our region, is 8.7 per 10,000 pregnancies (95% confidence intervals [CIs]: 8.03 to 9.72). Maternal and pregnancy characteristics are summarized in Table 1.

Table 1. Baseline characteristics of prenatally diagnosed isolated clubfoot.

Characteristics	n= 423	
Maternal characteristics		
Maternal age at diagnosis	31.5	(±5.2)
BMI (kg/m²)	25.5	(±4.8)
Nulliparous	215	(51%)
Family history of clubfoot	52	(12%)
1st degree	23	(5%)
2nd degree	16	(4%)
Pregnancy		
Gestational age at diagnosis (weeks)	20.4	(±1.2)
Singleton pregnancy	391	(92%)
Twin pregnancy, one affected	30	(7%)
Twin pregnancy, both affected	1	(0.5%)
Male/female	273/148 (65%/35%)	
Unilateral/bilateral	198/225 (47%/53%)	

Figure 1. Sankey diagram of prenatal diagnosed clubfoot, including the prenatal diagnostic pathway, outcome of prenatal diagnosis, pregnancy outcome and diagnosis at birth. NTD = neural tube defect, AMC = athrogryposis multiplex congenita, FADS = fetal akinesia deformation sequence.



Prenatal diagnostic findings

Of the 423 fetuses diagnosed with isolated clubfoot after the initial targeted ultrasound, 20 cases (5%) were reclassified as prenatal non-isolated clubfoot during pregnancy (Figure 1). In ten of these cases, additional structural anomalies were detected during follow-up ultrasound examinations, while in the other ten cases an underlying genetic anomaly was identified through invasive testing. The additional structural or genetic anomalies were minor in 4 cases (1%), and (potentially) major in 16 cases (4%). In another eleven cases (3%), the previously abnormal foot position was no longer visible on subsequent ultrasound examinations, and all infants were confirmed to have normal feet at birth, indicating a false-positive second-trimester diagnosis.

Genetic testing and yield

Prenatal invasive genetic testing was performed in 125 of the 423 cases (30%) (Table 2, Appendix A). Parents were significantly more likely to opt for invasive testing in cases of bilateral clubfoot compared with unilateral clubfoot (35%, n = 80/225 vs. 23%, n = 45/198; p = 0.004). Of the 125 cases tested, ten (8%) revealed abnormal results, leading to reclassification as prenatal non-isolated clubfoot. There was no significant difference in diagnostic yield between unilateral (11%, n = 5/45) and bilateral (6%, n = 5/80) clubfoot.

A subgroup analysis limited to cases of true isolated clubfoot, defined as those without additional structural anomalies on follow-up ultrasound or at postnatal examination, showed abnormal genetic test results in seven of 94 tested cases (7%). Chromosomal abnormalities were detected in six cases: two by rapid aneuploidy testing (n=2/94, 2%) and four by chromosomal microarray (n=4/57, 7%). In one case (n=1/20, 5%), a monogenic disorder was identified through prenatal exome sequencing.

Table 2. Outcome prenatal genetic testing in isolated clubfoot.

	Total n= 423	Unilateral n= 198	Bilateral n= 225	p-value
Invasive prenatal testing	125 (30%)	45 (23%)	80 (36%)	0.004
Detected genetic anomalies	10 (8%)	5 (11%)	5 (6%)	0.3
Pathogenic variant detected through				
Rapid aneuploidy testing	3/125 (2%)	1/45 (2%)	2/80 (3%)	1.0
Karyotyping	0/32 (0%)	0/10 (0%)	0/23 (0%)	-
Chromosomal micro-array analysis	5/80 (6%)	3/32 (9%)	2/48 (4%)	0.4
Exome sequencing	2/25 (8%)	1/13 (8%)	1/12 (8%)	1.0

Difference between unilateral and bilateral, tested with chi-square test.

Pregnancy outcomes

Six pregnancies were terminated after reclassification from prenatal isolated clubfoot to non-isolated clubfoot (Appendix B). Additionally, in five cases with prenatal isolated bilateral clubfoot parents decided for pregnancy termination.

Of 423 cases with prenatal isolated clubfoot at initial ultrasound, 387 pregnancies were ongoing and classified as isolated clubfoot after follow-up ultrasound and genetic testing. This included 176 unilateral (45%) and 211 bilateral cases (55%).

Stillbirth occurred in 5 cases (1%), all with bilateral clubfoot. Four were diagnosed with a genetic anomaly post-mortem (see Appendix B and C); for one, the cause of stillbirth was unknown. None of these cases had genetic testing during pregnancy. Consequently, 382 pregnancies resulted in live births, of which five children (1%) died in the neonatal period; two due to extreme prematurity and three due to missed anomalies (see Appendix C).

Postnatal reclassification

Among the 387 ongoing pregnancies, 47 children (12%) were found to have additional anomalies, developmental delay or a genetic anomaly after birth, resulting in reclassification of the diagnosis to postnatal non-isolated clubfoot (table 3 and Appendix C). These additional anomalies were minor in 10 cases (3%), and (potentially) major in 36 cases (9%). In one case, classification was not possible due to uncertain diagnosis of a parent-reported limb anomaly (case 4, appendix C).

Reclassification to postnatal non-isolated was more frequent in bilateral clubfoot (n = 33/211, 16%), than in unilateral clubfoot (n = 14/176, 8%; p = 0.02). In 29 cases (8%) the reclassification was attributed to identification of other structural anomalies or developmental delay, while in another 18 cases (5%), a genetic anomaly was diagnosed after postnatal genetic testing in 28 cases (Appendix A).

In 5 of the 18 cases with a genetic anomaly (27%), invasive prenatal testing with CMA had been performed during pregnancy and had demonstrated normal results. In five cases, a genetic anomaly was diagnosed postnatally that would not have been detected by our current protocol for prenatal genetic testing, including rapid aneuploidy testing, CNV-analysis, and SNV- analysis with exome sequencing. These included Prader-Willi syndrome, Beckwith-Wiedemann syndrome, spinal muscular atrophy, and two cases of myotonic dystrophy type 1. In three clubfoot cases a pathogenic genetic anomaly was detected postnatally, but these were unrelated to clubfoot, and therefore not included in the group with postnatal non-isolated clubfoot based on genetic anomalies. These included variants in *G6PD*, *PMP22* and *PKD1*.

Of the 387 ongoing pregnancies, isolated congenital clubfoot was confirmed postnatally in 290 children (75%). In 141 children (80%) with unilateral and 149 children (71%) with bilateral clubfoot, diagnosis was confirmed. A clinical relevant abnormal position of the foot could no longer be confirmed postnatally in 40 cases (10%). In 27 children (7%) no foot deformity was present, and in 13 children (3%) there was a positional clubfoot, not warranting any postnatal therapy. This results in a positive predictive value for prenatally isolated diagnosed clubfoot of 89% (95%-CIs 86;92). There was no difference between prenatally diagnosed unilateral (n = 18, 10%) and bilateral clubfoot (n = 22, 10%).

In 10 children (3%) another foot anomaly was diagnosed of which 7 needed conservative treatment and/or surgery (see Appendix D). Metatarsus adductus (n = 5) and vertical talus (n = 2) were the most common postpartum diagnosed foot deformities, other than clubfoot.

Table 3. Postnatal diagnosis in case of ongoing pregnancy

	Total n= 387	Unilateral n= 176	Bilateral n= 211	p-value
Isolated clubfoot*	**290 (75%)**	**141 (80%)**	**149 (71%)**	**0.03**
No clubfoot	**40 (10%)**	**18 (10%)**	**22 (10%)**	**0.09**
Other foot anomaly	**10 (3%)**	**3 (2%)**	**7 (3%)**	**0.23**
Postnatal non-isolated clubfoot*	**47 (12%)**	**14 (8%)**	**33 (16%)**	**0.02**
Multiple anomalies	17 (4%)	6 (3%)	11 (5%)	0.70
Chromosomal	9 (2%)	3 (2%)	6 (3%)	1.00
Monogenic	9 (2%)	2 (1%)	7 (3%)	0.70
Syndromic	12 (3%)	3 (2%)	9 (4%)	0.14

Syndromic non-isolated clubfoot: multiple related congenital and developmental anomalies without (known) chromosomal or genetic cause, including children that were not genetically tested. Multiple anomalies: additional anomalies that are (probably) unrelated to clubfoot.

Combined prenatal and postnatal diagnosis

When combining prenatal and postnatal follow-up, of the 423 cases diagnosed with isolated clubfoot at the initial targeted ultrasound, 295 cases (70%) were confirmed postnatally as isolated clubfoot, this includes five pregnancy termination with isolated clubfoot. Genetic testing was performed in 147 cases (n = 147/423, 35%): 125 prenatally and 28 postnatally, of which six already had prenatal genetic testing.

Abnormal genetic test results were found prenatally or postnatally in total in 28 cases (7%) with isolated clubfoot after the initial targeted ultrasound diagnosis. In a total of 67 cases (16%), the diagnosis changed from isolated to non-isolated during either prenatal follow-up or after birth, including abnormal genetic test results. The spectrum of findings responsible for this reclassification included minor anomalies without significant impact (n = 14, 3%) as well as major anomalies and/or genetic syndromes (n = 53, 13%) (Appendix B and C). In 51 cases (12%), the clubfoot diagnosis was rejected, either during prenatal follow-up or postnatally.

Discussion

This large population-based cohort over a 15-year period provides a comprehensive evaluation of the diagnostic accuracy, genetic yield, and outcome of prenatally diagnosed isolated clubfoot. Of the 423 cases classified as isolated clubfoot after the initial targeted ultrasound between 16 and 24 weeks, confirmation of isolated clubfoot at postnatal follow-up was achieved in 70%, in the other cases clubfoot diagnosis was false-positive or changed to non-isolated clubfoot or other foot anomalies during prenatal follow-up or postnatally.

Laterality played an important role in the diagnostic process: bilateral cases were more likely to undergo invasive prenatal testing and were more frequently reclassified postnatally as non-isolated clubfoot. However, laterality was not associated with differences in prenatal genetic yield or in the false positive rate (e.g., cases without clubfoot). Despite advances in prenatal imaging and genetic testing, a substantial proportion of cases (9%) were ultimately reclassified to postpartum non-isolated clubfoot with major additional structural anomalies and/or genetic anomalies. These findings underscore both the strengths and limitations of current prenatal diagnostic pathways and highlight the need for careful counseling regarding prognosis and the role of genetic testing.

In 47 of 387 cases (12%) with ongoing pregnancy with prenatal isolated clubfoot, diagnosis was changed to postnatal non-isolated directly at birth or at follow-up. Children with bilateral clubfoot compared to unilateral are more at risk of having their diagnosis changed to non-isolated postnatally.

In 29 cases (8%) the non-diagnosed non-isolated clubfoot was attributed to identification of other structural anomalies or developmental delay, while in 18 cases (5%), a genetic anomaly was found postnatally. The proportion of postnatal non-isolated clubfoot (n= 47/387, 12%) in our study is on average similar to previous research, but leans towards the higher side (11, 29). This may be attributed to earlier studies predominantly reporting on the presence of additional anomalies immediately postpartum, overlooking anomalies that may emerge later in a child's development. This becomes particularly important when accounting for neurodevelopmental outcomes (11).

In contrast, we observed several postnatal non-isolated clubfoot cases with minor additional anomalies (n=10), which, according to the electronic patient files, had no impact on (neuro)development. Although we reported all cases with multiple structural anomalies identified postnatally, regardless of their relation to congenital clubfoot, it is important to note that minor anomalies are unlikely to affect the prognosis and should be interpreted as such during prenatal counseling.

Another possible explanation for the proportion of postnatal non-isolated clubfoot is the absence of a standardised prenatal follow up programme for isolated clubfoot in the early years of this cohort study. If we examine the yearly proportion of postnatal non-isolated clubfoot cases, we see that in the last two years of the study, the proportion seems to be decreased to 7%.

The prenatal detection of additional anomalies remains a challenge and the possibility of missing additional structural or genetic anomalies is a substantial risk. Therefore, it remains essential to acknowledge certain inherent limitations of ultrasound: The quality of ultrasound is influenced by various factors, including the size and position of the fetus, the maternal body habitus, the resolution of the ultrasound machine, and the skills and experience of the sonographer. Secondly, not all defects can be effectively recognised by ultrasound, especially those that do not manifest as structural or gross functional abnormalities. Additionally, small structural abnormalities pose challenges for accurate prenatal diagnosis, as is the case with hand and foot deformities. (Inter)national guidelines often lack information on detailed assessment of the lower extremities with prenatal ultrasound (5, 32). Detecting more positional deformities in cases of clubfoot is important, as these may contribute to the early recognition of arthrogryposis multiplex congenita and its underlying causes.

Prenatal genetic testing was performed in 125 cases (30% of the total cohort), with abnormal results found in 10 cases (8%). While prenatal genetic testing is offered to all parents in case of isolated clubfoot, the number of invasive procedures and the diagnostic yield are influenced by various factors. Notably, sonographers may —sometimes unconsciously—steer parents toward invasive testing when soft markers or subjective, undefined findings are observed during ultrasound. For example, of the eight chromosomal abnormalities detected via invasive testing, four cases presented with sonomarkers (e.g. short nasal bone, choroid plexus cyst). The significant advancements in genetic testing over the study period (2007–2021) have also affected the rate of invasive procedures performed. The progression from only karyotyping to exome sequencing is evident in our center's data; since the introduction of prenatal exome sequencing for isolated clubfoot in 2021, uptake of invasive prenatal testing has increased, with 22 out of 40 parents (55%) opting for prenatal genetic testing that year.

Most studies report on the usefulness of prenatal CMA in isolated clubfoot (9, 16, 17, 22-25) and few address the added value of SNV-analysis with prenatal exome sequencing (26, 27). Chromosomal abnormalities detected with CMA are reported from 2.2% to 11% in isolated clubfoot and exome sequencing abnormalities in 4.3% to 10.5%. These results are similar with our cohort, with 6% and 8% abnormal test results, for CMA and exome sequencing, respectively. In line with previous studies,

we found no difference in prenatal genetic test results between bilateral and unilateral clubfoot.

Postnatal genetic testing revealed pathogenic variants in 18 cases. While invasive prenatal testing was performed in 31% (n=5/18) of these cases, the test conducted (CMA) was not the appropriate method for detecting the specific genetic syndromes involved. All cases took place before introduction of exome sequencing as prenatal diagnostic test for isolated clubfoot in our center. The question remains whether prenatal exome sequencing will reduce the likelihood of postnatal non-isolated clubfoot or whether physical examination will continue to play a critical role in determining which diagnostic tests should be performed. In our cohort, five cases of postnatal non-isolated clubfoot involved genetic anomalies that would not have been detected through prenatal CMA and exome sequencing, at the time. These findings underscore the importance of counseling parents that both normal ultrasound findings and normal genetic test results do not exclude the possibility of additional anomalies being identified later in pregnancy or after birth. Systematic use of exome sequencing, increases prenatal detection of genetic conditions. However, this approach also raises concerns about uncertain or incidental findings and the interpretation of variants with unclear clinical significance.

On the other hand, routine use of specific gene testing, such as for myotonic dystrophy, has shown limited added diagnostic value because due to the rarity of the disease, highlighting the need to balance potential benefits with clinical relevance and parental impact (16, 28). Careful evaluation of the clinical utility and psychological implications of expanded testing is therefore essential before routine implementation.

We plan a forthcoming study to explore the added value of exome sequencing in isolated prenatal clubfoot, focusing on ongoing challenges such as variant interpretation, unsolicited findings, and the clinical utility of exome sequencing in supporting reproductive decision-making.

In 10% children no true clubfoot was present at postnatal examination resulting in a positive predictive value of 89%, with no difference between unilateral and bilateral clubfoot initially diagnosed by ultrasound. This is in line with previous cohort studies and meta-analyses, acknowledging the inherent challenges in accurately diagnosing clubfoot prenatally due to the limitations of ultrasound (1, 11, 29). The relatively high false-positive rate is attributed to the difficulty to assess reducibility of the foot deformity during ultrasound examination, which is the main feature to differentiate between a positional clubfoot and a true clubfoot.

Differentiating between clubfoot, other types of foot anomalies, or even a normal foot position can be challenging, as clubfoot exists on a spectrum and many deformities appear similar on prenatal ultrasound. Definitive diagnosis often becomes clear only after birth, which should be discussed with parents during prenatal counseling to support informed decision-making and realistic expectations. Recent studies suggest that additional measurement during prenatal ultrasound of the angle between the foot and lower leg, or conduct multiple strict planes of the lower leg and feet, can enhance diagnostic accuracy, and help assessing the severity of clubfoot (30, 31).

Limitations of the study include its retrospective nature, resulting in non-uniform imaging quality over the 15-year study period. However, working according to a standard protocol for the second-trimester structural anomaly scan in 2012 has resulted in improved quality of prenatal screening. The evolution of genetic testing technologies as well as advances in ultrasound equipment may have introduced variability in the detection of additional structural and genetic anomalies. Nevertheless, the study's relatively recent timeframe provides a contemporary context compared to earlier reports and encompasses the full range of currently available prenatal tests. Our study also benefits from extensive postnatal follow-up, providing valuable prognostic data and resulting in relatively low rates of missing information.

Prenatally, all parents were offered follow-up ultrasounds and invasive genetic testing, thereby minimizing detection bias. Finally, the Dutch legal limit of 24 weeks for termination of pregnancy may limit the generalizability of our findings to healthcare settings with different legal or ethical frameworks.

In conclusion, our study sheds light on the complexities of prenatal diagnosis of congenital isolated clubfoot, emphasising the evolving landscape of genetic testing methods and targeted ultrasound examination. Further research, mainly on the utility of CMA and exome sequencing in case of prenatal isolated clubfoot, is essential to provide a comprehensive understanding of the effectiveness of combined genetic and advanced ultrasound examinations, minimising the risk of non-identified non-isolated clubfoot diagnoses postpartum. Counseling parents facing a prenatal diagnosis of clubfoot presents a unique challenge, balancing the possibility of no postpartum abnormalities whatsoever against a heightened risk of multiple anomalies or developmental delay postpartum. Parents' concerns about long-term implications and potential underlying neuro-muscular issues underscore the importance of thorough counseling and multidisciplinary approach.

Appendix A. Description of genetic screening and testing prenatally and postnatally.

Test timing and type	n= 423
First trimester screening	**139 (33%)**
cfDNA	89 (21%)
Combined testing	56 (13%)
Prenatal invasive testing	**125 (30%)**
Rapid aneuploidy testing	125 (30%)
Karyotyping	33 (8%)
CMA	80 (19%)
WES	24 (6%)
Specific genetic testing*	7 (2%)
Postnatal testing	**28 (7%)**
CMA	6 (1%)
WES	13 (3%)
Specific genetic testing	11 (3%)

*Specific genetic testing for myotonic dystrophy type 1 or spinal muscular atrophy type 1.
cfDNA: cell-free DNA testing for trisomy 13,18,21.
CMA: chromosomal microarray analysis.
WES: whole exome sequencing.

Appendix D. Description of cases with no confirmation of clubfoot postnatally.

	n = 50	Conservative treatment* versus surgery (n)
No clubfoot	27	-
Positional clubfoot	13	-
Other foot anomalies	10	7 versus 3
Metatarsus adductus	5	3 versus 0
Vertical talus (rocker bottom foot)	2	2 versus 2
Calcaneus foot	1	-
Posteromedial bowing	1	-
Varus foot	1	-

* Repositioning foot deformities with (serial) castings.

Appendix B. Case description when diagnosis changed from isolated clubfoot at initial targeted ultrasound to non-isolated clubfoot after follow-up ultrasound or invasive testing.

Case nr.	Laterality	Prenatal phenotype & classification	Genetic tests & timing (PN/PP)	Genetic test results (variant, classification, clinical syndrome)	Outcome
Multiple anomalies					
1	Unilateral	Ventriculomegaly 13mm. Minor.	PN: RAT, karyotyping	Normal	Neonatal demise due to peripartum asphyxia and prematurity (30w)
2	Bilateral	Ventriculomegaly 11mm. Minor.	Not tested		Live birth. Last FU at 2 years: normal development.
3	Unilateral	Ventriculomegaly 11mm. Minor.	Not tested		Live birth, no ventriculomegaly postpartum.
4	Unilateral	Aortic coarctation, VSD, bicuspid aortic valve. Major.	Not tested		Live birth. Cardiac surgery once, full cardiac function.
5	Bilateral	Contracture wrist. Minor.	PN: RAT, karyotyping	Normal	Live birth, normal wrist at postpartum examination.
Chromosomal					
6	Bilateral	Choroid plexus cyst, VSD, tricuspid regurgitation	PN: RAT, karyotyping	47,XY,+21, Down syndrome	Live birth
7	Bilateral	Short nasal bone	PN: RAT	47,XX,+21, Down syndrome	TOP 17w

Appendix B. (continued)

8	Unilateral	Sandal gap, short nasal bone, dolichocephalic	PN: RAT, array	47,XX,+21, Down syndrome	Live birth
9	Bilateral		PN: RAT, WES (CNV analysis)	Deletion 22q11.21 (approx. 2.5–2.7 Mb), de novo, 22q11.2 deletion syndrome	TOP 23w
10	Unilateral	Choroid plexus cyst, SUA, PRUV	PN: RAT, array	Deletion 22q11.2 (approx. 740 kb), de novo 22q11.2 deletion syndrome (C-D deletion)	Live birth, neurodevelopmental delay
11	Bilateral		PN: RAT, array	Deletion 15q11.2 (approx. 631 kb), susceptibility locus, maternal, 15q11.2 microdeletion syndrome (BP1-BP2 region)	Live birth, normal development
12	Unilateral		PN: RAT, array	Deletion 15q11.2 (approx. 960 kb), susceptibility locus, paternal, 15q11.2 microdeletion syndrome (BP1-BP2 region)	Live birth, normal development. No clubfoot. No family history of clubfoot.
13	Unilateral		PN: RAT, array	Deletion 17q12 (approx. 1.6 Mb), de novo, 17q12 recurrent deletion syndrome.	TOP 23w

Appendix B. (continued)

Monogenic					
14	Unilateral	VSD	PP: array, WES, episign methylation analysis	CHD7 c.4669A>G (p.Arg1557Gly), de novo, likely pathogenic, CHARGE syndrome	Live birth, neurodevelopmental delay
15	Bilateral	Hydrops, multiple contractures	PP: array, WES	SCN4A c.3911_3912+1del p.(?), compound heterozygous, likely pathogenic, SCN4A-related disorders	Stillbirth 31w, WES analysis on stored material after similar phenotype of fetus next pregnancy (nr 16) Both parents and one healthy sib heterozygous, one healthy sib no carrier.
16	Bilateral	Abnormal fetal movements	PN: RAT, array PP: WES	SCN4A c.3911_3912+1del p.(?), compound heterozygous, likely pathogenic, SCN4A-related disorders	TOP 23w. Sibling of case nr 15.
17	Bilateral	Abnormal fetal movements	PN: RAT, WES	PIEZO2 c. 570C>T (p. (Gly190=), paternal, VUS PIEZO2 c.492+2T>C, maternal, likely pathogenic, distal arthrogryposis type 5	TOP 22w, postpartum: flexion contractures of the wrists and elbows bilaterally; flexion contractures of the fingers bilaterally. Flexion contractures of the knees.
18	Bilateral		PN: RAT, WES	TRPV4 c.290C>G (p.Pro97Arg), paternal, likely pathogenic, congenital distal spinal muscular atrophy type 1	Live birth, distal muscle weakness

Appendix B. (continued)

Syndromic					
19	Unilateral	Severe hydrops	PN: RAT, karyotyping	Normal	Iatrogenic premature birth 28w, neonatal demise due to severe hydrops
20	Unilateral	vertebral anomalies. Twin pregnancy (other fetus had severe congenital anomalies diagnosed at initial ultrasound)	PN: RAT, karyotyping	Normal	TOP 21w, autopsy showed renal agenesis

PN: prenatal, PP: postpartum, RAT: rapid aneuploidy testing with QF-PCR or FISH for trisomy 13, 18 and 21. WES: whole exome sequencing, w: weeks of gestation, TOP: termination of pregnancy, VSD: ventrical septum defect, SUA: single umbilical artery, PRUV: persistent right umbilical vain, CF: clubfoot, VUS: variant of unknown significance, FADS: Fetal Akinesia Deformation Sequence. Subclassification of structural anomalies into major and minor categories: minor anomalies are those with low impact on quality of life and development, and are often unrelated to clubfoot; major anomalies are with potentially high impact on quality of life, (major) surgery, and/or developmental delay.

Appendix C. Case description of postnatal non-isolated clubfoot diagnosed after birth due to structural anomalies, genetic disorders or developmental delay.

Case Nr.	Laterality clubfoot	Phenotype,characteristics and classification	Timing of diagnosis	Genetic test & timing (PN/PP)	Genetic test results (variant, classification, clinical syndrome)
Multiple anomalies					
1	Unilateral	Missing two toes, longitudinal reduction defect. Major.	Birth	Not tested	
2	Bilateral	Camptodactyly. Minor.	Birth	PN: Karyotyping	Normal
3	Bilateral	Clinodactyly in two fingers. Minor.	Birth	Not tested	
4	Unilateral	Hand and foot deformities of unknown type. Unknown.	Birth	Not tested	
5	Unilateral	Amniotic band syndrome with bilateral oligodactyly. Minor.	Birth	Not tested	
6	Bilateral	Amniotic band syndrome with syndactyly, skin deformities of right foot, scalp injuries. Minor.	Birth	Not tested	
7	Bilateral	Hemodynamic significant persistent ductus arteriosus, pre-axial unilateral polydactyly. Major.	Birth	PP: array, WES	G6PD c.[292G>A; 466A>G]; p.[(Val98Met); (Asn156Asp)], pathogenic (incidental finding), G6PD deficiency class III

Appendix C. (continued)

8	Bilateral	Brachydactyly digiti 2 and 5. Minor.	Birth	Not tested	
9	Bilateral	Unilateral postaxial polydactyly. Minor.	Birth	Not tested	
10	Bilateral	Pulmonary valve stenosis, atrium septum defect. Major.	Birth	Not tested	
11	Unilateral	VSD, congenital hydrocele, hernia inguinalis. Minor.	Birth	Not tested	
12	Unilateral	Hemodynamic significant VSD, hypospadias. Major.	Birth	Not tested	
13	Bilateral	Unilateral cryptorchidism. Minor.	Birth	Not tested	
14	Bilateral	Vascular malformation of right arm, hernia inguinalis. Minor.	Birth	PN: Array PP: WES	Normal
15	Unilateral	Microcephaly with normal neurodevelopment. Major.	9 months	PP: WES	Normal
16	Bilateral	Benign external hydrocephalus. Major.	1 year	PN: Array	Normal
17	Bilateral	Sacrococcygeal teratoma. Major.	Birth	Not tested	
18	Unilateral	Laryngomalacia. Minor.	1 month	Not tested	

Appendix C. (continued)

19	Bilateral	Hypospadias, failure to thrive, several cortical kidney cysts. Major.	18 months	PN: Array PP: DNA sequence analysis of PKD1 gene.	PKD1 c.6643C>T p. (Arg2215Trp), paternal, pathogenic (unrelated to clubfoot), autosomal dominant polycystic kidney disease.
Chromosomal					
20	Unilateral	Neurodevelopmental delay (speech), schisis uvula	4 years	PP: Array (elsewhere)	22q11.2 deletion syndrome (exact annotation not available)
21	Bilateral	Short stature, neurodevelopmental delay (motor delay)	1 year	PP: WES (CNV analysis)	Deletion 22q11.2 (approx. 2.5 Mb), de novo, 22q11.2 deletion syndrome
22	Bilateral	Neurodevelopmental delay and auditory problems	1 year	PP: Array (elsewhere)	22q11.2 deletion syndrome (exact annotation not available)
23	Bilateral	Stillbirth at 25w. Typical facial features, elbow contracture	Fetal demise	PP: Karyotyping	47,XY,+21, Down syndrome
24	Bilateral	Stillbirth at 38w. Typical facial features.	Fetal demise	PP: RAT	47,XX,+21, Down syndrome
25	Bilateral	Stillbirth at 40w. No physical exam reported.	Fetal demise	PP: Array	Deletion 16p13.11p12.3 (approx. 3.1 Mb), de novo, pathogenic susceptibility region.

Appendix C. (continued)

Monogenetic					
26	Unilateral	Macrosomia, macroglossia.	Birth	PP: MS-MLPA test	Hypermethylation at 11p15.5 IC1 (H19/IGF2:IG-DMR), pathogenic, Beckwith-Wiedemann syndrome
27	Bilateral	Hypotonia, cryptorchidism	Birth	PP: MS-PCR test (for SNRPN region)	Abnormal methylation at 15q12 SNRPN promoter region, maternal uniparental disomy, pathogenic, Prader Willi syndrome
28	Bilateral	Hypotonia, facies myopathica, developmental delay (gross motor)	2 years	PP: WES, CTG repeat test in DMPK-gene	DMPK (3′UTR) CTG repeat expansion (n > 200), maternal carrier CTG expansion (n = 121), pathogenic, congenital myotonic dystrophy type 1

Appendix C. (continued)

29	Unilateral	Mother affected with myotonic dystrophy type 1. Multiple contractures, clinodactyly toes	Birth	PP: CTG repeat test in DMPK-gene	DMPK (3′UTR) CTG repeat expansion (n > 700), maternal carrier CTG expansion (n = 41-100, n>200), pathogenic, congenital myotonic dystrophy type 1
30	Bilateral	Hypotonia, failure to thrive, respiratory insufficiency. Deceased after 3.5 months	3 months	PN: Array, CTG repeat test in DMPK-gene PP: PCR for SMN1 exon 7 and 8	SMN1 exon 7 and exon 8 deletion, pathogenic, spinal muscular atrophy (type 1)
31	Bilateral	Oedema of hands, abnormal facial features, neurodevelopmental delay	1 year	PN: Array PP: WES	NSD1 c.6014G>A p. (Arg2005Gln), de novo, likely pathogenic, Sotos syndrome
32	Bilateral	Neurodevelopmental delay, epilepsy	1 year	PP: WES	WDR26 c.346A>T p. (Lys116), de novo, pathogenic, Skraban-Deardorff syndrome
33	Unilateral	VSD, hypospadias, auditory problems, cryptorchidism. Father affected with LEOPARD syndrome, without clubfoot.	Birth	PP: DNA sequence analysis of PTPN11 gene.	PTPN11 c.1403C>T p. (Thr468Met), paternal, pathogenic, LEOPARD syndrome

Appendix C. (continued)

34	Unilateral	Neurodevelopmental delay (IQ 59-70)	7 years	PP: Array, karyotyping	46,XY,der(3)t(3;8)(p26.3;p22),3p26.3(62075_2740118)x1,8p23.3p22(176452_17038594)x3 – 2.6 Mb deletion(3p), 16.8 Mb duplication(8p), de novo
35	Bilateral	Hydrocephalus, umbilical hernia, neurodevelopmental delay	6 months	PN: Array PP: WES (elsewhere)	ZFHX4 gene mutation (exact annotation not available)
36	Bilateral	Hypotonia, dysphagia, brain malformation (polymicrogyria). Deceased at 10 years.	Birth	PN: RAT, array PP: WES	PEX1 c.2097insT (p.Ile700Tyrfs*42) and c.2528G>A (p.Gly843Asp), pathogenic, Zellweger spectrum disorder
Syndromal					
37	Bilateral	Stillbirth at 24w, contractures of knees	Birth	PP: Karyotyping, PCR for SMN1 exon 7.	Normal
38	Bilateral	Distal arthrogryposis: clenched thumbs, ulnar deviation of hands	Birth	Not tested	

Appendix C. (continued)

39	Unilateral	Asphyxia, abnormal facial features, hyperlaxity, joint contractures, pes calcaneus (right foot), CF (left foot). Deceased after 3 weeks	Birth	Not tested	
40	Unilateral	Hypotonia, hyperlaxity and neurodevelopmental delay (motor delay)	1 year	PP: WES	Normal
41	Bilateral	Distal muscle weakness and EMG changes indicative of hereditary motor neuropathy	11 months	PP: WES	Normal
42	Bilateral	Meconium ileus, distal muscle weakness	6 months	PN: Array, WES PP: NGS for arthrogryposis	Normal
43	Bilateral	Neurodevelopmental delay (motor delay), visual and auditory problems	3 years	PP: Array, WES	Normal
44	Bilateral	Atrophy lower leg, bronchiolitis obliterans, hypo- and hyperpigmentation, pectus excavatum (unknown cause)	Birth	PN: Array PP: WES	Normal
45	Bilateral	Bilateral ptosis, hyperlaxity, hypotonia with neurodevelopmental delay (motor delay)	Birth	PN: WES	Normal
46	Bilateral	Cholestasis, hernia inguinalis, monokidney	2 months	PP: Array, WES	Normal
47	Bilateral	Neurodevelopmental delay with intellectual disability, obesity	2 years	PP: Array, WES	Normal

PN: prenatal, PP: postpartum, WES: whole exome sequencing, w: weeks of gestation, CNV: copy number variant, VUS: variance of unknown significance, MS-MLPA: Methylation-Sensitive Multiplex Ligation-dependent Probe Amplification, MS-PCR: Methylation-specific polymerase chain reaction, NGS: next generation sequencing, VSD: ventricular septal defect. Subclassification of structural anomalies into major and minor categories: minor anomalies are those with low impact on quality of life and development, and are often unrelated to clubfoot; major anomalies are with potentially high impact on quality of life, (major) surgery, and/or developmental delay.

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CHAPTER 6

Genetic analysis in fetuses with isolated clubfoot: diagnostic insights and added value.

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Abstract

This study evaluates the diagnostic genetic diagnostic yield in fetuses sonographically suspect of having isolated clubfoot. We conducted a retrospective study on all fetuses with apparently isolated clubfoot on initial ultrasound, examined between January 2021 and December 2024. Clubfoot was classified as isolated when no additional structural anomalies were observed on initial imaging. Among 218 cases, 140 (64%) were classified as isolated.

Prenatal genetic testing was performed in 64 of these cases (46%), of which 61 (95%) underwent both copy number variant (CNV) and single nucleotide variant (SNV) analysis. In 38 of the 61 (62%) cases targeted investigation of the *DMPK* gene was carried out too. Pathogenic or likely pathogenic causative variants were identified in six of the 61 (9.8%) pregnancies: two of the 26 tested (7.7%) with unilateral clubfoot and four of the 35 tested (11.4%) with bilateral clubfoot. These include SNVs in *TRPV4*, *PTPN11*, *BBS2*, and *MED13L* (4/61 = 6.6%) and two CNVs, a *de novo* 22q11.23 deletion, and a *de novo* 5q21.1q31.1 deletion (2/61 = 3.3%). One case remained unsolved due to the identification of a variant of uncertain significance (VUS) in PIEZO2. Three cases revealed unsolicited findings unrelated to the indication for testing.

Our findings highlight the diagnostic yield of prenatal CNV- and SNV-testing in cases of suspected isolated clubfoot, but does not support systemic testing for *DMPK*. Although broad genetic testing can support diagnosis and counseling, challenges remain in interpreting results and managing unsolicited findings.

Introduction

Congenital clubfoot (talipes equinovarus) is a spectrum with a complex deformity that affects the structure and alignment of the foot and ankle. It results in adduction, typically characterized by forefoot adduction, midfoot supination, hindfoot varus, and equinus positioning. Clubfoot has a prevalence of 1 to 3 per 1,000 live births, and has a prenatal detection of 30-77% (1-3). Clubfoot can be diagnosed during a fetal anomaly scan in the first and second trimester, and may present unilaterally or bilaterally, either as an isolated defect or in combination with other structural anomalies. In some cases, an underlying genetic disease is identified. Postnatal confirmation of clubfoot is required through physical examination. Its treatment typically follows the Ponseti method, involving serial casting, and in some cases surgical intervention (4).

Distinction between isolated and non-isolated clubfoot solely based on prenatal ultrasound examination can be challenging. Not all structural anomalies are visible at the time of examination, and some may only manifest later in pregnancy. Clubfoot can be an early symptom of syndromes involving the neuromuscular system, which may manifest later in pregnancy, such as arthrogryposis multiplex congenita (AMC) or neurodevelopmental disorders (4). Prenatal invasive testing should be offered to enable parents to opt for assessing a potential underlying genetic condition (1). A molecular genetic diagnosis helps to give future parents a tailored prognosis, thereby facilitating reproductive decisions and guiding perinatal and postnatal management.

Prenatal genetic testing for chromosomal and monogenic disorders can be performed using quantitative fluorescent-polymerase chain reaction (QF-PCR), chromosomal microarray analysis (CMA), and next generation sequencing (NGS)-based tests such as exome sequencing (ES), with the potential of analyzing Single Nucleotide Variants (SNVs) and Copy Number Variants (CNVs) from one set of data. The choice of test depends on local availability and protocols(1). Myotonic dystrophy type I (OMIM #160900) is regarded to be in the differential diagnosis of prenatal clubfoot too. Therefore, analysis of the *DMPK* gene is frequently carried out, even though authors such as Leyne *et al*. and Dap *et al*. already suggested in their cohorts that the added value of testing for *DMPK* variants is low (5,6).

The diagnostic genetic diagnostic yield for CMA in isolated clubfoot is reported to be 2.2-11% (4-9). In recent years, analysis of data generated by ES, being either SNV-analysis alone or SNV- and CNV-analysis simultaneously, has been implemented as a diagnostic tool for fetuses with any sort of structural anomalies on ultrasound (10-12). For isolated clubfoot specifically, fewer than one hundred cases have been reported in literature in which ES-SNV analysis was conducted

prenatally, with a diagnostic yield ranging from 4.3% to 25% (9,13,14). Further research is urgently needed to explore the diagnostic yield of high resolution genetic analysis in unilateral and bilateral cases, since the studies of Huang *et al*., Pan *et al*. and Yu *et al*. did not discriminate between these two categories (9,13,14). Therefore, the goal of this study is to explore the diagnostic yield of high resolution CNV- and SNV-analysis in case of apparently isolated clubfoot – in both unilateral and bilateral cases. We also took into account the yield of analysis of the *DMPK* gene.

Methods

Study design and participants

We performed a retrospective cohort study of all fetuses with prenatally isolated clubfoot diagnosed by ultrasound from January 2021 until December 2024 in the Fetal Medicine Unit (FMU) of Amsterdam UMC, a tertiary center. Cases were included when the initial targeted ultrasound examination –performed in the first or second trimester – resulted in a diagnosis of isolated clubfoot (15,16). A targeted ultrasound examination is a detailed and advanced fetal anomaly scan performed to assess fetal anatomy, after an anomaly is already suspected during a routine fetal anomaly scan. Clubfoot was considered isolated in case no other structural anomalies were found during this examination, with the exception of prenatal soft markers (e.g. plexus choroids cysts).

The sonographic diagnosis of clubfoot deformity was made when both long bones of the lower leg (tibia and fibula) were seen in the same plane as the sole of the foot throughout the entire examination. After the initial diagnosis of isolated clubfoot, parents were offered follow-up ultrasound examinations according to the care pathway that is carried out in the Amsterdam UMC in case of prenatally suspected contractures (17). Typically, the second scan in our center is performed approximately two weeks after the initial ultrasound. The aim is to examine the fetal movement and screening for multiple contractures or other associated structural anomalies. This evaluation takes place before the upper limit for termination of pregnancy of 24 weeks, as regulated by the Dutch law (17,18).

Additionally, a follow-up ultrasound is performed in the third trimester at 28-30 weeks of gestation to assess fetal growth, amniotic fluid levels, and signs of potential late development of neuromuscular symptoms (e.g. polyhydramnios, lunghypoplasia or micrognathia) (17).

Subsequently, all parents were offered prenatal counseling by a pediatric orthopedic surgeon to receive information on postnatal prognosis and treatment. Autopsy was offered in all cases of termination of pregnancy and was performed

when parental consent was obtained. In addition, all cases underwent external physical examination. Children with isolated clubfoot are not systematically referred for follow-up genetic evaluation unless there are additional findings or developmental delay postnatally.

Genetic Testing

The testing took place either at the Amsterdam UMC or at the department of Human Genetics of the Radboud university medical center in Nijmegen, the Netherlands. Genetic analysis was performed on DNA isolated from either chorionic villi or amniotic fluid. In all cases, rapid aneuploidy testing by QF-PCR was first performed, to rule out the most commonly occurring aneuploidies, followed by CNV analysis by genomewide Chromosomal MicroArray (CMA), and in case of a normal CMA result by genepanel SNV analysis from ES data (ES-SNV), or by simultaneous exomewide CNV- and genepanel SNV-analysis from ES data (ES-CNV/SNV). For a comprehensive description of simultaneous CNV- and SNV-analysis from ES data, see reference 12. CNV- and SNV-analysis for the fetus and the parents were preferably carried out simultaneously for optimal interpretation of the data. In addition, in part of the cases analysis of the *DMPK* gene was also requested by the clinical geneticist. Analysis of *SMN1* was not included in the exome sequencing pipeline used in this study and was not performed separately in any of the cases. However, targeted *SMN1* testing can be requested prenatally.

The resolution of the genomewide CNV-analysis by CMA was 75 kb, whereas the resolution of the exomewide ES-CNV analysis was up to a single exon level. ES-SNV analysis was limited to genes in the requested gene panel(s), which were selected by the clinical geneticist. The most requested gene panels were panels including genes related to fetal akinesia, movement, skeletal, cognitive and/or Mendelian disorders.

All results were reported according to the guidelines of the American College of Medical Genetics and Genomics (ACMG) and only included pathogenic (P) and likely pathogenic (LP) variants (19).

The clinical significance of variants was systematically evaluated by referring to databases, scientific literature, and ultrasound findings, based on the human phenotype ontology (20). The nomenclature of the detected variants was written in accordance with the International System for Cytogenomic Nomenclature (ISCN 2024) and the “Human Genome Variation Society” (HGVS) (https://varnomen.hgvs.org).

DMPK analysis was performed in accordance with the best practice guidelines, as previously published by Kamsteeg *et al*. (21).

Statistical Analysis

We characterized the data by using descriptive statistics, such as percentages and ratios, with differences assessed using chi-square. A significance level of $p < 0.05$ was applied for statistical significance. Statistical analyses were conducted using SPSS version 28 (IBM, Armonk, NY, USA). Categorical variables were compared using Fisher's exact test.

Data regarding maternal characteristics, obstetrical history, sonographic findings, prenatal screening, invasive testing, pregnancy and neonatal outcomes were collected from patient records. We contacted the general practitioners and parents by telephone to obtain missing information from hospital records. Cases where medical records or telephone questionnaires were not available were marked as lost to follow-up. This study was approved by the Medical Ethics Committee of Amsterdam UMC (W21_361 # 21.401) regarding general follow-up data on our FMU.

Results

A total of 218 cases of clubfoot were identified in our FMU between January 2021 and December 2024. Of these, 140 cases (64%) were isolated at the initial targeted anomaly scan and thus included in this study. Of these 140, 63 (45%) were diagnosed with unilateral clubfoot, and 77 (55%) with bilateral clubfoot. Pregnancy was terminated in 11 cases (8%). In total, parents opted for invasive prenatal testing in 64 of the 140 cases (46%). The characteristics of the 64 tested cases concerning the maternal age, family history of clubfoot, performed noninvasive prenatal testing (NIPT), gestational age at initial targeted anomaly scan, and pregnancy outcome are shown in Table 1.

Table 1. Baseline characteristics.

Maternal characteristics (n=64)	number (%)
Mean maternal age at diagnosis in years ±range	33.1±4.4
Family history of clubfoot	5 (7.8%)
NIPT test performed	55 (85.9%)
Gestational age at diagnosis	17+5± 3+4
Pregnancy outcome Live born Termination of pregnancy Neonatal death	 54 (84.4%) 9 (14.1%) 1 (1.5%)

In three cases, SNV-ES analysis was not performed because QF-PCR and CMA revealed no abnormalities, and the parents chose not to proceed with further testing. Finally, in 61 of the 64 (95%) cases ES was opted – all with a normal QF-PCR result.

The abnormal findings of the SNV- and CNV-analyses are listed in Table 2a. A pathogenic or likely pathogenic causative variant was identified in 6 of

the 61 cases (9.8%): in 2 of the 26 unilateral cases (7.7%) and in 4 of the 35 bilateral cases (11.4%). This difference was not statistically significant (p = 0.69). The abnormal SNV findings include SNVs in *TRPV4*, *PTPN11*, *BBS2*, and *MED13L* (4/61 = 6.6%). The father of case 1 had muscle weakness of the shoulder girdle and lower limbs, which following the prenatal diagnosis, is consistent with the diagnosis of *TRPV4*-related scapuloperoneal spinal muscular atrophy (SPSMA). In addition to the four SNVs, there were causative CNVs identified in two cases with bilateral clubfoot, a *de novo* 22q11 deletion, and a *de novo* 5q21.1q31.1 deletion (2/61 = 3.3%), the latter having been identified by NIPT, previously performed in the first trimester. In the case with the 22q11.2 deletion, the father's cousin also had clubfeet; however, no genetic testing was performed in that relative, and it was known that this was a *de novo* deletion.

ES-SNV analysis also revealed compound heterozygous variants in *PIEZO2* in case 7, of which the c.492+2T>C variant was likely pathogenic, and the c.570C>T variant was considered a variant of uncertain significance (VUS) (Table 2a). In three cases (3/61 = 4.9%), ES-SNV analysis revealed unsolicited findings unrelated to the indication for testing. One fetus and its mother carried a pathogenic variant in *BRCA2*; another fetus appeared compound heterozygous for two pathogenic variants in *FLG*; and a third fetus appeared homozygous for a pathogenic variant in *SERPINC1* (Table 2b).

In 38 of the 61 cases (62%), testing for myotonic dystrophy type 1, caused by a (CTG)n repeat expansion in the 3'UTR of the *DMPK* gene, was additionally performed, and none carried the expansion.

Postnatally, 5 of the 76 children (5.3%) with isolated clubfoot who had not undergone prior genetic testing received genetic evaluation, resulting in the diagnosis of trisomy 21 in one case. The other four were tested due to: (1) intrauterine fetal death at 39 weeks, (2) prematurity with pulmonary, thoracic, renal, and limb anomalies, (3) termination after abnormal fetal movements with clubfeet, and (4) paternal carrier status of a microduplication. The remaining 71 children were all clinically evaluated and did not show any signs of other underlying pathology.

Table 2a. Findings of ES-SNV (CMA negative) or ES-CNV/SNV analysis in 61 women who opted for invasive prenatal testing and ES. Classification following ACMG Criteria 2015.

Case	laterality	GA	NIPT	Inheritance	Variant: Nucleotide alteration, deduced protein change	ACMG criteria	Aggregated pathogenicity prediction	MAF	Pregnancy outcome	Pre-/postnatal findings
1	uni	18+2	Yes	AD	TRPV4: Chr12(GRCh37):g.110252312G>C NM_021625.5:c.290C>G p.(Pro97Arg) (paternal)	LP: PS3, PM2	Uncertain (0.6)	-	A, 41+1	Bilateral clubfeet, follow-up at 2 yrs: mild proximal and distal muscle weakness.
2	bi	20+2	No	DN	PTPN11: Chr12(GRCh37):g.112888150A>G NM_002834.5:c.166A>G p.(Ile56Val)	P: PS4, PS2, PM1, PM2, PM5, PP2, PP3, PP5	Deleterious (0.74)	1.240	TOP, 23+4	No additional findings
3	uni	20+3	Yes	AR (compound heterozygous)	BBS2: Chr16(GRCh37):g.56540122_56540123del NM_031885.5:c.627_628del p.(Cys210Serfs*20) (maternal) BBS2: Chr16(GRCh37):g.56532462G>A NM_031885.5:c.1546C>T p.(Gln516*) (paternal)	P: PVS1, PM2, PM3, PM5 P: PVS1, PM2, PM3, PM5	- -	0.000001859 -	TOP, 23+6	Postaxial polydactyly both hands, unilateral clubfoot

Table 2a. (continued)

Case	laterality	GA	NIPT	Inheritance	Variant: Nucleotide alteration, deduced protein change	ACMG criteria	Aggregated pathogenicity prediction	MAF	Pregnancy outcome	Pre-/postnatal findings
4	bi	20+4	Yes	DN	MED13L: Chr12(GRCh37):g.116401310_116401336inv NM_015335.5:c.6388-12_6402inv r.spl	LP: PVS1, PS2, PM2	-	-	TOP, 23+4	Down slanting palpebral fissures and hypertelorism. Notably low-set and posteriorly rotated ears. Curved nasal tip. Small chin and intact palate. Hands: slender, long fingers Feet: pronounced bilateral pes equinovarus. Camptodactyly of toes 3 to 5.
5	bi	19+6	Yes	DN	Seq[GRCh37] del(22)(q11.21 q11.21) Chr22:g.(18659584_18893887)_(21414817_21416012)del	P			TOP, 23+2	No additional findings
6	bi	14+3	Yes, abnormal: del(5)(q21.1q31.1)	DN	seq[GRCh37] 5q21.1q31.1(100147550_131411545)x1 NC_000005.9:g.(98262090_100147550)_(131411545_131528702)del	P			TOP, 19+6	No additional findings
7	bi	19+3	Yes	AR (Compound heterozygous)	PIEZO2: Chr18(GRCh37):g.10857132G>A NM_022068.4:c.570C>T p.(Gly190=) / r.spl? (paternal); PIEZO2: Chr18(GRCh37):g.10871249A>G NM_022068.4:c.492+2T>C p.? (maternal)	VUS: PM2 LP: PVS1, PM2	Deleterious (0.79) SpliceAI: Splice-Altering / strong (0.97) Deleterious (0.8) SpliceAI: Splice-Altering / strong (0.99)	6.503 e-7 -	TOP, 22+5	Abnormal quality of the fetal movement. Postnatally also contractures wrists, elbows, knees and fingers.

Table 2b. Unsolicited findings, unrelated to the indication for testing, identified by ES-SNV analysis in 61 women who opted for invasive prenatal testing and ES. Classification following ACMG Criteria 2015.

Case	laterality	GA	NIPT	Inheritance	Variant: Nucleotide alteration, deduced protein change	ACMG criteria	Aggregated pathogenicity prediction	MAF	Pregnancy outcome	Pre-/postnatal findings
8	uni	19+4	Yes	AD (heterozygous)	*BRCA2*: Chr13(GRCh37):g.32906762del NM_000059.3:c.1147del p.(Ile383fs) (paternal)	P: PVS1, PS4		1.859	A, 40+2	No clubfoot
9	uni	19+4	Yes	AR	*FLG*: Chr1(GRCh37):g.152285861G>A NM_002016.1:c.1501C>T p.(Arg501*) (maternal) *FLG*: Chr1(GRCh37):g.152277415G>C NM_002016.1:c.9947C>G p.(Ser3316*) (paternal)	LP: PVS1, PM2, PP5 LP: PVS1, PM2, PP5		0.01696 0.0004096	TOP, 23+6	No additional anomalies. Posteromedial bowing suspected.
10	bi	21+3	No	AR (homozygous)	*SERPINC1*: Chr1(GRCh37):g.173883708G>A NM_000488.4:c.391C>T p.(Leu131Phe) (both parents)	P: PS4, PM2, PP1, PP2, PP3	Deleterious (0.86)	1.115	A,41+3	Continued: not causal for clubfoot. Postnatally no clubfoot, but mild inversion of the foot

Discussion

In this study we investigated the diagnostic genetic diagnostic yield in fetuses with isolated clubfoot, providing valuable insights into the genetic etiology of this condition. Among the evaluated cases, we identified causative CNVs or SNVs in six cases (6/61 = 9.8%), four of which were SNVs (4/61 = 6.6%) and two CNVs (2/61 = 3.3%).

The variants in *TRPV4*, *PTPN11*, *BBS2*, and *MED13L* were all classified as pathogenic or likely pathogenic and causative for clubfoot. *TRPV4* variants are associated with a broad spectrum of neuromuscular disorders with clinical phenotypes that can vary even within the same family carrying identical variants(22,23). Variants in *PTPN11* are associated with Noonan syndrome (24-26), with clubfoot as one of the (rare) features. In addition, while a clubfoot is observed in only 1.8% of cases with Bardet-Biedl syndrome (*BBS*), renal anomalies (85%) and polydactyly (71%) are the most frequent findings (27,28). In the case with pathogenic variants in *BBS2*, the diagnosis of bilateral postaxial polydactyly had not been made prenatally.

Although polydactyly is generally considered a detectable anomaly during second-trimester scans, its visualization can be hampered by various factors, such as maternal obesity, suboptimal fetal position, or acoustic shadowing from uterine anomalies, particularly when the polydactyly lacks a bony content (29). Moreover, in the absence of other anomalies, subtle digital findings may be easily overlooked (29). This highlights the importance of systematic assessment of the hands and feet during prenatal imaging and the need for awareness that even seemingly isolated limb anomalies may have an underlying syndromic or genetic etiology.

Pathogenic heterozygous variants in MED13L are associated with an autosomal dominant syndrome characterized by intellectual disability and distinct facial features, with or without congenital heart defects (OMIM #616789) (30,31). Clubfoot has also been reported for patients with this condition (32).

Case 5 showed a de novo 22q11 deletion, that is linked to 22q11.2 deletion syndrome (OMIM #611867), a well-known pathogenic condition associated with clubfoot(5). Case 6 presented with a pathogenic de novo deletion in chromosome band 5q21.1q31, known to be associated with clubfoot and developmental disorders (33-36). This large deletion of 31–33 Mb includes the FBN2 gene, which is linked to arachnodactyly, joint contractures, kyphoscoliosis, and variable systemic involvement (OMIM #121050) (33-36). The extent of neurodevelopmental impairment could not be precisely predicted, but substantial impairment was considered likely based on the deletion size and gene content.

Case 7 remained unresolved due to a VUS. *PIEZO2* is associated with distal arthrogryposis with impaired proprioception and touch (DAIPT), and the fetus' phenotype was consistent with this condition, including the abnormal quality of the fetal movements (37,38). Although these findings suggest the *PIEZO2* variants are likely disease-causing, the c.570C>T variant remains classified as a VUS in the absence of RNA analysis confirming its predicted effect on splicing. This variant is predicted to be synonymous at the protein level while splice prediction tools suggests that this variant may result in a cryptic splice donor site in exon 6 (NM_022068.4). This case illustrates how postnatal follow-up can provide valuable insights into variant interpretation and supports the need for longitudinal phenotyping when initial classifications remain uncertain. These findings may contribute to future reclassification of the variant as likely pathogenic or pathogenic.

The diagnostic SNV-yield has been evaluated by three previous reports. Yu *et al*. analyzed data of 38 singleton pregnancies diagnosed with isolated clubfoot (14). Trio-based SNV-exome sequencing analysis was conducted after a normal CMA result. Pathogenic or likely pathogenic variants were identified in 4 of the 38 cases (10.5%), which is slightly higher than our finding of 6.6%. The genes involved in the study of Yu *et al*. were *BRPF1*, *ANKRD17*, *FLNA*, and *KIF1A* (14). These genes are associated with musculoskeletal and neurodevelopmental syndromes.

Another retrospective study by Huang et al., with 83 singleton pregnancies diagnosed with clubfoot (both isolated and non-isolated), identified clinically significant SNVs in 12% of cases, with a higher detection rate in non-isolated clubfoot (22.2%) compared to isolated cases (4.3%) (13). This latter finding being slightly lower than ours. Pathogenic SNVs were found in two of the 47 fetuses with isolated clubfoot, one in the TGM6 gene and another in the BRPF1 gene (13). In 2025, Pan et al. showed an SNV yield of 25% by ES data analysis in isolated clubfoot in a group of four fetuses with isolated clubfoot(9). This high percentage is likely due to the low number of tested fetuses with an isolated clubfoot(9). Together with our study, these three studies show that apparently isolated clubfoot can have an underlying genetic cause, highlighting the importance of parental counseling.

Genetic testing not only increases the likelihood of identifying an underlying genetic cause, but also the likelihood of identifying unsolicited findings. In the current study, we identified causative variants in 9.8% (n=6), and unsolicited findings, unrelated to the indication for testing in 4.9% of the fetuses (n=3). Depending on the type of unsolicited findings (fetal, parental or both, mild or severe phenotype), such findings can present ethical dilemmas and psychological burdens for parents, highlighting the importance of comprehensive pre-test

counseling to discuss possible outcomes and their implications(39).

As in none of the cases with *DMPK* analysis a causative repeat length was detected, the data of our cohort do not support routine *DMPK* analysis as part of the genetic work-up in isolated clubfoot, which is in accordance with previous reports (5,6,21).

A prenatal ultrasound diagnosis of isolated clubfoot should be handled with caution, as it may not always indicate an entirely isolated clubfoot (37). When using prenatal ultrasound techniques to detect fetal abnormalities, several limitations must be considered. Firstly, the sensitivity of ultrasound techniques is dependent on various factors, including fetal size and positioning, maternal body type, the resolution of ultrasound equipment, and the experience of the sonographer (40). Secondly, not all abnormalities are visible on ultrasound, especially those that do not manifest as clear structural or functional anomalies (e.g. intellectual disability or subtle neurodevelopmental disorders), leading to potential missed diagnoses (41-43). These conditions may only become apparent years after birth, often around school age, when developmental milestones and cognitive performance can be more accurately assessed (43).

As the current follow-up period is limited, it is possible that additional cases in the genetically untested group may eventually show signs of associated abnormalities that were not evident in the neonatal period. To date, only five cases underwent postnatal genetic testing, with trisomy 21 identified in one of them. However, since postnatal genetic testing was not systematically performed in our cohort, no definitive conclusions can be drawn regarding the overall genetic diagnostic yield. These findings underscore the importance of long-term follow-up and suggest that genetic abnormalities may be underestimated in cases with clubfoot initially classified as isolated.

Notably, among the five cases with a positive family history of clubfoot, one (20%) had a pathogenic variant identified, compared to 5 out of 59 cases (8.5%) without a family history. While this observation may indicate a potentially higher diagnostic yield in the presence of family history, causality was excluded in this case as the 22q11.2 deletion occurred de novo. In particular, isolated clubfoot often reflects multifactorial inheritance, and the presence of clubfoot in a relative does not necessarily imply a monogenic cause. These nuances should be clearly communicated during counseling and considered when deciding on genetic testing.

All clubfoot in our cohort were confirmed during targeted ultrasound examinations after referral because of an abnormal routine scan. Additional

anomalies were observed in two cases, and only during post-mortem examination.

Postnatal examinations after termination of pregnancies showed multiple contractures in the case with variants in *PIEZO2* - potentially due to the time interval between the last ultrasound and termination, as isolated contractures can progress to AMC – and polydactyly of both hands in the case with variants in *BBS* (2). This highlights the added value of genetic analysis in cases of isolated clubfoot, as, in the majority of our cohort, clubfoot was the only detectable phenotype of the underlying genetic disorder during prenatal ultrasound examination.

The strengths of this study include its focus on a well-defined cohort of cases with prenatally diagnosed isolated clubfoot, the detailed and systematic genetic testing, and the ability to differentiate between unilateral and bilateral cases. The study contributes to the limited existing data on comprehensive SNV analysis in isolated clubfoot cases. Although the number of cases remains limited, this study represents the largest prenatal cohort reported to date. The results support that, in cases of isolated clubfoot, prenatal genetic testing should include chromosomal, CNV- and SNV-analysis, using high-resolution genetic techniques.

In conclusion, our study demonstrates that prenatal CNV- and SNV-analysis yields a molecular diagnosis in approximately 10% of fetuses with apparently isolated clubfoot, supporting its diagnostic value even in the absence of other structural anomalies. Our findings show that both unilateral and bilateral cases may harbor causal pathogenic or likely pathogenic variants. This underscores the importance of offering genomic testing regardless of laterality. At the same time, challenges remain regarding the likelihood and interpretation of uncertain or unsolicited findings, and careful pre- and postnatal counseling is essential to ensure that testing contributes meaningfully to the parental decision.

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CHAPTER 7

Perinatal genetic diagnostic yield in a population of fetuses with the phenotype arthrogryposis multiplex congenita (AMC): a cohort study 2007-2021.

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Abstract

Arthrogryposis multiplex congenita (AMC) presents challenges for prenatal detection due to its heterogeneous etiology, onset, and phenotypical manifestations. This study aims to describe the genetic diagnostic yield in a population of fetuses with detailed phenotypic description over a 15-year period (2007-2021) at the Fetal Medicine Unit of Amsterdam UMC, the Netherlands. The fetal and neonatal phenotypes were classified into three clinical AMC Groups with the exception that Groups 1 and 2 were combined in the prenatal classification. Group 1 involves primarily limb involvement, Group 2 includes musculoskeletal involvement plus other system anomalies, and Group 3 involves musculoskeletal involvement with central nervous system disability, lethality, fetal akinesia deformation sequence and/or intellectual disability.

The cohort consisted of 64 consecutive cases, 13 in Groups 1+2 and 51 in Group 3. Perinatal genetic testing occurred in all cases: prenatally in 56 of the 64 (88%), and postnatally in 36 of the 64 (56%), and combined testing in 28 of the 64 cases (44%). The overall genetic diagnostic yield was 28% (18/64), and it increased over the 5-year periods from 14% to 50%. Whole exome sequencing had the highest yield (41.7%). The yield per phenotype was 30.8% (4/13) for AMC Group 1+2 and 27.4% (14/51) for AMC Group 3.

Detailed fetal phenotyping and perinatal genetic testing in all cases showed improved diagnostic yield over time, likely due to the introduction of Next-generation sequencing based tests. The availability of stored DNA will be beneficial for future investigations since further improvements in genetic testing possibilities are expected.

Introduction

Prenatal detection of arthrogryposis multiplex congenita (AMC) remains an ongoing challenge. AMC is a rare congenital condition characterized by multiple contractures affecting various joints (1). Detecting this condition before birth is complicated due to a variety of factors.

Firstly, the etiology of AMC varies, from genetic, environmental, to unknown factors (1,2). Secondly, the onset of AMC varies from the first through the third trimester of pregnancy. Thirdly, the expression of the spectrum AMC ranges from mild to severe. Postnatally, Hall *et al*. initiated the classification of AMC for therapeutic and prognostic purposes into three main groups dependent on involvement: Group 1 with primarily limb involvement, Group 2 with musculoskeletal involvement plus other system anomalies, and Group 3 with musculoskeletal involvement, central nervous system dysfunction and/or intellectual disability and/or lethality (3,4). Fetal akinesia deformation sequence (FADS) is one of the lethal forms of AMC with multiple contractures, abnormal facial profile and small thorax, and motility deterioration during pregnancy and after birth with a variety of causes(4). In line, this classification has been used prenatally to differentiate between the various phenotypes of AMC (5).

Management of all groups of AMC requires a multidisciplinary team of experts for perinatal parental counseling, aiming for care before and around birth, and throughout life. The quality of life of children within Group 1 and 2 is dependent on the cause of AMC. Two studies showed that, in general, these children have an acceptable level of independence and an overall high quality of life (6,7). The outcome and prognosis of children in Group 3 is also dependent on the etiology, however, in case of FADS the majority will die perinatally (8,9).

Prenatal detection of AMC by sonographic examination has shown little improvements over time. A study showed a prenatal detection of AMC in 28 of 107 children (26%) with Amyoplasia born after 1990 (10). In 2019, Dahan-Oliel *et al*. reported a prenatal detection of 53% (21/40) among infants with confirmed AMC Groups 1 and 2 (11). A prenatal detection of 37% was reported in 2024 within a cohort of 301 infants with AMC Group 1 and 2 by Lemin *et al*. (12). The integration of serial motor assessment of the fetus with structural anomaly scanning and evaluation by a multidisciplinary team further enhanced the prenatal detection to 100% in a high-risk population with AMC Groups 1, 2 and 3 (n=66) (13).

Not all causes of AMC can be explained by genetic abnormalities. Currently, more than 400 different genes have been associated with AMC (2). Advances in perinatal genetic testing have evolved over time. Karyotyping for aneuploidies has been available since the 1960s (14). Subsequent innovations have introduced Fluorescence In Situ Hybridization, FISH (1990s), Quantitative Fluorescence-Polymerase Chain Reaction, QF-PCR (2000), and Multiplex Ligation-dependent Probe Amplification, MLPA) (2002), enabling the identification of common aneuploidies in chromosomes 13, 18, and 21 (15-18).

Chromosomal microarrays, in use since 1995, marked a leap forward, allowing for the detection of DNA deletions and duplications (19). Sanger sequencing, a single-gene test widely used since 1977, has been largely replaced by next-generation sequencing (NGS) due to its greater speed and ability to sequence multiple genes simultaneously (20). The use of Whole Exome Sequencing (WES) has shown an increase since 2009 (21-23).

In this study, we aim to present the phenotype and genotype in a 15-year cohort of consecutive fetuses with prenatally suspected and postnatally confirmed AMC. The phenotypic descriptions were prenatally based on (serial) sonographic structural anomaly scans, with systematic or descriptive motor assessment. The postnatal phenotype was based on external examinations with or without neurological examinations and with or without post-mortem examination in case of termination of pregnancy or neonatal death. The performed genetic tests and the overall genetic diagnostic yield of the 15-year period were evaluated, as well as the yield per each 5-year period. This study includes recommendations for genetic testing in case of prenatally suspected AMC, ensuring alignment with the available resources and capabilities in a hospital setting.

Subjects and Methods

Inclusion and exclusion criteria

Inclusion criteria encompassed cases meeting the following criteria: prenatal suspicion of at least two contractures in different anatomical regions (e.g. a combination of contractures in ankle, knee, hip, fingers, wrist, elbow, shoulder) or contracture(s) in a single region with FADS-associated anomalies like facial anomalies, webbing, cerebral anomalies, hydrops, hypo-/akinesia and/or polyhydramnios with or without a family history of FADS/AMC. The suspicion of multiple contractures had to be confirmed in the postnatal period by at least a physical examination of the newborn or post-mortem evaluation in case of intrauterine fetal death or termination of pregnancy.

Postnatal evaluations occur directly after birth by a neonatologist in case of live-birth, and by an obstetrician, clinical geneticist, and/or pathologist in case of death. Excluded were those who did not fulfil the above mentioned criteria, for example contractures in one anatomic region (in e.g. isolated clubfeet).

Study population

In the Netherlands, pregnant women with suspected structural anomalies during a routine mid-trimester fetal ultrasound examination receive a targeted anomaly ultrasound examination in a tertiary Fetal Medicine Unit. In line, all cases underwent (serial) targeted anomaly scans in one of our two Fetal Medicine Units of the tertiary Amsterdam University Medical Center (UMC) in the Netherlands between January 1, 2007, and December 31, 2021. The Medical Ethical Committee of the Amsterdam UMC granted approval under reference number W21_361 # 21.401 and data were extracted at both UMC locations, VU University Medical Center (VUmc) and Academisch Medisch Centrum.

Data collection

For the description of the prenatal and postnatal phenotype and genotype, data were collected using the Fetal Medicine Units' Astraia database for ultrasound examinations, EPIC (Electronic Portfolio of International Credentials) for patient charts, and Genesis (GENetic Estimation and Inference in Structures samples) for genetic results. Also, it was evaluated if DNA was stored for potential future DNA re-assessment. Moreover, data extraction from location VUmc was supported by the perinatal database maintained by the Amsterdam UMC Expertise Center for FADS and AMC (24). This expertise centre on rare disease is acknowledged by Ministry of Health, Welfare and Sport since 2015, first at location VUmc and when both Amsterdam UMC locations merged, continued at location Amsterdam Medisch Centrum in 2021.

Phenotypic and genotypic classification

Postnatally, the phenotypes were divided into the three AMC Groups according to Hall *et al*. (4). However, prenatally, AMC Group 1 and 2 were merged due to sonographic limitations in distinguishing between AMC Group 1 and 2. For instance, it is challenging to detect the more pronounced muscle atrophy characteristic of Group 1 compared to Group 2 by using ultrasound.

Additionally, since intellectual disability cannot be detected through ultrasound examinations, the phenotypes of Hall *et al*. were modified for the prenatal period as follows:
•AMC Group 1+2: primary limb involvement + musculoskeletal involvement plus other system anomalies;
•AMC Group 3: musculoskeletal involvement with central nervous system involvement and/ or lethality within the spectrum of FADS (expanding contractures or abnormal/ worsening fetal movements or FADS related anomalies).

In cases where the prenatal or postnatal differential diagnosis had overlapping phenotyping Group 2 and 3, the phenotype was classified as Group 3 to emphasize the wide spectrum of AMC from milder to severe expressions.

The fetal structural anatomy in all cases were examined by targeted anomaly scans, and fetal movements by systematic motor assessment (sMA) or descriptively. At location VUmc, the majority of the fetuses underwent sMA. Details concerning the organization of the 7-step care pathway and the sMA evaluation can be read in the care pathway which was designed by a multidisciplinary team of our expertise center for AMC (24). The 15-minute motor assessment is conducted before a gestational age of 24 weeks to distinguish between the milder types of AMC and its most severe form (FADS), given the legal limit for pregnancy termination in the Netherlands.

During fetal movement recording, we evaluate differentiation (into movement patterns), quantity (frequency of general movements involving all limbs, trunk, and head), and quality (of general movements, isolated arm- and leg-movements concerning variation in amplitude, speed, joint participation, and direction). At location Academisch Medisch Centrum, the movements were mainly described descriptively. Movements were considered as abnormal if the sMA, evaluating three aspects, differentiation (D) into specific movement patterns, qualitative (QL) and quantitative performance (QN), during a sufficient long age-related observation period, revealed at least an abnormal qualitative performance (13,24). Movements evaluated descriptively were considered as abnormal if the sonographer documented that the movements were abnormal, reduced, or absent.

Prevalence calculation
The prevalence of AMC was estimated per 10,000 pregnancies in the North-West region of the Netherlands. The number of women receiving routine mid-trimester fetal ultrasound examination in this region was derived from not published, but digitised yearly audit files per prenatal ultrasound examination unit.

Moreover, we estimated the number of cases within the same region in which a targeted anomaly scan was either not performed, the mid-trimester fetal ultrasound examination did not lead to a prenatal referral, or in case the anomaly was not identified prenatally. These children were postnatally treated by the pediatric orthopedic surgeons and/or physiatrist and were described separately in this study. We used this number to identify missing prenatal cases with the AMC phenotype.

Genetic testing

The type and number of tests that were performed after counseling by the clinical geneticist and depending on parents' request, were examined. Genetic tests that were used included karyotyping, rapid aneuploidy testing (FISH, QF-PCR, or MLPA), chromosomal microarray, single gene testing, panel (various panels are available e.g. targeted Arthrogryposis panel and WES based FADS panel), and WES. WES based copy number variation (CNV) analysis was not implemented during the study period. Gene panels were updated yearly during the study period. The genes included in the latest FADS panel used in de study period are listed in Supplementary File 2. Findings per genetic test were presented for the prenatal phenotypes AMC Group 1+2 and for AMC Group 3. Additionally, the analysis is performed for the 15-year period as well as the three consecutive 5-year periods. For this study, all genotypic and phenotypic results were re-examined and genetic evaluation was updated with the present literature.

The results of prenatal genetic testing in women with more than one affected pregnancy with the same phenotypes and/or genotypes were initially assessed for each pregnancy individually, but subsequently the results were combined to form one case.

Statistics

Changes in prevalence of genetic tests and yield were compared by descriptive statistics in Excel, Microsoft 356 A3 for faculty. The genetic overall yield is evaluated in percentages. A chi-square analysis was performed to evaluate the differences in genetic diagnostic yield between the three 5-years intervals. A p-value <0.05 was considered statistically significant.

Results

Characteristics

In 64 women with in total 81 pregnancies, the inclusion criteria of their fetuses fulfilled the criteria of AMC, suspected by targeted anomaly scan and confirmed postnatally. The distribution of the phenotypes in the 64 women consisted of 13 with the prenatal phenotype within Group 1+2 and 51 within Group 3. The pregnancy outcomes are summarized in Table 1A and Supplementary Table 1A. There were eight women (cases 2008-3, 2009-3, 2010-1, 2012-6, 2012-10, 2015-2, 2017-7, 2020-1) with more than one pregnancy (total n=25), showing a similar prenatal phenotype and all within Group 3.

The distribution of the cases was 38 at location VUmc and 26 at location Academisch Medisch Centrum. The distribution of the fetal phenotype Groups 1+2 and 3 did not differ between both locations (p=0.28). Motor evaluation was available in 100% at location VUmc of which 84% (32/38) by sMA and 16% (6/38) descriptively, and in 92% (24/26) descriptively at location Academisch Medisch Centrum. In the remaining 2 cases there were no descriptives on fetal movements reported.

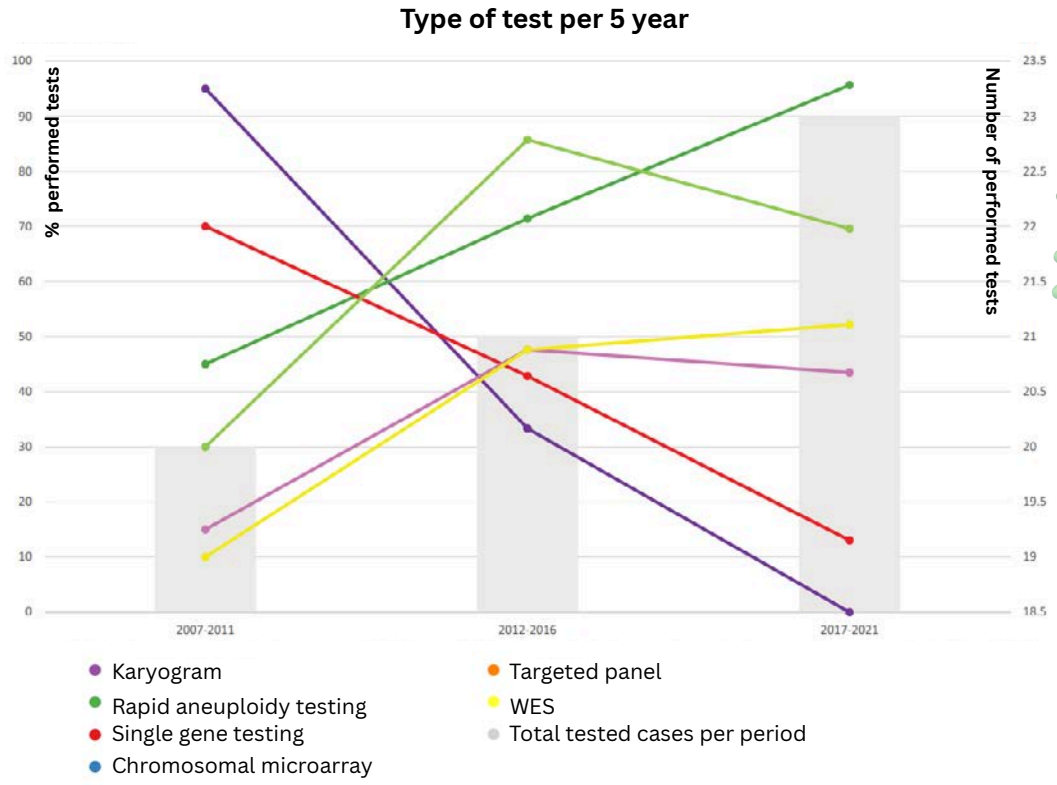


Figure 1 Genetic tests performed in a population of fetuses suspected of arthrogryposis multiplex congenita in a 15-year cohort, depicted per 5-year periods in numbers and percentages.

Table 1. A: Description of the prenatal and postnatal phenotype of 18 fetuses with AMC, classified according to Hall *et al*. [1]; B: Description of genotype based on perinatal genetic tests of 18 fetuses with AMC identified in the prenatal period at Amsterdam UMC during the period 2007–2021.

CASE	PRENATAL											POSTNATAL						
	Pregnancies with same phenotype	Consanguinity	Sonographic phenotype															
			Detection of the contractures (weeks)	Contractures CAL=arms and Legs	Enlarged nuchal translucency/fold	Hydrops	Pterygia	Amniotic fluid volume N = normal P = polyhydramnios O = oligohydramnios	CNS malformation	intrauterine growth restriction	Systematic motor assessment. If yes: differentiation(D),Quality (QL), quantity (QN): normal (nl)/ abnormal (abn) If no: descriptively	AMC group	Outcome A= alive at birth, IUFD = intrauterine fetal death, TOP = termination of pregnancy, N = neonatal death Gestational age at birth in weeks	Contractures CAL = arms and Legs	Additional anomalies CAL = arms and Legs	Autopsy	Age at last clinical evaluation. DPN = direct postnatal	AMC group
2008-1*	1	–	20 + 3	CAL: wrist, clubfoot Other: micrognathia	–	–	–	N	–	–	Yes 2x D, QL, QN nl	1 + 2	A, 40 + 4	CAL: fingers, wrist, clubfoot, clubfeet	Aortic root dilatation, pectus carinatum, kyphoscoliosis, normal cognitive development	–	16 years, diagnosis at age of 4	2
2008-3*, ***	5	+	13 + 3 12 + 2 10 + 5 10 + 6 11 + 0	CAL: wrists and clubfeet	+	+	+	N	–	–	No Absent fetal movements	3	TOP, 14 + 0 TOP, 12 + 6 TOP, 11 + 0 TOP, 11 + 6 TOP, 11 + 6	CAL: wrists and clubfeet	Cystic hygroma, lung hypoplasia, webbing elbows/knees – – – –	+ – – – –	DPN DPN DPN DPN DPN	3
2010-1*	3	–	21 + 3 18 + 5 13 + 0	CAL: wrists, knees and clubfeet	+	+	–	P	–	–	Yes Only in 2nd pregnancy: D, QL, QN abn	3	IUFD, 31 + 3 TOP, 23 + 6 IUFD, 13 + 3	CAL: wrists, knees and clubfeet	– – Hydrops	+ + +	DPN DPN DPN	3
2012-3	1	+	20 + 5	CAL: hips, knees, elbows, shoulders, wrists and fingers	+	–	–	N	Ventriculomegaly, choroid plexus cysts	–	No Absent movements	3	TOP, 23 + 0	CAL: hips, knees, elbows, shoulders, wrists and fingers	Micro-retrognathia	+	DPN	3
2012-10*	2	–	20 + 5 12 + 6	CAL: elbows, wrists, knees, clubfeet Other: short legs	+	–	–	N	–	–	Yes D abn QL abn isolated arm and leg movements variation reduced, general movements variation reduced, no in- and decreasing activity, abrupt movements QN nl	3	TOP, 23 + 5 TOP, 15 + 0	CAL: elbows, wrists, knees, clubfeet Other: short legs	Retrognathia, hitchhiker thumbs –	+ –	DPN DPN	3, diastrophic dysplasia
2015-2	2	+	13 + 6 20 + 1	1st pregnancy CAL: legs and arms 2nd pregnancy CAL: wrists, clubfeet Heart: VSD	+	–	–	N	–	–	No Absent movements	3	TOP, 14 + 6 TOP, 21 + 5	1st pregnancy CAL: legs and arms 2nd pregnancy CAL: wrists, clubfeet	Webbing Micrognathia, Coarctation of the aorta	+ +	DPN DPN	3, Nemaline myopathy-8 only in 2nd pregnancy
2016-3*	1	–	19 + 5	CAL: flexed wrists, clubfeet, Other: short femur	–	–	–	N	–	+	Yes D nl, QL of general movements abnormal (reduced participation in hands and feet), QN nl	1 + 2	A, 40 + 0 Died at age of 5 due to a septic shock after a RS virus pneumonia	CAL: flexed wrists, overlapping toes, clubfeet,	Other: feeding problems, bifid uvula, ptosis, small deepset eyes	–	5 years	2, Distal Arthrogryposis type 3 or 5

Table 1. (continued)

CASE	PRENATAL											POSTNATAL					
	Pregnancies with same phenotype	Consanguinity	Sonographic phenotype														
			Detection of the contractures (weeks)	Contractures CAL=arms and Legs	Enlarged nuchal translucency/ fold	Hydrops	Pterygia	Amniotic fluid volume N = normal P = polyhydramnios O = oligohydramnios	CNS malformation	intrauterine growth restriction	Systematic motor assessment. If yes: differentiation(D),Quality (QL), quantity (QN): normal (nl)/ abnormal (abn) If no: descriptively	AMC group	Outcome A= alive at birth, IUFD = intrauterine fetal death, TOP = termination of pregnancy, N = neonatal death Gestational age at birth in weeks	Contractures CAL = arms and Legs	Additional anomalies CAL = arms and Legs	Autopsy	Age at last clinical evaluation. DPN = direct postnatal	AMC group
2017-2	1	–	20 + 1	CAL: extended knees and abducted hips	–	–	–	N	–	–	No Normal overall movements, no movements in knees	2	A, 41 + 1	CAL: extended knees, abducted hips, ankle and finger contractures	Short neck, small mouth, mild retrognathia, ptosis, scoliosis, nl cognitive development. Walking	–	7 years, diagnosis in 5th month	2, Distal Arthrogryposis type 5
2017-4*, **	1	–	14 + 5	CAL: elbows, wrists, clubfoot Heart: abnormal aortic arch	+	–	–	N	–	–	Yes 2x, D nl, AL abn, worsening over time, QN nl	3	N, 38 + 6 Died after 4 months due to respiratory insufficiency	CAL: fingers, elbows, wrists, clubfoot	Heart: cardiomyopathy (open ductus and abnormal aortic arch)	–	4 months	3
2017-6	1	–	18 + 4	Fingers bilateral	–	–	–	N	–	–	No Normal movements	2	A, 38 + 6	CAL: fingers, elbows, hips	Heart : small VSD Normal cognitive development. Walking independently.	–	8 years	2
2018-4	1	–	19 + 5	CAL: arms, legs and clubfeet Other: empty stomach	+	+	–	P	–	–	No Absent movements	3	TOP, 22 + 3	CAL: arms, legs and clubfeet	Micrognathia, hypertelorism	–	DPN	3
2019-2*	1	–	19 + 5	CAL: clubfeet Other: open mouth.	+	+	–	N	–	–	Yes 2x D nl, QL abn and worsening over time, QN nl	3	N, 39 + 0 Child died at 2 months, respiratory insufficiency	CAL: wrist contractures and clubfeet	Floppy infant Other: retro/ micrognathia, renal cysts	–	2 months	3
2019-3	1	–	19 + 4	CAL: overlapping fingers, fixed elbows, wrists, knees and clubfeet Other: short limbs, Small thorax, hypospadias	–	–	–	N	–	–	No Decreased movements	3	TOP, 21 + 1	CAL: overlapping fingers, fixed elbows, wrists, knees and clubfeet	Webbing elbows, cleft palate	–	DPN	2/3 Distal arthrogryposis type 3 or 5
2019-4*	1	–	20 + 3	CAL: overlapping fingers, clubfeet Other: heart: VSD, micrognathia, omphalocele	–	–	–	P	Blake's pouch cyst	–	No Normal movements	3	TOP, 21 + 3	CAL: overlapping fingers, clubfeet	No cerebral anomalies	+	DPN	3
2020-1*	2	–	12 + 0 13 + 4	CAL: fixed arms, clubfeet	+	+	–	N	–	–	No Absent movements	3	TOP, 12 + 3 TOP, 14 + 3	CAL: fixed arms, clubfeet	– Webbing	– +	– DPN	3
2020-5	1	–	16 + 6	CAL: fingers, elbows, knees	+	–	–	N	–	–	No Absent fetal movements	3	TOP, 21 + 1	CAL: fingers, elbows, knees	Micrognathia, webbed neck	–	–	3

Table 1. (continued)

CASE	PRENATAL											POSTNATAL					
	Pregnancies with same phenotype	Consanguinity	Sonographic phenotype: Detection of the contractures (weeks)	Contractures CAL=arms and Legs	Enlarged nuchal translucency/fold	Hydrops	Pterygia	Amniotic fluid volume N = normal P = polyhydramnios O = oligohydramnios	CNS malformation	intrauterine growth restriction	Systematic motor assessment. If yes: differentiation(D),Quality (QL), quantity (QN): normal (nl)/ abnormal (abn) If no: descriptively	AMC group	Outcome A= alive at birth, IUFD = intrauterine fetal death, TOP = termination of pregnancy, N = neonatal death Gestational age at birth in weeks	Contractures CAL = arms and Legs	Additional anomalies CAL = arms and Legs	Autopsy	Age at last clinical evaluation. DPN = direct postnatal	AMC group
2020-8*	1	–	19 + 5	CAL: hands, clubfeet	–	–	–	N	–	–	Yes 2x, D, QL and QN abn	3	TOP, 21 + 5	CAL: hands, clubfeet	Micrognathia	+	DPN	3
2021-2*	1	–	19 + 3	CAL: Clubfeet Open mouth	–	–	–	N	–	–	Yes 2x. 19 wk D, QL and QN nl, 22 wk worsening: D nl, QL abn, absent variation in amplitude, speed, participation and direction, no in and decreasing activity only fluency, in isolated arm movements and general movements, QN nl	3	TOP, 22 + 5	CAL: Clubfeet Open mouth	Contractures wrists, elbows, fingers, hips and knees	–	DPN	2/3

B

CASE	Genetic tests A karyotyping B rapid aneuploidy C single gene testing D chromosomal microarray E1 panel E2 WES E3 genome-wide linkage scan and Sanger sequencing in research setting Bold for test detecting the mutation	Pre/postnatal testing? Pr=prenatal Po = postnatal Pr/Po testing both pre- and postnatal	Inheritance DN = de novo AR = autosomal recessive	Nucleotide alteration, deduced protein change	ACMG criteria P = pathogenic LP = likely pathogenic	Aggregated Pathogenicity Prediction (Franklin)	MAF (according to gnomAD v4.1.0)
2008-1*	A, **C**, D	Pr/**Po**	DN	NM_004612.4(TGFBR1):c.1013 A > G p.(Asn338Ser) Chr9(GRCh37):g.101907053 A > G	LP: PM1, PM2, PM6, PP2, PP3	Deleterious (0.87)	–
2008-3*, ***	A, B,C, E1, **E3**	Pr/**Po**	AR (Homozygous confirmed in 4 affected sibs, both parents heterozygous)	NM_000540.3(RYR1):c.6721 C > T p.(Arg2241*) Chr19(GRCh37):g.38987106 C > T	P: PVS1, PM2, PM3, PP5	–	0.0001611 (no homozygous alleles)
2010-1*	A, B, **E2**	Pr/ **Po**	AR (2 affected sibs, compound heterozygous) Both parents and one healthy sib heterozygous, one healthy sib no carrier.	NM_000334.4(SCN4A):c.274-2 A > G p.? Chr17(GRCh37):g.62049832 T > C (maternal) NM_000334.4(SCN4A):c.3911_3912+1del p.(Lys1304Ilefs*17) Chr17(GRCh37):g.62022041_62022043del (paternal) Confirmed in all 3 fetuses	P: PVS1, PM2, PM3, PP1 P: PVS1, PM2, PM3, PP1	– –	– 0.00001812 (no homozygous alleles)
2012-3	A, **C**, D	**Pr/** Po	AR (Homozygous, both parents heterozygous)	NM_005592.4(MUSK):c.1724 T > C p.(Ile575Thr) Chr9(GRCh37):g.113547944 T > C	LP: PS3, PM2, PP1, PP3, PP5	Deleterious (0.87)	0.00001178 (no homozygous alleles)
2012-10*	A, B, **C**, D, E1	Pr/**Po**	AR (Compound heterozygous) Both parents heterozygous	NM_000112.4(SLC26A2):c.931 T > C p.(Cys311Arg) Chr5(GRCh37):g.149360087 T > C (paternal) NM_000112.4(SLC26A2):c.1957T>A p.(Cys653Ser) Chr5(GRCh37):g.149361113 T > A (maternal)	LP: PM2, PM3, PP3, PP5 P: PS3, PM1, PM2, PM3, PP1, PP3, PP5	Deleterious (0.87) Deleterious (0.86)	0.000002478 (no homozygous alleles) 0.0001121 (no homozygous alleles)
2015-2	B, D, E1, **E2**	Pr/**Po**	AR (homozygous, both parents heterozygous) Only in 2nd pregnancy	NM_152393.4(KLHL40):c.360 C > A p.(Cys120*) Chr3(GRCh37):g.42727470 C > A	LP: PVS1, PM2	–	0.000006204 (no homozygous alleles)
2016-3*	B, D, **E2**	Pr/**Po**	DN	NM_022068.4(PIEZO2):c.2994 G > A p.(Met998Ile) Chr18(GRCh37):g.10762974 C > T	LP: PM2, PM5, PM6, PP5	Uncertain (0.57)	–
2017-2	B, D, **E2**	Pr/**Po**	AR (homozygous, both parents heterozygous)	NM_004826.4(ECEL1):c.1470 G > A p.(Trp490*) Chr2(GRCh37):g.233348162 C > T	P: PVS1, PM2, PM3, PP5	–	0.00002232 (no homozygous alleles)

Table 1. (continued)

CASE	Genetic tests A karyotyping B rapid aneuploidy C single gene testing D chromosomal microarray E1 panel E2 WES E3 genome-wide linkage scan and Sanger sequencing in research setting Bold for test detecting the mutation	Pre/postnatal testing? Pr=prenatal Po = postnatal Pr/Po testing both pre- and postnatal	Inheritance DN = de novo AR = autosomal recessive	Nucleotide alteration, deduced protein change	ACMG criteria P = pathogenic LP = likely pathogenic	Aggregated Pathogenicity Prediction (Franklin)	MAF (according to gnomAD v4.1.0)
2017-4*, **	B, **E1**	Pr/ **Po**	DN	NM_001005361.3(DNM2):c.1090 C > T p.(Arg364Cys) Chr19(GRCh37):g.10904493 C > T	LP: PM1, PM2, PM5, PM6, PP2, PP3	Deleterious (0.72)	–
2017-6	B, **D**	**Pr**	Maternal Mother and maternal grandfather both carrier of the duplication both affected with distal arthrogryposis	Arr[GRCh37] 6q24.3q25.2(145784222_153395582)x3 (gain of 7.6 Mb)			
2018-4	B, D, **E1**	**Pr**/Po	AR (Homozygous, both parents heterozygous)	NM_005592.4(MUSK):c.220 C > T p.(Arg74Trp) Chr9(GRCh37):g.113449410 C > T	LP: PM2, PM3, PP1, PP5	Uncertain (0.56)	0.000008059 (no homozygous alleles)
2019-2*	**E2**	**Po**	AR (Homozygous, both parents heterozygous)	NM_000287.4(PEX6):c.814_817dup p.(Val273Alafs*9) Chr6(GRCh37):g.42946073_42946076dup	P: PVS1, PM2, PP5	–	–
2019-3	B, C, D, E1, **E2**	Pr/ **Po**	DN	NM_022068.4(PIEZO2):c.8056 C > T p.(Arg2686Cys) Chr18(GRCh37):g.10671727 G > A	P: PS2, PM2, PM5, PP2, PP3, PP5	Deleterious (0.99)	–
2019-4*	B, D, **E2**	Pr/**Po**	AR (Compound heterozygous)	NM_024105.4(ALG12):c.200 C > G p.(Thr67Arg) Chr22(GRCh37):g.50307128 G > C (maternal) NM_024105.4(ALG12):c.437 G > A p.(Arg146Gln) Chr22(GRCh37):g.50304114 C > T (paternal)	LP: PM2, PM3, PM5, PP3 PS3, PM2, PM5, PP3, PP5	Deleterious (0.87) Deleterious (0.74)	– 0.0001233 (no homozygous alleles)
2020-1*	B, D, **E2**	**Po**	AR (2 affected sibs, both compound heterozygous)	NM_001164508.2(NEB):c.22205del p.(Asn7402Thrfs*11) Chr2(GRCh37):g.152381745del (maternal) NM_001164508.2(NEB):c.2014_2015del p.(Leu672Ilefs*30) Chr2(GRCh37):g.152548664_152548665del (paternal)	LP: PVS1, PM2 LP: PVS1, PM2	– –	– 6.197e−7 (no homozygous alleles)
2020-5	B, D, **E1**	**Pr**	AR (Compound heterozygous) Both parents heterozygous	NM_005199.5(CHRNG):c.397del p.(Ser133Profs*50) Chr2(GRCh37):g.233406130del (maternal) NM_005199.5(CHRNG):c.401_402del p.(Pro134Argfs*43) Chr2(GRCh37):g.233406134_233406135del (paternal)	P: PVS1, PM2, PM3, PP5 P: PVS1, PM2, PM3, PP5	– –	0.0001047 (no homozygous alleles) 0.00009046 (no homozygous alleles)
2020-8*	B, E1, **E2**	Pr/ **Po**	DN	NM_003289.4(TPM2):c.782 A > G p.(Tyr261Cys) Chr9(GRCh37):g.35683229 T > C	LP: PM2, PM6, PP2, PP3, PP5	Deleterious (0.86)	–
2021-2*	B, **E2**	Pr/ **Po**	AR (Compound heterozygous) Both parents heterozygous	NM_022068.4(PIEZO2):c.492+2 T > C p.? Chr18(GRCh37):g.10871249 A > G (maternal) NM_022068.4(PIEZO2):c.570 C > T p.(Gly190 =) / r.spl? Chr18(GRCh37):g.10857132 G > A (paternal)	LP: PVS1, PM2 VUS: PM2	Deleterious (0.8) SpliceAI: Splice-Altering/ strong (0.99) Deleterious (0.79) SpliceAI: Splice-Altering/ strong (0.97)	– 6.503e−7

Identified in the prenatal period at Amsterdam UMC during the period 2007–2021. The genotype is presented in Table 1B.

* For cases that were seen at location VUmc.

** For cases that were published by Tjon et al.

*** For one case that was published by McKie et al.

Classification following ACMG Criteria.

*** Published by McKie et al.

Prevalence

During the study period, a total of 485,000 pregnant women received a mid-trimester fetal ultrasound examination in the North-West of the Netherlands. The prevalence of AMC per 10,000 pregnancies with mid-trimester fetal ultrasound examination in North-West Netherlands was 1.7 (81 pregnancies of the 64 mothers /485,000). The paediatric orthopedic surgeons and physiatrists of Amsterdam UMC treated another four children with AMC that were not included in this prenatal cohort (one case with AMC in Group 1 and 3 in Group 2). They underwent routine mid-trimester fetal ultrasound examinations between 2007-2021 in the North-West region of the Netherlands. During this routine mid-trimester fetal ultrasound examinations, twice no anomalies were identified and therefore they were not referred to a Fetal Medicine Unit. The remaining two cases were referred because of isolated contractures (one clubfeet and one wrist contracture), but the targeted anomaly scans did not reveal other anomalies. Postnatally, examinations showed multiple contractures in all four children.

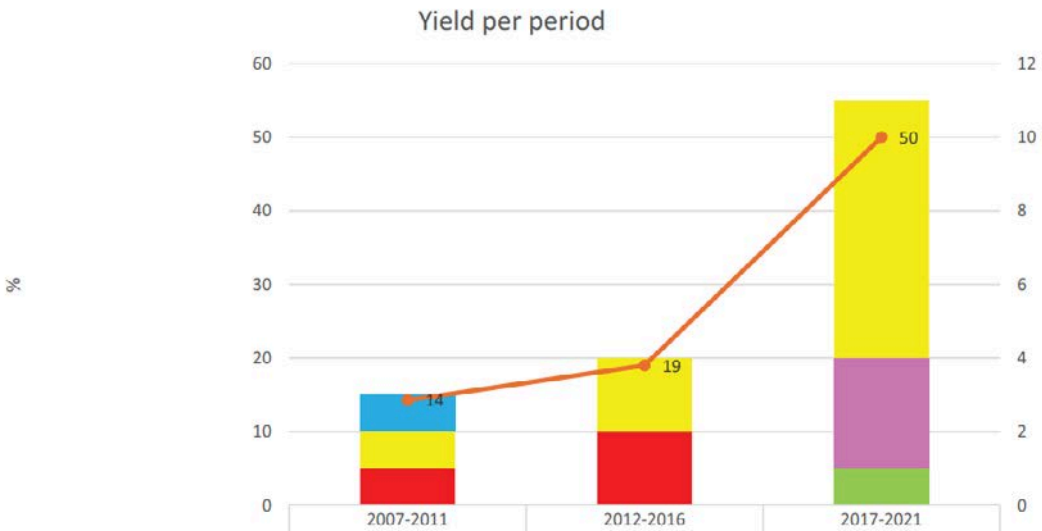
Prenatal and postnatal phenotype

The phenotype and pregnancy outcomes of the fetuses with a genetic diagnosis are summarized in Table 1A and for fetuses without a genetic diagnosis in Supplementary Table 1A.

The fetal phenotypes AMC Group 1+2 were suspected in 13 cases. Postnatal confirmation occurred in all, with 6 into Group 1 and the remaining 7 into Group 2. The outcome was live-born children in 9 (69%) and termination of pregnancy in 4 (31%). An AMC Group 3 phenotype was suspected in 51 cases. Postnatal confirmation occurred in all, with 49 cases classified in Group 3 , and 2 into Group 2 (Table 1A and Supplementary Table 1A). Of these 51 cases with a total of 68 pregnancies, the outcome was 3 live-born children (4%), 46 (68%) terminations of pregnancy, 12 (18%) intrauterine fetal deaths and 7 (10%) neonatal deaths.

Genetic tests

Perinatal genetic testing occurred in 100%. Prenatal genetic testing was performed in 88% (56/64). Postnatally, 56% (36/64) of the cases received first time genetic testing or extended testing (Table 1B and Supplementary Table 1). Combined pre- and postnatally testing occurred in 28 of the 64 cases (44%). The total number of tests and percentages of tests that were conducted per 5 year period are depicted in Figure 1. DNA samples from all cases have been stored and remain available for future re-assessment.



	2007-2011	2012-2016	2017-2021
Rapid aneupoloidy detection	0	0	0
Karyotype	0	0	0
GWLA in research setting	1	0	0
WES	1	2	7
Panel testing	0	0	3
Chromosomal microarray	0	0	1
Single gene test	1	2	0
Yield per period (%)	14	19	50

Figure 2. Perinatal genetic diagnostic yield in a cohort of 64 fetuses suspected of arthrogryposis multiplex congenita, depicted per 5-year periods in percentages (number of genetic abnormalities found per fetus). GWLA genome-wide linkage scan and Sanger sequencing in a research setting.

Genotype

The overall genetic diagnostic yield for the study population was 28% (18/64). The overall genetic diagnostic yield per 5-year interval is shown in Figure 2. An increase was seen in the genetic diagnostic yield with the performed tests between the three 5-year intervals (p=<0.00001) with the highest yield of 50% between 2017-2021. The genetic diagnostic yield per group and per test are summarized in Table 2. Panel testing and WES had the highest yield (13% and 41.7%). The yield per phenotype was 30.8% (4/13) for AMC Group 1+2 and 27.4% (14/51) for AMC Group 3. Variants were identified in the *ALG12*, *CHRNG*, *DNM2*, *ECEL1*, *KLHL40*, *MUSK* (2x), *NEB*, *PEX6*, *PIEZO2* (3x), *RYR1*, *SCN4A*, *SLC26A2*, *TGFBR1*, and *TPM2* genes. The variant in *RYR1* was identified through a genome-wide linkage scan and Sanger sequencing of the candidate gene. Additionally, genetic testing in this group revealed one maternally derived chromosomal abnormality, a microduplication 6q24.3q25.2. There are no cases that were diagnosed after re-analysis. Description of the genotype based on perinatal genetic tests are shown in Table 1B and Supplementary Table 1B.

Table 2. Genetic diagnostic yield per test in a cohort of 64 women with fetuses suspected for AMC groups 1, 2, and 3 during a 15-year period 2007–2021.

Group(s) with AMC - AMC 1,2,3 - AMC 1 + 2 - AMC 3	Genetic test(s)	Yield % (number of abnormalities/ group of fetuses)
AMC 1,2,3	Karyotyping	0 (0/26)
AMC 1,2,3	Rapid aneuploidy testing	0 (0/46)
AMC 1,2,3	Single gene testing	11.5 (3/26)
AMC 1,2,3	Chromosomal microarray	2.5 (1/40)
AMC 1,2,3	Panel testing	13 (3/23)
AMC 1,2,3	WES	41.7 (10/24)
AMC 1 or 2	All tests	30.8 (4/13)
AMC 3	All tests	27.4 (14/51)

Discussion

Targeted anomaly scans and motor evaluations in fetuses with multiple contractures, identified during the routine mid-trimester fetal ultrasound examinations, raised the prenatal suspicion of the AMC phenotype in 81 fetuses at our tertiary care center between 2007 and 2021. All parents opted for genetic evaluation before or after birth. Prenatal genetic testing was performed in the majority and combined pre- and postnatal testing in almost a half. A genetic underlying cause was identified in one-quarter of the cases during this 15-year period, increasing to half of the cases during the last 5-year period (2017-2021) due to the introduction of new genetic tests in diagnostics. Among the tests applied, NGS based tests, i.e. (WES based) panel testing and WES had the highest yield (13% and 41.7%).

Prevalence and phenotypic distribution

The finding of this study concerning the prenatal prevalence of 1.7 per 10,000 is in line with the previously reported live birth prevalence for the different AMC phenotypes. Reported prevalences are 1:10,000 for AMC Group 1 (including Amyoplasia), 1:3,000 for AMC Group 2 (with Distal Arthrogryposis), and 1:6,985-25,250 for AMC Group 3 (with FADS) (9,26,27).

In contrast to the prenatal period, distinction between the phenotypes of AMC Group 1, 2 and 3 was enabled postnatally by additional examinations, e.g. neurological examination. This resulted in a postnatal confirmation of AMC Group 1 in 46% (6/13). The low percentage of cases with Amyoplasia in Group 1 of this study (9%, 6/64) differs from prior reported postnatal AMC population, where Amyoplasia is the most frequent type of AMC (25-30% of all individuals with AMC) (1). A possible explanation is that Amyoplasia (e.g. atrophy) is not easily recognizable in the period of the routine mid-trimester fetal ultrasound examination. Probably, it is easier in the third trimester to recognize this atrophy. All cases with Amyoplasia in our cohort were diagnosed postnatally. Despite improvements in prenatal ultrasound techniques and the expertise of sonographers, it is still challenging to distinguish Amyoplasia and the other types of AMC.

It is emphasized that the fetal phenotypic description was not only supported by anatomical evaluation, but also by evaluation of the movements(13,24,28). In general, descriptive motor assessment is mostly applied for routine mid-trimester fetal ultrasound examinations (29). Despite the term FADS, absent movements are not frequent observed in fetuses with the phenotype AMC Group 3. The "A" in FADS stands for akinesia, which implies the absence of movements. However, based on two studies on consecutive decades of assessing fetal movements in FADS, we have observed that most fetuses with FADS do, in fact, move. In half of the cases, the quantity and differentiation of fetal movements are still within the normal range, while the qualitative performance is abnormal in all (13,24,28). In this study, akinesia was observed in 13 cases of which once by sMA and twelve times descriptively. The awareness has to remain that in case of late onset of AMC the motility does not have to be reduced (yet), even with the effort of serial ultrasound examinations to detect worsening over time.

Genetic findings

Perinatal genetic testing occurred in all cases of this cohort. However, globally, prenatal genetic testing is not always feasible due to social-economic circumstances. The knowledge of the underlying cause is of importance for counseling on the optimal care perinatally. Moreover, parents should be given the opportunity to consider the possibility of termination of pregnancy within the international varying abortion laws, even in case of congenital anomalies.

The overall genetic diagnostic yield in this population increased over the study period from 14% to 50%, largely due to the introduction of NGS based tests. This yield aligns with other studies in cohorts with AMC demonstrating a genetic diagnostic yield of 35-73% with WES, which is significantly higher than the 5% yield reported for chromosomal microarray (30-40). This highlights how genetic testing has evolved over the 15-year period with improvements in available genetic tests and it will continue further when more knowledge is obtained and novel tests are introduced. A new development in the diagnostic pathway is the use of Whole Genome Sequencing (WGS) to improve the diagnostic yield(23). WGS offers the advantage of detecting a broader range of genetic variations, including copy number variations, structural variants and non-coding variants, increasing the likelihood of a definitive diagnosis. Thereby, WGS can be used as a single test replacing most of the currently performed tests with a faster turnaround time.

The genetic diagnostic yield in fetuses with the prenatal phenotypes AMC 1+2 was 30.8% (4/13). Postnatally, all 4 fetuses were classified as AMC Group 2. Genetic testing in this group revealed one maternally derived chromosomal abnormality, a microduplication 6q24.3q25.2. The mother and maternal grandfather (who also carried the duplication) were affected with mild distal arthrogryposis and had normal cognitive development. The remaining cases had (likely) pathogenic variants in the *ECEL1*, *TGFBR1*, or *PIEZO2* gene. Additionally, there were 2 variants of unknown significance (VUS) in AMC Group 1+2, see Supplementary Table 1B. As expected, in the limited number of 6 fetuses with Amyoplasia no genetic anomaly was identified. Until now, no siblings or descendants have been reported with recurrent Amyoplasia (41). However, in research setting there are still ongoing efforts to identify genetic causes for these anomalies (1,41). A vascular origin during the embryonic period or epigenetic causes have been suggested (1,41).

In the AMC Group 3, we found in 27.4% (14/51) of the cases a causal (likely) pathogenic variant. These were in the *ALG12*, *CHRNG*, *DNM2*, *KLHL40*, 2x *MUSK*, *NEB*, *PEX6*, 2x *PIEZO2*, *RYR1*, *SCN4A*, *SLC26A2*, and *TPM2* genes. The majority of these genes are involved in skeletal muscle function (*DNM2, KLHL40*, *NEB, RYR1, SCN4A*, and *TPM2*) in 42.9% (6/14) or the neuromuscular junction function (*CHRNG* and *MUSK*) in 21.4% (3/14) of the cases, which is in line with previous observations(35). Additionally, there were 5 VUS's identified in AMC Group 3 cases, see Supplementary Table 1B. Comparable to the Group 1+2, attention has to be paid whether these VUS's could be associated with AMC Group 3 in the future. In one case was no underlying genetic anomaly identified, but the autosomal recessive Neu Laxova Syndrome was clinically suspected.

Among these genes, only three genes, *ALG12*, *KLHL40*, and *TGFBR1*, have not been listed in Kiefer and Hall's manuscript, which provides an overview of genes associated with the phenotype AMC in literature until 2019 (2). *ALG12* has been associated with congenital disorder of glycosylation, type Ig (OMIM 607143), a phenotype which includes generalized hypotonia, dysmorphic features, and progressive microcephaly, but also musculoskeletal malformations as overlapping fingers and pes equinovarus have been described (42,43). Mutations in *KLHL40* are a frequent cause of severe autosomal-recessive nemaline myopathy with multiple contractures (44). TGFBR1 has been reviewed by Baldo *et al*. in 2022 (45). They confirmed the association with the AMC phenotype by describing 14 neonatal cases with Loeys-Dietz Syndrome, a phenotype with variable expression of hypotonia and contractures.

Furthermore, three *PIEZO2* variants (two with dominant and one with recessive inheritance) were found in this cohort, once (*de novo*) prenatally suspected as AMC Group 2 and twice (one *de novo*, one recessive) as AMC Group 3. The diverse phenotypic expression underscores the importance of phenotypic evaluation over time, as goal to evaluate worsening. Autosomal dominant, loss-of-function variants in *PIEZO2* have been associated with the phenotypic overlapping features of Gordon syndrome (DA typ3), Distal Arthrogryposis type 5 and Marden Walker syndrome(46). The recessive *PIEZO2* variants is associated with loss-of function, causing Distal Arthrogryposis with impaired proprioception and touch (DAIPT) (47).

Strengths and limitations

The strength of this study is the systematic approach to evaluate phenotypic and genotypic characteristics. Tailored prenatal parental counseling by an obstetrician and clinical geneticists were performed, extended through a paediatric physiatrist and orthopedic surgeon in case of AMC Group 1+2 and paediatric neurologist and neonatologist in case of AMC Group 3. Our overview of the genetic diagnostic yield over the 15-year period clearly shows the limitations of the genetic tests that were available during the earlier period of the study. On the other hand, the high percentage of DNA storage will facilitate future re-assessment on individual parental request, potentially not only by WES but also by WGS.

Recommendations for clinical practice

We highly recommend other centres to develop a care pathway in case of prenatal suspected contractures, tailored to the centre's possibilities. A clear step-wise approach support the multidisciplinary team to plan examinations and counseling (24). Furthermore, a care pathway stimulates the multidisciplinary awareness to work according the Human Phenotype Ontology strategy, striving for a precise age-related descriptions of the phenotype and its relation with known pathogenic abnormalities (48-51). It is also advised to counsel parents about the benefits of WES based tests, if this test is available. The current study did not reveal any abnormal test results with karyotyping (0/26) or rapid aneuploidy testing (0/46), while chromosomal microarray had only a genetic diagnostic yield of 2.5% (1/40). On the other hand, the chance to identify a pathogenic chromosomal cause is higher when AMC is associated with multiple structural anomalies (49). Since nowadays, many DNA diagnostic laboratories offer WES based CNV testing most of these variants will be identified without the need of additional chromosomal microarray testing.

Specific molecular tests in AMC caused by congenital myotonic dystrophies (DMPK) and spinal muscular atrophies (SMA) can be applied(50). Our population with a modest population size showed that 11.5% (3/26) of the genetic causes were found by single gene testing. In line with our findings, Laquerriere *et al*. have demonstrated the additional value of WES over panel based testing in a large cohort of 315 AMC families (35). This study revealed a genetic diagnosis in 68 of 210 (32%) families. Of the 142 cases without a diagnosis after panel testing, an additional WES was performed in 111 families. In 24 of the 111 (21.6%) families a causal variant was identified by WES. This can be attributed to a wider clinical spectrum of the phenotype for these genes, as well as the identification of novel genes.

Conclusion

In conclusion, the importance of parental counseling on the possible genetic causes of AMC was highlighted in this 15-year cohort of fetuses suspected of having AMC. Advances in genetic testing techniques during the study period resulted in an increase of the genetic diagnostic yield into half of the cases between 2017-2021 due to the introduction of NGS based tests such as WES. Serial ultrasound examinations are essential to optimize the prenatal detection of AMC due to its variable cause, onset and expression before birth.

Supplementary documents

- Supplementary table 1A and 1B
- Supplementary file 2 with latest FADS panel

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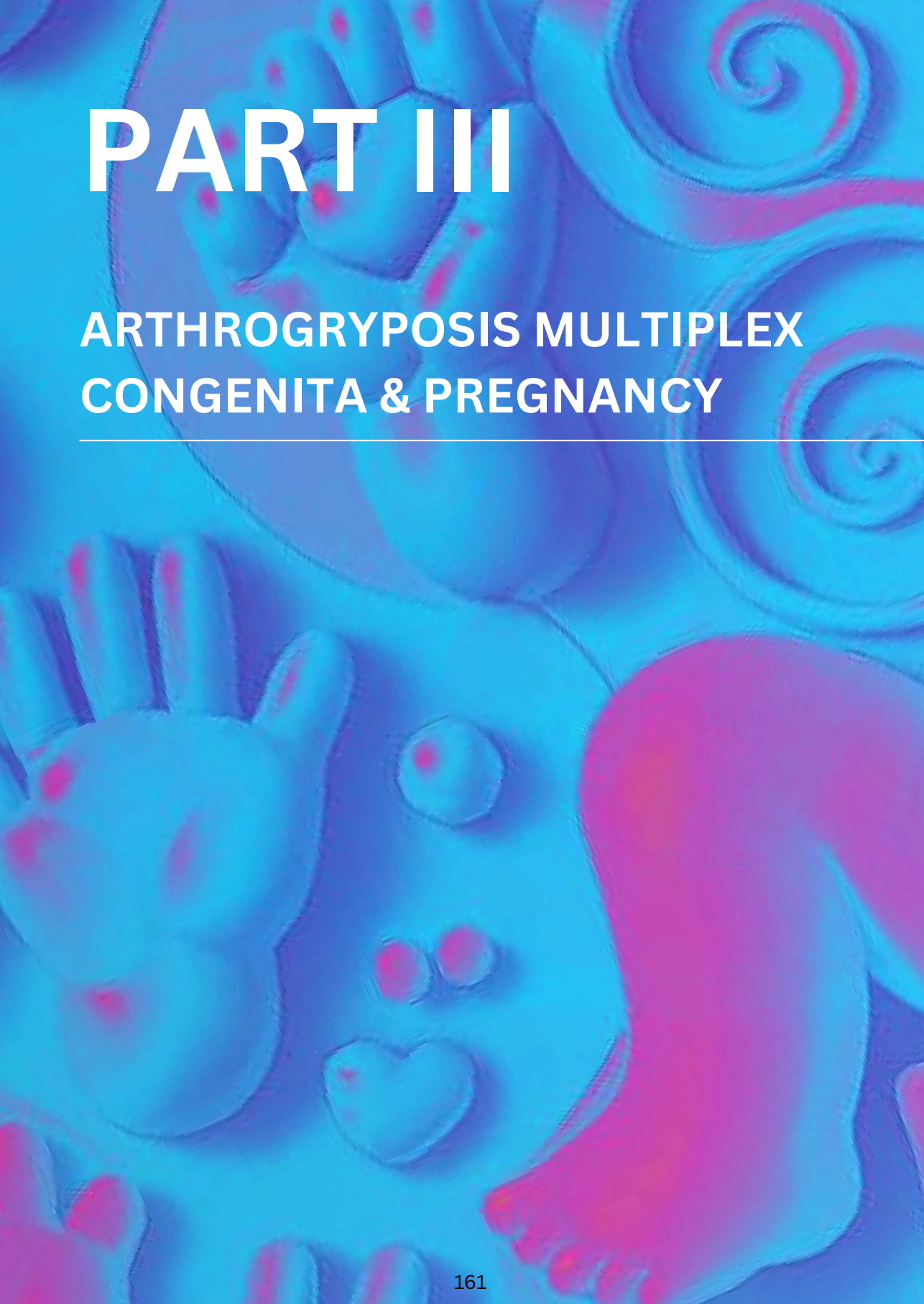
PART III

ARTHROGRYPOSIS MULTIPLEX CONGENITA & PREGNANCY



CHAPTER 8

Maternal, fetal and neonatal outcomes among pregnant women with arthrogryposis multiplex congenita: a scoping review.

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Abstract

Background The rarity of pregnancies in women with arthrogryposis multiplex congenita (AMC) could lead to healthcare providers having limited exposure to these cases. Consequently, they may be less familiar with the possibilities and challenges associated with pregnancies in women affected by AMC. AMC is an umbrella term for a disorder with multiple contractures at birth, having a broad spectrum of causes, onset and severity of expression. A clinical classification describing the phenotype is Group 1 with primary limb involvement, Group 2 with musculoskeletal involvement plus other system anomalies, and Group 3 with musculoskeletal involvement plus central nervous system dysfunction and/or intellectual disability. A scoping review was conducted to review available literature on documented cases of pregnancies in women with AMC, with the following aims: 1) to outline the maternal, fetal and neonatal outcomes; 2) to describe AMC stability during and after pregnancy (worsening of symptoms due to contractures, increased muscle weakness, pain or lung involvement); and 3) to summarize counseling aspects during pregnancy for expecting mothers who have AMC.

Results This scoping review included 27 manuscripts reporting on 43 women with 82 pregnancies, of whom 18 in Group 1, 20 in Group 2, 2 in Group 3, and 3 with an unknown type. Details on pregnancy-related outcomes could be depicted from 26 of the 43 women concerning 31 pregnancies. Among these pregnancies, 74% (23/31) had a cesarean section delivery, of which 74% (17/23) were elective. Children were born preterm before week 37 in 7 of 31 pregnancies (22%). A birth weight below the 10th percentile was seen in 6 of the 24 (25%) with a reported birth weight. The course of the pregnancy was uneventful in 16 of the 26 women (62%). Pregnancy had a limited negative influence on AMC stability except for three cases with a transient worsening of lung function.

Conclusion Gathering the information of the case histories revealed that the majority of the reported women had Distal Arthrogryposis with stable AMC during pregnancy and after delivery. The risk to have a cesarean section, preterm labour or a small for gestational age child is higher in this group than in the general population. Insights obtained by this review emphasized to offer (pre)pregnancy counseling and care by a multidisciplinary team tailored to the women's type of AMC, to ensure optimal preparation for both obstetric, genetic, neurologic, pulmonary and anesthetic care during pregnancy, delivery and postpartum period.

Background

Arthrogryposis multiplex congenita (AMC) is a group of rare diseases occurring in 1 in 3000-5200 live births (1,2). Healthcare providers have therefore a limited exposure to the possibilities and challenges associated with pregnancies in women affected by AMC. AMC is phenotypically characterized by multiple joint contractures manifesting in diverse anatomical regions and varying degrees of severity (1). Its etiology is varied and includes genetic and non-genetic factors, including neuromuscular conditions, maternal illnesses, and limited intrauterine space (1-3). The type of AMC was grouped into three groups depending on involvement according to Hall *et al*. (1): Group 1 with primary limb involvement, Group 2 with musculoskeletal involvement plus other system anomalies, and Group 3 with musculoskeletal involvement plus central nervous system dysfunction and/or intellectual disability.

Several evaluations in adults with AMC revealed a higher quality of life compared to the general population, despite the prevalent experiences of pain and fatigue in individuals with AMC (4-13). Notably, half of the adults with AMC lead independent lives with active engagement in work and social spheres, with the other half requiring some level of assistance (5,9). Given their productive lives, it is understandable that pregnancy-related questions such as child wish arise among women with AMC (4,14,15). A previous study showed a higher risk of adverse outcomes in pregnant women with physical (e.g. cerebral palsy), intellectual (e.g. DiGeorge syndrome), and sensory conditions (e.g. glaucoma) (16). Accessibility to maternity care remains limited for these women (17). Women with physical disabilities experience various challenges, including physical barriers, communication knowledge deficits with healthcare providers and limited accessibility to maternity care such as wheelchair accessible rooms and equipment adapted to their needs (17). Additionally, a survey among women with AMC has also emphasized the need for information on pregnancy-related topics (4).

Prompted by international patient support groups for AMC, a scoping review was conducted to evaluate whether pregnant women with AMC are at risk of complications for themselves, their fetus, or newborns to address the knowledge gap regarding pregnancy outcomes in women with AMC. Our focus was on maternal, fetal and neonatal outcomes, including maternal stability of AMC during and after pregnancy (worsening of symptoms due to the contractures, increased muscle weakness, pain or lung involvement), and counseling for women before and during their pregnancies. The insights gained by this literature review will increase awareness among healthcare providers and women with AMC about the possibilities and challenges during pregnancy.

Methods

A scoping review was conducted to better understand what is known about pregnancies among women with AMC (18-20). Specifically, we addressed the following aims: 1) outline the maternal, fetal and neonatal outcomes; 2) describe AMC stability during and after pregnancy (worsening of symptoms due to the contractures, for example increased muscle weakness, pain or lung involvement); and 3) summarize counseling aspects during pregnancy for expecting mothers who have AMC.

A systematic search of the literature was performed in the following databases: PubMed, Embase, and Web of Science. The timeframe within the databases was from inception to 5th August 2024 and conducted by the librarians. The search included keywords and free text terms for (synonyms of) 'arthrogryposis' combined with (synonyms of) 'pregnancy' combined with (synonyms of) 'data collection method'. Selection of manuscripts is done by applying all manuscripts related to maternal, fetal, and neonatal outcomes in pregnancy in women with AMC or counseling aspects (Figure 1). A full overview of the search terms per database can be found in the supplementary information (Additional File 1). No limitations on date or language were applied in the search. The PRISMA-ScR checklist for scoping reviews was used to guide the conduct of this review (Additional File 2) (20).

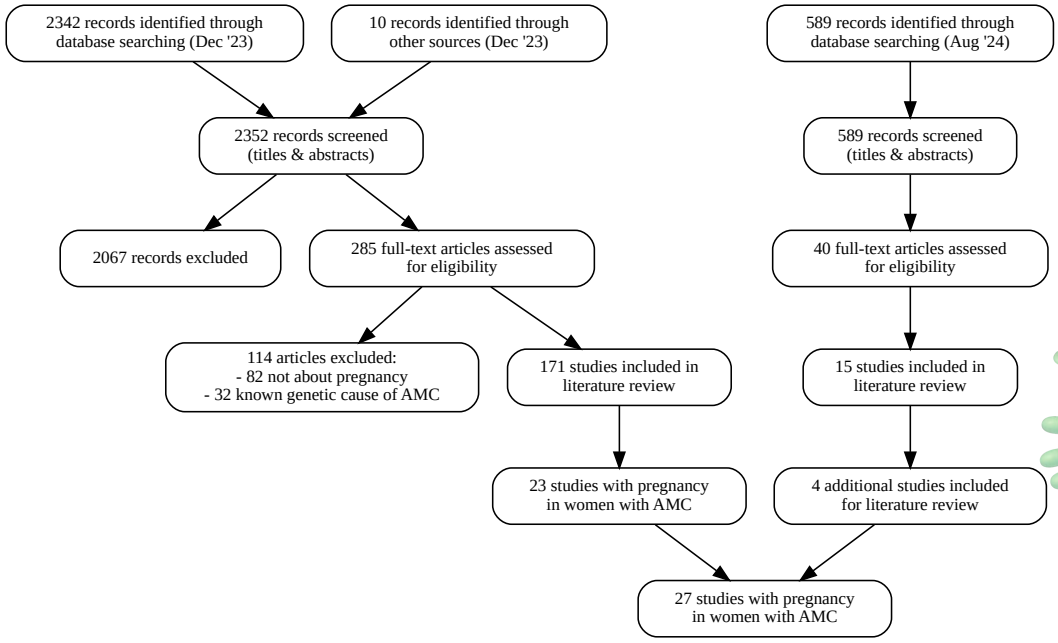

Figure 1 Flowchart of the literature search on pregnancies in women with AMC by using the PRISMA criteria

The Rayyan website (www.rayyab.qcri.org), which supports storage, multi-person selection, and grouping of the manuscripts, was used for the selection of manuscripts following a de-duplication process. Two investigators (AA and JIPdV) completed the screening process at the titles and abstracts, and full texts independently in a blinded fashion. Manuscripts that were not selected by both investigators were excluded for this review and a third reviewer was not consulted in case of disagreements. The residual manuscripts were assessed again on relevance by both investigators together. The quality of the included studies was not appraised. Data extraction was done by the two investigators.

The type of AMC was grouped into three groups depending on clinical involvement according to Hall *et al.* (1,21): Group 1 with primary limb involvement, Group 2 with musculoskeletal involvement plus other system anomalies, and Group 3 with musculoskeletal involvement plus central nervous system dysfunction and/or intellectual disability.

The information in these manuscripts was finally categorized in the following aspects:

1. Maternal, fetal and neonatal outcomes.
2. AMC stability during and after pregnancy: maternal difficulties were extracted from the included studies, such as worsening of symptoms due to the contractures, increased muscle weakness, pain, or lung involvement.
3. Counseling aspects in AMC: corresponding advice provided in the various manuscripts were gathered.

Results

The search yielded a total of 211 manuscripts about AMC and pregnancy; 27 met the inclusion criteria and were included in this review (Figure 1). These 27 reports included 43 women and 82 pregnancies (Table 1) (22-46).

Table 1. Maternal characteristics. Classifcation based on involvement of AMC with primary extremities (Group 1), with musculoskeletal and other system anomalies (Group 2), and with musculoskeletal plus central nervous system dysfunction and/or intellectual disability (Group 3) according to Hall *et al.* [1]. UK =unknown

Case	Author	Type of AMC AMC group (hall's classification) diagnosis clinical/ genetic diagnosis	Inheritance autosomal dominant (AD), autosomal recessive (AR),- (not inheritable), UK (unknown)	Body parts involved by AMC and mobility walking, wheelchair bound, imobilty	Age during pregnancy (years)
1	Moore et al., 1989	2, clinical	AD	Upper and lower limbs, fingers, hip and feet, craniofacial abnormalities. Walking	21
2	Moore et al., 1989	2, clinical Mother of case 1	AD	Upper and lower limbs, wrist, knees, foot, ankle and toes, craniofacial abnormalities. Walking	20
3	Hackett et al., 2000	1 (Amyoplasia), clinical	-	Upper and lower limbs, shoulders, elbows, wrists, fingers, hips, knees, feet and spine, scoliosis. Walking	22
4	Baty et al., 1988	1, DA (Type I), clinical	AD	Upper and lower limbs, wrist (limited), foot, ankle and toes. Walking	A.26 B. 29
5	Quance et al., 1988	3*, clinical	UK	Upper and lower limbs, spine (scoliosis), neck, pelvis, Facial and neuromuscular abnormalities. Wheelchair bound	21
6	Rozkowski et al., 1996	3*, clinical	UK	Upper and lower limb, pelvis, spine (kyphoscoliosis), neuromuscular abnormalitie.s Wheelchair bound	34
7	Gripp et al., 1996	1, clinical	AD	Upper and lower limbs, hips, knees and feet Walking, stiff gait	23
8	Spooner et al., 2000	2*, clinical	UK	Upper and lower limbs, hips, spine (kyphoscoliosis), narrow pelvis. Walking unaided, but use a wheelchair	26
9	Hardwick et al., 2002	2*, clinical	AR	Upper and lower limbs, hips and elbow, length 1.21 m Spine: limited neck extension and severe kyphoscoliosis. Walking with stich "swing-through gait", no mobility in the legs	27
10	Leeners et al., 2005	2 (Freeman-Sheldon syndrome), Clinical	AD	Feet. Walking	22
11	Duffy et al., 2007	2*, clinical	UK	Upper and lower limbs, spine severe thoracic kyphosis and lumbar lordosis, normal neck extension, Stature 0.91m, BMI 31. Walking (although little mobility in the legs)	27
12	Singhal et al., 2010	2*, clinical	UK	Upper and lower limbs, hands and feet, spine: no involvement neck, thoracic kyphosis and lumbar lordosis, short stature 1.3m. No walking, crawling	25

Table 1. (continued)

Case	Author	Type of AMC AMC group (hall's classification) diagnosis clinical/ genetic diagnosis	Inheritance autosomal dominant (AD), autosomal recessive (AR),- (not inheritable), UK (unknown)	Body parts involved by AMC and mobility walking, wheelchair bound, imobilty	Age during pregnancy (years)
13	Ko et al., 2013	2, Sheldon-Hall syndrome, Genetic: Heterozygous TPM2 mutation p.R133, autosomal dominant	AD	Upper and lower extremities, hands and feet, camptodactyly, short stature 1,53m, short neck sloping shoulders, facial anomalies, retrognathia. Independent walking	25
14	Castro et al., 2013	2,*, clinical	UK	Lower limbs, hips and spine. Lumbar scoliosis, pelvis, short stature 1.2m. Independent walking with crutches	36
15	Darwich et al., 2014	2*, clinical	UK	Limbs, spine severe kyphoscoliosis, restrictive lung disease, laryngeal surgery for vocal cord dysfunction, short stature 1,5m, weigh 30.8, BMI 13. Class 1 airway, small thyromental distance, stiff temporomandibular joint. Walking	26
16	Sadacharam et al., 2016	2*, clinical Diabetes, gastroesophageal reflux	UK	Upper and lower limbs and spine, severe thoracolumbar kyphoscoliosis. Reduced left lung volume, Class I airway, normal neck extension Walking	28
17	Guzman-Lopez et al., 2019	1*, clinical	UK	Upper and lower limbs, hips, coxofemoral prothesis. Wheelchair bound	19
18	De Burca et al., 2019	2, DA (type IIB), clinical Heterozygous TNNT3 mutation	AD	Upper and lower limbs, hip. Walking	30
19	Kawira et al., 1985	2, Klippel-Feil, clinical	AD	Upper and lower limbs, scoliosis, axillary pterygia, unusual facial appearance, joined vertebrae, axillary webbing, stature 1,43m, weigh 40kg. Walking	22
20	Pollazzon et al., 2021, Pt 2	1, DA (Type I), clinical and genetic: Variants in the TPM2 c.463G > A, p.(A155T)	AD	Upper and lower limbs. Walking	20
21	Pollazzon et al., 2021, Pt 3, mother of 37	1, DA (Type I), clinical and genetic: Variants in the TPM2 c.463G > A, p.(A155T)	AD	Upper limbs, scoliosis. Walking	UK
22	Pollazzon et al., 2021, Pt 8	2, DA (Type 2B), clinical and genetic: Heterozygous deletion in TNNI2 gene	AD	Upper and lower limbs, micro-, retrognathia. Walking	UK
23	Pollazzon et al., 2021, Pt 12	1 (Amyoplasia), clinical	-	Upper and lower limbs, scoliosis. Walking with orthoses	A. 25 B. UK
24	Llewellyn and Volikas, 2021 (conference abstract)	UK	UK	At least lower limbs + hip deformities. Wheelchair dependent	37
25	Sherlaw-Sturrock et al., 2022	2, DA (Type 5), clinical: heterozygous pathogenic variant in PIEZO2, autosomal dominant	AD	Upper and lower limbs, kyphosis. Progressive restrictive lung disease. Unknown mobility	2 × UK

Table 1. (continued)

Case	Author	Type of AMC AMC group (hall's classification) diagnosis clinical/ genetic diagnosis	Inheritance autosomal dominant (AD), autosomal recessive (AR),- (not inheritable), UK (unknown)	Body parts involved by AMC and mobility walking, wheelchair bound, imobilty	Age during pregnancy (years)
26	Serra et al., 2022	2, DA (Type 5), clinical: heterozygous pathogenic variant in PIEZO2, autosomal dominant	AD	Upper and lower limbs. Unknown mobility	UK
27	Tang et al., 2020	2, Sheldon Hall Syndrome, clinical	AD	Hands. Walking	UK
28	Tang et al., 2020	2, Sheldon Hall Syndrome, genetic, c.188G > A variant of TNNT3 gene, autosomal dominant	AD	Hands. Walking	UK
29	Tang et al., 2020	2, Sheldon Hall Syndrome), genetic, c.188G > A variant of TNNT3 gene, autosomal dominant	AD	Hands. Walking	UK
30	Böckel et al., 1984	2, clinical	AR	Upper and lower limbs, Multiple pterygium syndrome, short stature, 1,4m. Walking	UK
31	Carlson et al., 1985	UK	UK	UK	UK
32	Carlson et al., 1985	UK	UK	UK	
33	Stoll et al., 1996	1, DA (Type I), clinical	AD	Upper limbs, severe clinodactyly and camptodactyly (grandmother of proband). Walking	UK
34	Stoll et al., 1996	1, DA (Type I), clinical	AD	Clinodactyly and mild camptodactyly of the fifth fingers (mother of proband). Walking	19
35–43	Jiang et al., 2004	1, DA (Type I), clinical	AD	Upper and lower limbs, talipomanus and talipes equinovarus. Unknown mobility	UK

*Classifcation was not provided in the manuscript but could be interpreted from the provided clinical description

Table 2. (Pre)pregnancy outcomes of the mothers, fetuses and neonates. US =ultrasound examination, UK =unknown, GA =gestational age

Case	Number of pregnancies	Multidisciplinary team involved? yes/no/unknown = UK	Genetic testing during pregnancy?	Suspicion of fetus with amc?	AMC/ pregnancy related challenges during pregnancy?	Mode of delivery vaginal, emergency or elective cesarean section (EM SC/ EL SC)	Gestational age in weeks	AMC/ pregnancy related difficulties during delivery? yes/no, Including type of anesthesia
1	1	UK	No	No: serial US, GA unknown	No fetal movements till 24th week	Vaginal, forceps extraction for fetal distress	Term	No
2	3	UK	No	No 3x	No	Vaginal 3x	term 3x	No
3	1	Yes (occupational therapist, social worker, neonatologist, nurses, obstetricians, anesthetist)	No	No: serial US (no contractures, normal limb and body movements), GA unknown	Immobility due to pain and maternal discomforts	Induced, maternal indication, vaginal, vacuum extraction for failure of progress	37	No
4	2	UK	No	A. No B. Yes, US at 19 and 22 wks: wrist and finger contractures, 28 wks also feet	A. spontaneous premature rupture of membranes B. No	A. EM SC for fetal distress B. EL SC for cephalopelvine disproportion	A.35 B.37	No
5	1	Yes (obstetrician, anesthetist)	UK	No	No	EL SC, Maternal indication, Severe kyphoscoliosis and limited neck motility	38	Unsuccessful epidural, general anesthesia adapted intubation for expected Grade II into clinical grade III Airway
6	1	Yes, internal medicine, anaesthesiologist	UK	UK	UK	EL SC maternal indication kyphoscoliosis and respiratory compromise	38	Spontaneous active in labour 1 week before elective SC. Continuous spinal anesthesia
7	1	UK	No	Yes. US at 34 wks: abnormal position hands and feet, low set ears and hairline	No	EL SC on maternal indication, kyphoscoliosis	37	Uneventful Regional/ general anaesthesia not known
8	1	No	UK	UK	Anesthetic consultation 1 day prior to elective SC. SC was postponed 2 days to gather info	EL SC	38	Uneventful. Combined spinal epidural anesthesia, walking after 1 day
9	1	Yes: obstetrics, internal medicine, anesthetics thromboprophylaxis 1st trimester -6 wks postpartum, surveillance lung capacity	No, was declined	No, US at 16 weeks	Hospital admission for breathlessness at 16 and 29 weeks	EL SC maternal indication breathlessness	30	No. 'Awake' fibre optic intubation required, general anaesthesia

Table 2. (continued)

Case	Number of pregnancies	Multidisciplinary team involved? yes/no/unknown = UK	Genetic testing during pregnancy?	Suspicion of fetus with amc?	AMC/ pregnancy related challenges during pregnancy?	Mode of delivery vaginal, emergency or elective cesarean section (EM SC/ EL SC)	Gestational age in weeks	AMC/ pregnancy related difficulties during delivery? yes/no, Including type of anesthesia
10	1	UK	Amniocentesis and screening for viral infections unmarkable	Yes: contractures feet, hands, polyhydramnios, reduced rolling movements, small gastric bladder	No	EM SC, Fetal: Abnormal CTG and meconium stained fluid	38	No, General/ regional anesthetics not known
11	1	Yes (anesthetic, neonatal, obstetric and midwifery staff, clinical genetics)	Waived by the parents	No, ultrasound serial from 24 wks onwards	30 wk breathlessness Corticosteroids	EL SC, maternal indication breathlessness No thromboprophylaxis	31	No, general anesthesia
12	1	UK	UK	No, US at 36 weeks	No	Spontaneous labour, EM SC for cephalopelvenic dysproportion, narrow pelvic inlet	36	No, general anaesthesia
13	1	No	After pregnancy, daughter TPM2 mutation p.R133	Yes, US clenched hands, talipes equinovarus	No	EL SC Maternal, cephalopelvine, narrow pelvis	38	No, unknown general / local anesthesia
14	1	Yes (obstetrician, anesthetist and neonatologist)	No	No, serial US 1st, 2nd, 3rd trimester	No. Cervix length measurements and thromoboprofylaxis	EL SC maternal indication suspected cephalopelvine dysproportion, narrow pelvis and limited tight abduction. Vaginal delivery dissuaded for this reason	38	No, general anaesthesia
15	1	UK	UK	UK	Spontaneous preterm labor, Transverse lie fetus, fully dilated cervix	EM SC, maternal vocal cord surgery and failed epidural analgesia and the urgency of the situation	25	Epidural L4-5 insufficient for surgical anesthesia, mask induction and maintenance with nitrous oxide. Oxygen 50%, sevuflurane 1%, spontaneous breathing. No complications
16	1	Yes (gynaecologist and anesthesiologist)	UK	No	No	EM SC, Maternal indication, failed induction of labour, no fetal distress	37	Combined spinal and epidural L4-5, same as during labour
17	1	Yes (gynaecologist, anesthesiologist and cardiologist)	No	No, US at 22 wks	No	EL SC, maternal indication limited motility in hips. No thromboprofylaxis normal lung and heart function	38	No. Epidural

Table 2. (continued)

Case	Number of pregnancies	Multidisciplinary team involved? yes/no/ unknown = UK	Genetic testing during pregnancy?	Suspicion of fetus with amc?	AMC/ pregnancy related challenges during pregnancy?	Mode of delivery vaginal, emergency or elective cesarean section (EM SC/ EL SC)	Gestational age in weeks	AMC/ pregnancy related difficulties during delivery? yes/ no, Including type of anesthesia
18	1	No	UK	Yes, US 16 wks: contracted fingers, 30 min no finger extension, US 20 weeks no finger movements	No	EM SC, maternal indication, hip dislocations	36	No. General/ regional anesthesia not mentioned
19	1	UK	UK	Yes	No fetal movements during pregnancy, only "flitters"	Spontaneous immature twin delivery	20	-
20	1	UK	UK	Yes, clenched hands with overlapping fingers	UK	UK	40	UK
21	1	UK	UK	UK	UK	UK	UK	UK
22	1	UK	No	UK	Oligohydramnios, decreased fetal movements during pregnancy	Vaginal	41	UK
23	2	UK	UK	UK	UK	UK	UK	UK
24	1	Yes, also anesthesiologist	UK	UK	UK	EL SC	38/40	Spinal anesthesia after a MRI lumbar spine to exclude neurological abnormalities
25	2	UK	UK	UK	UK	Vaginal EL SC due to breech position	2×term	UK
26	1	UK	No	Yes, flexed wrists and clubfeet	Hypertension	EL SC due to maternal indication (preeclampsia)	38	UK
27	1	UK	UK	UK	UK	UK	UK	UK
28	1	UK	UK	UK	UK	UK	UK	UK
29	1	UK	Yes	Yes	UK	Termination of pregnancy due to US abnormalities (clubfeet)	UK	-
30	5	UK	UK	No	UK	UK	UK	UK
31	2	UK	UK	UK	UK	UK	UK	UK
32	UK	UK	UK	UK	UK	UK	UK	UK
33	1	UK	UK	UK	UK	UK	UK	UK
34	1	UK	UK	UK	UK	UK	UK	UK
35–43	40	UK	UK	UK	UK	UK	UK	UK

Maternal, fetal and neonatal outcomes

Median maternal age during pregnancy was 25 years (range: 19-37 years). AMC group distribution was 18 women in Group 1, 20 women in Group 2, two in Group 3, and three with an unknown type. Regarding physical ability, at least 26 women (60%) were able to achieve independence in mobility with or without aids. The assumed inheritance (based on the clinical presentation and family history) was known in 29 of the 43 women: autosomal dominant in 27, and autosomal recessive in 2. Genetic causes were found in nine women. Characteristics of the women concerning AMC groups, inheritance, involved body parts and maternal age during gestation are presented in Table 1.

Details on pregnancy-related outcomes could only be depicted from the first 26 women with in total 31 pregnancies (Table 2). Among these pregnancies, 74% (23/31) had a cesarean section delivery, of which 74% (17/23) were elective. The remaining 26% (8/31) of deliveries were vaginal, with an uncomplicated labour in 5 cases, forceps extraction in 1 case, and vacuum extraction in another. Children were born preterm (before week 37) in 7 of 31 pregnancies (22%). A birth weight below the 10th percentile was seen in 6 of the 24 (25%) with a reported birth weight. The course of the pregnancy was uneventful in 16 of the 26 women (62%), without reported pain, premature labour, lung problems, or difficulties during analgesia. Only one of the manuscripts reported on hypertensive disorder of pregnancy. Miscarriage percentage, or fertility issues were not reported in the manuscripts. Maternal and fetal outcomes are presented in Table 2.

The obstetric outcomes of cases 1–26 were categorized by group. In Group 1 (n = 7), one woman (14%) had a vacuum-assisted vaginal delivery, three (43%) underwent cesarean sections, and the mode of delivery was unknown for the remaining three (43%). In Group 2 (n = 16), four women (25%) had vaginal deliveries, 11 (69%) underwent cesarean sections, and one experienced a preterm labour at 22 weeks. In Group 3 (n = 2), both women had cesarean sections.

AMC was suspected prenatally in 9 of the 82 pregnancies (11%), with contractures in hands and feet in 5, only in the hands in 3, and only clubfeet in 1 (Table 2). The 43 women gave birth to at least 71 liveborn children, with two neonatal deaths as a result of AMC in one case and in another case due to cardiorespiratory failure in a newborn with osteogenesis imperfecta and fractures. Postnatally, AMC was diagnosed after birth in 49% of the liveborn children (35 of the 71). Neonatal feeding problems or a pharyngeal obstruction were reported in cases 7, 10 and 13. Admission to a Neonatal Intensive Care Unit (NICU) was mentioned once, due to respiratory distress after a labour at 38 weeks. All neonatal outcomes are presented in Table 3.

AMC stability during and after pregnancy

Lung problems were mentioned in three manuscripts on three women, of whom two were grouped into Group 2 and one in Group 3. In one case (case 9) with a severe kyphoscoliosis, admission at 16 weeks gestational age was reported due to this problem. The uterine fundus was at xyphoid level. Antenatal corticosteroids were administered due to breathlessness and dosage of inhaled agonist and steroid was increased along with chest physiotherapy and upright position during sleep enabled continuation of the pregnancy till a gestational age of 29 weeks. Case 11 also reported breathlessness during pregnancy. Another woman (case 6) underwent an elective section cesarean due to a kyphoscoliosis and respiratory compromise. From these 3 women with maternal difficulties, none had a child affected by AMC. No reports were found in the included manuscripts on fatigue during or after pregnancy, hyperemesis, gestational diabetes, use of medication such as pain reliever, or anemia. Comments on challenges caused by maternal AMC during and after pregnancy are presented in Tables 2 and 3, respectively.

Counseling aspects in AMC and pregnancy

The utilization of pre-pregnancy counseling, a medical consultation before pregnancy aiming to optime health and to address potential pregnancy-related risks, was described in one manuscript(37). Counseling advice to perform before, during and after pregnancy from the included manuscripts are grouped in Table 4.

Table 3. Postnatal outcomes, including maternal and neonatal complications

Case	Difficulties (maternal)	Difficulties (neonatal)	Child with AMC? (number/total children)	Birth weight (gram, percentile)
1	No	No	1/1	3700, 75th
2	No	No Died on day 5 of another autosomal dominant disease osteogenesis imperfecta, born with fractures and unexplained cardiorespiratory failure No	3/3 3 × affected hands, feet, facial anomalies	3100, 10th 2700, 3rd 3100, 10th
3	No	No	0/1	2740, 14th
4	No	No	2/2 fingers, hips, feet wrists, hands, feet and also limited knee movements and micrognathia	2120, 25th 2180, 10th
5	No	No	0/1	Appropriate growth for gestational age
6	No	No	0/1	UK
7	No	feeding difficulties, gastrostomy tube, 18 month	1/1elbows, wrist, fingers, hips, knees, feet facial, limited mouth opening, Identical to the mother	2400, 25th
8	No, discharged 3 days postoperatively	No	0/1	UK, healthy
9	No, walked with crutches within 2 days after operation. Thromboprofylaxis for 6 weeks postpartum	No, thrived well, home after 6 weeks	0/1	1300(girl), 10-50th
10	No	Intubation of the fetus due to functional pharyngeal obstruction, followed by tracheostomy	1/1, feet, hands, polyhydramnios, reduced rolling movements, small gastric bladder Functional pharyngeal obstruction caused by the Freeman-Sheldon Syndrome	3420, 50-90th
11	No	No, thrived well	0/1	1100, < 3rd
12	No	No	0/1	2500 gr, 10-50th
13	No	Feeding problems due to micrognathia. 10 days after birth 1 week hospitalized because of aspiration pneumonia, 4 weeks after birth resuscitation because breathing difficulties 1 h after bottle feeding, not successful, child died. Suspected aspiration pneumonia	1/1, Hands, feet, facial abnormalities, triangular face, downslanting palpebrae fissures, small mouth	2560, 3rd-10th
14	No	No, follow-up through 6 months	0/1	2755, 5-10th
15	No, postpartum +6 days home	No	UK	UK
16	No, postpartum normal	No, stable	0/1	UK
17	No, after 3 days to home	No	0/1	3020, 10-50th
18	No	No	1/1, Distal arthrogryposis, arachnodactyly, and hip dislocation	2470, 10-50th
19	No	2 × Death caused by immaturity	2/2 Contractures feet, knees, hips, hands, mild scoliosis, retrognathia, pterygium colli	340 and 420 (twin)
20	UK	No	1/1	3124, 10-25th
21	UK	UK	1/1	UK
22	UK	No	1/1 upper and lower limbs	2430, < 3rd
23	UK	UK	0/2	UK
24	UK	UK	UK	UK
25	UK	UK	1/1	3700, 62nd 3120, 17th

Table 3. (continued)

Case	Difficulties (maternal)	Difficulties (neonatal)	Child with AMC? (number/total children)	Birth weight (gram, percentile)
26	No	NICU admission due to respiratory distress	1/1	2460, 5th
27	UK	UK	1/1, hand	UK
28	UK	UK	1/1, hand	UK
29	UK	-	1/1, hand	UK, termination of pregnancy
30	UK	No	0/5	UK
31	UK	UK	UK	UK
32	UK	UK	UK	UK
33	UK	UK	1/1 Clinodactyly and camptodactyly	UK
34	UK	UK	1/1 Upper and lower limbs, fingers, feet, hips	3600, > 10
25–43	UK	UK	16/31	UK

Discussion

This study makes a significant contribution in filling the knowledge gap concerning pregnancy-related topics in 43 women with AMC . The outcomes of pregnancy were reviewed in 82 pregnancies published during a 40-year period from 1984 to 2024. This information can serve as an important support for healthcare professionals who provide care for women with AMC and for the AMC community.

Maternal, fetal and neonatal outcomes

The characteristics of the women with AMC affect an about equal distribution of the AMC Groups 1 and 2. Notably, only two women were diagnosed with Amyoplasia, which is in contrast with the typical distribution observed in live-born children with AMC, where about a third has Amyoplasia (1). This suggests that the review may not fully represent the general population of individuals with AMC. The low occurrence of AMC Group 3 with musculoskeletal involvement plus central nervous system dysfunction, namely 2 of the 43 women (5%), might be attributed to the severity of these conditions. For example, these individuals are typically more severely impacted, and may therefore have lower pregnancy rates. Regarding physical ability, at least 26 women (60%) achieved mobility independence, aligning with the 52% observed in a cohort of 177 individuals with AMC (9).

Table 4. Overview of advice mentioned in the included articles from the literature search AMC and pregnancy

Period	Counselling aspect	Explanation
Prepregnancy	Genetic counseling	Geneticists informs about the types of AMC and update on possible genetic tests [1]
	Prepregnancy counselling	Multidisciplinary approach [8, 35] Contraceptive advice [35] Discussing facts and challenges of pregnancy and AMC [37] Respiratory function test if applicable [37]
Pregnancy	Medical history	Obstetric history (prior pregnancies, mode and time of delivery, birth weight) [24, 25] Prior operations, including type of anesthesia and possible advices [24, 27] Family history [30] Use of medication [30] Mobility: independent walking, walking with aids, wheelchair bound, immobility [27]
	Physical examination	Physical examination with extra focus of members of multidisciplinary team (e.g. gynaecologist, internist, pulmonologist, anesthesiologist) [25, 27] Weight, height, BMI [25] Extremities including mobility (including range of movements of the joints) [8] Cardiovascular and respiratory system [40] Head and neck area (e.g. micrognathia or high arched palate), including Mallampati score and neck mobility [24, 25, 39] Shoulders (e.g. deformity of the scapula) [25] Spine (e.g. scoliosis, spina bifida, sacral agenesia or vertebral anomalies) [25] Cardiovascular system (e.g. heart diseases) [25] Respiratory system (e.g. tracheoesophageal fistula or hypoplastic lungs) [25] Genitourinary system (e.g. rectal or labial defects) [25] Abdomen (e.g. inguinal hernia) [25] Venous access evaluation [30]
	Home management	Needs for home management dependent of mobility (aids) [8] Local occupational therapist (for home modifications) [8] Social worker [8]
	Tromboprophylaxis	Tailored counselling about using thromboprophylaxis during and after the pregnancy [28, 31]
	Cervix length measurements	Suggested in relation to increased risk of preterm labour [34]
	In case of breathlessness (AMC related)	Monitoring cardio-respiratory condition [31] Peak expiratory flow rates measurements [31, 37] Chest radiography (signs of infection?) [34] Evaluation of the uterine fundus in relation to diaphragm [34] Steroids for maternal lungs [34] Chest physiotherapy [34] Advice upright sleeping position to reduce elevation of the diaphragm [34] In case of worsening: counselling about continuation or terminating the pregnancy [34]
	Serial ultrasound investigations	Healthcare providers should be aware of recurrent AMC in the fetus [8, 37]: - Features due to limited motility: joint contractures (e.g. clubfoot), micrognathia, decreased fetal movements, altered amniotic fluid - Associated anomalies: brain and hearth anomalies, heart, joint webbing - Fetal growth restriction
	Prenatal testing	First trimester test for aneuploidies [37] Genetic counselling about prenatal invasive testing: risk calculation for fetal AMC [24, 37] Genetic testing update possibilities(chromosomal or monogenic) [31]
	Anesthetic	
	Anesthetic assessment	Early in pregnancy anesthetic assessment [30, 39] Expected difficulties during administration of analgesia [30, 39] Craniofacial evaluation: cleft palate, laryngeal stenosis, craniosynosthosis, micrognathia Spinal abnormalities: scoliosis, spina bifida, or sacral agenesis could have abnormal cerebrospinal fluid dynamics [25, 39] Expected anesthetic problems during infusion placement (e.g. due to joint contractures or scarring), or insertion the catheter of the regional analgesia (e.g. due to spinal anomalies) [25] Choice of anesthesia technique should be tailored to the individual patient's anatomy, overall health, and the specific surgical procedure to optimize safety and efficacy [25]

Table 4. (continued)

Period	Counselling aspect	Explanation
	General versus regional analgesia	Weigh the potential difficulties and risks [27, 31, 34]: - Additional risks during general analgesia compared to regional analgesia are: difficulties during intubation due to a limited neck mobility and problems related to the decreased cardiopulmonary function - Patients with AMC could react unpredictable on medications (e.g. muscle relaxants and inhalation anesthetics). Therefore, proper dosing and careful monitoring are crucial [25, 37] - Spinal analgesia could be challenging in patients with AMC who have spine deformities (e.g. scoliosis) Therefore, identifying and targeting nerves for blocks may be more difficult due to the altered anatomy
Delivery	Mode of delivery	Counselling about the mode of delivery, individualized and dependent of maternal and fetal investigations [8]
	Timing of delivery	In general term age. Challenge in case of for example maternal pulmonary discomfort (e.g. breathlessness) before term age, while the fetus is a good condition [31] A pulmonary function test is suggested after 28 weeks gestational age in symptomatic patients and an electrocardiogram in asymptomatic patients and adjustments in more upright sleeping position [34]
Postpartum	Maternal	
	Home management	Modifications to a bassinet to enable self-sufficient care of the newborn [8, 34] Social service provision: need for carers and housing [34]
	Thromboprophylaxis	Continuation of 6 weeks, in line with recommendation of the Royal College of Obstetricians and Gynaecologists [31]
	Physical examination (neonatal)	Joint stature including range of motion, features of AMC, general physical evaluation, and birth weight [24]

Details on pregnancy-related outcomes could be depicted from 26 of the 43 women (cases 1-26) with in total 31 pregnancies. The mode of delivery among these women was in one-quarter a vaginal delivery (8 of the 31 pregnancies) and in the remaining 74% a cesarean section. The percentage of cesarean sections among cases 1-26 was higher than in the general American population (30-32%) (47). Among this group, the distribution of elective and emergency cesarean section was 17 (74%) and 6 (26%), respectively. The main reason for an elective cesareans were suspected cephalopelvic disproportion and for the emergency cesarean section lack of progress during labour. Breathlessness caused by the AMC and the expanding gravid uterus was the reason to perform an elective cesarean section three times (cases 6, 9 and 11). The reported percentage of preterm deliveries (< 37th week) among cases 1-26 was 22% (7 of the 31), with a median at 31 weeks (range 20-36 weeks). This finding is also higher than in the worldwide general population observed 12% (48).

One-quarter (6 of the 24) of all infants with a reported birth weight had a birth weight below the 10th percentile. A recent study confirmed a smaller weight in 206 American children with AMC in comparison to typically developing children during the first 36 months of life (49). A higher maternal risk of adverse outcomes was also observed in a recent retrospective study among 2074 women with a physical, intellectual, and sensory disability (16). This study showed higher rates of cesarean sections and premature rupture of membranes in women with a disability compared to those without (16).

These 43 women gave birth to at least 71 liveborn children, with two neonatal deaths as a result of AMC in one case and in another case due to the coexisting osteogenesis imperfecta (cases 2 and 13). One woman had a spontaneous and immature delivery of twins affected by AMC at 20 weeks (case 9). Another woman with Sheldon Hall syndrome terminated the pregnancy due to a pathogenic variant in the *TNNT3* gene (case 29). In this case, prenatal ultrasound examinations had shown abnormalities and prenatal invasive testing confirmed that the fetus was also affected. Additionally, prenatal invasive testing was also reported in another case, but the results were not discussed and the author did not mention which genetic tests were applied (case 10).

Sonographic structural examination led nine times to a prenatal suspicion of fetal AMC. Additional descriptive fetal motor assessment was described in two manuscripts (33,41). Maternal perceived fetal movements were mentioned in six cases (1, 3, 10, 18, 19 and 22) and serial ultrasound investigations were performed in six cases (1, 2, 4, 11, 14 and 18). Serial examinations are the advised manner to observe if the phenotypical features of AMC worsen over time(50-52). Finally, AMC was diagnosed after birth in 49% of the liveborn children (35 of the 71). This high percentage can be explained by the high percentage of autosomal dominant inheritance in this population.

Over time, new genetic techniques have been developed. While there are nine women with a proven genetic abnormality in the current study, we assume that more women had a genetic disorder who have never been tested. In this population, the inheritance of 14 of the 43 mothers was not known. A genetic diagnosis could help to confirm the genotype of AMC and to estimate the recurrence rate(53). Recently, Laquierriere *et al*. emphasized the additional value of Whole Exome Sequencing to targeted exome sequencing in a population of unrelated parents from children with AMC (54). Therefore, a close collaboration is crucial between clinical geneticist and obstetrician who should be up to date on new genetic testing possibilities.

AMC stability during and after pregnancy

Stability of AMC during and after pregnancy did not deteriorate in most of the included cases, as far as this was described in the included manuscripts. There were three exceptions. Three women experienced breathlessness during pregnancy (cases 6, 9 and 11). In all cases it was caused by the combination of small stature and scoliosis. Only one other manuscript described immobility and pai) (8(. The latter is unexpected since pain is commonly experienced in adult populations with AMC and also in pregnant women without AMC (9,55,56).

Counseling aspects in AMC and pregnancy

A few aspects for (pre) pregnancy counseling for women with AMC will be highlighted. Firstly, the importance of a multidisciplinary approach was emphasized by various authors (8,25,27,31,34,37,39,40,44). The team should be tailored to the type of AMC (e.g. gynecologist, neurologist, anesthesiologist, rehabilitation doctor, neonatologist, physiotherapist and/or social worker). Secondly, while a pre-pregnancy counseling was described in only one manuscript, we emphasize its importance (37).

A pre-pregnancy counseling should focus on the understanding of the impact of AMC on pregnancy and vice versa. This stepwise approach evaluating the opportunities and challenges is similar to individuals with other relatively rare chronic diseases like systemic lupus erythematosus and kidney disease (57-59). Providing women with AMC a (pre)pregnancy counseling is advantageous in preparing them for potential challenges during pregnancy, for example the respiratory system's impairment leading to maternal discomforts such as breathlessness, potential anesthetic difficulties and increased risk of thromboembolisms caused by decreased mobility (37). It is advisable to have medical follow-up examinations for pregnant women with AMC in a secondary or tertiary healthcare center, according to existing comorbidities. The accessibility of the airway should always be checked in patients with AMC since limitations have been reported in 25% of these patients (60). Regional anesthesia could be advantageous in these cases, but it could also be challenging in case of a scoliosis (60). A difficult intubation was described in two of the included manuscripts (25,38). The total number of cases with a general anesthesia is unknown. In case of severe airway obstruction, resorting to a tracheostomy may be a final option in patients with AMC (60). Nothing related to optimize intraoperative position or intravenous access was reported in any of the cases, despite the significance of these aspects (60).

Strengths and limitations

The strength of our literature search lies within the systematic approach of evaluating case reports concerning women with AMC with a pregnancy. The obtained knowledge facilitates information and advice in detail for professional healthcare providers and women with AMC. Most information in this rare disorder was obtained concerning women with AMC group 1 and 2, especially Distal arthrogryposis of various types (1 and higher) and limited to Amyoplasia. A limitation is that despite the substantial period of manuscript search (1984-2024) the number of included cases is still modest. Moreover, not all manuscripts have been set-up with the goal in mind to examine the influence of AMC on pregnancy and vice versa.

Therefore, no details on obstetrical outcome parameters could be given in a considerable proportion. We are aware of the Bamshad classification on arthrogryposis making precise distinction between various forms of distal arthrogryposis based on neurological examination and genetic findings (62). The manuscripts of our study examined a period lacking this detailed information. On the other hand, all present individual data of the included women with AMC and their pregnancy outcome are listed systematically.

Future research

Future research with a larger sample size should strive to register prospectively influence of AMC on pregnancy and vice versa in women with different types of AMC. With this purpose in mind, a minimal common data set for an AMC register, inclusive pregnancy outcome has been designed by means of a multidisciplinary Delphi procedure inclusive patients with AMC (63). Furthermore, more information is needed on aspects during delivery (e.g. leg positioning during vaginal or operative labour, pain relief during a vaginal labour) and postpartum period (e.g. breastfeeding instructions with the affected limbs).

Conclusion

This scoping review is an initial step in addressing the knowledge gap on the obstetrical outcome in women with AMC. The findings of this review underscore the importance of (pre-)pregnancy counseling concerning the mode of delivery, possibility of preterm birth, and stability of AMC (worsening of symptoms due to contractures, increased muscle weakness, pain or lung involvement). The relevance of the obtained information is great concerning women with Distal Arthrogryposis. Further prospective studies are needed to provide more information in a populations with a wider spectrum of AMC, especially Amyoplasia. The wide spectrum of the AMC phenotypic expression and underlying causes will always necessitate a multidisciplinary tailored approach.

Supplementary documents

- Additional File Table 1 Query developed to search literature for pregnancy related topics in women with AMC, December 2021 and August 2024
- PRISMA-ScR Checklist

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CHAPTER 9

Arthrogryposis multiplex congenita (AMC) and counseling before and during pregnancy: a questionnaire study

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Abstract

Background Pre-pregnancy counseling in women with arthrogryposis multiplex congenita (AMC) is not implemented as standard care. Prior surveys revealed that the majority of adults with AMC live independently with or without support, are working, and socially active. Although many of them underwent pregnancies, literature is scarce concerning women with AMC and pregnancies. This study enquires information of women with AMC and their preferences to optimize counseling and guidance concerning aspects related to (pre-)pregnancy, childbirth and parenthood. Women with AMC, being members of an international AMC patient support group or Canadian register, were invited anonymously via newsletter in mail or announcement on social media.

Results A total of 53 women with confirmed AMC participated in this questionnaire study. Pregnancies were reported in 64.2% (34/53) of the women and 26 women had multiple pregnancies and delivered in total 45 children. A third (15/45) of the children were born vaginally and the remaining per caesarean section. None of the children had AMC. Four women reported on complications during application of local analgesia in labour and in 3 while positioning on the operating table.

Of the 47 women who answered this question, 95.7% (45/47) expressed a wish for standard pre-pregnancy counseling. As optimal circumstances they prefer counseling at or above 18 years of age, by a gynaecologist, at the outpatient clinic and for those with a relationship together with their partner. The importance of multidisciplinary team of physicians was emphasized as well as information from peer support groups and websites. Women expressed concerns about lack of knowledge on AMC in healthcare workers, discontinuity in care and attitude towards treatment and acknowledged the importance of this study.

Conclusions This study provides unique insights from women with AMC regarding pregnancy and parenthood. Participants emphasized the importance of tailored care. Their preferences strongly advocate for implementing pre-pregnancy counseling from the age of 18, ideally by a multidisciplinary team including a gynecologist. Incorporating these perspectives is essential to improve reproductive care.

Background

Pregnancy-related questions arise in women diagnosed with the rare condition arthrogryposis multiplex congenita (AMC), which affects approximately 1 in 3000-5000 newborns (1). AMC is an umbrella term for conditions with heterogeneous phenotypes and underlying causes (1). These conditions are characterized by contractures (joints lacking full normal motion) in various anatomical regions (1,2). The affected joints restrict movements, often cause physical impairment and chronic back or joint pain, resulting in limitations in daily functioning (1,3). Pregnancy-related questions may emerge in individuals with AMC, a condition in which the majority live independently, pursue education or employment, and actively engage in social activities (4-8). Approximately half of the people with AMC manage this independency without assistance and the other half require extra aids such as power mobility or braces (3,7).

Pre-pregnancy counseling is not routinely offered to women with AMC, despite the reported challenges in pregnancies with AMC, caused by immobility, associated anomalies (e.g. abnormal orofacial development) and occasionally impaired respiratory system (9). This is in contrast to other chronic medical conditions, such as diabetes, hypertension, and thyroid disease for which awareness already exists that optimizing the healthcare before and during pregnancy can reduce the risk of adverse effects for both mother and child (10,11). In our recent scoping review, we provided an overview of key perinatal care aspects, along with additional recommendations, including potential anesthesiologic challenges that should be anticipated (12).
Currently, there is a lack of literature on pregnancy-specific challenges in women with AMC (2). Sawatzky *et al.* previously highlighted this gap using a scoping review and Delphi study to identify outcome measures for adults with AMC (2). Our recent scoping review further confirmed this knowledge gap, revealing only 27 published reports covering 82 pregnancies in 43 women with AMC, primarily with distal arthrogryposis (12). These two scoping reviews emphasized the need to understand not only clinical outcomes but also the informational needs and preferences of women with AMC regarding (pre-)pregnancy, childbirth, and parenthood.

This study aims to fill that gap by evaluating how to optimize counseling and guidance of women with AMC concerning aspects related to (pre-)pregnancy, childbirth, and parenthood, including the anticipated challenges of physical caregiving tasks, breastfeeding, and organizing support for newborn care, which may be affected by the functional limitations associated with AMC. The questionnaire encompasses the characteristics of the women with AMC (general and medical background, education, work, and social life), experience during earlier pregnancies, information before pregnancy, during pregnancy, delivery and after birth, as well as personal opinions and advices. The findings of this study will empower healthcare providers to implement personalized (pre-) pregnancy counseling and tailored healthcare that align with the preferences of women with AMC**.**

Methods

Study design

A questionnaire was initially developed by the researchers (A. Arduç and J.I.P. de Vries) and adapted after comments of the co-investigators, including the representatives of the patient support groups (Spain, the Netherlands, United Kingdom and United States of America) and the coordinator of the Canadian adult AMC register. In line with recommendations of other AMC questionnaire studies, questions are kept as accessible as possible by formulating short questions in laymen's terms, using a positive approach concerning pregnancy and ability with AMC, and limiting duration to about 45 minutes to complete (2,29). The distribution of questions per six themes was: (1) 2 questions on general background, 16 on medical background, 6 on social life, education and work; (2) 14 on experience during earlier pregnancies; (3) 14 on information before pregnancy; (4) 8 on information about pregnancy and delivery, including topics for the birth plan; (5) 5 on information about period after birth, and (6) 1 open question to share opinions and advices (Questions are presented in Additional File Table 1).

Research ethics

The Medical Ethics Committee of the Amsterdam UMC agreed upon the questionnaire study and assessed that this study is not subject to the Medical Research Involving Human Subjects Act (2022.0579).

Data collection

Women with AMC who are members of the patient support groups for AMC (including the Netherlands (www.spierziekten.nl), Spain (www.artrogriposis.org), United Kingdom (https://www.arthrogryposis.co.uk), and United States of America

(www.amcsupport.org)) and the Canadian register (Adult AMC International Registry, B. Sawatzky, M.D., Ph.D.) were invited to fill in the questionnaire. Female members with AMC were approached via an email and/or an announcement on the social media of the patient support groups, or via an email by the Canadian register. The email and announcement contained information of the study and a link to the questionnaire. It was not traceable for the patient support groups, register, or researchers which women chose to participate in the study. The anonymous questionnaire was available in Dutch, English, Spanish, and French via the secured website Castor (www.castoredc.com). Data collection was conducted from April to December 2023.

Study population

The study population included women with AMC of 16 years or older, who were invited by one of the before mentioned patient support groups or Canadian register. Exclusion criteria were not being able to read Dutch, English, Spanish, or French. The population size was calculated to become approximately 70 women with AMC, as we expected that we could reach out to 220 women with AMC through the European patient support groups, and that a third of them would answer. The latter is in line with 34% completion of a questionnaire by people with AMC from the English patient support group (6).

Despite the fact that the AMC diagnosis was not derived from medical charts, the AMC could be phenotypically confirmed in all women by combining information of three questions concerning involvement of two or more anatomical regions, functional abilities and operations.

Statistical analysis

The collected data was transferred and organized in Excel, Microsoft 356 A3 for faculty. Descriptive analysis was performed using Excel. Data was presented as numbers with percentages and medians and ranges. Answers were adapted to the number of participants that responded to each question, as number of answers/total answers. The survey design permitted non-mandatory responses, leading to minor variations in the response denominators across different questions. The survey design permitted non-mandatory responses, leading to minor variations in the response denominators across different questions. All free text field data was manually categorized.

Results

The study population includes 53 women with AMC. Initially, 71 women started this study. Seventeen women, however, answered the characteristics section alone and were therefore excluded from the result analysis. These 17 women were evenly distributed over the various countries, with similar age ranges and severity of AMC concerning affected joints compared to the remaining participants. Another woman was excluded due to not having the diagnosis of AMC herself, although her child did. Below the main aspects are highlighted per theme. The survey and all results of the survey are demonstrated in Additional File tables 1-8.

Theme 1. Characteristics

The median age in 5-year period of the women was 36-40 years (range 16-20 – 71-75 years). Participants reported their current country of residence: 40 out of 53 lived in Europe, 12 in North America, and 1 in New Zealand. Reported joint involvement, operations, and mobility impairment by AMC, confirmed that all women fulfilled the phenotypic description of presence of contractures in more than one anatomic region. Underlying cause of AMC was known in 14/53 (1 Amyoplasia, 6 genetic or non-genetic inheritable, and 7 environmental). Genetic tests have been performed in 19/53 (36%) women in the period 1980-2023. Living independently was reported by 62.3% of participants (33/53), including those living alone (n = 3), with a partner without children (n = 12), or with a partner and children (n = 18). The remaining women were living with parents or other family members in 22.6% (12/53), or with help from other people in 15.1% (8/53). Women were employed in 67.9% (36/53). Reasons not to work were being a student (n = 4), retirement (n = 5), having limitations by AMC (n = 5), taking care of children (n = 2), and searching for a job (n = 1). In 69.8% (37/53) women reported to have a live partner. Having energy for hobbies and sports, such as physical and creative activities was reported in 58.5% (31/53). The quality of life of the women with AMC concerning physical, mental and social functioning was scored with a median of 7 (range 1-10).

Theme 2. Experience during earlier pregnancies

Pregnancies were reported in 34 of the 53 women (64.2%). From the 34 women with pregnancies, a total of 26 women gave birth to 45 children, who were healthy and unaffected by AMC. A third (15/45) of the children were born vaginally and the remaining per caesarean section. The majority of cesarean sections were planned: 18 out of 30 (60%) were scheduled early in the pregnancy, and 5 (16.7%) were planned closer to the time of delivery. Two cesarean sections were unplanned (6.7%), and 5 (6.7%) women could not recall the details. The reported experience during earlier pregnancies are listed in Table 1.

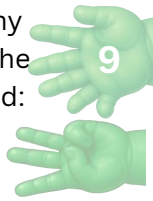

Table 1. Experience during earlier pregnancies of women with AMC, survey study in 2024

	Number/ completed questions (percentage)
Ever been pregnant	34/53 (64.2%)
Yes	26/53 (49.1%)
Yes and gave birth to alive child	19/53 (35.8%)
No, reasons:	
Own choice ($n=15$); advice health care provider ($n=2$); future wish to get pregnant ($n=1$); not able to have intercourse ($n=1$)	
Total number of alive children	45
Number of (alive) children per woman	12/26 (46.2%)
1	11/26 (42.3%)
2	2/26 (7.7%)
3	0
4	1/26 (3.8%)
5	
Location of delivery	45/45 (100%)
Hospital	3/26 (11.5%)
Midwife assisted	18/26 (69.2%)
Doctor assisted	5/26 (19.2)
Midwife and doctor assisted	
Vaginal delivery	10/15 (66.7%)
Uneventful	2/15 (13.3%)
Difficulties application epidural/spinal	0/15
Difficulties application iv drip	3/15 (20%)
Vacuum/ forcipal extraction	
Caesarean section	27/30 (90%)
Uneventful	2/30 (6.7%)
Difficulties application epidural/ spinal	0/30
Difficulties application iv drip	3/30 (1%)
Difficulties position on operation table	
The course of pregnancy lead to refrain from future pregnancies	21/34 (61.8%)
No	12/34 (35.3%)
Yes	1/34 (2.9%)
Do not know yet	
Change in mobility during or after pregnancy	18/26 (69.2%)
Cause increase in pain	7/18
Difficulties walking	8/18
Worsening of joint complaints	4/18
Complaints like fatigue and swelling of the legs	4/18
Gestational age at birth	35/45 (77.8%)
Term birth	7/45 (15.6%)
Preterm birth	3/45 (6.7%)
Unknown	
Extra help from own network (friends or family)	14/34 (41.2%)
During pregnancy	17/34 (50.0%)
After pregnancy	13/34 (38.2%)
No extra help	
Extra help for housekeeping/household	12/34 (35.3%)
During pregnancy	18/34 (52.9%)
After pregnancy	15/34 (44.1%)
No extra help	
More assistance required with self-care	13/34 (38.2%)
During pregnancy	11/34 (32.4%)
After pregnancy	18/34 (52.9%)
No extra help	
Adaptions/help for breastfeeding	8/26 (30.8%)
Yes	18/26 (69.2%)
No	
Adaptions/help for formula feeding	6/26 (23.1%)
Yes	20/26 (76.9%)
No	

Table 1. (continued)

	Number/ completed questions (percentage)
Adaptions/help for childcare	
Yes	12/26 (46.2%)
No	14/26 (53.8%)

Theme 3. Information before pregnancy

Participants talked with healthcare providers about the wish to have children in 57.7% (30/52), sexuality in 37.3% (19/51), and fertility in 49.0% (25/51). Women reported that they did talk with their own healthcare providers about the wish to have children, but the healthcare providers could not answer questions related to the three topics in 36.7% (11/30) child wish, 15.8% (3/19) sexuality, 20% (5/25) fertility, respectively. Women found it difficult to talk about these topics (21.2%; 35.3%; 19.6%) and healthcare providers had no time to talk about it (3.8%; 11.8%; 7.8%). Other women did not feel the need to talk about it with their healthcare providers (13.5%; 17.6%; 17.6%). Participants had an active sex life without pain [60.8% (31/51)] or with some extra pain [23.5% (12/51)]. Two women (aged 54–60 years) reported that sex was very painful. Both had contractures in the upper and lower limbs and had undergone multiple lower limb surgeries. They were independently mobile without assistive devices. There were no women with absent sex life due to pain.

The preferences concerning a pre-pregnancy counseling are shown in Table 2. The three other most preferred ways to be informed about pregnancy and AMC include: by other patients with AMC [59.6% (31/52)], internet or websites [54.8% (28/52)], and a meeting with presentations by healthcare providers [51.9% (27/52)]. Approximately half of the women [49.0% (25/51)] had ever heard of pre-pregnancy counseling. Most women [95.7% (45/47)] found pre-pregnancy counseling to be useful as standard care. In 73.6% (39/53) women expressed openness to genetic counseling regarding new possibilities in genetic testing. Curiosity [59.0% (23/39)] as well as family planning [35.9% (14/39)] are the main arguments for this.

Table 2. Preferences concerning information before pregnancy expressed by women with AMC, survey study in 2024

	Number/completed questions (percentage)
Preferred age to get information about fertility and future child wish	2/51 (3.9%)
15–16 years old	4/51 (7.8%)
17–18 years old	32/51 (62.7%)
> 18 years old	2/51 (3.9%)
Open answer: Age-appropriate information from nursery or primary school onwards	11/51 (21.6%)
Open answer: not specified	
Preferred company during pre-pregnancy counselling	14/50 (28.0%)
Alone (without anyone else)	26/50 (52.0%)
Together with my partner	1/50 (2.0%)
Together with my parents	1/50 (2.0%)
I don't know	8/50 (16.0%)
Open answer: not specified	
Preferred healthcare provider to give pre-pregnancy counselling	17/50 (34.0%)
General practitioner	39/50 (78.0%)
Gynaecologist	9/50 (18.0%)
Anesthesiologist	5/50 (10.0%)
Rehabilitation doctor/physiatrist	13/50 (26.0%)
Midwife	7/50 (14.0%)
Neurologist	20/50 (40.0%)
Clinical geneticist	11/50 (22.0%)
Combination of doctors	2/50 (4.0%)
Open answer: trauma surgeon	7/50 (14.0%)
Open answer: not specified	
Preferred appointment for pre-pregnancy counselling	27/50 (54.0%)
Outpatient clinic	11/50 (22.0%)
By phone (with or without seeing each other/video-interactive meeting)	2/50 (4.0%)
In hospital	1/50 (2.0%)
Open answer: At mutual insurance company	9/50 (18.0%)
Not specified	
Preferred topics during pre-pregnancy counselling	33/50 (66.0%)
Fertility	29/50 (58.0%)
Medication to be stopped before pregnancy	37/50 (74.0%)
Influence of pregnancy and parenthood on daily functioning with AMC	37/50 (74.0%)
Influence of AMC on baby's and own health	39/50 (78.0%)
Most common difficulties during pregnancy in women with AMC	37/50 (74.0%)
Possibilities of genetic testing before/during pregnancy and chance for heredity	29/50 (58.0%)
Possibility of 20-week ultrasound during pregnancy to exclude AMC	35/50 (70.0%)
Information on labour and preparing "Birth plan"	31/50 (62.0%)
Breastfeeding and possibility of referral to occupational therapist for advice	32/50 (64.0%)
Possibilities to deploy help to maximize the independency around pregnancy	1/50 (2.0%)
Open answer: Emotions that may arise, maternal mental health	1/50 (2.0%)
Open answer: Guidance and public subsidies for IVF treatment	

Theme 4. Information about pregnancy, delivery and period after delivery

An overview of preferences for receiving information concerning pregnancy, birth plan and after delivery is depicted in Table 3. Women prefer to discuss their birth plan with their healthcare providers multiple times before and during the pregnancy. Thirty-three women (71.7%, 33/46) appreciate the possibility to have a separate consultation with an anesthesiologist. Eleven women (23.9%, 11/46) described that they had a consultation with an anesthesiologist during a prior pregnancy.

Theme 5. Information about period after birth

The expected extra help or aids after birth are presented in Table 3.

Table 3. Preferences concerning information about pregnancy, delivery and period after birth of women with AMC, survey study in 2024

	Number/ completed questions (percentage)
Feel supported if during pregnancy gynaecologist collaborates with my general practitioner for AMC	26/47 (55.3%)
Yes, early in pregnancy	18/47 (38.3%)
Yes, before the delivery	11/47 (23.4%)
No	1/47 (2.1%)
Open answer: Don't have a doctor for AMC	
Expected healthcare provider(s) to be in need during pregnancy (other than obstetric caregiver)	25/47 (53.2%)
General practitioner	18/47 (38.3%)
Rehabilitation doctor	7/47 (14.9%)
Neurologist	11/47 (23.4%)
Orthopaedic surgeon	7/47 (14.9%)
Social worker	10/47 (21.3%)
Psychologist	23/47 (48.9%)
Anesthesiologist	18/47 (38.3%)
Clinical geneticist	18/47 (38.3%)
Paediatrician	18/47 (38.3%)
Multidisciplinary team with tailored care for own situation with AMC	1/47 (2.1%)
Open answer: Occupational therapist	
Topics birth plan	29/46 (63.0%)
Location of labour	21/46 (45.7%)
Mode of delivery: spontaneous vaginal delivery	15/46 (32.6%)
Mode of delivery: induced labour	31/46 (67.4%)
Mode of delivery: caesarean section	22/46 (47.8%)
Discussion about at which pregnancy week	31/46 (67.4%)
Possibility for pain relief	28/46 (60.9%)
Which healthcare provider present during labour	25/46 (54.3%)
If vaginal delivery: position during delivery	30/46 (65.2%)
Breast and formula feeding	36/46 (78.3%)
Extra care after birth	
Preferred mode of delivery adapted to your AMC	12/46 (26.1%)
Vaginally	19/46 (41.3%)
Caesarean section	7/46 (15.2%)
No preference, but would prefer to make choice during labour	4/46 (8.7%)
Not applicable, don't have the wish to become pregnant	4/46 (8.7%)
Open answers: No preference, but would prefer to discuss the options in consultation and receive information about what is possible and can be facilitated (e.g. adaption of delivery table)	
Expect to need extra support for	**Agree (number/ completed questions (percentage))**
Physical care of myself and child from own network after dellivery	33/46 (71.7%)
Households after delivery	41/46 (89.1%)
Breastfeeding after delivery	24/46 (52.2%)
Formula feeding	23/46 (50.0%)
Care for child	26/46 (56.5%)

Theme 6. Opinions and advices

Reported opinions, ideas and advice are categorized for healthcare, knowledge on AMC and organization aspects and summarized concerning all 53 women in Table 4. Advice around pregnancy given by the women with a prior pregnancy are categorized for knowledge on AMC and suggestions for support and presented in Table 5. To illustrate concerns of women with AMC, we cite below from the free text answers, and all individual answers are listed in Additional File Table 9.

One participant with one vaginal birth and one caesarean, and history of surgery on the hips, knees and feet mentioned, “Having hip problems is not synonymous with having to give birth by caesarean section.”

A woman that chose not to have a child partially due to lack of educated doctors around AMC and fearful of how her body would adapt to pregnancy. “Women with AMC need to be given the same counseling and information about pregnancy and childbirth as able bodied women. The medical field has a lot of internalized ableism around this and seldom initiated conversations about this with me.” – With better resources, I may have chosen differently”.

A few participants shared their experience of feeling unsupported during her pregnancy despite early efforts to arrange care. They described difficulties in accessing referred specialists and a lack of communication with the healthcare provider, which left them uncertain about how her pregnancy would progress.

Another comment was about the importance of assistance in finding a way to hold, breastfeed and formula feed the child(ren) “This can mean using feet to change a diaper, or even the mouth, using the mouth or chin to lift and position the baby or the need to have our kids placed on/next to us by our partners or personal assistants” .

A further remark was given on the well-prepared organization during pregnancy “It was helpful to have additional ultrasounds to identify if there were any contractures. Not enough gynecologists and obstetricians have accessible offices and my OB expressed anxiety over treating me due to complications. The anesthesiologist was fantastic and was prepared for my needs”.

Table 4. Ideas for ideal pre-pregnancy counseling, experienced and suggestions by women with AMC, survey study in 2024.

*Elaboration in form of a quote in the text (Results theme 6)

Ideas for ideal pre-pregnancy counselling (*n* = 44)	
With history of pregnancy (*n* = 31)	**Without history of pregnancy (*n* = 13)**
Healthcare provider Patient tailored information by most frequent visited doctor (*n* = 1) Concern about knowledge of gynaecologist (*n* = 1) * Tailored specialist: first GP and afterwards gynaecologist (*n* = 1)	Healthcare provider Tailored specialist: first GP and afterwards gynaecologist (*n* = 2)
Knowledge Doctor with knowledge of AMC (*n* = 4) More academic research on AMC in general (*n* = 1)	Knowledge Availability of an updated list of genes involved in AMC (*n* = 1)
Organisation During teens (*n* = 1) Age 18 + (*n* = 1) Peer contact with fellow AMC'er that experienced pregnancy (was not available during own pregnancy) (*n* = 1) Online counselling (*n* = 2) "In a place that builds trust" (*n* = 1) Deciding when you want to have children (*n* = 1) Multidisciplinary approach (*n* = 2) Need for counselling, since experienced current absence (*n* = 1) Organisation of care for patient self and child (*n* = 2)	Organisation Personalized care: awareness is asked for over treating / undertreating / "ableism" (*n* = 2)*
Other topics Limitations (*n* = 1) Exploring possible emotions that arise after birth (*n* = 1) Expectations (*n* = 1) Fertility, inherit, risks (*n* = 1)	Other topics Consequences of weight gain during pregnancy (*n* = 1)
No additional ideas (n = 15)	No additional ideas (n = 8)
Expressing contentment towards the study (n = 2)	
Opinions, experiences, and suggestions (*n* = 25)	
With history of pregnancy (*n* = 17)	**Without history of pregnancy (*n* = 8)**
Experiences with organisation Consultant referrals were made but not realised (*n* = 1)* Referred for counselling but in absence of knowledge no counselling was realized (*n* = 1) Delivery room areas and bathroom not accessible (*n* = 1)* Caesarean prevented visiting her children in incubator for 24 h after birth (*n* = 1)	Experiences with organisation Wish for peer contact (*n* = 1) Refusal of pre-pregnancy counselling (*n* = 1) Lack of educated doctors on AMC and pregnancy (*n* = 1)*
Opinions Contacting a geneticist who specialized in AMC was very helpful (*n* = 1) Website lactancia.org very useful (*n* = 1)	Opinions None
Suggestions Multidisciplinary team, adaptions and recourses for people with reduced mobility. Training and information for health professionals (*n* = 1)	Suggestions For future research: differentiate between major and minor surgeries (*n* = 1) Increase of awareness for the need of pre-pregnancy counselling (*n* = 1)
Requesting information sources (*n* = 3) a.o.: wish for online or research forums	Requesting information sources and tools (*n* = 2)
Expressing gratitude and contentment towards the study (*n* = 9)	Expressing gratitude and contentment towards the study (*n* = 2)
No additional comments (*n* = 2)	No additional comments (*n* = 2)

* Elaboration in form of a quote in the text (Results theme 6)

Table 5. Advices given by 31 women with AMC and experienced pregnancies, survey study in 2024

To women with AMC	To healthcare providers
Knowledge on AMC and pregnancy • Seek counseling and preparation for childbirth and breastfeeding, ask everything (without feeling burdened) (n=5) • a.o.: reach out to someone who has been there, midwife workshops, more child delivery: "Having hip problems is not synonymous with having to give birth by caesarean section"* but also "Do not try vaginally, just go for the caesarean section" Follow research updates on AMC (and baby movement) (n=1)	• More knowledge of AMC amongst treating doctors (n=5). ◦ a.o: to prevent the need for repeating, explaining, to prevent anxiety among healthcare providers during treatment, more knowledge on challenges with intravenous line insertion, mobility problems after pregnancy, delay in recovery after CS. • More knowledge on physical disabilities and understanding of patients moving differently, experienced as safe (n=4) • a.o.: training and preparation for healthcare personnel to care for patients with physical disabilities / AMC (n=2) • Assist in finding way to hold, breastfeed/formula feed, and change child(ren) (n=1) • Inform patient of limitations and problems (n=2) • Personalized care: awareness is asked for overtreating /undertreating /"ableism" (n=8) • a.o.: balance between care and intruding in bonding time, do not judge someone on their ability to carry a child because of the physical disability, do not overdo it, allow relaxation, "More humanity"(n=3) • Diagnostics: additional ultrasounds, prior genetic counseling, (free) genetic testing (n=7) • Facilities: accessibility in offices, delivery room and maternity ward, privacy for extra assistance in private room if possible (n=3) • Active support on mental wellbeing and experienced care (n=1) • Listen to your patient, she knows her body better than anyone (n=2)
Supportive help/treatment • Look for a gynaecologist that is supportive and inspiring (n=1) • Ask for and accept any extra assistance from friends, family and professionals (n=2) • Try gentle exercising, such as swimming (n=3), control weight as much as possible (n=1) • Enjoy the pregnancy and your children, live the precious experience (n=3) • Expressing contentment towards their pregnancy and childbirth (n=2) • No additional advices (n=5) • a.o. since miscarriage in first trimester (n=1)	

*Elaboration in form of a quote in the text (Results theme 6)

Discussion

This study focused on the preferences of an international cohort of 53 women with AMC on pregnancy-related topics. The majority of women experienced a normal sex life, two third became pregnant and half of the women delivered children. These findings emphasize the importance of the focus on possibilities of pregnancy and parenthood in AMC. Their experiences and advices regarding standard implementation of (pre-)pregnancy counseling and experiences around pregnancy care are explicit.

This population of women with AMC was reached through patient support groups and one AMC register. The underlying cause in this population was found in about a quarter of the women. While prior studies report that Amyoplasia represents about one-third of AMC, in this study only one woman (2%) had Amyoplasia (1). Furthermore, a genetic or non-genetic heritable cause was found in 6 women (31%), which is also lower than the genetic diagnostic yield that was reported in prior studies with individuals with AMC (up to 52.7-65.2%) (13,14). However, the lower genetic diagnostic yield may be partially explained by the fact that genetic testing in many participants was performed before the widespread clinical implementation of advanced sequencing techniques such as next generation sequencing based tests (whole exome sequencing) (15).

Educational level, work, and quality of life experience of the participants are in line with prior studies (3,16). Studies of Altiok and Nouraei showed also a high quality of life among individuals with AMC (3,16). The level of independency of the participants was also similar to previous quality of life and AMC studies (51.8-75%) (3,8).

Pregnancies were reported in two third of this study population and half delivered children of which one third vaginally and two third per caesarean sections. While this caesarean rate is higher than in the general population, it aligns with findings in other populations of women with neuromuscular disorders or physical disabilities (17). Awater *et al*. reported similarly elevated caesarean rates in a cohort of 178 women with hereditary neuromuscular disorders, primarily due to obstetric indications such as abnormal fetal presentation, concerns about maternal muscle function, or pelvic anatomy (17). The deliveries were uneventful in two third of the vaginal deliveries and in 90% of the caesarean sections. A few had applications problems with the epidural during labour and none with introduction of intravenous drip. The fact that the majority of cesarean sections were planned suggests they were performed due to maternal indications. In this population, none of the born children had AMC.

The received information before pregnancy concerning pregnancy-related aspects has been experienced as insufficient among the participants. Half of the women had heard of the possibility of pre-pregnancy counseling, but nearly all would (have) preferred it as a standard procedure. The lack of doctor-patient communication concerning sexuality and fertility were mentioned by the participants. This is in line with other reports on women with a physical impairment who experience that healthcare providers pay limited attention to sexual activity, and fail to inquire about the effect of the impairment on sexuality and the wish to get pregnant (7,18). Healthcare providers might feel ambivalence and discomfort to discuss these topics (19,20). Absence of such information prevents some women to not fulfil their wish to have children (21). In our study, this was also reported by one of the participants. The most optimal implementation of pre-pregnancy counseling was considered to be performed above 18 years of age, provided by a gynaecologist, at the outpatient clinic, and together with their partner. It is important to know that the majority of women with AMC is interested to be counseled again about new possibilities for genetic testing, such as preimplantation genetic testing.

During pregnancy, women prefer to receive information and care by a multidisciplinary team. This team will be tailored per woman with AMC. Moreover, half of the women expected care of a general practitioner and anesthesiologist in addition to a gynaecologist. A third of the women who had a prior pregnancy reported that they had been referred for consultation to an anesthesiologist. One women emphasized the safety that was felt when having a well-prepared anesthesiologist with knowledge of AMC. Given the potential for anaesthetic difficulties, there was a strong desire for thorough preparation (9). The majority of topics in the birth plan for timely preparation of the delivery were considered as important, but the main topic was to discuss the possible extra care needed after birth (78%).

During pregnancy, the increased body weight and growing uterus may affect posture and balance, increase joint strain, and can lead to fatigue. Although physical proportions generally normalize within the postpartum period (6-8 weeks after delivery), persistent fatigue related to circulatory and hormonal adaptations may delay musculoskeletal recovery. After birth, the majority of the women in this study expected to need help of their own network for physical care of themselves and their child, and for the households. However, our study showed that approximately half of the women who had delivered indeed needed extra help after the pregnancy with physical care or housekeeping and even less for self-care, breastfeeding, and formula feeding. Functional limitations associated with AMC may affect the ability to perform early parenting tasks independently. Some women described inventive adaptations, while others highlighted the essential role of partners or support networks in day-to-day infant care. These findings emphasize the need to include practical parenting support and planning as a formal part of pre-pregnancy and postpartum counseling for women with AMC. Addressing these challenges proactively may empower women and reduce anxiety during the early stages of parenthood.

With the obtained knowledge on the possibilities to deliver vaginally or per caesarean section we can improve our counseling. Women with AMC have to be aware of a higher chance of operative delivery in comparison to the global average of a general population, two third versus one third (30-32%) (22). Specific patient oriented preparation with an understanding of AMC has to be made.

During the counseling before and during pregnancy, the challenge on immobility by increased pain during pregnancy in nearly 70% has to be discussed. This pain will be primarily due to the physiological changes of the musculoskeletal system adapting to the growing uterine content (23). In case of advancing immobility, there is a risk factor for thromboembolism, and it should be evaluated if anticoagulation medication is needed (24).

The individual remarks concerning pregnancy-related topics revealed information which was not derived from the multiple choice questions. Some women expressed their concern about the knowledge on AMC by healthcare providers. They also gave examples illustrating lack of knowledge caused by not reading the medical chart prior to the consultation, by no communication from one healthcare to another. Simply reading of the charts by the healthcare provider prior to a visit is suggested to reduce the burden for women with AMC preventing to repeat their long and well known history at each visit.

Several examples were given to illustrate experienced discrimination because of the physical challenges, one woman explicitly expressed her concern of ableism. The definition of ableism is persistent disability-related discrimination, which often results in psychological disadvantages for people with disabilities (25).

Women with AMC emphasize the importance to evaluate opportunities and not just obstacles during pre-pregnancy counseling and care during and after pregnancy. The balance between facilitating adequate care, and prevent over- and undertreatment is delicate. Women with AMC would appreciate more familiarity with pregnancy in women with disabilities to improve easiness in posing open pregnancy-related questions without prejudice and in a wheelchair accessible setting. In terms of perceived satisfaction with healthcare and counseling, women with physical disabilities -including those with spinal cord injury or cerebral palsy- have expressed similar concerns (26,27).

One of the strengths of this study is the focus on the preferences and ideas concerning pre-pregnancy counseling provided by women with AMC. The collaboration with patient support groups and one AMC register from nine countries in three continents facilitated this study. The clear answers on the many topics on pregnancy-related questions paves the way to arm healthcare givers and women with AMC with new information.

Despite valuable insights from our study, we must acknowledge its limitations. The study population is still limited. We cannot exclude recall and selection bias. However, we did not question specific details on for example medication use, but essentials amongst others number of pregnancies and gestational age at birth in a population with a median age of 36-40 years. Moreover, the open questions gave a wealth of information of individual experiences.

The findings suggest several implications for clinical practice, including the importance of implementing standard pre-pregnancy counseling, providing information through a multidisciplinary team, and ensuring healthcare providers are adequately informed about AMC-specific challenges, such as anesthetic risks, physical limitations, and postpartum care needs. While the preferences reported in this study were not analyzed by region, it is important to acknowledge that healthcare experiences may vary based on healthcare system structure, access to services, and cultural expectations. Future research should aim to explore these differences systematically, ideally through large, international registries that combine patient-reported experiences with verified clinical and genetic data. Such registries would enable meaningful subgroup analyses- by AMC subtype, severity, or healthcare system- and guide the development of evidence- based guidelines for reproductive care in women with AMC. We attributed to a Delphi method among medical professionals and people with AMC experience to define common data elements which should be recorded in the international register on AMC (28). Comparative studies including other groups of women with chronic illnesses or disabilities may also provide valuable insights into shared needs and gaps in current care models. Ultimately, these efforts can help bridge the knowledge and practice gaps and promote equitable, person-centered care for this underrepresented population.

Conclusion

Women with AMC reported their experience with pregnancies and specific wishes to be informed about pregnancy-related topics. A lack of information on pregnancy-related topics was experienced and their wish is to implement pre-pregnancy counseling as standard care, preferable at the age of 18 years or older, by a gynaecologist in collaboration with a clinical geneticist and general practitioner, at the outpatient clinic, and with their partner. Women with AMC acknowledged the importance of this study. The close collaboration with the patient support groups and registers will further support future research for increasing knowledge among healthcare providers and implementation of standard pre-pregnancy counseling.

Supplementary documents

- Additional File Table 1. Questionnaire arthrogryposis multiplex congenita (AMC) and Pregnancy, survey study among women with AMC 2024
- Additional File Tables 2-8. Results Survey AMC and pregnancy in 2024

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CHAPTER 10

Maternal Experience of fetal movements from a Child with AMC: MECA survey.

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Abstract

Objective The prevailing assumption is that fetal movements are always absent or reduced in pregnancies affected by arthrogryposis multiplex congenita (AMC), leading to the belief that mothers do not perceive or perceive less fetal movements during affected pregnancies. This study aims to investigate the maternal perception of fetal movements in pregnancies with a child diagnosed with AMC and to challenge this assumption. Additionally, it seeks to expand current knowledge on the perception by comparing with pregnancies with children not affected by AMC.

Methods A survey-based study was conducted in collaboration with international patient support groups. The survey included mothers with at least one child diagnosed with AMC. The questionnaire covered not only the presence of movements, but also other aspects such as daily movements, consistency throughout the pregnancy, and perceived normalcy. A subgroup comparison was made between mothers who had both an affected and non- affected pregnancy, as well as by pregnancy order and its impact on clinical follow-up.

Results A total of 170 mothers participated in this survey and 118 (70%) of them had both an affected and non-affected pregnancy and 52 (30%) had pregnancies with AMC-affected children alone. Most (77%) perceived fetal movements during AMC-affected pregnancies, though fewer described them as daily (66%), stable (51%), or normal (44%) compared to unaffected pregnancies.

Conclusion This study showed that fetal movements can be perceived by the majority of mothers of children with AMC. The presence of fetal movements should not rule out the possibility of AMC in case of fetal contractures.

Introduction

AMC (arthrogryposis multiplex congenita) is an umbrella term for rare conditions characterized by joint contractures (joints lacking full normal motion) present at birth with a prevalence of 1 in 3,000-5,200 newborns (1-4). The affected joints can limit joint motility and daily functioning (5). AMC has various underlying causes, including genetic, syndromic, infectious, environmental factors, as well as other causes that are still unknown (1). Several attempts have been made to classify the heterogeneous diagnoses of AMC after birth to provide clearer insight into the prognosis (1,6,7). One classification system divides AMC into three phenotypic groups based on the affected body parts: primarily limbs, limbs and other body parts, and limbs including central nervous system (CNS) involvement (1). This phenotypical classification can also be applied in the prenatal period by using ultrasound examinations (8).

Despite the varied etiology of AMC, reduced or absent fetal movements are considered to be the common feature in the development of contractures (1,9). Embryonic and fetal movements are essential for the development of the musculoskeletal and neurological system (9). The movements are spontaneous expressions of the developing nervous system and are necessary for the proper development of soft tissue pliability around the joint, which allows for appropriate motion after birth (1,10). In uncomplicated pregnancies the earliest movements seen during prenatal ultrasound examinations appear at 7 weeks of gestation as slow, small sideways bending of head and or rump (10). Between 7-8.5 weeks, these movements progress to include sideways bending together with small movements in the extremities (10). By 9 weeks, all body parts become active with general movements varying in speed, amplitude, direction, and participation of all body parts (10). These general movements remain recognizable throughout gestation and after birth (11).

In a longitudinal prospective study concerning fetuses with two or more contractures, it was found that fetuses developing AMC without CNS involvement exhibited reduced mobility in their general movements which was limited to the affected joints, while fetuses with CNS involvement demonstrated a decreased motility across all body parts (12). The latter study demonstrated the wide spectrum of motility observed by prenatal ultrasound examinations, which varied depending on the affected body parts and the underlying cause (12).

The average gestational age of the first maternal perception of fetal movements in uncomplicated pregnancies is around 19.5 weeks with a broad range from 14 to 26 weeks and beyond (13). This timing may be delayed by higher maternal age, higher body mass index, nulliparity and anterior placental position (13,14). Once fetal movements are perceived, they typically become part of the mother's daily awareness of the pregnancy (13). In a healthy pregnancy, fetal movements are expected to be felt every day, and these movements often follow a somewhat consistent pattern, particularly in the third trimester. This consistency, referred to as "stable" fetal movements, implies that the movements are perceived regularly and similarly over time.

Since reduced fetal movements are thought to play a key role in the development of contractures in AMC, an important question is whether mothers always notice reduced, absent, or delayed fetal movements during pregnancies affected by AMC? Two questionnaire-based studies involving mothers of children with AMC demonstrated that fetal movements perceived as "normal" by the mothers can occur (3,15). In the questionnaire study by Dahan-Oliel *et al*. 2019, it was reported that 7 of the 36 (20%) mothers perceived normal movements during pregnancy (3). Similarly, Lemin *et al*. 2024 conducted a survey among 301 biological parents of children with AMC, revealing that 32% of mothers perceived normal fetal movements (15). In both of these studies, the primary focus was to collect a wide range of information on prenatal and postnatal outcomes by telephone or online questionnaire and the maternal perception of fetal movement was only a small topic. Dahan-Oliel *et al*. evaluated the perceived fetal movements through open-ended questions while Lemin *et al.* used one question (How would you describe your baby's movement during your pregnancy?) with a multiple-choice answer option. Neither study explored further details of the movements such as daily occurrence or consistency over the length of the pregnancy, nor did either study make a direct comparison in mothers having both AMC-affected and unaffected pregnancies.

The aim of this study was to expand the current knowledge on the spectrum of maternal perceived movements during pregnancies of children with postnatally confirmed AMC. It focused on key movement aspects commonly monitored during routine prenatal check-ups, including presence of movements, daily occurrence, consistency of movements throughout pregnancy, and perceived normalcy. These aspects were assessed for all included mothers and separately analyzed for those who had both AMC-affected and non-affected pregnancies, allowing for a comparative evaluation of their perception.

In addition, the aspects of the perceived movements were also evaluated for the first and subsequent pregnancies for mothers who had both AMC-affected and non-affected pregnancies. The set-up of the study was performed in close collaboration with the patients support groups. Our hypothesis was that fetal movements could also be perceived by the mothers in pregnancies with AMC-affected fetuses. The specific research questions guiding this study were:

- How are fetal movements perceived during pregnancies with AMC-affected children, considering aspects such as the presence of movements, daily occurrence, consistency throughout the pregnancy, and whether they were perceived as normal?
- Did mothers perceive fetal movements in both AMC-affected and non-affected pregnancies?
- Did the birth order (first pregnancy vs. subsequent pregnancies) affect maternal perception of fetal movements of an affected child with AMC?
- Did maternal perception of fetal movements in AMC-affected pregnancies influence the application of follow-up ultrasound examinations during pregnancy?
- What advice do mothers with an AMC-affected child offer to healthcare providers, to other mothers with a suspected AMC-affected fetus, and to patient support groups, based on their perception?

Methods

Development of the questionnaire

To explore maternal perceptions of fetal movements in pregnancies affected by AMC, we developed the *Maternal Experience of fetal movements from a Child with AMC* (MECA) survey. This questionnaire has been developed by three researchers from the Obstetrics and Gynecology department (AA, JS, and JIPdV). We designed the questions to align with those used in two previous studies that examined maternal perception of fetal movements in AMC (3,15). The patient support group representatives, medical advisers and contact persons of the register were asked to evaluate the questionnaire and provided additional information and questions. The questionnaire was translated from English into Dutch, Spanish, German and French by professional translators except for the Dutch version by the researchers themselves. The questionnaire was kept as short at possible (estimated duration to complete 15-30 minutes) with no/ limited medical jargon to keep it accessible for the participants. All questions are presented in Additional File 1.

The MECA survey included 111 questions divided into 6 chapters. The first chapter was the introduction of the survey and contained background information. Characteristics of mother and child were asked in chapter 2 and 3, respectively. Chapter 4 concerned questions on maternally perceived movements during the pregnancy of the Child with AMC and whether the experience influenced the application of follow-up ultrasound examinations (in addition to the routine ultrasound examinations). All aspects of fetal movement—such as their presence, daily occurrence, consistency throughout pregnancy, and perceived normalcy—are described by the participants and were based on their personal experience. The categories of abnormal fetal movements were aligned with those used in the survey by Dahan-Oliel *et al*. to allow for comparison (3). These included no movements and abnormal (decreased movements, in one place only, and reduced strength/power). Chapter 5 posed the same questions on the perception during a pregnancy of a child without AMC and only appeared when a mother had answered that she had been also pregnant with a child not affected by AMC. In chapter 6, mothers had the opportunity to share their advice and recommendations for professional health care providers, for pregnant women with a child suspected of having AMC, and for patient support groups for AMC.

Participants

Participating mothers, with a minimum age of 16 years, were recruited with the assistance of patient support groups and adult registry. These mothers with a history of a pregnancy of a child with AMC were informed through anonymous study announcements shared via newsletters and social media channels. Mothers self-selected to participate by responding to these public calls. Exclusion criteria were non-fluency in English, French, Spanish or Dutch, mother's current age over 70 years, and pregnancy prior to 1975 when fetal sonography was not widely utilized.

Collaborating AMC patient support groups (Netherlands (www.spierziekten.nl), Canada/United States (www.amcsupport.org), Spain (www.artrogriposis.org), Germany (https://arthrogryposis.de/) and United Kingdom (www.arthrogryposis.co.uk) and a Canadian patient registry coordinator were asked to send an invitation to their members, with a link to the questionnaire on the Castor platform (www.castorredc.com). No personal email lists were used, and no identifiable information was collected during recruitment. The questionnaire was accessible between May and July, 2024.

Due to the nature of recruitment, neither a response rate calculation nor sample size calculation was feasible, as an unknown number of mothers of children with AMC were contacted. The anticipated sample size was 90 participants, including 50 mothers with at least one child with AMC and another 40 mothers with both at least one child with AMC and at least one child without AMC. The maternal perception of movements during pregnancies of children with AMC and without AMC were evaluated separately. In case of several qualifying pregnancies, the data of the mother's oldest child with, and without, AMC was evaluated. All answers were translated into English (if necessary) by the researchers.

Research ethics

The Medical Ethics Committee of the Amsterdam UMC agreed upon the questionnaire study and assessed that this study is not subject to the Medical Research Involving Human Subjects Act (reference number 2024.0036).

Statistical analysis

The collected data were transferred and organized in Excel, Microsoft 365 A3 (Redmond, WA, USA). Analyses were performed using SPSS software (IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY, USA: IBM Corp; 2021). Data were descriptively presented as numbers with percentages and five-year medians, mean and/or ranges. Answers were adapted to the number of mothers that answered the question, as number of answers/total answers. The survey design permitted non-mandatory responses to each question, leading to minor variations in the response denominators across different questions. The qualitative responses provided in the free-text field of the last chapter, along with additional free-text contributions, were analyzed using thematic analysis and categorized accordingly.

Results

In total 184 mothers participated in this survey. There were 14 mothers excluded, one was not the biological parent, 9 only answered the first question concerning the maternal characteristics, and 4 mothers were 70 years or older and/or the pregnancy was before 1975. An overview of the answers of all participants is presented in Additional File 2.

Participant characteristics

The included mothers (n=170) had at delivery a median five year's age range of 31-35 years (ranged from 16-20 to 41-45 years). The mothers reside in Germany (70), the United States of America (40), Spain (28), the Netherlands (13), the United Kingdom (9), Switzerland (4), and one each from Canada, Sweden, Croatia, Australia, Chile, and Belgium.

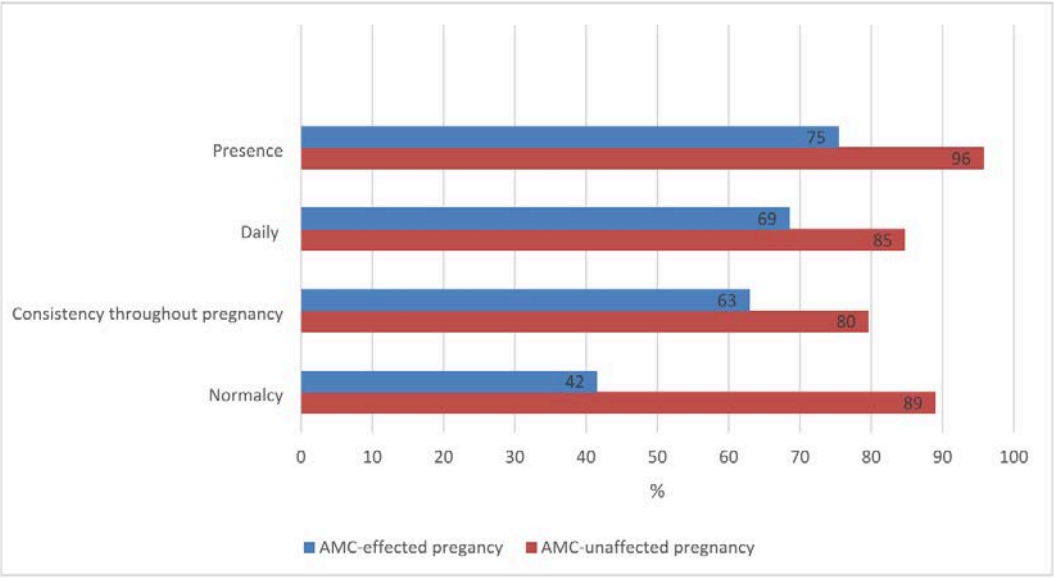
Family sizes varied (n=170): 51 (30%) mothers had one child, 60 (35%) had two, 38 (22%) had three, 13 (8%) had four, six (4%) had five, one (0.5%) had seven, and another one (0.5%) eight children. The birth order of the oldest child with AMC in each family (n=170) was 83 (49%) first-borns, 57 (34%) second, 19 (11%) third, six (3%) fourth, three (2%) fifth, one (0.6%) was sixth, and one unknown (0.6%). There were 10 twin pregnancies of which, AMC affecting both babies in 3 pregnancies and one baby in 7 pregnancies. Out of 170 mothers, 166 (98%) had one child with AMC, while four (2%) had two children with AMC. The children's current ages ranged from 0–4 years to 40–44 years.

The suspicion of AMC was made prenatally in 34 (21%) of the 162 mothers who responded to this question, at a median gestational age of 19 weeks (range 11-36 weeks). In the remaining 128 cases (79%), AMC was diagnosed after birth. The mode of delivery was provided for 161 respondents, a vaginal delivery in 61 pregnancies (38%) and a cesarean section in the remaining 100 (62%). The phenotypic description of multiple contractures confirmed the diagnosis in all children. The 17 underlying genetic causes and level of independency of the children are available in Additional File 2.

Perception of fetal movements

The questions concerning the perception during AMC-affected pregnancies was answered by 162 of the 170 (95%) mothers and their answers are presented in Table 1. Fetal movements were perceived by 125 of the 162 mothers (77%), of whom 44 (35%) perceived normal movements and another 44 (35%) abnormal movements. Absent movements were experienced by 31 of the 162 mothers (19%). From the 170 mothers with a child with AMC, 118 (69%) had also a pregnancy of an unaffected child, either before or after the pregnancy of the child affected by AMC, which is depicted in Figure 1. Fetal movements in pregnancies with AMC were less often perceived as present, daily, stable, and normal compared to pregnancies without AMC.

Figure 1. Maternal perception of fetal movements during pregnancies with and without AMC-affected children in 118 mothers of the 170 from the MECA survey in 2024.



The experience based on the order of pregnancy was also evaluated separately in Figure 2. Among the 118 mothers with both affected and non-affected pregnancies, 37 (31%) had the affected pregnancy in their first pregnancy, while 81 (69%) had the affected pregnancy in a subsequent pregnancy. Mothers perceived from a first child with AMC less presence and normalcy of fetal movements than of the subsequent child with AMC.

The influence of the perceived movements (normal or not) on the follow-up ultrasound examinations was evaluated separately in the subgroup with 118 mothers with affected and non-affected pregnancies and in the remaining 52 with only pregnancies with AMC-affected children (Table 2a-b).

Additionally, of 118 mothers, 24 (20%) responded to the question of whether fetal movements were assessed during ultrasound examinations. One (0.8%) mother could not recall. Of the remaining 23, 20 (87%) reported that the healthcare providers looked specifically for fetal movements during ultrasound examination. Among these, four (20%) mothers reported that fetal movements appeared normal.

Figure 2. Maternal experienced fetal movements grouped per first and subsequent pregnancy, MECA survey on 118 mothers having both a child with and without AMC

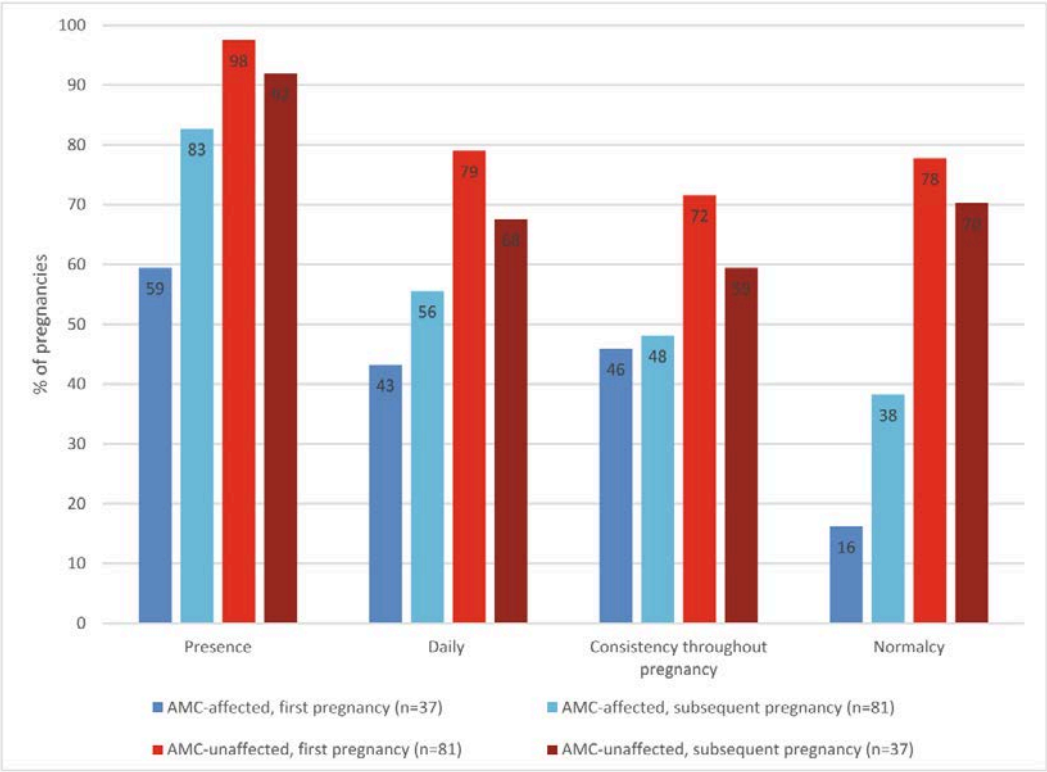


Table 1. Maternal experience of fetal movements of children with AMC from 162 of 170 mothers answering the MECA survey in 2024.

Maternal experienced child Movements in case of AMC	Yes/total number of mothers who answered questions (%)
Presence?	
Yes	125/162 (77)
No	31/ 162 (19)
Do not remember	6/162 (3.7)
Daily movements?	83/125 (66)
Stable until birth?	76/125 (51)
Decreased during pregnancy?	27/124 (22)
Were the movements normal?	
Yes	44/124 (35)
No	44/124 (35)
In case of abnormal movements: why abnormal?*	
Reduced in number	36/44 (82)
In one place only	14/44 (32)
Strength/power reduced	8/44 (18)
None of above	5/44 (11)

*Multiple answers possible

Table 2a. Influence of maternal perceived movements on application of follow-up ultrasound examinations, subgroup of 118 mothers who gave birth to children with and without AMC, MECA survey 2024.

Experienced movements (n)	Extra follow-up ultrasound examination (%)	Prenatal suspicion of AMC (%)	Birth order of AMC pregnancy	
			AMC as first pregnancy (%)	AMC as subsequent pregnancy (%)
Mothers experienced "normal" movement (37)	20 (54)	6 (16)	6 (16)	31 (84)
Mothers experienced abnormal movement (34)	21 (62)	8 (24)	5 (15)	29 (85)
Mothers experienced no movement (23)	NA	4 (17)	21 (91)	2 (9)
Could not compare with other pregnancies (15)	NA	NA	NA	NA
Do not remember (9)	NA	NA	NA	NA

NA=not answered

Table 2b. Influence of maternal perceived movements on application of follow-up ultrasound examinations, subgroup of 52 mothers who gave birth to only child(ren) with AMC (including one mother with two children with AMC), MECA survey 2024.

Experienced movements (n)	Extra follow-up ultrasound examination (%)	Prenatal suspicion of AMC (%)
Mothers experienced "normal" movement (7)	7 (100)	1 (14)
Mothers experienced abnormal movement (10)	9 (90)	4 (40)
Mothers experienced no movement (8)	NA	2 (25)
Could not compare with other pregnancies (18)	NA	NA
Do not remember (18)	NA	NA

NA=not answered

Qualitative results: advice for healthcare providers, other mothers, and patient support groups.

The responses concerning the advice were clustered and presented for all participants in Table 3. Additionally, participants were given the opportunity to share other thoughts or experiences through a separate open-ended question. Representative quotes of this last question are provided in Supplement File 2.

The broad spectrum of perceived movements is illustrated in the group mothers concerning their maternal experience of fetal movements from their child with AMC. One mother reflected on the normalcy: "Truly, my AMC'er moved a lot—more than my first three pregnancies. But after he was born, I realized that what I had felt were all kicks and knees rather than punches." Another mother described the limited perceived movements: "In hindsight, without any point of comparison, I noticed that the movements in my belly were very soft. It felt more like a tickling sensation." A third mother commented on the unusual nature of the movements she perceived: "My AMC'er never 'kicked.' I only felt large movements, like his whole body shifting or rearranging itself inside my belly. I didn't think much of it at the time, as the ultrasounds appeared normal and nothing abnormal was detected."

Several participants highlighted the limited awareness and knowledge of AMC among healthcare providers. One mother shared: "A clubfoot is an indicator of AMC. We knew our son had a clubfoot, but we were never informed that it could be a sign of something more serious." Another parent recounted her son's delayed diagnosis and inadequate treatment postnatally due to the limited knowledge of the healthcare provider: "My son was diagnosed with AMC only about a year after his birth. Until then, we were under the care of a pediatric orthopedist who seemed unfamiliar with the condition and was experimenting with treatments. Casting his legs for several weeks proved counterproductive, as it further restricted his mobility." Another mother who also expressed her concern regarding the limited knowledge on this rare condition: "There are very few doctors who are knowledgeable about AMC."

Table 3. Advice of the participants for health care providers, other mothers of fetuses suspected for AMC and patient support groups, MECA survey 2024.

For who? (number answered)	Clustered advice	Number (%)
Healthcare providers (59)*	• Offer detailed ultrasound examinations, including evaluation of the fetal movements and/or limb position	15 (25)
	• Improve communication and support for parents	16 (27)
	• Understanding of the wide spectrum of AMC	9 (15)
	• Take pregnant women seriously	7 (11)
	• More knowledge and training on AMC	5 (8)
	• Avoiding pressure towards termination of pregnancy	4 (7)
	• More info on postnatal therapy	3 (5)
Other mothers (44)*	• Check your experienced fetal movements over time	14 (32)
	• Insufficient information - insist on more and ask questions	9 (20)
	• Discuss whether movements can be stimulated or benefit of early birth	5 (11)
	• Realize the wide spectrum - don't worry too much	4 (9)
	• Become a member of a patient support group	4 (9)
	• Make your own decision regarding continuation of pregnancy	2 (5)
	• Realize that not all healthcare providers know AMC	1 (2)
	• Other: personal experiences and actions	5 (11)
Patient support group (17)*	• Share info on (other) patient support groups	7 (41)
	• Share info on AMC experts	4 (24)
	• Share info on resources	3 (18)
	• Other: personal experiences and actions	3 (18)

*Multiple answers possible

Discussion

This MECA survey confirmed our hypothesis that most mothers perceived fetal movements during pregnancies with a child affected by AMC. The perception of normal fetal movements should not rule out AMC in fetuses with contractures. By examining multiple aspects of these movements, a deeper understanding was gained of the broad spectrum of perceived movements in case of AMC in the fetus. Notably, maternal perception of fetal movements -regarding presence, daily occurrence, consistency throughout pregnancy, and perceived normalcy -was often present but lower in first pregnancies with a child affected by AMC compared to subsequent AMC pregnancies, suggesting that prior pregnancy experience may influence how fetal movements are interpreted.

Maternal perception of fetal movements

The presence of fetal movements was perceived in nearly eighty percent of pregnancies with AMC versus nearly one hundred percent of non-affected pregnancies. This challenges the common assumption that all mothers of children with AMC perceive abnormal movements during pregnancy. More specifically, among the 170 mothers surveyed, fetal movements were perceived as *normal* by one third, as *abnormal* by another third, and as *decreased* by approximately one fifth. These findings are in line with the two previous mentioned studies, reporting *normal* fetal movements in 20-32%, *abnormal* in 28%, and *decreased* in 31% (3,15).

Our study showed that a fifth perceived *absent* movements, approximately twice the reported percentages in the two latter studies (8-12%) (3,15). Daily and consistent movements were perceived less frequently by the mothers in pregnancies of children with AMC compared to those without AMC. While AMC can affect fetal movement, many mothers still perceive movements, like during their unaffected pregnancies. This suggests that most fetuses with AMC can express spontaneous motility, but the characteristics of these perceived movements may be dependent on the specific joints that are affected and the underlying cause of AMC. The daily felt movements and stable felt movements throughout pregnancy being slightly more reduced in the AMC fetuses can be explained by the variance in development of AMC throughout pregnancy.

Impact of pregnancy order on maternal perception

Mothers did perceive fetal movements, but these were less frequently as present, daily, and normal when the AMC-affected pregnancy was their first, compared to when it was a subsequent pregnancy. These differences suggest that maternal perception of fetal movements might be influenced by the lack of prior pregnancy experience. Despite these patterns, many mothers were able to describe their perception consistently with their fetus affected by AMC, regardless of pregnancy order, as also observed by Lemin *et al.* (15). Another study by Brown *et al*. compared the maternal perception of fetal movements and real-time ultrasound observation of fetal movement in pregnant women above 34 weeks gestational age without suspicion of a congenital abnormality in their fetus (16). This study found no significant relationship between parity and accuracy of fetal movement perception (16). This indicates that healthcare providers should not assume that mothers with previous pregnancies are inherently more accurate in recognizing fetal movements, nor should they dismiss the concerns of first-time mothers for inexperience. Maternal concerns -regardless of parity -should be taken seriously.

Healthcare providers response and follow-up ultrasound examinations

The rate of follow-up ultrasound examinations was only 13 percentage points higher in pregnancies with perceived *abnormal* movements compared to those with *normal* movements (62% versus 54%) in the group of mothers who had AMC-affected and non-AMC affected pregnancies. This relatively small difference indicates that maternal concerns about fetal movement patterns do not consistently lead to additional ultrasound imaging, possibly due to the lack of specific guidelines for evaluating suspected contractures or AMC (19). Conversely, follow-up ultrasound examinations were notably more frequent in pregnancies exclusively affected by AMC. This observed difference may, however, be influenced by the smaller sample size in this group or a recall bias. A cohort study by Tjon *et al*. has demonstrated that the chance to detect AMC is enhanced by serial ultrasound examinations extending the structural anatomical evaluation with assessment of the fetal movements (12). However, no study has ever examined the relationship between ultrasound findings and maternal perception of fetal movements in AMC.

Recommendations to health care providers, mothers, patient support groups.
The worries concerning the knowledge gap on AMC and communication problems are not new. The AMC knowledge gap has been highlighted by many authors, concerning fetuses, youth and adults with AMC (15,17,18). In this study we reached out to healthcare providers specifically concerning the wide spectrum of perceived fetal movements from absence to normal movements in mothers of children with AMC. The mothers were straightforward in their recommendations to other pregnant women with a fetus suspected of AMC and stressed the importance of the role of patient support groups in providing practical, mental and knowledge support. They helped many parents in finding their way to take care of their healthy child with a physical condition.

Strengths and limitations
A major strength of this study is its focus on maternal perception, providing detailed insight into fetal movement perception in pregnancies with and without AMC. While previous studies included only mothers from one center or only English-speaking mothers, we were able to reach out also to Spanish, Dutch and German speaking mothers. This study confirmed prior findings on movement variability and contributed new knowledge regarding the various aspects of the perceived fetal movements. The main limitation is the reliance on self-reported data, which introduces the potential for recall bias. One mother carrying twins was unsure which fetus she felt moving. Additionally, 52 mothers had experienced only pregnancies of children affected by AMC—preventing direct within-subject comparisons. Another limitation is the lack of objective clinical data from medical records, though we collected information to confirm the phenotype of AMC by reporting on presence of multiple contractures in various anatomical regions and levels of independence in activities of daily living. Finally, our study may not adequately sample all categories of AMC since women whose pregnancies ended in perinatal loss or termination would be underrepresented in parent support groups.

Future directions
This study highlights the need for greater awareness of fetal movements in fetuses with AMC. Future research should prospectively investigate the relationship between serial ultrasound examinations and maternal perception of fetal movements in pregnancies with contractures, while also examining how these perceptions correlate with the type or diagnosis of AMC—for example, acknowledging that general fetal movement may not be reduced in distal arthrogryposis forms of AMC.

Given the rarity of AMC, centralized hospital-based care pathways should be developed, with collaboration across centers and patient support groups (20,21). Establishing an international AMC registry integrating medical professionals, patient organizations, and research initiatives will be critical in improving detection, counseling, and long-term outcomes for affected families (22).

Conclusion

This study highlights that most mothers of children with AMC perceive fetal movements despite the contractures and expected common feature of affected motility. Elucidating various regularly evaluated motility aspects -such as presence, daily occurrence, consistency throughout pregnancy, and perceived normalcy- our findings underscore the wide spectrum of maternally perceived fetal activity in pregnancies affected by AMC. Mothers who experienced pregnancies of children with AMC gave the advice to overcome the knowledge gap concerning the rare AMC disorder to ameliorate examinations and counseling. Given the rarity of AMC, it is essential for healthcare providers to have this knowledge on the fetal movements in AMC, to prevent ruling out AMC in case of normal perceived fetal movements in fetuses with contractures.

Supplementary documents

- Supplementary material 1: question list.
- Supplementary material 2: overview of all answers per question.

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CHAPTER 11

English summary

Introduction

Congenital anomalies occur in approximately 2.5% of all newborns. Limb anomalies are among the most common subtypes (about 45 per 10,000 births according to EUROCAT), with a 2:1 ratio between upper and lower limbs. Major defects, such as the absence of an arm or leg due to a reduction defect, are relatively well detected prenatally. However, more subtle anomalies, such as polydactyly (additional finger[s]) or syndactyly (fusion of fingers), have low detection rates (4–19%). As a result, parents can be confronted with a certain limb anomaly after birth. This thesis explores in three parts how ultrasound, genetics, and counseling together can improve the quality of prenatal care in case of suspected limb anomalies.

Part I. Prenatal identification of limb anomalies

Chapter 2 describes a retrospective cohort (2007–2021) including 199 prenatal and 362 postnatal cases of upper limb anomalies. Subtle digital anomalies were often diagnosed after birth, partly because finger assessment is not a standard component of structural ultrasound examinations. Chromosomal or monogenic abnormalities were identified in 76 (38.2%) of the prenatal cases compared with 31 (8.6%) of the postnatal cases. Most isolated anomalies did not have a genetic cause, whereas non-isolated anomalies were more frequently associated with syndromic or genetic conditions. This highlights the difficulty of recognizing finger anomalies prenatally and the particular value of genetic testing in non-isolated anomalies.

Chapter 3 evaluates, in a retrospective study, the impact of the introduction of the 20-week anomaly scan (since 2007) and the 13-week scan (since 2021) on termination rates in 300 prenatally detected upper limb anomalies (2000–2023). Of these, 133 (44.3%) were isolated and 167 (55.7%) were non-isolated. Genetic abnormalities were found in 44% of fetuses and were strongly associated with pregnancy termination. Termination was rare in cases of isolated polydactyly or syndactyly (0–1.6%), more frequent in isolated reduction defects (23%), and much higher in non-isolated anomalies (47–74%).

The introduction of the 13- and 20-week scans led to earlier prenatal detection of upper limb anomalies (median 20.4 to 14.9 weeks) and earlier termination of pregnancy (20.5 to 15.0 weeks). Although overall termination rates remained stable, an increase in the termination rate was observed over time in isolated reduction defects.

Chapter 4 introduces the PRELLIM classification (PREnatal Lower Limb IMpairment), the first systematic prenatal classification specifically developed by a multidisciplinary team for sonographically detectable lower limb anomalies. A literature review confirmed that no prenatal classification system for lower limb anomalies previously existed.

The PRELLIM classification distinguishes between isolated and non-isolated anomalies and further subdivides isolated cases into six subgroups: absent/short, duplication, fusion, contracture, bowing, and other. It hopes to enhance diagnostic consistency, improve interdisciplinary communication, and support prenatal counseling and decision-making.

Part II. Prenatal identification of contractures

Chapter 5 focuses on clubfoot, the most common congenital contracture (prevalence ~1/1000 births). In this population-based cohort of 423 fetuses prenatally diagnosed with isolated clubfoot between 2007 and 2021, 387 represented ongoing pregnancies with a prenatal diagnosis of isolated clubfoot. Among these 387 cases, the diagnosis was confirmed postnatally as truly isolated in 290 (75%), reclassified as non-isolated in 47 (12%) (including both structural and genetic anomalies), refuted in 40 (10%), and in the remaining 10 cases (3%) another type of foot anomaly was observed.

Bilateral cases were more likely than unilateral ones to be reclassified as non-isolated, yet laterality did not influence the genetic yield or false-positive rate. Invasive prenatal testing was performed in 30% of cases, with an overall abnormal result in 8%. Among truly isolated cases, the prenatal genetic diagnostic yield was approximately 7%.

These findings underscore that, despite advances in ultrasound and genetic testing, distinguishing isolated from non-isolated clubfoot prenatally remains difficult. Parents should also be counseled about the possibility of false-positive results and the risk that additional structural anomalies may become apparent later in pregnancy or after birth.

Chapter 6 analyzes genetic testing outcomes in 140 fetuses with isolated clubfoot (63 unilateral, 77 bilateral). Invasive genetic testing was performed in 46% of cases, most commonly using chromosomal microarray (CMA) and exome sequencing (ES). Pathogenic or likely pathogenic variants were identified in 6 of 61 cases (9.8%), in 2 of the 26 unilateral cases (7.7%) and in 4 of the 35 bilateral cases (11.4%). Three incidental findings were also reported. These results show that even apparently isolated clubfoot can harbor a relevant genetic diagnosis, regardless of laterality.

Routine *DMPK* testing, however, was not useful, since no causative *DMPK* variants were found.. These findings underscore the importance of comprehensive genetic counseling in prenatally detected contractures.

Chapter 7 describes a cohort of 64 consecutive fetuses with prenatally suspected and postnatally confirmed arthrogryposis multiplex congenita (AMC) (2007–2021). Most cases belonged to AMC group 3 (n=51), characterized by limb involvement next to central nervous system involvement, with a lethal course, or fetal akinesia deformation sequence (FADS). The remaining 13 cases fell into AMC groups 1+2, with group 1 limited to limb contractures and group 2 including additional anomalies in other organ systems.

All cases underwent genetic testing (from karyogram to whole exome sequencing): prenatally in 88%, postnatally in 56%, and in 44% both pre- and postnatally. The overall diagnostic yield was 28%, rising from 14% in 2007–2011 to 50% in 2017–2021, likely due to the introduction of exome sequencing. Whole exome sequencing had the highest yield (41.7%).

Part III. Arthrogryposis multiplex congenita & pregnancy

Chapter 8 presents a scoping review of 27 publications describing pregnancies in 43 women with AMC. Most cases involved AMC group 2 (musculoskeletal plus other anomalies), followed by group 1 (primary limb involvement) and group 3 (including central nervous system dysfunction and/or intellectual disability).

Details on pregnancy-related outcomes could be depicted from 26 of the 43 women concerning 31 pregnancies. Among these pregnancies, 74% (23/31) had a cesarean section delivery, of which 74% (17/23) were elective. Children were born preterm before week 37 in 7 of 31 pregnancies (22%). A birth weight below the 10th percentile was seen in 6 of the 24 (25%) with a reported birth weight. The course of the pregnancy was uneventful in 16 of the 26 women (62%). Pregnancy had a limited negative influence on AMC stability except for three cases with a transient worsening of lung function. Complications were as expected related to mechanical limitations (such as a narrow pelvis or severe spinal deformity) and respiratory comorbidity. Consequently, most women delivered by planned cesarean section, often for maternal indications. Anesthesiological complications have been reported in association with spinal, airway, and thoracic abnormalities.

Our findings emphasize the importance of a multidisciplinary approach and tailored counseling to assess risks and support parents.

Chapter 9 reports on an international survey of 53 women with AMC from nine countries. Two-thirds had experienced pregnancy; one-third delivered vaginally, while the remainder had (mostly planned) cesarean sections. None of the children were affected by AMC.

Most women reported good quality of life and active participation in work and social activities. However, many experienced gaps in medical support: limited knowledge of AMC among professionals, discontinuity of care, and reluctance to address topics such as sexuality, fertility, and family planning.

Almost all respondents (96%) expressed the wish for a standard preconception consultation from the age of 18 years, preferably with a gynecologist, in a multidisciplinary setting, and for those in a relationship together with their partner. Genetic counseling and practical postpartum support were also considered essential. Peer support and reliable online resources were frequently mentioned as valuable additions.

This study highlights that women with AMC have a desire for parenthood and a positive perspective on it, but they need more tailored, AMC-specific guidance. Implementing preconception consultation and multidisciplinary counseling may improve reproductive care.

Finally, **chapter 10** analyzes maternal perception of fetal movements in AMC pregnancies using the international MECA survey (n=170). The majority of mothers (77%) reported that fetal movements were felt during their pregnancy with an AMC-affected child. This demonstrates that normal fetal movements do not exclude an AMC diagnosis in case of known fetal contractures, while many healthcare believe that normally experienced fetal movements could not be possible in AMC-affected pregnancies.

Conclusion

This thesis demonstrates that while the prenatally identification of limb anomalies and contractures by ultrasound has significantly improved, subtle anomalies remain challenging to recognize. Advances in imaging, comprehensive genetic testing, and structured counseling can strengthen prenatal care for these pregnancies. In addition, specific attention is required for women with AMC who wish to conceive or are pregnant. A multidisciplinary approach is essential to enhance the guidance of future parents and women with AMC.

CHAPTER 12

Dutch summary

Inleiding

Aangeboren afwijkingen komen voor bij ongeveer 2,5% van alle pasgeborenen. Ledemaatafwijkingen behoren tot de meest voorkomende subtypen (ongeveer 45 per 10.000 geboorten volgens EUROCAT), met een verhouding van 2:1 tussen bovenste en onderste ledematen. Grote afwijkingen, zoals het ontbreken van een arm of been door een reductiedefect, worden prenataal relatief goed gezien met echoscopisch onderzoek. Subtielere afwijkingen, zoals polydactylie (extra vinger[s]) of syndactylie (fusie van vingers), worden minder goed opgespoord (detectieratio's 4–19%). Hierdoor worden ouders soms pas na de geboorte geconfronteerd met een ledemaatafwijking. Dit proefschrift onderzoekt in drie delen hoe echoscopisch onderzoek, genetisch onderzoek en counseling gezamenlijk de kwaliteit van prenatale zorg kunnen verbeteren bij een vermoeden van een ledemaatafwijking bij de foetus.

Deel I. Prenatale identificatie van ledemaatafwijkingen

Hoofdstuk 2 beschrijft een retrospectief cohort (2007–2021) met 199 prenatale en 362 postnatale casus van afwijkingen van de bovenste ledematen. Subtiele digitale afwijkingen werden vaak pas na de geboorte vastgesteld, mede omdat beoordeling van de vingers geen standaardonderdeel is van structurele echo-onderzoeken naar aangeboden afwijkingen.

Chromosomale of monogenetische afwijkingen werden gevonden in 76 (38,2%) van de prenatale casussen, vergeleken met 31 (8,6%) van de postnatale casussen. De meeste geïsoleerde afwijkingen hadden geen genetische oorzaak, terwijl niet-geïsoleerde afwijkingen vaker geassocieerd bleken met syndromale of genetische aandoeningen. Dit benadrukt de uitdaging van het prenataal herkennen van vingerafwijkingen en de toegevoegde waarde van genetisch onderzoek bij met name niet-geïsoleerde afwijkingen.

Hoofdstuk 3 beoordeelt in een retrospectieve studie de impact van de introductie van de 20-wekenecho (sinds 2007) en de 13-wekenecho (sinds 2021) op het aantal zwangerschapsbeëindigingen bij 300 prenataal vastgestelde afwijkingen van de bovenste ledematen (2000–2023). Van deze casus waren 133 (44,3%) geïsoleerd en 167 (55,7%) niet-geïsoleerd. Genetische afwijkingen werden gevonden in 44% van de foetussen en er werd dan vaker gekozen voor een zwangerschapsbeëindiging.

Voor een zwangerschapsbeëindiging werd zelfden gekozen in het geval van geïsoleerde polydactylie of syndactylie (0–1,6%), vaker bij geïsoleerde reductiedefecten (23%), en veel vaker bij niet-geïsoleerde afwijkingen (47–74%).

De introductie van de 13- en 20-wekenecho leidde tot een vroegere herkenning van afwijkingen aan de bovenste ledematen in de zwangerschap (mediaan van 20,4 naar 14,9 weken) en tot eerdere keuze voor zwangerschapsbeëindiging (20,5 naar 15,0 weken). Hoewel het aantal totale zwangerschapsbeëindigingen in ratio gelijk bleef, werd er een stijging waargenomen in zwangerschapsbeëindigingen bij geïsoleerde reductiedefecten.

Hoofdstuk 4 introduceert de PRELLIM-classificatie (PREnatal Lower Limb IMpairment), het eerste systematische prenatale classificatiesysteem dat specifiek ontwikkeld is door een multidisciplinair team voor echoscopisch detecteerbare afwijkingen van de onderste ledematen. Een literatuuronderzoek bevestigde dat er eerder geen prenataal classificatiesysteem voor dergelijke afwijkingen bestond.

De PRELLIM-classificatie maakt onderscheid tussen geïsoleerde en niet-geïsoleerde afwijkingen en verdeelt de geïsoleerde groep verder in zes subtypes: afwezig/kort, verdubbeling, fusie, contractuur, kromming en overige. Het doel van de classificatie is diagnostische consistentie te verbeteren, de interdisciplinaire communicatie te versterken en prenatale counseling en besluitvorming te ondersteunen.

Deel II. Prenatale identificatie van contracturen

Hoofdstuk 5 richt zich op klompvoet (talipes equinovarus), de meest voorkomende congenitale contractuur (prevalentie ~1/1000 geboorten). In dit populatie-gebaseerde cohort van 423 foetussen met prenataal gediagnosticeerde geïsoleerde klompvoet tussen 2007 en 2021, betroffen 387 doorlopende zwangerschappen met prenatale geïsoleerde klompvoeten. Van deze 387 casussen werden er 290 (75%) postnataal bevestigd als werkelijk geïsoleerd, werden er 47 (12%) uiteindelijk geclassificeerd als niet-geïsoleerd (inclusief structurele en genetische afwijkingen), waren er 40 (10%) fout-positief (er was een normale stand van de voet (en) na geboorte) en bleek de overige 10 casus (3%) een andere voetafwijking.

Casus met bilaterale klompvoeten hadden een grotere kans op herclassificatie als niet-geïsoleerd dan unilaterale gevallen. Daarentegen had lateraliteit geen invloed op de genetische opbrengst of de fout-positieve ratio. Invasief prenataal onderzoek werd uitgevoerd in 30% van de casussen, met een afwijkende uitslag in 8%. Bij werkelijk geïsoleerde casussen was na herclassificatie de genetische opbrengst ongeveer 7%.

Deze bevindingen onderstrepen dat, ondanks vooruitgang in echoscopie en genetische diagnostiek, het onderscheid tussen geïsoleerde en niet-geïsoleerde klompvoet prenataal moeilijk blijft. Ouders moeten worden voorgelicht over de mogelijkheid van fout-positieve bevindingen en het risico dat bijkomende structurele afwijkingen later in de zwangerschap of na de geboorte pas zichtbaar kunnen worden.

Hoofdstuk 6 analyseert genetische testresultaten van 140 foetussen met geïsoleerde klompvoet (63 unilateraal, 77 bilateraal). Invasief genetisch onderzoek werd verricht in 46% van de casussen, meestal met chromosomale microarray (CMA) en exoomsequencing (ES). Pathogene of waarschijnlijk pathogene varianten werden gevonden in 6 van de 61 casussen (9,8%): in 2 van de 26 unilaterale gevallen (7,7%) en in 4 van de 35 bilaterale gevallen (11,4%). Ook werden drie toevallige bevindingen gerapporteerd, die niet gerelateerd zijn aan de indicatie voor testen. Deze resultaten laten zien dat zelfs op echo schijnbaar geïsoleerde klompvoeten een relevante genetische diagnose kunnen verbergen, ongeacht de lateraliteit.

Routinematig *DMPK*-onderzoek bleek niet zinvol, aangezien er geen oorzakelijke *DMPK*-varianten werden gevonden. Deze resultaten benadrukken het belang van zorgvuldige genetische counseling bij prenataal vastgestelde contracturen.

Hoofdstuk 7 beschrijft een cohort van 64 opeenvolgende foetussen met prenataal vermoede en postnataal bevestigde arthrogryposis multiplex congenita (AMC) (2007–2021). De meeste casussen behoorden tot AMC-groep 3 (n=51), gekenmerkt door ledematenafwijkingen naast centrale zenuwstelselafwijkingen, een lethaal beloop of de foetale akinesie-deformatiesequentie (FADS). De overige 13 casussen behoorden tot AMC-groepen 1+2, waarbij groep 1 beperkt is tot contracturen van de ledematen en groep 2 bijkomende afwijkingen in andere orgaansystemen omvat.

Alle casussen ondergingen genetisch onderzoek (van karyogram tot whole exome sequencing): prenataal in 88%, postnataal in 56% en bij 44% zowel pre- als postnataal. De totale diagnostische opbrengst met alle genetische testen was 28%, met een stijgende trend van 14% in 2007–2011 tot 50% in 2017–2021, waarschijnlijk door de introductie van exoomsequencing. Exoomsequencing had de hoogste opbrengst (41,7%).

Deel III. AMC en zwangerschap

Hoofdstuk 8 presenteert een scoping review van 27 publicaties met beschrijvingen van zwangerschappen bij 43 vrouwen met AMC. De meeste casussen betroffen AMC-groep 2 (musculoskeletaal plus andere afwijkingen), gevolgd door groep 1 (primair ledematen) en groep 3 (centrale neurologische disfunctie en/of verstandelijke beperking).

Zwangerschapsuitkomsten konden worden afgeleid voor 26 van de 43 vrouwen, betreffende 31 zwangerschappen. Van deze zwangerschappen beviel 74% (23/31) per keizersnede, waarvan er 74% (17/23) electief waren. Kinderen werden prematuur geboren vóór 37 weken in 7 van de 31 zwangerschappen (22%). Een geboortegewicht <P10 werd gezien in 6 van de 24 casussen (25%) waarbij het geboortegewicht bekend was. Het zwangerschapsverloop was ongecompliceerd bij 16 van de 26 vrouwen (62%).

Zwangerschap had een beperkte negatieve invloed op de stabiliteit van AMC, behalve bij drie vrouwen met een tijdelijke verslechtering van de longfunctie. Complicaties hielden verband met mechanische beperkingen (zoals verwacht een smal bekken of ernstige wervelkolomafwijkingen) en respiratoire comorbiditeit. Hierdoor bevielen de meeste vrouwen via een geplande keizersnede, vaak op maternale indicatie. Anesthesiologische complicaties zijn beschreven in verband met spinale, luchtweg- en thoracale afwijkingen.

Onze bevindingen benadrukken het belang van een multidisciplinaire aanpak en op maat gemaakte counseling om risico's te beoordelen en ouders te ondersteunen.

Hoofdstuk 9 beschrijft een internationale enquête onder 53 vrouwen met AMC uit negen verschillende landen. Twee derde van deze vrouwen had een zwangerschap doorgemaakt; een derde beviel vaginaal en de rest via (veelal geplande) keizersnede. Geen van de kinderen had AMC.

De meeste vrouwen rapporteerden een goede kwaliteit van leven en actieve deelname aan werk en sociale activiteiten. Wel bleken er hiaten in de medische zorg: beperkte kennis over AMC bij professionals, discontinuïteit in zorg, en terughoudendheid om onderwerpen als seksualiteit, vruchtbaarheid en gezinsplanning te bespreken.

Bijna alle respondenten (96%) gaven aan behoefte te hebben gehad aan een standaard preconceptieconsultatie vanaf 18 jaar, bij voorkeur bij een gynaecoloog, in een multidisciplinaire setting en – voor vrouwen met een partner – samen met die partner. Genetische counseling en praktische postpartumondersteuning

werden eveneens als essentieel beschouwd. Lotgenotencontact en betrouwbare online informatiebronnen werden frequent genoemd als waardevolle aanvullingen.

Deze studie onderstreept dat vrouwen met AMC een duidelijke kinderwens hebben en positief staan tegenover zwangerschap, maar behoefte hebben aan specifiek op AMC toegespitste begeleiding. Implementatie van preconceptiezorg en multidisciplinaire counseling kan de reproductieve zorg verbeteren.

Hoofdstuk 10 analyseert de maternale perceptie van foetale bewegingen in AMC-zwangerschappen aan de hand van de internationale MECA-enquête (n=170). De meerderheid van de moeders (77%) meldde dat zij foetale bewegingen voelden tijdens hun zwangerschap met een kind dat AMC had. Dit toont aan dat normale foetale bewegingen een AMC-diagnose niet uitsluiten in geval van bekende contracturen, terwijl veel zorgverleners denken dat normaal ervaren foetale bewegingen niet horen bij een foetus met verdenking op AMC.

Conclusie

Dit proefschrift toont aan dat hoewel de prenatale identificatie van ledemaatafwijkingen en contracturen door middel van echoscopie aanzienlijk is verbeterd, subtiele afwijkingen moeilijk herkenbaar blijven. Vooruitgang in beeldvorming, uitbreiding van genetisch onderzoek en gestructureerde counseling kunnen de prenatale zorg voor deze zwangerschappen versterken. Daarnaast is specifieke aandacht nodig voor vrouwen met AMC die een kinderwens hebben of zwanger zijn. Een multidisciplinaire aanpak is essentieel om de begeleiding van toekomstige ouders en vrouwen met AMC te optimaliseren.

CHAPTER 13

General discussion & future perspective

The prenatal detection of congenital limb anomalies continues to pose significant challenges. Despite progress in imaging techniques and introduction of new genetic tests, detection limitations persist, not only for distal and subtle anomalies but also more serious anomalies in upper and lower limbs with or without associated features. This thesis explores how prenatal care for fetuses with limb anomalies can be improved through three key pillars: refining the role of prenatal imaging by focusing on timing and technique with knowledge on normal developmental milestones and classification of underlying causes of limb anomalies; integrating genetic testing to increase diagnostic yield and clarifying the potential spectrum of prognosis of a certain anomaly; and enhancing counseling by supporting informed decision-making and addressing the ethical and emotional dimensions of prenatal detection. The three pillars use the information from the various chapters in Part I concerning the extremities, Part II on contractures, and Part III on arthrogryposis multiplex congenita (AMC) and pregnancy. Together, the evaluation of the three key pillars aim to strengthen the diagnostic process, guide reproductive choices, and improve outcomes for both affected children and their families.

1. Role of prenatal imaging

Imaging technique

The role of prenatal imaging was firstly investigated in **chapter 2**, where we evaluated a prenatal cohort of 199 fetuses and a postnatal cohort of 362 children with upper limb anomalies in a period between 2007 and 2021 (1). Sonographically suspected upper limb anomalies were included, such as transverse and longitudinal reduction defects, polydactyly, and syndactyly at our fetal medicine unit. Postnatally, children with the same listed anomalies were evaluated separately, those who were identified after referral to the Congenital Hand Team of Amsterdam UMC (1). Cases were grouped per affected axis (proximodistal, radioulnar or unspecified) according to the Oberg–Manske–Tonkin (OMT) classification for upper limb anomalies (2). We found an estimated overall detection rate of 33.8% and the highest detection rate for transverse reduction defects 57%, then 46% for longitudinal reduction defects, 31% for polydactyly, and the lowest for syndactyly with 17% (1). These percentages are in line with prior studies with overall prenatal detection rates for upper limb anomalies ranging between 22.8% and 42% for the period 1990–2010 (3-11). The prenatal detection of anomalies involving the entire upper limb have higher rates (70%–100%), whereas those limited to the digits show lower sensitivities (4%–19%) (10,11).

Postnatally, we found that 56 out of 71 (78.9%) of reduction defects were only confined to the digits (1). In line, Ruscutti *et al*. reported in a cohort of 188 cases with prenatally or postnatally detected upper limb anomalies examined in the United Kingdom between 2012 and 2023 that, in the majority of cases, anomalies involving the digits were missed prenatally (22). These anomalies were polydactyly, upper limb hypoplasia with clinodactyly and symbrachydactyly, a transverse defect only involving the digits, and a longitudinal ulnar deficiency (22). As current (inter)national guidelines for first (FTAS) and second-trimester anomaly scans (STAS) do not include a specific evaluation of the digits, it is likely that such anomalies, such as polydactyly with or without bone structure and syndactyly, are frequently overlooked during routine fetal structural assessments (12-15,22).

Across the studies included in this thesis, the distinction between isolated and non-isolated anomalies consistently proved to be an informative imaging feature in the prenatal assessment of limb anomalies. Non-isolated cases, defined by the presence of additional structural anomalies, are more often associated with adverse outcomes than isolated cases (e.g., perinatal death) (1,7,11). Prenatal identification of isolated or subtle cases can be considered as more challenging, as we assume that sonographers may tend to be more vigilant and thorough in their assessment when a non-isolated anomaly is already detected, prompting a more detailed evaluation for other abnormalities.

The case description in the introduction was a patient who was reassured after the routine 20-week scan, which showed no abnormalities. However, her child was later born with a congenital transverse reduction defect of the upper limb – several digits were missing from one hand – raising the question whether this anomaly should have been detected prenatally. First, from a developmental point of view, anomalies such as reduction defects, as well as polydactyly and syndactyly, are typically established by the end of the first trimester. Therefore, the transverse limb reduction defect of the hand should theoretically be detectable during the FTAS or STAS with two-dimensional (2D) ultrasound techniques, assuming adequate imaging conditions. Imaging quality of the prenatal ultrasound can be hampered by limitations such as the rising percentages of maternal obesity, oligohydramnios, suboptimal fetal positioning (29). The expansion of three-dimensional (3D) ultrasound techniques or fetal MRI can then sometimes serve as complementary options to optimize visualization (30).

More subtle distal anomalies, such as the isolated digital reductions in the introduction, are more likely to be missed. This may also reflect a knowledge gap among sonographers, who tend to focus primarily on more prominent, serious, and widely recognized anomalies, such as those affecting the fetal heart or brain.

Timing

During an extended period from 2000 to 2023, we examined the gestational age when upper limb anomalies were detected in **chapter 3**. This retrospective cohort study with 300 fetuses showed that the majority of the anomalies (reduction defect, syndactyly, and polydactyly) were detected between a gestational age of 18 and 22 weeks, which is the recommended period for the STAS. However, notable number of anomalies in this study were visualized between 12-15 weeks, which covers the recommended period of the FTAS (12+3 to 14+3 weeks). The introduction of the FTAS in September 2021, which is still in research setting in the Netherlands, have resulted in an earlier detection. This study also showed a decrease of the median gestational age at diagnosis for all limb anomalies together from 20.4 weeks (range: 12.7–29.1) before 2007, to 19.4 (range: 8.3–36.0) between 2007 and August 2021, and to 14.9 (range: 11.0–23.1) after September 2021. These findings highlight the possibility of earlier detection due to an additional anomaly scan at the end of the first trimester.

The period between the late first trimester and the early second trimester is considered as the optimal timing for evaluating the upper limbs, particularly the fetal digits (10). At this stage, the fetal digits are more often extended, providing a more accurate assessment of the digits (10). However, this window is not necessarily ideal for the evaluation of other organ systems: for instance, the fetal brain structures are still developing.

In line with an earlier gestational age at detection, we also demonstrated that the timing of termination of pregnancy (TOP) shifted to an earlier gestational age in this study period. The median gestational age at termination was 20.5 weeks before the introduction of STAS and it decreased to 15.0 weeks after the introduction of the FTAS.

Prenatal phenotype

Whereas for the upper limb anomalies the existing postnatal OMT classification was used to categorize the phenotype of upper limb anomalies, we introduced in **chapter 4** the PREnatal Lower LIMb impairment (PRELLIM) classification for lower limb anomalies (23).

This system focuses on isolated anomalies seen on prenatal ultrasound, with subgroups absent/short (e.g. reduction defect), duplication (e.g. polydactyly), fusion (e.g. syndactyly), but also on contractures (e.g. clubfoot), bowing (e.g. posteromedial bowing), and other anomalies (e.g. lymphangioma, overgrowth). Firstly, our literature review confirmed that no prenatal classification existed for lower limb anomalies. Existing postnatal systems are less suitable for prenatal use, as they largely rely on clinical findings after birth, many of which cannot be visualized on ultrasound and are therefore not applicable in the prenatal setting. Secondly, the application in a cohort of 123 fetuses seen at our fetal medicine unit with lower limb anomalies in a period between 2007 and 2024, showed that the PRELLIM classification is a practical tool that supports consistent categorization and will facilitate a clearer communication in both a clinical and research setting.

Distinguishing between isolated and non-isolated limb anomalies remains a prenatal challenge. **Chapter 5** highlights these difficulties in the context of isolated clubfoot. In this retrospective cohort of 423 fetuses evaluated between 2007 and 2021, an initial diagnosis of isolated clubfoot was reclassified as non-isolated in 20 cases (5%)—10 due to additional anomalies detected on follow-up ultrasound and 10 following abnormal genetic test results. After birth, another 47 cases (12%) were reclassified as non-isolated due to structural or genetic anomalies or developmental delay. In 40 children (10%), the prenatal diagnosis of clubfoot was not confirmed postnatally, and in 10 children (3%) another foot anomaly was diagnosed. These findings align with previous studies and underscore the inherent uncertainty of prenatal diagnosis during mid-gestation.

As development continues in the second half of pregnancy, additional anomalies may appear or earlier findings may resolve, making the diagnosis more reliable after birth (24,25). A useful tool as the PRELLIM classification can help to differentiate between for example the wide spectrum of clubfoot and other associated lower limb anomalies, such as posteromedial bowing.

Although anomalies like limb reductions or polydactyly can be detectable early in pregnancy, the full phenotypic picture of some other conditions, for example AMC, can have an onset throughout the whole course of pregnancy. Knowledge of the AMC classification system, the variability in onset and expression, and the broad range of underlying causes encourages a multidisciplinary approach involving obstetricians, clinical geneticists, physiatrists, orthopedic surgeons, plastic surgeons, neurologists, pediatricians and other specialists.

One widely used clinical framework for AMC is the postnatal classification proposed by Judith Hall, which categorizes AMC into three primary groups based on clinical phenotyping (26). Subsequent classification steps can be based on pre- and postnatal genetic evaluation or postnatal assessments of developmental milestones conducted by medical specialists. Group 1 includes cases with contractures predominantly confined to the extremities, often involving the hands and feet, without other major anomalies (26). Group 2 consists of individuals with joint contractures in combination with other systemic abnormalities, such as craniofacial malformations (26). Group 3 encompasses cases in which the contractures are associated with involvement of the central or peripheral nervous system, including conditions linked to the fetal akinesia deformation sequence (FADS).

In more severe or lethal forms, such as FADS, contractures can worsen or deteriorate to other joints, and can be accompanied by secondary features, such as lung hypoplasia, polyhydramnios and abnormal facial features (26). Therefore, a single-point ultrasound assessment may miss evolving signs, and serial examinations are essential to monitor this phenotypic progression over time (27,28).

Even in case of isolated findings such as a clubfoot or wrist contracture serial ultrasound evaluations are crucial to refining the fetal phenotype over time (27,28). These isolated findings may be the first feature of AMC, with the potential to evolve into more severe forms such as those seen in Groups 2 and 3 (27,28). The progression of contractures and hypo-/akinesia over time increases the suspicion of a Group 3.

2. Role of genetic testing

Genetic testing plays a pivotal role in the diagnostic work-up and counseling when congenital limb anomalies are suspected. The likelihood of an underlying genetic abnormality increases when multiple anomalies are present. This was confirmed in **chapter 2**, where the majority (95-96%) of cases with isolated upper limb anomalies – both prenatally and postnatally – were not associated with a chromosomal or monogenic cause, nor linked to a recognisable syndrome (1). This is consistent with findings in the literature, which show that the diagnostic yield of genetic testing is significantly higher in non-isolated or syndromic cases with limb anomalies (11,22).

In contrast, non-isolated cases more frequently (86%) had an identifiable genetic or syndromic etiology, underscoring the importance of thorough anatomical assessment to guide appropriate genetic evaluation (1). Although the absence of additional anomalies during ultrasound examination reduces the probability of a genetic cause, genetic testing remains appropriate to exclude rare genetic conditions.

The utility of genetic testing was evaluated in case of prenatally suspected isolated clubfoot between 2021 and 2024 in **chapter 6**. Next-Generation Sequence (NGS) tests, including SNV- and CNV-analysis from exome sequencing (ES) data, can be applied as a targeted panel of genes known to be associated with a certain phenotype, or as whole exome sequencing (WES), which analyzes all protein-coding regions of the genome, irrespective of relevance to the detected anomaly. In this retrospective study with 140 cases of isolated clubfoot, we found pathogenic or likely pathogenic causal variants with SNV- and CNV-analysis in 6 out of the 61 (9.8%) tested fetuses. These include 4 SNV variants (6.6%). In addition, there were two CNVs identified (3.3%). Furthermore, there were one variant of uncertain significance (VUS) and three unsolicited findings unrelated to the indication for testing.

To date, three studies have evaluated the genetic diagnostic yield of SNV analysis using ES (SNV-ES) in fetuses with isolated clubfoot. Yu *et al*. reported a 10.5% (4/38) diagnostic yield from SNV-ES, after CNV analysis which was non-diagnostic in 38 cases of isolated clubfoot, which is higher than our finding of 6.6%(43). Huang *et al*. found an even higher yield of 12% of SNVs in 83 cases of clubfoot, with higher detection in non-isolated cases (22.2%) compared to isolated (2/47, 4.3%) (44). Pan *et al*. (2025) reported a 25% yield with SNV-ES in a small cohort of only four isolated cases (45). Together with our results, these studies show that SNV- analysis from ES data has an average added diagnostic value of 7.3% (11/150) in isolated clubfoot.

The comparable number of additional clinically relevant findings highlights the importance of thorough pre-test and post-test counseling when considering ES, which is in line with the statement of the International Society for Prenatal Diagnosis (ISPD) from 2022 (69). For example, in **chapter 6**, we described a case involving compound heterozygous variants in *PIEZO2* (c.570C>T, paternal; c.492+2T>C, maternal). *PIEZO2* is associated with distal arthrogryposis with impaired proprioception and touch (DAIPT), and the fetal phenotype was consistent with this condition (46).

Although these findings strongly suggest a causal relationship, the c.570C>T variant remains classified as a VUS due to the absence of RNA analysis to confirm its predicted splicing effect. The latter should be the driving force to publish in detail the phenotype in cases with VUS. This paves the way for future genotype-phenotype evaluations shifting this VUS into a pathogenic variant.

The diagnostic genetic yield of AMC was also evaluated in 64 prenatal cases with multiple contractures during the period between 2007 and 2021 in **chapter 7** (31). A significant higher yield of 50% during the third period between 2017 and 2021 was found in contrast to the first and second five-year period(31). The latter is achieved by the introduction of NGS based tests as the SNV-ES, including targeted panel-based NGS and broader exome-wide sequencing.

In contrast to isolated clubfoot, the diagnostic yield of genetic testing in cases with AMC has been studied more often, with reported rates ranging from 35 to 73% (32-42). The mean genetic yield of SNV-ES comes closest to the yield (50.3%) that was given in the study of Laquerriere *et al.* (37). Their reported genetic yield was comparable to the 50% observed in our cohort (31). In the cohorts of Bayram *et al*. and Pergande *et al*., however, higher yields were reported, with 58.3% and 73%, respectively (34,39). The differences in genetic diagnostic yield between the included studies, can be due to different sample sizes and different AMC types (see below the comments on chapter 7).

In addition, Laquerriere *et al*. have also demonstrated the added diagnostic value of WES over targeted panel-based NGS testing in a large cohort of 315 families affected by AMC (37). In this study, a genetic diagnosis was established in 68 of 210 families (32%) following targeted panel testing. Among the 142 families without a diagnosis after targeted panel testing, exome-wide sequencing was subsequently performed in 111 cases, resulting in the identification of a causal variant in 24 families (21.6%).

The prenatal phenotypic classification in **chapter 7** was based on detailed ultrasound findings of both structural anomalies and fetal motor assessment (31). Due to prenatal imaging limitations, AMC Groups 1 and 2 were merged into a combined group. Within this cohort, 13 fetuses (20.3%) were classified in AMC Group 1+2 and 51 fetuses (79.7%) as AMC Group 3. The genetic diagnostic yield was 30.8% (4/13) in AMC Group 1+2 and 27.4% (14/51) in AMC Group 3.

The Human Phenotype Ontology (HPO) supports standardized detailed phenotypic annotation facilitating computational genetic analyses (70,71). Notably, AMC-specific phenotypic traits have recently been added to the HPO system, a significant milestone achieved by the international AMC Consortium, marking an important step toward improved integration of phenotypic and genetic data in rare disease diagnostics (66).

3. Role of counseling

Parental decision-making

Once a limb anomaly is identified, involvement of a multidisciplinary team is essential to guide the evaluation for associated anomalies, plan follow-up ultrasound examinations, and determine the need for genetic evaluation. The timely provision of relevant information by healthcare providers is a critical step in empowering parents (5). It enables them to better understand the condition and the expected quality of life of their unborn child. In addition, it supports a greater sense of autonomy during a period often marked by uncertainty and loss of control. For some parents, a prenatal diagnosis provides an opportunity to prepare emotionally and practically for the birth of a child with a congenital anomaly.

Although most congenital limb anomalies do not require immediate postnatal intervention, their diagnosis can significantly influence perinatal care and delivery planning. In the majority of cases, excluding severe forms such as AMC or specifically FADS, there is no strict need for specialized neonatal resuscitation or immediate life-sustaining procedures directly in the postnatal period (1). Therefore, a limb anomaly in itself does not generally dictate the need for delivery in a hospital based on vital risks.

However, timely postnatal evaluation by a multidisciplinary team, including radiologists, geneticists, and orthopedic or plastic surgeons, is often essential (1). Early imaging, such as targeted postnatal radiographs or ultrasound of the limbs, can help refine the diagnosis and support classification of the anomaly, particularly in cases where prenatal imaging was inconclusive or distorted by fetal positioning or technical limitations. In certain conditions (e.g., reduction defects, polydactyly requiring surgical removal, or syndactyly) surgical interventions are typically planned during infancy or early childhood.

For complex limb abnormalities, further (postnatal) genetic testing and longitudinal follow-up may be required to evaluate associated syndromes or developmental delays. In this light, prenatal counseling should include not only a discussion of prognosis and associated findings but also practical information about possible surgeries, timeline of care, and quality of life. This supports both realistic parental expectations and optimal organization of neonatal services.

The severity of the anomaly or the anticipated prognosis may lead to a decision for a TOP, which is legally permitted up to a gestational age of 24 weeks in the Netherlands. In this thesis, we evaluated the TOP rates in case of upper limb isolated and non-isolated reduction defects, syndactyly and polydactyly during the period 2000-2023 in **chapter 3**. Time trend analyses indicated a statistically significant upward trend in TOP rates for only isolated reduction defects since 2000, with a notable increase following the introduction of the STAS and FTAS. In contrast, TOP was rare for isolated syndactyly (0/4) and polydactyly (1/63). These findings suggest that parental decision-making may be influenced by the severity of the condition. TOP rates in isolated reduction defects were similar among cases with a normal (24.1%) and abnormal (25%) genetic outcome. Ruscutti *et al*. showed in their retrospective study on 188 children of which 158 with prenatally detected upper limb anomalies, a genetic anomaly in 23 out of the 66 (35%) terminated pregnancies (22).

Several factors may account for the observed increase in TOP rates for isolated reduction defects in our study. Reduction defects may be perceived by both parents and healthcare providers as being associated with greater functional impairment and psychosocial burden compared to other upper limb anomalies, potentially influencing decisions toward TOP. Societal and cultural factors can also play a significant role in parental choices. Over the last decades, societal attitudes may have evolved toward lower tolerance for congenital anomalies, with increasing emphasis on perceived quality of life (17).

Parental perceptions of life with limb deficiencies may have become less accepted for example due to social media, despite the fact that many upper limb anomalies can be corrected with prostheses and still allow for a good quality of life. Additionally, the emotional burden associated with such decisions on quality of life with the found anomaly, continuation or termination of pregnancy is considerable (17).

Prior research highlights the critical importance of timely, coordinated, and tailored counseling by a multidisciplinary team to guide families through this complex decision-making process (17,20,21). A study conducted in the United Kingdom and Ireland investigated parental experiences upon first learning that their child had a congenital upper limb anomaly (48). Among 261 respondents, the anomaly was suspected prenatally in 41% of cases and identified postnatally in 57%, and in 2% pre- or postnatally not known (48). Notably, 51% of all parents stated they would have preferred to receive information about the diagnosis before birth (48). Among those who received a prenatal diagnosis, 84% reported that they preferred to learn about the anomaly at that time (48). In general, prenatal detection can reduce parental anxiety, enhance preparation for the birth of a child with a congenital condition, and potentially shorten the duration of postnatal hospitalization (49,50).

Understanding AMCs etiology

The broad spectrum and varying etiologies of AMC have been elucidated in **chapter 7**. A common assumption is that fetal movements are always absent or reduced in AMC, leading to the belief of health care professionals that mothers never perceive movement during AMC-affected pregnancies (26). Lack of knowledge on this aspect hampers counseling of the parents. To explore motility in fetuses with AMC in more detail, we retrospectively assessed maternal perception of fetal movements concerning 170 pregnancies with AMC in **chapter 10**. Among these, 118 mothers had both affected and unaffected pregnancies. Most (77%) perceived fetal movements in their AMC-affected pregnancies, though fewer reported them as daily (66%), stable (51%), or normal compared to unaffected pregnancies (44%). These findings challenge the assumption that perceived movement excludes AMC, and emphasize that the presence of fetal movements – despite joint contractures – does not rule out the diagnosis AMC. Given the rarity of AMC, understanding the variability in fetal movement perception is essential for healthcare providers to perform follow-up ultrasound examinations irrespective of the presence of absence of felt movements and for accurate parental counseling in case of contractures. Women from patient support groups who were invited to participate in this survey shared their concern about lack of knowledge on the rare disease their fetus was suspected for.

Beyond childhood: long-term outcomes in adults with AMC

While many studies focus primarily on short-term outcomes in individuals with limb anomalies, such as neonatal and pediatric treatment options, literature on long-term needs and reproductive challenges remains limited. Existing literature shows that most adults with AMC live independently (with or without support), work, are socially active, and that many of them express a wish to have children (52-59).

Women with AMC reached out to us because they could not find reliable information about pregnancy outcomes in adults with AMC. Therefore, we firstly presented a scoping review of 27 manuscripts describing 43 women with AMC who gave birth to in total 82 children in **chapter 8** (60). Among 26 women with available pregnancy outcome, the course of pregnancy was uneventful in 16 cases (62%). AMC remained clinically stable in most women, with only three reporting a transient worsening of lung function. Given the broad phenotypic spectrum and heterogeneous causes of AMC, a multidisciplinary and individualized approach to care is essential. To complement these findings, we conducted an additional questionnaire in **chapter 9** among adult women with AMC, distributed via patient support groups and the Canadian adult AMC registry. Of the 53 participants, 34 (64%) had experienced a pregnancy, resulting in the birth of 45 children. One-third delivered vaginally, and two-thirds by cesarean section. The women were clear in their message: all women with AMC should be offered a pre-pregnancy counseling, ideally after the age of 18, involving both the obstetrician and partner in an outpatient setting. They emphasized the importance to evaluate opportunities and not only obstacles.

Currently, pre-pregnancy counseling is not routinely offered to women with AMC, despite the known challenges these pregnancies may present due to immobility, associated anomalies (such as orofacial abnormalities), and, in some cases, impaired respiratory function(61). This stands in contrast to other chronic medical conditions, such as diabetes, hypertension, and thyroid disease, where there is awareness that optimizing care before and during pregnancy can significantly reduce risks for both mother and child (62,63).

Another important answer of the women with AMC was their wish to have genetic evaluation in case this has been performed too long ago. This knowledge support geneticists to offer this opportunity to retest in case women ask for pre-pregnancy counseling to be informed of the underlying cause for themselves and chances of inheritance for their child.

Concerns were also raised about healthcare professionals' limited knowledge of AMC, lack of continuity in care, and prevailing attitudes toward individuals with disabilities, in line with other studies on individuals with physical disabilities (64,65). These findings highlight the need for tailored counseling for individuals with AMC, beginning in the prenatal period and continuing throughout their life and future pregnancies.

Future Directions

We highly recommend other centers to develop a prenatal care pathway for limb anomalies, tailored to the center's possibilities. A clear stepwise approach can support the multidisciplinary team in planning ultrasound examinations, selecting appropriate genetic testing, and providing tailored counseling: the three key pillars of this thesis.

1. Imaging

To enhance the prenatal detection of limb anomalies, we advocate for a detailed evaluation of the fetal limbs with the FTAS and STAS. In the coming years, prenatal care for congenital limb anomalies is expected to undergo further transformation, driven by advances in imaging, genomics, and counseling. The future role of the FTAS is still evolving. Ongoing evaluations may influence whether this scan secures a permanent place in routine prenatal care in the Netherlands.

A classification such as the PRELLIM should be further evaluated for usefulness and if there are anomalies that may not be adequately represented in the current version. In line with this, a prenatal classification system should also be developed for upper limb anomalies, as the postnatal OMT classification is currently applied. Presently there is a study in progress on clubfeet whether prenatal ultrasound examination can provide further details into the classification system to estimate the severity and identify children who are at higher risk of developing therapy-resistant or recurrent clubfoot. Addressing this knowledge gap is important, as previous studies have shown that approximately 10% of cases result in therapy-resistant or recurrent clubfoot (24).

While ultrasound examinations will remain the cornerstone of early detection, the integration of artificial intelligence (AI) may further enhance prenatal detection by linking phenotypic, genotypic, treatment, and outcome parameters to support parental counseling. However, to realize the full potential of AI, its development must be grounded in high-quality, structured datasets.

Without registries, AI applications will remain limited in utility. For example, national efforts such as the introduction of the Dutch clubfoot registry in 2021 may offer new possibilities as model for the development of more robust data infrastructures. Furthermore, international initiatives such as the emerging AMC registries aim to gather standardized prenatal and postnatal data with the application of HPO terms across the different AMC groups (66). Once sufficiently populated, these registries could offer essential insight into prognosis, therapeutic strategies, recurrence risk, pre-pregnancy counseling, and treatment planning for this heterogeneous disorder. AI may also support the development of open access training for fetal motility assessment in case of contractures to distinguish between isolated contractures and the various forms of AMC.

2. Genetics

From a genetic point of view, we encourage healthcare providers to integrate prenatal phenotype and genotype with postnatal findings and to publish on changes in phenotypic presentation over time in relation to the genetic findings. This combined approach can increase the diagnostic yield of current genetic testing, as prenatal observations may provide early clues that complement postnatal phenotypic evaluation.

The current lack of publications on fetal phenotyping and genetic diagnostic yield has been acknowledged by the HPO organization (47). The restricted knowledge on prenatal age-related phenotype development, results in prenatal testing mainly limited to broad targeted panels, covering only genes known to be associated with a certain phenotype (47,69). Consequently, some diagnoses can be missed prenatally when the performed tests are unable to detect a certain diagnosis (e.g. myotonic dystrophy or spinal muscular atrophy). Therefore, further phenotype and (postnatal) genotype evaluation is essential in cases of unsolved cases, and healthcare providers should inform future parents on these limitations (67).

Despite the limited clinical application of SNV analysis on the entire exome before birth, we do have the opportunity to start research on whole genome sequencing (WGS) with the available stored material during the perinatal period. Such a study, however, can only be undertaken with parental consent and within a rigorous international research framework. While WGS is poised to supplement or eventually replace ES in selected cases, research on genetic WGS yield in people with AMC is still restricted (68). By offering access to both coding and non-coding regions of the genome, WGS may increase diagnostic genetic yield, particularly in complex or previously unexplained cases. However, it also introduces new challenges, including the interpretation of variants of uncertain significance.

In addition, offering DNA storage will facilitate future re-assessment on individual parental request, potentially not only by ES but also by WGS. This approach could be valuable in cases initially classified as “isolated,” such as clubfoot, where the long-term neurodevelopmental outcome is uncertain at the time of diagnosis.

The preservation of biological material enables future genetic testing in cases where developmental concerns arise postnatally. However, routine long-term DNA storage also raises ethical and practical considerations, including environmental impact (e.g., CO_2 footprint of biobanking). An alternative strategy may involve scheduling standardized follow-up with a clinical geneticist several years after birth, such as at five years of age, to reassess outcomes and offer genetic testing if indicated. This may help address the risk of underreporting of neurodevelopmental impairment, which remains a relevant concern in prenatal counseling.

3.Counseling

To optimize counseling, education should not only focus on parents but also on healthcare professionals. Members of the multidisciplinary team need to be aware of the specific challenges in detecting and monitoring limb anomalies. For example, sonographers should be educated about the importance of follow-up scans in both isolated and combined extremity anomalies, and about the added value of carefully assessing fetal movements over time. Increasing this awareness may improve early recognition and more accurate prognostication.

To support shared decision-making, the availability of clear and accessible patient information is crucial. The PRELLIM framework developed in this thesis could serve as a foundation for improving communication. Linking specific diagnoses to example ultrasound images or schematic illustrations may help parents better understand the anomaly. In addition, innovative tools such as 3D printed models of fetal limbs, based on actual ultrasound or MRI data, could enhance parental understanding and engagement. Digital platforms or mobile applications could further support this process by integrating visual examples, background genetic information, treatment options, and outcome data tailored to the individual case. Importantly, all such resources should be co-developed with patients.

By improving the quality and accessibility of counseling and patient information, we can foster a more informed, empathetic, and collaborative prenatal care experience for families facing a diagnosis of limb anomaly. In addition, effective counseling for congenital limb anomalies, including AMC, requires a nuanced, multidisciplinary approach that begins early and extends across the reproductive journey.

Although our survey on wishes concerning pre-pregnancy counseling was performed internationally, the numbers were too limited to examine differences per country. National evaluation will support specific wishes and explore the possibilities which are important to recognize since healthcare experiences can vary depending on system structure, access to services, and cultural expectations. Future research in collaboration with patient support groups should address these differences through large, international registries that integrate clinical and genetic data with patient-reported outcomes. Such registries would allow for meaningful subgroup analyses, for example by AMC subtype, disease severity, healthcare system. Healthcare providers and individuals with limb anomalies should closely collaborate in research and development of care pathways. These collaborations have already led to a care pathway for contractures, but pathways for upper and lower extremities still need to be developed (28).

Conclusion

This thesis helps to understand the current prenatal care in case of congenital limb anomalies by bridging imaging, genetics, and parental counseling in a multidisciplinary setting including the support of patient support groups. It calls for a tailored approach that recognizes both the benefits and limitations of current diagnostic tools. The ultimate goal is not merely (earlier) detection, but a better understanding of developmental aspects of anomalies over time.

The motivation for this thesis is perhaps best illustrated by the story of the mother who, after receiving reassurance following a normal STAS, gave birth to a child with a transverse limb reduction defect. Her experience highlights the limitations of current prenatal screening protocols, which can fail to identify certain anomalies. This thesis underscores a broader issue: how can we refine screening protocols, and are parents adequately informed about both their potential and their limitations? Families confronted with limb anomalies, whether detected prenatally or postnatally, need structured, multidisciplinary approaches that combine fetal imaging, genetic diagnostics, longitudinal phenotyping, and individualized counseling to ensure optimal care and guidance.

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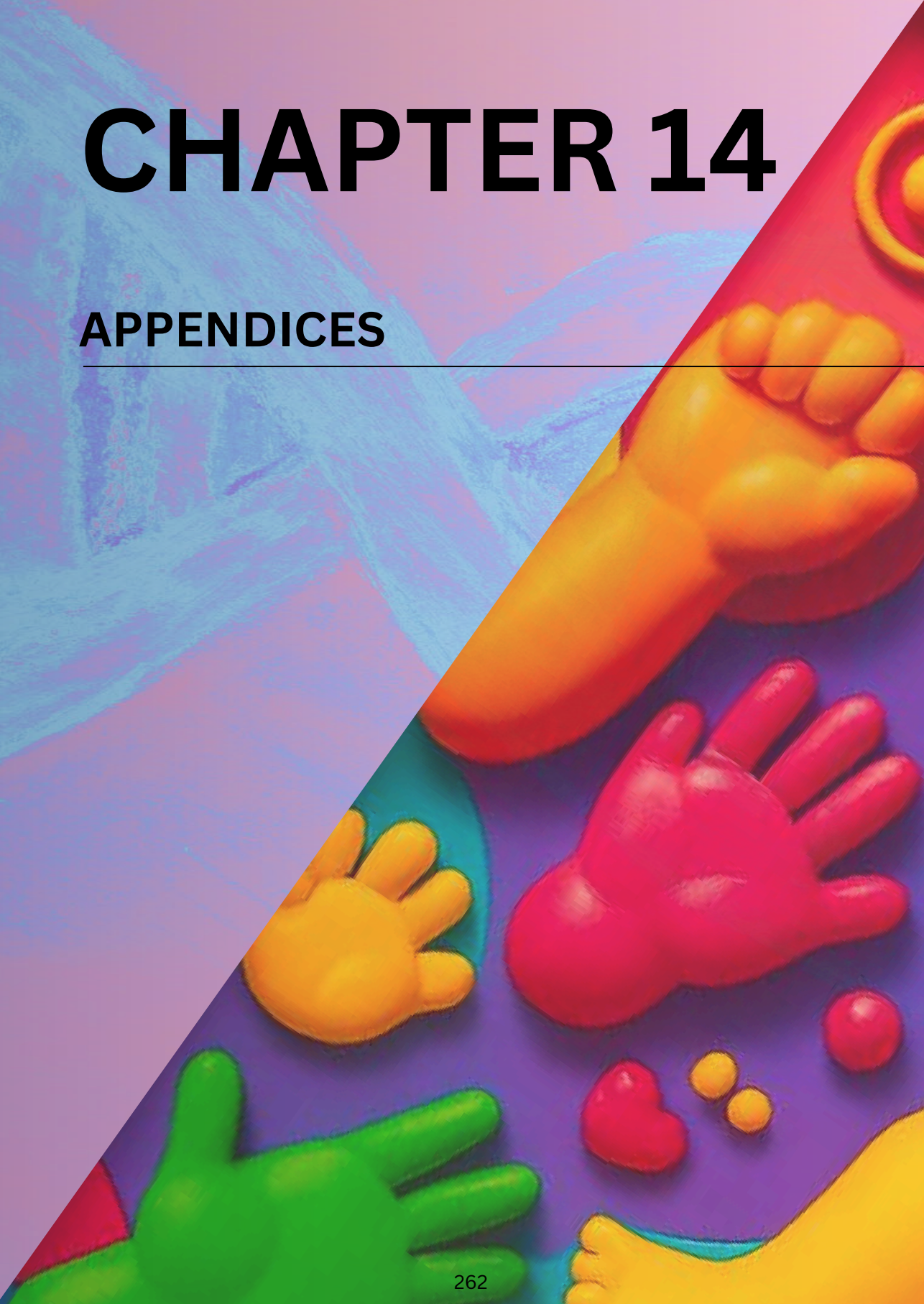
CHAPTER 14

APPENDICES



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PhD portfolio

Courses	ECT	Year
Basic Course Legislation and Organization, eBROK NFU	1,0	2021
Castor EDC Training Beginners, Clinical Research Unit AMC	0,1	2021
Scientific Data Visualization: Tables and Graphs, Amsterdam UMC	0,1	2022
Counseling prenatal screening, SPSAO	1,0	2021
Ultrasound training, second trimester anomaly scan, SPSAO	1,0	2021
Ultrasound training, first trimester anomaly scan, SPSAO	1,0	2023

Seminars, symposia & meetings		
Weekly seminars fetal medicine unit, Amsterdam UMC	6,0	2021-2026
Monthly seminars fetal medicine unit, Amsterdam UMC	2,0	2021-2026
Nationwide seminars with update on prenatal screening, CLBPS	0,1	2021
Quarterly seminars Expertise centrum FADS en AMC	0,1	2021-2026
Regional case discussion prenatal screening, SPSAO	0,5	2021-2026
Research retreat, Amsterdam Reproduction and Development	1,5	2022-2024

(Inter)national conferences		
Research retreat, Amsterdam Reproduction & Development	1,5	2022-2024
24th IGO Doelen conference Rotterdam, the Netherlands	1,0	2023
FMF 20th World Congress Valencia, Spain	1,0	2023
Cursus Prenatale Geneeskunde, Erasmus MC	1,0	2023
4th International Symposium on Arthrogryposis	1,0	2024
ISPD 29th International Conference on Prenatal Diagnosis, Capetown	1,0	2025

Oral presentations		
Negenmaandenbeurs, Amsterdam RAI; genetische diagnostiek bij afwijkingen aan bovenste ledematen	1,0	2023
24th IGO Doelen conference, Rotterdam; echoscopische afwijkingen van de bovenste extremiteiten	1,0	2023
Phd Symposium Jill Tjon, Amsterdam UMC; Serial fetal motor assessment in clubfeet, AMC and FADS: case presentations, Amsterdam UMC	1,0	2023
Cursus Prenatale Geneeskunde, Erasmus MC; Spectrum van onderste extremiteit afwijkingen: van “simpele” polydactylie tot lethale contracturen	1,0	2024
4th International Symposium on Arthrogryposis, Montreal; Knowledge gap AMC & pregnancy. Step 1: literature review	1,0	2024
4th International Symposium on Arthrogryposis, Montreal; Prenatal detection of contractures	1,0	2024
4th International Symposium on Arthrogryposis, Montreal; Genetic diagnostic yield in fetuses with AMC Cohort study, 2007-2021	1,0	2024
4th International Symposium on Arthrogryposis, Montreal; Maternal Experience of movements from a Child with AMC (MECA survey)	1,0	2024
Pre-congress 4th International Symposium on Arthrogryposis, Montreal; Systematic motor assessment in fetuses with single arthrogryposis, AMC and FADS	1,0	2024
ERN-ITHACA Winter School, Paris; Systematic motor assessment in fetuses with AMC and FADS	1,0	2025

Poster presentations		
FMF 20th World Congress Valencia, Spain, Phenotype-to-genotype description of prenatal suspected and postnatal discovered upper limb anomalies: a retrospective cohort study.	0,5	2023
ISPD 29th International Conference, Capetown; A practical prenatal ultrasound classification system for lower limb anomalies –PRELLIM classification.	0,5	2025
ISPD 29th International Conference on Prenatal Diagnosis, Capetown; Genetic analysis in fetuses with isolated clubfoot: diagnostic insights and added value	0,5	2025

Teaching		
Ultrasound for midwives, trainees and residents	0,5	2023
Supervising scientific internship Bachelor of Medicine (2 students)	2,0	2021-2025
Supervising scientific internship Master of Medicine (4 students)	4,0	2021-2025
Lectures at minor foetale echoscopie	2,0	2022-2023
Guest lectures at studentenorganisatie voor Gynaecologie, Obstetrie en Kindergeneeskunde	2,0	2022-2023

Parameter of Esteem		
Prize for best trainee presentation,4th International Symposium on Arthrogryposis, Montreal	0,5	2024

About the author

Arda Arduc was born in 1991 in Schiedam, the Netherlands. After completing grammar school at SG Spieringshoek, he began a bachelor's degree in Biomedical Sciences in 2010. In his second year, in 2012, he was also admitted to the Medicine program. In the years that followed, he successfully completed both bachelor's degrees and subsequently continued with a Master's in Medicine which he completed in 2018. Throughout his medical training, he worked as a nursing assistant at the VUmc, a role that offered him countless clinical experiences and early exposure to Obstetrics and Gynecology.

After completing his Master's degree in Medicine, he began his career as a physician in the Department of Obstetrics and Gynaecology at OLVG West in Amsterdam. In 2021, he began his PhD trajectory at Amsterdam UMC, where his work and research was centered on the prenatal detection of congenital limb anomalies and arthrogryposis multiplex congenita. As part of his PhD, he also worked as a physician-sonographer, further strengthening his clinical expertise in fetal imaging.

Alongside pursuing his PhD, he launched his own cosmetic clinic in 2023 named: ARDA clinic, where he works one to two days a week as a cosmetic doctor, allowing him to combine his medical expertise with aesthetic practice.

In the free time he has left, he enjoys going to the theatre or opera, and he re-energises by windsurfing and being close to nature