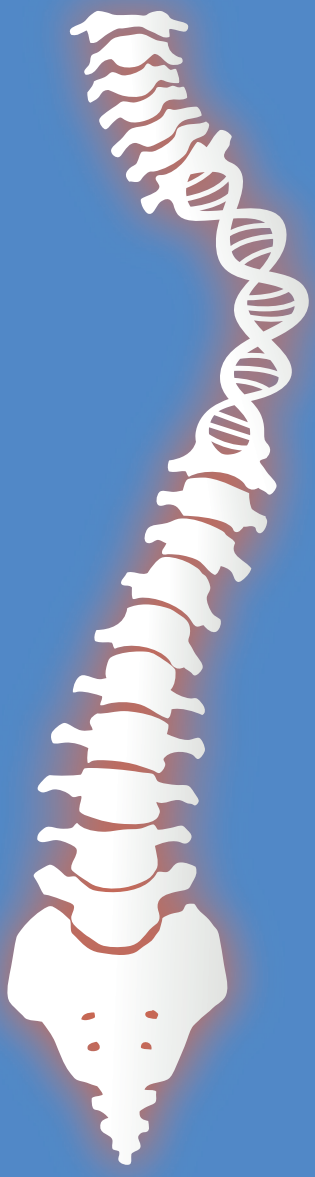
The 22q11.2 Deletion Syndrome as a Model for Idiopathic Scoliosis



Jelle F. Homans

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Jelle Frederik Homans

Colofon

The research in this thesis was financially supported by: K2M research grant - Scoliosis Research Society.

The reproduction of this thesis was supported by: Dutch Spine Society - Nederlandse Orthopaedische Vereniging - Castor EDC - Chipsoft - InSpine Netherlands B.V. - Loopvisie B.V. - Sectra Benelux

ISBN: 978-94-6380-672-5
Layout: Fenna Schaap
Printed by: Proefschriftmaken.nl

The 22q11.2 Deletion Syndrome as a Model for Idiopathic Scoliosis

Het 22q11.2 Deletie Syndroom als Model voor Idiopathische Scoliose
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. H.R.B.M. Kummeling, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 23 januari 2020
des middags te 4.15 uur

door

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geboren op 11 mei 1989 te Hengelo

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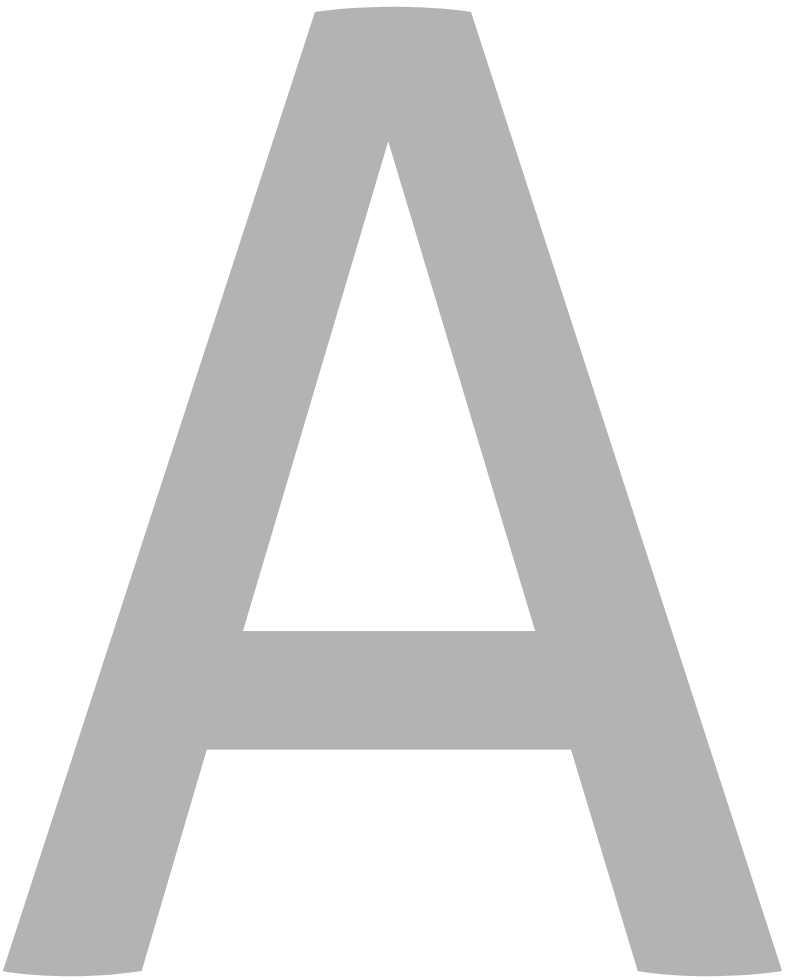
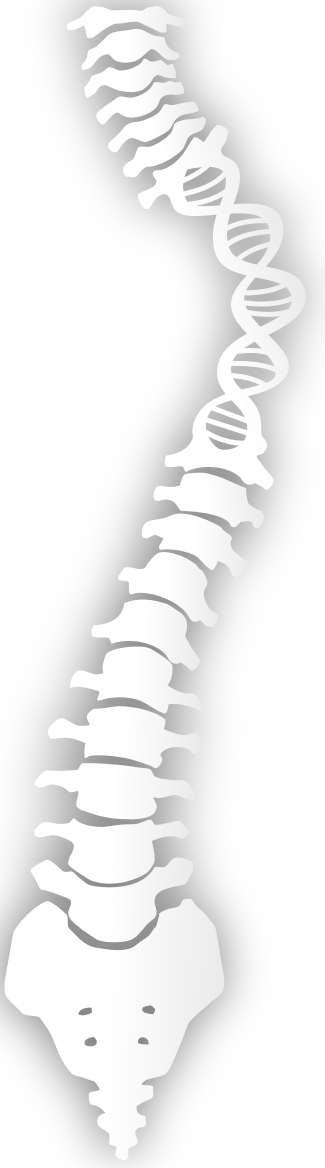
Table of Contents

Chapter 1.	Introduction, Aims and Outline of this Thesis	9
Section A	**Orthopedic Manifestations in the 22q11.2 Deletion Syndrome**	
Chapter 2.	Orthopedic Manifestations within the 22q11.2 Deletion Syndrome: A Systematic Review	21
Chapter 3.	Club Foot in Association with the 22q11.2 Deletion Syndrome: An Observational Study	47
Chapter 4.	Scoliosis in Association with the 22q11.2 Deletion Syndrome: An Observational Study	57
Chapter 5.	The Role of 22q11.2 Deletion Syndrome in the Relationship between Congenital Heart Disease and Scoliosis	69
Section B	**The 22q11.2 Deletion Syndrome as a Model for Idiopathic Scoliosis**	
Chapter 6.	The 22q11.2 Deletion Syndrome as a Model for Idiopathic Scoliosis - A Hypothesis	83
Chapter 7.	The 22q11.2 Deletion Syndrome as a possible Model for Idiopathic Scoliosis	95
Chapter 8.	The Influence of Arm Position during Imaging of the Sagittal Profile of the Spine	109
Chapter 9.	Different Scoliotic Curve Patterns develop based on pre-existent Differences in Sagittal Alignment in Patients with 22q11.2 Deletion Syndrome: A Perspective	121
Section C		
Chapter 10.	Summary, Future Perspectives and Final Conclusions	135
Chapter 11.	References	149
Chapter 12.	Nederlandse Samenvatting	173
Chapter 13.	List of Abbreviations and Definitions	187
Chapter 14.	List of Publications, Manuscripts, Acknowledgements and Curriculum Vitae	191



CHAPTER 1



Introduction, Aims and Outline of this Thesis

Introduction

Scoliosis, a three-dimensional (3-D) deformation of the spine and trunk, is a classic orthopedic disorder (Figure 1).[1,2] The most common type of scoliosis, is referred to as adolescent idiopathic scoliosis (AIS) and occurs in 1-4% of the general population.[2] Many years of dedicated research have been performed into the etio-pathogenesis of AIS, however, until now, not one specific cause was found for this classic orthopedic enigma.[2] Recent research has shed light on the important role of the unique human upright spinal biomechanics on the rotatory stability of the spine.[3–7] Besides idiopathic scoliosis, there are multiple forms of scoliosis that have a known cause; e.g. congenital and neuromuscular scoliosis (Figure 1).

In case of a progressive curve, conservative and/or operative scoliosis treatments may become necessary. If the curve has a Cobb angle of over 20 degrees and a patient has sufficient growth remaining, the indicated treatment is brace therapy.[2] The brace has to be worn during a large part of the day and night. The ultimate goal of brace therapy is to prevent progression and the need for surgery.[8] According to the multicenter study by Weinstein et al. the rate of treatment success is 72% after brace treatment, as compared to 48% with observation only.[8] The question remains if an outcome with a curve of < 50 degrees

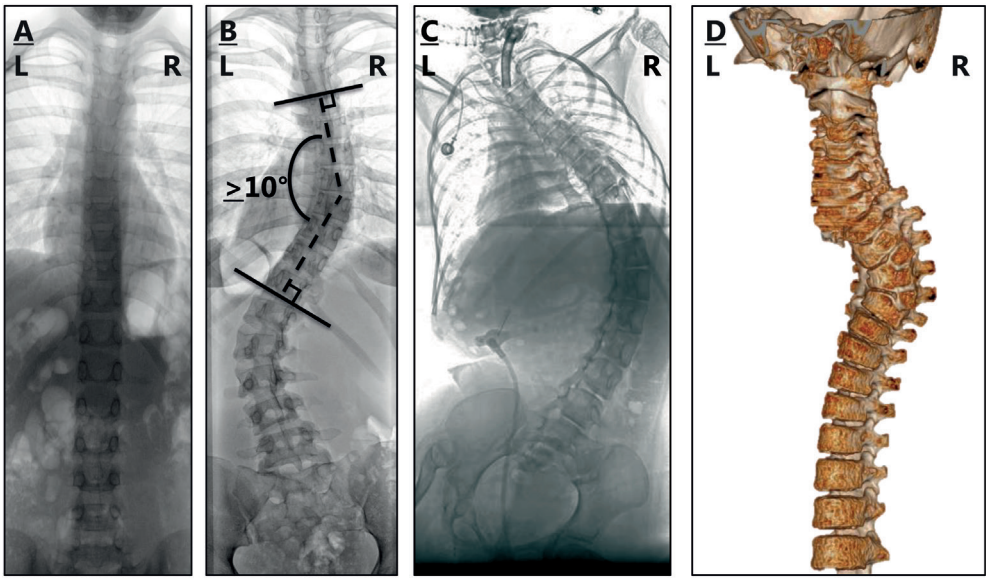


Figure 1: A patient without scoliosis (A) and multiple forms of scoliosis (B-D) are shown.
A: A patient without scoliosis.
B: A patient with adolescent idiopathic scoliosis (S shape, right thoracic, left lumbar). Scoliosis is classically defined as a Cobb angle (the angle between the two most tilted vertebrae) of at least ten degrees.
C: A patient with neuromuscular scoliosis (C-shape, long curve).
D: A patient with congenital scoliosis (multiple congenital anomalies in the upper thoracic region).

should be considered as a success of brace treatment. Although a dose-effect relationship has been established, there is still debate on how many hours per day the brace should be worn, which patients really benefit from it, which type of brace is best, and at what stage of the disease the brace should be prescribed. If a curve exceeds 45-50 degrees, surgery is recommended.[2,9] The primary goal of surgery is to stop progression of the curve. Moreover, secondary goals of surgery are to achieve a maximum correction in 3-D, to improve balance of the trunk and to protect the unfused discs.[10,11] Besides impact to the patient and family, the diagnosis, monitoring and possible treatment of the scoliosis has major (economic) impact to society as well (the average surgical costs including the first three months of follow-up after posterior spinal fusion for AIS are $124,360).[12]

For over a century, dedicated research has been performed in the field of idiopathic scoliosis. This has elucidated the role of genetics, metabolic factors and the Utrecht scoliosis research line has presented evidence that unique biomechanics act on the fully upright posture of man, that make the spine a rotationally unstable construct.[2–4,13–26]

In order to study the development and treatment of a disease, a commonly used approach is to look for models, i.e. study animals with the same phenotype as humans. However, as of this moment there is no mammal known in nature, besides man, that develops an idiopathic scoliosis.[27,28] As a substrate there is a long history in experimental setups that intent to induce a scoliosis in animals.[27] The first known animal study was performed by Von Lesser in 1888. He performed a unilateral dissection of the phrenic nerve, which caused a thoracolumbar scoliosis in rabbits.[29] Many animal models followed and this led to the discovery of specific genes in the development of scoliosis as shown by multiple zebrafish studies.[27,30–33] However, the induced scoliosis models are difficult to translate to humans illustrated by the fact that scoliosis in humans is not caused by one gene, but appears to be the result of a multifactorial pathway (i.e. combination of central nervous system, environmental, genetic, metabolic factors).[2,27]

The Unique Human Upright Posture

Although bipedalism has always (back to the time of the dinosaurs) been a normal variant in nature, human bipedalism differs in a biomechanically very principle sense from all other vertebrates (Figure 2).[3] Humans, as opposed to all other animals, have the ability to stand fully upright without flexion in the hips and/or knees, due to a lordosis that already starts in the pelvis and continues into the lumbar spine.[19,34,35] In the posteriorly (backward) tilted vertebrae there is less rotational stability as compared to anteriorly tilted vertebrae (Figure 2).[3,4,18,20] Thus, the more posteriorly tilted vertebrae (and depending on the mechanical resistance of the stabilizing structures as the discs), the more segments are prone to rotate, i.e. the first step towards a rotational deformity.[3,5,18]

The sagittal shape of the spine develops from a global kyphosis in utero into a double S-shape with a pelvic, lumbar and cervical lordosis.[36] However, how the sagittal shape of the pediatric spine develops is not very well studied or defined.[37,38] In a study by Janssen et al. (2009) it was shown that there are differences in the sagittal spinal alignment between asymptomatic young men and women.[20] Women had more backward tilted vertebrae as compared to men. During the peak of growth spurt, spines of girls are more backwardly tilted as compared to boys, as was shown by Schlösser et al.[6] When comparing the sagittal profile during the early phase of thoracic and lumbar scoliosis (Cobb angle of 10-20 degrees), it was shown that, there was a different sagittal profile of the lumbar scoliosis as compared with the thoracic scoliosis.[7] Moreover, Brink et al. showed that the pelvic incidence (PI), as described by Duval Beaupère, was significantly higher in lumbar than in thoracic scoliosis, and controls.[35,39] These studies indicated that pre-existing differences in spino-pelvic morphology, the sagittal spinal alignment and biomechanical factors are part of the etio-pathogenesis of AIS.

In 1968 Wilson and Jungner published the general principles and practice of screening for a disease on behalf of the World Health Organization. These principles consist of ten criteria, including the following; the disease should be an important health problem, there should be an accepted treatment and natural history should be understood.[40] The burden of disease of

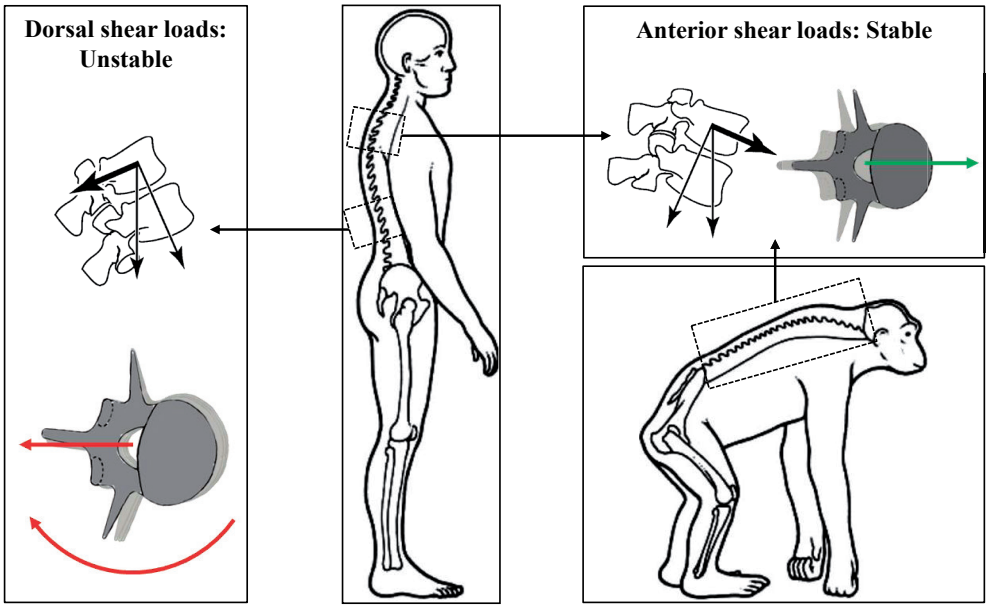


Figure 2: Unique differences between humans and all other animals.
Humans as opposed to other animals have the center of gravity above the pelvis and posteriorly tilted vertebrae. These posterior tilted vertebrae have less rotational stability as compared to anterior tilted vertebrae due to the dorsal shear loads. Image compiled from Castelein et al.[3]

a scoliosis is tremendous because it starts at such a young age and has its effects throughout life as a never ending chronic disease. The ultimate goal in scoliosis care is to be able to perform secondary prevention (prevent disease progression) and, if possible, primary prevention (prevent disease onset). In order to reach these goals, causative mechanisms should be identified and early intervention should be performed. However, since we do not have models for the disease, the patients themselves are the only source of data. One of the major problems in current scoliosis research is that the disorder is only observed in the more established cases, not at- or before- the onset of symptoms. Therefore, it is impossible to determine whether specific factors that are shown to be related to scoliosis are causative, an epiphenomena, or rather a secondary consequence of the scoliosis. As discussed, animal models can be used in order to identify specific factors and/or genes.[27] However, the interaction between the unique human sagittal profile and potential other triggering factors is investigated in man, and etiological factors should be identified before disease onset. There are other fields of medicine that have this same challenge and, especially in the field of schizophrenia research, a new approach has been used: The use of a subset of the population, with a high risk for a disease, as a "model" for the general population.[41] In this thesis it is investigated, whether patients with the 22q11.2 deletion syndrome (22q11.2DS) can be used as such a model to study the development of idiopathic scoliosis in the general population.

The 22q11.2 Deletion Syndrome (22q11.2DS)

22q11.2DS is the most common microdeletion syndrome and occurs in 1:3000-6000 newborns and ±1:1000 fetusus.[42–45] This syndrome, in which patients lack 46 protein coding genes on the 22q11.2 region, is caused by a meiotic error between low copy repeats (LCR's) at the 22nd chromosome (Figure 3, 4). The typical 3 megabase (Mb, ~85% of the patients) 22q11.2 deletion is due to a recombination between LCR22A-LCR22D (Figure 4).[46] Moreover, nested proximal deletions can occur (i.e. LCR22A-LCR22B or LCR22A-LCR22C) in which patients have the major phenotypic characteristics that occur in the typical deletion. If patients have nested distal deletions (i.e. LCR22B-LCR22D or LCR22C-LCR22D), there are less overlapping phenotypic features.[47,48]

One of the challenging factors of this condition is that it is characterized by broad variable phenotypic expression. This is already shown by the fact that until approximately 1990-2000, multiple phenotypical diagnoses (i.e. DiGeorge syndrome, Velo-Cardio-Facial syndrome, Shprinten syndrome) existed for the same genotypic condition (the 22q11.2 deletion).[48] Core features of the syndrome are palatal abnormalities, in particular velopharyngeal insufficiency, congenital heart disease (CHD), primarily conotruncal defects, hypocalcemia as a result of hypoparathyroidism, immunodeficiency, scoliosis and developmental delay with cognitive deficits and behavioral differences such as ADHD, autism, anxiety and psychosis including schizophrenia.[48] Furthermore, in a study by Vergaelen et al. (2017) it was shown that 80% of the adult 22q11.2DS study population had more fatigue as compared to the

general population and more than 90% scored above the mean of physical fatigue score of the general population.[49]
An intriguing factor of schizophrenia in 22q11.2DS is that it has many similarities with idiopathic schizophrenia. This led to founding of the International 22q11.2DS Brain and Behaviour Consortium: The consortium demonstrated that 22q11.2DS can be used as a neurogenetic model for schizophrenia.[41]

Orthopedic manifestations are important factors of 22q11.2DS as well. In 1997, Ming et al. reported that 36% of 108 patients had at least one skeletal anomaly, including scoliosis. Moreover, it was shown that the vast majority of patients with 22q11.2DS has congenital cervical anomalies, that in rare case necessitate cervical spinal fusion due to neurological symptomatology.[50,51] So far, there has been relatively little attention for the orthopedic manifestations in 22q11.2DS, while at the same time, the orthopedic surgeon might be the first medical specialist to see patients with this condition. In order to increase the awareness of the orthopedic involvement in 22q11.2DS, this thesis focuses (Section A) on the different orthopedic manifestations in 22q11.2DS.

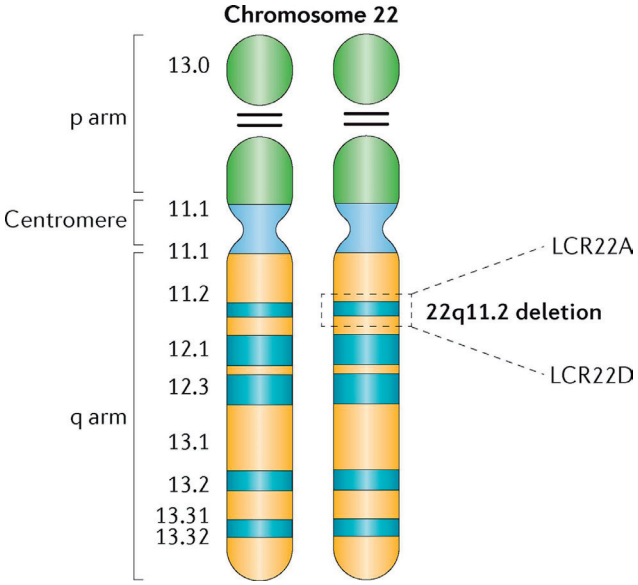


Figure 3: The 22q11.2 deletion
The 22q11.2 deletion occurs on the long arm of one of the chromosomes. The typical, 3 Megabase, deletion occurs between low copy repeats (LCRs) 22A and 22D. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Reviews Disease Primers 22q11.2 deletion syndrome Donna M McDonald-McGinn et al. 2015;1: 15071:1–19.[48]

At the University Medical Center Utrecht (UMCU) and the Children's Hospital of Philadelphia (CHOP), both specialized 22q11.2DS clinics, some patients have been treated for presumed AIS, that later turned out to suffer from the 22q11.2DS and therefore by definition had not AIS but a syndromic scoliosis. However, the scoliosis in those patients strikingly resembled AIS which led to the primary goal of this thesis (Section B), to investigate whether the 22q11.2DS can be used as a model to truly study (certain aspects of) the earliest phases in the development of idiopathic scoliosis in the general population.

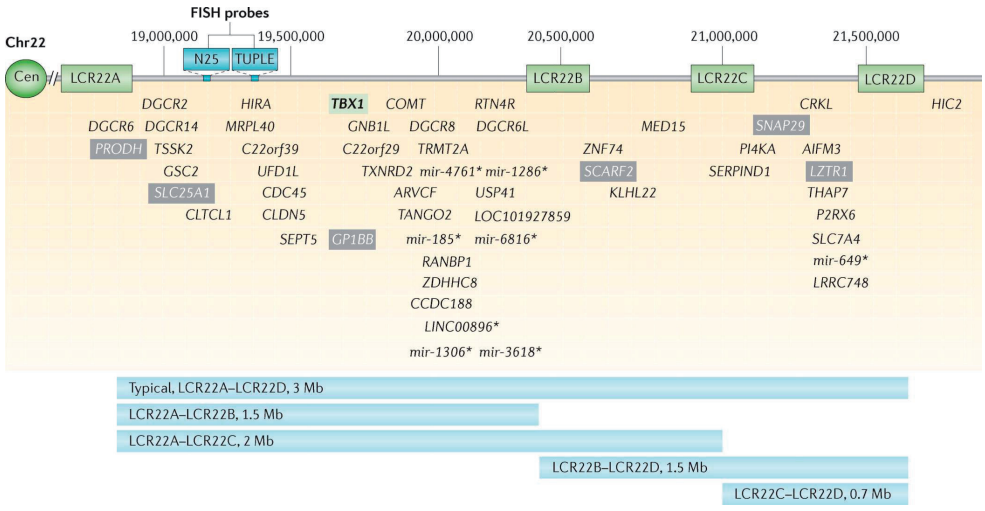


Figure 4: Low copy repeats and genes within the 22q11.2 deletion
A schematic representation of the typical 22q11.2 deletion between low copy repeats (LCRs) 22A and 22D, including the deleted genes. The protein-coding and selected non-coding (*) are depicted at their relative position along the chromosome. Moreover, the proximal and distal nested deletions are shown (LCR22A-LCR22B, LCR22A-LCR22C, LCR22B-LCR22D and LCR22C-LCR22D). The commercial probes for fluoresence *in situ* hybridization (FISH) targets between LCR22A and LCR22B. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Reviews Disease Primers 22q11.2 deletion syndrome Donna M McDonald-McGinn et al. 2015;1: 15071:1–19[48]

Aims and Outline of this Thesis

This thesis is divided into three sections. Section A focuses on different orthopedic manifestations within 22q11.2DS. Section B focuses on the question whether 22q11.2DS can serve as a model for the development of scoliosis in the general population. Section C contains the summary, future perspectives, final conclusions and the Dutch summary.

Section A

Chapter 2. Orthopedic Manifestations within the 22q11.2 Deletion Syndrome: A Systematic Review

Cervical abnormalities occur in nearly all patients with 22q11.2DS.[50,51] However, there is very little known on (other) orthopedic manifestations of 22q11.2DS, while these can have major consequences for a patient, both in terms of early recognition of the syndromic nature of the presenting problems, as well as in terms of early and adequate treatment. The occurrence of orthopedic manifestations in 22q11.2DS is important to recognize, the possible combination with other phenotypical features might lead to the suspicion of 22q11.2DS. Moreover, the possibility of hypocalcemia, bleeding disorders, cervical instability, and psychiatric disease are important features that need to be recognized as part of the syndrome by anyone that is involved in its treatment. Last, once a patient is diagnosed with 22q11.2DS it is important for other caregivers to know which orthopedic manifestations can occur in patients with 22q11.2DS. Therefore, the objective of **chapter 2** is to provide a systematic review on the musculoskeletal manifestations in 22q11.2DS, in order to enhance awareness and improve the orthopedic care for patients with 22q11.2DS.

Chapter 3. Club Foot in Association with the 22q11.2 Deletion Syndrome: An Observational Study

As shown in **chapter 2**, there is a broad range of club foot prevalence (1.1-13.3%) in current 22q11.2DS literature.[52] This is most likely due to the fact that there is no study dedicated to the prevalence of club foot in this condition. Moreover, it is unknown whether club foot is associated with other congenital anomalies in 22q11.2DS, such as congenital heart disease (CHD) and cleft palate. In **chapter 3** we determine the prevalence of club foot in 22q11.2DS and we investigate whether club foot within 22q11.2DS is associated with CHD and/or cleft palate.

Chapter 4. Scoliosis in Association with the 22q11.2 Deletion Syndrome: An Observational Study

In **chapter 2** it was shown that knowledge of the prevalence and clinical characteristics of scoliosis associated with 22q11.2DS is limited and diverse. In earlier reports on patients with 22q11.2DS, in which scoliosis was not the primary outcome, the prevalence was described in a wide range from 0.6% to 60%.[52–56] There are numerous reports on the relation between

CHD and scoliosis in the general population, suggesting that embryologic thoracic anatomy or thoracic surgery in infancy may lead to scoliosis.[57–61] Interestingly, CHD is one of the most common abnormalities in 22q11.2DS (>60%).[48,62] The first aim of **chapter 4** is to determine the prevalence of scoliosis in 22q11.2DS. Second, to investigate whether there is an association between CHD and scoliosis in 22q11.2DS. Last, we investigate the characteristics of scoliosis in 22q11.2DS, we will elaborate on these in **chapter 9**.

Chapter 5. The Role of 22q11.2 Deletion Syndrome in the Relationship between Congenital Heart Disease and Scoliosis

Already for over four decades, a relationship between CHD and scoliosis was suggested, for which several possible mechanisms have been proposed.[63–65] However, in **chapter 4** we could not identify an independent association between CHD and scoliosis in the 22q11.2DS population. Therefore, we hypothesized, that in the previous literature on the relationship between CHD and scoliosis, 22q11.2DS could have been a confounder for both the development of CHD as well as scoliosis. Elaborating on that hypothesis, in **chapter 5**, we determine the prevalence of scoliosis in adult patients with CHD with and without 22q11.2DS.

Section B

Chapter 6. The 22q11.2 Deletion Syndrome as a Model for Idiopathic Scoliosis - A Hypothesis

Based on **chapter 4** in which we showed that 48-49% of the patients with 22q11.2DS has a scoliosis that in most cases resembles idiopathic scoliosis, the general hypothesis was formulated that 22q11.2DS can be used as a model for scoliosis. In **chapter 6** we lay out the path towards investigating this hypothesis.

Chapter 7. The 22q11.2 Deletion Syndrome as a possible Model for Idiopathic Scoliosis

In **chapter 4** it was shown that the majority of patients with 22q11.2DS have a scoliosis that resembles AIS in terms of curve pattern. In **chapter 6** we described which studies need to be performed to test the hypotheses that a (subset) of the patients with 22q11.2DS can be used as a model to study the development of scoliosis in the general population. In **chapter 7** we systematically study whether ambulant, non-congenital, 22q11.2DS scoliotic patients (age >4) have an idiopathic-like curve based on morphology and progression rate. Second, we study whether the intraspinal anomalies are comparable with idiopathic scoliosis.

Chapter 8. The Influence of Arm Position during Imaging of the Sagittal Profile of the Spine

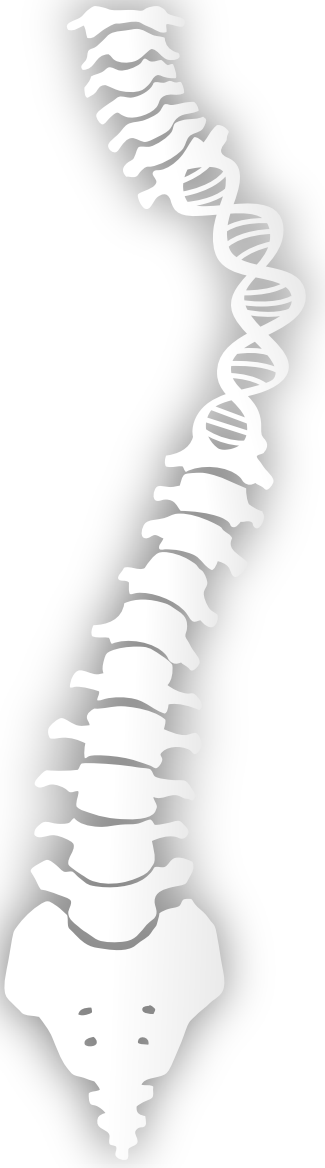
The studies described in **chapter 4** and **chapter 7** were performed in the Children's Hospital of Philadelphia (CHOP) and University Medical Center Utrecht (UMCU). In these two

centers, different arm positions during radiography are used (CHOP: hand-on-wall position, UMCU: hands-on-cheek position). As described in **chapter 1** the sagittal profile is of major importance in relation to the etio-pathogenesis of scoliosis. Therefore, in **chapter 8** we investigate which arm position, that can also be applied in the small gantry of biplanar radiography, is most representative of the natural free-standing position and should be used during acquisition of lateral spinal radiographs in order to better compare the lateral radiographs throughout the world.

Chapter 9. Different Scoliotic Curve Patterns develop based on pre-existent Differences in Sagittal Alignment in Patients with 22q11.2 Deletion Syndrome: A Perspective

In 2014, Schlösser et al. showed that there are differences in the sagittal alignment between thoracic, (thoraco)lumbar scoliosis and controls in patients with a small scoliosis (Cobb angle 10-20 degrees).[7] According to the dorsal shear force theory these differences should be present before the onset of scoliosis.[3] This proof-of-concept compares the sagittal alignment of thoracic, (thoraco)lumbar scoliosis and controls before the onset of scoliosis in the 22q11.2DS population.

CHAPTER 2



Orthopedic Manifestations within the 22q11.2 Deletion Syndrome: A Systematic Review

Homans JF, Tromp IN, Colo D, Schlösser TPC, Kruyt MC, Deeney VFX, Crowley TB, McDonald-McGinn DM, Castelein RM.

Based on: Orthopaedic manifestations within the 22q11.2 Deletion syndrome: A systematic review. *Am J Med Genet A*. 2018;176:2104–2120

Abstract

Purpose: The 22q11.2 Deletion Syndrome (22q11.2DS) is the most common microdeletion syndrome with an estimated prevalence of 1:4000 live births. 22q11.2DS is known to have wide phenotypic variability, including orthopedic manifestations. The purpose of this systematic review is to increase the awareness of orthopedic manifestations associated with 22q11.2DS.

Methods: This systematic review was performed according to the PRISMA Guidelines. Original epidemiological studies on the prevalence of orthopedic manifestations within 22q11.2DS were systematically searched for in PubMed and EMBASE. The included articles were scored according to a risk-of-bias tool, a best-evidence synthesis was performed and the prevalence data was extracted.

Results: 69 published manuscripts described 58 orthopedic manifestations in a total of 6055 patients. The prevalence of at least one cervical or occipital anomaly is 90.5-100% (strong evidence). Fourteen studies (n=2264) revealed moderate evidence for a wide scoliosis prevalence of 0.6-60%. Two studies demonstrated that 5-6.4% of all patients with 22q11.2DS required surgical scoliosis correction. Fifteen studies (n=2115) reported a 1.1-13.3% prevalence of clubfoot with moderate evidence. Other reported orthopedic manifestations are patellar dislocation (10-20%), juvenile idiopathic arthritis (3.8%), impaired growth and skeletal anomalies like polydactyly (1.0-3.7%), syndactyly (11-11.8%), butterfly vertebrae (11.1%) and supernumerary (13) ribs (2-19%).

Conclusions: Orthopedic findings are important manifestations of the 22q11.2DS, both in bringing patients to diagnostic attention and in requiring surveillance and appropriate intervention. Data on these manifestations are scattered and incomprehensive. Routinely screening for cervical anomalies, scoliosis and upper and lower limb malformations is recommended in this vulnerable group of patients.

Introduction

The 22q11.2 deletion syndrome (22q11.2DS) is the most common microdeletion syndrome. The estimated prevalence of 22q11.2DS lies between 1:3000 – 1:6000 live births and more recently has been identified in 1:1000 unselected fetuses.[42,43,45,66] Given the wide variability of the phenotype and the limited knowledge of the condition by medical practitioners, the incidence is probably much higher than previously estimated.[48,62] 22q11.2DS is known to be the most common cause of multiple conditions originally described clinically, e.g. DiGeorge syndrome, velocardiofacial syndrome, conotruncal anomaly face syndrome, Cayler cardiofacial syndrome and a subset of patients with Opitz G/BBB syndrome.[67–72]

Orthopedic manifestations are highly prevalent, with cervical abnormalities identified in almost all patients and scoliosis in up to 60% of patients with 22q11.2DS.[50,56] These manifestations can have significant consequences for counseling and treatment, such as awareness of possible cervical instability, scoliosis surgery and club foot.[51,55] The orthopedic disorders may be overshadowed by the more vital general and psychiatric conditions that are part of the phenotypic heterogeneity of this syndrome.

On the other hand, orthopedic surgeons may be the first specialists to see the patient with 22q11.2DS, should a child for example, present with clubfoot or polydactyly in infancy or with scoliosis in later childhood. Thus, the orthopedic surgeon should be aware of the syndromal nature of the orthopedic problem. Currently an overview of the prevalence and clinical significance of these orthopedic manifestations is lacking. Therefore, the objective of this study is to provide a systematic review on the musculoskeletal manifestations in 22q11.2DS, in order to enhance awareness and improve the care for these patients.

Material and Methods

The systematic review was conducted in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement criteria.[73]

Search strategy and study selection

A search strategy was designed to select studies on 22q11.2DS and orthopedic manifestations. The search was conducted in PubMed and EMBASE on March 1st 2017. Title and abstracts were searched using synonyms of 22q11.2DS, 52 orthopedic terms and phenotype/clinical manifestations. The syntax can be seen in appendix I. Duplicates were removed. Abstracts were checked for relevance by two independent authors (JH and IT) using the predetermined in- and exclusion criteria (Figure 1). Agreement was reached by consensus. Full-text articles were reviewed if inclusion was unclear from title and abstract. Articles in Dutch, English, French, German and Spanish were included and there were no publication date or status restrictions.

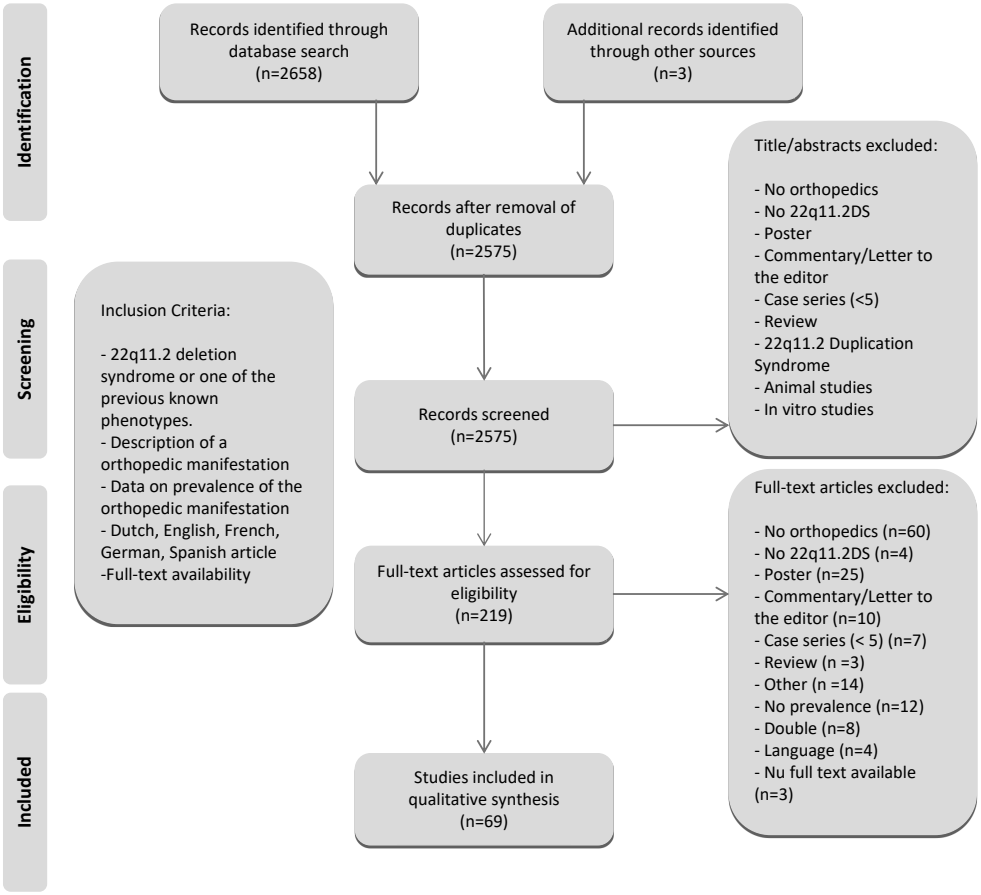


Figure 1: PRISMA flow diagram [Moher et al. 2009][73]

All original studies regarding 22q11.2DS that might possibly discuss orthopedic manifestations were considered for inclusion. Reviews, case reports and case series (< 5 patients) were excluded. The reference sections of all included papers were hand-searched for additional articles relevant for this systematic review.

Assessment of risk-of-bias

For the included studies a risk-of-bias assessment was performed with a critical appraisal that was specifically designed for this systematic review (Table I). This critical appraisal was based on critical appraisal recommendations for observational and prevalence studies.[74,75] It consisted of twelve items and was completed by two authors (JH and IT). The articles were ranked as follows: 0-4 points high risk of bias, 5-8 points moderate risk of bias, 9-12 points low risk of bias.

Table 1: Critical appraisal was performed using a twelve-item scoring list for description and validity of the orthopedic manifestations described in the studies.

Item	Scoring	
Population:		
1. Was the sample representative of the target population?	1 = yes	0 = no or unclear description
2. Was the diagnosis genetically confirmed in all cases?	1 = yes	0 = no or unclear description
3. Were study participants recruited in an appropriate way/was the study approved by the ethical board?	1 = yes	0 = no or unclear description
4. Was there a calculation for an adequate sample size?	1 = yes	0 = no or unclear description
5. Were the study subjects and the setting described in detail?	1 = yes	0 = no or unclear description
6. Was the data analysis conducted with sufficient coverage of the identified sample?	1 = yes	0 = no or unclear description
Outcome:		
7. Are the outcomes assessed based on existing definitions or diagnostic criteria. (objective and validated tools)?	1 = yes	0 = no or unclear description
8. Were all participants screened/diagnosed using validated diagnostics?	1 = yes	0 = no or unclear description
9. Was the condition measured reliably?	1 = yes	0 = no or unclear description
Statistics:		
10. Was there appropriate statistical analysis?	1 = yes	0 = no or unclear description
Confounding:		
11. Are all important confounding factors/subgroups/differences identified and accounted for?	1 = yes	0 = no or unclear description
Conflict of interest:		
12. Was there conflict of interest?	1 = no	0 = yes or unclear description

To avoid duplication bias, all studies were reviewed for potential overlap in study groups (named as double in the flow chart), especially because several studies originated from the same institution. In case of possible duplication bias for studies from the same research group, the study with the lowest risk of bias for that manifestation was included.

Best evidence synthesis

Due to broad heterogeneity in study design, sample size and primary outcome of the different studies, a best-evidence synthesis for each orthopedic manifestation was conducted.[76] The synthesis was based on the risk-of-bias-assessment. The evidence for prevalence of orthopedic manifestations was scored as strong, moderate or weak (Figure 2). The best available level of evidence was given for each orthopedic manifestation. Therefore, studies that had a lower level of evidence were excluded for that specific manifestation. If there was only one study that reported an orthopedic manifestation with high risk-of-bias, this

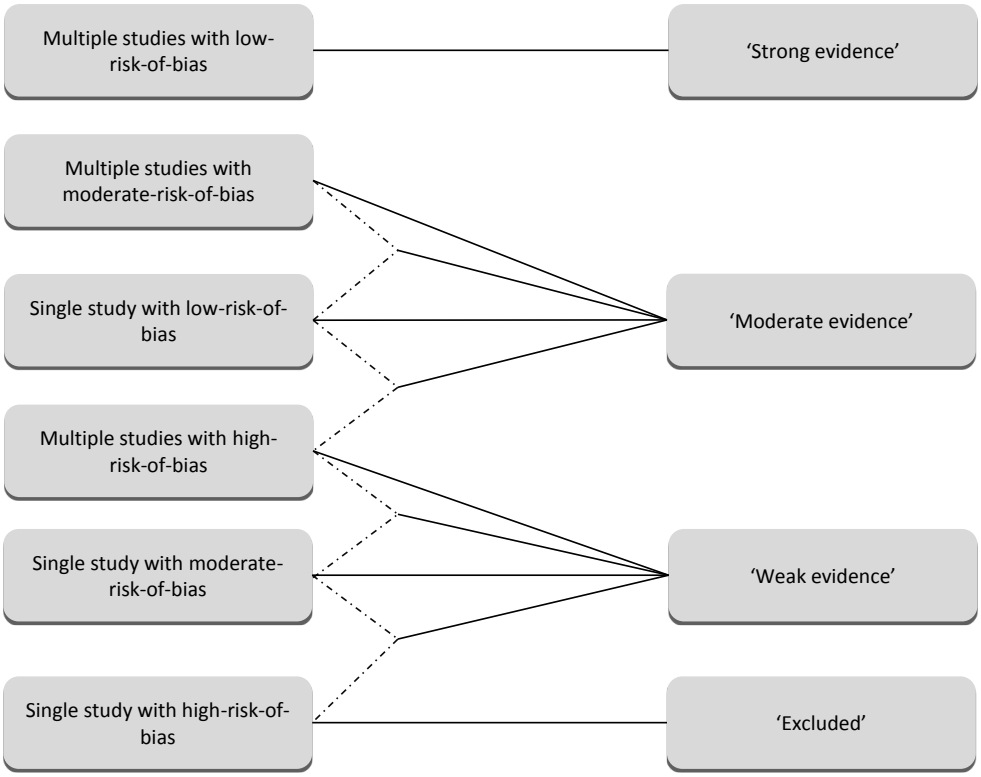


Figure 2: A best-evidence synthesis was performed for each orthopedic manifestation.

orthopedic manifestation was excluded. In table II-IV, the level of evidence and number of studies that report a specific orthopedic manifestation runs from top to bottom.

Data collection and statistical analysis

Data was extracted based on a pre-developed extraction form by one author (JH or IT) and controlled by a second author (JH or IT). Study design, study population, sample size, hospital and orthopedic manifestation were extracted. Orthopedic aspects concerned clinical as well as radiological features. In addition, any data on the treatment, outcome and prognosis of these manifestations was collected. Based on the results the clinical recommendations are summarized in Figure 3.

Results

Study selection and quality assessment

A total of 69 studies, describing 6055 patients, were included. The PRISMA flowchart is shown in Figure 1. All included studies are shown in Appendix II. Three articles were added after hand searching of the reference sections.

The studies consisted of one prospective, eight retrospective, 55 cross-sectional studies and five case series. Eight studies were excluded because of duplication bias issues. The two studies of Ricchetti et al. (2004 and 2008) partially described same groups. However, these papers used different radiographic techniques to screen the same population for cervical spine anomalies, therefore both were included.

Study demographics

Different musculoskeletal manifestations in 22q11.2DS are displayed in Table II-IV. A total of ten manifestations were reported with high level of evidence, 37 with moderate evidence and the remaining with weak evidence. The most common manifestations as well as manifestations with conservative or surgical treatment options are outlined.

Cervical spine

Fifteen studies concerning 500 patients considered the occiput and cervical spine. After best-evidence synthesis ten studies with 408 patients were included. The studies show broad heterogeneity regarding sample size, age of inclusion and risk of bias (Appendix II and Table II).

There was strong evidence that 90.5 to 100% of patients had at least one occipital-cervical anomaly on (advanced) imaging.[50,77] A common feature was platybasia, which is defined as flattening of the base of the skull, with a cranial base angle of more than the usual 136°, as defined by Ricchetti et al.[50] At the first cervical vertebra the most common manifestation was a dysmorphic shape or open arch. [50,51,77,78] Regarding the second cervical vertebra, the most common manifestations were a dysmorphic dens and the so-called C2 “Nike swoosh”. This term is used for an upswept lamina and posterior elements (Figure 4).[50]

A frequently reported anomaly on flexion-extension radiographs is increased segmental motion (56%).[50] In some cases this is accompanied by adjacent level vertebral fusions.[50,77] Ricchetti et al. showed that spinal canal and cord dimensions in the cervical spine were reduced in 22q11.2DS compared to age-matched controls, even in areas without bony anomalies.[51] Clinical consequences of these findings are unclear. One patient described by Ricchetti et al. and a patient described by Boot et al. had neurological symptoms, requiring a high cervical decompression and occipital-cervical spondylodesis which led to resolution of the symptoms.[51,79]

Table 2: Cervical manifestations within the 22q11.2 Deletion Syndrome

Orthopedic manifestation	Number of studies	Strong evidence	Moderate evidence	Weak evidence
At least one cervical/occipital anomaly on X-ray	2	90.5-100%		
Occipital abnormalities				
Platybasia	4	11.5-91.2%		
Basilar impression on CT/MRI	3		2.2%-9.4%	
Basilar impression on X-ray	1		2.5%	
Chiari type 1 malformation on CT/MRI	1			15.8%
Cervical manifestations on X-ray				
Dysmorphic shape C1	3	12.5-75%		
Open posterior arch C1	3	41-66.7%		
Fusion C2-C3 Posterior elements and vertebral body	3	12.7-21.4%		
Dysmorphic dens	2	23.8-58.2%		
'C2 swoosh'	2	43-59%		
Fusion C2-C3 Posterior elements only	2		20-21.1%	
Hypoplastic arch	1		38.1%	
Occipitalization	1		2.5%	
Motion on X-ray				
Increased segmental motion	1		56%	
Occipitoatlantal motion	1		44%	
Atlantoaxial motion	1		10%	
C2-3 motion	1		6%	
C3-4 motion	1		15%	
Cervical manifestations on CT/MRI				
Dysmorphic shape C1	2	12.5-75%		
Open posterior arch C1	2	56.3-66.7%		
'C2 swoosh'	2	31.3-59.4%		
Anterior arch cleft C1	2		64-83%	
Fusion C2-C3 Posterior elements and body	2		9.4-32.1%	
Occipitalization	2		15.6-15.8%	
Subluxation C1	2		5.3-6.3%	
Fusion C1/C3	2		2.2-5.3%	
Open anterior arch C1	1		68.8%	
Dysmorphic dens	1		65.6%	
Fusion C2-C3 Posterior elements	1		18.8%	
Fusion C1/C2	1		15.4%	
Open posterior arch C2	1		6.3%	

Orthopedic manifestation	Number of studies	Strong evidence	Moderate evidence	Weak evidence
C1-C2 anomalies	1			97.7%
Posterior arch cleft C1	1			96%
Defect of posterior arch C1	1			92.5%
Anterior and posterior clefting ring C1	1			88.8%
Neurologic				
Neurological symptoms requiring surgery	2		2.4-12.5%	
Spinal canal encroachment	1		7.4%	
Spinal cord impingement	1		11.1%	
Neurological symptoms	1		3.8%	
Other				
Degenerative disk disease	1			7.7%
Torticollis	1			3.2%

Presently, routine radiographic evaluation of the cervical spine, including flexion and extension in combination with clinical examination is recommended (Figure 3). In the case of neurologic signs and/or symptoms suggestive of spinal canal encroachment or impingement, advanced imaging such as (dynamic) MRI is recommended.[51]

Thoracic/lumbar spine and the ribs

There were 22 studies considering either the thoracolumbar spine or the ribs, with a total of 2763 patients. After best evidence synthesis eighteen studies (2440 patients) of moderate or weak evidence remained.

Scoliosis was described in fourteen studies (2264 patients), with a prevalence of 0.6-60% (Table III). Two studies (Bassett et al. and Liu et al.) included patients from the age of seventeen years. All the other studies included either patients of 0-65 years, or the age range was not described. In none of these studies the type of scoliosis was described. Neither was there one study that described whether there was a relation with the thoracotomy of the patients with 22q11.2DS. Curiously, there was only one study that described scoliosis as primary outcome.[80] This study described 20 patients, three patients (15%) had a scoliosis of which one needed surgery. The patient that required surgery had poliomyelitis during his early childhood.[80] In the other studies, where scoliosis was not the primary outcome, it was often unclear how scoliosis was diagnosed and if this was done systematically.

In 2014 the University Medical Center Utrecht (UMCU) began standardizing radiological evaluation for scoliosis within the 22q11.2DS population. Since 2014, 84 patients with an age range of 6-19 years have been evaluated (mean age: 11.2 years). Of these, 37 (45%) had confirmed scoliosis (Cobb angle > 10 degrees). Importantly, within this cohort, age and

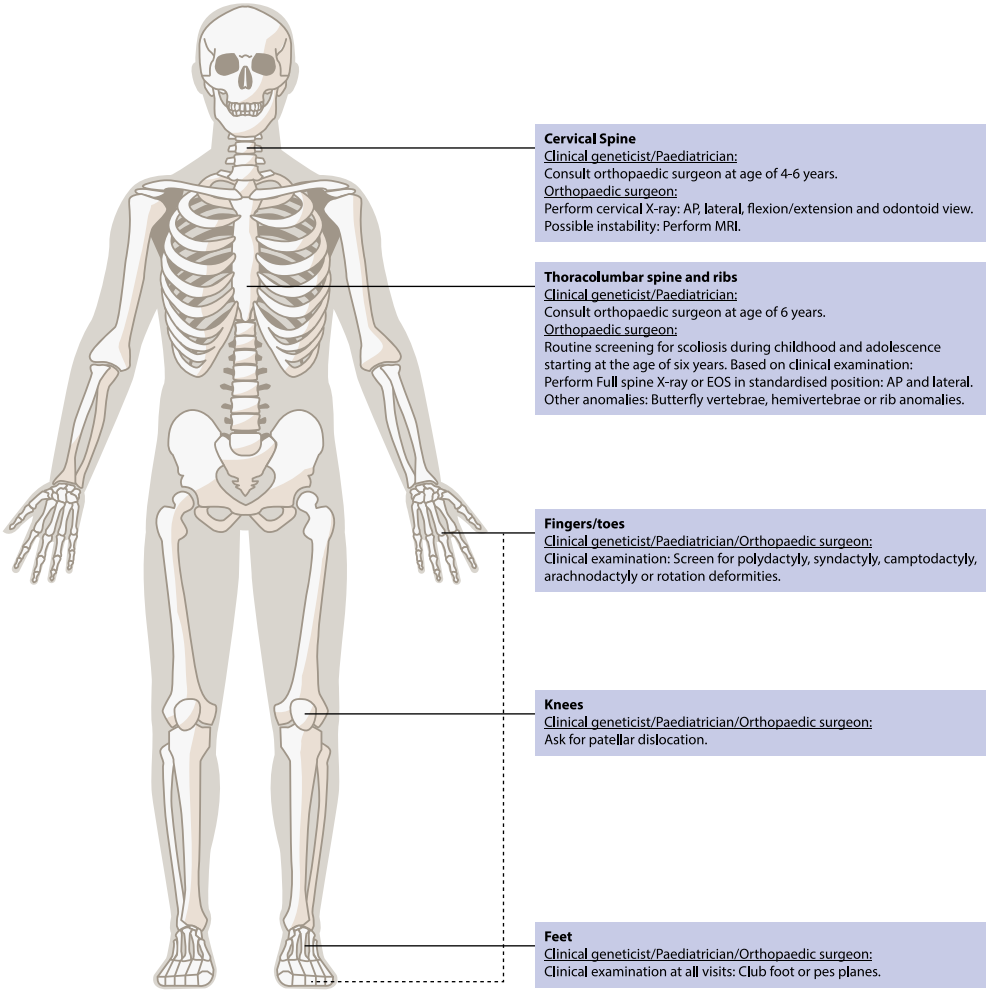


Figure 3: Clinical recommendations for the screening of orthopedic deformities. Abbreviations: AP: Anterior-Posterior, MRI: Magnetic Resonance Imaging

cardiac surgery could not be identified as independent risk factors for the development of scoliosis based on an independent t-test.

Two studies with moderate evidence reported a 5-6.4% prevalence of surgical repair for scoliosis, but no outcome data was provided.[55,80] Other reported anomalies of the axial skeleton are shown in Table III. These include rib anomalies such as 13 ribs and hemi- or butterfly vertebrae.[54]

Table 3: Thoracic/lumbar spine manifestations and rib anomalies

Orthopedic manifestation	Number of studies	Moderate evidence	Weak evidence
Chest/spinal X-ray findings			
Scoliosis	14	0.6-60%	
Scoliosis requiring surgery	2	3-6.4%	
Rib anomalies	2	2-19%	
Abnormal vertebrae	2	1.1-1.5%	
Hemivertebrae	2		1.6-4.7%
Butterfly vertebrae	1		11.1%
Fusion T1-T2	1		11%
Vertebral and/or scoliosis	1		9%
Scoliosis requiring intervention (not defined)	1		5.6%
Coronal cleft	1		1.6%
Spine bone malformation	1		0.6%

Upper and lower limb malformations

Following best-evidence synthesis, out of 33 studies reporting either upper or lower extremity deformities, 26 studies with 2445 patients were included (Table IV). Only the Ming et al. study reported on limb deformities as their primary outcome.[54] The most commonly described extremity manifestation is pes equinovarus (clubfoot). This manifestation was described, with moderate evidence, in fifteen studies (2115 patients), with a prevalence of 1.1-13.3%. Patellar dislocation was present in 10-20% of the patients in weak evidence studies. Many other anomalies were reported, such as polydactyly and overfolded toes, these are listed in Table IV.

Table 4: Orthopedic manifestations within the extremities

Orthopedic manifestation	Number of studies	Moderate evidence	Weak evidence
Upper limb malformations			
Camptodactyly	3	3-20%	
Polydactyly	2	1.0-3.7%	
Syndactyly	2	11-11.8%	
Clinodactyly fifth finger	1		0.9%
Overfolding finger	1		1.1%
Ulnar rotation of digits	1		0.9%
Scapular deformity	1		0.9%
Lower limb malformations			
Pes equinovarus (club foot)	15	1.1-13.3%	
Overfolded toes	4	2.8-36.7%	
Syndactyly	3	1.9-11%	
Foot eversion	3	0.9-2.5%	
Metatarsus adductus	2	0.9-1%	
Patellar dislocation	3		10-20%
Polydactyly	2		0.9-7.1%
Toe contractures	2		0.9%-10%
Clinodactyly	1		11%
Rocker Bottom feet	1		5.6%
Hammertoes	1		2.5%
Lateral deviation great toe	1		0.9%
Unclear upper/lower limb			
Polydactyly	7	1-25%	
Arachnodactyly	2		7.1-35%
Camptodactyly	1		2%
Syndactyly	1		1.1%

Growth

There are 26 studies on growth, with a total of 3114 patients. The recent study of Levy-Shraga et al. (2017) is a low risk-of-bias study consisting of 48 patients. They performed a retrospective study with a total of 384 visits of all patients. The height of patients with 22q11.2DS was plotted on growth charts for the general population and was lower for all ages. Height-SDS of patients with congenital heart disease was significantly lower compared to patients without a congenital heart disease (-1.5 SDS versus -0.6 SDS, p=0.036).[81] There was no correlation between height and weight, palatal disorders or recurrent infections.[81]

Arthritis and other skeletal abnormalities

The association of juvenile idiopathic arthritis with 22q11.2DS has been reported in the literature.[82–84] Two reports were case series where the prevalence could not be extracted. Sullivan et al. reported a 3.75% prevalence of juvenile idiopathic arthritis which is 50-150 times higher than the prevalence of juvenile rheumatoid arthritis in the general population.[85] A noteworthy additional manifestation in patients with 22q11.2DS is bone density. Two case control studies with a low risk of bias report (significant) reduction in bone mass in comparison to healthy controls.[86,87] Stagi et al. demonstrated a bone mineral density z-score of -0.90 ±1.01 vs 0.01 ± 0.87; P<0.001, with an increase in severity in adults. However, they did not find a significant difference for fracture occurrence. A slight bone loss in patients with 22q11.2DS was reported by Ficcadenti et al. (z-score 22q11.2DS: -0.20 ± 0.33; controls 0.67 ± 0.14; P: 0.037).[86] Neither reported osteoporosis.

Discussion

The 22q11.2 Deletion Syndrome provides a challenging condition for many specialists, including the orthopedic surgeon. Due to the complexity of various, sometimes serious, clinical manifestations across all ages, the orthopedic manifestations might be overlooked and thus adequate early, less invasive treatment may be averted. On the other hand, a patient with 22q11.2DS may primarily present with an orthopedic disorder and in that

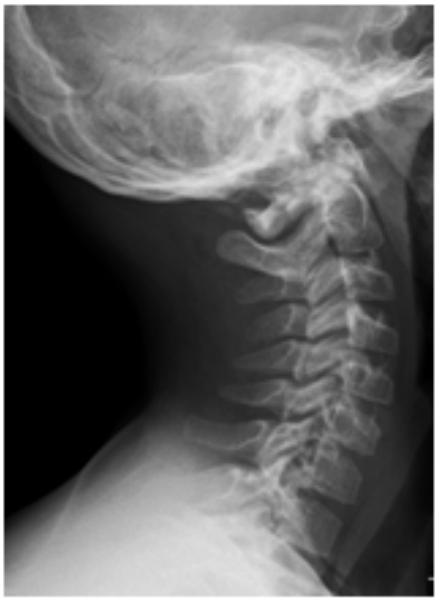


Figure 4: A patient with the 22q11.2 deletion syndrome and diagnosed with a 'C2-swoosh', which is an upswept lamina and posterior elements

case recognition of the syndrome is important because of possible co-existing pathology such as immune compromise, bleeding disorders and heart defects. This systematic review was performed for a better understanding of the orthopedic manifestations associated with 22q11.2DS and contains 69 studies with 6055 patients, describing 58 orthopedic manifestations.

The most frequently reported musculoskeletal manifestations were cervical spine anomalies, scoliosis and club foot. The prevalence of at least one anomaly of the cervical spine, as shown by two studies with strong evidence, is 90.5-100%. The clinical implications of these anomalies remain uncertain.[50] There are a few case reports that show progressive neurology of patients with cervical anomalies. Therefore, we recommend a neurological examination as part of the routine work-up of the patients with 22q11.2DS. If a patient shows neurological symptoms or signs of other features suggestive of spinal cord involvement, MR imaging should be performed at a low threshold. In some cases it is advised to refrain from collision sports. This advice is dependent on the congenital anomaly on the one hand and the age, experience of the athlete, level of participation and desires of the athlete and the parents on the other hand.[88]

Scoliosis is another important spinal deformity, with surgical intervention on 5-6.4% of all patients with 22q11.2DS.[55,80] The evidence of the prevalence on scoliosis within 22q11.2DS is limited. There is only one study that has evaluated this feature specifically (20 patients, prevalence 15%).[80] Furthermore, the study of Bassett et al. 2005 is the only study that describes the prevalence of scoliosis within adults (45%), however scoliosis is not their primary outcome. Within the preliminary results of the UMCU, there is a prevalence of 45% of scoliosis (mean age 11.2 years). Many studies, including the preliminary UMCU results, evaluated 22q11.2DS children before the end of their growth, which leads to an underestimation because most patients will develop scoliosis during their growth spurt, identical to adolescent idiopathic scoliosis.[2,89] The above described prevalence of scoliosis is many times higher than the prevalence of scoliosis within the general population (1-4%).[2] The optimal treatment of scoliosis in these patients could not be derived from this review. Although the curve type may resemble an idiopathic curve, it seems reasonable to regard the scoliosis as syndromal with increased surgical risks like infection, profuse bleeding and cardiopulmonary complications.[90,91]

Scoliosis can occur at any age during spinal growth. Screening at only one age could result in missing the diagnosis at a later stage. Besides, when idiopathic curves are still small, conservative (brace) treatment can prevent surgical procedures in later life.[2,8,92] Therefore, we recommend screening for scoliosis during childhood and adolescence. Based on clinical examination a full spine X-ray or EOS should be performed at a low threshold.

Upper and lower limb deformities were frequently reported, although only the report of Ming et al specifically focuses on these associated features. Based on our in- and exclusion criteria we identified nineteen upper and lower limb deformities, as listed in Table IV. However, due to these criteria we did exclude some of the possible deformities

associated with 22q11.2DS, such as Sprengel Anomaly, which is a uni- or bilateral elevation of the scapula.[93] Therefore, we recommend a full physical examination of all patients with 22q11.2DS, in order to identify possible orthopedic deformities. The most common limb malformations are pes equinovarus (clubfoot) and patellar dislocation.

A majority of the upper and lower limb manifestations might be the result of the increased joint laxity of patients with 22q11.2DS and thus related to the hypotonia of patients with 22q11.2DS. Another possible, genetic, explanation is a mutation in the SCARF2 gene on the non-deleted allele resulting in the presence of the autosomal recessive Van den Ende-Gupta syndrome. Within this autosomal recessive disorder, skeletal manifestations like polydactyly, syndactyly and joint contractures are present.[94] Based on the available literature, no strong statement can be made concerning the best treatment in association with the 22q11.2DS, but it is expected that standard interventions should be successful.

Growth retardation appears to be another key feature of 22q11.2DS. The studies that have growth as a primary outcome describe that the mean length of adults with 22q11.2DS is shorter than compared to the general population and approximately at the 10-20th percentile of the World Health Organization Child Growth Standards.[81,95,96] One of the reasons of growth retardation could be orthopedic (e.g. scoliosis). However, obviously, reduced body length can be secondary to multiple, inherent conditions like chronic (heart) disease, feeding difficulties or growth hormone deficiency.[97] Therefore, if an orthopedic surgeon is the first specialist who sees a patient with growth retardation, he or she should be aware of the possible underlying syndromal nature of this problem. On the other, for the pediatrician it is important to realize, that within 22q11.2DS a scoliosis could contribute to a short stature.

It is important for physicians to be aware of the fact that rheumatoid arthritis is associated with 22q11.2DS.[83] When suspected, early diagnosis and medical treatment could possibly reduce the joint destructive effects of arthritis. The prevalence of arthritis is mostly based on case series and therefore remains unclear.

There are limitations within this systematic review. Of the 69 included studies, only a minority had one of the orthopedic manifestations as their primary outcome. Therefore, in most reports, the methodology was not specifically reviewing orthopedic manifestations. It was often unknown whether the orthopedic outcome was based on existing diagnostic criteria, whether all patients were screened and whether the condition was measured reliably. Elaborating on that, it seems possible that there is an underreporting of the orthopedic manifestations. A second limitation is the variability in study design and lack of systematic follow-up.

Within the included articles there was no study that systematically described the treatment of orthopedic manifestations. This should be one of the main subjects of future orthopedic studies regarding this condition.

We recommend scoliosis screening starting during childhood. Second, we recommend cervical spine X-rays, including flexion/extension X-rays at the age of four-six years. In case of possible instability a MRI of the cervical spine is recommended. Third, there are multiple

(orthopedic) conditions that have to be monitored during birth and growth, such as clubfoot and finger/toe abnormalities. Therefore, we recommend a full physical (orthopedic) examination of all patients with 22q11.2DS. The general recommendations based on this systematic review are shown in Figure 3. Depending on the infrastructure of the hospital this follow-up can be done by an orthopedic surgeon or a pediatrician.

Conclusion

This systematic review attempts to shed light on the broad spectrum of orthopedic manifestations associated with the 22q11.2 Deletion Syndrome. However, a definite answer on the specific prevalence and prognosis of these manifestations cannot be provided. Of the 58 orthopedic manifestations noted within the included articles, the most common is an occipital-cervical spinal anomaly, followed by scoliosis in up to 60% and clubfoot in up to 13% of the patients. However, reports in the literature are fragmented, incomprehensive and large prospective studies are lacking. Further (multicentric) prospective research to focus on orthopedic manifestations that have the prospect of either conservative or surgical treatment options is required. Based on the current literature, we recommend cervical spinal anomaly screening at the age of four to six years and routine scoliosis screening during childhood and adolescence starting at the age of six years.

Supplementary online material

Appendix I syntax

The search was performed on march 1st 2017

Pubmed:

("22q11 Deletion Syndrome"[mh] OR 22q11*[tiab] OR del22q*[tiab] OR DiGeorge[tiab] OR di-george[tiab] OR Velocardiofacial[tiab] OR velo-cardio-facial[tiab] OR VCF-syndrome[tiab] OR (Conotruncal[tiab] AND anomal*[tiab] AND face[tiab]) OR CTAF[tiab] OR "Autosomal dominant Opitz"[tiab] OR "opitz G"[tiab] OR G/BBB[tiab] OR GBBB[tiab] OR "G BBB"[tiab] OR sedlackova[tiab] OR Cayler[tiab] OR catch22[tiab] OR "catch 22"[tiab] OR shprintzen[tiab] OR "thymic aplasia"[tiab]) **AND** ("Spinal Curvatures"[mh] OR spine[mh] OR "guidelines as topic"[mh] OR consensus[mh] OR scolios*[tiab] OR kyphoscol*[tiab] OR scoliotic[tiab] OR cervica*[tiab] OR spine*[tiab] OR spina*[tiab] OR vertebr*[tiab] OR hemivertebr*[tiab] OR Orthopedics[mh] OR "Orthopedic Procedures"[mh] OR "Musculoskeletal System"[mh] OR "Musculoskeletal Development"[mh] OR "Growth and Development"[mh] OR Extremities[mh] OR "arthritis"[mh] OR orthoped*[tiab] OR orthopaed*[tiab] OR musculoskelet*[tiab] OR musculoscelet*[tiab] OR skelet*[tiab] OR skelat*[tiab] OR bone*[tiab] OR joint*[tiab] OR growth*[tiab] OR limb*[tiab] OR extremit*[tiab] OR arthrit*[tiab] OR artrit*[tiab] OR polyarthrit*[tiab] OR arthrosynovitis[tiab] OR shoulder*[tiab] OR elbow*[tiab] OR cubitus[tiab] OR cubiti*[tiab] OR wrist*[tiab] OR hand[tiab] OR hands[tiab] OR finger*[tiab] OR thumb*[tiab] OR digit*[tiab] OR hip[tiab] OR hips[tiab] OR coxa*[tiab] OR knee*[tiab] OR genu*[tiab] OR patella*[tiab] OR ankle*[tiab] OR foot[tiab] OR feet[tiab] OR pes[tiab] OR pedis[tiab] OR phenotyp*[tiab] OR ((clinical[tiab] OR physical*[tiab]) AND (finding*[tiab] OR spectrum[tiab] OR feature*[tiab] OR variable*[tiab] OR variabil*[tiab] OR symptom*[tiab] OR characteristic*[tiab] OR manifestation*[tiab])))

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NOT "case reports"[Publication Type]

Embase:

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OR 'vertebra'/exp OR 'practice guideline'/exp OR 'consensus'/exp OR scolios*:ab,ti OR kyphoscol*:ab,ti OR scoliotic:ab,ti OR cervica*:ab,ti spine*:ab,ti OR spina*:ab,ti OR vertebr*:ab,ti OR hemivertebr*:ab,ti OR orthopedics/exp OR 'orthopedic surgery'/exp OR 'musculoskeletal system'/exp OR body growth/exp OR postnatal growth/exp OR limb/exp OR arthritis/exp OR orthoped*:ab,ti OR orthopaed*:ab,ti OR musculoskelet*:ab,ti OR musculoscelet*:ab,ti OR skelet*:ab,ti OR skelat*:ab,ti OR bone*:ab,ti OR joint*:ab,ti OR growth*:ab,ti OR limb*:ab,ti OR extremit*:ab,ti OR arthrit*:ab,ti OR artrit*:ab,ti OR polyarthrit*:ab,ti OR arthrochondritis:ab,ti OR arthrosynovitis:ab,ti OR shoulder*:ab,ti OR elbow*:ab,ti OR cubitus:ab,ti OR cubiti*:ab,ti OR wrist*:ab,ti OR hand:ab,ti OR hands:ab,ti OR finger*:ab,ti OR thumb*:ab,ti OR digit*:ab,ti OR hip:ab,ti OR hips:ab,ti OR coxa*:ab,ti OR knee*:ab,ti OR genu*:ab,ti OR patella*:ab,ti OR ankle*:ab,ti OR foot:ab,ti OR feet:ab,ti OR pes:ab,ti OR pedis:ab,ti OR phenotyp*:ab,ti OR ((clinical:ab,ti OR physical*:ab,ti) AND (finding*:ab,ti OR spectrum:ab,ti OR feature*:ab,ti OR variable*:ab,ti OR variabil*:ab,ti OR symptom*:ab,ti OR characteristic*:ab,ti OR manifestation*:ab,ti)))

Filters:
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NOT [animals]/lim
NOT 'case report'/de

Appendix II: Result all studies

Abbreviations: d=days, m=month, y=year, N.R.= Not Reported, Ref.= reference

Author, Year of Publication	Ref.	Study design	Sample size	Age of inclusion	Hospital	Orthopedic manifestations	Critical appraisal
[Aglony et al., 2004]	98	Cross-sectional	9	3-6y	Multiple in Chile	clubfoot	4
[Arvystas et al., 1984]	99	Cross-sectional	13	3y6m-11y	Montefiore Medical Center, Bronx	platybasia	5
[Bassett et al., 2005]	55	Cross-sectional	78	>17y	Multiple in Toronto, Canada	degenerative disk disease, scoliosis, scoliosis requiring surgery, patellar dislocation, growth	8
[Boot et al., 2015]	79	Case series	5	30-54y	Multiple: Canada and the Netherlands	basilar impression, occipitocervical spondylodesis, patellar dislocation	3
[Botto et al., 2003]	44	Retrospective cohort	43	0-6y	Multiple in Atlanta	rib anomalies, hemivertabrae, polydactyly of upper limb	4
[Brauner et al., 2003]	100	Cross-sectional	39	2.5-20y	Hôpital Necker Enfants Malades, Paris	clubfoot, growth	8
[Breviere et al., 1999]	101	Cross-sectional	111	0-45y	Hôpital Saint-Antoine, Lille	scoliosis, scoliosis requiring bracing, scoliosis requiring surgery	3
[Cayler et al., 1969]	102	Cross-sectional	14	N.R.	Sutter Cummunity Hospital, Sacramento	polydactyly of lower limb, clubfoot, overfolded toes	3
[Choi et al., 2005]	103	Retrospective cohort	61	0-12y	University of Ulsan College of medicine, Seoul	Polydactyly location not described, growth	8
[Davies et al., 2001]	82	Case series	5	4-22y	Great Ormond Street Hospital for sick children,London Mattel Children's Hospital, Los Angeles	fusion posterior elements C2-C3, polyarticular arthritis, extended oligoarticular arthritis	6
[Derbent et al., 2003]	104	Cross-sectional	30	1m-9y	Baskent University Faculty of Medicine, Ankara	platybasia, fusion vertebral body C2-C3, fusion vertebral body T1-T2, camptodactyly of the upper limb, syndactyly of upper limb, overfolded toes, syndactyly of lower limb, clinodactyly of lower limb	6
[Digilio et al., 2003]	56	Cross-sectional	132	N.R.	Bambino Gesù Hospital, Rome	scoliosis, clubfoot	5
[Digilio et al., 2001]	105	Cross-sectional	73	0.3-16.3y	Bambino Gesù Hospital, Rome	growth	7

Author, Year of Publication	Ref.	Study design	Sample size	Age of inclusion	Hospital	Orthopedic manifestations	Critical appraisal
[Ficcadenti et al., 2015]	86	Cross-sectional	8	7-16.4y	Salesi Hospital, Ancona	Bone density, growth	9
[Fomin et al., 2010]	106	Cross-sectional	14	8m-18y11m	Hospital das Clínicas of Universidade, São Paulo	clubfoot	7
[Friedman et al., 2016]	84	Cross-sectional	29	0-57y	Sheba Medical Center, Tel Hashomer	patellar dislocation, arthritis	4
[Gaspar et al., 1999]	107	Cross-sectional	12	0-45y	Egas Moniz Hospital, Lisboa	scoliosis	6
[Goldberg et al., 1993]	108	Cross-sectional	75	N.R.	Montefiore Medical Center, Bronx	scoliosis, growth	5
[Grassi et al., 2014]	109	Cross-sectional	60	14d-20y3m	Instituto da Crianca, HC-FMUSP, São Paulo	clubfoot	7
[Guzman et al., 2012]	95	Retrospective cohort	239	0-18y	Multiple in Chile	growth	8
[Habel et al., 2012]	96	Retrospective cohort	818	0-37y	The Children's Hospital of Philadelphia, Great Ormond Street Hospital for Children, London	growth	7
[Hamidi et al., 2014]	78	Cross-sectional	16	<18y	Children's Hospital Lonson Health Sciences Centre, Ontario	platybasia, open posterior arch C1, fusion vertebral body C2-C3, dysmorphic C1, anterior arch cleft of C1, fusion C1-C2, upswept C2 lamina	9
[Havkin et al., 2000]	110	Cross-sectional	36	3-14y	Upstate Medical University, New York	platybasia	7
[Hultman et al., 2000]	111	Cross-sectional	41	0.5-15.2y	Scottish Rite Children's Medical Center, Atlanta	chiari type malformation, C1 variations, occipitalization C1, open posterior arch C1, subluxation C1, fusion C1/C3	7
[Kitsiou-Tzeli et al., 2004]	112	Cross-sectional	17	10d-15y	Multiple in Greece	arachnodactyly of upper limb, growth	5

Author, Year of Publication	Ref.	Study design	Sample size	Age of inclusion	Hospital	Orthopedic manifestations	Critical appraisal
[Konen et al., 2008]	[113]	Cross-sectional	76	4-17y	The Hospital for Sick Children, Toronto	C1-C2 anomalies, basilar impression, anterior arch cleft C1, defects of posterior arch C1, posterior arch cleft C1, anterior and posterior clefting C1 ring5, fusion, C1/C3, upswept posterior element C2, fusion posterior elements and body C2-C3	7
[Kyburz et al., 2008]	[114]	Retrospective cohort	49	N.R.	University Children's Hospital Zurich	growth	6
[Lee et al., 2013]	[115]	Cross-sectional	190	0.34-32y	Ajou University Medical Center, Suwon	torticollis	6
[Levy-Mozziconacci et al., 1996]	[116]	Cross-sectional	49	0-15y	Hôpital d' enfants de la Timone, Marseille	polydactyly location not described, camptodactyly location not described, overfolded toes	5
[Levy-Mozziconacci et al., 1994]	[117]	Cross-sectional	7	0-5y	Hôpital d' enfants de la Timone, Marseille	growth	3
[Levy-Shraga et al., 2017]	[81]	Retrospective cohort	48	0-9.8y	Sheba Medical Center, Ramat gan	growth	9
[Lima et al., 2010]	[118]	Cross-sectional	60	1-54y	Rikshospitalet, Oslo	scoliosis, clubfoot, overfolded toes, polydactyly location not described	7
[Lipson et al. 1991]	[119]	Cross-sectional	38	9m-30y	The children's hospital, Sydney	scoliosis, talipes, vertical talus, growth	4
[Liu et al., 2014]	[120]	Cross-sectional	18	18-46y	Queen Mary hospital, Hong Kong	scoliosis, rocker bottom feet	6
[Matsuoka et al., 1998]	[53]	Cross-sectional	180	1m-35y	Multiple in Japan	butterfly vertebrae, scoliosis, polydactyly of upper limb, clubfoot, foot eversion, abnormality hip joint	7
[Mehraein et al., 1997]	[121]	Cross-sectional	40	0-25y7m	Clinical Genetics Department University of Marburg and Pediatric Cardiology department University of Mainz	clubfoot, foot eversion, postaxial polydactyly, syndactyly of lower limb, hammer toes, postaxial polysyndactyly, polyarticular arthritis	7

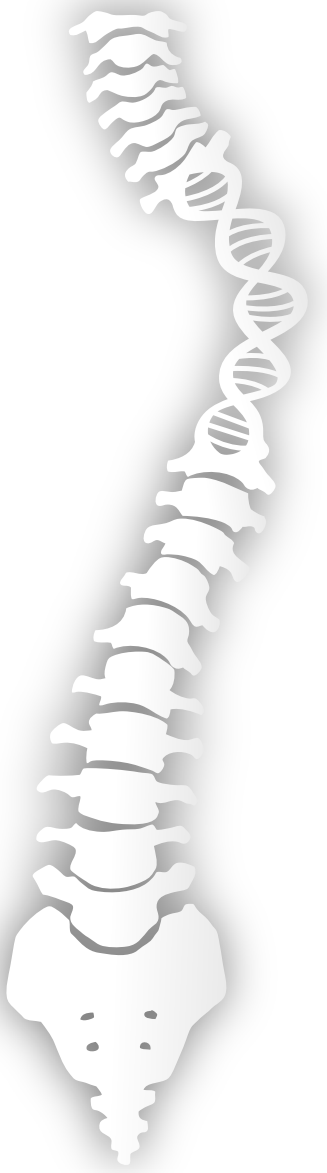
Author, Year of Publication	Ref.	Study design	Sample size	Age of inclusion	Hospital	Orthopedic manifestations	Critical appraisal
[McDonald-McGinn et al., 2005]	[122]	Cross-sectional	370	0-52y	The Children's Hospital of Philadelphia	growth	4
[Meinecke et al. 1986]	[123]	Case series	8	N.R.	Multiple: the Netherlands, Germany, Switzerland, USA	growth, clubfoot, overriding toe, base angle	4
[Ming et al., 1997]	[54]	Cross-sectional	108	1d-36y	The Children's Hospital of Philadelphia	at least one cervical anomaly, anomaly on chest/spinal x-ray, butterfly vertebrae, hemivertebrae, coronal cleft, scoliosis, scoliosis requiring surgery, upper limb malformations, polydactyly of upper limb, clinodactyly of 5th finger, ulnar rotation of digits, lower limb malformations, polydactyly of lower limb, clubfoot, overfolded toes, syndactyly of lower limb, foot eversion, metatarsus adductes, lateral deviation great toe, hypoplastic scapula	6
[Monteiro et al., 2013]	[124]	Cross-sectional	194	10d-33y	Multiple Brazilian hospitals	scoliosis, clubfoot, cortical dysplasia	8
[Del Carmen Montes et al., 2013]	[125]	Cross-sectional	32	7d-31y	Los Hospitales de Ninos y Privado, Cordoba	scoliosis requiring surgery	5
[Morava et al., 2002]	[80]	Cross-sectional	20	N.R.	Children's Hospital of New Orleans	scoliosis, clinodactyly 5th finger, arachnodactyly location unclear	5
[Motzkin et al., 1993]	[126]	Cross-sectional	18	6-42y	Montefiore Medical Center, Bronx	growth	5
[Muller et al., 1988]	[127]	Cross-sectional	13	1d- 15.5y	Medizinische Hochschule, Hannover	growth	6
[Oh et al., 2007]	[128]	Cross-sectional	16	10m- 19y	Sutter memorial hospital, Sacramento	growth	6
[Oskarsdottir et al., 2005a]	[129]	Cross-sectional	33	0.01-19.4y, median 6.7y	Queen Silvia Children's hospital, Göteborg	growth	8

Author, Year of Publication	Ref.	Study design	Sample size	Age of inclusion	Hospital	Orthopedic manifestations	Critical appraisal
[Oskarsdottir et al., 2005b]	[130]	Cross-sectional	100	0.01-19.4y, median 6.7y	Queen Silvia Children's hospital, Göteborg	rib malformations, vertebral and/or scoliosis, polydactyly of upper limb, camptodactyly of upper limb, clubfoot, metatarsus adductus	7
[Poirsier et al., 2016]	[131]	Cross-sectional	747	birth-65y, mean 9.8y	31 French laboratories	spine bone malformations, scoliosis, clubfoot	7
[Radford et al., 1988]	[132]	Cross-sectional	26	N.R.	Princes Charles hospital, Chermside	clubfoot, congenital dislocation hip, abnormal digits, bone exostoses, hyplplastic right pelvis	3
[Rakonjac et al., 2016]	[133]	Cross-sectional	11	mean 89.9m	University Children's Hospital, Belgrade	clubfoot	8
[Repetto et al., 2009]	[134]	Cross-sectional	208	0-39y, mean 5.2y	Multiple in Chile	scoliosis, growth	8
[Reynaud et al., 2011]	[135]	Prospective cohort	102	0-48y	Hôpital d' enfants de la Timone, Marseille	growth	8
[Ricchetti et al., 2008]	[51]	Cross-sectional	32	mean age 8.94y	The Children's Hospital of Philadelphia	dysmorphic C1, occipitalization C1, basilar impression C1, open posterior arch C1, open anterior arch C1, anterior and posterior clefting, dysmorphic dens C2, open posterior arch C2, C2 swoosh, fusion posterior elements C2-C3, fusion posterior elements and body C2-C3, spinal cord encroachment, spinal cord impingement, neurologic symptoms requiring surgery	9
[Ricchetti et al., 2004]	[50]	Cross-sectional	79	1.6-46.0y	The Children's Hospital of Philadelphia	at least one cervical anomaly, platybasia, dysmorphic shape C1, open posterior arch C1, hypoplastic/dysmorphic arch C1, dysmorphic dens C2, C2 swoosh, fusion C2-C3 (posterior elements only, posterior elements and vertebral body), occipitoatlantal motion, atlantoaxial motion, C2-C3 motion	10

Author, Year of Publication	Ref.	Study design	Sample size	Age of inclusion	Hospital	Orthopedic manifestations	Critical appraisal
[Ryan et al., 1997]	[136]	Cross-sectional	558	N.R.	23 European centres	abnormal vertebrae on chest/spinal vertebrae, scoliosis, clubfoot, syndactyly location unclear, polydactyly location unclear	5
[Sandrin-Garcia et al., 2007]	[137]	Cross-sectional	29	N.R.	Craniofacial Anomalies Rehablitation Hospital, Bauru and Division of Medical Genetics, University Hospital, Preto	scoliosis	5
[Seaver et al., 1994]	[138]	Cross-sectional	6	0-7y	Steele Memorial Children's Research Center, Tucson	growth	2
[Shprintzen et al. 1978]	[139]	Case series	12	N.R.	Montefiore Medical Center, Bronx	growth	3
[Shprintzen et al., 1981]	[140]	Cross-sectional	39	N.R.	Multiple in USA	growth	6
[Spruijt et al., 2014]	[141]	Cross-sectional	24	4.0-13.1y	University Medical Center, Utrecht	platybasia, basilar impression	9
[Stagi et al., 2010]	[87]	Cross-sectional	28	6.1-42.9y median 12.5y	Anna Meyer Children's Hospital, Florence	growth	11
[Sullivan et al., 1997]	[83]	Cross-sectional	80	N.R.	The Children's Hospital of Philadelphia	polyarticular arthritis	7
[Tarquinio et al., 2012]	[142]	Retrospective cohort	188	N.R.	Rady Children's Hospital, San Diego	growth	8
[Vantrappen et al., 1999]	[143]	Cross-sectional	130	"age varied widely"	University Hospital Leuven	scoliosis, camptodactyly, syndactyly, clinodactyly	3
[Veerapandiyan et al., 2011]	[77]	Cross-sectional	26	6-23y	Wake forest University health sciences, Winston-Salem	at least one cervical anomaly, platybasia, dysmorphic shape C1, open posterior arch C1, hypoplastic/dysmorphic arch C1, dysmorphic dens C2, C2 swoosh, fusion C2-C3,	11
[Verhagen et al., 2012]	[144]	Case series	8	4d-33y	Erasmus Medical Center, Rotterdam	overfolded toes	6

Author, Year of Publication	Ref.	Study design	Sample size	Age of inclusion	Hospital	Orthopedic manifestations	Critical appraisal
[Wichajarn et al., 2014]	[145]	Retrospective cohort	20	0-16y	Faculty of Medicine, Khon Kaen	polydactyly unclear location, growth	6
[Wilson et al., 1993]	[146]	Cross-sectional	44	"children"	Multiple in Great Britain	coronal cleft, clubfoot, pectus excavatum, right lobster claw deformity	3
[Wozniak et al., 2010]	[147]	Cross-sectional	13	N.R.	Poznan University of Medical Sciences	clubfoot, extentend oligoarticular arthritis	4

CHAPTER 3



Club Foot in Association with the 22q11.2 Deletion Syndrome: An Observational Study

Homans JF, Crowley TB, Chen E, McGinn DE, Deeney VFX, Sakkers RJB, Davidson RS, Castelein RM, McDonald-McGinn DM.

Based on: Club foot in association with the 22q11.2 deletion syndrome : An observational study. *Am J Med Genet A*. 2018;176:2135–2139

Abstract

Objective: The 22q11.2 Deletion Syndrome (22q11.2DS) occurs in ~1:3000 - 6000 individuals. Features less typically associated with 22q11.2DS, such as orthopedic manifestations, may be overlooked or may not lead to appropriate diagnostic testing. Club foot has a general population prevalence of ~1:1000 and has been occasionally described in association with 22q11.2DS. Our hypothesis is that the prevalence of club foot is higher in patients with 22q11.2DS.

Materials and methods: We performed a retrospective review in two specialized 22q11.2DS centers to determine the prevalence of club foot. "True club foot" requires treatment (either conservative or surgical), therefore we only included those patients with proof of treatment. We investigated whether congenital heart disease (CHD) and/or cleft palate were associated with the presence of club foot within 22q11.2DS.

Results: The records of 1466 patients were reviewed. Of these, 48 (3.3%) had confirmation of club foot (95% Confidence Interval: 2.4-4.3): 22 (46%) had a bilateral, 12 (25%) left, and 14 (29%) right club foot. Within our study, neither a CHD and/or a cleft palate were associated with a club foot.

Conclusion: The prevalence of club foot in 22q11.2DS is 30 times higher than that observed in the general population. This suggests the diagnosis of club foot, especially in the face of other typically associated abnormalities of 22q11.2DS, should provoke consideration of 22q11.2DS as an underlying diagnosis, particularly in the neonatal setting.

Introduction

The 22q11.2 Deletion Syndrome (22q11.2DS) is the most common microdeletion syndrome in humans, with a prevalence of one in 3000-6000 live births and one in 1000 pregnancies.[42–45,66,148] Within a subset of the patients with 22q11.2DS, congenital anomalies need treatment in the neonatal period of their life. The most severe congenital anomalies include congenital heart disease (CHD) e.g. Tetralogy of Fallot, and palatal deficiencies such as cleft palate.[48] However, these are just a few of the large number of (congenital) clinical characteristics that can be part of the 22q11.2DS. [48,62] Recently, 69 orthopedic manifestations have been described as being part of the 22q11.2DS. One of these manifestations, (congenital) club foot, has attained little attention so far.[52]

Club foot (Figure 1, 2), can be identifiable in utero (Figure 3) and it contains four characteristic features which can be remembered through the acronym CAVE: **c**avus (a high medial longitudinal arch), forefoot **a**dductus, hindfoot **v**arus and hindfoot **e**quinus (Figure 1) .[149–151] The prevalence of congenital isolated club foot in the general population differs among multiple ethnic populations, but is approximately 1.2-6 per 1000 individuals. Within the group of patients with isolated club foot the male:female ratio is 2:1 and half of the patients have a bilateral club foot.[151–155] In studies on club foot within 22q11.2DS the prevalence ranges from 1.1-13.3%, which seems to be higher as compared to the general population.[52] However, none of these studies had club foot as their primary outcome nor was it explained

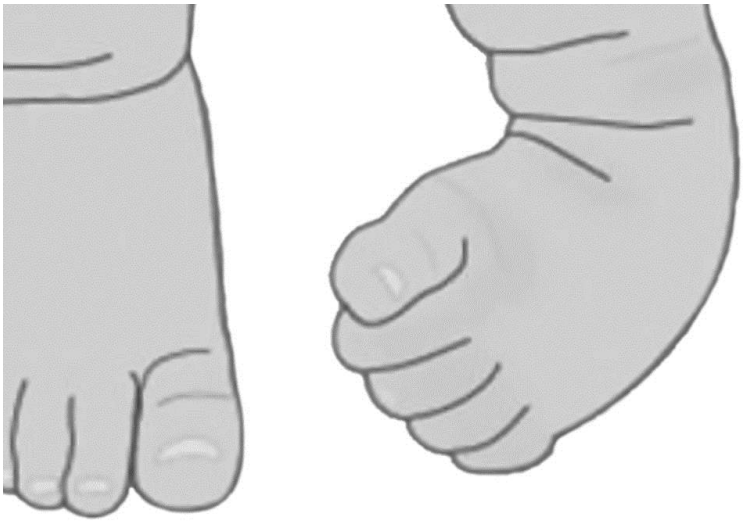


Figure 1: An illustration of a left club foot. The four characteristics of club foot can be seen: cavus (a high medial longitudinal arch), forefoot adductus, hindfoot varus and hindfoot equinus

how the club foot was diagnosed.[52] Currently, it is unknown whether the club foot within 22q11.2DS is typically associated with other severe congenital anomalies, such as CHD and cleft palate or whether the club foot can occur as a single entity. CHD and/or cleft palate will lead to genetic testing and subsequently bring the diagnosis of 22q11.2DS into light. However, if the prevalence of club foot within 22q11.2DS is increased and it occurs without the presence of these congenital malformations, a club foot in combination with other, subtle, 22q11.2DS phenotypic features might lead to the suspicion of 22q11.2DS.
First, we wanted to investigate the prevalence of club foot within 22q11.2DS. Second, we investigated whether club foot within 22q11.2DS is associated with CHD and/or cleft palate. Our hypothesis is that the prevalence of club foot is higher in 22q11.2DS as compared to the general population. Moreover, since scoliosis within 22q11.2DS is not associated with CHD and the prevalence of club foot is increased in other syndromes (e.g. Down Syndrome) we hypothesized that the club foot within 22q11.2DS is not associated with CHD and/or cleft palate.[156,157]

Material and Methods

After Institutional Review Board approval was obtained, a retrospective analysis based on longitudinal collected data was performed in two specialized 22q11.2DS centers. The research was conducted according to the STROBE criteria.[158] The patients were evaluated by the multidisciplinary team at the "22q and You" center at the Children's Hospital of Philadelphia (CHOP, inclusion: January 1999 – June 2018**)** or by the multidisciplinary 22q team at the University Medical Center Utrecht (UMCU, inclusion: January 2014 – May 2018).

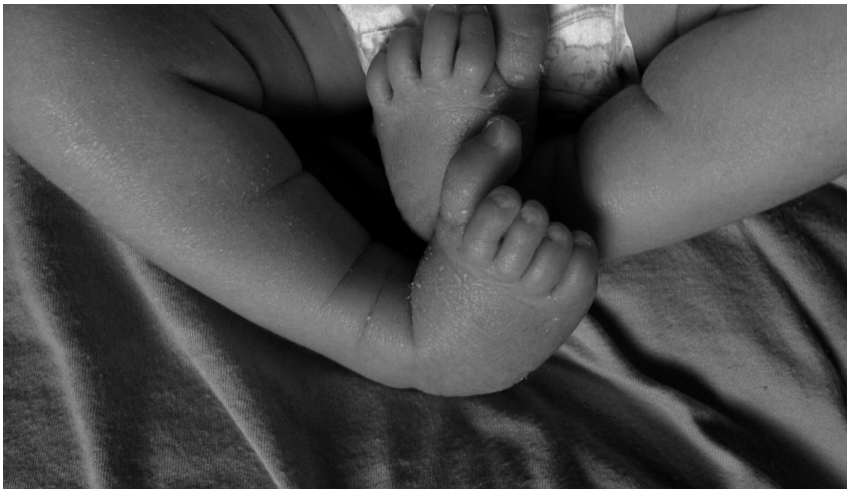


Figure 2: A patient with the 22q11.2 deletion syndrome and a bilateral club foot

All patients were diagnosed with a 22q11.2 deletion using fluorescent in situ hybridization, array comparative genomic hybridization, multiplex ligation probe amplification or SNP microarray. Patients with a known genetic disorder in addition to the 22q11.2 deletion were excluded. "True club foot" needs treatment (either conservative or surgical) and therefore, in order to prevent false positive cases (e.g. patients with another congenital malformation of the foot), we only included patients whom had proof of treatment of the club foot and thus the clinical diagnosis of club foot.[149–151]

Baseline characteristics (age, gender, presence and type of CHD and the presence of a cleft palate) were collected. Cerebral palsy and spina bifida are known to have a strong association with club foot and therefore the 22q11.2DS cases were screened for these anomalies.[149,151] The patients with 22q11.2DS with a club foot were compared with the non-club foot patients with 22q11.2DS with respect to the presence of CHD and cleft palate. These characteristics were chosen since these congenital anomalies would definitely lead to hospital referral (and genetic testing) within the first year and subsequently reveal the 22q11.2DS diagnosis. The CHDs were graded according to the grading scale described by Billett and colleagues: Simple, moderate or complex and for further analyses they were dichotomized (present or absent).[159] Cleft palate was considered a dichotomous outcome; present or absent.

Statistical analysis

The 95% confidence intervals (CI) for the prevalence estimates were calculated. Baseline differences between the patients with and without club foot were compared with the two-tailed Fisher's exact test. All statistical analyses were conducted with the Statistical Package for the Social Sciences (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA: IBM Corp). A p-value of <0.05 was considered statistically significant. There was missing data on CHD and cleft palate within the group of patients without a club foot and there was no missing data within the group of patients with a club foot. In order to investigate whether the missing data had influence on the statistical significance of the findings on the possible association between CHD and/or cleft palate and club foot we performed a sensitivity analysis: First, we imputed the missing data as "event" (e.g. presence of CHD or cleft palate). Second, we imputed the missing data as "no event" (e.g. no CHD/cleft palate). Next, we performed the Fisher's exact test multiple times in which we either considered all the missing data as "event" or "no event".

Results

Prevalence of club foot

At the time of analysis, the CHOP database consisted of 1332 patients evaluated in the 22q and You Center (a multidisciplinary clinic for patients with a chromosome 22q11.2 abnormality). All patients were seen by a clinical geneticist and/or an orthopedic surgeon. Seventy-four percent of the CHOP cohort was Caucasian. Within the UMCU cohort 134 patients were seen by the pediatrician and orthopedics (ethnicity unknown). The total cohort consisted of 1466 patients of whom 51.0% were male. Out of the total cohort 48 patients (3.3%) had a confirmed club foot (95% CI: 2.4-4.3). Out of this group, two patients had cerebral palsy and one patient had spina bifida. Thirty-seven patients were male (77%, 95% CI: 63-87%, P<0.005) which corresponds to a male:female ratio of 3.4:1. Twenty-two patients (46%, 95% CI 31-61%) had bilateral club feet (ratio: 1:0.8) and the remainder had either a left (n=12) or right (n=14) club foot (left:right ratio of 1:1.2).

Congenital anomalies and club foot

The presence of a CHD or a cleft palate was not associated with a club foot (Table I). Second, a separate category was made (the presence of either a CHD, a cleft palate, or both) and this category could not be identified as a risk factor for club foot as well. Third, there was no association between CHD, cleft palate and the multiple sub-categories of club foot (bilateral, left or right club foot).

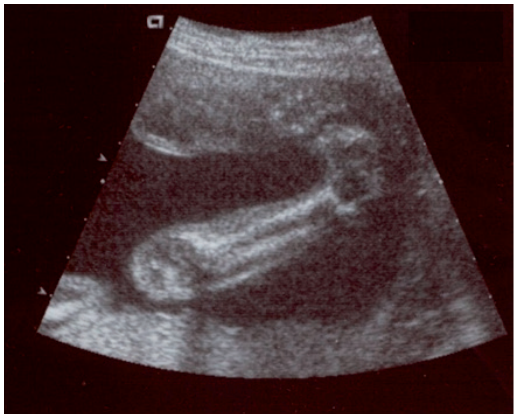


Figure 3: A patient with the 22q11.2 deletion syndrome and a prenatal ultrasound of a club foot

Table 1: Congenital anomalies in association to club foot. Categorical values are expressed as the number and the ratio in %. CHD: congenital heart disease.

	Patients without club foot	Patients with club foot	P-value
Presence of a CHD	837 (65.5%)	28 (58.3%)	0.354
Presence of a cleft palate	257 (19.1%)	11 (22.9%)	0.462
Presence of either a CHD or a cleft palate	903 (72.9%)	32 (66.7%)	0.327

Missing data

In the group without club foot there was missing data on the presence of CHD and cleft palate: 140 patients (9.5%) and 73 patients (5.0%) respectively. First, the missing data was imputed as "event": the p-values were 0.154, 1.00, 0.125 for CHD, cleft palate and CHD and/or cleft palate respectively. Second, the missing data was imputed as "no event": the p-values were 1.00, 0.446 and 1.00 respectively. Subsequently, our sensitivity analysis revealed that the missing data had no effect on the statistical significance as shown in Table I.

Discussion

The 22q11.2DS is the most common microdeletion syndrome and is characterized by broad phenotypic heterogeneity including multiple congenital anomalies, such as tetralogy of Fallot and cleft palate. [48,62] Due to these conditions, often requiring urgent medical/surgical attention, possible orthopedic features tend to be overshadowed, as shown by the fact that there are no studies on the treatment of orthopedic manifestations within 22q11.2DS.[52,156] Club foot has occasionally been mentioned in previous research, but no study had club foot as their primary outcome of interest.[52] Our research has shown that club foot is definitely associated with 22q11.2DS with a prevalence of 3.3%. The majority (74%) of the CHOP cohort is Caucasian and since the prevalence of club foot within the general Caucasian population is ~1:1000 patients, the prevalence of club foot occurs approximately 30 times more often within 22q11.2DS as compared to the general Caucasian population. Moreover, the bilateral:unilateral and male:female ratio is comparable with the general population.[151,153–155] Last, we did not find a relation between the presence of club foot and the presence of a CHD and/or a cleft palate. We chose CHD and cleft palate since these major congenital anomalies would definitely lead to genetic testing and subsequently reveal the diagnosis of 22q11.2DS. However, it is important to note that 58.3% and 22.9% of the patients with club foot had a CHD or cleft palate respectively.

Within our cohorts there were multiple patients that were diagnosed with 22q11.2DS at a later age, however they could have been diagnosed with 22q11.2DS in the neonatal period because of the combination of a club foot at the prenatal ultrasound and other congenital

malformations. One patient, whose father had a history of repaired ventricular septal defect and cleft palate, was discharged to home from an outside hospital neonatally, without genetic testing and proper physical examination. Afterwards, this patient was transferred emergently to our hospital, in cardiac extremis due to a previously unrecognized diagnosis of an interrupted aortic arch type B (a malformation associated with 22q11.2DS). Another patient was found prenatally to have club foot, but no other features. Postnatally the child had stridor but no doctor considered the diagnosis (or any diagnosis for that matter). When the child was a toddler, he was finally referred to the clinical geneticist and 22q11.2DS was confirmed.

In this study we examined the possible relationship between one of the most extreme associated congenital clinical features of the 22q11.2DS (CHD and/or cleft palate) in relation to the club foot. However, this is just a small portion of all the associated anomalies within 22q11.2DS.[48,62] Stone et al. performed a long term study on the associated anomalies found in patients with presumed idiopathic club foot.[155] These features include developmental and mild cardiovascular abnormalities, abnormalities that are also part of the 22q11.2DS. In other words, if a patient with a club foot is identified, it could be important to identify whether the patient truly has an idiopathic club foot or other possible (mild) syndromic features as well. For example, if a patient has associated anomalies such as developmental delay, characteristic facial features and/or a CHD, careful examination should follow to determine whether the combination of symptoms leads to the suspicion of the 22q11.2DS and/or another syndrome. [48]

In patients with idiopathic club foot the etiology is unknown.[150] It has been related to the intra-uterine position, environmental factors such as smoking, or abnormal muscle, soft tissue, bone and vascular malformations.[149,150] Moreover, there is definitely a genetic component regarding the development of club foot within 22q11.2DS: multiple genes (e.g. *PITX1*, *TBX4*) are associated with the development of club foot and within identical twins there is 33% concordance. [150,160] Interestingly, *TBX1* is one of the deleted genes within the 22q11.2 region.

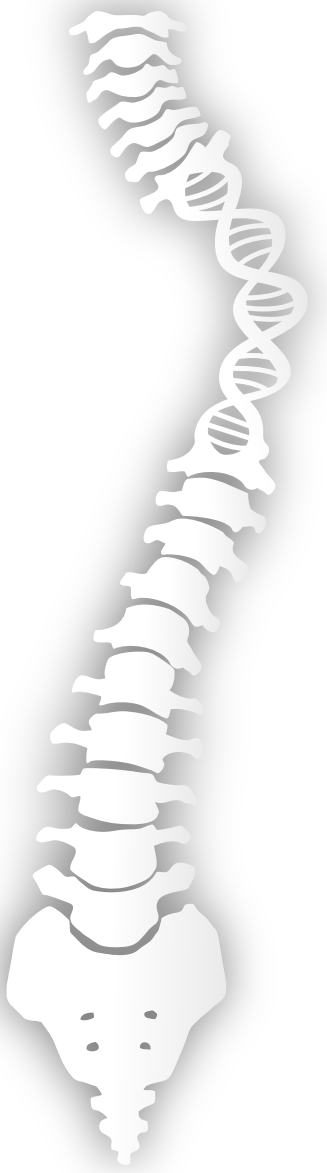
Multiple (family) studies on club foot have provided (genetic) insights in the development of idiopathic club foot.[160] Despite this valuable research, the etiology of club foot is still largely unknown and therefore we propose an alternative possibility in order to gain more knowledge on the development of idiopathic club foot. Given the fact that the club foot male:female ratio and bilateral:unilateral ratio in 22q11.2DS is comparable to the general population and the fact that there was no relation with CHD and/or cleft palate it seems to be that the 22q11.2 deletion itself is a risk factor for developing an “idiopathic-like” club foot. Since the prevalence of club foot within 22q11.2DS is increased, further research on the club foot within 22q11.2DS could lead to more insight in the prenatal differences and possible risk factors for developing a club foot. Subsequently, this might provide insights in the development of club foot in the general population analogous to schizophrenia research within 22q11.2DS.[48]

There are a number of limitations within our study. First, this study was performed retrospectively and we only classified patients as having a club foot if we could find proof of the treatment. As a result, we excluded patients without a letter of orthopedic treatment. However, patients could have received the treatment in an outside hospital after referral to one of our specialized 22q11.2DS centers. Therefore, the prevalence of 3.3% could be an underestimation of the true prevalence of club foot within 22q11.2DS. On the other hand, the study was conducted in two tertiary expertise centers for 22q11.2DS. Therefore it is possible that patients with major conditions, such as club foot, could have been referred to the CHOP or UMCU. However, in none of the referral letters the club foot was the specific cause for referral to one of the specialized centers. Moreover, we only examined the association between the major congenital phenotypic features (CHD and cleft palate) and club foot. In order to further determine whether other associated features within the first year, such as feeding difficulties and seizures, are associated with club foot a 22q11.2DS prospective study should be performed. At last, we had 9.5% missing data regarding CHD and 5.0% missing regarding cleft palate, for which we performed a sensitivity analysis, which showed that are findings were robust.

Conclusion

The 22q11.2 deletion syndrome is a challenging condition, characterized by a high diversity in (congenital) phenotypic features. Club foot is definitely one of these features, since the prevalence is approximately 30 times higher as compared to the general population. Moreover, the major congenital phenotypic features, congenital heart disease and cleft palate, which will lead to referral to the hospital and the clinical geneticist, could not be identified as risk factors for club foot within the 22q11.2 deletion syndrome.

CHAPTER 4



Scoliosis in Association with the 22q11.2 Deletion Syndrome: An Observational Study

Homans JF, Baldew VGM, Brink RC, Kruyt MC, Schlösser TPC, Houben ML, Deeney VFX, Crowley TB, Castelein RM, McDonald-McGinn DM.

Based on: Scoliosis in association with the 22q11.2 deletion syndrome: An observational study. *Arch Dis Child*. 2019;104:19–24

Abstract

Objective: The 22q11.2 Deletion Syndrome (22q11.2DS) is the most common microdeletion syndrome in humans. It is characterized by wide phenotypic variability, including congenital heart disease (CHD), immunodeficiency and scoliosis. However, little is known regarding the prevalence and characteristics of scoliosis in patients with 22q11.2DS. The objective of this study is to assess the prevalence of scoliosis, its characteristics and the association with CHD in patients with 22q11.2DS.

Design: This prevalence study is based on physical examination and questionnaires of the world's largest 22q11.2DS longitudinal collected database (n=1393, Childrens's Hospital of Philadelphia) and was augmented with the scoliosis prevalence based on radiography in a smaller cohort (cross-sectional, University Medical Center Utrecht).

Patients: Patients with a laboratory confirmed 22q11.2 deletion that visited the specialized outpatient clinics were considered for inclusion.

Main outcome Measures: 1) The prevalence of scoliosis, 2) its association with CHD, 3) the similarity between 22q11.2DS curve patterns and adolescent idiopathic scoliosis (AIS) curve patterns.

Results: Within the Philadelphia cohort, the prevalence of scoliosis in patients older than 16 years (n=317) was 48% (n=152). A similar prevalence (49%) was shown for the younger Utrecht cohort (n= 97). The occurrence of scoliosis was not associated with the presence of CHD. Sixty-three percent of patients with scoliosis had a scoliotic curve pattern that resembled AIS.

Conclusions: Clinicians should be aware that scoliosis is highly prevalent (48-49%) in association with 22q11.2DS, irrespective of other clinical features (e.g. the presence of CHD). Furthermore, 22q11.2DS may provide insights into the causes of adolescent idiopathic scoliosis.

Background

The 22q11.2 Deletion Syndrome (22q11.2DS) is the most common microdeletion syndrome in humans, with a prevalence of one in 3000-6000 live births and one in 1000 pregnancies.[42–45,66,148] It is characterized by a broad spectrum of clinical features, including congenital heart disease (CHD), psychiatric illness (most notably schizophrenia), and orthopedic manifestations including scoliosis.[48] The epidemiology of scoliosis, however, has received little attention and remains largely unknown.

Scoliosis can have serious sequelae throughout life such as impaired pulmonary development, disc degeneration, back pain and ultimately the need for extensive and high risk scoliosis surgery.[2,89,161] If scoliosis is diagnosed at an early stage, it may successfully be treated in a non-operative manner.[8] At this moment knowledge of the prevalence and clinical characteristics of scoliosis associated with 22q11.2DS is limited. In earlier reports on patients with 22q11.2DS, in which scoliosis was not the primary outcome, the prevalence was described in a wide range from 0.6% to 60%.[52–56]

One of the most common abnormalities within the 22q11.2DS is CHD (>60%).[48,62] Interestingly, the 22q11.2DS is the second most common genetic cause of major CHD after Down syndrome.[62] There are numerous reports on the relation between CHD and scoliosis in the general population, all suggesting that embryologic thoracic anatomy or thoracic surgery in infancy may introduce a spinal imbalance.[57–61] However, the relation between this common cardiac pathology and scoliosis in the 22q11.2DS population has not been described before. Since scoliosis also occurs in patients with 22q11.2DS without CHD, we hypothesize that the pathogenesis of scoliosis in 22q11.2DS is partly independent from the above mentioned suggested mechanisms. Moreover, we hypothesize that a subset of the observed scoliosis in 22q11.2DS may be of a biomechanically similar type as observed in adolescent idiopathic scoliosis (AIS). The current study addresses three questions:

1. What is the prevalence of scoliosis in patients with 22q11.2DS at any age?
2. What is the association between scoliosis and CHD in 22q11.2DS?
3. What are the characteristics of scoliosis in 22q11.2DS, and to what extent do these characteristics resemble the scoliotic curve pattern of AIS?

Material and Methods

Outline

Two cohorts from specialized 22q11.2DS clinics were included, from the Children's Hospital of Philadelphia (CHOP), USA and from the University Medical Center Utrecht (UMCU), The Netherlands. As the screening protocol and infrastructure differs between the two cohorts the results are presented separately. Within CHOP a retrospective analysis was performed in order to determine the prevalence of clinical evident scoliosis throughout age until skeletal maturity. Within UMCU a cross-sectional analysis was performed in order to determine the radiological proven prevalence of scoliosis. We excluded patients with a known genetic disorder in addition to the 22q11.2 deletion. The local Ethical Review Boards of both hospitals approved this study and waived the necessity of explicit (parental) informed consent, since data were collected during standard care and since data were handled anonymously.

Study population CHOP

All patients from birth till last follow-up visiting the outpatient clinic from January 1999 to September 2017 were eligible. The patients had been seen at least once by the clinical geneticist, which included a full physical examination. The presence of scoliosis was based on systematic physical examination (Adam's forward bend test) performed by the clinical geneticist, as well as on questionnaires (patients were asked whether they are diagnosed with a scoliosis, whether they consult an orthopedic surgeon and if they had undergone scoliosis surgery).

Baseline characteristics (age, gender and the presence and type of CHD) were collected. Patients were coded in a longitudinal manner across age groups of two years, based on their last visit, to demonstrate the development of scoliosis throughout age. Patients who were lost to follow-up were removed from the consecutive age groups. In order to determine the definitive prevalence of scoliosis a separate analysis was performed on all patients older than 16. If a patient is over 16 there is only a slight chance of progression of major scoliotic curves, but this will not influence the definitive prevalence of scoliosis.[89] For the same reason, the possible relation between CHD and scoliosis was investigated in patients older than 16. The CHDs were graded according to the grading scale described by Billett *et al.*: Simple, moderate or complex.[159] Both the presence and grade of a CHD were investigated as a risk factor for the occurrence of a scoliosis.

Study population UMCU

All patients between six and 18 years visiting the outpatient clinic from January 2014 to June 2017 were eligible. Standing posterior-anterior and lateral full spine radiographs were made as standard care of all the patients with 22q11.2DS from the age of six, regardless of the suspicion of a scoliosis. Patients without spinal radiographs were excluded. If a patient had

multiple visits during the study period, the presence of scoliosis was based on the full spinal radiograph during the last outpatient visit. A scoliosis is defined as a deviation exceeding ten degrees in the coronal plane.[162]

Radiological assessment

The radiological characteristics of the 22q11.2DS scoliosis were assessed on all available posterior-anterior and lateral radiographs of patients with 22q11.2DS with scoliosis from either the CHOP or UMCU cohort. If a patient had multiple radiographs, the first radiograph in which the scoliosis was determined was used. The magnitude of the main curve was determined with the Cobb angle.[163] Coronal balance, sagittal balance, kyphosis (T5-T12) and lordosis (T12-S1) were measured according to the guidelines of the Scoliosis Research Society (Figure 1).[162]

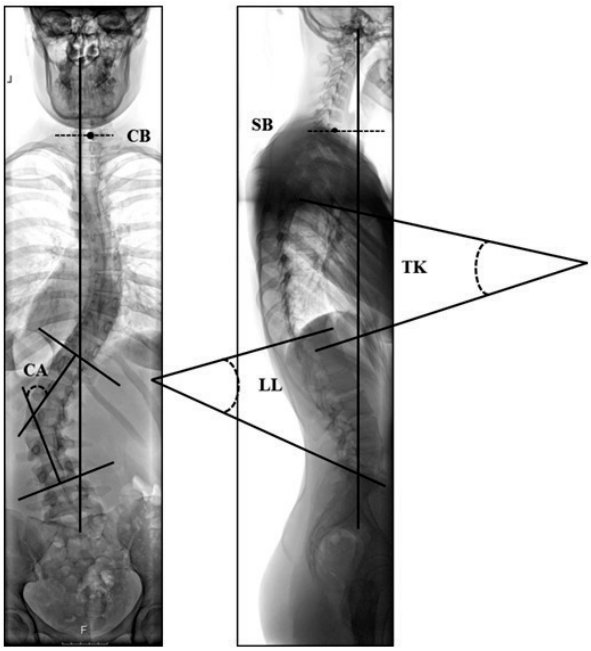


Figure 1: The radiological assessments measured according to the guidelines of the Scoliosis Research Society.[162]

CB: coronal balance, measured in millimeters from the center of C7 to the vertical line drawn from the center of S1. CA: Cobb angle, measured in degrees from the upper endplate of the most tilted vertebra to the lower endplate of the most tilted vertebra. SB: sagittal balance, measured in millimeters from the center of C7 to the vertical line drawn from the posterior-superior corner of S1.TK: thoracic kyphosis, measured in degrees from the upper endplate of T5 to the lower endplate of T12. LL: lumbar lordosis, measured in degrees from the upper endplate of T12 to the endplate of S1.

To compare with the type of scoliosis that normally occurs in the general population, AIS, the two –step concept developed by Spiegel *et al.* was utilized. The first step is to determine whether there is a 'typical' (AIS-like) curve pattern or 'atypical' (not resembling AIS) curve pattern (Figure 2). [164] The second step is to determine whether the curve pattern has no atypical features. Atypical features are features that are not commonly seen in AIS curves (e.g. a curve until the fifth lumbar vertebra).[164] For this analysis patients with a congenital spinal malformation were excluded.

Statistical analysis

The 95% confidence intervals (CI) for the prevalence estimates were calculated. Baseline differences between the patients with and without scoliosis were compared with the Fisher's exact test. Continuous variables were compared by *t* test if applicable or with the Mann-Whitney test if not normally distributed. All statistical analyses were conducted with the Statistical Package for the Social Sciences (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA: IBM Corp). A p-value of <0.05 was considered statistically significant.

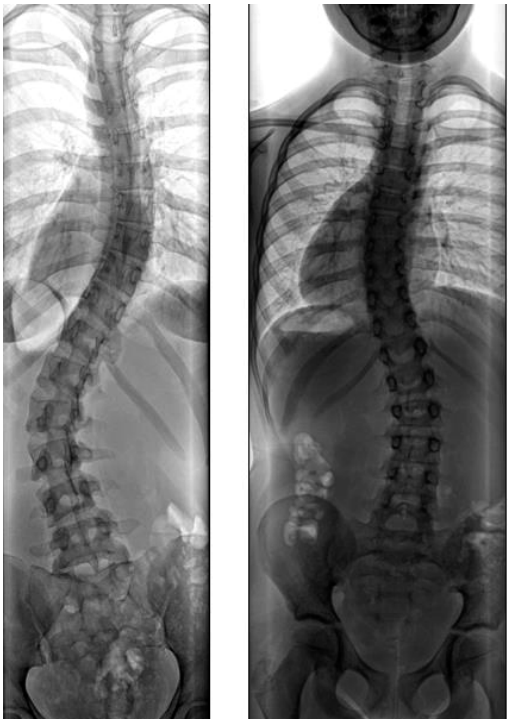


Figure 2: Total spine radiographs from two patients with the 22q11.2 deletion syndrome.
The left image shows a "typical" right thoracic/left lumbar curve. The right image shows an "atypical" left thoracic/right lumber curve.

Results

Prevalence of Scoliosis CHOP

The CHOP database consisted of 1393 patients evaluated in the 22q and You Center, a multidisciplinary clinic for patients with a chromosome 22q11.2 abnormality. In total, 308 patients were excluded, since (1) they had no recorded spinal examination (n=249), or (2) they had the 22q11.2 Duplication Syndrome and/or another genetic disorder (n=59) besides the 22q11.2 deletion. A total of 1085 patients were included with a laboratory confirmed 22q11.2 deletion (using fluorescent *in situ* hybridization, array comparative genomic hybridization, multiplex ligation probe amplification, or SNP microarray) and spinal examination. Mean age was 11.3 years (standard deviation: 8.8) and 48% were female (Table 1). The overall prevalence of scoliosis within this cohort was 20% (95% CI 18-23%). As can be seen in Figure 3, the prevalence increased throughout age. Within the group of patients older than 16 (n=317) the prevalence of scoliosis was 48% (95% CI 42-54%) (Figure 3, Table 1), resembling the definitive prevalence of scoliosis.

Table 1: Patient characteristics

Categorical values are expressed as the number and the ratio in %. Continuous values are expressed as mean with standard deviation. SD: standard deviation. CHD: congenital heart disease. *: There were 14.5 % missing. **: There were 8.2% missing. ***: Multiple grades of CHD were compared with no CHD.

	All patients	Patients without scoliosis	Patients with scoliosis	P-value
All CHOP patients:	1085 (100%)	864 (80%)	221 (20%)	
Female	525 (48%)	410 (47%)	115 (55%)	0.427
Age in years (SD)	11.4 (8.8)	10.1 (8.8)	16.4 (7.1)	**0.000**
Presence of a CHD*	588 (63%)	461 (64%)	127 (61%)	0.481
CHOP patients older than 16 years	317 (100%)	165 (52%)	152 (48%)	
Gender (female)	165 (52%)	87 (53%)	78 (51%)	0.802
Age (SD)	21.7 (8.5)	24.3 (8.9)	18.9 (7.1)	**0.000**
Presence of a CHD**	171 (59%)	82 (56%)	89 (61%)	0.297
Divided per grade:				
No CHD	120 (41%)	65 (44%)	55 (38%)	
Grade 1	51 (18%)	28 (19%)	23 (16%)	0.93***
Grade 2	75 (26%)	34 (23%)	41 (28%)	0.23***
Grade 3	45 (15%)	20 (14%)	25 (17%)	0.266***
All UMCU patients	97 (100%)	49 (51%)	48 (49%)	
Female	53 (55%)	23 (47%)	30 (67%)	0.124
Age in years (SD)	11.6 (3.6)	11.2 (3.5)	12.1 (3.6)	0.193
Presence of a CHD	36 (37%)	15 (31%)	21 (44%)	0.181

Prevalence of Scoliosis UMCU

A total of 121 consecutive patients (aged >6 years) visited the UMCU 22q11.2DS outpatient clinic in the study period. Twenty-four patients were excluded (11 patients were too young, five had other genetic disorders besides 22q11.2DS, four did not have a laboratory confirmed 22q11.2 deletion, four had incomplete radiological charts). The remaining 97 patients, with a mean age of 11.6 years (standard deviation 3.6), constituted the study population of the UMCU (Table 1). Scoliosis was present in 48 (49%, 95% CI 39-60%) of these patients. Of the 14 patients aged above 16, the prevalence of scoliosis was 57% (95% CI 33-79%).

Congenital heart disease and scoliosis

In the group of patients older than 16, within the CHOP cohort, CHD was present in 59% of the patients. The presence of CHD, as well as the grade of the CHD was not associated with the development of scoliosis in patients with 22q11.2DS, as can be seen in Table 1.

Radiological assessment of the 22q11.2DS scoliosis

The radiographs of 137 patients with 22q11.2DS (CHOP and UMCU) with scoliosis could be analyzed (Table 2). The median coronal balance and mean sagittal balance revealed that the majority of patients were in good balance. There were eight patients with a congenital malformation (seven butterfly vertebra, one hemivertebra). In this series, after exclusion

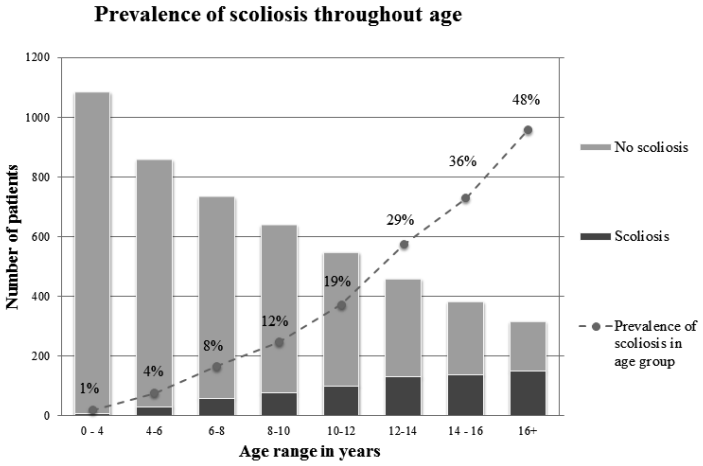


Figure 3: The prevalence of scoliosis throughout age

The prevalence of scoliosis within the 22q11.2 Deletion Syndrome (the Children's Hospital of Philadelphia cohort). Y: years.

Table 2: Radiological parameters.
Categorical values are expressed as the number and the ratio in %. Continuous values are expressed as mean with standard deviation, unless specified otherwise. Moreover, the range of the continuous radiographic parameters are shown. SD: standard deviation. IQR: Inter Quartile Range. mm: millimeter.*: 5% missing. **: 21% missing. ***: 18% missing.****: 8 patients excluded due to a congenital malformation.

		Patients with scoliosis
All patients	N	137
Female	N (%)	73 (53%)
Age in years	Mean (SD)	11.1 (4.5)
First Cobb angle:	Range	10 until 74 degrees
	Median (IQR)	18 (14-26)
Coronal balance*	Range	0-50 mm
	Median (IQR)	11 mm (5-15)
Sagittal balance**	Range	-93 until +78 mm
	Mean (SD)	-2 mm (40)
Thoracic kyphosis (T5-T12)***	Range	4 until 59 degrees
	Mean (SD)	26 (11)
Lumbar lordosis (T12-S1)***	Range	13 until 85 degrees
	Median (IQR)	52 (45-63)
Typical curve pattern****	N (%)	81 (63%)
Patients with a typical curve pattern and typical features	N (%)	19 (24%)

of the patients with a congenital malformation, 63% (n=81) of patients had a typical (AIS-like) curve pattern. However, further analyses of these curve patterns showed that 76% had atypical features in their curve.

Treatment

Although the type and success of scoliosis surgery was not the topic of this study, it shows the severity of scoliosis within 22q11.2DS. Analogous to the CHD analysis, we looked at CHOP patients older than 16. It became evident that 26 out of the 152 scoliosis patients (17%, 95% CI: 12-24%) older than 16 from the CHOP cohort had undergone scoliosis surgery, whereas the remainder were managed conservatively.

Discussion

The 22q11.2DS is the most common microdeletion syndrome and is characterized by broad phenotypic heterogeneity including multiple congenital anomalies, complex medical conditions and psychiatric illness (most notably 25% schizophrenia).[48,62] Due to these conditions, often requiring urgent medical/surgical attention, possible orthopedic

manifestations tend to be overshadowed. Our study demonstrates that nearly half of the patients with 22q11.2DS aged 16 and older have a scoliosis. Moreover, and importantly, CHD was not associated with the development of scoliosis – giving all patients with 22q11.2DS an increased risk for developing scoliosis. Sixty-three percent had a typical scoliotic curve that resembles AIS. Thus, it seems possible to study certain aspects of the development of AIS, for which no valid animal model exists, in the 22q11.2DS.[27]

In previous studies reporting on patients with 22q11.2DS, the prevalence of scoliosis ranged from 0.6-60%.[52–56] This is most likely due to the fact that either scoliosis was not the primary outcome in these studies and/or most often the patients were young and thus had not yet developed their spinal deformity.[53,54,56,80,136] In contrast, Bassett *et al.* reported scoliosis in 47.4% of 78 adults, however it was not known whether the scoliosis was investigated systematically. Thus, ours is the first study that specifically investigated the prevalence of scoliosis in patients with 22q11.2DS. This study does not provide a definitive answer regarding an optimal, uniform, screening protocol for scoliosis within 22q11.2DS. In order to do so, at first we should know the effectiveness of brace treatment and scoliosis surgery. Based on the literature, we expected to identify a relationship between scoliosis and CHD.[57–61] However, in our study the presence of CHD was not noted to be a risk factor. There are multiple theories on the relation between CHD and scoliosis. First, an enlarged CHD heart might put mechanical stress on the spine.[57,64,165,166] Second, surgery on an immature thoracic cage may result in an alteration in growth of the bone with consequent deformity of the thorax and scoliosis.[59,167] Yet, in our group of patients with moderate or complex CHD (grade 2 and 3), in which patients often require a sternotomy or thoracotomy, the CHD was not associated with scoliosis. Third, a common genetic predisposition could play a role as well. As mentioned earlier, 22q11.2DS has broad phenotypic variability and 22q11.2DS itself is the second most common genetic cause for having CHD.[62] With this in mind, perhaps a subset of patients previously reported in the literature with both CHD and scoliosis actually had unrecognized 22q11.2DS. This theory would suggest that the CHD was not actually the risk factor for scoliosis, but rather the 22q11.2 deletion could have been a confounder. This concept is supported by a more recent study that did not demonstrate an increased risk for scoliosis in patients following sternotomy/thoracotomy.[168]

To better understand the scoliosis associated with the 22q11.2DS, curve patterns and features were scrutinized using the method described by Spiegel *et al.*[164] Over 60% of the patients with scoliosis had typical curve patterns resembling AIS. The majority of these AIS-like curve patterns had atypical features. However, the exact significance of this finding is indefinite, as the incidence of “atypical” curve features in AIS patients is unknown. Consequently, it is impossible to compare these results with findings in AIS patients. In our cohort, a subset of the patients developed a left thoracic curve, which resembles juvenile (age four –ten years) idiopathic scoliosis. [169] In addition, a subset of the patients with 22q11.2DS has a right descending aorta, which is strongly correlated with a left thoracic curve.[64] In order to combine juvenile with adolescent scoliosis and right descending with left descending aortas, research

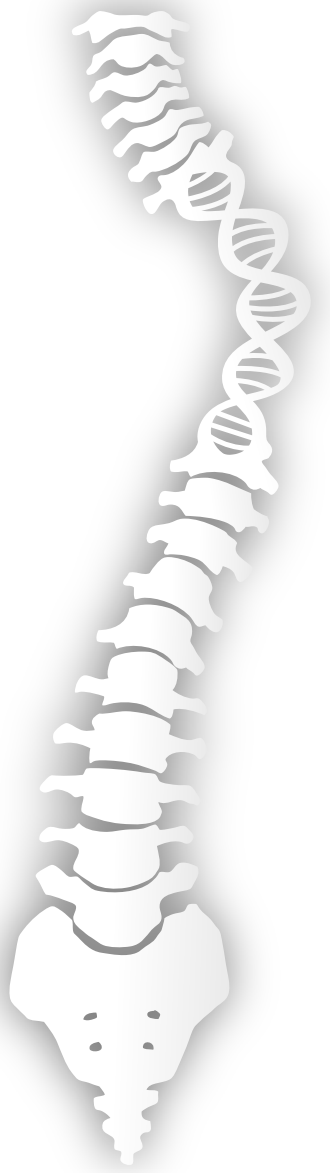
should be performed on the typical biomechanical characteristics of an idiopathic curve, independent of age. By means of these biomechanical characteristics it might be possible to truly identify the similarities and possible differences between idiopathic scoliosis and 22q11.2DS scoliosis. No uniform cause has ever been found for the development of AIS.[2] AIS is more common in girls than in boys with a ratio of 2.7 for spinal curves exceeding ten degrees and 4.5 for curves exceeding 20 degrees.[170] In our cohorts, the ratio between girls and boys with scoliosis is equal (Table 1). Based on the similarities between the 22q11.2DS scoliosis and AIS scoliosis, the 22q11.2 deletion may serve as a research model in order to amplify unknown cues or mechanisms that cause AIS, analogous to the way the 22q11.2DS is successfully used to study certain aspects of schizophrenia.[48]

There are a number of limitations within our study. First, the study was conducted in two tertiary expertise centers for 22q11.2DS. It is possible that patients with more demanding conditions, such as a serious scoliosis, were more likely to be referred. Furthermore, the two cohorts have different age groups. Since most patients from the UMC cohort have not yet reached spinal maturity, the final prevalence of scoliosis may even be higher than the reported 49%. However, with a group of 317 patients older than 16 at CHOP, resembling the definitive prevalence of scoliosis, we showed that at least half develop scoliosis.

Conclusion

Our data confirms that scoliosis is definitely associated with the chromosome 22q11.2 microdeletion syndrome, with a prevalence of at least 48-49% of patients who have reached skeletal maturity. Thus, it is imperative that clinicians be aware of the association, irrespective of other clinical features. Alternatively, (spine) surgeons should be aware that patients with what may look like an idiopathic scoliosis may have this underlying syndrome, which warrants further pediatric consultation because of possibly serious concomitant pathology such as CHD, chronic infection, dysphagia, and endocrinopathies. Furthermore, 22q11.2DS may provide insight into the causes of adolescent idiopathic scoliosis in the general population.

CHAPTER 5



The Role of 22q11.2 Deletion Syndrome in the Relationship between Congenital Heart Disease and Scoliosis

Homans JF, de Reuver S, Heung T, Silversides CK, Oechslin EN, Houben ML, McDonald-McGinn DM, Kruyt MC, Castelein RM, Bassett AS.

Based on: The Role of 22q11.2 Deletion Syndrome in the Relationship between Congenital Heart Disease and Scoliosis. **Under Review.** 2019

Abstract

Background context: For over four decades, clinicians and researchers have suggested a relationship between congenital heart disease (CHD) and scoliosis, attributed to either the disease itself or to the long-term effects of cardiac surgery on the immature thoracic cage. However, no study has yet accounted for 22q11.2 deletion syndrome (22q11.2DS), which is known for a scoliosis risk of 50% and is the second most common cause of CHD after Down syndrome.

Purpose: To determine the prevalence of scoliosis in patients with CHD with and without 22q11.2DS.

Study design/setting: Cross-sectional.

Patient sample: A well-characterized existing database of 315 adults with CHD (primarily tetralogy of Fallot), with (n=86) and without (n=229) 22q11.2DS, matched by sex and CHD severity, and excluding other known syndromic diagnoses.

Outcome measures: Presence of scoliosis (Cobb angle >10 degrees).

Methods: We systematically determined the presence of scoliosis in all included patients using thorax radiographs, blind to genetic diagnosis. Besides 22q11.2DS, other suspected risk factors for scoliosis were tested with a regression analysis: thoracotomy before the age of 12 years, severe CHD type and sex.

Results: The prevalence of scoliosis in adults with CHD and 22q11.2DS (n=46, 53.5%) was significantly greater than in those without 22q11.2DS (n=18, 7.9%, p<0.0001). The presence of a 22q11.2 deletion (odds ratio [OR] 25.4, 95% confidence interval [95%CI] 11.2–57.4, p<0.0001), a history of thoracotomy before the age of 12 years (OR 3.5, 95% CI 1.6–8.1, p=0.0027) and most complex CHD class (OR 2.3, 95% CI 1.1–4.7, p=0.0196), but not sex, were significant independent predictors of scoliosis. In the 22q11.2DS group, a right-sided aortic arch was associated with a left thoracic scoliotic curve (p=0.036).

Conclusions: The prevalence of scoliosis in the CHD population without a 22q11.2 deletion approximates that of the general population. While in the CHD population with a 22q11.2 deletion, the prevalence of scoliosis approximates that of the general 22q11.2DS population. Paediatric surgical approach and severity of CHD were weaker independent contributors as compared to the 22q11.2 deletion. The results support the importance of a genetic diagnosis of 22q11.2DS to the risk of developing scoliosis in individuals with CHD. The 22q11.2 deletion may represent a common etio-pathogenesis pathway for both CHD and scoliosis, possibly involving early laterality mechanisms.

Introduction

For over four decades, researchers and clinicians have suggested a relationship between congenital heart disease (CHD) and scoliosis (a three-dimensional rotational deformity of the spine and trunk[1,2]), for which several possible mechanisms have been proposed.[63–65] These included biomechanical forces, for example due to altered aortic configuration during development[63,64] or effects of cardiac surgery on an immature thoracic cage disturbing symmetrical growth.[60,171,172]

Scoliosis can have important consequences, including respiratory dysfunction and in severe cases necessitating brace therapy or spinal surgery.[2] The majority of patients have adolescent idiopathic scoliosis (AIS), which has an estimated general population prevalence of 1-9%, and for which the cause is still largely unknown.[2,173] It is widely accepted however that genetic as well as biomechanical factors play an important role in the etio-pathogenesis of AIS. There is a higher concordance of scoliosis in monozygotic twins (73%) and dizygotic twins (36%) than in unrelated individuals.[14] Notably, recent reports indicate that rare pathogenic copy number variants (CNVs) play a role in the development of AIS,[26,174] as they do in CHD.[175] Also, in nature AIS only occurs in fully upright bipedal man.[3,4,27]

The 22q11.2 deletion associated with 22q11.2 deletion syndrome (22q11.2DS), formerly known as DiGeorge syndrome or velocardiofacial syndrome, is a prime example of a rare pathogenic CNV.[48] The deletion has an estimated prevalence of 1 in 3000 live births and is characterized by early and later onset conditions, including CHD and scoliosis.[48] In the current study, we used data obtained from an adult CHD cohort to test the hypothesis that the higher prevalence of scoliosis in CHD is related to an underlying 22q11.2 deletion, while accounting for pediatric cardiac surgery and CHD severity.

Methods

Study Population

To determine the scoliosis prevalence in the adult (> 17 years) CHD population, patients were included from an existing sample followed at an specialized adult CHD hospital.[176–179] The data are part of ongoing studies approved by the local Research Ethics Board.

Figure 1 shows the sample derivation and individuals included and excluded from the current study. We used data available from an existing database for a well-characterized sample of adults with CHD, including CHD type (mostly tetralogy of Fallot),[178] cardiac surgical history, laterality of aortic arch and presence of musculoskeletal anomalies.[176–179] CHD complexity was classified as simple, moderate and severe, following the 2018 guidelines from the American Heart Association and American College of Cardiology.[180] We confined the sample to adults with CHD and sufficient molecular genetic data (mostly standard clinical genetic testing and/or research-based genome-wide microarray),[176–179] to determine presence or absence of a 22q11.2 deletion.[48,176] We used these molecular data to determine individuals confirmed to

have the typical chromosome 22q11.2 deletion, i.e. at least including the low copy repeat region (LCR)22A-LCR22B and most commonly involving the 2.5 megabase LCR22A-LCR22D region,[48,176] (the 22q11.2DS group), and a comparison group comprising those confirmed to have no typical 22q11.2 deletion (the no 22q11.2DS group). The comparison group was selected in a 2-3 to 1 ratio, matching for sex and CHD severity class, by a research-analyst blind to scoliosis status.

Exclusion criteria were: absence of a thorax radiograph obtained between 17 and 40 years of age, presence of an atypical nested distal (e.g. LCR22B-LCR22D, LCR22C-LCR22D) chromosome 22q11.2 deletion,[48,176] congenital spinal anomalies or variants (e.g. hemivertebra, butterfly vertebrae, Klippel-Feil), or a documented genetic or other syndromic disorder other than 22q11.2DS (e.g., VACTERL, CHARGE, Klinefelter, Goldenhar, Pallister Killian, hemihypertrophy or fetal ethanol syndromes) (Figure 1). After these exclusions the sample comprised 315 adults with CHD, either with or without a 22q11.2 deletion.

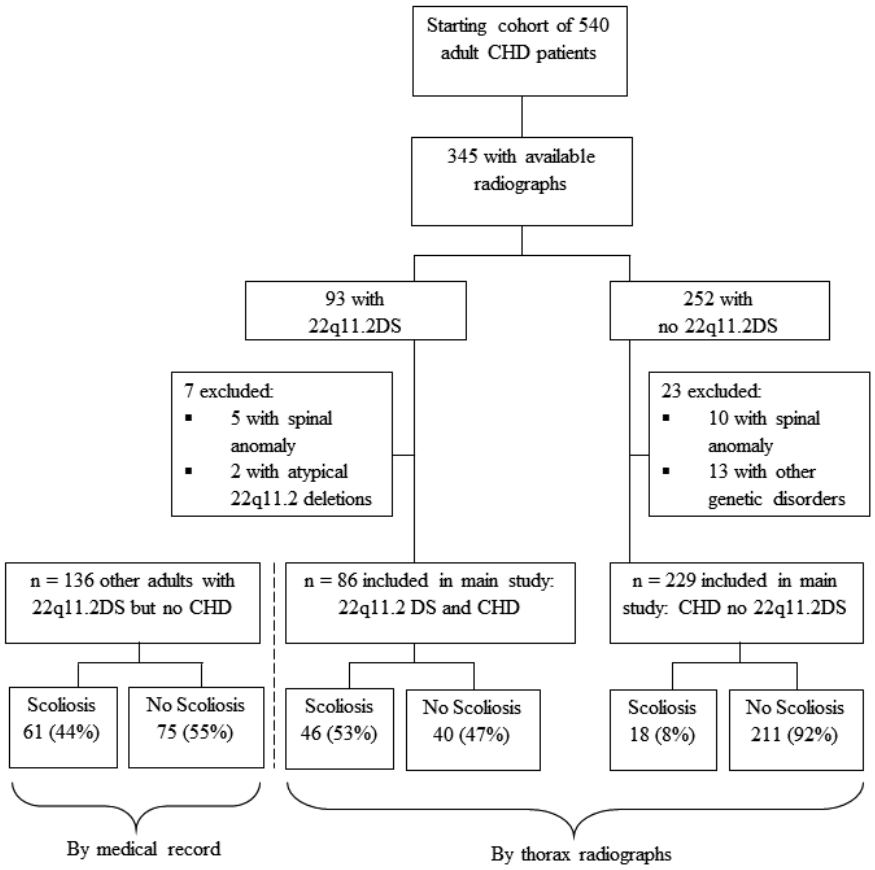


Figure 1: Flowchart of the sample studied.

Thorax Radiograph Assessment

One trained observer, who was blinded to 22q11.2 deletion status and medical history, assessed the earliest upright thorax radiograph available at the adult CHD hospital. The observer first screened each radiograph for the presence of congenital spinal anomalies (if present, patients were excluded, n=15, Figure 1) and signs of surgery in the past, including sternotomy wires for cardiac surgery (Figure 2) or spondylodesis material indicating surgically corrected scoliosis. The radiographs were then analyzed according to the Scoliosis Research Society: the observer recorded the number of thoracic vertebrae and (visible) lumbar vertebrae, the presence of scoliosis (a lateral deviation of the spine, defined as a Cobb angle (the angle between the two most tilted vertebrae) >10 degrees), the number of curves and the most severe (i.e. major) curve, the convexity of the curve, the apex and the number of involved vertebrae.[9,163] The laterality of the aortic arch was also assessed (Figure 2) and these data checked against those previously recorded in medical records.

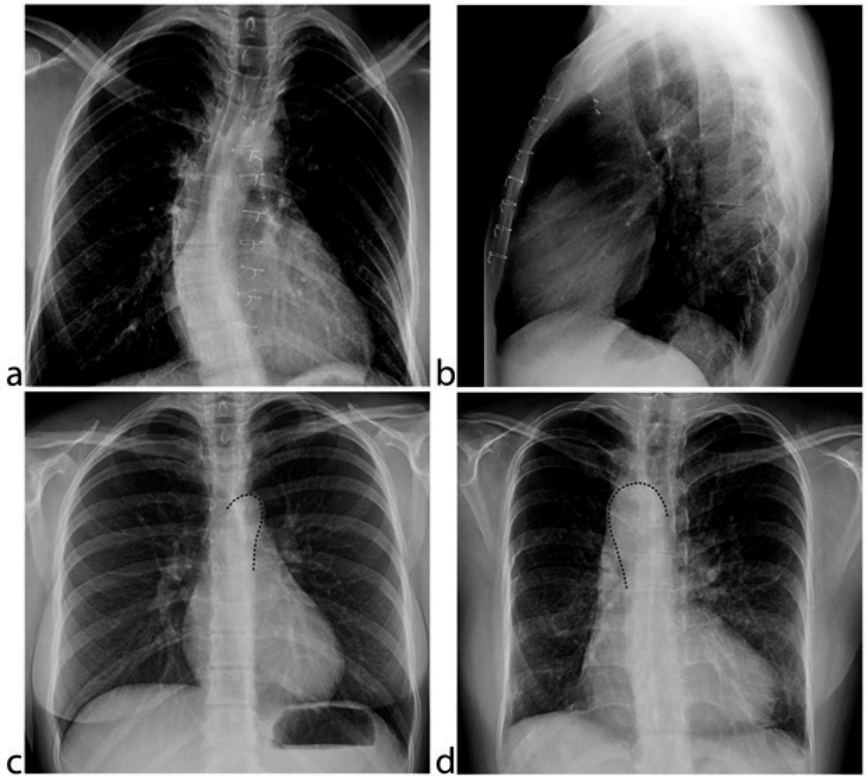


Figure 2: Findings during thorax radiography assessment.
In the case of a history of sternotomy, sternal wires can be visible from a coronal view (a) and sagittal view (b) of the thorax radiograph. While the aortic arch is normally left-sided (c), in this group of patients with CHD the aortic arch is sometimes right-sided (d). Dashed guidelines are drawn over figures 2c and 2d to indicate the aortic arch position

Scoliosis in patients with 22q11.2DS without CHD

We also determined the prevalence of scoliosis in a cohort of 136 adults with 22q11.2DS who had no CHD on echocardiogram, (as recommended in the 22q11.2DS practical guidelines[62]) using available medical records data. Comparable data were available for the cohort with 22q11.2DS and CHD, allowing evaluation of these records-based data and the determination of scoliosis based on thorax radiographs.

Statistical Analysis

Descriptive statistics were performed using Fisher's Exact test and if normally distributed (determined with Shapiro-Wilk's test) the means of continuous variables were compared using independent samples t-test. Mann-Whitney U tests were used if distribution was non-normal. The main analysis used a logistic regression model to assess possible contributory factors to the development of scoliosis in CHD: presence of a 22q11.2 deletion, sex, CHD severity class and thoracotomy under age of 12 years. The variance inflation factor and tolerance methods were used to determine that there was no multicollinearity between variables. Post-hoc chi-square (*X2*) tests and degrees of freedom (*df*) for the regression model and odds ratios (OR) and 95% confidence intervals (95%CI) for every predictor were reported. Statistical analysis was done in SPSS 25.0 for Windows (IBM, Armonk, NY, USA) and/or SAS. The statistical significance level was set at 0.05, two-tailed.

Results

A total of 315 patients with a CHD formed the main sample studied: 86 with and 229 without 22q11.2DS (Table 1). By design, there were no significant between-group sex or CHD severity class differences. Mean age at thorax radiography was significantly older in the no 22q11.2DS group (Table 1). While the majority of patients had a sternotomy before the age of 12 years, a significantly greater proportion of those in the no 22q11.2DS group had thoracotomy whereas the 22q11.2DS group was enriched for those who had no cardiac surgery before age 12 years or where there was uncertainty about the surgical approach (Table 1).

Scoliosis in adults with CHD

Of the 64 individuals with scoliosis within the CHD cohort studied, the scoliosis prevalence was significantly greater in the 22q11.2DS group (n=46, 53.5%, 95%CI: 42.7-64.2) than in the no 22q11.2DS CHD group (n=18, 7.9%, 95%CI: 4.3-11.4; p<0.0001).

The logistic regression model was highly significant (*X2*=94.6, *df*=4, p<0.0001). Consistent with our hypothesis, the presence of a 22q11.2 deletion was the most significant predictor of scoliosis (OR 25.4, 95%CI: 11.2-57.4; p<0.0001), followed by thoracotomy before the age of 12 years and CHD severity (Table 2). A secondary analysis using the same predictors (except 22q11.2 deletion) but restricting to the 229 adults with CHD and no 22q11.2DS,

showed that the regression model remained significant (*X2*=13.4, *df*=3, p=0.0039) but only thoracotomy before age 12 years was a significant predictor of scoliosis (OR 4.4, 95%CI: 1.5-13.2; p=0.0078); CHD severity was non-significant (OR 2.0, 95% CI: 0.7-5.6, p=0.183). A further secondary analysis examined the model to predict scoliosis in only patients with 22q11.2DS (*X2*=8.6, *df*=3, p=0.035); this showed no significant predictors for scoliosis, with a trend only for CHD severity (p=0.057).

Table 3 presents further details of the scoliosis in this cohort; there were no significant differences found between the two groups on the parameters assessed. Only a minority had scoliosis surgery, non-significantly fewer in the 22q11.2DS than the no 22q11.2DS group (Table 3). Six of those who did not have scoliosis surgery had thoracic scoliosis with a Cobb angle greater than 45 degrees, all in the 22q11.2DS group; no individual in the no 22q11.2DS group had a Cobb angle over 40 degrees (Table 3).

Table 1: Characteristics of the 315 adults with congenital heart disease (CHD) studied, comparing those with and without 22q11.2 deletion syndrome (22q11.2DS).

Significant findings are indicated in bold font. [a]By design, the no 22q11.2DS group was matched a priori to the 22q11.2DS group by sex and CHD severity class

[b]CHD severity class was determined following the 2018 guidelines from the American Heart Association and American College of Cardiology;[180] mild and moderate severity classes were combined given small numbers for the mild subgroup. [c]The surgical approach was determined based on the medical records. The patients could either fall in the sternotomy only group (first category), the lateral thoracotomy group with or without a sternotomy (second category) or in the group in which it was uncertain whether the patients had surgery paediatric cardiac and/or it was uncertain what kind of surgical approach was used (third category).

Variables	22q11.2DS (n=86)	No 22q11.2DS[a] (n=229)	p-value
Female sex (%)	39 (45.4%)	108 (47.2%)	0.8009[a]
Mean age in years at time of thoracic radiograph (SD)	22.7 (5.0)	26.9 (6.4)	**<0.0001**
CHD severity class[b]			
Mild-moderate	56 (65.1%)	139 (60.7%)	0.5163[a]
Severe	30 (34.9%)	90 (39.3%)	
Cardiac surgery before age 12 years[c]			
Sternotomy only	55 (64.0%)	124 (54.2%)	0.1270
Thoracotomy (with/without sternotomy)	15 (17.4%)	83 (36.2%)	**0.0016**
No cardiac surgery or uncertain cardiac surgical approach	16 (18.6%)	22 (9.6%)	**0.0339**

Table 2: Factors contributing to scoliosis risk in 315 adults with congenital heart disease (CHD). OR: Odds ratio, 95%CI: 95% confidence intervals; significant findings are indicated in bold font. Likelihood ratio for regression model: X^2=94.6, df=4, $p < 0.0001$

			Logistic regression analysis			
Predictor variables	**Total (n=315)**	**Scoliosis (n=64, 20.3%)**	**No scoliosis (n=251, 79.7%)**	**OR**	**95% CI**	**P**
22q11.2 deletion syndrome	86	46 (53.5%)	40 (46.5%)	25.4	11.2 – 57.4	**<0.0001**
Thoracotomy before age 12 years	98	24 (24.5%)	74 (75.5%)	3.5	1.6 – 8.1	**0.0027**
Severe CHD	120	32 (26.7%)	88 (73.3%)	2.3	1.1 – 4.7	**0.0196**
Female sex	147	35 (23.8%)	112 (76.2%)	1.7	0.9 – 3.3	0.1309

Scoliosis convexity and aortic arch laterality

With respect to the 51 adults with CHD and thoracic scoliosis, the majority had the typical scoliotic curve convexity to the right, with no significant difference between the 22q11.2DS and no 22q11.2DS groups (Table 3). Amongst the 35 individuals with 22q11.2DS and a major thoracic scoliotic curve, there were 21 with a normal left-sided aortic arch, 15 (71%) of whom had a right convex scoliosis curve. There were 14 with a right-sided aortic arch, 5 (36%) with a right convex scoliosis curve, demonstrating a significant association between right-sided aortic arch and left convex thoracic curve (p=0.036).

Scoliosis in adults with 22q11.2DS without CHD

There was a clinically determined history of scoliosis in 61 of 136 adults with 22q11.2DS but no CHD (44.9%, 95%CI: 36.8-53.2%). Of the 86 patients with 22q11.2DS and a CHD, 69 had both data from medical records and radiography available to assess for the presence of scoliosis; there was agreement for 61 of these 69 (88.4%).

Table 3: Radiographic parameters of the scoliosis in the 64 adults with scoliosis in the CHD cohort studied, comparing those with and without 22q11.2 deletion syndrome (22q11.2DS).
IQR= interquartile range; T=level of thoracic vertebra

Variables	22q11.2DS with scoliosis (n=46)	No 22q11.2DS with scoliosis (n=18)	p-value
Major scoliosis curve type (total n=64)			
Cervicothoracic	0 (0%)	1 (5.6%)	0.2812
Thoracic	35 (76.1%)	16 (88.9%)	0.3200
Thoracolumbar	3 (6.5%)	1 (5.6%)	1.0000
Lumbar	8 (17.4%)	0 (0%)	0.0930
Scoliosis surgery	6 (13.0%)	5 (27.8%)	0.2667
Subset with major thoracic scoliosis without surgery (n=41)	**(n=30)**	**(n=11)**	
Median degree of Cobb angle (IQR)	21.3 (17-41)	23.7 (20-28)	0.9758
Range	11-111	13-40	
Median number of vertebrae involved (IQR)	6 (5-7)	6 (6-8)	0.3309
Range	4-9	4-8	
Apex of curve (vertebra)	T6 (T4-T8)	T8 (T6-T9)	0.0675
Range	T3-T9	T3-T10	

Discussion

For the past four decades, the role of CHD in development of scoliosis has been noted as a partial explanation of the enigma of scoliosis pathogenesis but in no previous study was a major risk factor for both entities taken into account: the 22q11.2 deletion. The current study provides the first evidence of the significant impact of the 22q11.2 deletion in the development of scoliosis in a cohort of adult patients with CHD. Importantly, the prevalence of scoliosis in the no 22q11.2DS CHD cohort was found to be nearly similar to the prevalence of scoliosis in the general population. [2,173]

The results are consistent with previous studies reporting high prevalence of scoliosis in 22q11.2DS of about 50%,[156] compared with general population expectations of about 1-9%.[2,173] The scoliosis prevalence in the general population varies greatly, with estimates from 0.5-5.2% based on physical examination.[181] However, in two independent studies using thorax radiographs and a definition of scoliosis as a Cobb angle of >10 degrees, the scoliosis prevalence in the general population was reported as 9.3% and 13.4% respectively.[173,182] The scoliosis prevalence of 8% we found in the no 22q11.2DS CHD population in this study thus appears comparable to that of the general population when assessed radiographically. Taken together, the results may indicate that in previous studies unrecognized 22q11.2DS could be a confounder for reported associations between CHD and the development of scoliosis.

AIS is more common in females,[170] whereas in the 22q11.2DS population in this, as other studies, the prevalence of scoliosis is about equal between females and males.[156] In the general population, early onset scoliosis (age <10 years) comes closer to a 1:1 female:male ratio.[169,183] Prospective studies in 22q11.2DS, investigating the differences between males and females in the development of scoliosis and between patients with and without a scoliosis, might therefore help shed light on the scoliosis development in the general population.[184]

The type of scoliosis, both in the 22q11.2DS and no 22q11.2DS group is comparable to that of the general population with the majority having a major thoracic curve.[185] This finding supports the hypothesis that the 22q11.2 deletion population, which has a high risk to develop an idiopathic-like scoliosis, can be used as a model to study the development of scoliosis.[156,184]

Recent studies have suggested a general role for CNVs in the development of AIS.[26,174] Sadler et al. reported that 16p11.2 duplications explain nearly 1% of AIS cases, in a study restricted to patients without major development impairment or major congenital anomalies.[26] Given that 22q11.2DS population is characterized by broad phenotypic heterogeneity, with developmental impairment and congenital anomalies (e.g. CHD) as common features, many patients with 22q11.2DS and scoliosis may have been excluded from the Sadler et al. study. Nonetheless they reported two patients with 22q11.2DS in their cohort of 1197 AIS patients, reinforcing the importance of considering clinical genetic testing by microarray in AIS, as in CHD.[175]

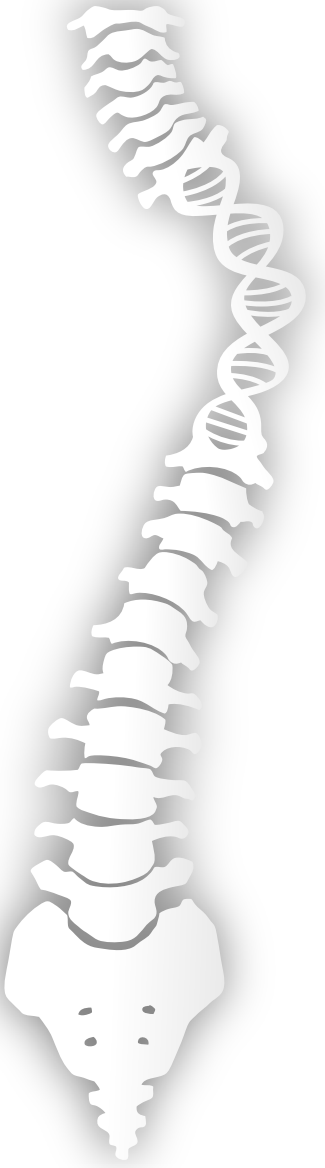
In the current study, for the main regression model, and in the secondary analysis of the no 22q11.2DS group, pediatric thoracotomy was a significant predictor of scoliosis. This could be explained by the fact that a thoracotomy is an asymmetrical procedure on an immature thoracic cage, which may lead to a disturbance of symmetrical growth and an increased scoliosis risk, as proposed by others.[60,171,172] However, in the literature, results are mixed as to whether and which type of cardiac surgery, including sternotomy, is associated with scoliosis risk.[60,64,168,171,172] Further studies, taking genetic syndrome status into account, are needed.

A right-sided aortic arch is rare with an estimated incidence of 0.1% in the general population, yet in 22q11.2DS a right-sided aortic arch is relatively common.[186] In the current study of CHD, in patients with 22q11.2DS we found that a right-sided aortic arch was associated with a left convex curve in patients with a major thoracic scoliosis. There was a similar finding in a previous study of 119 patients with CHD where all eight scoliosis patients with a right-sided aortic arch had a left convex thoracic curve.[64] This phenomenon may be explained by the principle of inverted organ anatomy and spinal lateralization. In patients with scoliosis, one study found that scoliotic curve convexity was predominantly to the right in patients with normal organ anatomy (situs solitus), and to the left in patients with situs inversus totalis.[187] Also, laterality of the center of mass in the thorax is related to slight spinal rotation in the opposite direction in patients without scoliosis.[188] Although no causality can be concluded

based on these former studies, our findings make the biomechanical theory of an aortic arch to the right side increasing the chance of a left convex scoliotic curve appear plausible. As the first study to report the scoliosis prevalence in patients with CHD, while taking 22q11.2DS into account this study had several advantages but also some limitations. The study was based on a relatively large existing database of patients with CHD, in which all patients had genetic testing to confirm or rule out the 22q11.2 deletion.[176–179] Moreover, in order to find the prevalence of scoliosis not caused by congenital spinal malformations, patients with a congenital spinal malformation were excluded. However, since the radiographs were made in order to visualize the thorax, the radiographs did not capture the entire spine. Therefore, it might be possible that patients with an undetectable lumbar congenital malformation remain in the sample. We excluded radiographs of patients under age 17 years and older than 40 years, in order to find the definitive prevalence of scoliosis: The younger age group may not yet have developed a scoliosis, whereas scoliosis in the older age group may be related to degenerative scoliosis.[189] We also excluded patients with other syndromic forms of CHD; scoliosis is known to occur more often in other syndromes.[190]
In our determination of the scoliosis prevalence in patients with 22q11.2DS without CHD a limitation was that radiographs were not available, thus prevalence was based on medical records. However, there was a high level of agreement between medical records data and thorax radiographs for the 22q11.2DS group with CHD.
In conclusion, the results of this study support the importance of clinical genetic testing for a chromosome 22q11.2 deletion in patients with CHD, and the relevance of 22q11.2DS in understanding the risk for scoliosis in the CHD population. With respect to the CHD population without 22q11.2DS, the scoliosis prevalence is comparable to that of the general population, with a slightly increased risk for those who underwent a thoracotomy as a child. These findings suggest that the 22q11.2 deletion may represent a common genetic pathway for the development of CHD and scoliosis. Future studies using this genetic model may help determine the pathogenesis of both of these complex developmental conditions.

B

CHAPTER 6



The 22q11.2 Deletion Syndrome as a Model for Idiopathic Scoliosis - A Hypothesis

Based on: Homans JF, Reuver S De, Breetvelt EJ, Vorstman JAS, Deeney VFX, Flynn JM, McDonald-McGinn DM, Kruyt MC, Castelein RM.

Based on: The 22q11.2 Deletion Syndrome as a model for idiopathic scoliosis – a Hypothesis . *Med Hypotheses*. 2019;127:57–62.

Abstract

Adolescent idiopathic scoliosis (AIS), defined as a lateral deviation of the spine of at least ten degrees, is a classic enigma within orthopedics and affects 1-4% of the general population. Despite (over) a century of intensive research, the etiology is still largely unknown. One of the major problems within all existing AIS research is the fact that most patients come to medical attention after onset of the curve. Therefore, it is impossible to know whether current investigated parameters are causative, or an effect of the scoliosis. Moreover, up until now there is no known animal model that captures the core features of AIS. In order to identify causal pathways leading to AIS we propose another approach, which has been of great value in other medical disciplines: To use a subset of the population, with a higher risk for a certain disease as a “model” for the general population. Such a “model” may allow the identification of causative mechanisms that might be applicable to the general population. The 22q11.2 deletion syndrome (22q11.2DS) is the most common microdeletion syndrome and occurs in ~1:3000-6000 children and 1:1000 pregnancies. Nearly half of the population of patients with 22q11.2DS develop a scoliosis that in most cases resembles AIS as far as age at onset and curve pattern. We postulate that within 22q11.2DS certain causal pathways leading to scoliosis can be identified and that these are applicable to the general population.

Introduction

Scoliosis is a three-dimensional (3-D) rotational deformity of the spine and trunk which has major consequences for the patient in terms of self-image, pain and the serious impact of possible invasive treatments (brace therapy and/or scoliosis surgery).[1,2] A scoliosis is defined as a lateral deviation of the spine of at least ten degrees Cobb angle (Figure 1).[191] Several known causes for scoliosis exist (congenital and neuromuscular scoliosis). However, the majority of patients have an adolescent idiopathic scoliosis (AIS), for which the cause is, to a large extent, unknown. The majority of AIS patients are healthy and well-functioning up to the age of the pubertal growth spurt. AIS affects 1-4% of the general population and is a classic enigma within orthopedics.[2] Although recent research has elucidated the role of genetics and the biomechanics of the upright human spine, the true cause of this disorder, and thus the potential for prevention, has remained largely undiscovered.[2,4,13,14,192,193] As a result, surgery is the main treatment option in AIS patients with curves exceeding 45-50 degrees.[2,10] There are two important reasons why there is such a variety of theories and why the etio-pathogenesis, is still to a large extent unraveled:

1. Patients with AIS are identified as such after the onset of the scoliosis. Therefore, it is impossible to identify causative factors of the curve onset. As a consequence, within current research, it is unknown whether correlated parameters are the cause, the consequence or an epiphenomenon of the scoliosis.[2,193]
2. There is no animal that, without experimental intervention, develops a scoliosis. Specific genes are known to play a role in the development of scoliosis, as shown by the curvature developed within e.g. the mutant guppy syndrome curveback or POC-5 zebrafish.[30,31] However, as shown by multiple large genetic studies it clear that the development of idiopathic scoliosis is not limited to one gene and/or pure mendelian inheritance.[2] On the contrary, the development of idiopathic scoliosis is known to be multifactorial (a combination of genetic, metabolic, the central nervous system, biomechanics and environmental factors).[2] Thus, in order to investigate the (combination of) multiple pathways leading to scoliosis and to understand the development of idiopathic scoliosis we have to investigate man: Only humans carry the body's center of gravity straight above the pelvis due to a pelvic and lumbar lordosis. All other animals, quadrupedal and bipedal alike, carry the body's center of gravity in front of the pelvis. Man has a unique biomechanical loading of the spine that introduces dorsal shear forces, that have been shown to cause a loss of rotational stability (Figure 2).[3,4,18]

There is a large gap of knowledge in the etio-pathogenesis of scoliosis, which needs to be bridged in order to reach the next step in scoliosis care: Primary prevention (prevent the development of scoliosis) and/or secondary prevention (identify the patients in an early stage in order to prevent surgery). Therefore, we propose another possibility, which has been of great value in other medical disciplines: To prospectively investigate a subset of the population, with a higher risk for a certain disease, as a model for the general population. For

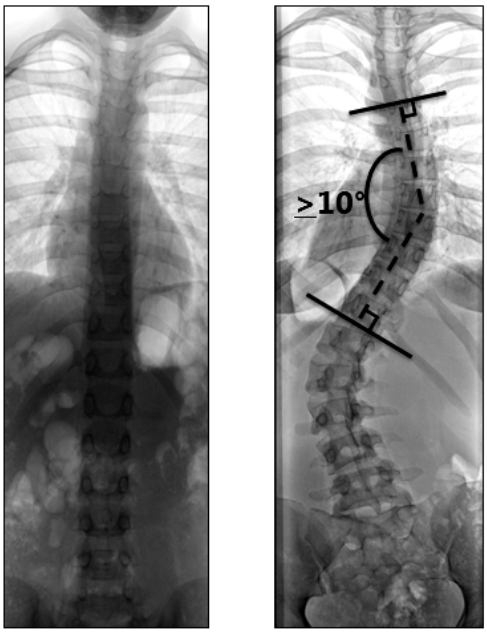


Figure 1: A scoliosis (right image) is diagnosed as curve ≥ 10 degrees.

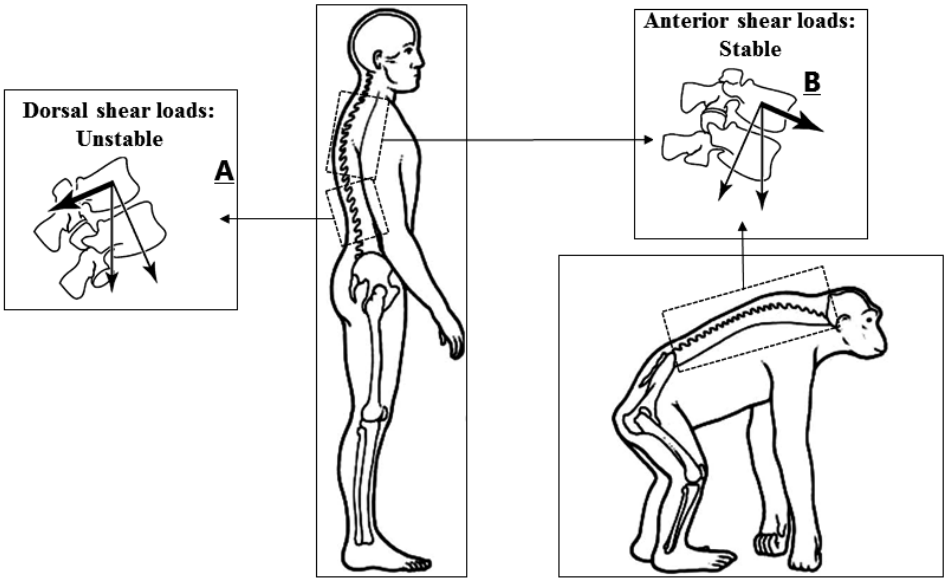


Figure 2: There are unique differences between human and all other animals.
Humans have the center of gravity straight above the pelvis, while all other animals (including the bipedal ones) carry the body's center of gravity in front of the pelvis, leading to different biomechanical circumstances. Certain parts of the human spine experience dorsally directed shear loads (A), while other parts and all other animals only have anteriorly directed shear loads (B). The dorsal shear loads have been shown to decrease rotational stability in the affected segments. Compiled from Castelein et al.[3]

AIS, patients with the 22q11.2 deletion syndrome (22q11.2DS) could be such a population to study, since 50% develops a scoliosis and the majority of patients has a scoliosis resembling AIS as far as age at onset and curve pattern. [156]

In humans, 22q11.2DS, is the most common microdeletion syndrome with a prevalence of ~1 in 3-6 thousand live births and 1 in 1000 unselected pregnancies.[42–45] Patients with 22q11.2DS, prior to the identification of the chromosomal etiology, may have been diagnosed with a variety of clinical described entities such as the DiGeorge syndrome, velocardiofacial syndrome or conotruncal anomaly face syndrome.[62] The clinical features associated with this condition vary greatly within and between individuals.[48] Numerous clinical features are now known to be associated with 22q11.2DS including common conditions such as congenital heart disease (CHD, 25-60%), endocrinopathies such as hypocalcemia (55%), immunodeficiency (77%), cognitive deficits (>95%) and psychiatric illness including schizophrenia (25%), and less frequently associated problems such as congenital diaphragmatic hernia and imperforate anus.[48,194] On the other hand, the clinical features of 22q11.2DS can be relatively mild and the diagnosis tends to be missed. In fact, we treated multiple patients for presumed AIS that later turned out to suffer from 22q11.2DS. Scoliosis is present in about 50% of patients with 22q11.2DS, compared to about 1-4% in the general population.[156,195] We postulate that the population of individuals with 22q11.2DS can be used as a model to study scoliosis in a unique, prospective manner, starting from genetic risk to the emergence of first signs of (spine) abnormalities. We hypothesize that these insights will be informative for our understanding of the causal pathways leading to scoliosis in the general population.

Lessons Learned from other High-Risk Populations

In the field of gynecology, the Sjögren-Larsson syndrome is proposed as a model for preterm labor and in the field of psychiatry 22q11.2DS is regarded as a model for idiopathic schizophrenia.[196,197] The 22q11.2 deletion is found to be the most prevalent and strongest single genetic risk factor for developing schizophrenia. The correlation between 22q11.2DS and schizophrenia has long been established; multiple studies confirm that approximately one out in four patients with 22q11.2DS develop schizophrenia.[48,196,198] On the other hand, within the general population, out of all patients with schizophrenia only one in 100-200 have the 22q11.2 deletion.[48,199] This led to the establishment of the International 22q11.2DS Brain and Behavior Consortium (a large group of international experts representing 22 clinical and five genomic sites) that aims to identify causal mechanisms leading to schizophrenia within 22q11.2DS and elaborating on that, investigate if these causal mechanisms are applicable to the general population. The large a-priori chance of conversion to schizophrenia within 22q11.2DS, leads to a dramatic decrease in required sample size to identify causative mechanisms of schizophrenia within 22q11.2DS.[41] Using this approach, multiple studies revealed that several parameters, such as prematurity, lower global neurocognitive

performance, poorer premorbid functioning and a decrease in intelligence quotient years before the onset of schizophrenia, pose an increased risk of developing schizophrenia at a later stage.[41,198,200]

Obviously, preterm labor and schizophrenia are two disorders very distinct from scoliosis, however the onset of all three are thought to be multifactorial. By the use of a model as proposed, we can prospectively study one or more causative factors within a subgroup, and possibly extrapolate these findings to the general population.[2,48,197]

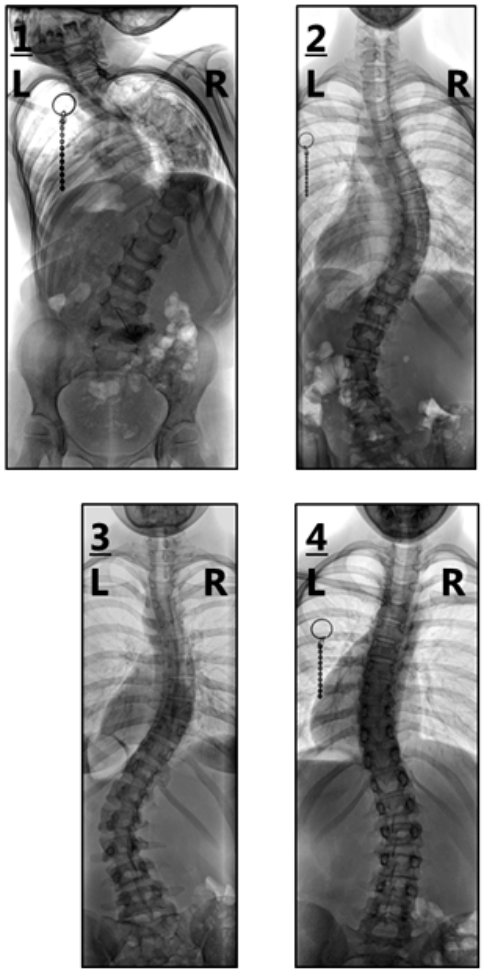


Figure 3

1: A five year old spinal muscular atrophy patient with a scoliosis neuromuscular scoliosis (C-shape, right thoracic)

2: A 14 year old patient with an adolecent idiopathic scoliosis (S-shape, right thoracic, left lumbar)

3: A 16 year old 22q11.2 Deletion Syndrome patient with a scoliosis (S-shape, right thoracic, left lumbar)

4: A seven year old 22q11.2 Deletion Syndrome patient with a scoliosis (S-shape left thoracic, right lumbar)

Neuromuscular versus Idiopathic Scoliosis

Neuromuscular scoliosis is a distinct spinal curvature which is caused by a disorder of the muscles and/or central nervous system. Common causes are cerebral palsy, myelodysplasia, spinal muscular atrophy (SMA) or Duchenne's muscular dystrophy.[201] These patients do not have the ability to maintain postural balance, are often wheelchair bound, and develop a C-curved scoliosis already at a very early stage of development (Figure 3). Both the underlying condition, the age at onset and the curve type are very different from AIS. Moreover, in neuromuscular scoliosis (e.g. Duchenne and spinal muscular atrophy) the risk of curve progression and subsequent surgical treatment is much higher as compared to AIS.[2,202]

Scoliosis within 22q11.2DS

Scoliosis is an important part of the multi-morbidity seen in association with 22q11.2DS, with a prevalence of about 50%.[156] In 22q11.2DS, as well as in the general population, scoliosis usually develops during the growth spurt.[2,156] Moreover, the majority of patients with 22q11.2DS have an idiopathic-like curve pattern. Lastly, although during development gross motor milestones like crawling, cruising, walking are slightly behind peers and siblings, patients with 22q11.2DS, in general are fully ambulant.[203]

This leads to our hypothesis that within 22q11.2DS causal pathways resulting in scoliosis can be identified and that these may also play a role in the general population.

Testing the Hypothesis

In order to test the hypothesis that the scoliosis in patients with 22q11.2DS can serve as a model for idiopathic scoliosis, four important factors, of the development of idiopathic scoliosis, should be determined within the 22q11.2DS population:

1. Does the scoliosis in 22q11.2DS behave like AIS?
2. What is the prevalence of intraspinal anomalies in 22q11.2DS?
3. What is the neuromuscular status of patients with 22q11.2DS as compared to AIS?
4. What is the condition of essential soft tissue structures, such as the intervertebral discs (IVD)?

1. Does the scoliosis in 22q11.2DS behave like AIS?

In order to investigate whether the 22q11.2DS is comparable with AIS, both the curve pattern and the progression rate of patients with 22q11.2DS should be compared with AIS. This is illustrated by the fact that within neuromuscular scoliosis both of these factors are very different as compared to AIS. The majority of patients with 22q11.2DS have an idiopathic-like curve scoliosis pattern and a relatively mild scoliosis as shown by the fact that 16% of all 22q11.2DS scoliosis patients eventually require scoliosis surgery.[52,156] Within AIS, 13.2% of the patients require brace and/or surgical treatment (2.4% of all the AIS patients

require surgical treatment).[170] It is not possible to compare the progression rate of AIS and 22q11.2DS scoliosis based on the need for surgical treatment. With the introduction of brace therapy, the need for scoliosis surgery in AIS decreased dramatically.[8] In 22q11.2DS, associated symptoms such as CHD and psychological status, can influence the compliance for brace therapy. Therefore, we should focus on the rate of progression. According to a recent systematic review by Negrini et al. the pooled estimated progression prevalence (defined as >5 degrees curve progression) within juvenile and adolescent idiopathic scoliosis was 49% and the rate of scoliosis progression ranged from 2.2 to 9.6 degrees per year. We hypothesize that the patients with 22q11.2DS with an idiopathic-like curve have a comparable progression rate as in idiopathic scoliosis.

2. What is the prevalence of intraspinal anomalies in 22q11.2DS?

In a recent systematic review it was shown that, approximately ten percent of all AIS patients have intraspinal anomalies as shown on MRI.[204] In some cases this is linked to the development of scoliosis (e.g. a tethered cord) and subsequently in that case it is not deemed as AIS. However, how the majority of the intraspinal anomalies found in AIS relate to the development of idiopathic scoliosis remains unclear.[204] Therefore, there is no consensus on whether all AIS patients, prior to surgery, should receive an MRI or only the patients with atypical curves or abnormal neurologic findings.[204] From the point of view of our hypothesis it would be preferable if the scoliosis patients with 22q11.2DS have a similar percentage and/or a similar sort of intraspinal anomalies as AIS patients and not anomalies that are directly related to scoliosis development. However, it is currently unknown whether patients with 22q11.2DS, with an idiopathic-like curve, have a similar rate of intraspinal anomalies as compared to the AIS population.

3. What is the neuromuscular status of patients with 22q11.2DS as compared to AIS?

Although AIS patients are -by definition- considered to be normal apart from their spinal deformity, various subtle differences that may be cause or effect, with the normal population have been described. As discussed, there is a large difference between AIS patients and neuromuscular scoliosis patients with regards to their postural balance and body control. However, in AIS, there are small differences with respect to the neuromuscular status as compared to the general, non-scoliotic population. For example, in a gait analysis study, there was a significantly higher postural instability in AIS that included limb load symmetry, sway length and velocity in anteroposterior and latero-lateral directions. [205] Once again, it cannot be determined if these differences are the cause or the effect of the disorder. It is currently unknown whether the subtle neuromuscular differences (as present in AIS) also occur between patients with 22q11.2DS with and without a scoliosis. More research should be performed on the possible neuromuscular differences in patients with 22q11.2DS with and without scoliosis, in order to, analyze whether these differences are causative or an effect of the scoliosis.

4. What is the condition of essential soft tissue structures, such as the intervertebral discs (IVD)?

In AIS patients, it was demonstrated that the curves were characterized by a much greater deformation in the intervertebral discs (IVD) as compared to the vertebral bodies.[206,207] The increase in curve magnitude, during adolescent skeletal growth and maturation, occurs mostly through disc wedging during the rapid growth spurt and vertebral wedging occurs later and to a lesser extent.[208] In other words, within the general population it is known that the intervertebral disk plays an important role in the development of scoliosis. Yet, whether there are primary IVD differences between the population that does and does not develop a scoliosis is unknown. Within 22q11.2DS, we should analyze the possible disc property differences of patients with and without scoliosis. Hereafter, with intensive monitoring of the patients with 22q11.2DS starting at a young age, we have the opportunity to analyze possible differences in the disc properties before the onset of the scoliosis.

Patients with 22q11.2DS are prone to develop scoliosis; in 22q11.2DS nearly half of the patients develop scoliosis, while within the general population scoliosis occurs in 1-4%. The major question is why do 50% of the patients with 22q11.2DS develop scoliosis and moreover what are the differences between the patients with 22q11.2DS with and without a (progressive) scoliosis. There may be small differences between the patients with 22q11.2DS and the general population (e.g. a slight delay in milestone development). Yet, as opposed to AIS, within 22q11.2DS we have the opportunity to compare the parameters before the onset of the scoliosis and thus truly determine whether these parameters are the cause or the consequence of the scoliosis. Our hypothesis is that the 22q11.2DS scoliosis behaves the same as compared to AIS and by prospectively identifying differences between the patients with 22q11.2DS with and without a scoliosis, we can identify causal mechanisms between these groups and subsequently expand these findings to the general population.

Discussion

Scoliosis has severe consequences for the patient in terms of self-image and pain and in severe cases possible cardiopulmonary compromise.[1,209] Moreover, surgical treatment as well as brace therapy, that consists of rigid and constraining braces that have to be worn extensively in an emotionally vulnerable period of life, is a severe burden on the patient. Last, apart from the impact of the spinal deformity on the quality of life of the patient, scoliosis patients are also a considerable economic burden to society: it is the spinal deformity most frequently seen by general practitioners, pediatricians and orthopedic surgeons, and current therapies are very costly.[2,210,211] Therefore, the ultimate goal of scoliosis care is to prevent the development and/or deter progression, thereby eliminating the need for brace/surgical treatment.

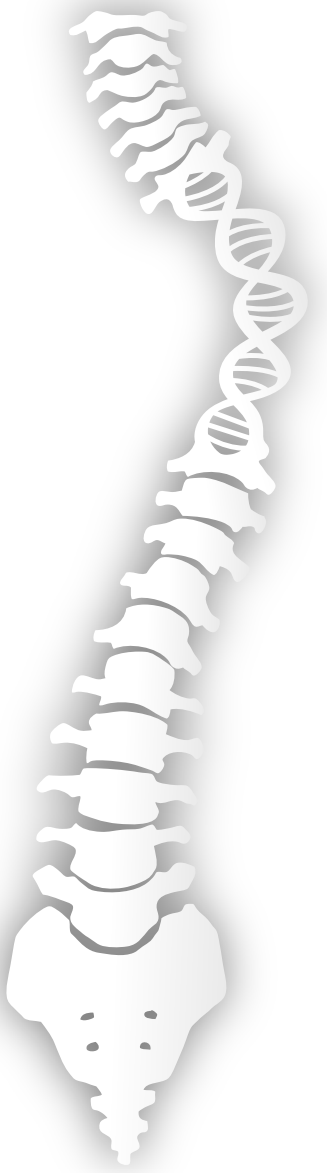
The first step to prevent the development of scoliosis within a patient is to identify the etio-pathogenesis of this deformity. It is well recognized that the development of scoliosis is multifactorial, and in order to truly elucidate its cause, new approaches are needed.[2]

The identification of causal pathways leading to scoliosis within 22q11.2DS, will lead to a large improvement in care for the population of patients with 22q11DS, both in primary and secondary prevention. At the same time, we hypothesize that the causative mechanisms leading to scoliosis within 22q11.2DS are applicable to the general population and thereby, this can lead to the improvement of care for a disease troubling 1-4% of the general population. The majority of patients with 22q11.2DS have an idiopathic-like curve scoliosis pattern and a relatively mild scoliosis as shown by the fact that 16% of all patients with 22q11.2DS eventually require scoliosis surgery.[52,156] To investigate the differences between patients with 22q11.2DS with and without a scoliosis and with a non-progressive and (rapid) progressive scoliosis will be the next step. Within the 22q11.2DS population we have the opportunity to analyze metabolic, the central nervous system, biomechanics and environmental factors, but also genetic factors: Possible differences within the deletion and/or genetic variances outside of the 22q11.2 deletion.

From a scientific perspective, a limitation of the 22q11.2DS population as a model for the general population could be that congenital heart disease (CHD) is common (25-60 %) in patients with 22q11.2DS.[48,156,194] The limitation would be that already four decades ago, a correlation between the appearance of CHD and the development of scoliosis in the general population was shown.[58,63,64] Multiple theories were formed for why CHD leads to a scoliosis. First, different biomechanical forces, due to altered aortic configuration, could possibly cause an increased risk in developing scoliosis.[63,64] Second, surgery on an immature thoracic cage may result in altered growth and an increased scoliosis risk.[58,59,171] Yet, in a recent study, no relation between a thoracotomy/sternotomy for CHD and scoliosis was found.[168] In other words, there are conflicting results on the correlation between CHD and scoliosis. Moreover, genetic testing of (all) patients was not performed in any of these studies. Subsequently, it is unknown whether (a subset of) the included patients in these studies may have had 22q11.2DS.[58,59,63,64,171] This is important because 22q11.2DS is the second greatest risk factor for CHD and the symptoms of 22q11.2DS can be mild, leading to underdiagnoses of 22q11.2DS.[48,62] Interestingly, in a recent study, in which all the patients had the 22q11.2DS diagnosis there was no association between CHD and scoliosis.[156] In other words, it is possible that actually 22q11.2DS was the reason that these patients developed both a CHD and a scoliosis.

Patients with 22q11.2DS are at an increased (~25 times fold) risk for the development of scoliosis. The major question is what are the factors that determine whether a scoliosis develops, or not. Moreover, what are the factors that lead to a progressive scoliosis that necessitates surgery in 16% of the patients with 22q11.2DS. Within 22q11.2DS we have the opportunity to truly investigate this multifactorial pathway and, in the end, possibly, extrapolate these results to the general population.

CHAPTER 7



The 22q11.2 Deletion Syndrome as a possible Model for Idiopathic Scoliosis

Homans JF, Kruyt MC, Schlösser TPC, Houben M,L Deeney VFX, Crowley T, McDonald-McGinn DM, Stücker R, Pasha S, Castelein RM.

Based on: The 22q11.2 Deletion Syndrome as a possible model for Idiopathic Scoliosis. **under Review.** 2019

Abstract

Purpose: A problem in idiopathic scoliosis (IS) etiology research is that patients are identified after curve onset. In order to distinguish between cause and effect of the disorder, there is a need to study the morphology of the spine before disease onset. Such a study is not possible in the general population and other medical disciplines have developed a new approach: use a subset of the population, with a higher risk for the disease, as a "model" for the general population. Our goal is to explore whether scoliosis in 22q11.2 deletion syndrome (22q11.2DS) can be used as a model for IS.

Methods: All ambulant patients with 22q11.2DS (age >4) with non-congenital scoliosis were identified. According to previously developed criteria, we determined whether patients had a curve that resembled typical morphology of idiopathic scoliosis. For individuals with at least one-year follow-up the curve progression (degrees/year) was calculated. Lastly, MRI reports were screened for intraspinal anomalies.

Results: 185 patients were included (female=92), median Cobb angle 16° (interquartile range: 13-25) and 43% had a thoracic curve. 182 patients (98.4%) had an idiopathic-like curve pattern. Forty-eight patients had at least one-year follow-up, of whom 26 (54%) had a progressive curve. Median progression in that group was 2.5 degrees/year. Lastly, four out of 38 patients (10.5%) had intraspinal abnormalities.

Conclusion: This study provides the first evidence that ambulant patients with 22q11.2DS with scoliosis can, possibly, be used as a patho-anatomic model in order to study the changes in spinal morphology before the onset of scoliosis.

Introduction

Idiopathic scoliosis (IS) is a classic enigma in orthopedics; despite decades of research the cause of this disease is still not fully unraveled.[2] Recent research has shown important findings concerning the role of genetics and the biomechanics of the upright human spine, yet the true cause of this disorder, and thus the potential for prevention, has remained largely undiscovered.[2,4,13,14,192,193] One of the problems in etiology research is that patients are identified only after onset of the curve. As a consequence, it is unknown whether the many factors that have been found to be correlated with scoliosis are causative, or rather an effect of the deformity.[2,193] Moreover, there unfortunately is no suitable animal model for IS.[27] This problem is not unique to scoliosis research, and especially in psychiatry, an innovative approach has led to important new insights: the use of a subset of the population, with a high risk for a certain disease, as a "model" for the general population.[41] Although this approach obviously also has its scientific limitations, the important advantage is that the population at risk can be identified before the onset of the disease.

The 22q11.2 deletion syndrome (22q11.2DS), the most common cause of DiGeorge syndrome, velocardiofacial syndrome, conotruncal anomaly face syndrome, Cayler Cardiofacial syndrome, and a subset of patients with autosomal dominant Opitz G/BBB syndrome, is the most common microdeletion syndrome in man with an estimated prevalence of 1:3000-6000 live births, with an incidence of scoliosis of around 50%.[48] It was recently hypothesized that 22q11.2DS could be used as a model for IS.[184] A subset of the patients with 22q11.2DS have full spine radiographs before the onset of the spinal deformity, and thus, if 22q11.2DS could serve as a model this group can provide unique insights in the development of scoliosis since there is the possibility of studying the pre-scoliotic spine. The main goal of this study is to determine whether scoliosis observed in 22q11.2DS corresponds to idiopathic curves as far as curve pattern, curve behavior and the occurrence of intraspinal abnormalities as found on MRI. We hypothesize that 22q11.2DS scoliotic patients will have a morphological idiopathic-like curve with a progression rate comparable to IS and that the occurrence of intraspinal anomalies is comparable with the intraspinal anomalies found in IS.

Methods

Population

A retrospective cohort study was performed (Figure 1). For the radiographic analysis of the curve pattern and curve progression the patients with 22q11.2DS databases from two specialized 22q11.2DS and scoliosis centers were searched for all patients with 22q11.2DS with whole spine radiographs. Ambulant patients with a laboratory confirmed (FISH,MLPA, CGH or SNP-array) 22q11.2 deletion, full spine coronal X-ray, age >4 years (juvenile and adolescent idiopathic scoliosis, determined as Cobb angle > 10 degrees) on first radiograph

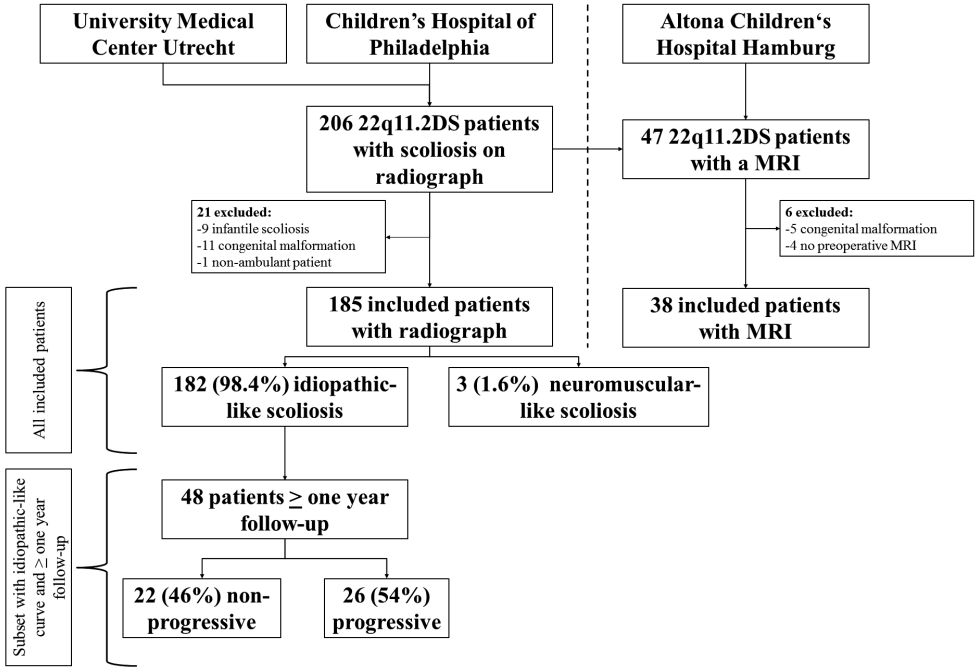


Figure 1: Flowchart of the excluded and included patients.
22q11.2DS = 22q11.2 Deletion syndrome

were included. Patients with, infantile idiopathic scoliosis, congenital scoliosis and non-ambulant patients were excluded. Moreover, sitting/suspension radiographs were excluded for sagittal analysis. Gender, age at time of radiograph(s), and data on the presence / absence of co-morbidity was collected. For curve progression analysis, all patients with at least one year of radiographic follow-up were included (Figure 1). For MRI analysis, three cohorts from specialized 22q11.2DS clinics were used. All scoliosis patients with an MRI, obtained due to a fast-progressive curve or as pre-operative screening purposes, were included.
The local Ethical Review Boards of the hospitals approved this study and waived the necessity of explicit (parental) informed consent, since data were collected during standard care and were handled anonymously.

Radiographic analysis
One trained observer (JH) analyzed all the radiographs. The researcher was blinded for clinical data at the moment of radiographic analysis and all the radiographs were analyzed in chronological order, from first to most recent radiograph.
At first, the curve location of the primary curve (thoracic, (thoraco)lumbar), coronal balance (absolute difference from neutral), sagittal balance, kyphosis and lordosis, Risser stage and open/closed triradiate cartilage were measured according to the Scoliosis Research Society criteria.[162] Next, curve morphology was determined based on the first radiograph according to

the criteria by Abul-Kasim et al.: the combination of a Cobb to Cobb curve length > 8 vertebrae, a C-shaped curve and the location of the lower-end vertebrae at the lowest or second lowest lumbar vertebrae was determined as a non-idiopathic curve pattern (Figure 2).[212]

Our goal was to identify whether the morphological idiopathic-like 22q11.2DS scoliosis behaves comparable with IS scoliosis. Therefore, in the group considered as idiopathic-like and with at least one-year follow-up, the presence of curve progression was based on the difference in magnitude of the major curve between the first scoliosis radiograph and the last radiograph. An increase of > 5 degrees major Cobb angle was considered progressive.[213] If a patient was indicated for brace therapy and/or surgery, the curve was considered progressive. Finally, the progression rate per year was calculated based on the first and last radiograph. If a patient received brace therapy or surgery, the progression rate was determined from the first radiograph until the last radiograph before the start of treatment.

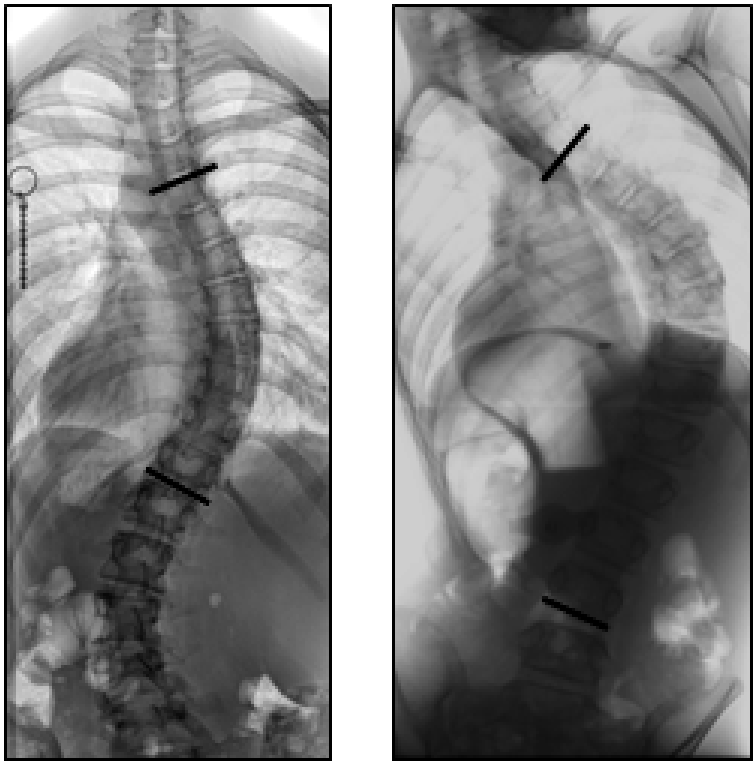


Figure 2: Differences between idiopathic-like and neuromuscular curves
Left image: An idiopathic-like curve: An S-shaped curve. The apex of the major curve is located at T9, with a curve length of 8 vertebrae. **Right image:** A neuromuscular curve: A C-shaped curve, with a curve length of 12 vertebrae and a lower-end vertebra located at L4.

MRI analysis

The whole spine MRI reports of patients with 22q11.2DS without congenital anomalies were screened in three university hospitals. In the hospitals the MRI's were assessed by musculoskeletal (child) radiologists. The MRI's were made because of rapid progression and/or for pre-operative screenings purposes. The reports from the MRI's were screened on the presence/absence of anomalies as described in a recent systematic review (Table 3).[204]

Statistical analysis

The categorical variables, gender and progressive/non-progressive scoliosis, were compared by the Fisher's exact test. Continuous variables were compared by t-test or by Mann-Whitney U test, for normally and non-normally distributed data, respectively. All statistical analyses were conducted with the Statistical Package for the Social Sciences (IBM Released 2012. IBM SPSS Statistics for Windows, V.21.0: IBM). A p-value of <0.05 was considered significant.

Results

Radiographic results

A total of 206 patients with 22q11.2DS were diagnosed with a scoliosis based on a whole spine radiograph, after exclusion a total of 185 patients were included for analysis (Flowchart, Figure 1). The baseline characteristics and age distribution can be seen in Table 1 and Figure 3 respectively. In total, based on the first radiograph, there were three patients (1.6%) with a neuromuscular curve pattern and 182 patients (98.4%) with an idiopathic curve pattern. At the moment of analysis, 15 patients (8.1%) were in brace treatment and 17 (9.2%) had been surgically treated.

Progression

The subset of patients with 22q11.2DS with an idiopathic-like curve and at least one year of radiographic follow-up consisted of 48 patients (Table 2). Out of the 48 patients there were 22 (46%) patients with a non-progressive and 26 (54%) patients with a progressive curve (Table 2). The median follow-up as well as the median progression per year differed between the two groups; 2.5 versus 4.4 year ($p=0.018$) and -0.14 versus 2.5 degrees/year ($p<0,001$) respectively.

Both the patients with non-progressive scoliosis and progressive scoliosis were normo-kyphotic and in good, overall, coronal and sagittal balance ($p>0.05$, Table 2). Progression for IS patients before treatment is generally in the order of 2.2-9.6 degrees per year according to the systematic review and meta-analysis of Di Felice et al.[213], for the progressive 22q11.2DS population this rate was 2.5 degrees.

Table 1: Patient characteristics of the 185 ambulant 22q11.2 deletion syndrome patients with a non-congenital scoliosis.

^The references values of the idiopathic and neuromuscular scoliosis are from Abul-Kasim et al., reprinted by permission of SAGE Publications, Ltd.212 *;seven missing, **The combination of a Cobb to Cobb curve length > 8 vertebrae, a C-shaped curve and the location of the lower-end vertebrae at the lowest or second lowest lumbar vertebrae is determined as a non-idiopathic (neuromuscular) curve pattern. [212]

		Total	female	Male	^Idiopathic scoliosis	^Neuromuscular/ neuropathic scoliosis
General						
All patients		185	92	93	77	21
Age at first X-ray		11.6 (4.2)	11.3 (4.3)	12.0 (4.2)		
Congenital heart defect*	Yes	111 (62%)	56 (51%)	55 (50%)		
	No	67 (38%)	(30 (45%)	37 (55%)		
Curve Characteristics						
Magnitude of major curve		16 (13-25)	17.5 (13-26.5)	15 (12-25)		
location of the major curve						
- Thoracic		79 (437%)	36 (46%)	43 (54%)	52 (68%)	5 (24%)
- Thoracolumbar		54 (29%)	(31 (57%)	23 (43%)	13 (17%)	12 (57%)
- Lumbar		52 (28%)	25 (48%)	27 (52%)	12 (16%)	4 (19%)
Curve length	3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	4	25 (14%)	9 (36%)	16 (64%)	0 (0%)	0 (0%)
	5	55 (30%)	27 (49%)	28 (51%)	1 (1%)	0 (0%)
	6	42 (23%)	25 (60%)	17 (40%)	17 (22%)	0 (0%)
	7	30 (16%)	14 (47%)	16 (53%)	27 (35%)	0 (0%)
	8	15 (8%)	6 (40%)	9 (60%)	29 (38%)	2 (10%)
	9	9 (5%)	6 (67%)	3 (33%)	3 (4%)	4 (19%)
	10	6 (3%)	4 (67%)	2 (33%)	0 (0%)	6 (29%)
	11	1 (1%)	1 (100%)	0 (0%)	0 (0%)	6 (29%)
	12	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	13	1 (1%)	0 (0%)	1 (100%)	0 (0%)	3 (14%)
	15	1 (1%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Curve shape	C	58 (31%)	24 (43%)	33 (57%)	63 (82%)	21 (100%)
	S	127 (69%)	67 (53%)	60 (47%)	14 (18%)	0 (0%)
Location of lower-end vertebra	Above L4	114 (62%)	50 (44%)	64 (56%)	62 (81%)	5 (24%)
	L4	67 (36%)	41 (61%)	26 (39)	15 (19%)	8 (38%)
	L5	4 (2%)	1 (25%)	3 (75%)	0 (0%)	8 (38%)
Curve length in vertebrae	>8	18 (10%)	11 (61%)	7 (39%)	3 (4%)	19 (90%)
	< 8	167 (90%)	81 (49%)	86 (51%)	74 (96%)	2 (10%)
Criteria for neuromuscular/ neuropathic scoliosis fulfilled**	yes	3 (2%)	2 (67%)	1 (33%)	0 (0%)	16 (76%)
	no	182 (98%)	90 (49%)	92 (51%)	77 (100%)	5 (24%)

MRI results

After exclusion (Figure 1), a total of 38 patients (female =21) were included for the analysis of the MRI whole spine. The mean age during MRI was 12.7 (SD: 4.6). There were four patients (10.5%), with five abnormalities in total (11.5%). The different abnormalities were tonsillar herniation (n=1, 3mm below foramen magnum with preserved cerebro-spinal-fluid space), extra dural cyst at the neural foramen T10/T11 (n=1), intraspinal lipoma (n=1) and vertebral body abnormality (n=2, one vertical cleft T7 and one partial fusion T12-L1) as can be seen in Table 3.

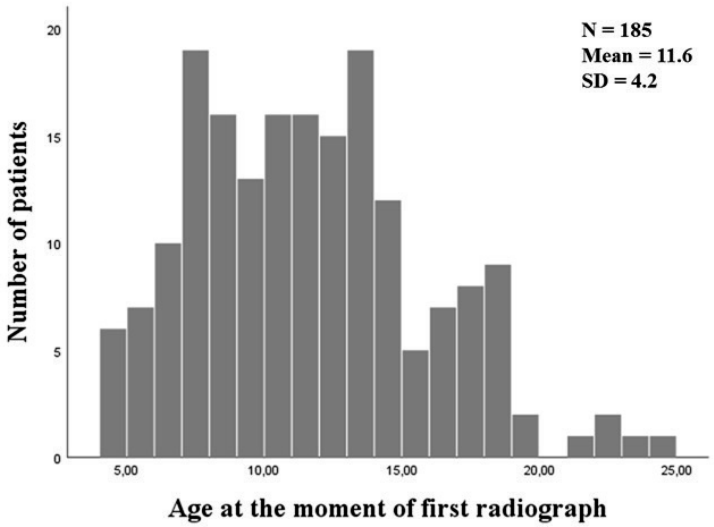


Figure 3: The age distribution at the moment of scoliosis diagnosis

Table 2: All the 22q11.2 deletion syndrome patients with at least one year follow-up and an idiopathic-like curve.

The patient are divided into non progressive scoliosis (<5 degrees curve progression) and progressive scoliosis (>5 degrees progression). *; one missing, **; 16 missing, ***; 13 missing, ^; 12 missing, ^^; 15 missing,^^^; 6 missing

		Total	Non progressive scoliosis	Progressive scoliosis	p-value
General (n=48)					
All patients		48	22 (46%)	26 (54%)	
Gender (female)		27 (55%)	14 (52%)	13 (48%)	0.393
Age at first X-ray		9.8 (2.7)	9.2 (2.9)	10.3 (2.6)	0.189
Congenital heart defect	Yes	31 (67%)	12 (39%)	19 (61%)	0.355
	No	16 (33%)	9 (56%)	7 (44%)	
Length of follow-up		3.4 (2.3-5.1)	2.5 (1.3-4.7)	4.4 (2.9-5.4)	**0.013**
Progression per year		1.6 (-0.2 - 2.8)	-0.14 (-1.3 - 0.82)	2.5 (1.4-5.0)	**0.000**
Radiographic					
Characteristics first radiograph					
Magnitude of major curve		15 (12.3-22.0)	15 (14-18.3)	16 (12-23.2)	0.597
Site of the major curve					
- Thoracic		17 (35%)	5 (29%)	12 (71%)	
- Thoracolumbar		13 (27%)	8 (62%)	5 (38%)	0.234
- Lumbar		18 (38%)	9 (50%)	9 (50%)	
Coronal balance in millimeter*		11.5 (9.0)	12.1 (8.8)	11.1 (9.3)	0.705
Sagittal vertical axis in millimeter		8.8 (40.7)	9.7 (45.8)	8.2 (38.6)	0.923
Kyphosis T5-T12***		26.1 (9.2)	25.8 (8.1)	26.3 (10.0)	0.865
Lordosis T12-S1 ^		54.8 (11.6)	56.7 (11.7)	53.4 (11.5)	0.415
Risser stage^^	0	28 (85%)	10 (44%)	18 (56%)	0.537
	1	1 (3%)	0	1	
	2	0	0	0	
	3	1 (3%)	0	1	
	4	1 (3%)	1	0	
	5	2 (6%)	1	1	
Triradiate^^^	open	34 (81%)	18 (53%)	16 (47%)	1.000
	closed	8 (19%)	4 (50%)	4 (50%)	

Table 3: The intraspinal anomalies as found in idiopathic scoliosis are shown.
The idiopathic scoliosis data is compiled from the systematic review and meta-analysis as performed by Heemskerk et al.204 * Hemangioma or lipoma in vertebral body. ^Patient 1: Partial fusion of the left aspect of the T12 and L1 vertebral bodies, mild osteophytic ridging at T10-T11 and T11-T12 with Schmorl's nodes and decreased disc height at these levels. Patient 2: Vertical cleft in the midline of the T7 vertebral body.

Anomalies	Number (n)	Prevalence IS (%)	Number in 22q11.2DS (n)
Total number of patients	8622		38
Isolated syrinx	318	3.7	
Isolated Arnold-Chiari malformation	259	3.0	
Arnold-Chiari malformation with syrinx	218	2.5	
Tethered cord	49	0.57	
Tonsilar herniation	20	0.23	1
Dural ectasia	33	0.38	
Cerabral or intra/paraspinal tumors	22	0.26	
Diastematomyelia	19	0.22	
Abnormal position of conus	10	0.12	
Extra- or intradural cysts	6	0.07	1
Intraspinal lipoma	5	0.06	1
Discopathy	4	0.05	
Hydrocephalus	3	0.03	
Vertebral body abnormality	3*	0.03	2^
Hydromyelia	2	0.02	
Craniocervical junctional narrowing	1	0.01	
Cerebellar angioma	1	0.01	
Dandy-Walker syndrome	1	0.01	
Arteriovenous fistula	1	0.01	
Not specified	88	1.0	

Discussion

This is a first step to relate scoliosis in 22q11.2DS to IS in terms of curve morphology, curve progression, and the occurrence of intraspinal abnormalities on MRI. We found a striking resemblance between scoliosis in 22q11.2DS and IS; both in curve pattern (98.4%) and curve progression. Moreover, although in a small sample size, the prevalence of MRI anomalies was 10.5%, which is comparable with IS (11.4%).

The curve pattern of the 22q11.2DS population was analyzed in terms of resembling IS, this analysis was performed in three different steps. The first step was the morphology (curve pattern). There are multiple classification systems that describe a curve pattern in scoliosis. The King and Lenke classification systems are based on bending radiographs and are mainly created for surgical planning and not to distinguish between neuromuscular and idiopathic scoliosis.[185,214] Abul-Kasim et al. showed that the vast majority of neuromuscular curves are easy to distinguish from idiopathic-like curves, based on the shape, length, and lower end vertebra of the curve.[212] In our study we determined the location of the major curve based on the apex and we did not provide Lenke types, since bending radiographs are only made for pre-operative surgical purposes. As compared to the results of Abul-Kasim et al. (Table 1), patients with 22q11.2DS and an idiopathic-like curve had more often a S-shape and a thoracolumbar curve instead of a C-shape or a thoracic curve. These differences might account for the fact that in the 22q11.2DS population there were more patients in which L4 was the lower end vertebra. Importantly, none of the 22q11.2DS idiopathic-like curves continued until L5, which often occurs in neuromuscular curves (Table 1). General parameters, such as coronal balance, sagittal balance, kyphosis and lordosis are no part of the criteria as made by Abul-Kasim et al. However, as can be seen in Table 2, the patients with 22q11.2DS are in good overall balance and have normal kyphosis.

The second step was the curve behavior in terms of progression over time. According to a recent systematic review by Di Felice *et al*. the pooled estimated progression prevalence (defined as >5 degrees curve progression) in juvenile and adolescent idiopathic scoliosis is 49% and the rapidity of scoliosis progression ranges from 2.2 to 9.6 degrees per year.[213] The chance of curve progression is related to multiple factors, such as age of onset, gender, location of the major curve and magnitude of the major curve.[215] Our study demonstrated that the progression rate in the group of idiopathic-like scoliosis, in general, is comparable with the progression rate as in IS. Further studies with a larger sample size should be executed in order to analyze the effect of known risk factors (i.e. age of onset) on the chance of curve progression in 22q11.2DS. It is important to note that there was a difference in length of follow-up between the patients with and without a progressive scoliosis in our cohort, in other words the non-progressive curves might still develop into progressive curves. Yet, as shown in a recent study on scoliosis prevalence in 22q11.2DS, 16% of all the patients older than 16 with scoliosis, require scoliosis surgery which suggests that only the minority of patients with 22q11.2DS has a severe progressive curve as opposed to neuromuscular scoliosis.[156]

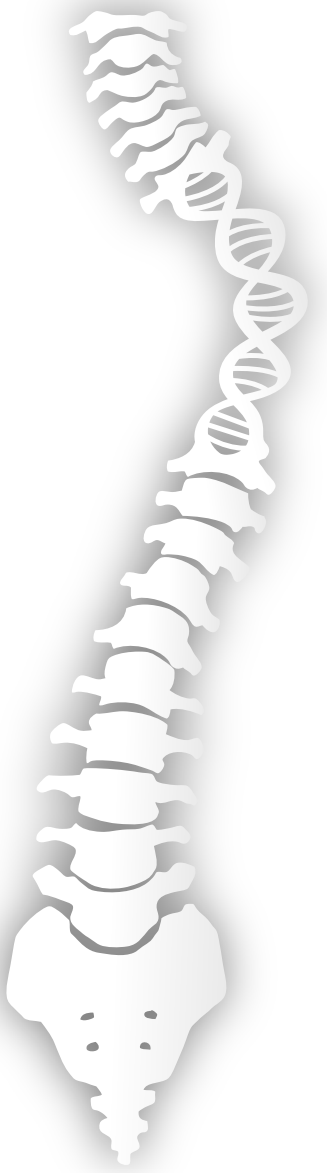
The third step was to assess if relevant intraspinal anomalies can be the cause of scoliosis in 22q11.2DS. In the overall IS population intraspinal anomalies occur in 11.4%, although most of the abnormalities are subtle such as a lipomatous filum terminale. This is the first study to retrospectively report instraspinal abnormalities in the (scoliotic) 22q11.2DS population. All MRIs were obtained due to a history of rapid curve progression and/or pre-operative screenings purposes. In the IS population the need for screening through MRI remains debated. Based on this study it cannot be recommended whether all 22q11.2DS scoliosis patients should receive a MRI, although it seems to appropriate to use the same criteria as the IS population.

AIS is the most common form of scoliosis.[2] As can be seen in Figure 3, it seems that scoliosis in 22q11.2DS, develops at a slightly earlier age as compared to the general population. The earlier diagnosis of scoliosis in 22q11.2DS can be due either to the intensive scoliosis screening and/or due to the fact the patients with 22q11.2DS simply develop the scoliosis at an earlier age as compared to the general population. This is possibly due to the presence of more risk factors. 22q11.2DS is a multisystem syndrome, in which nearly all medical specialist can be involved.[48,62] For example, approximately half of the patients have hypocalcaemia, due to hypoparathyroidism, and moreover lower bone mineral density (BMD) has also been reported.[48,216] At the same time, it is known that patients with AIS have lower BMD as well.[217–220] The curve pattern and curve behavior of scoliosis in 22q11.2DS, in general, has large similarities with IS. This supports the idea patients with 22q11.2DS can be used as a model to study the patho-anatomy of scoliosis development in the general population. Moreover, factors that are known to relate to AIS (such as lower bone mineral density) should be further investigated in 22q11.2DS. It is very likely that certain factors, such as lower BMD, have a different absolute value in 22q11.2DS as compared to the general population and therefore can clearly be risk factors for the development of scoliosis in 22q11.2DS. The major question is, in 22q11.2DS and in AIS, why do only a subset of patients develop scoliosis and moreover, why do half of the patients with either idiopathic scoliosis or scoliosis in 22q11.2DS have a progressive curve?

Conclusion

This study shows that the progressive to non-progressive ratio of scoliotic curves in 22q11.2DS and the rate of progression is comparable with the ratio and rate in IS, and that intraspinal abnormalities occur at a similar rate as IS. This study provides the first insights in the curve behavior of 22q11.2DS and can be used for clinical applications. Moreover, this study is the first exploration in a new line of research that is already applied in other fields of medicine: identify a subset of the population, with a higher risk for a certain disease, that can be studies before the onset of the disease, as a “model” for the general population. This study explores the idea that ambulant patients with 22q11.2DS without congenital vertebral abnormalities can be used, from a scientific point of view, as a patho-anatomy model for scoliosis in the general population.

CHAPTER 8



The Influence of Arm Position during Imaging of the Sagittal Profile of the Spine

Homans JF, Brink RC, Lee TT, Kiers H, Gielis WP, Kruyt MC, Zheng Y-P, Castelein RM, Abelin-Genevois K.

Based on: The influence of arm position during imaging on the sagittal profile of the spine. **under Review**. 2019

Abstract

Background: The sagittal spinal alignment is an important aspect for clinicians to consider in the evaluation and treatment of patients. However, in the natural standing position (i.e., hands at the side) the arms inhibit adequate visualization of the spine. It is currently unclear which of the (numerous) arms positions as described in the literature, approaches most adequately the natural position. Using three-dimensional (3-D) ultrasound, it is possible to test which position corresponds most adequately with the natural standing position. **Purpose:** The goal of this study is to compare the hands-on-cheek and hands-on-wall position with the natural standing position and to determine which position provides the most "functional representation" of the natural standing position using 3-D ultrasound.

Study-Design: A cross-sectional study.

Patient sample: Healthy volunteers.

Outcome measures: The main study parameters were the thoracic and lumbar sagittal angles and overall statistical shape modelling (SSM) of the spine.

Methods: Three standing positions were used in a standard order (first natural, second hands-on-cheek and third hands-on-wall. Volunteers were examined using 3-D ultrasound. After scanning, sagittal ultrasound images were reconstructed and the multiple positions were compared.

Results: Sixteen volunteers (female: male ratio 1:1) were included. Both the hands-on-cheek and hands-on-wall positions gave an underestimation of the thoracic kyphosis (38.9±6.5, 38.3±7.8° respectively), as compared to the natural position (43.5±7.4°), p=0.004. SSM showed the largest difference between the control and hands-on-wall position (natural, cheek and wall position: 0.48, -0.16 and -0.33 respectively ($p \leq 0.002$)).

Conclusions: This is the first study to describe the sagittal alignment in healthy volunteers using 3-D ultrasound. The sagittal spinal alignment differs between the different positions that are used during radiography. Based on SSM, the hands-on-cheek position resembles the natural position the most and we therefore recommend to use this position for lateral radiography of the spine.

Introduction

The sagittal spinal alignment is an important aspect for the clinician to consider in the evaluation and treatment of spinal pathologies and/or deformities such as (degenerative) scoliosis.[2,3,19,39,221–223] It is important to establish accurate and consistent methods for the assessment of the sagittal profile, since surgical corrections result in changes in the coronal as well as the sagittal plane.[224–227] Ideally, for (radiographic) imaging patients should stand in a comfortable, functional, and natural posture, with the arms hanging at the sides (Figure 1). However, in this natural position, the arms prevent adequate visualization of the spine on a lateral radiograph. Patients may therefore be instructed to stand in one of many possible adjusted positions with conventional radiography.[228–231] If radiography is performed with the, increasingly available, biplanar radiography (EOS®), patients stand on a small platform during the radiography. Moreover, using EOS® technology a new position has been introduced with the patients standing with their hands and forearms against the wall, which enables simultaneous visualisation of maturation aspects of the bones of the hands.[232]

Previous studies on the optimal sagittal position for the lateral radiograph were hampered due to the position of the arms or the use of external markers.[231,233–235] Three-dimensional (3-D) ultrasound imaging enables us to determine the sagittal alignment of the spine both in the natural standing position with relaxed arms at the side and in the positions as proposed for radiographic protocols in literature. Therefore we designed a 3-D ultrasound evaluation of the spinal sagittal alignment in healthy volunteers. The goal of this study is to compare the hands-on-cheek and hands-on-wall position with the natural standing position and to determine which position provides the most "functional representation" of the natural standing position. Due to the larger change in hands-on-wall-position as compared to the hands-on-cheek position (90 versus 30-45 degrees), and that the hands-on-wall position can lead to either pushing or leaning against the wall we hypothesize that the hands-on-cheek position resembles the natural position most.

Methods

Study population

The local Medical Research Ethics Committee has granted approval for this study. We included healthy adults (18 years or older) after written informed consent. Exclusion criteria were systematically checked: any spine health issue or previous spinal surgery as well as disabilities to stand in one or more of the positions. Baseline characteristics were collected: age, sex, body weight and length of the subjects.

Different positions

Three standing positions were used in a standard order (first natural, second hands-on-cheek and third hands-on-wall, Figure 1).

1. Natural position: Freestanding, feet at shoulder width and a relaxed standing position with arms hanging on the sides with the ventral side of hands and fingers lightly touching the lateral side of the upper legs.
2. Hands-on-cheek position: Freestanding, feet at shoulder width with finger tips overlying ipsilateral cheeks.
3. Hands-on-wall position: Feet at shoulder width and forearms placed on a wall in front of the volunteer, with the elbows and shoulders in 90° flexion. The volunteers were instructed to gently position the elbows against the wall.

Scanning procedure

First, the volunteer was instructed how to stand and to look straight forward. Next, two researchers checked whether the volunteer was standing appropriately. Next, in order to register a possible change in center of pressure (CoP) position, the volunteer was asked to stand in the natural position and was instructed, after 15 seconds, to change to the hands-on-cheek or hands-on-wall position.

Figure 1: The three different positions are shown.
From left to right the natural, hands-on-cheek, and hands-on-arm positions, respectively

3-D ultrasound measurements

In this study, healthy adults were examined using 3-D ultrasound (Scolioscan®, Model SCN801, Telefield Medical Imaging Ltd, Hong Kong) to compare the different positions. Imaging of the spine was achieved through freehand scanning using linear ultrasound probe (center frequency of 7.5 MHz, width of 7.5 cm), combined with a sensor to detect the position and orientation of the probe.[236,237] After scanning, the B-mode image with corresponding orientation and position were used for 3-D ultrasound volume reconstruction, and the volumes were transferred to customized software for post-processing and generating sagittal ultrasound images. With use of customized software the slices where bilateral laminae could be visualized were generated [238], followed by generating a sagittal profile of the spine based on the laminae which were manually identified (Figure 2).[239] Next, global sagittal parameters were calculated (thoracic kyphosis and lumbar lordosis). Kyphosis was defined as the tangent between the laminae of T4/T5 and T11/T12. Lordosis was defined by the intersection angle of the tangent between T12/L1 and L4/L5. Additionally, the apex of the thoracic kyphosis as well as the number of levels that are posteriorly inclined were determined. A level was posteriorly inclined, if the line between the two lamina of the consecutive levels, was angulated posteriorly.[7]

The intra- and inter-observer variability for different scans are very reliable as shown in a previous study (intraclass correlation coefficient (ICC) 0.97 versus 0.94 respectively).[240] Moreover, the ICC for the intra-observer reliability for the sagittal reconstruction is 0.99 and 0.97 for the thoracic angles and 0.98 and 0.97 for the lumbar angles, the ICC for the inter-observer reliability for the sagittal reconstruction is 0.95 and 0.94 respectively.[241] Last, there is a good agreement between the ultrasound angles adjusted with linear equations and the radiographic angles.[238,239,241]

Center of Pressure (CoP) measurements

During ultrasound scanning the volunteers were instructed to stand on a force plate (Kistler 9286 AA). In order to make the hands-on-wall position similar to that position in EOS®, the force plate was positioned in such a way that the distance of the center from the force plate to the wall of the Scolioscan® was the same as the distance from the center of the EOS® cabin to the wall. Bioware 3.24 software was used to collect the force plate data. The sample frequency was 200 samples/s. The data were low-pass filtered using a 3rd order bidirectional Butterworth filter with a cut-off frequency of 10 Hz. All CoP based measurements were calculated using Matlab (version 7.1.1). The position of the volunteers as well as the stability (movement during scanning period) was collected.

Statistical Shape modelling (SSM)

To capture subtle variations of sagittal spinal alignment, SSM was used (Figure 3 and 4). A shape model determines the mean shape of the volunteers. Next, modes of variations were determined. Each mode describes a distinct shape variation from the population

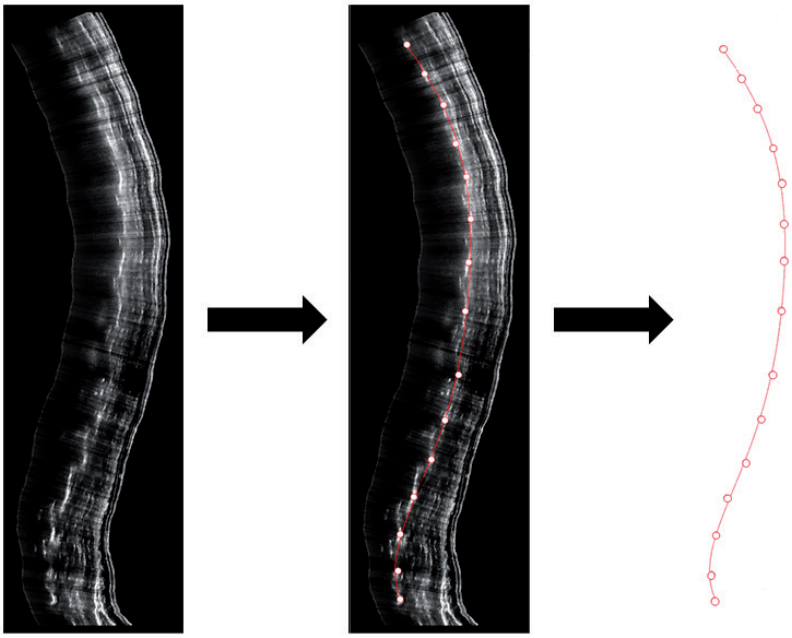


Figure 2: Sagittal ultrasound image before (left) and after (center/right) imaging processing.
The white dots indicate the laminae from L5 (lowest) to T3 (top).

mean, ranging from large to subtle variations. Each scan was given a value for each shape mode, where 0 represented the mean population shape and a positive or negative value represented the deviation (given in standard deviations) in that mode. We built a shape model, using 14 points, each placed on a lamina (L5 to T4), to describe 85% of the shape variation. The final model consisted of six shape modes. All SSM was performed using BoneFinder®.[242,243]

Statistical analysis

The outcomes were thoracic and lumbar sagittal angles (kyphosis and lordosis), level of kyphosis apex, the number of posteriorly inclined segments, CoP and SSM. The difference of the sagittal angles, CoP and between the control, cheek and wall position was analysed with paired t-tests, since paired differences were normally distributed (visually checked using histograms and Q-Q plots). Possible differences in stability were analysed with one-way repeated measures ANOVA. All statistical analyses were conducted with the Statistical Package for the Social Sciences (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA: IBM Corp). An alpha-level of 0.05 was used to define statistical significance.

Results

Population

Out of 25 participants, nine had to be excluded due to insufficient imaging quality (motion artefacts and/or no skin contact in the midline due to lumbar musculature). Sixteen volunteers (eight females, eight males) with a mean age of 26 years were included. Baseline characteristics are shown in Table 1.

Table 1: Baseline characteristics of the included healthy volunteers.
SD: standard deviation.

	Total	Female	Male	p-value
Number of patients	16	8	8	
Age (years)	26 (SD: 6)	26.9 (SD: 8)	26 (SD:2)	0.686
Length (cm)	178 (SD: 9)	175 (SD:7)	182 (SD:10)	0.129
Weight (kg)	72 (SD: 9)	67 (SD: 5)	78 (SD: 8)	0.007
BMI (kg/cm2)	22.6 (SD: 1.8)	21.7 (SD:1.4)	23.5 (SD: 1.8)	0.04

Table 2: Differences between the multiple positions.
SD: standard deviation, CoP: Center of Pressure.*ANOVA between the three positions.

	Control position	Cheek position	Wall position	Control vs. cheek (p-value)	Control vs. wall (p-value)
Kyphosis (T4-T12)	43.5° (SD 7.4)	38.9° (SD 6.5)	38.3° (SD 7.8)	0.004	0.004
Lordosis (T12 – L5	34.6° (SD 13.9)	35.8° (SD 14.0)	37.5° (SD 10.3)	0.781	0.340
Apex kyphosis	7.5 (SD 0.9)	6.8 (SD 1.2)	7.3 (SD 0.9)	0.019	0.509
Number of dorsally inclined segments	8.7 (SD 1.0)	9.4 (SD 1.2)	8.4 (SD 1.0)	0.016	0.528
CoP in centimeter from the midline	-3.09 (SD:1.99)	-3.10 (SD:2.02)		0.417	
	-2.07 (SD: 1.94)		-2.07 (SD: 1.96)		0.811
Movement during scanning period in centimeters per second	1.24 (SD:0.43)	1.31 (SD:0.45)	1.41 (SD: 0.50)		0.671*
Statistical shape modelling	0.48 (SD: 0.92)	-0.16 (SD: 0.91)	-0.33 (SD: 1.04)	0.001	0.002

Global spinal parameters and CoP data

Concerning kyphosis, both the hands-on-cheek (38.9±6.5°; p=0.004) and the hands-on-wall position (38.3±7.8°; p=0.004) gave an underestimation as compared to the natural position (43.5±7.4°). Furthermore, in the wall-on-cheek position the mean location of the thoracic apex was 0.7+0.9 levels higher (p=0.019) and there was a larger number of posteriorly inclined segments (p=0.016). There was no statistical difference between the lordosis in the control, hands-on-cheek and hands-on-wall positions (Table 2). Last there was no difference in the CoP data among the multiple positions both in position as well as stability during scanning period (Table 2). Moreover, there was no difference in stability during standing in the natural position or standing in the hands-on-cheek/hands-on-wall position and no difference in stability between standing and scanning in the natural, hands-on-cheek/hands-on-wall position (p=0.225, p=0.292, p=0.148 respectively).

SSM

In mode 1, which describes 50% of the total shape variance (between all participants and positions), the mean mode values differed significantly between the control (-0.48+0.92) and cheek (-0.16+0.91; p=0.001) and wall (-0.33+1.04; p=0.002) positions, respectively. The wall position and, in lesser extent the cheek position, show a decreased kyphosis and higher apex of the kyphosis as compared to the control position (the mean value of mode 1 for the three positions is shown in Figure 3). Moreover, the shape mode differed significantly between males (mean -0.09±0.36) and females (1.05±0.97; p=0.007; Figure 4).

Table 3: Differences between female and male volunteers.
SD: standard deviation.

	Total	Female	Male	p-value
Kyphosis (T4-T12)	43.5° (SD 7.4)	43.4° (SD 8.8)	43.6° (SD 6.1)	0.969
Lordosis (T12 – L5	34.6° (SD 13.9)	28.3° (SD 15.5)	40.1° (SD 9.1)	0.069
Apex kyphosis	7.5 (0.9)	7.9 (0.8)	7.1 (0.8)	0.094
Number of dorsally inclined segments	8.7 (1.0)	8.6 (1.1)	8.8 (1.0)	0.724
Statistical shape modelling	0.48 (SD: 0.92)	1.05 (SD: 0.97)	-0.09 (SD: 0.036)	0.007

Figure 3: Shape modelling of differences between multiple positions (8 female, 8 male). Dark-grey (right) = natural, light-grey (middle) = hands-on-cheek position and black (left) = hands-on-wall position.

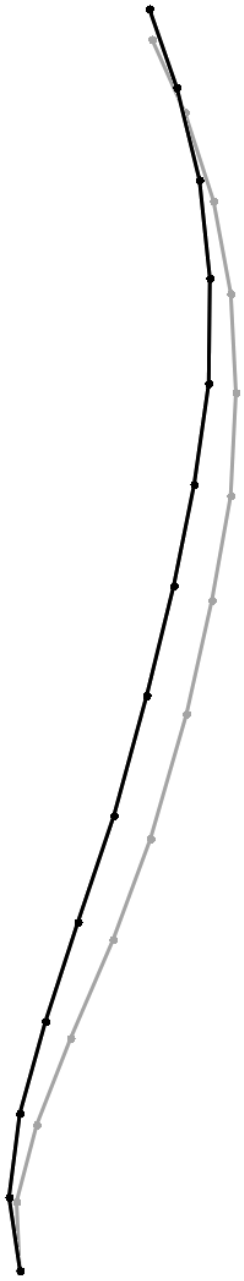


Figure 4: Shape modelling of differences between the eight females (grey) and eight males (black).

Discussion

Standardized positioning during lateral radiography is important to measure the (closest to natural) global sagittal alignment, compare outcomes and provide references. With the use of 3-D ultrasound technique it is now possible to visualize the posterior bony spinal structures in the most natural, freestanding position. This is the first study to compare the sagittal alignment, in either natural position, to the frequently used alternative positions (hands-on-cheek and hands-on-wall positions).[231,232,234] Using global spinal parameters, the thoracic kyphosis, kyphosis apex level and number of posteriorly inclined segments showed minor differences among the three positions.

An important difference between EOS® technology and conventional radiographs is the size of the gantry and the platform of the EOS®; as a consequence, the majority of the positions (e.g. 30 degrees flexion in shoulder and elbows fully extended) that are described for conventional radiography cannot be used.[232] From a scientific perspective, in order to truly compare the sagittal radiographs and thus results between studies (both with EOS® and conventional radiography) the same standing position should be used. Therefore, in this study we compared the two positions that are most commonly used in the EOS® system, since they can also be applied for conventional radiography. In the hands-on-cheek position the shoulders are in 30-45 degrees flexion. This is comparable with the fists on clavicle position. Marks et al. described a large variation in the instruction of the radiograph technician towards the patient within the fists on clavicles position.[234] Moreover, Pasha et al. described that it is important that the knuckles or fingertips should touch the clavicles and not the acromion in order to avoid the visibility of the sagittal cervical spine.[232]

Our study showed that kyphosis both in the hands-on-cheek and hands-on-wall position decreased as compared to the natural position, particularly in the hands-on-wall position. This can also be seen in the SSM in which level T4 was slightly higher in the hands-on-cheek and hands-on-wall position as compared to the natural position (Figure 3). This is in agreement with the study by Marks et al. in in which multiple positions were compared with the natural position based on markers attached to the back.[234] In other words, when volunteers are instructed to stand in hands-on-cheek/hands-on-wall position they bring their fingers to the cheek/to the wall, they stand up straighter and slightly extend their thoracic spine.

The most important parameter, SSM, differed significantly between the natural position and the two alternatives, in which the hands-on-cheek position corresponded most closely to the natural position. SSM has been adapted by many research group to study the influence of joint morphology and enables quantification of subtle shape variations, which are lost when using predefined measurements.[244,245] In the current study we used SSM to quantify the global spine morphology in the sagittal plane. SSM showed clear differences between the three positions and between males and females. Whereas the global spinal parameters showed only differences between the positions and not between females and males. In other words, with SSM it is possible to identify more subtle differences of the sagittal alignment.

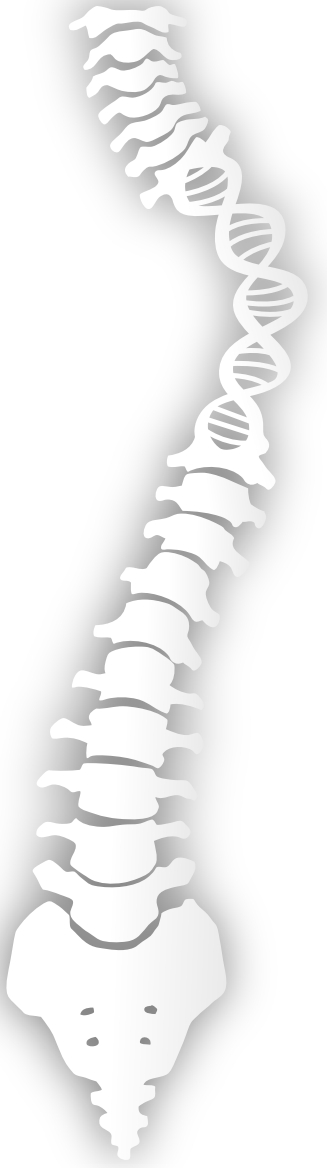
One of the limitations of our study is that we had to exclude nine patients. Using 3-D ultrasound it is necessary to have skin contact within the whole scanned region. Some volunteers were too muscular to ensure skin contact in the lumbar region of the spine. This resulted, in combination with motion artefacts, in nine exclusions. In previous studies using Scolioscan® patients were instructed to stand against chest and hips supports in order to grant stability during scanning. However, this was not possible for the purpose of this study, since our goal was to compare multiple standing positions with the natural standing position. Therefore, our volunteers stood on a force plate, and as shown in Table 2, there is no difference in stability during scanning in the multiple positions. Interestingly, in hands-on-wall position, which can be considered as a supported position, there was no increase in stability.

Lumbar lordosis as measured using 3-D ultrasound was much smaller as described in radiographs.[246–248] There are several reasons for this discrepancy. First, the ultrasound lumbar lordosis is measured using L4-L5, whereas the largest part of the lordosis is observed between level L4 and S1.[246–248] Unfortunately, S1 is not visible on most ultrasound scans. Second, all ultrasound angles are based on the posterior structures, whereas the radiographic angles are based on the vertebral bodies. Another limitation is that we were not able to measure the SVA. Using ultrasound it is not possible to visualize the vertebral bodies and for that reason the shape method is used to assess the alignment of the thoracic and lumbar spine. Even though, shape modelling enabled us to measure the shape of the spine, instead of measuring the balance of a vertebra as relative to the sacral end plate. Moreover, the goal of this study was to compare natural to standardized postures with arms flexed, and not to describe absolute angular values. With use of this method it is possible to identify more subtle differences.

Conclusion

In the present healthy control study, we showed that the sagittal spinal alignment in the most recommended radiographic positions differs from the natural freestanding position. With use of statistical shape modeling, we could accurately identify subtle regional and global sagittal differences. The hands-on-cheek position overlaps more with the natural position as compared to the hands-on-wall position. Based on this finding, we recommend to use the hands-on-cheek position for lateral radiography of the spine in order to most closely correspond to the natural position. Additionally, this is the first study to describe the sagittal alignment in healthy volunteers using 3-D ultrasound. This opens a large range of diverse possibilities to use 3-D ultrasound, for screening on spinal abnormalities as well as assessment of the normal spinal development.

CHAPTER 9



Different Scoliotic Curve Patterns develop based on pre-existent Differences in Sagittal Alignment in Patients with 22q11.2 Deletion Syndrome: A Perspective

Homans JF, Schlösser TPC, Pasha S, Kruyt MC, Castelein RM.

Based on: Different Scoliotic Curve Patterns develop based on pre-existent Differences in Sagittal Alignment in Patients with 22q11.2 Deletion Syndrome: A Perspective. **Submitted.** 2019

Background

The sagittal alignment of the human spine develops from a global kyphosis in utero, into a double S-shape with a cervical, lumbar and pelvic lordosis at adulthood.[36] This configuration leads to unique spinal biomechanics, due to the fact that human is the only species that carries its body's center of gravity directly above the pelvis (Figure 1). All other vertebrates, bipedal and quadrupedal alike, have a center of gravity that lies in front of the pelvis due to a lack of pelvic and lumbar lordosis, and an inability to simultaneously extend hips and knees. It has previously been shown that the human situation leads to a posteriorly inclined segment of varying length, height and inclination angle. This posteriorly inclined segment is subject to so-called posteriorly or dorsally directed shear loads. These posteriorly directed shear loads lead to a decreased rotational stiffness, making the exposed segments vulnerable to develop a progressive rotational deformity: a scoliosis (Figure 1).[3,4] Unfortunately, little is known on how the sagittal shape of the spine develops throughout growth. Schlösser et al. (2014) showed that there is a significant difference between the sagittal profiles of boys and girls during the peak of pubertal growth velocity, and also between patients with early thoracic scoliosis *vs.* (thoraco)lumbar scoliosis *vs.* controls.[6,7] In agreement with the posterior shear load theory, thoracic scoliosis had a longer, more proximal, posterior inclined segment of the spine as compared to lumbar scoliosis and controls, whereas the lumbar scoliosis had a steeper posterior inclined thoracolumbar segment as compared to thoracic scoliosis

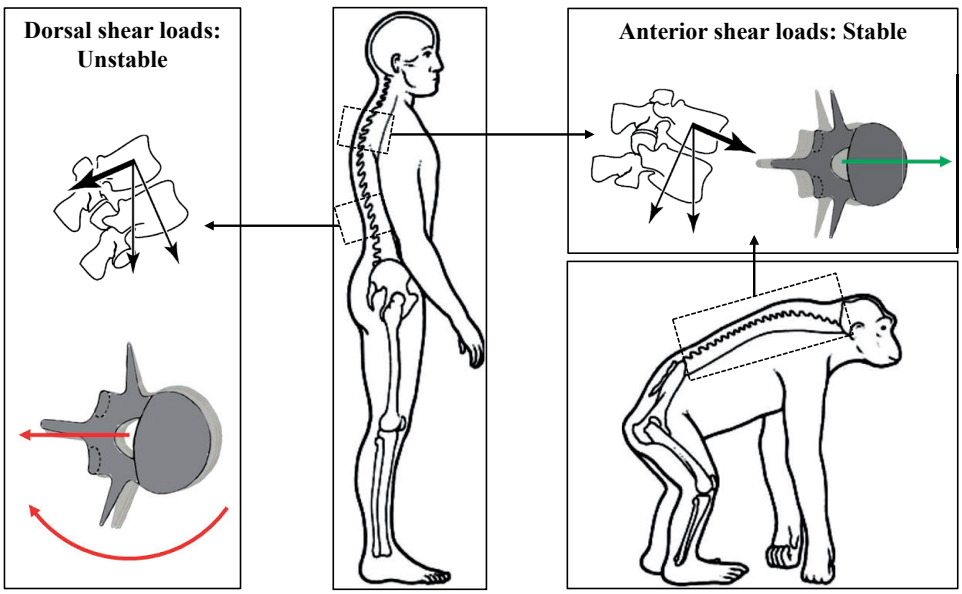


Figure 1: Unique dorsal shear forces in human
Human as opposed to all other (bipedal) animals have the center of gravity above the pelvic. Therefore, certain vertebrae of the human spine are dorsally inclined (left). These vertebrae have a decrease in rotational stability as compared to anteriorly inclined vertebrae (right). Image compiled from Castelein et al. [3]

and controls.[7] Moreover, Brink et al. (2018) showed in a 3-D CT study that there is a higher pelvic incidence in lumbar as compared to thoracic scoliosis, emphasizing the role of overall spino-pelvic alignment and biomechanics.[35] However, as in practically all clinical scoliosis research, the observed differences apply to subjects with an already established (small) scoliosis. The question whether these observed differences are indeed present before the onset of scoliosis (and thus more likely part of its etiology), has so far remained unanswered. If the rotationally unstable, dorsally inclined segment is likely to play a role in its etiology, the difference in sagittal profile should be present before the onset of scoliosis.[3]

A novel approach

In the general population it is not possible to screen the sagittal profile of all children with radiographs in order to identify possible differences between children that do and do not develop a scoliosis. Therefore, it was previously hypothesized that a subset of the population with a high risk of developing scoliosis can be used as a proxy for the general population.[184] In the 22q11.2 deletion syndrome (22q11.2DS) half of the patients develop a scoliosis and moreover this scoliosis has large phenotypic similarities with idiopathic scoliosis.[249] The goal of this pilot study is to determine whether the biomechanical differences that were observed in previous studies between thoracic scoliosis *vs.* (thoraco)lumbar scoliosis *vs.* controls, can be identified before the onset of the deformity. This prospective case series will be used for a *lege-artis* powered prospective study to test the hypothesis of the dorsal shear theory.

Methods

All patients with 22q11.2DS starting at the age of six and visiting the 22q11.2DS clinic in the University Medical Center Utrecht (UMCU) were eligible. All patients with 22q11.2DS receive standardized AP and lateral radiography of the spine starting at the age of eight years in 2014 and from the age of six in 2016. This scoliosis screenings protocol is part of good clinical practice since there is high chance of scoliosis development (~50%).[156] We included all patients that had a straight spine in the coronal plane at first X-ray (age > 6 years), had at least one follow-up visit at least 12 months after the initial radiograph. We excluded all patients with a scoliosis at first X-ray, or lack of standardized full length upright coronal and sagittal radiographs at first visit. Based on the most recent coronal radiograph it was determined whether patients fell in the non-scoliotic control group, thoracic scoliosis group or (thoraco)lumbar scoliosis group, based on the definitions of the Scoliosis Research Society.[162] Care was taken to obtain all radiographs in a standardized manner by instructing personnel on a regular basis and explaining the importance of a standardized position (patients were instructed to stand in the hand-on-cheek position, Figure 2).

Figure 2: All patients are instructed to stand in the standardized hand-on-cheek position

Baseline

Age and sex were collected during first visit and last follow-up.

Radiological analysis

The first lateral radiographs of all included patients were analyzed in Surgimap by one observer according to a previously validated protocol.[7] The observer was blinded for outcome (whether the patient fell in the control group, thoracic scoliosis group or (thoraco) lumbar scoliosis group). The radiographic parameters that were determined can be seen in Table 1 and Figure 3.

Table 1 Explanation of the measurements

Global spinal parameters		
Thoracic kyphosis	TK	This angle is measured from the upper end plate of T4 to the lower endplate of T12 using the Cobb method
Lumbar lordosis	LL	This is angle is measured from the upper endplate of T12 to the endplate of S1 using the Cobb method.
Inclination of the spine		
Number of declive vertebrae	#DV	Number of vertebrae of which the inferior endplate is posteriorly inclined as compared to the horizontal.
Declive length	DL	Length of the part of the spine that is posteriorly inclined segment, normalized for T1-L5 as can be seen in Figure 1.
Declive segment inclination	DI	Angle between a line through the centroids of the cranial and caudal end level of the part of the spine that is posteriorly inclined and the vertical as can be seen in Figure 1.
Pelvic parameters		
Pelvic incidence	PI	Angle between the perpendicular to the sacral plate and the line connecting the sacral end plate midpoint to the hip axis.
Pelvic tilt	PT	Angle between the line connecting the midpoint of the sacral plate to the hip axis, and the vertical.
Sacral slope	SS	Angle between the superior end plate of S1 and the horizontal.

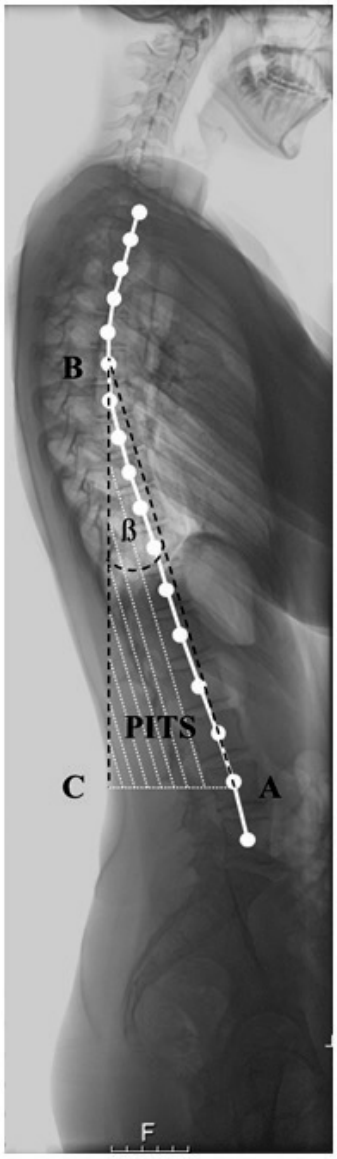


Figure 3: Explanation of the parameters considering the inclination of the spine
A vertebra is considered posteriorly inclined based on the angle of inferior endplate with the horizontal. The number of posteriorly inclined vertebrae (declive vertebrae (#DV)) is the number of vertebrae that have a posteriorly inclined inferior endplate, in this case from **A** to **B** = 11. The declive length (DL) of the spine is the length of the spine that is posteriorly inclined (the length of the white line from centroid **A** to **B**) divided by the total length of the thoracolumbar spine (length of the total white line). The declive inclination (DI) is the angle (ß) between the centroids of the cranial and caudal end level of the part of the spine that is posteriorly inclined (**A** and **B**) and the vertical (from **B** to **C**). The posterior inclined triangle surface (PITS) is depicted with the white dashed line and was calculated by using DL and DI: ½ * ((SIN(ß) * DL) * (COS(ß)*DL))

At first, global spinal parameters (thoracic kyphosis, lumbar lordosis, PI, pelvic tilt and sacral slope) were measured. Hereafter, all individual vertebral bodies were segmented, by indication of its four corners (the anterior and posterior point of each endplate was manually selected). Surgimap automatically calculates the centroid of each vertebra and determines whether a vertebra is posteriorly or anteriorly inclined (based on the angle of inferior endplate with the horizontal line). Since there is variety in length between the individuals, normalization was performed for each length measurement (length of posteriorly inclined segment divided by the total length of the spine; Table 1, Figure 3). In order to identify a single value that predicts onset of scoliosis (irrespective of apex height) or not, the surface of the triangle defined by the posterior inclined segment was calculated (Figure 3). After analyzing the radiographs, the data was extracted and matched with the outcome of the patients: Patients were divided into three outcome groups, based on the most recent coronal radiograph: main thoracic scoliosis (apex between T2- disc T11-12), (thoraco)lumbar scoliosis (apex between T12 and L4) and controls (Cobb angle < 10 degrees).
In a previous study it was shown that the ICC for inter- and intra-observer reliabilities were 0.96 and 0.94 for general sagittal spinopelvic parameters, 0.98 and 0.96 for normalized distance measurements and 0.99 and 0.99 for vertebral inclination.

Statistical analysis
This is a first explorative study and therefore (to prevent both type-1 and type-2 errors) only descriptive statistics were done. Means with standard deviations (SD) and medians with interquartile range (IQR) of the parameters as shown in Table 1 are shown.

Results

Study population
Based on our in- and exclusion criteria, we included 31 22q11.2DS patients out of our database of 150 patients. Baseline characteristics are shown in Table 2. Out of 31 patients, five developed a thoracic scoliosis and seven developed a (thoraco)lumbar scoliosis (mean Cobb angle 19 and 16 degrees respectively), after a mean follow-up of 3.4 years.

Sagittal parameters before the onset of scoliosis
We did not statistically compare the different groups. Based on the means and medians there are differences between thoracic and (thoraco)lumbar scoliosis present before the development of scoliosis in patients with 22q11.2DS. The patients with (thoraco)lumbar scoliosis had a more posteriorly inclined lower segment of the spine, a larger thoracic kyphosis, larger lumbar lordosis and a larger PI as compared to those with thoracic scoliosis and controls as can be seen in Figure 4 and Table 3. Patients with thoracic scoliosis had a longer and more proximally extending posteriorly inclined segment as compared to (thoraco)

lumbar scoliosis and controls (Figure 4, Table 3). The posterior inclination triangle surface (PITS) has a different shape for thoracic as compared to (thoraco)lumbar scoliosis (Figure 4). Nevertheless, the PITS reflects an overall risk factor for scoliosis: it was considerably higher in the group of scoliosis patients (0.059, SD: 0.020, thoracic and thoracolumbar scoliosis combined) as compared to controls (0.043, SD:0.022).

Future Prospective Studies

We performed a power calculation (Nominal Power 0.8, α 0.025) based on the major outcome: the (normalized) length of the dorsally inclined segment (thoracic scoliosis versus controls and thoracic versus (thoraco)lumbar scoliosis). Due to the small sample size in this study we worked with the means and SDs of the study by Schlösser et al.[7] Given the distribution of a) scoliosis versus non-scoliosis (1:1) and b) curve location thoracic versus (thoraco)lumbar (1:1) scoliosis in 22q11.2DS we will need a total of approximately 60 (30 controls, 15 thoracic and 15 (thoraco)lumbar scoliosis) 22q11.2DS patients in order to prove a pre-existent relationship between the length of the dorsally inclined segment and the scoliosis. Moreover, in a prospective study, the PITS can be used in order to identify the dorsal shear theory as a causal factor in the development of scoliosis. Based on the preliminary data in this study we would need a sample size of 50 patients with 22q11.2DS (Nominal Power 0.8, α 0.05).

Discussion

This is the first study to indicate that differences in sagittal alignment between thoracic scoliosis, (thoraco)lumbar scoliosis and non-scoliotic adolescents already exist before onset of scoliosis. In the ideal situation these data should be obtained from the general population. Yet, it would be extremely difficult and unethical to do such a study in the general population. Therefore we chose an approach that can be considered second best and that is already applied in other fields of medicine (especially in psychiatry); use a subset of the population

Table 2 Baseline parameters
SD= standard deviation

	Total	No scoliosis	Thoracic scoliosis	(thoraco)lumbar scoliosis
Number of patients	31	19	5	7
Gender (female)	12 (39%)	8 (42%)	1 (20%)	3 (43%)
Age	10.5 (SD: 2.3)	10.5 (SD: 2.4)	10.7 (SD: 3.2)	10.2 (SD: 1.7)
Length Follow-Up	3.4 (SD: 1.4)	3.2 (SD: 1.4)	4.0 (SD: 1.0)	3.8 (SD: 1.6)
Cobb Angle major curve		<10 degrees	19 (SD: 8)	16 (SD: 4)

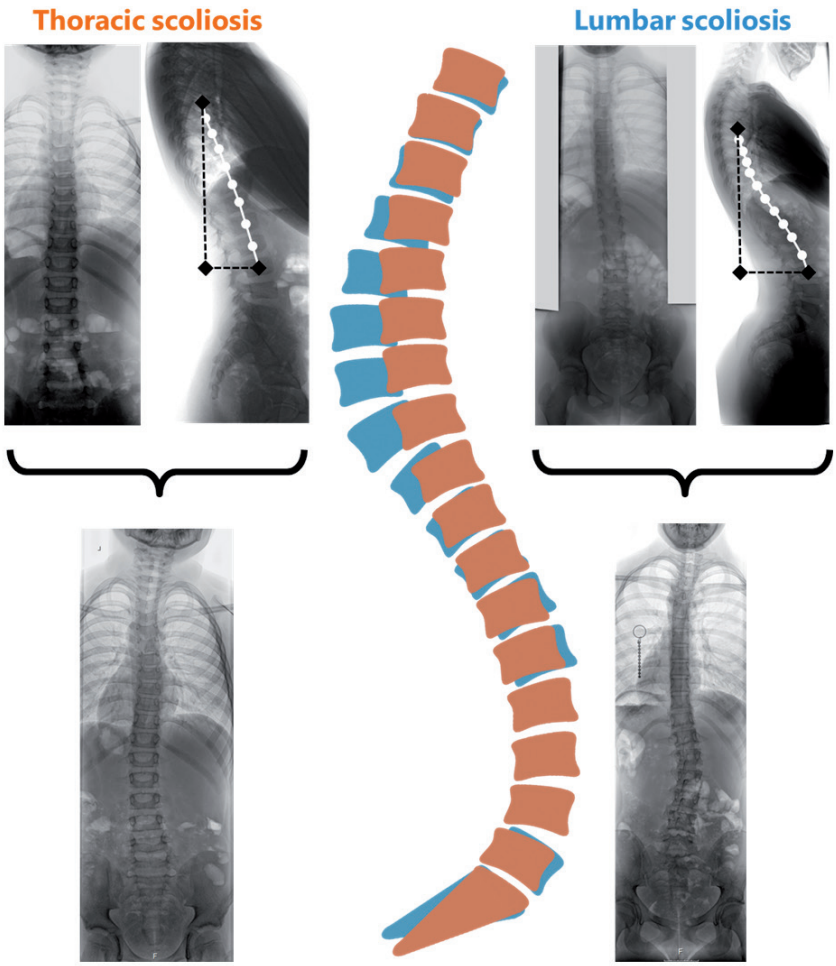


Figure 4
Differences in sagittal alignment between a patient with a thoracic scoliosis (left) and a lumbar scoliosis (right) before (top) the development of scoliosis (bottom). In the middle you can see an overlay of the differences between the thoracic (orange) and lumbar (blue) scoliosis before the onset of scoliosis: Thoracic scoliosis patients have a longer, more proximal, posterior inclined segment. Lumbar scoliosis patient have a steeper, shorter, posterior inclined segment. The patients with thoracic scoliosis had another shape of the posterior inclined triangle surface (PITS, triangle with black and white dashed lines) as compared to patients with lumbar scoliosis.

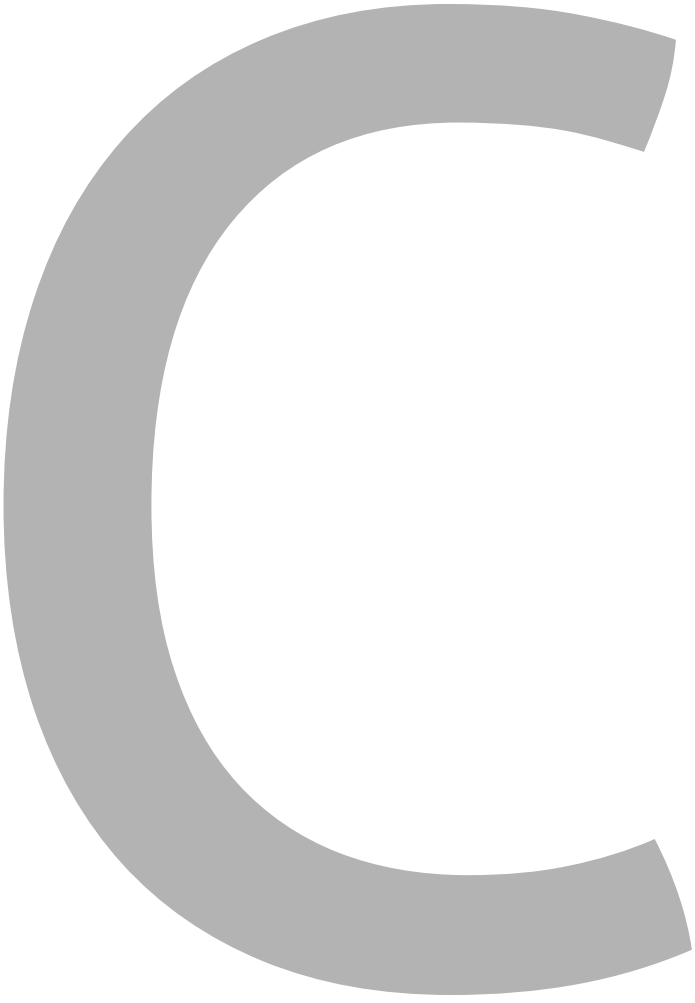
with a high risk for that disease, as a proxy for the general population.[41] We performed this study in a cohort of patients with 22q11.2DS An important factor in this approach is that patients with 22q1.2DS have a scoliosis that strongly resembles idiopathic scoliosis as far as curve pattern and curve behavior (both chance of progression and speed of progression) is concerned.[249] An additional advantage is that there is a major decrease in required sample size in 22q11.2DS, since there is a ~50% chance of development of scoliosis.[156]

In 2005, the dorsal shear force hypothesis was introduced by Castelein et al.[3] Dorsally inclined vertebrae are potentially prone to start rotating and contribute to the development of a scoliosis, the location of these dorsally inclined vertebrae depends on the individually determined sagittal profile (Figure 1). The growing child's individually determined sagittal profile is not well known. We identified the parameters that most likely predict the onset of scoliosis. The values for these parameters are different for thoracic scoliosis, (thoraco) lumbar scoliosis and controls. We combined these parameters into a single parameter (PITS) in order to identify differences between scoliosis (both thoracic and (thoraco)lumbar) and controls. In order to prove a causal relation between dorsally inclined vertebrae and the development of scoliosis, the next step would be to perform a controlled trial where patients with 22q11.2DS with a certain dorsal inclination or PITS are randomized to receive a treatment to lower the inclination (e.g. a de-lordating brace) or not. If such an intervention successfully prevents scoliosis, level 1 evidence has been achieved.

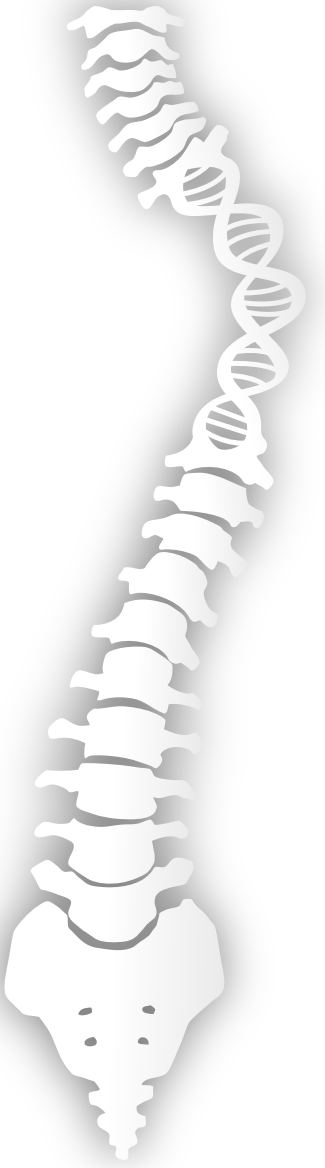
Our proof-of-concept study is the first to indicate that the differences in different parameters and PITS are present already *before* the onset of any scoliosis. Moreover, this study shows that it is feasible to, from a scientific perspective, use a high-risk population as a proxy for the general population.

Table 3 Mean and median values of the spino-pelvic sagittal parameters
IQR=interquartile range, SD= standard deviation

	Means			**Medians**		
	No scoliosis	thoracic scoliosis	(thoraco)lumbar scoliosis	No scoliosis	thoracic scoliosis	(thoraco)lumbar scoliosis
Pelvic parameters						
Pelvic incidence	38,6 (SD:11,9)	36,3 (SD:4,4)	50,9 (SD:33,6)	37,7 (IQR:30,4-46,7)	37,7 (IQR:37,3-38,1)	41,2 (IQR:33-48,8)
Pelvic tilt	5,8 (SD:6,7)	7,4 (SD:5,9)	16,3 (SD:32)	5 (IQR:1,8-10,7)	4,6 (IQR:3,4-10,5)	4,4 (IQR:-2,5-18)
Sacral slope	32,8 (SD:10,5)	28,9 (SD:5,7)	34,6 (SD:8,4)	31,3 (IQR:25-39,2)	29,3 (IQR:25,3-32,7)	39,1 (IQR:28,2-40,7)
Global spinal parameters						
Thoracic kyphosis	27,6 (SD:11,6)	28 (SD:5,4)	32 (SD:7,7)	25,4 (IQR:23,6-33,5)	28,7 (IQR:27,2-29,5)	30 (IQR:25,4-37,1)
Lumbar lordosis	48,3 (SD:11,7)	47 (SD:7,8)	55,7 (SD:8,5)	49,6 (IQR:-39,7-53,7)	44,9 (IQR:41,7-53,3)	58 (IQR:49,2-59,8)
Inclination of the spine						
Number of declive vertebae	9,3 (SD:2)	11 (SD:1,2)	10,1 (SD:0,9)	9 (IQR:8-11)	11 (IQR:11-12)	10 (IQR:9,5-11)
declive length	0,551 (SD:0,123)	0,664 (SD:0,092)	0,593 (SD:0,071)	0,5 (IQR:0,5-0,6)	0,7 (IQR:0,6-0,8)	0,6 (IQR:0,5-0,6)
Declive inclination	15,9 (SD:4,6)	16,7 (SD:2,1)	19,7 (SD:6,5)	16,5 (IQR:14,5-19,4)	16,4 (IQR:16,3-18,3)	17,7 (IQR:15,3-23,1)
Posterior inclined triangle surface	0,043 (SD: 0,022)	0,062 (SD: 0,018)	0,057 (SD:0,023)	0,045 (IQR:0,029-0,061)	0,069 (IQR: 0,055-0,076)	0,057 (IQR: 0,043-0,063



CHAPTER 10



Summary, Future Perspectives and Final Conclusions

Summary

In the introduction of this thesis (**chapter 1**) we emphasized the unique biomechanical posture of human, the posterior shear loads and its role in the development of idiopathic scoliosis. Second, we discussed the difficulties that current etiologic as well as pathogenesis research is facing: in general, scoliosis patients reach the outpatient clinic after curve onset. Hereafter, we described an alternative approach in order to perform etio-pathogenesis research (the use of a subset of the population). Last, we faced the current lack of knowledge on orthopedic manifestations in the 22q11.2 deletion syndrome (22q11.2DS). Below is a summary of the major results of the different studies that were performed in this thesis.

Orthopedic Manifestations within the 22q11.2 Deletion Syndrome

22q11.2DS is characterized by broad phenotypic heterogeneity. For a long period of time extensive research has been performed on 22q11.2DS, mainly in the field of congenital heart disease (CHD) and schizophrenia.[41,48,200,250–259] Until now, compared to those research lines, only little research has been performed on orthopedic manifestations in the syndrome. We started this thesis with an overview on the different musculoskeletal manifestations in 22q11.2DS.

Which musculoskeletal manifestations are present in 22q11.2DS?

We performed a systematic review in order to identify all possible musculoskeletal manifestations in 22q11.2DS. This search led ultimately to 69 articles in which we identified 58 musculoskeletal manifestations. The vast majority of these manuscripts did not have a musculoskeletal manifestation as a primary outcome, subsequently the evidence for the majority of the orthopedic manifestations was moderate or weak.[52] This led, most probably, to a wide prevalence range for multiple manifestations (i.e. scoliosis 0.6-60%). However, this systematic review demonstrated that orthopedic manifestations are an important feature of 22q11.2DS (despite the lack of dedicated research). Many of these musculoskeletal manifestations need awareness and possible treatment (i.e. club foot, congenital cervical anomalies and scoliosis). Hereafter we focused on two of those orthopedic manifestations.

What is the prevalence of club foot in 22q11.2DS and is associated with CHD and/or cleft palate?

An intriguing finding of the systematic review performed in **chapter 2** was a prevalence range of 1.1-13.3% of club foot, whereas in the general population the prevalence is 0.1%. If there is truly such a high prevalence of club foot in 22q11.2DS, the occurrence of club foot in a newborn should possibly lead to genetic testing. However, none of the studies on club foot in the systematic review was dedicated towards investigating the prevalence of club foot. Therefore, in order to, truly identify the possible connection between club foot and 22q11.2DS we investigated the prevalence of club foot in the world's largest cohort

of patients with 22q11.2DS. We observed a prevalence of 3.3%, which is 30 times higher than the prevalence in the general population. Moreover, we investigated whether club foot occurred more often in combination with other major congenital defects: CHD and cleft palate. The clinical implication of such an association would be that the combination of a club foot and other (neonatal) 22q11.2DS phenotypic characteristics should possibly lead to genetic testing. However, we did not identify an association between club foot on the one hand and CHD/cleft palate on the other hand.

What is the prevalence of scoliosis in patients with 22q11.2DS and is there an association between scoliosis and CHD?

Analogous to the prevalence on club foot, our systematic review (**chapter 2**) also showed a large spread on the prevalence of scoliosis (0.6-60%). It is important to know the true prevalence of scoliosis, since it can have major consequences for patients including screening at the age of growth and possible (brace and surgical) treatment. The main reason for surgery is that, if a curve exceeds 45-50 degrees it can progress even after growth has ended. Subsequently debilitating curves exceeding 100 degrees can occur with possible effects such as trunk imbalance, pain and cardiopulmonary problems (Figure 1). This study is a combination of a cross-sectional (UMCU) and retrospective (CHOP) design and led to the insight that nearly half of the 22q11.2DS population develops a scoliosis. Moreover, 8% of the CHOP population (>16 years) needed scoliosis surgery. For over 40 years, an association

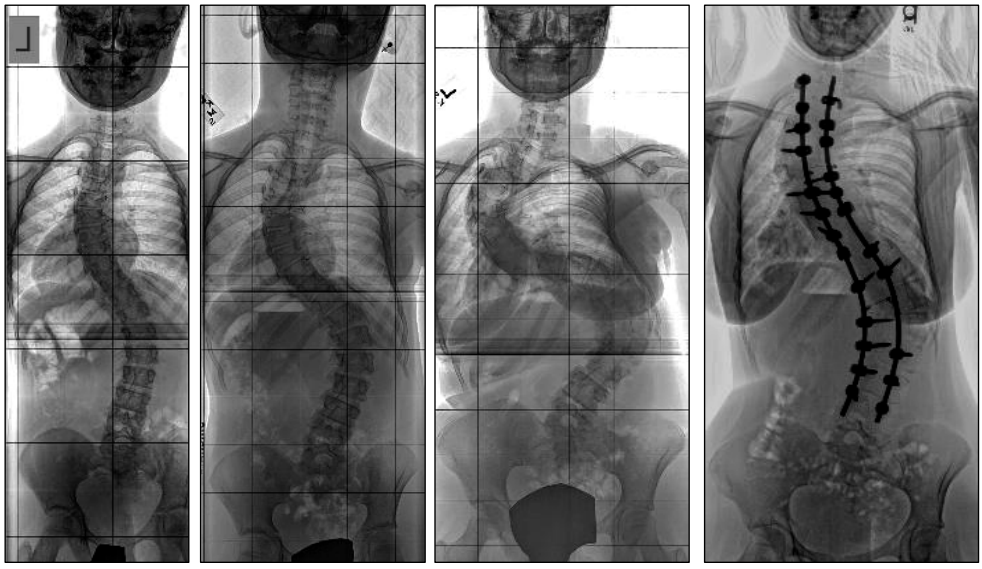


Figure 1: Progression of scoliosis

If a progressive scoliosis is left untreated (radiographs of the same patient below, chronologically from left to right) this can lead to curves exceeding 100 degrees, with subsequently the need for major surgery.

between CHD and scoliosis has been described. Since CHD is one of the major phenotypic features of 22q11.2DS, we investigated whether, in 22q11.2DS, there was an association between CHD and scoliosis. Interestingly we did not find it. Therefore, we continued the research on CHD and scoliosis in a next study (**chapter 5)**.

What is the prevalence of scoliosis in a cohort of patients with CHD with and without the 22q11.2 deletion, matched for CHD and gender?

Elaborating on **chapter 4**, we looked further into the possible association between CHD and scoliosis. Due to the large variation in phenotypic appearance of patients with 22q11.2DS and the fact that none of the current studies on scoliosis in CHD performed genetic testing on (all) patients, we thought that this possible described association in the literature was actually due to the 22q11.2 deletion. Elaborating on that, we proposed that in studies which demonstrated a relationship between CHD and scoliosis, (unrecognized) patients with 22q11.2DS may well have been the common denominator (Figure 2). We performed a study in a cohort of patients with CHD in which it was known whether they had the 22q11.2 deletion or not (all patients underwent genetic testing for the 22q11.2 deletion). In this adult CHD population, we identified the presence of scoliosis on thorax radiographs and found that half (53.5%) of the CHD 22q11.2DS population had a scoliosis. Interestingly, the CHD population without 22q11.2DS had a scoliosis prevalence of only 7.9%. In the multivariate regression we showed that the 22q11.2 deletion was by far the largest risk factor for the development of scoliosis, followed by CHD type and a thoracotomy. If we examine the prevalence of scoliosis, in the general population, based on thorax radiographs, there is 9-13% prevalence and thus the effect of CHD is likely to be low.[173,182] These findings suggest that the 22q11.2 deletion represents a common pathway for both CHD and scoliosis.

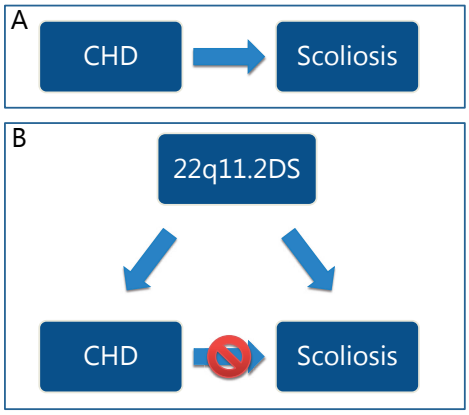


Figure 2: The association between congenital heart defects (CHD), the 22q11.2 deletion syndrome (22q11.2DS) and scoliosis

For over a period of 40 years an association between CHD and scoliosis was described (A). Our hypothesis is that (B) actually the 22q11.2DS is a confounder in this presumed association.

The 22q11.2 Deletion Syndrome as a Model for Scoliosis in the General Population

An important question towards the etio-pathogenesis of idiopathic scoliosis is the interaction between biomechanical, central nervous system, environmental, genetic and metabolic factors.[2] In order to truly investigate the interaction between protective and provocative factors we believe we should analyze the very early and initiating factors in humans. Elaborating on this idea, given the relatively low prevalence of scoliosis, and the ~50% scoliosis prevalence in 22q11.2DS the second section of this thesis focuses on the question whether, from a scientific perspective, 22q11.2DS could be used as model to study the development of scoliosis in the general population.

In **chapter 6** the hypothesis that 22q11.2DS can be used as a model to study the development of scoliosis in the general population is introduced. Moreover, multiple questions were formulated that should be answered in order to determine whether 22q11.2DS can be used as a scoliosis model:

1. Does scoliosis in 22q11.2DS behave in a comparable manner as idiopathic scoliosis?
2. What is the prevalence of intraspinal anomalies in 22q11.2DS as compared to idiopathic scoliosis?
3. How is the neuromuscular status of patients with 22q11.2DS as compared to the general population?
4. What is the condition of the essential tissue structures (such as IVD) in patients with 22q11.2DS?

The goal of **chapter 7** was to answer the first two questions. In order for 22q11.2DS to be possibly suitable as a model for scoliosis, the curve characteristics should be similar to idiopathic scoliosis. We identified all ambulant patients with 22q11.2DS with non-congenital scoliosis and scoliosis onset of at least juvenile age. First, we determined the characteristics of the curve. Second, we determined the prevalence and rate of progression in 22q11.2DS scoliosis. Last, we identified the prevalence of intraspinal anomalies in pre-operative and fast progressive 22q11.2DS scoliosis cases. We showed that, in our included sample, nearly all patients had an idiopathic-like curve pattern (98.4%). Moreover, approximately half of the 22q11.2DS population had a progressive curve (54%, defined as progression of >5 degrees Cobb angle) with a median progression in that group of 2.5 degrees/year. The prevalence and rate of progression was comparable with idiopathic scoliosis. Last, the type and frequency of intraspinal anomalies seen in 22q11.2DS were comparable with idiopathic scoliosis.

Which arm position provides the most "functional representation" of the natural sagittal standing position?

Already in **chapter 1** we introduced the importance of the sagittal shape of the spine in relation to scoliosis. In **chapter 8**, we showed the first explorative results implying that

22q11.2DS might be used as a model to study the development of scoliosis in the general population. This study was performed in two 22q11.2DS clinics (CHOP and UMCU). In these two clinics there is different positioning protocol during radiography. In CHOP, radiographs are made with biplanar radiography (EOS®) and patients stand in the hands-on-wall position (**Figure 1, chapter 9)**. In UMCU, with conventional radiography, patients have the arms on the hands-on-cheek position (**figure 1, chapter 9)**. In order to truly compare sagittal results between multiple centers we should use the same position. This will make collaborative, multicenter, research easier and enlarger the chance on identifying causative mechanisms. We showed, in healthy volunteers, that the sagittal spinal alignment differs between the positons and the natural position. Although the differences are small, the hands-on-cheek position had the most overlap with the natural standing position.

Are there sagittal alignment differences between thoracic scoliosis, (thoraco)lumbar scoliosis and controls before the development of scoliosis?

After validation of 22q11.2DS as a model for scoliosis the ultimate goal is to find true etiological factors and subsequently identify possible preventive measurements. **Chapter 10** provides an example of such research. According to the dorsal shear force theory there should be sagittal alignment differences before the onset of scoliosis.[3] The proof-of-concept in **chapter 10** is the first prospective sagittal alignment study before the onset of scoliosis and indicates that there are indeed sagittal alignment differences between thoracic scoliosis versus (thoraco)lumbar scoliosis versus controls before onset of scoliosis. Patients with thoracic scoliosis had a longer and more proximally extending posteriorly inclined segment, before the development of scoliosis (Figure 3). Moreover, patients with (thoraco)lumbar scoliosis had a steeper posterior inclined segment as compared to thoracic scoliosis and controls (Figure 3). This study was performed in 31 patients with 22q11.2DS, based on the power calculation a study of 60 patients would be needed to prove a causal relationship between sagittal profile and scoliosis. Last, the posterior inclination triangle surface (PITS) reflects an overall risk factor for scoliosis: it was considerably higher in the group of scoliosis (thoracic and thoracolumbar scoliosis combined) as compared to controls.

Ethical considerations

A question that should be asked is whether it is ethical to use a subset of the population as a scientific model for the general population. All knowledge acquired in this thesis is based on data that was gathered by means of good clinical practice. However, if we intend to gather (more) data before onset of scoliosis (i.e. balance parameters, muscle strength) by means of research activities, this will be an extra burden for patients that can already have many medical issues. Importantly, the first patients that will benefit from the obtained knowledge, are patients with the 22q11.2 deletion. It is imperative that as soon as research (especially in this group of patients) has revealed differences between certain patients and or has revealed etiological factors, we take the next step: focus towards treatment and/or prevention. If the

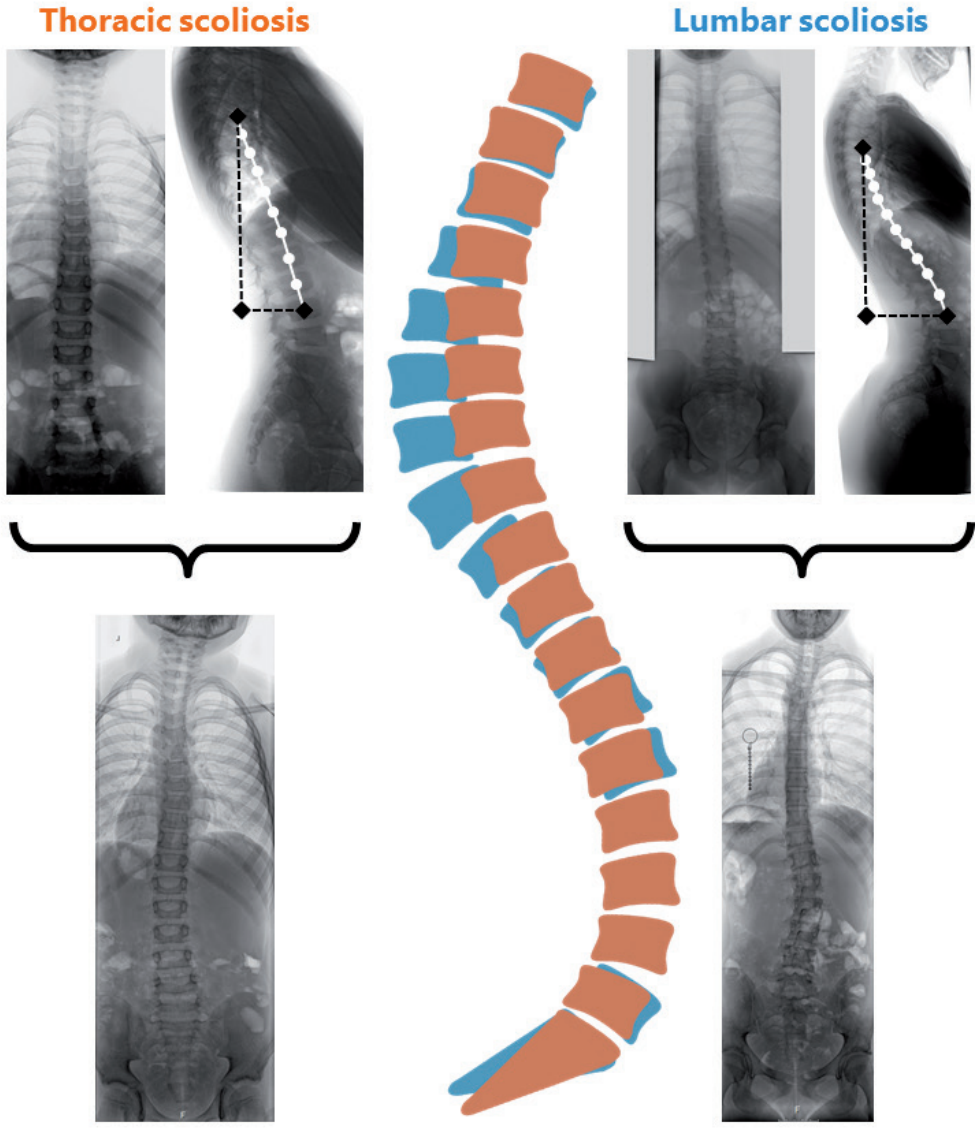


Figure 3: Differences in sagittal alignment between thoracic and lumbar scoliosis before curve onset
Differences in sagittal alignment between a patient with a thoracic scoliosis (left) and a lumbar scoliosis (right) before (top) the development of scoliosis (bottom). In the middle you can see an overlay of the differences between the thoracic (orange) and lumbar (blue) scoliosis before the onset of scoliosis: Thoracic scoliosis patients have a longer, more proximal, posterior inclined segment. Lumbar scoliosis patient have a steeper, shorter, posterior inclined segment. Last, the patients with thoracic scoliosis had another shape of the posterior inclined triangle surface (PITS, triangle with black and white dashed lines) as compared to patients with lumbar scoliosis.

"model" approach works, it is very likely that more grants and research time will be devoted towards patients with this condition, which will lead to more knowledge (and hopefully better care) for patients with 22q11.2DS. For our care and research we try to minimize the burden for the patient and constantly evaluated the need and intensity of the screening program. In CHOP and the UMCU (Wilhelmina's Children's hospital) we have a dedicated multidisciplinary 22q11.2DS team and dedicated 22q11.2DS outpatient clinic in order to give the patients (and parents) optimal care. By means of this specialized outpatient clinic we try to improve efficiency of the care for this vulnerable group of patients. Last, both in CHOP and the UMCU we have a weekly multidisciplinary 22q11.2DS scientific meeting in which we discuss past, current and future research, including the possible impact for patients.

Future Perspectives

Section A: Orthopedic Manifestations in the 22q11.2 Deletion Syndrome

Whereas our research on the orthopedic manifestations in 22q11.2DS was only observational, future research should focus on the true implications of this knowledge. For example, multiple studies are performed on the cervical spinal anomalies of patients with 22q11.2DS and as a result, in general, all patients with 22q11.2DS are screened between four and eight years of age. Yet, in the few case reports in which a patient with 22q11.2DS developed neurological symptoms based on cervical anomalies, the patients were older. This obviously lead to the question whether patients with 22q11.2DS should be screened (only) at a later age and/or whether patients should have a second screening at a later age. Since there is possible cervical instability in a subset of the patients, screening is important: in some hospitals, based on the anomaly, patients are advised to refrain from collision sports. Therefore, if needed, it seems more appropriate to perform a second screening at a later age instead of postponing the first moment of screening. Although the anomalies are congenital, the effect of these anomalies seems to have the possibility to develop over time throughout growth and development. Elaborating, it is important that patients (and parents) know the (subtle) effects of slowly developing myelopathy.

Although it will be challenging to have sufficient power, it is important to investigate whether current gold standard treatments for certain orthopedic manifestations, i.e. club foot and scoliosis, have the same effectiveness in 22q11.2DS as in the general population. These results would have important implications; at first for the healthcare provider; should there be more follow-up appointments? Should there be another (post-)treatment? Do we provide false (high) expectations to patients and parents? On the other hand, this will be important for patients and parents; can we expect the same results from a certain treatment as in the general population? For example: It is unclear whether conservative (brace) treatment for scoliosis in the 22q11.2DS population leads to the same results as in AIS. If conservative

treatment does not lead to a slower progression and or stop of curve progression the need for scoliosis screening will become different. At this moment, with CHOP and UMCU data combined, we do not have the power to determine whether brace treatment is effective in 22q11.2DS scoliosis.
The CHOP and UMCU are one of the few centers in the world in which the orthopedic surgeon is a standard member of the multidisciplinary team who sees the patient with 22q11.2DS multiple times between the age of six to 18 years. The musculoskeletal system is an important part of the body. Therefore it is important to make sure that the patients are in an optimal physical condition. Based on this thesis and the large number of possible musculoskeletal manifestations in 22q11.2DS, an orthopedic surgeon as a (standard) member of the specialized 22q11.2DS teams throughout the world should at least be (re) considered if not mandated.

Section B: The 22q11.2 Deletion Syndrome as a Model for Idiopathic Scoliosis

In section B of this thesis, we worked on the first two questions to answer the hypothesis whether 22q11.2DS can be used as a scientific model for scoliosis. The last two questions (IVD properties and neuromuscular status) should still be answered. For example, we know from existing literature that the deformation of AIS is mostly in the disc (and not in the vertebral body).[208,260] Logically, the same phenomenon should be seen in 22q11.2DS scoliosis. The same accounts for the neurological status of patients with 22q11.2DS and how/if this relates to scoliosis.

All-in all, we know that scoliosis in the general population is the result of an interaction between multiple pathways and risk factors.[2] Most likely, different risk factors have a different contribution in the overall risk of development of scoliosis (i.e. in the AIS population, being female, is clearly a risk factor). It we consider all factors as being on a balance; you will have risk factors on the one side and protective factors on the other side. It is very likely that the fulcrum of these factors is different in 22q11.2DS as compared to the general population (for example, in 22q11.2DS there is no difference in scoliosis prevalence between gender), however it can still shed light on different protective and risk factors. In the proof-of-concept study (**chapter 9**) we do show that the previously identified relation between sagittal pattern and the early forms of scoliosis is also present before onset of scoliosis. This finding indicates that we are on the right track and that we can use 22q11.2DS as a model for scoliosis in the general population.

An intriguing factor of 22q11.2DS is that scoliosis and schizophrenia, which at first sight seems to be very distinct, both occur 25 times more often as compared to the general population. [261,262] Moreover, both phenotypes in 22q11.2DS have large similarities with that disease in the general population and both diseases are natural to humans only, there are no existing natural animal models to date.[27,263] Interestingly, in the general population there

is a clustering of scoliosis and schizophrenia.[264] Within 22q11.2DS it is unknown whether there is clustering of scoliosis and schizophrenia. This is important to investigate: If there is indeed a clustering of scoliosis and schizophrenia in 22q11.2DS, this would lead to the suspicion of a combined etiological pathway. The other way around, if there is no clustering, this is more evidence that the 22q11.2DS itself is a multiplier effect on disease(s) that are exclusive to humans. This multiplier effect might be derived from the development of low copy repeats during evolution from hominids to human and which contributes to *de novo* cases of the 22q11.2 deletion.[265]

This thesis focused on whether 22q11.2DS could be used as a model for idiopathic scoliosis from a phenotypic and biomechanical perspective. Another approach is to focus on this question from a genotypic perspective. Currently, nearly 40% of all current etiological AIS research is focused on genetics. It is evident that genetics play a role in the development of scoliosis; there is a higher concordance of scoliosis in monozygotic twins (73%) than dizygotic twins (36%).[14] Moreover, first-degree relatives of AIS patients have an increased risk (6-11%) of developing AIS as compared to the general population.[266]
Genetic research in the cohort of scoliosis patients with 22q11.2DS could focus on a number of aspects. As discussed in the introduction, the typical deletion occurs between LCR22A and LCR22D, which involves ~3 Mb. The remaining 15% have “nested” deletions. To date, most of the variable effects of the deletion remain unexplained. A first step would be to identify whether the length of deletion has an effect on the development of scoliosis. Second, we can investigate whether there are variants at the other 22nd chromosome (on the 22q11.2 region) that causes the scoliosis (recessive condition). Last, we can investigate whether the common sequence variants as found in current genetic AIS studies occur more often in patients with 22q11.2DS with scoliosis as compared to patients with 22q11.2DS without scoliosis.

Despite the multiple large genetic studies that are ongoing, the genetic variants for the development of AIS are poorly understood. It is most likely a complex polygenic model, in which there is also large genotypic heterogeneity.[2] In current genetic (AIS) studies, the genome is considered in two-dimensional (2-D) linear fashion: There are very long lists of the genome and its variants and it is investigated whether there are less or more variants in the cases as compared to the controls. The same 2-D approach accounted for a long period of time in scoliosis (research), with the introduction of radiograph. Moreover, the official diagnosis of scoliosis is only in 2-D (a Cobb angle of at least 10 degrees). However, in recent years, it was shown (again) that it is important to analyze scoliosis in a 3-D fashion.[267] The same reasoning can be applied to genetic research: Two meters of DNA has to be folded into the nucleus and therefore sequence and interactions should be considered in 3-D.[268] Recently there is more interest in this 3-D organization of the genome and the role of the 3-D organization towards the functionality of genes. In 2-D certain regions can be very far apart;

yet in 3-D, the genome is actually folded and thus regions can be very close. This folding of the genome is nonrandom and results in structural units called topologically associating domains (TADs). These TADs are conserved among species, cell types and tissue and it is thought that they actually represent structural units of genome.[268,269] A possible explanation for the role of the 22q11.2 deletion in the development of scoliosis could be that not the specific genes itself, but the whole 22q11.2 region plays a role in the development. For example, in AIS, there can be many variants at the location at the 22q11.2 deletion, that may not be identifiable with genome-wide association studies (GWAS), but does have an effect on the TAD and thus on the functionality of a set of genes. Due to the 22q11.2 deletion the TAD can be changed and thus this deletion can have effects beyond the 2-D location of the 22q11.2 deletion.[270] In general, future research on genetics in AIS will most likely focus more on the 3-D architecture of the genome. Moreover, it is very well possible that in there, there lies a special role in the 22q11.2 deletion, since it is a prime example of a copy number variant, it can possibly change a TAD and the scoliosis associated with 22q11.2DS scoliosis has large similarities with idiopathic scoliosis.

In this thesis we focused on the question as to whether or not 22q11.2DS could be used as a model for scoliosis in the general population. This line of thought was derived from our collaborating partners in the field of schizophrenia research. Genetic schizophrenia research is far ahead as compared to genetic scoliosis research; in a recent schizophrenia GWAS there were over 40,000 patients with schizophrenia included, while in recent scoliosis GWAS there were <8,000 included.[271,272] By collaborating with psychiatric genetic experts we have the possibility to learn from their research and possible accelerate the genetic research in scoliosis.

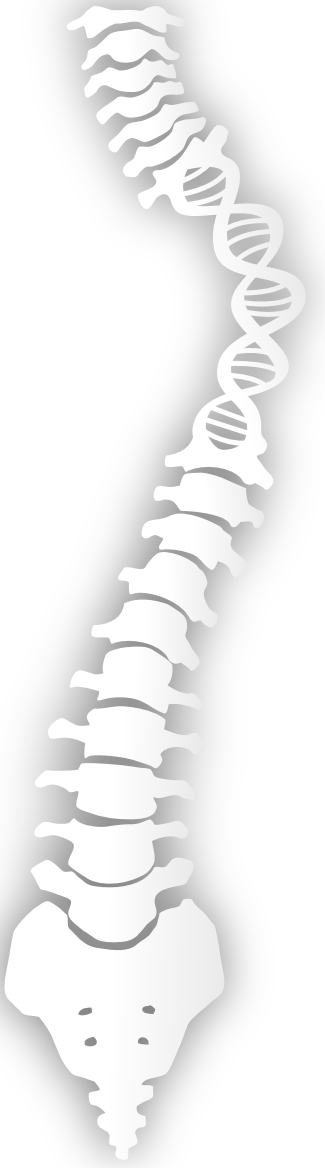
We showed that, phenotypically, 22q11.2DS can be used as a model for scoliosis in the general population. There are multiple syndromes (including Down and Prader-Willi syndrome) in which there is a higher prevalence of scoliosis.[273,274] Moreover, in Prader-Willi the majority of the patients have an idiopathic curve pattern as well.[273] The trajectory performed as in this thesis can be explored for other conditions: Interestingly in section A we showed that there was no association between CHD and scoliosis, whereas there is a higher prevalence of CHD in Down's syndrome as well.[275] In the end, combining different (syndromic) patient groups could shed light on common etio-pathogenesis pathways, such as CHD and scoliosis and provide insights into the development of scoliosis that are not possible to derive if you only study idiopathic scoliosis patients.

Final Conclusions

Over a century of dedicated research has been performed on the etiology of scoliosis. One of the main problems in that research is that patients only present following the onset of scoliosis and thus truly causative research is impossible. In this thesis, we introduced a new way of research in orthopedics that is already applied in other fields of medicine: using a subset of patients with a common condition as a model for the general population.

We started this thesis with a more general section concerning orthopedic manifestations in 22q11.2DS. This thesis shows that there are at least 58 musculoskeletal manifestations present in 22q11.2DS, of which multiple possibly need (surgical) intervention. The presence of club foot and scoliosis occurs 25-30 times more often as compared to the general population. Already for over forty years it has been shown that there is a clear association between CHD and scoliosis. However, we showed that the 22q11.2 deletion might actually be a confounder in this presumed association. The second part of this thesis focuses on the question whether 22q11.2DS might be used as a model for scoliosis in the general population. This research clearly is not complete; however the first steps have been taken. We revealed that, phenotypically, 22q11.2DS can be used as a model for scoliosis in the general population.

CHAPTER 11



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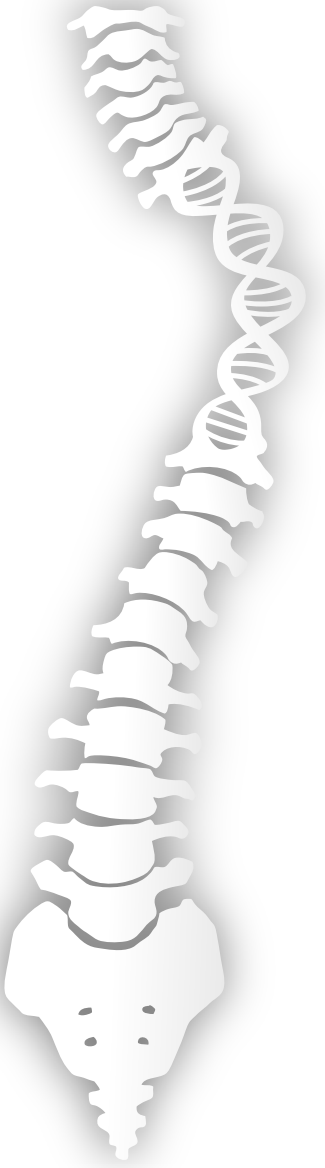
CHAPTER 12

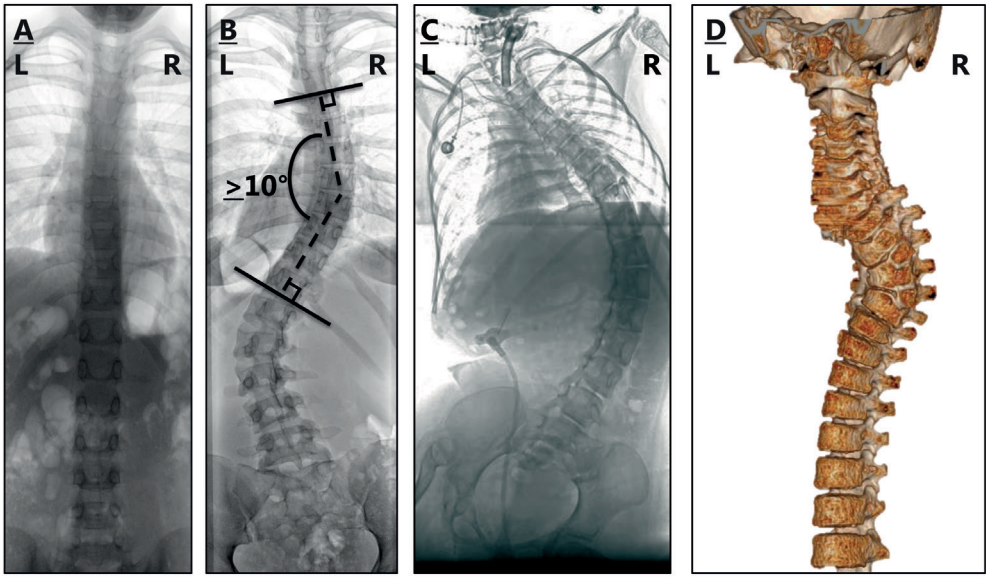


Nederlandse samenvatting

Samenvatting

Scoliose, een driedimensionale (3-D) deformatie van de wervelkolom en de romp, is een klassieke orthopedische deformiteit (Figuur 1).[1,2] Er zijn verschillende soorten scoliose, de meest voorkomende is adolescente idiopathische scoliose (AIS) en komt voor bij ongeveer 1-4% van de bevolking.[2] Ondanks decennialang onderzoek naar de etiologie van de idiopathische scoliose is er nog geen specifieke oorzaak gevonden en de algemene gedachte is dat AIS een multifactoriële oorzaak heeft.[2] Naast idiopathische scoliose zijn er verschillende types scoliose die wel een duidelijke oorzaak hebben, zoals congenitale of neuromusculaire scoliose (Figuur 1).

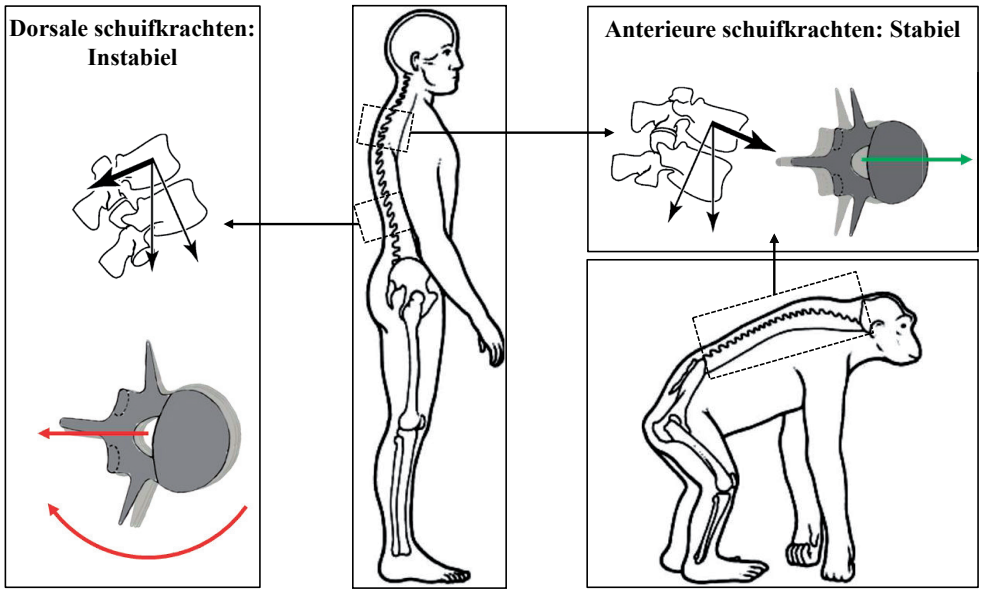
In de introductie van dit proefschrift (**hoofdstuk 1**) is de rol van het unieke biomechanische postuur van de mens en diens rol in de ontwikkeling van idiopathische scoliose benadrukt (zie Figuur 2). Daarnaast is een grote uitdaging in het huidige etiologisch onderzoek besproken: patiënten komen naar de scoliose polikliniek omdat ze een scoliose hebben ontwikkeld. Daarom is bij al het klinisch etiologisch onderzoek dat dan start niet mogelijk om te bepalen of de factoren die onderzocht worden oorzaak, gevolg of een epi-fenomeen zijn van de



Figuur 1: Een patiënt zonder (A) en meerdere patiënten met (B-D) een scoliose
A: Een patiënt zonder scoliose.
B: Een patiënt met een adolescente idiopathische scoliose (S-vorm, rechts thoracaal, links lumbaal). Een scoliose is gediagnosticeerd indien de Cobb hoek (de hoek tussen de meest gekantelde wervels) ten minste tien graden is.
C: Een patiënt met een neuromusculaire scoliose (C-vorm, lange curve).
D: Een patiënt met een congenitale scoliose (meerdere congenitale afwijkingen in de thoracale regio).

scoliose. Een mogelijke nieuwe benadering van deze uitdaging is het identificeren van een hoog-risico populatie op een bepaalde ziekte en deze populatie vervolgens bestuderen als een model voor de bevolking. In het Universitair Medisch Centrum Utrecht (UMCU) en het *Children's Hospital of Philadelphia* (CHOP) zijn verschillende kinderen behandeld waarvan gedacht werd dat ze AIS hadden. Echter, in een later stadium bleek dat deze kinderen het 22q11.2 deletie syndroom (22q11.2DS) hadden. Het doel van dit proefschrift is om te beoordelen of de scoliose binnen 22q11.2DS als model voor de bevolking gebruikt kan worden.

22q11.2DS is het meest voorkomende syndroom ná Down en komt voor bij 1 op 3000-6000 pasgeborenen.[42–45] Dit syndroom kenmerkt zicht door een brede fenotypische variabiliteit. Er wordt al uitvoerig onderzoek gedaan binnen 22q11.2DS: dit onderzoek richt zich voornamelijk op congenitale hart afwijkingen (CHD) en schizophrenie.[41,48,200,250–259] Tot nu toe, vergeleken met die onderzoekslijnen, is er slechts weinig onderzoek gedaan naar de orthopedische manifestaties binnen dit syndroom. Daarom richt het eerste deel van dit proefschrift op de orthopedische manifestaties binnen het 22q11.2DS.



Figuur 2: Uniek biomechanisch verschil tussen mensen en alle andere diersoorten
Mensen hebben in tegenstelling tot alle andere dieren het lichaamszwaartepunt boven het bekken en wervels die naar posterieur gericht zijn. Deze, naar posterieur gerichte, wervels hebben vanwege de dorsale schuifkrachten minder rotatoire stabiliteit in vergelijking tot naar anterieur gerichte wervels. Afbeeldingen overgenomen vanuit een studie van Castelein et al.[3]

Sectie A: Orthopedische manifestaties binnen het 22q11.2DS

Welke musculoskeletale afwijkingen zijn aanwezig binnen het 22q11.2DS?

In **hoofdstuk 2** is een systematische review van de literatuur verricht, met als doel om alle mogelijke musculoskeletale manifestaties die binnen 22q11.2DS voorkomen te ontdekken. De zoekopdracht leidde tot 69 artikelen, waarin 58 verschillende musculoskeletale manifestaties worden besproken. In het grootste gedeelte van de geïncludeerde artikelen was de musculoskeletale manifestatie niet het primaire onderwerp. Als gevolg daarvan was het bewijs van het grootste gedeelte van de manifestaties matig tot zwak.[52] Wat deze review echter wel laat zien is dat orthopedische manifestaties een belangrijk onderdeel zijn van 22q11.2DS. Het is van belang dat de orthopedisch chirurg op de hoogte is van de andere mogelijke aandoeningen (zoals een CHD). Tegelijkertijd is het van belang dat andere medisch hulpverleners op de hoogte zijn van deze orthopedische aandoeningen en dat deze aandoeningen mogelijk behandeling nodig hebben (zoals bijvoorbeeld instabiele cervicale afwijkingen, scoliose en klompvoet). Vervolgens is op twee van deze orthopedische manifestaties (klompvoet en scoliose) gefocust.

Wat is de prevalentie van klompvoet in 22q11.2DS en is het geassocieerd met CHD en/of schisis?

Een interessante bevinding van de systematische review die is uitgevoerd in **hoofdstuk 2** is dat de prevalentie van klompvoet binnen 22q11.2DS tussen 1.1% en 13.3% ligt. In de algemene populatie is de prevalentie van klompvoet 0.1%. Echter, geen van de klompvoet-studies zoals besproken in **hoofdstuk 2**, was primair gericht op de diagnose van klompvoeten. Indien er inderdaad een dergelijk hoge prevalentie van klompvoet binnen 22q11.2DS is, zou het aanwezig zijn van een klompvoet (in combinatie met andere (grote) aangeboren aandoeningen binnen 22q11.2DS) mogelijk moeten leiden tot genetisch onderzoek naar de 22q11.2 deletie. Daarom hebben we in **hoofdstuk 3** de eerste studie gedaan specifiek gericht naar klompvoeten in het grootste cohort van 22q11.2DS patiënten ter wereld. In dit cohort vonden we een klompvoeten prevalentie van 3.3%, hetgeen 30 keer hoger is dan de prevalentie in de algemene bevolking. We vonden geen associatie tussen klompvoeten en CHD/schisis.

Wat is de prevalentie van scoliose in 22q11.2DS en is het geassocieerd met CHD?

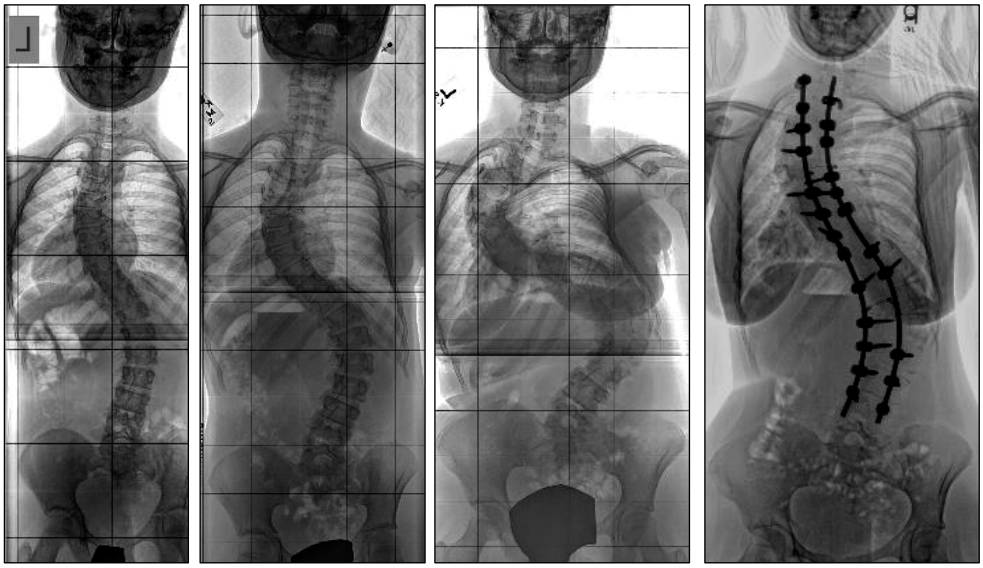
Net als de prevalentie van klompvoeten binnen 22q11.2DS, liet de systematische review (**hoofdstuk 2**) ook een grote spreiding in prevalentie van scoliose binnen 22q11.2DS zien (0.6%-60%). Het is belangrijk om de daadwerkelijke prevalentie van scoliose te weten, aangezien scoliose grote consequenties voor een patiënt kan hebben; screening gedurende de groei en mogelijk conservatieve evenals operatieve behandeling. De voornaamste reden voor chirurgie is dat, wanneer een curve groter is dan 45-50 graden, er ook na het einde van de groei kans is op progressie van de bocht. Daardoor kunnen er bochten groter dan 100

graden ontstaan (Figuur 3), welke neveneffecten als pijn, balans, long en hartproblemen met zich meebrengen. De studie in **hoofdstuk 4** is een combinatie van cross-sectioneel (UMCU) en retrospectief (CHOP) onderzoek, en toont aan dat bijna de helft (48-49%) van alle kinderen met 22q11.2DS een scoliose ontwikkelt. Bovendien, als we kijken naar alle patiënten van ten minste 16 jaar in CHOP, dan zien we dat 8% van deze patiënten scoliose chirurgie nodig heeft.

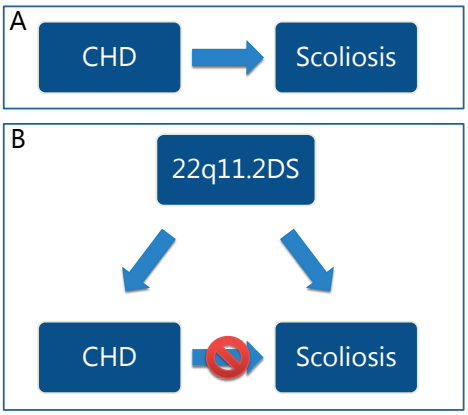
Gedurende de afgelopen 40 jaar wordt er in de literatuur een associatie beschreven tussen CHD en scoliose. Aangezien CHD vaak binnen 22q11.2DS voorkomt hebben we onderzocht of er een associatie was tussen scoliose en CHD in ons cohort, deze vonden we deze niet. Daarom hebben we het onderzoek naar de mogelijke associatie tussen CHD en scoliose voortgezet in **hoofdstuk 5**.

Wat is de prevalentie van scoliose in een cohort van CHD patiënten met en zonder de 22q11.2 deletie?

Voortbordurend op de inhoud van **hoofdstuk 4**, is in **hoofdstuk 5** onderzoek gedaan naar de mogelijke associatie tussen CHD en scoliose. Door de grote fenotypische variatie tussen 22q11.2DS patiënten en het feit dat in geen enkele huidige studie naar scoliose binnen CHD patiënten er genetisch onderzoek is gedaan naar (alle) patiënten, was onze gedachte dat de associatie zoals beschreven in de literatuur eigenlijk veroorzaakt wordt door de 22q11.2 deletie. Met andere woorden, ons idee was dat in studies die een relatie tussen CHD en



Figuur 3: Een voorbeeld van een progressieve scoliose
Indien een progressieve bocht niet behandeld wordt kan dit leiden tot een bocht van meer dan 100 graden (zie bovenstaande röntgenfoto's, verloop over de tijd van links naar rechts bij eenzelfde patiënt), welke ingrijpende scoliose chirurgie nodig heeft.



Figuur 4: De associatie tussen congenitale hartafwijkingen (CHD), het 22q11.2 deletie syndroom (22q11.2DS) en scoliose.
Gedurende de afgelopen 40 jaar wordt er een associatie beschreven tussen CHD en scoliose (A). Onze hypothese (B) is dat 22q11.2DS echter een *confounde*r is in deze mogelijke associatie

scoliose lieten zien, (niet herkende) patiënten met 22q11.2DS eigenlijk de oorzakelijke factor waren (Figuur 4). We hebben een studie uitgevoerd in een cohort CHD patiënten, waarbij het bij elke patiënt bekend was of deze patiënt wel/niet de 22q11.2 deletie had. In dit cohort CHD patiënten hebben we de aanwezigheid van scoliose onderzocht op de aanwezige thorax röntgenfoto's. In het cohort CHD patiënten met 22q11.2DS had de helft (53.5%) van de patiënten een scoliose. In het cohort CHD patiënten zonder 22q11.2DS was er een scoliose prevalentie van 7.9%. In de multivariate regressie hebben we aangetoond dat de 22q11.2 deletie verreweg de grootste risicofactor was, gevolgd door type CHD en de wijze van chirurgische benadering van de hartafwijking. Wanneer we kijken naar de prevalentie van scoliose in de algemene bevolking, gebaseerd op thorax röntgenfoto's, vinden we een prevalentie van 9% tot 13%. Met andere woorden het effect van CHD op de ontwikkeling van scoliose is vermoedelijk klein.[173,182] Deze studie suggereert dat de 22q11.2 deletie in de gezamenlijke oorzakelijke keten ligt van zowel CHD als scoliose.

Sectie B: Het 22q11.2 Deletie Syndroom als een Model voor Scoliose in de Bevolking

Een belangrijke vraag met betrekking tot de etio-pathogenese van idiopathische scoliose is hoe de interactie tussen het centraal zenuwstelsel, biomechanische, omgevings-, genetische en metabolische factoren werkt.[2] Gezien het unieke biomechanische postuur van de mens (Figuur 2), zijn wij van mening dat de interactie tussen beschermende en uitlokkende factoren het beste in de mens geanalyseerd kan worden. Hierop volgend, gezien de relatief lage prevalentie van scoliose in de algemene bevolking en een scoliose prevalentie van ongeveer 50% in patiënten met 22q11.2DS, is de hypothese dat vanuit een wetenschappelijk oogpunt 22q11.2DS gebruikt kan worden als een proxy voor de bevolking.

In **hoofdstuk 6** formuleren we meerdere vragen die beantwoord moeten worden om deze hypothese te testen:

1. Gedraagt scoliose binnen 22q11.2DS zich op dezelfde manier als idiopathische scoliose?
2. Wat is de prevalentie van intraspinale afwijkingen binnen 22q11.2DS in vergelijking tot idiopathische scoliose?
3. Hoe is de neuromusculaire status van 22q11.2DS patiënten vergeleken met de algemene bevolking?
4. Wat is de status van essentiële structuren zoals de tussenwervelschijf bij 22q11.2DS patiënten?

Het doel van **hoofdstuk 7** is om de eerste twee vragen te beantwoorden. Om te beoordelen of 22q11.2DS geschikt kan zijn als proxy voor idiopathische scoliose moeten de karakteristieken van de 22q11.2DS scoliose overeenkomen met die van idiopathische scoliose. We hebben alle ambulante 22q11.2DS patiënten vanaf een leeftijd van vier jaar met niet-congenitale scoliose in CHOP en het UMCU geïdentificeerd. Ten eerste hebben we de curve karakteristieken geanalyseerd. Ten tweede hebben we de prevalentie van progressieve bochten evenals de snelheid van progressie bepaald. Tot slot hebben we de prevalentie van intraspinale afwijkingen in een groep van preoperatieve en snel progressieve 22q11.2DS patiënten bepaald. Wij hebben aangetoond dat in onze subgroep nagenoeg alle 22q11.2DS patiënten een idiopathisch curve patroon hebben (98.4%), bovendien komt de prevalentie en de snelheid van progressie (54% en 2.5 graden per jaar) overeen met de algemene bevolking. Tot slot komt de frequentie en het type intraspinale afwijkingen overeen met idiopathische scoliose.

Welke arm positie komt het meeste overeen met de "natuurlijke armpositie" gedurende een laterale röntgenfoto?

In de introductie van dit proefschrift, **hoofdstuk 1**, is het belang van het sagittale profiel van de wervelkolom in relatie tot scoliose besproken. In **hoofdstuk 8** hebben we de eerste resultaten laten zien dat 22q11.2DS mogelijk als model voor idiopathische scoliose gebruikt kan worden. De studie in **hoofdstuk** 8 is uitgevoerd in twee 22q11.2DS centra (CHOP en UMCU). In deze twee centra is er een verschillende methode voor het maken van de laterale röntgenfoto: in CHOP worden de röntgenfoto's gemaakt met EOS® waarbij patiënten met de handen tegen de wand van de cabine staan (**Figuur 1, hoofdstuk 9**). In het UMCU worden conventionele röntgenfoto's gemaakt, waarbij de handen tegen de jukbeenderen worden gehouden (**Figuur 1, hoofdstuk 9**). Om het sagittale profiel goed te kunnen vergelijken tussen verschillende centra moet in elk centrum dezelfde positie gebruikt worden. Dit maakt het makkelijker om grote multicentrische studies te doen en daarmee vergroot het de kans op het identificeren van oorzakelijke mechanismen. Middels het gebruik van 3-D echo hebben we in **hoofdstuk 9**, met kleine verschillen, aangetoond dat wanneer de handen

op de jukbeenderen worden geplaatst dit de meeste overlap heeft met de “natuurlijke armpositie”(armen langs de zij).

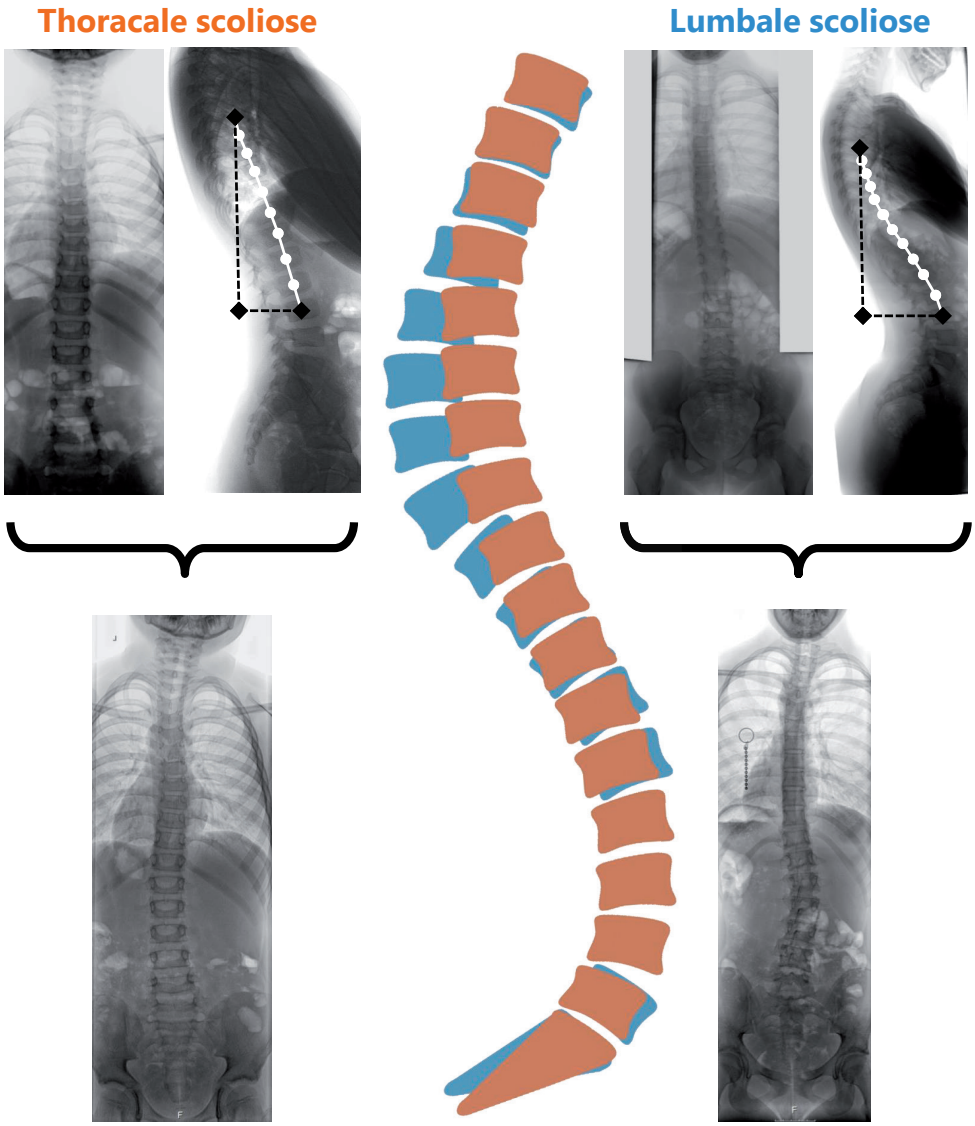
Zijn er verschillen in het sagittale profiel tussen thoracale scoliose, (thoraco)lumbale scoliose en controles voor de ontwikkeling van de scoliose?

Na validatie van 22q11.2DS als model voor de bevolking, is het volgende doel om etiologische factoren te ontdekken om hier vervolgens mogelijk preventieve maatregelen op toe te passen. In **hoofdstuk 10** wordt een voorbeeld van een dergelijk onderzoek gegeven. Volgens de dorsale schuifkrachten theorie (Figuur 2), moeten de sagittale verschillen aanwezig zijn voor de ontwikkeling van scoliose.[3] In **hoofdstuk 10** is de eerste prospectieve *proof-of-concept* studie uitgevoerd waarbij onderzocht is of er inderdaad sagittale verschillen zijn tussen verschillende types scoliose en controles voor de ontwikkeling van de scoliose. Voor de ontwikkeling van de scoliose hebben patiënten met een thoracale scoliose een langer en meer proximaal gedeelte van de wervelkolom dat naar posterieur is gericht (Figuur 5) in vergelijking tot (thoraco)lumbale scoliose en controles. Aan de andere kant hebben patiënten met een (thoraco)lumbale scoliose een posterieur segment dat méér naar achteren was gericht dan de thoracale scoliose en de controles voor de ontwikkeling van de scoliose (Figuur 5). Deze studie is uitgevoerd bij 31 22q11.2DS patiënten. Gebaseerd op de power calculatie hebben we tenminste 60 patiënten nodig om deze verschillen significant aan te tonen. Tot slot, het oppervlak van de posterieure inclinatie driehoek lijkt een algemene risicomaat voor de ontwikkeling van scoliose te zijn. Patiënten met een scoliose (thoracaal dan wel (thoraco)lumbaal) hebben een groter oppervlak van deze driehoek in vergelijking tot controles (Figuur 5).

Ethische overwegingen

Het is belangrijk om goed te overwegen of het ethisch verantwoord is om patiënten met 22q11.2DS als model voor de bevolking te gebruiken. Alle kennis die vergaard is in dit proefschrift is gebaseerd op gegevens die verzameld zijn op basis van goede klinisch zorg. Echter, wanneer we (meer) onderzoeksgegevens willen verzamelen (bijvoorbeeld vóór de ontwikkeling van de scoliose, zoals balans en spierkracht parameters), zal dit een extra belasting zijn voor patiënten die over het algemeen al veel zorg nodig hebben. Het is belangrijk dat de eerste patiënten die voordeel hebben bij dit onderzoek patiënten met 22q11.2DS zijn. Bovendien is het essentieel dat wanneer er oorzakelijke factoren worden gevonden er direct een volgende stap gezet wordt: de focus verplaatsen naar (mogelijke) behandeling en/of preventie.

Indien de gekozen benadering (22q11.2DS als model voor de algehele bevolking) inderdaad leidt tot meer inzichten voor de algemene bevolking zal dit waarschijnlijk leiden tot betere zorg voor de patiënten met 22q11.2DS: er zal dan meer tijd en onderzoeksgeld gestoken worden naar scoliose in de 22q11.2DS populatie. Dit zal naar alle waarschijnlijkheid leiden tot meer kennis en (hopelijk) betere zorg voor de 22q11.2DS patiënten.



Figuur 5: Verschillen in het sagittale profile van thoracale en lumbale scoliose zijn aanwezig voor de ontwikkeling van de deformiteit.
Er zijn verschillen in het sagittale profiel tussen patiënten met een thoracale scoliose (links) en een lumbale scoliose (rechts). In het midden is een schematische weergave van deze verschillen te zien. Patiënten met een thoracale scoliose (oranje) hebben een langer en meer proximaal gelegen posterior gericht segment. De lumbale scoliose patiënten (blauw) hebben een korter, meer naar achter gericht, posterieur segment. Middels de wit/zwarte stippellijn is de posterieure inclinatie driehoek aangegeven. Deze verschilt in vorm tussen de thoracale en lumbale scoliose.

In het UMCU zijn we constant bezig om de belasting voor zowel zorg als onderzoek zo laag mogelijk te houden voor de 22q11.2DS patiënten. In het UMCU is er een speciaal multidisciplinair team gericht op zorg voor patiënten met 22q11.2DS. Hiermee proberen we optimale (multidisciplinaire) zorg aan de patiënten (en ouders) te geven. Met dit team proberen we de zorg voor deze kwetsbare patiënten zo efficiënt mogelijk te houden en maken. Er is een wekelijkse multidisciplinaire 22q11.2DS onderzoek vergadering waarin afgerond, huidig en toekomstig onderzoek, inclusief de mogelijke impact voor patiënten, wordt besproken.

Toekomstperspectieven

Sectie A: Orthopedische Manifestaties binnen 22q11.2DS

Het uitgevoerde onderzoek in dit proefschrift was observationeel, de focus van toekomstig onderzoek zal moeten liggen op de implicaties van de opgedane kennis. Zo zijn er meerdere studies naar de cervicale afwijkingen binnen 22q11.2DS met als resultaat dat het wordt geadviseerd radiologische screening van de nek uit te voeren tussen de 4 en 8 jaar. Echter, in de *case reports* die gepubliceerd zijn over de ontwikkeling van neurologische symptomen waarop een operatie aan de nek nodig was, waren de patiënten ouder. Deze discrepantie leidt tot de vraag of 22q11.2DS patiënten op een latere leeftijd gescreend moeten worden? En moet dit dan een tweede screeningsmoment zijn en/of moet de initiële screening pas later plaats vinden? Afhankelijk van de afwijkingen wordt in sommige ziekenhuizen geadviseerd dat de kinderen geen hoge-impact sporten (als skiën, rugby) mogen doen. Daarom lijkt het het meest verstandig om de initiële screening te laten staan en mogelijk een tweede screeningsmoment toe te voegen. Bovendien is het van groot belang dat de ouders en patiënten inzicht verwerven in de kenmerken van langzaam ontwikkelende myelopathie. Ten tweede is het van belang te onderzoeken of de huidige orthopedische behandelingen (zoals voor klompvoet) tot dezelfde resultaten leiden binnen de 22q11.2DS populatie in vergelijking tot de algemene bevolking. Deze bevindingen zijn belangrijk voor zowel de dokter als de patiënt. Voor de dokter: moeten er meer follow-up momenten zijn? Is er een andere (na-)behandeling nodig? Geven we valse (hoge) verwachtingen aan patiënten en ouders? Voor de patiënt en ouders: zijn de resultaten het zelfde als voor de algemene bevolking? Zo weten we nu nog niet of conservatieve (brace) behandeling bij scoliose dezelfde effectiviteit heeft binnen als 22q11.2DS in vergelijking tot de algemene bevolking. Indien brace behandeling niet leidt tot langzamere en/of een stop van de progressieve is het minder noodzakelijk om te screenen voor scoliose. Gebaseerd op CHOP en UMCU data is het op dit moment nog niet mogelijk om te bepalen of brace behandeling zinvol is voor de 22q11.2DS scoliose.

Het UMCU is een van de weinige ziekenhuizen ter wereld waar de orthopedische chirurg een standaard onderdeel is van het multidisciplinaire 22q11.2DS team. In het UMCU

worden de patiënten tussen de leeftijd van zes en 18 jaar door de orthopedie gezien. Het musculoskeletale systeem is een belangrijk onderdeel van het lichaam. Het is belangrijk dat de patiënten in een zo optimaal mogelijke lichamelijke conditie komen. Gebaseerd op resultaten van dit proefschrift en het grote aantal mogelijke musculoskeletale aandoeningen binnen 22q11.2DS dient het toevoegen van een orthopedisch chirurg als standaard onderdeel van de multidisciplinaire 22q11.2DS teams ten minste te moeten worden (her) overwogen.

Sectie B: Het 22q11.2DS als een Model voor Scoliose in de Bevolking

In sectie B hebben we ons op de eerste twee vragen van de hypothese gericht. Vraag 3 en 4 met betrekking tot de discus eigenschappen en neuromusculaire status moeten nog worden beantwoord. Bijvoorbeeld, het is al beschreven dat bij er AIS voornamelijk deformatie zit in de discus en niet in het wervellichaam. Ditzelfde zou gezien moeten worden binnen 22q11.2DS. Hetzelfde geldt voor de neuromusculaire status van 22q11.2DS patiënten en hoe zich dit verhoudt tot de scoliose.

Als we naar de balans kijken tussen risicofactoren aan de ene kant en beschermingsfactoren aan de andere kant is het heel goed mogelijk dat binnen 22q11.2DS de verhouding tussen deze risicofactoren en beschermingsfactoren anders is dan binnen AIS. Zo is bijvoorbeeld, binnen 22q11.2DS er geen verschil in risico op scoliose tussen mannen en vrouwen. Toch kan etiologisch onderzoek binnen 22q11.2DS wel een licht doen schijnen op de aanwezigheid en de werking van de verschillende risicofactoren en beschermingsfactoren. Zo laten we in de *proof-of-concept* studie in **hoofdstuk 9** zien dat de beschreven associatie tussen sagittaal profiel en type scoliose (thoracaal versus lumbaal versus controle) aanwezig is voor de ontwikkeling van de scoliose. Deze bevinding toont aan dat we op de juiste weg zijn en dat 22q11.2DS als een model voor de algemene bevolking gebruikt kan worden.

Een intrigerend aspect van 22q11.2DS is dat scoliose en schizofrenie binnen 22q11.2DS allebei 25 keer vaker voorkomen in vergelijking tot de algemene bevolking.[261,262] Bovendien hebben beide aandoeningen binnen 22q11.2DS sterke fenotypische overeenkomsten met de uiting van de aandoening in de algemene bevolking en zijn het aandoeningen die allebei alleen bij mensen voorkomen.[27,263] In de algemene bevolking is er een clustering van scoliose en schizofrenie.[264] Het is op dit moment onbekend of deze clustering er ook binnen 22q11.2DS is. Dit is belangrijk om te onderzoeken: Als er inderdaad een clustering is van scoliose en schizofrenie is leidt dit tot de verdenking op een gezamenlijke etiologische oorzaak. Aan de andere kant, indien er geen clustering plaatsvindt, is dit meer bewijs dat 22q11.2DS op zichzelf een *multiplier* effect is van ziektes die enkel bij mensen voorkomen. Dit *multiplier* effect kan zijn oorsprong vinden in de ontwikkeling van de *low copy repeats* in de evolutie van de orang-oetan naar de mens, welke ook de basis vormt voor de 22q11.2 deletie.

Deze thesis heeft zich gericht op de vraag of 22q11.2DS fenotypisch gezien als een model voor idiopathische scoliose gebruikt kant worden. Een andere benadering is om deze vraag vanuit een genetisch perspectief aan te vliegen. Op dit moment is bijna 40% van het etiologisch onderzoek naar AIS gericht op genetica. Gezien het feit dat er een hoge concordantie is van scoliose binnen monozygote en dizygote tweelingen (73% en 36% respectievelijk) is het duidelijk dat er een genetische oorsprong aan AIS ligt.[14]

Er vinden veel grote genetische studies plaats naar AIS, het is het meest waarschijnlijk dat er een complex poly-genetisch model ten grondslag aan de ontwikkeling van scoliose ligt. Daarbij is er waarschijnlijk veel genotypische variatie.[2]

Genetisch onderzoek naar scoliose binnen 22q11.2DS kan zich op een aantal aspecten richten. Zoals besproken in **hoofdstuk 1** heeft 85% van de patiënten met 22q11.2DS de typische deletie tussen *low copy repeats* (LCR)22A en LCR22D. De overige 15% van de patiënten heeft een kleinere deletie (bijvoorbeeld tussen LCR22A en LCR22B of LCR22B en LCR22D. Een eerste stap in het genetisch onderzoek naar scoliose binnen 22q11.2DS zou zich kunnen richten op de lengte van de deletie: mogelijk heeft de lengte van de deletie invloed op het risico op de ontwikkeling van scoliose. Daarnaast kan er worden gekeken of er varianten op het andere chromosoom 22, op locatie q11.2, een effect hebben op het risico op scoliose. Tot slot kan er worden onderzocht of de genetische varianten die vaker voorkomen bij AIS ook vaker voorkomen bij de 22q11.2DS patiënten met een scoliose in vergelijking tot patiënten met 22q11.2DS zonder scoliose. Daarnaast wordt in huidige genetische (AIS) studies het genoom in 2-D bekeken: Er zijn lange lijsten van het genoom en de daarbij horende varianten. Vervolgens wordt onderzocht of er meer of minder varianten aanwezig zijn bij patiënten met in vergelijking tot patiënten zonder scoliose. Ook binnen het onderzoek naar AIS is er lange tijd in 2-D gekeken, met name sinds de introductie van de röntgenfoto. Echter, binnen het etiologisch onderzoek is recentelijk (opnieuw) het belang van het beoordelen van de wervelkolom in 3-D aan het licht gekomen.[267] Ook binnen het genetisch onderzoek is het belangrijk het DNA in 3-D te bekijken, namelijk: er zit twee meter aan DNA in de celkern gevouwen.[268] Op dit moment komt er meer aandacht voor de 3-D organisatie van het genoom in de celkern en de rol van deze 3-D organisatie met betrekking tot de functionaliteit van genen. Bepaalde regio's kunnen in 2-D erg ver van elkaar liggen, maar vanwege de vouwing kunnen deze regio's in 3-D dicht bij elkaar liggen. De vouwing van het genoom is niet-toevallig (*non-random*), deze 3-D structuur is georganiseerd in zogenoemde *topologically associating domains* (TADs). Deze TADs komen overeen tussen verschillende species en de gedachte is dat deze TADS eigenlijk structurele gebieden van het genoom zijn.[268,269] Het is goed mogelijk dat hierin de 22q11.2 deletie (en/of variaties in het 22q11.2 gebied) een belangrijke rol speelt.

In deze thesis hebben we ons gefocust op de vraag of 22q11.2DS als een model voor scoliose in de algemene bevolking gebruikt kan worden. Deze gedachtegang hebben we overgenomen van onze samenwerkingspartners op het gebied van schizofrenie onderzoek. Het genetisch schizofrenie onderzoek ligt ver voor in vergelijking tot het genetisch scoliose onderzoek, zo

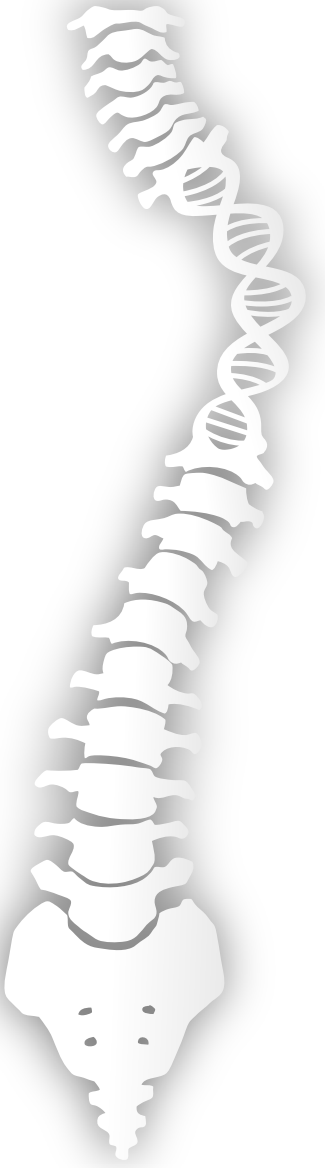
waren er in een recente schizofrenie GWAS studie 40.000 patiënten geïncludeerd, terwijl dit bij een recent scoliose onderzoek er <8.000 waren.[271,272] Door samen te werken met experts op het gebied van genetica-psychiatrie onderzoek hebben we de mogelijkheid om het genetisch onderzoek binnen scoliose te versnellen.
We hebben aangetoond dat 22q11.2DS, fenotypisch gezien, gebruikt kan worden als proxy voor de algemene bevolking. Echter, er zijn meerdere syndromen (o.a. syndroom van Down en Prader-Willi syndroom) waarbij er een hogere prevalentie is van scoliose.[273,274] Bovendien, heeft bij het Prader-Willi syndroom ook de meerderheid van de patiënten een idiopathisch curve patroon.[273] Het pad dat voor deze thesis is gekozen kan ook verkend worden voor andere syndromen. Het combineren van gegevens van verschillende (syndromale) patiëntengroepen kan leiden tot meer inzichten in de ontwikkeling van idiopathische scoliose, inzichten die mogelijk niet te verkrijgen zijn wanneer je alleen onderzoek doet naar idiopathische scoliose patiënten.

Eindconclusies

Een van de grote problemen in het huidige etiologische onderzoek naar idiopathische scoliose is dat patiënten zichzelf presenteren bij de orthopedisch chirurg na ontwikkeling van de scoliose. Met dat gegeven als startpunt is het onmogelijk om daadwerkelijk etiologisch onderzoek te doen. In deze thesis is er een nieuwe methode van etiologisch onderzoek binnen de orthopedie geïntroduceerd, een methode die al in andere medische disciplines wordt gebruikt: een subset van de populatie als model voor de bevolking.

Het eerste gedeelte van deze thesis richt zich op orthopedische manifestaties binnen 22q11.2DS. Deze thesis toont aan dat er ten minste 58 musculoskeletale manifestaties binnen 22q11.2DS zijn, waarvan meerdere mogelijke (chirurgische) interventie nodig hebben. Een klompvoet en scoliose komen 25-30 keer vaker voor binnen 22q11.2DS in vergelijking tot de algemene bevolking. Er wordt reeds gedurende 40 jaar een associatie tussen congenitale hartafwijkingen en scoliose beschreven, gebaseerd op ons onderzoek lijkt het echter dat de 22q11.2 deletie een *confounder* is in deze associatie. Het tweede gedeelte van deze thesis richt zich op de vraag of 22q11.2DS als een model voor scoliose in de algemene bevolking gebruikt kan worden. Hiervoor zijn de eerste stappen gezet, echter is dit onderzoek niet ten einde. In deze thesis is aangetoond, dat fenotypisch gezien, 22q11.2DS als een model voor scoliose in de algemene bevolking gebruikt kan worden.

CHAPTER 13

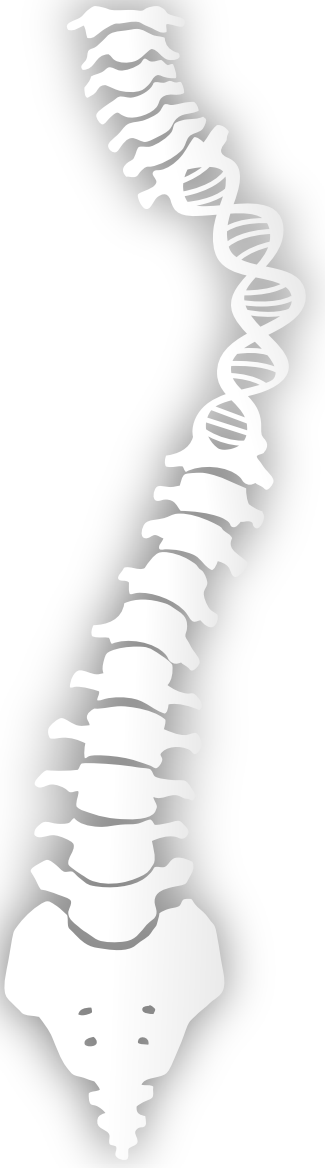


List of Abbreviations and Definitions

2-D	Two-dimensional
22q11.2DS	22q11.2 Deletion Syndrome
3-D	Three-dimensional
95%CI	95% confidence interval
AIS	Adolescent idiopathic scoliosis (10-16 years old)
ANOVA	Analysis of variance
Apex	The most laterally deviated vertebra or disc in a scoliotic curve in the coronal plane
BMD	Bone mineral density
CAVE	Acronym for the four characteristics of a club foot: **c**avus (a high medial longitudinal arch), forefoot **a**dductus, hindfoot **v**arus and hindfoot **e**quinus
CGH	Comparative genomic hybridization
CoP	Center of pressure
CHOP	Children's Hospital of Philadelphia
CI	Confidence interval
CHD	Congenital heart defect or congenital heart disease
CNV	Copy number variants
Cobb	Angle between lines drawn on endplated of the end vertebrae
Cobb end vertebrae	The cranial and caudal vertebrae that bound a scoliotic curve in the coronal plane
CT	Computed tomography
df	Degrees of freedom
DI	Declive segment inclination: angle between a line through the centroids of the cranial and caudal end level of the part of the spine that is posteriorly inclined and the vertical
DL	Declive length: length of the part of the spine that is posteriorly inclined segment, normalized for T1-L5
e.g.	Exempli gratia
Fig.	Figure
FISH	Fluorescence in situ hybridization
GWAS	Genome-wide association studies
Idiopathic	A disease that is not linked to any physical impairment or previous medical history (in greek: ἴδιος; *idios* "one's own" and πάθος; *pathos* "suffering"
i.e.	Id est
IIS	Infantile idiopathic scoliosis (0-3 years old)
IS	Idiopathic scoliosis
IVD	Intervertebral disc
IQR	Inter quartile range
ICC	Intraclass correlation coefficient
JIS	Juvenile idiopathic scoliosis (4-9 years old)
LCR	Low copy repeat
LL	Lumbar lordosis

Mb	Megabase
mm	Millimeter
MRI	Magnetic resonance imaging
OR	Odds ratio
P	
PI	Pelvic incidence
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
PT	Pelvic tilt
Scoliosis	A curvature of the spine of at least ten degrees in the coronal plane
SD	Standard deviation
SMA	Spinal muscular atrophy
SNP	Single nucleotide polymorphism
SPSS	Statistical Package for the Social Sciences
SS	Sacral slope
SSM	Statistical shape modelling
T	Level of thoracic vertebra
TAD	Topologically associating domain
TK	Thoracic kyphosis
UMCU	University medical Center Utrecht
Vs	Versus
Y	Years
#DV	Number of vertebrae of which the inferior endplate is posteriorly inclined as compared to the horizontal.

CHAPTER 14



List of Publications, Manuscripts, Acknowledgements and Curriculum Vitae

List of Publications and Manuscripts

This thesis is based upon the following publications and manuscripts:

Chapter 2: Homans JF, Tromp IN, Colo D, Schlösser TPC, Kruyt MC, Deeney VFX, Crowley TB, McDonald-McGinn DM, Castelein RM. Orthopaedic manifestations within the 22q11.2 Deletion syndrome: A systematic review. *Am J Med Genet A*. 2018;176:2104–2120

Chapter 3: Homans JF, Crowley TB, Chen E, McGinn DE, Deeney VFX, Sakkers RJB, Davidson RS, Castelein RM, McDonald-McGinn DM. Club foot in association with the 22q11.2 deletion syndrome : An observational study. *Am J Med Genet A*. 2018;176:2135–2139

Chapter 4: Homans JF, Baldew VGM, Brink RC, Kruyt MC, Schlösser TPC, Houben ML, Deeney VFX, Crowley TB, Castelein RM, McDonald-McGinn DM. Scoliosis in association with the 22q11.2 deletion syndrome: An observational study. *Arch Dis Child*. 2019;104:19–24

Chapter 5: Homans JF, de Reuver S, Heung T, Silversides CK, Oechslin EN, Houben ML, McDonald-McGinn DM, Kruyt MC, Castelein RM, Bassett AS. The Role of 22q11.2 Deletion Syndrome in the Relationship between Congenital Heart Disease and Scoliosis. **Under Review.** 2019

Chapter 6: Homans JF, Reuver S De, Breetvelt EJ, Vorstman JAS, Deeney VFX, Flynn JM, McDonald-McGinn DM, Kruyt MC, Castelein RM. The 22q11.2 Deletion Syndrome as a model for idiopathic scoliosis – a Hypothesis . *Med Hypotheses*. 2019;127:57–62.

Chapter 7: Homans JF, Kruyt MC, Schlösser TPC, Houben ML, Deeney VFX, Crowley T, McDonald-McGinn DM, Stücker R, Pasha S, Castelein RM. The 22q11.2 Deletion Syndrome as a possible model for Idiopathic Scoliosis. **under Review.** 2019

Chapter 8: Homans JF, Brink RC, Lee TT, Kiers H, Gielis WP, Kruyt MC, Zheng Y-P, Castelein RM, Abelin-Genevois K. The influence of arm position during imaging on the sagittal profile of the spine. **under Review**. 2019

Chapter 9: Homans JF, Schlösser TPC, Pasha S, Kruyt MC, Castelein RM. Different Scoliotic Curve Patterns develop based on pre-existent Differences in Sagittal Alignment in Patients with 22q11.2 Deletion Syndrome: A Perspective. **Submitted.** 2019

Other papers:

1. Brink RC, **Homans JF**, Schlösser TPC, van Stralen M, Vincken KL, Shi L, Chu WCW, Viergever MA, Castelein RM, Cheng JCY. CT-based study of vertebral and intravertebral rotation in right thoracic adolescent idiopathic scoliosis. *Eur Spine J*. 2019;Epub:1–9.

2. **Homans JF**, Kruyt MC, Schlösser TPC, Colo D, Rogers K, Shah SA, Flynn JM, Castelein RM, Pasha S. Changes in the Position of the Junctional Vertebrae After Posterior Spinal Fusion in Adolescent Idiopathic Scoliosis. *J Pediatr Orthop.* 2019;Epub:1–7.
3. Hundersmarck D, Slooff WBM, **Homans JF**, van der Vliet QMJ, Moayeri N, Hietbrink F, de Borst GJ, Öner FC, Muijs SPJ, Leenen LPH. Blunt cerebrovascular injury: incidence and long-term follow-up. *Eur J Trauma Emerg Surg.* 2019;Epub:1–10.
4. de Reuver S, Brink R, **Homans JF**, Kruyt MC, van Stralen M, Schlösser TPC, Castelein RM. The changing position of the center of mass of the thorax during growth in relation to pre-existent vertebral rotation. *Spine (Phila Pa 1976).* 2019;44:679–684.
5. Campbell IM, Sheppard SE, Crowley TB, McGinn DE, Bailey A, McGinn MJ, Unolt M, **Homans JF**, Chen EY, Salmons HI, Gaynor JW, Goldmuntz E, Jackson OA, Katz LE, Mascarenhas MR, Deeney VFX, Castelein RM, Zur KB, Elden L, Kallish S, Kolon TF, Hopkins SE, Chadehumbe MA, Lambert MP, Forbes BJ, Moldenhauer JS, Schindewolf EM, Solot CB, Moss EM, Gur RE, Sullivan KE, Emanuel BS, Zackai EH, McDonald-McGinn DM. What is new with 22q? An update from the 22q and You Center at the Children's Hospital of Philadelphia. *Am J Med Genet Part A.* 2018;176:2058–2069.
6. **Homans JF**, Slooff BM, de Jong PA, Buckens CF. Letter to the Editor: The Parkland Carotid and Vertebral Artery Injury Survey. *J Neurosurg.* 2016;124:1878–1879.

Acknowledgements

Prof. Dr. R.M. Castelein, beste René. Vanaf het eerste moment dat we elkaar uitgebreid spraken heb je mij het vertrouwen gegeven om met deze nieuwe tak van scoliose onderzoek aan de slag te gaan. Hartelijk dank daarvoor. Hoe verder ik in mijn promotie-traject kwam hoe meer ik me realiseerde dat het niet standaard is dat je je promotor wekelijks spreekt. Door de vele ontmoetingen, snelle feedback op zowel ideeën als manuscripten en continue blik op de rode draad is dit proefschrift geworden tot wat het nu is. Daarnaast wil ik je graag bedanken voor het vertrouwen dat je me hebt gegeven door je (inter)nationale netwerk met mij te delen. Dit resulteerde in bezoeken aan (en gezamenlijke papers met) o.a. Philadelphia, Wilmington, Montreal en Toronto.

Prof. D.M. McDonald-McGinn, Dear Donna, during our first skype session in December 2016 we discussed the first paper (that was submitted in March 2017) and my first visit to CHOP. You gave me the opportunity to visit CHOP twice, this was a great experience. I think that you have a wonderful 22q Clinic, both in terms of patient care and research. I could not have obtained the studies as presented in this thesis without your support, thank you very much!

Dr. M.C. Kruyt, beste Moyo, hartelijk dank voor het vele brainstormen evenals de altijd aanwezige kritische blik op de onderzoeksplannen en de daaruit volgende manuscripten. Dit heeft zeker gezorgd voor een hogere wetenschappelijke kwaliteit van de studies die ik heb uitgevoerd.

Dr. M.L. Houben, beste Michiel, hartelijk dank voor de overlegmomenten, zowel in de wekelijkse 22q11 researchmeeting als daarbuiten. Je staat altijd open voor nieuwe (wetenschappelijke) ideeën, waarbij de patiënt overduidelijk op één staat. Veel dank voor je belangrijke bijdrage aan dit proefschrift.

All studies performed in this thesis were done in collaboration with international colleagues. A special thanks to Blaine Crowley, dr. Davidson, dr. Deeney, dr. Flynn and Saba Pasha, from the Children's Hospital of Philadelphia; dr. Rogers and dr. Shah from the Nemours/Alfred I. duPont Hospital for Children; prof. Bassett from The Dalglish 22q Clinic for Adults, Toronto, dr. Abelin-Genevois from Centre Medico Chirurgical et de Réadaptation des Massues - Croix Rouge, Lyon; Prof. Stücker from Altona Children's Hospital, Hamburg; Prof. Zheng and Timothy Lee from The Hong Kong Polytechnic University and last but not least the (Dutch) international colleagues Elemi Breetvelt and Jacob Vorstman from the Hospital for Sick Children, Toronto.

Dr. T.P.C. Schlösser, beste Tom, hartelijk dank voor al je (wetenschappelijke) adviezen. Zeker in de beginfase van mijn promotie-traject hadden we veelvuldig contact. Hierdoor heb ik een goede en snelle start van mijn onderzoek gehad, dank!

Dr. R.C. Brink, vriend, collega, paranimf. Van samenwonen aan de Goedestraat via Q03.2.305, naar wonen in Maarssen en Baarn. Of het nu gaat over het gezamenlijk leggen van een laminaat, inkopen van vuurwerk, vullen van 1000 enveloppen of opzetten van een studie, ik ken niemand die dit zo nuchter, efficiënt en humoristisch tegelijkertijd kan uitvoeren. Boven alles bedankt voor onze goede vriendschap.

Haa Cor, papa, ik vind het ontzettend leuk dat jij mijn paranimf bent. Sommige dingen zijn zo-vader-zo-zoon en daar ben ik best trots op. Samen met mama staan jullie altijd voor het hele gezin klaar.

Alle collega's van de orthopedie (in het bijzonder de onderzoekscollega's Bruce, Jasmijn, Lorenzo, Rob, Sebastiaan, Steven, Chella, Floris, Jonneke, Justin, Koen, Chien, Erin, Esmee, Isabel, WP, Mechteld, Hilde, Pauline, Jasper, Margot en Mattie) dank voor alle afleiding die nodig was voor het maken van dit proefschrift: van organisatie researchmeeting naar orthofeest, van frog-of-the-day (-week-year) naar Q-meeting, van Hide-Out naar Nijeveen, van pingpong naar 07:15 in zwembad de Krommerijn.

Collega's van de 22q11 polikliniek en de wekelijkse 22q11 onderzoeksmeeting, hartelijk dank voor de fijne samenwerking de afgelopen drie jaar.

Simone Sienema, veel dank voor alle hulp rondom het werken in het UMCU en organisatie rondom inplannen van bijeenkomsten met René.

Alle studiedeelnemers, dank voor uw deelname aan het onderzoek.

Lieven familie, schoonfamilie en vrienden (in het bijzonder mama, papa, Willemijn, Joris, Japke, Svea, Lotta, Silke, Tjalling, Guus, Douwe, Eva, Emma, Wietske, Erik-Jan, Joppe en iedereen met wie ik nog niet zo lang geleden op het Markermeer was), hartelijk dank voor alle steun en afleiding tijdens dit promotie-traject.

Lieve Dolfine, #Team Kosters-Homans, dank voor je onvoorwaardelijke steun, je bent er altijd voor mij. Ik heb ongelofelijk veel zin in de toekomst samen met jou!

Curriculum vitae

Jelle Homans was born on May 11th 1989 in Hengelo, the Netherlands and grew op as the youngest of five children. In 2007 he graduated from high school (VWO (Gymnasium), *De Grundel*, Hengelo). After high school, he studied bass trombone at the conservatory for one year in Utrecht and started his medicine study (BSc and MSc) at the Utrecht University in 2008. In January 2016 he worked as a non-training orthopedic resident at *Flevoziekenhui*s in Almere. In December 2016 he started as a full-time researcher at the University Medical Center Utrecht under supervision of prof. R.M. Castelein, dr. M.C. Kruyt and dr. M.L. Houben. A part of his PhD was performed at the Children's Hospital of Philadelphia under direct supervision of prof. D.M. McDonald-McGinn. During his PhD program, Jelle Homans continued to work in previously established (inter)national collaborations including the Children's Hospital of Philadelphia (Philadelphia, USA) and Alfred I DuPont Hospital for Children (Wilmington, USA). Furthermore, he initiated new collaborations with Toronto General Hospital (Toronto, Canada), Hospital for Sick Children (Toronto, Canada) and the Antona Children's Hospital (Hamburg, Germany). Besides collaborating with orthopedic surgeons, he also collaborated with a variety of (medical) specialists including clinical geneticists, genetic counselors, pediatricians, plastic surgeons, ENT surgeons and psychiatrists. The new research line described in this thesis led to two grants of the Scoliosis Research Society (small exploratory grant and standard research grant).