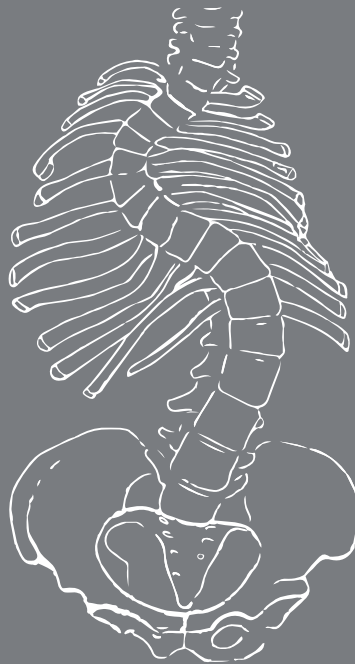


Thesis for doctoral degree (Ph.D.)  
2015

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# Inheritance and genetics in idiopathic scoliosis



Anna Grauers



**Karolinska  
Institutet**

From the Department of Clinical Science, Intervention and  
Technology (CLINTEC), Division of Orthopedics and Biotechnology  
Karolinska Institutet, Stockholm, Sweden

# **INHERITANCE AND GENETICS IN IDIOPATHIC SCOLIOSIS**

Anna Grauers



**Karolinska  
Institutet**

Stockholm 2015

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Cover artwork: Elisabet Einarsdóttir and Tommy Sund. Adapted from: Braus H. Anatomie des Menschen: ein Lehrbuch für Studierende und Ärzte.

Published by Karolinska Institutet.

Printed by AJ E-print AB.

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ISBN 978-91-7549-904-8



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(CLINTEC), Division of Orthopedics and Biotechnology**

## **Inheritance and genetics in idiopathic Scoliosis**

THESIS FOR DOCTORAL DEGREE (Ph.D.)

which, by due permission of Karolinska Institutet, will be publicly defended in the English language in Hillarpsalen, Retzius väg 8, Karolinska Institutet.

**Saturday 23 May 2015 at 10 am**

By

**Anna Grauers**

MD

*Principal Supervisor:*

Associate professor Paul Gerdhem  
Karolinska Institutet  
Department of Clinical Science, Intervention and  
Technology (CLINTEC)  
Division of Orthopedics and Biotechnology

*Co-supervisor(s):*

Professor Juha Kere  
Karolinska Institutet  
Department of Biosciences and Nutrition

Associate professor Elísabet Einarisdóttir  
Karolinska Institutet  
Department of Biosciences and Nutrition

*Opponent:*

Professor Jack Cheng  
The Chinese University of Hong Kong  
Department of Orthopaedics and  
Traumatology

*Examination Board:*

Professor Hans Tropp  
Linköping University  
Department of Clinical and Experimental  
Medicine

Professor Jens Ivar Brox  
University of Oslo  
Department of Physical Medicine and  
Rehabilitation

Associate professor Erik Björck  
Karolinska Institutet  
Department of Molecular Medicine and  
Surgery

**Stockholm 2015**



To my grandmother Ulla Hall



## ABSTRACT

Idiopathic scoliosis is the most common spine deformity, affecting approximately 3% of children and adolescents. Its aetiology is still unknown. However, relatives of individuals with idiopathic scoliosis have a higher risk of developing scoliosis compared to the general population. The aim of this thesis was to improve our understanding of the hereditary and genetic background of idiopathic scoliosis.

Self-reported data on scoliosis in twins (n=64,578) in the population-based Swedish Twin Registry were analysed to estimate the relative importance of genetic effects on the phenotypic variance – that is, the heritability of scoliosis. Using structural equation modeling, we estimated that 38% of the phenotypic variance of scoliosis is due to additive genetic effects and 62% to unique environmental effects.

In ScoliGeneS, an ongoing multi-centre study, we included individuals with idiopathic scoliosis and controls. The importance of a family history of scoliosis was investigated in 1,463 individuals with idiopathic scoliosis. Among those treated with a brace or surgery for scoliosis, 53% reported one or more relatives with scoliosis compared to 46% of the untreated, indicating a higher risk of treatment in the presence of a family history of scoliosis (odds ratio 1.32; 95% confidence interval 1.06–1.64). The prevalence of back problems was investigated in 1,069 adults with idiopathic scoliosis and in 158 controls. Back problems were reported in 64% of the individuals with scoliosis compared to 29% of the controls ( $p < 0.001$ , adjusted for sex, age and smoking). No differences between untreated and treated individuals with idiopathic scoliosis regarding the prevalence of back problems in adulthood were seen.

Four common single-nucleotide variants, previously shown to be associated with idiopathic scoliosis, were genotyped in 1,739 individuals with idiopathic scoliosis from the ScoliGeneS cohort and in 1,812 controls. In addition, the protein-coding regions of the genome – the exome – was sequenced in pooled samples (10x10) from 100 surgically treated patients in the ScoliGeneS cohort. We found a strong association of idiopathic scoliosis with a common previously known variant downstream of the *LBX1* gene (OR=1.53;  $p = 7.0 \times 10^{-18}$ ). We identified twenty novel variants by exome sequencing after filtering and an initial genotyping validation. No significant association was found with idiopathic scoliosis in the large cohort of 1,739 cases and 1,812 controls.

In summary, inherited factors are of importance in the development and progression of idiopathic scoliosis. A genetic variant downstream of the *LBX1* gene is strongly associated with idiopathic scoliosis. We were unable to find genes of similar or stronger effect.



## SAMMANFATTNING

Idiopatisk skolios är ett tillstånd där ryggen kröks och roterar i frontalplanet. Det är den vanligaste förekommande ryggsdeformiteten; ca 3% av alla barn och ungdomar drabbas. Av dessa får de flesta en liten krök av ryggraden men cirka en tiondel utvecklar en kraftig deformation av rygg och bröstorg. Detta kan leda till ryggbesvär och nedsatt lungfunktion förutom en psykisk påverkan. För att förhindra utvecklingen av en kraftig deformitet korsettbehandlas barn som har tillväxt kvar. I de fall detta inte hjälper eller om kröken är för stor erbjuds patienterna steloperation och kirurgisk korrigerande av deformiteten. Idag finns inga säkra sätt att på individnivå förutspå vem som kommer att utveckla en kraftig och därmed behandlingskrävande skolios. Detta innebär att ett stort antal barn och ungdomar följs av sjukvården under uppväxten med upprepade läkarbesök och röntgenkontroller.

Den specifika orsaken till idiopatisk skolios är fortfarande okänd. Det är väl känt att släktingar till skoliospatienter har en högre risk att själva få skolios. Detta projekt syftar till att studera den ärftliga och genetiska bakgrunden till idiopatisk skolios.

Med hjälp av frågeformulärsdata har vi studerat skoliosförekomsten hos 64578 tvillingar i svenska tvillingregistret. Vi fann att heritabiliteten, dvs den relativa betydelsen av genetiska effekter på variationen i en sjukdom, var 38% för skolios.

I en multi-center studie inkluderade vi individer med idiopatisk skolios och studerade förekomsten av släktingar med skolios hos en grupp på 1463 individer. 53% av de som behandlats för sin skolios hade en eller flera släktingar med skolios jämfört med 46% av de obehandlade (odds ratio 1.32; 95% CI 1.06-1.64). Förekomsten av ryggproblem studerades i en grupp på 1069 vuxna som fått skolios som barn och jämfördes med 158 kontroller. Bland vuxna med idiopatisk skolios hade 64% ryggproblem jämfört med 29% av kontrollerna ( $p < 0.001$ , justerat för kön ålder och rökning). Däremot hade ryggbesvären ingen större effekt på deras vardag. Vi fann heller ingen skillnad i ryggbesvär mellan korsettbehandlade, opererade och obehandlade individer.

Vi genotypade fyra vanligt förekommande basparsvariationer i 1739 individer med idiopatisk skolios och 1812 populationsbaserade kontroller och fann att en variant i närheten av genen *LBX1* (OR=1.53;  $p=7.0 \times 10^{-18}$ ) var starkt associerad till idiopatisk skolios. Vi sekvenserade de proteinkodande regionerna i genomet, exomet, i poolade prover från 100 stycken patienter med allvarlig skolios. Vi identifierade ett flertal nya varianter efter filtrering och validering i en mindre grupp patienter. Däremot fann vi ingen signifikant association av dessa varianter till idiopatisk skolios i den stora kohorten på 1739 patienter och 1812 kontroller.

Sammanfattningsvis har vi visat att ärftliga orsaker bidrar till utvecklingen av idiopatisk skolios och att förekomsten av släktingar med skolios ökar risken för att få en behandlingskrävande skolios. I genetiska studier har vi visat att en variant i närheten av *LBX1* genen är starkt associerad till idiopatisk skolios.

## LIST OF SCIENTIFIC PAPERS

- I. **Grauers A**, Rahman I, Gerdhem P.  
Heritability of scoliosis.  
*Eur Spine J.* 2012; 21: 1069-1074.
- II. **Grauers A**, Danielsson A, Karlsson MK, Ohlin A, Gerdhem P.  
Family history and its association to curve size and treatment in 1463 patients with idiopathic scoliosis.  
*Eur Spine J.* 2013; 22: 2421-2426.
- III. **Grauers A**, Topalis C, Möller H, Normelli H, Karlsson MK, Danielsson A, Gerdhem P.  
Prevalence of back problems in 1069 adults with idiopathic scoliosis and 158 adults without scoliosis.  
*Spine.* 2014; 39: 886-892.
- IV. **Grauers A**, Wang J, Einarsdottir E, Simony A, Danielsson A, Åkesson K, Ohlin A, Halldin K, Grabowski P, Tenne M, Laivuori H, Dahlman I, Andersen M, Christensen SB, Karlsson MK, Jiao H, Kere J, Gerdhem P.  
Candidate gene analysis and exome sequencing confirm *LBX1* as a susceptibility gene for idiopathic scoliosis.  
*Manuscript submitted.*

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## **LIST OF ABBREVIATIONS**

DZ	Dizygotic
LBX1	Ladybird homeobox 1
MRI	Magnetic resonance imaging
MZ	Monozygotic
PCR	Polymerase chain reaction
ScoliGeneS	The 'Scoliosis and Genetics in Scandinavia' project
SNV	Single-nucleotide variant
TLSO	Thoracolumbosacral orthosis

# 1 BACKGROUND

## 1.1 INTRODUCTION

Spinal deformities were already well known in ancient Greece. Hippocrates (460–370 BC) explicitly described scoliosis in *On the Articulations*. Claudius Galen of Pergamon (130–200 AD), another Greek physician, is credited with assigning the term ‘scoliosis’, derived from the Greek word for ‘crooked’, to this phenomenon (1).

Examples of skeletons exhibiting idiopathic scoliosis have been found throughout history. Richard III, the last Plantagenet king of England, was killed in battle on 20 August 1485 at the age of 33. In 2012, when his skeleton was excavated in Leicester, it was discovered to have a severe but well-balanced right convex thoracic scoliosis (2). In a supine position the Cobb angle was estimated at 75 degrees. There were no vertebral anomalies or other skeletal signs indicating a neuromuscular or connective tissue disorder. Taken together with descriptions of a humpback and short stature in real life, it is not unreasonable to believe that he suffered from idiopathic scoliosis.

## 1.2 CLINICAL MANIFESTATION

### 1.2.1 Clinical presentation

Scoliosis, the most common form of spinal deformity, is defined as a lateral deviation and structural rotation of the spine. A scoliotic spine can develop due to vertebral malformations, neuromuscular disorders, tumours, or various other syndromes. The most common form, however, idiopathic scoliosis, refers to the entity without any associated disorders and of unrecognised cause.

Idiopathic scoliosis affects otherwise healthy children and adolescents during growth. It usually presents as a rib hump visible at forward bending, together with unlevelled shoulders and asymmetrical waist, Figure 1. Most often the deformity is discovered in school-screening programmes or by close relatives.

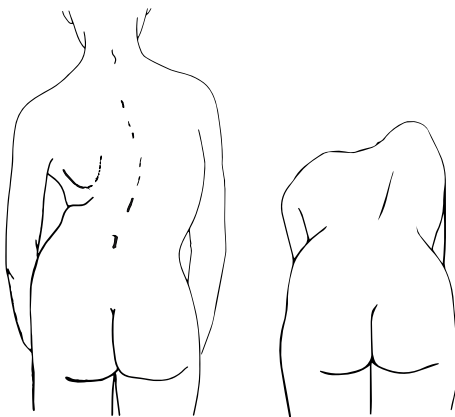


Figure 1. Left: drawing of a right convex thoracic scoliosis. Note waist asymmetry. Right: Adams test (forward bending test) .Visible rib hump at forward bending indicates a scoliosis (3). Artwork: Elísabet Einarsdóttir and Tommy Sund.

The diagnosis is confirmed by a standing spinal radiograph showing a lateral curvature of the spine exceeding 10 degrees according to Cobb (4), Figure 2. A thorough medical history and clinical examination are required to exclude associated disorders. Adams' test (forward bending test) is used to estimate rotation of the trunk – that is, the rib hump, Figure 1. When no signs of any associated disorder are found the scoliosis is said to be idiopathic.

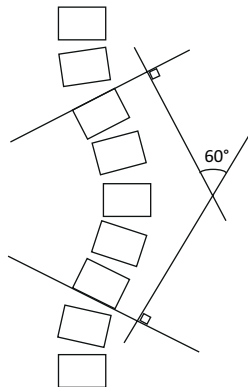


Figure 2. The Cobb angle is measured between the vertebrae with the largest inclination. Artwork: Elísabet Einarsdóttir.

### 1.2.2 Prevalence

The prevalence of idiopathic scoliosis is approximately 2–3% worldwide (5-7). Most individuals have small curvatures, girls and boys being equally affected. Approximately 10% progress to a moderate or severe curve (6, 7). Among those with severe curves the percentage of boys is less than 10% (8).

### 1.2.3 Curve patterns

There is a high variability in the clinical manifestation or phenotype of idiopathic scoliosis: the apex of the major curve may be thoracic, thoracolumbar or lumbar and the convexity may be either left or right-sided, with compensatory curvatures above and below, as can be seen in Figure 3. Some patients have double major curves – two curves of similar size, Figure 3. The spine can be in or off balance – that is, the head of the patient is not centralised on top of the pelvis. The most common form is a right thoracic convexity with a compensatory left lumbar convexity. A left thoracic convexity is uncommon and more often associated with asymptomatic neural axis abnormalities (9). A double major curve and lumbar curves tend to be discovered at larger Cobb angles since the typical “rib hump” may be absent. Several classification systems based on the radiographical image of the deformity have been described, the Lenke classification being the most renowned (10).

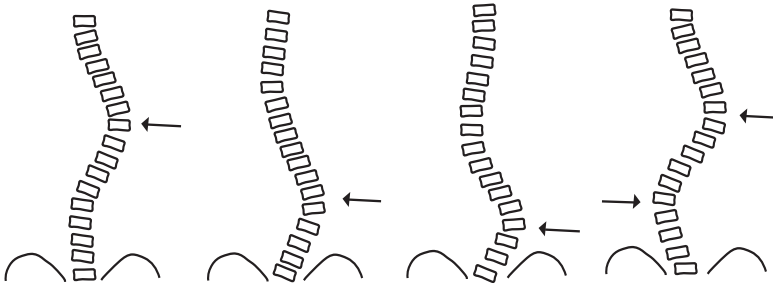


Figure 3. Curve patterns according to the level of the apex of the major curve (arrows). From left to right: thoracic, thoracolumbar, lumbar and double major. Artwork: Elísabet Einarsdóttir.

#### 1.2.4 Age at onset

Idiopathic scoliosis was originally classified according to its age of presentation: infantile (0–3 years), juvenile (4–9 years), or adolescent ( $\geq 10$  years) (11). Infantile idiopathic scoliosis has a different clinical course, involving a higher percentage of boys and spontaneously resolving, and will not be further considered in this thesis (12). Asymptomatic neural abnormalities are more common in scoliosis with juvenile onset (13). Recently a modified classification – early versus late onset – has been suggested in consideration of the different treatment regimens needed (14).

#### 1.2.5 Progression

Scoliosis is most often discovered through school-screening programmes or by the parents. It is difficult to identify the individuals in whom the scoliosis will progress. A young age at onset, large curvature at presentation, a thoracic curve pattern, and skeletal immaturity increase the likelihood of progression (15, 16), as shown in Table 1. Thoracic curves in the skeletally immature individual have the highest risk of progression, 58–100% (16–18). When the individual stops growing, the risk of progression diminishes. At skeletal maturity, curves less than 30 degrees carry a very small risk of progression. In contrast, curves that reach 50 degrees continue to progress throughout adulthood, at a rate of approximately  $1^\circ$  per year (17). To detect progression, idiopathic scoliosis patients are frequently followed in the outpatient clinic, through spinal inspection and spinal radiographs until they have terminated growth.



Table 1. Risk of curve progression in relation to curve size and age at clinical presentation (19). The percentages in the contingency table refer to the risk of progression in an individual presenting in the clinic with this particular combination of age and curve size.

Cobb Angle (degrees)	Age at onset of curvature (years)		
	10-12	13-15	16-
<20	25%	10%	0%
20-29	60%	40%	10%
30-59	90%	70%	30%
>60	100%	90%	70%

### 1.3 TREATMENT

Treatment of scoliosis is recommended to prevent progression into a severe deformity. Today, in patients with remaining growth, the general recommendation is brace treatment for curves of 25–40 degrees and surgical treatment for curves >45 degrees. In skeletally mature adolescents, surgical treatment is recommended for curves >50 degrees.

#### 1.3.1 History

Various treatments for scoliosis have existed throughout history (1, 20). Hippocrates advocated an extension apparatus for intermittent traction. Claudius Galen of Pergamon refined the technique; otherwise no major developments were seen until the 16th century when Ambrose Paré (1510–1590) invented the first supportive brace, an iron corset. In 1865, William Adams, the same Adams as in the Adams’ test, advocated a light steel spinal support, partial recumbency, and light gymnastic exercises (3). In 1874, Lewis Albert Sayre described the “plaster-of-Paris bandage” – a cast that was moulded on the body while the patient was suspended off the ground in a frame – and recommended gymnastic exercises.

In 1911, Russell Hibbs performed the first invasive procedure in the treatment of spinal deformities (21). His aim was to achieve a bony fusion of the vertebrae in order to halt the progression of the deformity. No surgical correction of the deformity was possible; instead the patients were immobilised in casts under traction to correct the deformity preoperatively. Postoperatively they were immobilised for approximately 6 months to aid fusion. Deaths, high infection rates, and pseudarthrosis were major drawbacks, as was recurrence of the deformity when the cast was removed.

A parallel development was that of removable casts, used to hold moderate curvatures in skeletally immature patients. In the 1950s, Joseph Risser invented the Risser frame, a metal frame in which the patient was placed supine and the deformity was corrected by a combination of traction and pressure on the rib hump, after which a lighter, contoured cast was moulded onto the body (20). The cast, which was repeatedly changed to accommodate growth, was worn until the patient stopped growing. Increased knowledge of the natural

history of idiopathic scoliosis and the invention of removable spine orthoses instead of casts paved the way for the principles of bracing, which are still used today.

### 1.3.2 Bracing

The objective of bracing is to correct the deformity while the patient is still growing, thereby preventing further structural changes that could lead to a progression. The brace needs to be worn until skeletal maturity, when the risk of progression greatly diminishes. For many individuals, this means several years.

Many different brace designs exist, most of which use external forces (passive correction) to restore the alignment of the spine, though some stimulate active correction as well, as the patient tries to move the spine away from pressures within the brace. The brace chosen in clinical practice depends on the particular clinic's traditions and knowledge, and varies with the geographical location. Below is a brief summary of the braces most frequently used in Sweden, both previously and today.

The first widely used orthotic brace for spinal deformities was the Milwaukee brace, a so-called C TLSO (cervicothoracolumbosacral orthosis), shown in Figure 4. In 1958 Walter Blount described its use in non-operatively treated scoliosis (22). The Milwaukee brace is constructed of a pelvic girdle in leather, a metal superstructure, and a chin rest. Traction is achieved through the chin rest, and lateral forces through chest pads attached to the superstructure. Albeit successful in scoliosis treatment, the brace is rather awkward and uncomfortable with its chin rest. In response to patient demands, low-profile underarm orthoses were invented – so-called TLSOs (thoracolumbosacral orthosis).

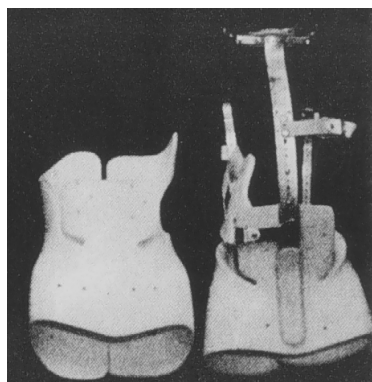


Figure 4. Left: Boston type brace. Right: Milwaukee brace.

In 1972, Hall and Miller, in Boston, developed the first prefabricated TLSO from plastic materials – the Boston brace (20), shown in Figure 4. The brace template is manufactured using moulds of individuals without scoliosis and adapted to the scoliotic patient using lateral pads pushing on the apical vertebrae. On the side of the concavity there are open areas to

allow active movement towards this side. This type of brace and the principle of correction are the most widely used today although different models and manufacturers exist.

Night-time braces were introduced in 1979 (20). The principle is that of over-correction to enable part-time wear. The Charleston brace uses over-bending, while the Providence brace uses both lateral and rotational forces (20). Preliminary data from a randomised study show results in comparison with full-time TLSOs (23), but there have been no controlled studies on night-time bracing versus observation only (24).

Bracing imposes a significant psychosocial burden (25). Its efficacy in preventing curve progression has recently been much questioned. However, one prospective non-randomised cohort study and one partly randomised study show that full-time bracing is effective in preventing progression to curvatures warranting surgery (26-28). In the partly randomised study, Weinstein et al. showed that the number of patients needed to treat (NNT) with full time bracing to avoid one surgery was 3.0 (95% CI, 2.0 to 6.2) (27). In addition, they found that the rate of success was strongly correlated to time spent wearing the brace, corroborating previous studies (29, 30).

In Sweden the current state of art is brace treatment with full-time wearing of a TLSO for curves of 25–40 degrees for remaining growth. The wearing of a night-time brace and physiotherapy are currently under investigation (31).

### 1.3.3 Physical exercise

The effect of physiotherapy on the progress of idiopathic scoliosis is controversial (32). Its popularity has fluctuated over time and is different in different parts of the world. The principle is that the patient performs daily exercises to correct and hold the curvature. Physical exercise as an adjunct to bracing is widely recommended, not to prevent progression but rather to prevent side-effects.

### 1.3.4 Surgery

Surgical treatment of scoliosis is restricted to severe curves. The general recommendation is surgery for curves > 45 degrees in patients with remaining growth and for curves > 50 degrees in adolescents with terminated growth. Today, the objective of operative treatment is a surgical correction of the deformity implying a spinal fusion. Below is a brief summary of the development of modern surgical techniques.

#### 1.3.4.1 *The golden rod*

The treatment of severe spinal deformities was revolutionised in the 1960s when Paul Harrington introduced the first instrumented spinal fusion technique, the so-called Harrington rod (33). Distraction on the concave side and compression on the convex side of the spine were achieved by anchoring a stiff metal rod to the top and bottom of the curvature on either side of the spine, Figure 5. In addition, decortication, facetectomies and autologous transplantation of bone from the iliac crest along the spine were performed to achieve a bony fusion. The patients were braced six months postoperatively to enhance stability and aid fusion. In comparison with the non-instrumented fusion of Hibbs described above, the technique was very successful. The progression of the curvature was halted, although only

small correction was possible and the patients ended up with a stiff, often flat back. The Harrington procedure was the gold standard in spine surgery for several decades.



Figure 5. Pre and postoperative radiograph of an idiopathic scoliosis patient operated on with a Harrington procedure.

#### *1.3.4.2 Sublaminar wires*

In the 1970s, Eduardo Luque started to use sublaminar wires on most operated levels in combination with metal rods along the spine in neuromuscular scoliosis (34). The wires are sequentially attached to the rods and the curvature reduced. This construction is more stable than the Harrington rod, eliminating the need of postoperative bracing. However, the use of sublaminar wires entails a risk of spinal cord injury, which is why other techniques usually are preferred for idiopathic scoliosis.

#### *1.3.4.3 Segmental techniques*

The increasing demand for three-dimensional correction of the deformity resulted in the invention of segmental techniques. Cotrel and Dubousset described the first method in 1984 and this was followed by many others (35, 36). Basically, the technique involves placing anchoring devices (hooks) in the vertebrae at several levels (segments), subsequently attached to metal rods lining the spine. Correction is possible by rotating a bent metal rod in the anchoring devices before fixation and fusion. The pedicle screw was originally described by Roy-Camille in 1970 (37). The technique of employing bilateral pedicle screws as anchoring devices on all operated levels further increased the possibility of three-dimensional correction and was popularised by Suk et al. in the 1990s (38), Figure 6. Hybrid techniques, using both screws and hooks, are commonly used today.

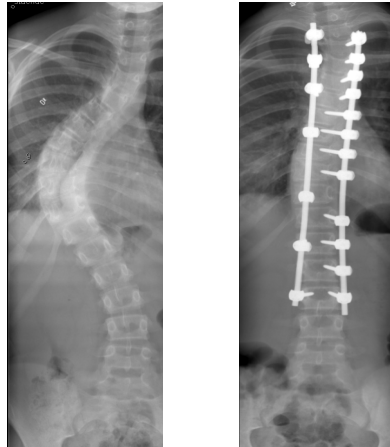


Figure 6. Pre and postoperative radiograph of an idiopathic scoliosis patient operated on with correction and posterior spinal fusion; segmental technique with bilateral pedicle screws on most levels.

#### 1.3.4.4 Approaches in segmental techniques

Posterior approaches, where the spine is approached from the back, are most commonly used in scoliosis surgery in Sweden. Anterior techniques, where the spine is approached from the lateral side by a thoracotomy or thoracoabdominal approach, enable correction and fusion of lesser levels than do posterior approaches (39, 40), Figure 7. However, decreased lung function is seen after anterior approaches to thoracic curves; consequently, it is only recommended for selected patient categories (41). Decreased lung function is not seen after anterior approaches to thoracolumbar and lumbar curvatures.

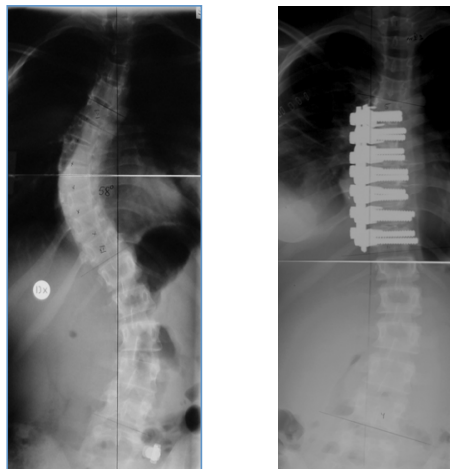


Figure 7. Pre and postoperative radiograph of an idiopathic scoliosis patient operated on with correction and anterior spinal fusion.

#### *1.3.4.5 Non fusion techniques*

Spinal fusions have the most undesirable effects in younger children who have not by far reached their full height. As the child continues to grow the deformity can be aggravated both above and below the fused levels and can also increase and rotate in the fused area, a phenomenon called crankshafting. The latter is due to the continued anterior spinal growth in the presence of a posterior fusion. In addition, the thorax will not reach a size large enough to enable a normal lung function (42, 43). The height of the thoracic spine must be greater than 20 cm at skeletal maturity to avoid severe restrictive lung disease (44).

The “growing rod” technique was developed to account for these problems (45). Anchoring devices are placed in a couple of segments at the top and bottom of the deformity, interconnected by two partly overlapping metal rods. Every 4–6 months, depending on the patient, the metal rods can be distracted to accommodate growth. Wound infections, stiffness, rod breakage and repeated anaesthesia are major concerns. In 2012, Cheung et al. reported on magnetic growing rods that can be lengthened without surgery, so far with promising results (46). A new concept, VEPTR (Vertical Expandable Prosthetic Titanium rib), addressing thorax insufficiency syndromes in patients with fused ribs and congenital scoliosis was described by Campbell et al. in 2004 (47). In addition to expanding the thorax by pushing the ribs apart on the concave side, VEPTR has been shown to successfully reduce the spinal curvature. Elongation to accommodate growth is performed every 4–6 months. Different methods of reversible blocking of growth in moderate deformities have been reported: staples bridging the growth plate and tethers interconnecting segmental screws, both on the convexity side of the deformity (42). However, these methods suffer the same problem as does bracing: the difficulty of predicting who will benefit from treatment.

#### *1.3.4.6 Complications*

The prevalence of neural complications is approximately 1% (48), spanning from resolving nerve root affection to paralysis. The latter is believed to be due to stretching of the spinal cord during the correction manoeuvre or decreased blood circulation. To permit early identification and decrease the risk of neural complications, intraoperative neurophysiological monitoring is regularly used. Deep infections and implant loosening are uncommon with modern titanium-alloy implants and prophylactic perioperative antibiotics (49). However, when they do occur, they may require repeated surgery and can sometimes lead to a loss of the correction.

#### *1.3.4.7 Hospital stay and postoperative regimes*

The most common surgical procedure for idiopathic scoliosis in Sweden is correction and posterior spinal fusion using segmental techniques. The mean duration of surgery is 4.2 hours, number of operated vertebrae 11, blood loss 1.2 litres, and hospital stay nine days (50). As a rule of thumb, school children are usually absent from school for approximately 4 weeks.

## **1.4 LONG-TERM PROGNOSIS**

The objective of all treatments is to alter the natural history of a disorder. Historically, untreated scoliosis was believed to cause decreased lung function, cor pulmonale and premature death (51-53). However, these studies were performed on scoliosis of various aetiologies, including early-onset, paralytic, neuromuscular, congenital, syndromic, and idiopathic scoliosis. More recent studies on the long-term prognosis of untreated idiopathic scoliosis have not shown any increased mortality (18, 54).

In a 50-year follow-up of untreated idiopathic scoliosis, Weinstein et al. found that patients with thoracic curves with a Cobb angle larger than 70 degrees were associated with decreased lung function compared to controls, measured as decreased vital capacity. Only patients with curves larger than 100 degrees had a significant impairment (54).

Both Weinstein et al. and Mayo et al. have shown that untreated individuals with idiopathic scoliosis have more back pain than do healthy controls, but that this pain was not related to curve type or severity (54, 55). In addition, it had no substantial impact on working life and social activities (54, 56). A large curve is often perceived as a cosmetic issue and is assumed to have a psycho-social impact (25). Compared to controls, scoliosis patients are less satisfied with their body appearance (54).

Danielsson et al. have studied long-term outcome in brace and surgically treated patients comprehensively (57, 58). Like the untreated idiopathic scoliosis patients, brace and surgically treated patients had more back pain compared to controls but not such that it had any substantial effect on their daily activities, and no major differences in sociodemographic variables were found. This was supported by Dickson et al. (59). Likewise, the brace and surgically treated patients were more dissatisfied with their appearance as compared to controls.

## **1.5 AETIOLOGY AND PATHOGENESIS**

The pathogenesis of scoliosis, both idiopathic and syndromic, is poorly understood. It is not unreasonable to believe that an existing deformity produces an asymmetrical loading of the growing spine, which in turn causes asymmetrical growth of the vertebrae. But how does it start? And why is it progressive in some but not in others?

Biomechanical, neural, metabolic and hormonal changes have been reported in idiopathic scoliosis. Whether these are primary or secondary to the deformity is difficult to prove. Various theories based on these findings have been suggested. To describe them all in detail is beyond the scope of this thesis. Highlights are listed below.

In 1959, Marie Thillard discovered that pinealectomised chicken developed scoliosis (60). This was repeated in bi-pedalised rats and a deficiency of melatonin was suggested to be causative of idiopathic scoliosis (61, 62). Further studies showed that adolescent idiopathic scoliosis patients had normal melatonin levels (63), and that pinealectomised monkeys did not develop scoliosis (64). Instead, a melatonin-signaling pathway dysfunction affecting certain cell types, notably osteoblasts, was suggested (65, 66). Calmodulin, a calcium-binding

receptor protein, regulates contractile properties in platelets and muscles, and interacts with melatonin. Increased levels of calmodulin in platelets and an asymmetrical distribution of calmodulin in paraspinal muscles compared to healthy controls have been described in idiopathic scoliosis patients (67, 68).

Dickson et al. reported on the fact that vertebral bodies were wedged in the sagittal plane in idiopathic scoliosis patients, causing an apical lordosis in thoracic curvatures. He suggested that this lordosis, in a region that is normally kyphotic, created a rotation of the spine and, secondarily, a lateral spinal curvature (69). On MRI scans of idiopathic scoliosis patients it has been shown that the spinal cord is shorter in relation to the vertebral column (70), and that there is an increased prevalence of cerebellar tonsillar ectopia (71), as well as an uncoordinated growth of the vertebral bodies in relation to the dorsal elements (72), compared to controls. This has led to theories postulating a relative anterior spinal overgrowth (RASO) or an uncoupled neuro-osseous growth as a cause of idiopathic scoliosis (73).

As previously described, the risk of curve progression in idiopathic scoliosis is related to skeletal immaturity. It has also been shown that girls with adolescent idiopathic scoliosis are taller (74-76) and have a higher growth velocity during puberty compared to healthy controls (77-79). Subsequently, bone mineral density, growth, and sex hormones have been studied in the pathogenesis of idiopathic scoliosis. Cheung et al. showed that adolescent idiopathic scoliosis girls had lower bone mineral density than did healthy controls, and a higher bone turnover rate (75). In the same cohort, Hung et al. found that low bone mineral density in the femoral neck was associated with curve progression (80).

Gerdhem et al. showed a decreased level of COMP, cartilage oligomeric matrix protein, in serum in idiopathic scoliosis patients compared to controls (81). COMP has previously been associated with growth velocity in juvenile rheumatoid arthritis patients (82). In addition, raised levels of growth hormone (GH) and insulin-like growth factor 1 (IGF-1) have been associated with idiopathic scoliosis (83, 84), as well as lower circulating levels of leptin, the “satiety” hormone (85). Oestrogen levels have also been studied, but with inconclusive results (86).

## **1.6 HEREDITY AND GENETICS**

It has long been known that hereditary factors play a role in the aetiology of idiopathic scoliosis. Inheritance of scoliosis in five generations was described by Garland in 1934 (87).

In 1968 Wynne-Davis and in 1973 Riseborough and Wynne-Davis reported on the familial occurrence of idiopathic scoliosis in one British and one American cohort (88, 89). The proportions of study participants having a relative with idiopathic scoliosis were 27 and 26%, respectively. The prevalence of scoliosis among first-degree relatives was 7 and 15.8%, which is significantly higher than in the general population. Tang et al. showed a sibling recurrence risk of scoliosis of 18% in a Chinese cohort of 415 female adolescent idiopathic scoliosis patients with Cobb > 20 degrees (90).



In addition, several twin studies have reported a higher concordance of idiopathic scoliosis (meaning that both twins have the disorder) in monozygotic compared to dizygotic twin pairs, indicating a genetic influence (91-93).

As a consequence there has been a vast amount of genetic research on idiopathic scoliosis. A short description of different approaches in genetic research as well as a summary of the findings on idiopathic scoliosis are given below.

### 1.6.1 Genetic approaches

Sequencing allows us to determine the nucleotide sequence of a DNA strand, and thus potentially discover new mutations or genetic variants. Sequencing a whole genome, however, produces immense amounts of data and requires large amounts of downstream bioinformatic analysis. Severe phenotypes could be assumed to be due to mutations in protein-coding genes rather than in the non-coding parts of the genome. One option could then be to sequence only the protein-coding parts, the so-called exome, which constitute approximately 1% of the genome, Figure 8.

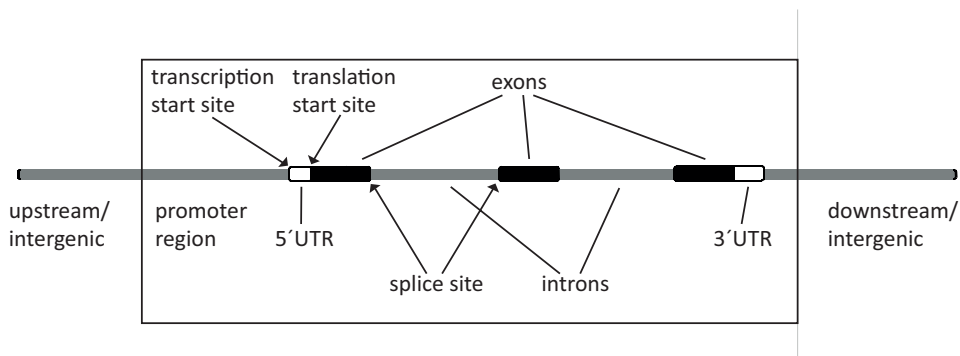


Figure 8. Gene anatomy. Intergenic regions: areas between genes. Transcription start site: starting point for the RNA transcription (from DNA template). Translation start site: starting point for protein translation (from mRNA template). Exons: retained in mRNA, basis for protein translation. Promoter region: regulatory region important for initiation of transcription. 5'UTR and 3'UTR: non-coding start and endpoint of mRNA. Splice site: sequence that guides splicing of exonic RNA. Introns: sequence removed during splicing. Artwork: Elísabet Einarsdóttir.

Genotyping, in contrast to sequencing, depends on the knowledge of known variations – for example, SNVs (single-nucleotide variants), with known positions in the genome, Figure 9. An assay is set up to test for the specific variation/s, meaning that one tests which of the possible alleles or versions of the variation the individual has at that specific point. Compared to sequencing, this is a very efficient method of finding out if a certain known variation is associated with a disease. Genotyping can be used in both genome-wide and candidate-gene approaches.

In genome-wide approaches, millions of variations throughout the genome can be assayed simultaneously. This approach is useful when one has no prior hypothesis of what region or gene might be involved in the disease. However, it is expensive as it results in massive amounts of data, and the criteria for significance of the data are often quite stringent due to a need for multiple testing correction. If there is a hypothesis of what gene/s might be involved in the disorder, one can elect to test variations in this specific area only – a so called candidate-gene approach. The latter approach is more straightforward and allows for a more detailed analysis of a candidate gene, but it is highly dependent on the initial assumptions of the study design. It would also not be helpful for discovering completely new and previously unsuspected disease mechanisms.

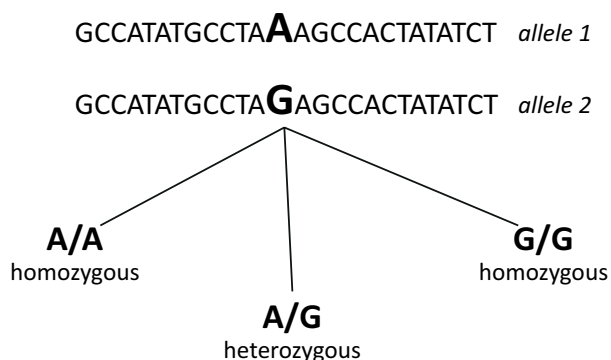


Figure 9. Upper part: DNA sequence illustrating two possible alleles at a specific point in the genome. This type of variation is called single-nucleotide variation (SNV). Lower part: the resulting three possible genotypes. Artwork: Elísabet Einarsdóttir.

Genotyping is used in both association and linkage studies. In association studies one compares the frequency of specific versions/forms/alleles of genetic variants in cases and controls. Association studies can establish whether common known genetic variants are associated with a disorder, even if they only have a weak effect on the phenotype or low penetrance. The existence of a variant in an individual is usually not diagnostic for the disease, but rather indicates an (often subtle) increased disease susceptibility. Even if a specific variant increases the person's susceptibility to a disease by only 5%, this can be a very important modulator of disease risk in the population if the variant is common.

Linkage studies, on the other hand, analyse the cosegregation of a phenotype and a mutation in families. Both large and small families can be used. DNA markers or SNVs are analysed either at a certain point of interest or genome-wide in each individual in the family. It is then possible to link a region of the genome with the phenotype. Both parametric and non-parametric models can be used to calculate the probability that a certain region in the genome cosegregates with the phenotype. In parametric models, specific assumptions on the mode of inheritance and penetrance are made. In non-parametric models only the amount of sharing of the phenotype and variant of interest is tested. The advantage of linkage studies is that one does not need to know what one is looking for in advance, and a study of multiple families

could yield a linkage signal in common even if the disease-causing mutations underlying the linkage signal differed between families. A limitation is that a strong correlation between the phenotype and genotype is needed (a high penetrance), making linkage a more powerful approach for phenotypes of more classical Mendelian inheritance (e.g. recessive or dominant). This type of study can have diagnostic value for members of families carrying a rare, monogenic disease, but the relevance of such findings for the general population is unclear.

### 1.6.2 Linkage and inheritance models

Several genome-wide linkage studies have been performed on idiopathic scoliosis families (94). Both autosomal dominant, X-linked dominant and autosomal recessive models of inheritance have been suggested. Different chromosomal regions have shown linkage in different subsets of families. Gao et al. suggested linkage to the chromosomal region 8q12, and fine-mapping of this area revealed the *CDH7* gene (95). Mutations in *CDH7* are responsible for the CHARGE syndrome, in which a high percentage of patients develop scoliosis. This finding could, however, not be replicated in another subset of families (96). Edery et al. suggested linkage to the regions 3q12.1 and 5q13.3 in a multigenerational family (97). In a follow-up using exome sequencing of three affected members of this family, a novel rare missense variant in *POC5*, a centripolar protein, was discovered. In a zebrafish model this variant caused spine deformity (98).

### 1.6.3 Candidate gene association studies

Inspired by the speculations on the pathogenesis, candidate genes related to bone metabolism, connective tissue, the melatonin-signaling pathway, growth and sex hormones have been investigated in idiopathic scoliosis (94). Hampered by a small sample size, most of these associations have not been replicated in later larger studies (94, 99-103).

Recent studies have shown an association between *IL-17RC* (interleukin 17 receptor C), promoting the production of pro-inflammatory cytokines, genes correlated with peak height velocity during puberty, *DOTIL* and *C17orf67*, and idiopathic scoliosis (104, 105). In addition, variants in *TGFBI* (transforming growth factor beta 1) have shown association with idiopathic scoliosis in a Russian cohort (106).

### 1.6.4 Genome-wide association studies (GWAS)

Four genome-wide association studies (GWAS) in adolescent idiopathic scoliosis have been reported. Sharma et al. found an association of variants in the proximity of *CHLI* and in *DSCAM* to idiopathic scoliosis in a GWAS of 419 family trios (107). Subsequent candidate gene studies in Asian populations have not replicated these findings (108, 109).

In 2011, Takahashi et al. performed a large GWAS in a Japanese population and found an association with a variant downstream of the *LBX1* (ladybird homeobox 1) gene (110). This finding was later replicated in both Chinese Han and Caucasian populations (111, 112). The function of *LBX1* is largely unknown but it has been shown to be expressed in dorsal spinal neurons and hindbrain, muscle precursor cells, and certain cardiac crest cells (113-117). A

recent study reported a clinical case involving scoliosis and myopathy due to a microduplication in the chromosomal region of 10q24.31 affecting exclusively *LBX1* (118).

Kou et al. found an association of *GPR126* (G-protein coupled receptor 126), to idiopathic scoliosis in a GWAS in Japanese, Han Chinese and European ancestry populations (119). This finding has been replicated in a small Chinese candidate gene study (120). A knockdown of *GPR126* in zebrafish caused delayed ossification of the developing spine (119).

In a GWAS of severe cases of adolescent idiopathic scoliosis in Japanese and Chinese populations, Miyake et al. found an association to the variant rs12946942 on chromosome 17q24.3 near the genes *SOX9* and *KCNJ2* (121). Mutations within these genes are associated with campomelic dysplasia and Andersen-Tawil syndrome, both demonstrating a scoliotic phenotype in addition to other symptoms.

Ward et al. identified 53 variants associated with curve progression of adolescent idiopathic scoliosis in a GWAS that is not yet published and validated them in a Caucasian cohort (121). They suggested that these variants could be useful for predicting progression of scoliosis. However, the association of these variants to progression of scoliosis has not been replicated in either a Japanese or a French-Canadian cohort (123, 124).

#### 1.6.5 Exome sequencing

Baschal et al. sequenced the exomes of three affected individuals in a multigenerational family with dominant Mendelian inheritance of idiopathic scoliosis. They identified a rare missense variant in *HSPG2*, coding for an extracellular matrix protein, also known as perlecan. They further sequenced exons of *HSPG2* in 100 independent idiopathic scoliosis patients and found 21 other potentially damaging variants in *HSPG2* (125). Buchan et al. exome-sequenced a cohort of 91 individuals with severe idiopathic scoliosis and compared the results with 337 controls (126). Using a gene burden analysis they found that variants within the *Fibrillin 1* and *2* genes were associated with idiopathic scoliosis. Mutations in *Fibrillin 1* are known to be associated with Marfan syndrome, in which a high percentage of patients develop scoliosis.

#### 1.6.6 Other approaches

Fendri et al. compared mRNA expression in primary osteoblasts from vertebrae in adolescent idiopathic scoliosis patients and healthy controls and found 145 genes differentially expressed in osteoblasts from the patients (127). The most significant changes in expression levels were observed in homeobox genes as well as in *ZIC2*, *FAM101A*, *COMP* and *PITX1*. These genes interact in the biological pathways of bone development, particularly in the differentiation of skeletal elements and the structural integrity of the vertebrae (127).

Buchan et al. reported rare copy number variations (CNVs) in a cohort of 143 idiopathic scoliosis patients (128). The affected genes have not previously been investigated in idiopathic scoliosis.

## **2 AIMS**

The general aim of this thesis is to improve our understanding of the hereditary and genetic background of idiopathic scoliosis. The specific aims are as follows:

To estimate the heritability of scoliosis.

To investigate whether a family history of idiopathic scoliosis is associated with the severity of the disorder.

To describe the self-assessed prevalence of back problems in adults with idiopathic scoliosis.

To investigate the genetics of idiopathic scoliosis by performing a candidate gene study of genetic variants previously found to be associated with idiopathic scoliosis and by exome sequencing of patients with a severe phenotype.

### 3 SUBJECTS AND METHODS

An overview of the study populations and the methods used in this thesis is given in Table 2 and in the following sections. Details may be found in the corresponding papers.

#### 3.1 STUDY POPULATIONS

##### 3.1.1 The Swedish Twin Registry (Paper I)

The population-based Swedish Twin Registry contains information on the largest collection of twins in the world – currently approximately 85,000 mono and dizygotic twins. In the late 1990s, all twins born in 1958 or earlier were asked to participate in the Screening Across the Lifespan Twin (SALT) study, a telephone interview on health status and diseases (129). In the mid 2000s, the Swedish Twin Studies of Adults: Genes and Environment (STAGE) was conducted. This consisted of a Web survey focusing on the health and behaviour of, and environmental influences on, all twins born between 1959 and 1985 (130). Zygosity was assigned by intrasimilarity questions or by genotyping (129). In total 104,349 individual twins, alive and living in Sweden during the study periods were contacted.

Both studies included the question “Do you have or have you had scoliosis?” The question was answered by a total of 64,587 individual twins, who constitute the first study population in Paper I.

##### 3.1.1.1 *Twins treated for idiopathic scoliosis in the National Patient Register*

All of the twins in the Swedish Twin Registry – that is, not only those participating in the aforementioned surveys – were matched against the National Patient Register using International Classification of Diseases (ICD) codes for the primary diagnosis of idiopathic scoliosis. One hundred and sixty-one twins were registered as inpatients with idiopathic scoliosis in the National Patient Register (1964–2008). Information on zygosity was available for 152 of these twins, who also constitute the second study population in Paper I.

##### 3.1.2 The ‘Scoliosis and Genetics in Scandinavia project’, ScoliGeneS (Paper II–IV)

In 2004 the ScoliGeneS project was launched in Sweden at Skåne University Hospital in Malmö, with the objective of studying the health, heredity and genetics of patients with idiopathic scoliosis. The study’s inclusion and exclusion criteria are shown in Table 3. In 2006, the project was transformed into a multi-centre study with the inclusion of Sahlgrenska University Hospital in Gothenburg and Karolinska University Hospital in Stockholm. In 2009, two more Swedish sites were added to the project – Sundsvall and Härnösand County Hospital and Umeå University Hospital – while in 2012, a Danish site was added – Denmark’s Middelfart Hospital. The project is still ongoing. In this thesis the cohort included until Dec 2013 is used.

Table 2. An overview of study populations and methods used in this thesis.

Paper	Research questions	Study population	Study design	Data	Methods
I	What is the concordance and heritability of scoliosis in the Swedish Twin Registry? What is the concordance of idiopathic scoliosis in the National Patient Register?	Population-based twins (n= 64587) Twins treated for idiopathic scoliosis in the National Patient Register (n=152)	Cross-sectional study	Self-assessed questionnaire data, Inpatient diagnosis data from the National Patient Register	Pair and probandwise concordances, heritability estimates
II	Does a family history of scoliosis increase the risk of treatment in a patient with idiopathic scoliosis?	Idiopathic scoliosis patients (n=1463)	Cross-sectional study	Self-assessed questionnaire data	Group comparisons
III	Do adult idiopathic scoliosis patients have more back problems than population-based controls? Does the prevalence of back problems differ between untreated, brace and surgically treated adult idiopathic scoliosis patients?	Idiopathic scoliosis patients (n=1069) Population-based controls (n=159)	Case control study	Self-assessed questionnaire data	Group comparisons
IV	1. Can we replicate previously described idiopathic scoliosis risk variants in a Scandinavian cohort? 2. Are there novel rare variants associated with idiopathic scoliosis in a Scandinavian cohort?	1 & 2. Idiopathic scoliosis patients (n =1739), population-based controls (n=1812) 2. Surgically treated idiopathic scoliosis patients (n = 100)	Case control study	DNA samples	1. Candidate gene association study, Sanger sequencing 2. Exome sequencing, association study as follow-up

Table 3. Inclusion and exclusion criteria of the ScolioGeneS project.

Inclusion criteria	Exclusion criteria
Idiopathic scoliosis	Neuromuscular disorder
Cobb $\geq$ 10 degrees	Syndromic scoliosis
Age at onset 4–20 years	Congenital scoliosis
Untreated; brace or surgically treated	Degenerative scoliosis
Standing posteroanterior radiograph of the spine before age 27	Metabolic disorders (except diabetes mellitus)
	Thoracic surgery in childhood
	Abnormal MRI of the spine (if performed)
	Mental retardation
	Juvenile rheumatoid arthritis
	Mb Scheuermann
	Abnormal neurological examination

Eligible patients were identified and recruited in several ways: 1) Outpatient and inpatient records were searched for all patients formerly observed or treated for the primary diagnosis of idiopathic scoliosis using ICD codes. These individuals were contacted by regular mail. 2) Patients currently under observation and treatment were approached during their visits to the hospital. 3) Individuals included in the Gothenburg Scoliosis Database, a prospectively collected cohort of patients formerly treated or observed at Sahlgrenska University Hospital, were contacted by regular mail. 4) Surgically or brace-treated patients from a prospectively collected Danish cohort were contacted by regular mail (131).

In December 2013, 4404 patients had been invited to join the study. Of these, 1244 did not answer, 955 declined to participate, and 320 were excluded due to signs of non-idiopathic scoliosis, incomplete medical records, or age at onset of less than four. This left 1885 individuals eligible and available for inclusion in the ScolioGeneS project. A flow-chart describing the study populations used in Papers II–IV is shown in Figure 10. Group characteristics are shown in Table 4.

### 3.1.3 Population-based controls (Paper III)

To recruit a cohort of population-based controls, 421 individuals 16–69 years of age were randomly selected from the Swedish population register. They were contacted by mail only and 202 individuals agreed to participate. Individuals who reported that they had scoliosis ( $n = 9$ ), who were younger than 20 ( $n = 12$ ), or 66 or older ( $n = 23$ ), were excluded. The remaining 158 individuals were used as controls in Paper III.



Figure 10. Flow chart of the ScolioGeneS cohort and the study populations included in Paper II-IV.

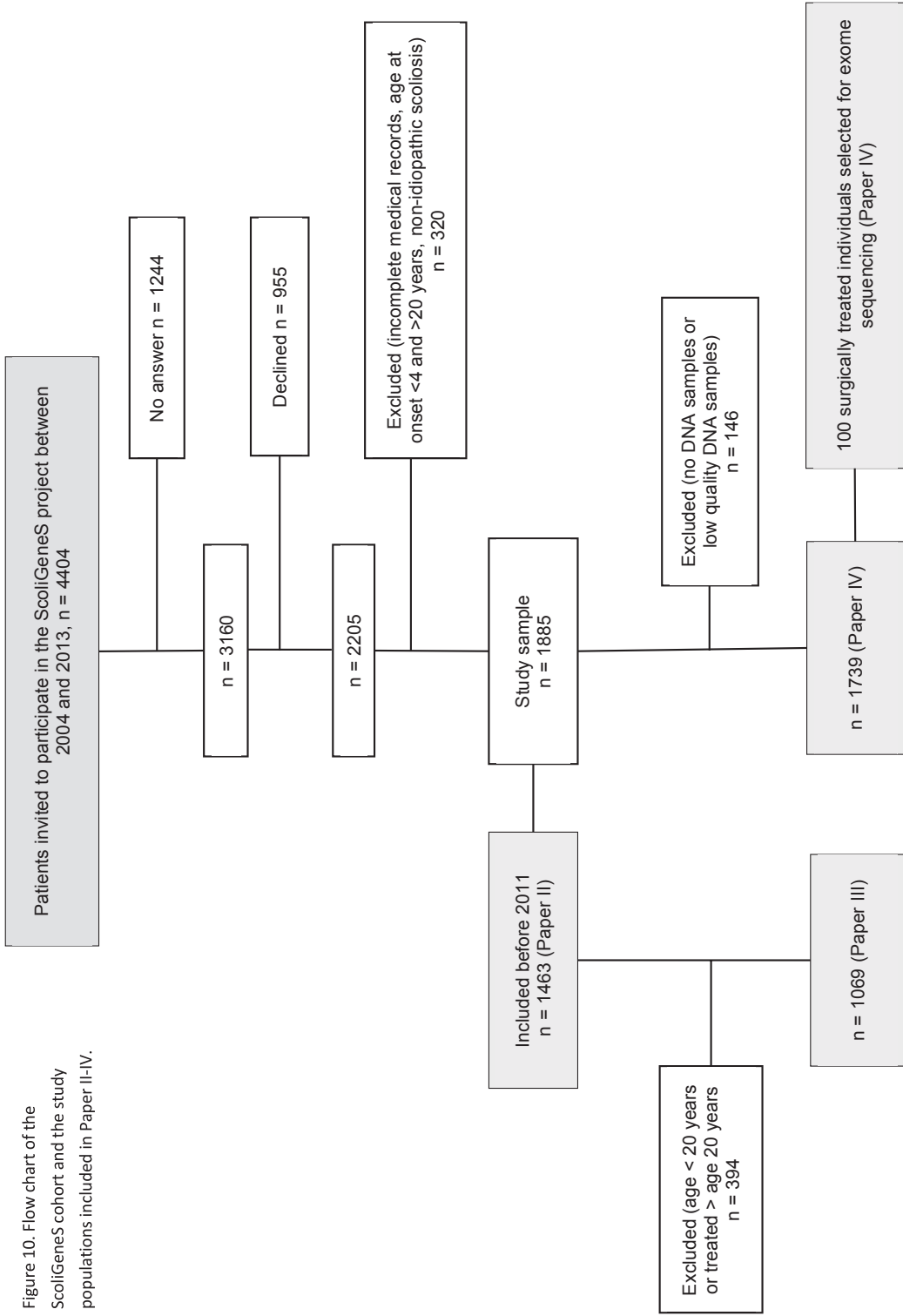


Table 4. Descriptive data of the ScoliGeneS cohort. Data is presented as mean (standard deviation).

<b>The ScoliGeneS cohort</b>				
	<b>All (Paper IV)</b>		<i>Paper II</i>	<i>Paper III</i>
	n	Cobb	n	n
<b>All</b>	1739	39(17)	1463	1069
Female	1498	39(17)	1273	946
Male	241	40(18)	190	123
Juvenile (onset 4–9 years)	213	40(19)	198	159
Adolescent (onset 10–20 years)	1526	39(17)	1265	910
Untreated	495	25(12)	502	374
Brace-treated	641	33(8)	552	451
Surgically treated	603	56(14)	409	244

n = number of patients. Cobb = Cobb angle of the largest curve measured on the last available radiograph before the age of 27 in untreated patients, on the last radiograph before bracing in brace-treated patients, and on the preoperative radiograph in surgically treated patients.

### 3.1.4 Population based controls (Paper IV)

DNA samples from women who participated in either of two population-based studies on bone mass and osteoporosis in southern Sweden were used as controls in Paper IV (132, 133). DNA was available from 2011 individuals. In both of these studies, dual-energy x-ray absorptiometry (DXA) was part of the research protocols, and this was used to evaluate scoliosis (134). We chose to exclude all individuals showing any sign of a curved spine on DXA (n=199). However, we used DNA samples from individuals without DXA information (n=69), since the prevalence of idiopathic scoliosis in the population is low. In total, DNA samples from 1812 population-based controls were used in Paper IV.

## 3.2 QUESTIONNAIRES

All patients in the ScoliGeneS cohort, except the Danish, were asked to answer a self-assessment questionnaire on age at onset, family history of idiopathic scoliosis, back problems, smoking, and occupational strain, shown in Table 5. The population-based controls in Paper III answered the same questionnaire.

## 3.3 SAMPLES

All of the patients in the ScoliGeneS cohort were asked to give a venous blood sample, except for a few cases who gave a saliva sample instead. All of the samples were anonymised and stored in a biobank at the Department of Orthopaedic Surgery at Karolinska University Hospital in Huddinge and at the Karolinska Institutet biobank. DNA was extracted according to standard protocols.

Table 5. Self-assessment questionnaire on ancestry, heredity, back problems, occupational strain, and smoking.

At what age did you get the diagnosis of idiopathic scoliosis? ..... years

Were you born in Sweden? Yes  No

If you were not born in Sweden, where were you born? .....

Was your mother born in Sweden? Yes  No

If she was not born in Sweden, where was she born? .....

Was your father born in Sweden? Yes  No

If he was not born in Sweden, where was he born? .....

Do any of your close relatives have scoliosis? Yes  No  Don't know

Mother

Father

Maternal grandmother

Maternal grandfather

Paternal grandmother

Paternal grandfather

How many sisters do you have? ..... (No.)

How many brothers do you have? ..... (No.)

If you have siblings, do any of them have scoliosis? Yes  No  Don't know  Age (years)

Sister 1    .....

Sister 2    .....

Sister 3    .....

Sister 4    .....

Sister 5    .....

Brother 1    .....

Brother 2    .....

Brother 3    .....

Brother 4    .....

Brother 5    .....

Do you have any children? Yes  No

How many daughters do you have? ..... (No.)

How many sons do you have? ..... (No.)

If you have children, do any of them have scoliosis?				Yes	<input type="checkbox"/>	No	<input type="checkbox"/>	Don't know	<input type="checkbox"/>	Age (years)
Daughter	<input type="checkbox"/>	Son	<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>	.....
Daughter	<input type="checkbox"/>	Son	<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>	.....
Daughter	<input type="checkbox"/>	Son	<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>	.....
Daughter	<input type="checkbox"/>	Son	<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>	.....
Daughter	<input type="checkbox"/>	Son	<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>	.....

Do you have any other relatives with scoliosis (circle)?

1. Uncle
2. Aunt
3. Other

Do you know anything about the treatment of your relatives? Please fill in:

Relative	No treatment	Brace	Surgery	Other
.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Do you experience any back problems? Yes  No

If so, how often?

1. Daily
2. Once a week
3. Once a month

Do your back problems compromise your activity level? Yes  No

Do you go to school or study? Yes  No

Do you work for living? Yes  No

Which of the following best describes your activity level at work (including household work):

1. Mostly sedentary work
2. Light work including some walking and no heavy lifting
3. Moderately heavy work including a lot of walking and lifting
4. Heavy work

Do you smoke? Yes  No

### 3.4 ETHICS

Ethical approval was obtained for all parts of this thesis. For the genetic studies, all of the patients signed informed consents.

### 3.5 CONCORDANCE AND HERITABILITY

Twin studies are used to assess the genetic influence of a trait by comparing similarities for the trait in monozygotic and dizygotic twin pairs. A twin pair is denoted ‘concordant’ when both twins are affected and ‘discordant’ when only one twin is affected by a disorder. A higher proportion of concordant pairs in monozygotic compared to dizygotic twins indicates that genetic factors are important in the development of a trait. ‘Pairwise concordance’ is the probability that both twins in a pair will have a certain characteristic, given that one of the pair has the characteristic. Pairwise concordance is calculated as follows (135):

concordant pairs / (concordant + discordant pairs)

‘Probandwise concordance’ is the probability of a twin being affected given that his/her twin partner is affected. In other words the absolute risk of developing a trait for the twin of a person with the trait. Probandwise concordance is calculated as follows (135):

$2 \times \text{concordant affected pairs} / (2 \times \text{concordant affected pairs} + \text{discordant pairs})$ .

In a large sample of twins it is possible to quantify the relative importance of environmental and genetic effects (heritability) on variation in a trait (136).

The ‘phenotypic variance’ can be attributed to genetic shared environmental and unique environmental effects’. ‘Genetic variance’ reflects additive (A) and/or dominant (D) genetic effects. ‘Additive genetic effects’ refers to when both alleles contribute to the effect across all responsible loci in the genome. ‘Dominant genetic effects’ refers to the non-additive effects of one allele at loci responsible for the effect. ‘Shared environmental effects’ (C) refers to experiences common to both twins – for example, living in the same household when growing up. ‘Unique environmental effects’ or ‘random effects’ (E) refers to events happening to only one twin – for example, a fracture.

Assuming that monozygotic (MZ) twins have identical genomes, and that dizygotic (DZ) twins share half of their genes on average, the correlations in genetic effects between twins can be set to 1 for MZ and 0.5 for DZ twins. Assuming that twin pairs from both zygositys share early household environment, the correlations in shared environmental effects can be set to 1. Structural equation modeling can then be used to test different models for variance partitioning and estimate the relative size of the contributing genetic and environmental variance (137).

In Paper I, both pair and probandwise concordances for scoliosis were calculated for twins in the Swedish Twin Registry and in the National Patient Register. In addition heritability estimates of scoliosis in twins in the Swedish Twin Registry were performed using structural equation modeling (137).

### 3.6 GENETIC METHODS

Below is a general description of the genetic methods used in this thesis.

#### 3.6.1 Polymerase chain reaction (PCR)

PCR is a process of enzymatic replication of DNA used for amplification. The DNA sample is heated to separate the two DNA strands. Primers complementary to the ends of the DNA locus of interest, polymerase enzyme, and nucleotides are added. The temperature is lowered and a complementary DNA strand of the region of interest is built. The sample is heated and the DNA strands separated again. There are now four copies of the region of interest. When the cycle is repeated the number of copies increases exponentially. Amplification of DNA using PCR is necessary in most genetic methodologies and is used in Paper IV.

#### 3.6.2 Sanger sequencing

In order to determine the nucleotide sequence of DNA, Sanger sequencing uses chain termination nucleotides in the replication process. PCR is most commonly used to amplify the DNA sequence of interest. Thereafter, fluorescently labeled chain-terminating nucleotides (one colour for each type of nucleotide) are added in combination with ordinary nucleotides to the PCR. When a chain-terminating nucleotide is randomly incorporated into the DNA strand the replication stops, resulting in different lengths of DNA strands with a fluorescently labeled nucleotide at the end. The DNA strands are separated on a gel, yielding one band for each possible PCR product, and scanned, whereafter the colour and thus the sequence can be read in a so-called electropherogram.

In Paper IV, Sanger sequencing was used to sequence the 5'UTR, non-coding exon and promoter regions of *LBX1* in pooled samples from 100 surgically treated patients in the ScoliGeneS cohort.

#### 3.6.3 Genotyping

Different types of genotyping assays can be set up to determine which of the previously known common alleles or variants an individual has at a specific point in the genome. In paper IV we used MALDI-TOF (Matrix Assisted Laser Desorption-Ionisation-Time Of Flight) analysis, performed on a MassARRAY Platform from Sequenom at the Mutation Analysis Facility at the Karolinska University Hospital in Huddinge, Sweden.

In each DNA sample, the locus of interest is amplified by PCR. Thereafter, extension primers designed to hybridise directly adjacent to the variant locus are added, followed by mass-modified nucleotides (slightly different weights for each type of nucleotide). The DNA strands are then separated and the extended primers analysed using mass spectrophotometry (MALDI-TOF). The difference in mass can then be translated to different genotypes.

In Paper IV, four single-nucleotide variants previously reported to be associated with idiopathic scoliosis in Asian or Caucasian populations were genotyped in 1739 cases from the ScoliGeneS cohort and in 1812 population-based controls. In addition, genotyping was used in the same cohort to validate the variants found in the exome sequencing.

#### 3.6.4 Exome sequencing and next generation sequencing

In exome sequencing the objective is to sequence only the protein-coding parts of the genome – the so-called exome. DNA samples (individual or pooled) are fragmented and prepared in a standardised way to create DNA libraries, and the exonic regions are enriched for. In Paper IV, “in solution capturing” was used: short DNA strands designed to hybridise with the exonic regions and labeled with magnetic beads were mixed with the DNA libraries. A magnet was used to capture the beads (with exonic DNA attached) and excess DNA was washed away. The enriched samples were amplified using PCR and sequenced using next-generation sequencing.

Methods enabling parallel sequencing reactions are referred to as high-throughput sequencing or next-generation sequencing. In Paper IV we used the Illumina platform at the Science for Life Laboratory in Stockholm. Single strands of DNA were attached to the bottom of a flow cell and amplified – a process called DNA clustering. In one flow cell there is room for millions of DNA clusters. DNA polymerase and fluorescently labeled nucleotides (one colour per nucleotide) were added and complementary strands were created in all DNA clusters at the same time. For each reaction (or incorporation of a nucleotide) a photo was taken of the flow cell. Using computerised analysis of all colour signals in every photo, the DNA sequence in each DNA cluster – that is, the ‘sequencing read’ – was established.

Using bioinformatic software, the sequencing reads were mapped to the human reference genome. A number of filtering approaches were subsequently used to evaluate the reads and identify putative novel variants. In paper IV, we exome-sequenced pooled samples (10 x10) from 100 surgically treated idiopathic scoliosis patients. Exome-sequencing data from 100 pre-eclampsia and 100 obesity patients were used to filter out normal variation that is unlikely to contribute to scoliosis. Novel or rare missense, nonsense and splice-site variants were chosen for initial genotyping validation.

### 3.7 STATISTICS

In Paper I, the Chi-square goodness of fit test was used to compare nested models (ACE and AE), and Akaike’s information criterion (AIC) was used to compare goodness of fit between non-nested models (ACE and ADE).

In Paper II, associations in contingency tables were evaluated using the Chi-square test. For continuous variables, the Mann-Whitney U test was used for group comparisons. For comparison of age at onset, the cohort was divided into two groups: 4–9 years (juvenile) and 10–20 years (adolescent), as well as into four groups: 4–8, 9–12, 13–16 and 17–20 years.

In Paper III, hypotheses of variables in contingency tables were evaluated using logistic regression and analysis of covariance. Normal distribution was determined by visual inspection and by use of the Shapiro-Wilk test. For age comparisons the cohort was divided into two age groups, 20 to 44 and 45 to 65. Data on occupational strain were dichotomised into sedentary/light and moderate/heavy work.

In Paper IV, allelic associations were evaluated using the Chi-square test.

Analyses were carried out using the Mx statistics software, IBM SPSS Statistics version 20–22 or PLINK v1.07.



## 4 RESULTS AND DISCUSSION

An overview of the results of this thesis is presented in Table 6 (see page 35) and in the following sections. Details may be found in the corresponding papers.

### 4.1 TWIN STUDIES

Pair and probandwise concordances for MZ and DZ twin pairs in the Swedish Twin Registry are shown in Table 7. Previous twin studies of idiopathic scoliosis have shown higher pairwise concordances for monozygotic (0.76–0.92) compared to dizygotic twins (0.36–0.63) (92, 93). However, these studies have been based on smaller clinical or case-series of twin pairs. In a population-based study of self-assessed idiopathic scoliosis from the Danish Twin Registry, Andersen et al. calculated pair and probandwise concordances of 0.13 and 0.25 in MZ and 0.0 and 0.0, in DZ twins, respectively, which is in parity with our results (91). One might argue that the higher concordance figures in the previous clinical or case-series studies might be due to sampling biases.

Table 7. Pair and probandwise concordances of self-assessed scoliosis in twin pairs in the Swedish Twin Registry.

	All zygosity	Monozygotic	same-sex Dizygotic
Complete pairs (n)	22470	6853	7779
Discordant pairs (n)	1645	476	546
Concordant pairs (n)	94	50	24
Pairwise concordance	-	0.11	0.04
Probandwise concordance	-	0.17	0.08

Complete pairs (n): number of pairs where both twins have answered the scoliosis question.

Discordant pairs (n): number of pairs where one twin has scoliosis and the other does not.

Concordant pairs (n): number of pairs where both twins have scoliosis.

The use of self-assessment data is an obvious limitation of this study since the presence and type of scoliosis is not verified in the study participants. However, when we analysed twins recorded as inpatients in conjunction with the primary diagnosis of idiopathic scoliosis in the National Patient Register we found a pairwise concordance of 0.08 for MZ and 0.0 for same-sex DZ twins. No concordant same-sex dizygotic pairs were found. The probandwise concordance was 0.15 for MZ and 0.0 for same-sex DZ twins. These results are in parity with the concordances in the self-assessed data. In addition, we excluded all twins aged 50 and older in the self-assessed data ( $n = 34,585$ ), but the results did not change substantially (data not shown). It therefore seems that degenerative scoliosis does not influence the results significantly.

Due to sample size constraints, previous twin studies of idiopathic scoliosis have not been able to report heritability estimates – the proportion of phenotypic variance attributed to genetic effects. Using structural equation modeling we estimated the heritability of scoliosis in the Swedish Twin Registry at 38%, Table 8.

Table 6. An overview of the results presented in this thesis.

Paper	Research questions	Study design	Results	Conclusions
I	What is the concordance and heritability of scoliosis in the Swedish Twin Registry? What is the concordance of idiopathic scoliosis in the National Patient Register?	Cross-sectional study	Pairwise concordance was 0.11 for MZ and 0.04 for same-sex DZ twins. Additive genetic effects were estimated at 0.38 (0.18–0.46). Pairwise concordance for idiopathic scoliosis in the Swedish Inpatient Register was 0.08 for MZ and 0.0 for same-sex DZ twins.	Overall genetic effects account for 38% of observed phenotypic variance in scoliosis. The remaining 62% is due to environmental influences.
II	Does a family history of scoliosis increase the risk of treatment in a patient with idiopathic scoliosis?	Cross-sectional study	When the patient had a relative with scoliosis the OR for being treated was 1.32 (1.06–1.64), compared to not having a relative with scoliosis.	A family history of scoliosis increases the risk of needing treatment.
III	Do adult idiopathic scoliosis patients have more back problems than population-based controls? Does the prevalence of back problems differ between untreated, brace-treated and surgically treated adult idiopathic scoliosis patients?	Case control study	The prevalence of back problems in adulthood was 64% in idiopathic scoliosis patients and 29% in population-based controls ( $P < 0.001$ ). There was no significant difference between untreated, brace-treated and surgically treated patients.	Adults with idiopathic scoliosis have a higher prevalence of back problems than controls. Treatment was not related to prevalence of back problems in adulthood.
IV	1. Can we replicate previously described idiopathic scoliosis risk variants in a Scandinavian cohort? 2. Are there novel rare variants associated with idiopathic scoliosis in a Scandinavian cohort?	Case control study	1. A variant close to <i>LBX1</i> showed a strong association with idiopathic scoliosis ( $p=7.0 \times 10^{-18}$ ). 2. We identified twenty novel variants by exome-sequencing after filtering and an initial genotyping validation. No association with idiopathic scoliosis was found in the large cohort of 1739 cases and 1812 controls.	We confirm <i>LBX1</i> as a susceptibility gene for idiopathic scoliosis in a Scandinavian population and are unable to find evidence of other genes of similar or stronger effect.

A model partitioning the phenotypic variance in additive genetic effects (A) and unique environmental effects (E) was found to be the most favourable, according to the test statistics and the principle of parsimony, Table 8.

Table 8. Estimation of genetic and environmental effects on the phenotypic variance of scoliosis in the Swedish Twin Registry.

Fitted model	Genetic effects (A)	Shared environmental effects (C)	Unique environmental effects (E)	Chi-square goodness of fit
ACE	0.38 (0.18–0.46)	0.00 (0.00–0.17)	0.62 (0.54–0.70)	18.00
AE	0.38 (0.18–0.46)	–	0.62 (0.54–0.70)	18.00

Results are presented as proportion of variance (95% confidence interval).

ACE: model implying additive genetic effects (A), shared environmental effects (C), and unique environmental effects (E).

AE: nested model implying additive genetic effects (A) and unique environmental effects (E) only.

The AE-model was found to be the most favourable according to the principle of parsimony, since there was no difference in chi-square goodness of fit between the ACE and the AE models. Sex-specific models or models implying a dominant genetic effect did not have significantly better goodness of fit (data not shown).

The validity of twin studies is limited by their general assumptions of random mating, no gene–environment interaction, and equal environments when growing up, and may not hold if any of these assumptions is violated (138). In addition, twins differ in their developmental environments from singletons and may not be representative of the general population. Whether the prevalence of idiopathic scoliosis is different in twins and singletons is unknown. Tang et al. reported a sibling recurrence risk of 17% in a cohort of 415 Chinese adolescent idiopathic scoliosis girls, identical to the twin recurrence risk of scoliosis in the present study (90). However, Tang et al. estimated the heritability of idiopathic scoliosis at 87% in the same cohort, substantially higher than in the present study. On the other hand, their study was not population-based and a Cobb angle of 20 degrees was required for inclusion. They furthermore used a different method for estimating heritability by comparing sibling recurrence risk in cases and the prevalence of idiopathic scoliosis in healthy controls.

In summary, in the largest population-based study of scoliosis to date, we confirm the role of a genetic predisposition in the aetiology of idiopathic scoliosis, although not as strong as previously stated.

## 4.2 THE SCOLIGENES COHORT

### 4.2.1 Family history

In the ScoliGeneS cohort, a family history of scoliosis was associated with being treated for and having a Cobb angle > 40 degrees, as shown in Table 9. In addition, we found a small but significant increase in maximum curve size between patients with and without a family history of scoliosis (median 35°, interquartile range 25 compared to median 32°, interquartile range 23,  $p = 0.02$ ).

Table 9. The number (%) of idiopathic scoliosis patients with a family history of scoliosis in relation to sex, age at onset, curve severity, and treatment. A family history of scoliosis is defined as having at least one relative with scoliosis. The p-value refers to the Chi-square test.

Study participants (n =1463)	Family history of scoliosis	P-value	OR(CI)
Female	647 (51%)	0.939	1.01 (0.75–1.37)
Male	96 (51%)		
Juvenile	111 (56%)	0.110	1.28 (0.95–1.73)
Adolescent	632 (50%)		
Cobb < 40 degrees	441 (48%)	0.017	1.30 (1.05–1.60)
Cobb => 40 degrees	302 (55%)		
Untreated	232 (46%)	0.026 *	1.32 (1.03–1.68)*
Brace	293 (53%)	0.033 †	1.32 (1.02–1.73)†
Surgery	218 (53%)		
Untreated	232 (46%)	0.011	1.32 (1.06–1.64)
Treated with brace or surgery	511 (53%)		

\* Comparison between brace-treated and untreated patients

† Comparison between surgically treated and untreated patients

OR (CI): odds ratio (confidence interval)

One might speculate that a family history of scoliosis would predispose patients to treatment with brace and/or surgery, explaining the difference with reference to heredity. The difference in maximum curve size indicates that this is not the case. In addition, this difference might be underestimated as being due to treatment. Miller et al. found a correlation between the degree of lateral curvature and the number of affected relatives supporting our results (139).

We found no significant differences in neither family history between females and males, nor between juvenile and adolescent idiopathic scoliosis patients, as shown in Table 9. In addition, we subdivided the patients into four age groups (4–8, 9–12, 13–16 and 17–20 years) according to the age at onset, but found no difference in the proportion of study participants with a family history of scoliosis (data not shown). An early onset of disease is usually an indication of a stronger genetic background than is a late onset. We could not, however, find an association between a positive family history and an early onset in our cohort.

A major limitation of this study is the fact that the diagnosis of scoliosis in relatives was not confirmed using radiographs or medical records. However, one of the main objectives of Paper II was merely to investigate whether a simple question about heredity in the clinical setting would yield information on the risk of progression and future treatment.

In summary, a family history of scoliosis was associated with larger curve size and need of treatment in idiopathic scoliosis and may suggest that a closer monitoring of these patients is warranted.

#### 4.2.2 Back problems

In paper III, we found a significantly higher prevalence of back problems in adult idiopathic scoliosis patients (n = 1069) compared to population-based controls (n=158), as shown in Table 10 (see page 39). This finding is in line with previous long-term studies of idiopathic scoliosis reporting significantly more back pain in idiopathic scoliosis patients than in controls but with no large functional consequences (55, 57, 58). In this study, 30% of the individuals with idiopathic scoliosis had back problems compromising their activity level, compared to 15% of those without scoliosis. On the other hand, the individuals with idiopathic scoliosis were gainfully employed to a higher extent than were the individuals without scoliosis, and there was no statistically significant difference in occupational strain between the two groups. In a previous population-based study of Swedish adults, the prevalence of low back pain was higher (41%) than in the present study (29%), but the mean age at investigation was also higher (140).

We found no statistically significant differences in the prevalence of back problems among idiopathic scoliosis patients when comparing untreated, brace-treated, and surgically treated patients, Table 11. One of the aims of surgical treatment of idiopathic scoliosis is to decrease back pain and improve function in adulthood. In this study the prevalence of back problems was similarly increased in both treated and untreated patients. However, the majority of surgically treated patients are operated on using a Harrington procedure (n =213, 87%). Modern implants allow for better correction of the deformity, which might improve the long-term outcome in comparison to untreated patients. Interestingly, Weinstein et al. found no correlation between the degree of curvature and the prevalence of back pain in a long-term follow-up of untreated patients (54).

Table 11. Prevalence of back problems in the ScoliGeneS cohort in relation to gender, age at onset, and previous treatment (n=1069). Data are shown as number (%) of individuals.

	Back problems n (%)	p-value
Men (n=123)	71 (58%)	0.10 *
Women (n=946)	617 (65%)	
Juvenile (n=159)	109 (69%)	0.20†
Adolescent (n=910)	579 (64%)	
Untreated (n=374)	258 (69%)	0.06‡
Brace-treated (n=451)	274 (61%)	
Surgically treated § (n=244)	156 (64%)	

P-value: level of significance

\* Corrected for age (20–44 years or 45–65 years), smoking, treatment (untreated, brace-treated, or surgically treated) and diagnosis (juvenile or adolescent).

† Corrected for gender, age (20–44 years or 45–65 years), smoking and treatment (untreated, brace-treated and surgically treated).

‡ Corrected for gender, age (20–44 years or 45–65 years), smoking and diagnosis (juvenile and adolescent).

§ Implant type: Harrington rod, n = 213 (87%); segmental fixation, n = 28 (11%); noninstrumented fusion in situ, n = 3 (1%). Approach: posterior, n = 232 (95%); anterior, n = 9 (4%); anterior and posterior, n = 3 (1%).

Table 10. Prevalence of back problems in the ScolliGeneS cohort (n=1069) compared to population-based controls (n=158). Data is shown as number (%) of individuals.

	Age at investigation mean (SD) years	Frequency of back problems*				Occupational strain†			
		Back problems compromising the level of activity	Daily	Weekly	Monthly	Gainfully employed	Sedentary/ light	Moderate/ heavy	Smokers
Idiopathic scoliosis (n=1069)	41 (9)	688 (64%)	320 (30%)	245 (37%)	168 (25%)	257 (38%)	678 (73%)	246 (27%)	122 (11%)
Controls (n=158)	45 (13)	46 (29%)	24 (15%)	12 (26%)	14 (30%)	20 (44%)	82 (66%)	42 (34%)	18 (11%)
p-value ‡		<0.001 <sup>§</sup>	0.001 <sup>§</sup>	0.57 <sup>§</sup>	0.003 <sup>§</sup>	0.13 <sup>§</sup>	0.65 <sup>  </sup>		

\* Denominator is the number of individuals who have indicated the frequency of back problems (670 individuals with scoliosis and 46 individuals without scoliosis).

† Denominator is the number of individuals who have indicated the occupational strain (924 individuals with scoliosis and 124 individuals without scoliosis).

‡ p-value: level of significance

§ Corrected for gender, age (20–44 years or 45–65 years) and smoking.

|| Corrected for gender and age (20–44 years or 45–65 years).

In addition, we found no statistically significant differences in the prevalence of back problems between sexes in the study cohort overall, as shown in Table 11, nor within the untreated, brace-treated, and surgically treated groups (all  $P > 0.14$ , corrected for smoking, diagnosis [juvenile and adolescent], and age). This corroborates findings in earlier long-term follow-ups of untreated and surgically treated patients (55, 141). To the best of our knowledge, the impact of gender on the prevalence of back problems in adulthood in brace-treated patients has not been studied previously.

Regarding age at onset, we found no statistically significant differences in the prevalence of back problems between adolescent and juvenile idiopathic scoliosis in the study cohort overall, Table 11, nor within the untreated, brace-treated, and surgically treated groups (all  $P > 0.17$ , corrected for sex, smoking, and age). The relations between the age at the onset and the long-term prevalence of back problems have previously been studied by Lange et al. in brace-treated patients, reporting similar findings (142).

The current study has several limitations: the questions on back problems were not validated and the results may not be applicable to idiopathic scoliosis patients in general. The validated questionnaire SRS22r, widely used in scoliosis research today, was not yet available in Swedish when this study was initiated. The rationale for using the term “back problems” was the wish to include any type of problem that individuals themselves associated with their back – not only back pain. However, it makes comparisons with other studies using the term “back pain” more difficult. The use of a control group answering the same questions attenuates this limitation. Another limitation of this study is the low response rate: 47% of the idiopathic scoliosis patients and 48% of controls. Nonresponse bias has often been associated with poor health but not always a poor outcome. Sohlberg et al. did not find any significant differences in outcome between responders and nonresponders in the Norwegian Spine Registry (143).

The major strength of this study is its size: it is large enough to describe minor populations within the idiopathic scoliosis population, such as male and juvenile patients, that have not been studied in detail previously. In summary, adults with idiopathic scoliosis have a higher prevalence of back problems than do individuals without scoliosis. Treatment, sex, and juvenile or adolescent onset of diagnosis were not related to the prevalence of back problems in adulthood.

#### 4.2.3 Genetic studies

In Paper IV, four single-nucleotide variants previously reported to be associated with idiopathic scoliosis were genotyped in 1739 cases and 1812 controls. An intergenic variant, rs11190870, downstream of the ladybird homeobox 1 gene, *LBX1* showed a strong association with idiopathic scoliosis ( $7.0 \times 10^{-18}$ ), Table 12. The other three tested variants showed weaker or no association, as can be seen in Table 12. In subgroup analyses of the association of rs11190870, there seemed to be a stronger association with females than males, and with right thoracic curve types compared to all other curve types together, yielding higher point estimates for the odds ratios. However, these differences were not significant (data not shown).

Table 12. Association of candidate genes with idiopathic scoliosis in a Scandinavian cohort of 1739 cases and 1812 controls.

SNP name	Gene	A1	A2	RAF		P-value	OR (95%CI)
				cases	controls		
rs6570507	<i>GPR126</i> intron	A	G	0.32	0.28	$1.6 \times 10^{-4}$	1.22 (1.10–1.35)
rs11190870	<i>LBX1</i> downstream	T	C	0.69	0.59	$7.0 \times 10^{-18}$	1.53 (1.39–1.69)
rs12946942	Intergenic	T	G	0.09	0.08	0.005	1.27 (1.08–1.51)
rs10510181	<i>CHL1</i> upstream	A	G	0.36	0.36	0.97	1.0 (0.91–1.1)

A1: allele 1 (risk allele)

A2: allele 2

RAF: risk allele frequency

OR: odds ratio

CI: confidence interval

Because we observed strong association of the *LBX1* variant (rs11190870) with idiopathic scoliosis, we performed Sanger sequencing to discover further genetic variation at this locus. We could, however, not find any variants in the 5'UTR, non-coding exon or promoter regions of *LBX1* (data not shown). Previous studies sequencing coding regions of *LBX1* have not found any potentially damaging variants (111). When scrutinizing our exome-sequencing results for the *LBX1* exons we found only one synonymous variant that was as common in our control data set. Chettier et al. found strong association with a variant upstream of *LBX1* and with an imputed haplotype including rs11190870. They suggest that the *LBX1* region is a highly conserved locus and might have a regulatory function (144). Our study confirms the *LBX1* region as a susceptibility locus for idiopathic scoliosis, although the mechanism of action remains elusive.

Genetic variation at the *LBX1* locus likely explains only a small part of the heritability of idiopathic scoliosis. We hypothesised that scoliosis could be caused by a range of rare mutations affecting important structural genes. Such mutations may not be adequately captured by genome-wide association studies, leading us to choose an exome-sequencing approach in the current study. We identified several novel potentially damaging variants when exome-sequencing pooled samples (10 x10) in 100 surgically treated idiopathic scoliosis patients from the ScoliGeneS cohort. After filtering and initial genotyping validation, 20 variants were chosen for follow-up in a case-control setting. However, we failed to find significant association of any of the exonic variants with idiopathic scoliosis in 1739 cases and 1812 population-based controls (data not shown).

One might argue that we did not find any associations because exome-sequencing is designed for finding rare variants, but association studies, which we used for validation, are less powerful when dealing with rare variants. Another limitation of our study is the use of a pooled exome-sequencing strategy in the initial variant discovery phase, increasing the need for filtering and making the study of singleton variants difficult. Also, variants found in only one pool, and variants with a very low frequency, despite being found in several pools, had to be discarded to reduce the number of false-positive variant calls. Buchan et al. sequenced the



exomes of 91 individuals with severe idiopathic scoliosis and found, in a gene burden analysis, that *Fibrillin 1* and *2* genes were associated with idiopathic scoliosis (126). In a gene burden analysis all individuals having a variant in a specific gene (and not necessarily the same variant) are taken into account in an association analysis. Unfortunately, a gene burden analysis was not possible in our material due to the pooling strategy.

In summary, we confirm *LBX1* as a susceptibility gene for idiopathic scoliosis in a Scandinavian population and are unable to find evidence of other genes of similar or stronger effect.

## 5 CONCLUDING REMARKS

Considering the results in this thesis, the classification of idiopathic scoliosis according to age at onset in juvenile (4–10 years) and adolescent (10–18 years) seems arbitrary. We found no differences regarding long-term outcome, genetic association studies or family history of scoliosis between these two groups.

The present thesis corroborates the understanding of idiopathic scoliosis as a complex disease with a polygenic background. Presumably idiopathic scoliosis can be due to a spectrum of risk variants, ranging from very rare or even private to very common on the population scale. The risk effect of the variants could also range from quite severe to very mild and even undetectable in practice. A synergy of the effects of all these variants with each other and environmental factors is to be expected. In addition, there are probably various inheritance patterns spanning from a dominant monogenic disease in some families to a multifactorial polygenic disease in the major population of idiopathic scoliosis patients. The definition of disease is wide and incorporates a variety of different phenotypes in curve size, curve pattern and age at onset, further increasing the difficulty of finding underlying genetic mechanisms. Possibly these different phenotypes represent different subgroups of disease and could have different genetic backgrounds.

Approaches to addressing common genetic variants that increase disease susceptibility as well as novel rare causative variants have been tested in this thesis project. A future strategy might be studying families with monogenic idiopathic scoliosis in order to find a causative mutation. A possible finding will not explain the specific genetic background in the general idiopathic scoliosis population but might reveal biological pathways that are important in all or most forms of idiopathic scoliosis. In addition there are several genetic syndromes, of both known and unknown causes, in which scoliosis is part of the phenotype. Further studies of these disorders could add information to the pathogenetic mechanism of scoliosis development. Yet another possibility is international collaboration in collecting large cohorts of idiopathic scoliosis patients. A large sample size would better enable us to find association with rare variants. Drawbacks of this strategy might include less consistency in the diagnosis and population-specific differences. It has been suggested that genetic variation for complex traits could be caused by mutations altering the amount of gene expression. Future approaches studying mRNA expression, methylation, and protein expression could be rewarding.

A major concern in idiopathic scoliosis is the absence of perfect means by which to predict risk of progression, leading to frequent follow-ups, radiographs, and brace treatments – possibly an unnecessary burden for the particular individual. Every effort to improve the identification of at-risk individuals is of importance, since this will ultimately lead to an earlier diagnosis and possibly better preventive and therapeutic options. While it has not revealed the aetiology or pathogenesis of this complex disorder, this thesis project has improved our understanding of inheritance and genetics in idiopathic scoliosis.

## 6 ACKNOWLEDGEMENTS

I sincerely would like to express my gratitude to everyone who has contributed to this thesis. In particular:

**Paul Gerdhem**, principal supervisor. It has been an honour and a pleasure to work with you. With you as a supervisor, I would happily do a second thesis project.

**Juha Kere**, co-supervisor and genetic visionary. Thank you for teaching and sharing your ideas and great experience in genetics with me. I still look forward to that Finnish sauna.

**Elísabet Einarsdóttir**, co-supervisor and the dream of every PhD student: a geneticist, linguist and art director in one person. Thank you for your efforts in teaching me genetics as well as in helping me compile this thesis.

**Aina Danielsson**, co-author. Thank you for all your work in collecting patients and reviewing manuscripts, and for sharing your vast knowledge in idiopathic scoliosis with me. I much appreciate our collaboration.

**Magnus Karlsson**, co-author. Your instant feed-back on all of the manuscripts has always been highly insightful. Thanks for bringing focus to my analyses and enhancing in writing.

**Acke Ohlin**, co-author and nestor of spine surgery. I sincerely appreciate your great experience in idiopathic scoliosis as well as your linguistic talents. Thanks for all your encouragement and work reviewing the manuscripts and thesis compilation.

**Hong Jiao** and **Jingwen Wang**, co-authors. Without your bioinformatical expertise and efforts this project would not have gone far. Thank you for your helpful collaboration on manuscript writing and on lab work, and for your kind and patient explanations.

**Klas Halldin, Pawel Grabowski, Ane Simony, Mikkel Anderssen, Steen Bach Christensen, Hannele Laivuori, Ingrid Dahlman, Christos Topalis, Helena Normelli, Kristina Åkesson, Max Tenne**, co-authors. Your dedicated work recruiting patients and controls was indispensable. I look forward to future collaboration.

**Hans Möller**, co-author, and **Tomas Reigo, Kourosh Jalalpour, Peter Endler, Panayotis Savvides, Daniel Bring, Anastasios Charalampidis**, all colleagues at the spinal surgery unit at Karolinska University Hospital. You have made me eat, sleep, and live spine surgery during a whole year as a spinal fellow. You have recruited patients, commented on my research, supported, and encouraged me and my career in every way. I am much indebted to you all and sincerely enjoy your company.

**Iffat Rahman**, co-author and structural equation-modeling expert, and **Per Näsman**, statistician. Thank you both for patiently helping me with calculations, statistical advice and trouble-shooting.

**Yvonne Haglund Åkerlind**, mentor, for inspiration and valuable advice on research, career and life.

**Åsa Fröberg**, colleague and friend. As my sounding board in research, career and life, you have helped me with big and small decisions. You are most essential to me.

**Li Tsai**, for crucial advice at the right moment and for your support for this thesis project.

**Luigi Belcastro**, for keeping track of the project, patients, samples, files, and the rest of us. Your good spirits never fail, whether you are faced with organising samples in the freezer or sorting out who is who in a messed-up file.

**Elias Diarbakerli**, for your help in recruiting patients, cleaning and organising databases, as well as your congenial company and for a good collaboration.

**Ann-Christin Eriksson, Cathrin Berggren, Tarja Savoulidis**. Without your care and practical arrangements I would never have managed. Thank you.

**Helene Fils**, for your patience in posting letters, checking diagnosis codes, and proof-reading databases. Thank you for your invaluable assistance.

**The department of research and development of Västernorrland County Council**, for supporting and encouraging research in Sundsvall, by practical and financial means – and especially **Jeanette Sundberg**, for your kind and devoted help with all the practical arrangements, and **Erling Englund**, for explaining and solving problems in SPSS software.

**Åsa Lindberg, Annette Rafstedt**, Malmö, **Anne Dohsé, Carina Gille, Åsa Jalking, Ingalill Pehrsson**, Gothenburg, **Unni Ivefors**, Sundsvall, **Sabine Süllow Barin, Ellinor Lekkerimäki, Nora Andaloussi Atallah, Maria Wikzén, Margareta Fahlén** and **Luisa Güntner Johansson**, Stockholm, for all your work in inviting, examining, and sampling patients, as well as collecting questionnaires and organising databases. I am most grateful to you all.

**Zekija Beganovic** and **Sigvard Karlsson** for meticulously tracking down medical records and x-ray films in the archives of Skåne University Hospital in Malmö.

**Siv Fougner**, for patiently requesting medical records and x-rays from all over Sweden. No request has ever been too large for you.

**Margareta Wall**, for providing me with all those lists of former inpatients and outpatients.

**Elisabeth Dungner, Tiina Skoog**, and **Ingegerd Fransson** for your kind and accurate help with the lab work.

**Merja Ylioikarainen**, bibliometrical expert. No reference is too old or obscure for you to get hold of, and no endnote problem too difficult. Thank you!

**Tommy Sund**, graphic-design and burger pro. Thank you for excellent help with the illustrations and the lay-out of this thesis! The knowledge that I did not need to fight these issues on my own gave me peace while writing.

**Ulf** and **Eva Sigurdsson**. Your five-star all-inclusive hotel with the ocean view, private terrace, bike, dinners, lunchboxes, and delightful company made my endeavours in the archives of Skåne University Hospital in Malmö a lot more pleasant.

**Gunnar Sandersjöö.** Your skilful surgical technique is an endless source of inspiration. I much appreciate your congenial company and wonderful sense of humour.

**Fredrik Andersson, Sigmund Norberg, Johan Nilsson, Mats Wadsten, Thomas Wikström, Göran Sjärdén, Arkan Sayed Noor, Lennart Bengtsson, Carl-Håkan Ohlson, Eskild Pedersen, Martin Kjellberg, Fredrik Lundh, Björn Knutsson, Nader Mafi, Afshin Ammouzandeh, Bakir Kadum, Sarwar Mahmood, Daniel Björkman, Johan Dalén, Emma Granstrand, Ammar al Azzavi, Anna-Sara Magnusson, Emmanouil Bonatos, Nikolaos Perisynakis, Sebastian Mukka, Mark Kruse, Per Vestin, David Edlund, Zolt Császár, Hans Engstedt, and Hoda Carlsson,** all colleagues in Sundsvall, past and present. Thanks for giving me the opportunity to do research, for sharing my workload while I have been away, and for always supporting and encouraging me. I very much appreciate your good spirits and the friendly working atmosphere in Sundsvall. Especially: **Björn Knutsson**, for never giving up on my becoming a spine surgeon, teaching me all you know, whilst having fun and always encouraging me in research and career; and **Emma Granstrand**, roommate and dear friend, for your hilarious comments and unconditional support whenever needed.

**The research school for clinicians in epidemiology at Karolinska Institutet**, director **Ylva Trolle Lagerros** and **my course mates**, for well-organised, high-quality education and inspiring discussions about research and career. Especially: **Carina Grönhagen** and **Nikolas Kartalis**.

**My family**, for your ever-lasting encouragement and support. Especially my mother **Catharina Hall** and my parents-in-law **Leif** and **Ingegerd Berglund**, for spending weeks taking care of the kids, cooking dinners, filling the freezer, and doing all those small things that make everyday life so much easier. Thank you very, very much!

**Ulla Hall**, grandmother and lifelong companion in skiing, hiking, boating, theatre, opera, and art museum visits. When in Stockholm during this thesis project you have housed me, fed me, washed my clothes, chastised me when I worked too much, and everyday at dinner asked me what I learned that day. Thank you! I wish everybody had a grandmother like you.

**Olof** and **Gustaf Grauers**, true miracles. I waited long but not in vain. You make me so much more efficient.

**Markus Grauers**, the master of care and support. You are my context, my strength and the source of my zest for life. I look forward to an endless stream of skiing holidays.

This work was financially supported by the **Swedish Research Council**, the **regional agreement on medical training and clinical research (ALF)** between Stockholm County Council and Karolinska Institutet, the **Department of Research and Development of Västernorrland County Council**, the **Karolinska Institutet research funds**, the **Research and Development Council of Region Skåne**, the **Swedish Society of Spinal Surgeons**, the **Joint Committee of the Northern Health Region of Sweden (Visare Norr)**, the **Scoliosis Research Society**, the **Alfred Österlund Foundation**, the **Emil Andersson Foundation** and the **Swedish Orthopaedic Association**.

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