Mortality and morbidity in patients with osteogenesis imperfecta in Denmark

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Mortality and Morbidity in Patients with Osteogenesis Imperfecta in Denmark

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List of included papers


1. LIST OF ABBREVIATIONS

- BMD: Bone mineral density
- BMP1: Bone morphogenetic protein 1
- BV: Bone volume
- CI: Confidence interval
- COL1A1: Collagen type 1 alpha 1
- COL1A2: Collagen type 1 alpha 2
- CPR: Chronic obstructive pulmonary disease
- CPRC: Danish Civil Registration Register
- CREE31: USR response element binding protein 3 like 1
- CRTAP: Cartilage associated protein
- CSA: Cross-sectional area
- CT: Computed tomography
- DI: Dentinogenesis imperfecta
- DNA: Deoxyribonucleic acid
- DNP: Danish National Prescription Register
- DXA: Dual energy x-ray absorptiometry
- ECG: Electrocardiogram
- EDR: Excess death rate
- EF: Ejection fraction
- FEV1: Forced expiratory volume in 1 second
- FEV1/FVC: Ratio of Forced expiratory volume in 1 second to Forced vital capacity
- FIBD10: FIBD binding protein 10
- FVC: Forced vital capacity
- HA: Hyaluronic acid
- HQRQ: Health-related quality of life
- HR: Hazard ratio
- HRpQCT: High-resolution peripheral quantitative computed tomography
- ITIM: Interferon induced transmembrane protein 5
- IQR: Interquartile range
- NDR: Osteogenesis imperfecta
- NFR: Danish National Patient Register
- NSAID: Non-steroidal anti-inflammatory drugs
- NYHA: New York Heart Association Functional Classification
- OI: Osteogenesis imperfecta
- OR: Odds ratio
- P3H1: Prolyl 3-oxoglutarate dioxygenase 2
- PCL: Membrane bound transcription factor peptidease, site 2
- PCL: Perilubertinase
- P3H1: Peptidylprolyl isomerase B
- PCL: Plastin 3
- PCL: Oxygen tension
- PTH: Parathyroid hormone
- PCL: Receptor activator of nuclear factor kappa B ligand
- PCL: Randomised controlled trial
- PCL: Standard deviation
- PCL: Serpin family H member 1
- PCL: Serpin family H member 1
- PCL: Sub-hazard ratio
- PCL: Sp7 transcription factor
- PCL: Sp7 enriched proteoglycan
- PCL: Standard deviation of (1/trabecular number)
- PCL: Trabecular number
- PCL: Trabecular thickness
- PCL: Trabecular thickness
- PCL: Trabecular thickness
- PCL: Total lung capacity
- PCL: Transmembrane protein 388
- PCL: Tissue volume
- PCL: Maximal oxygen uptake
- PCL: Volumetric bone mineral density
- PCL: The plateau of oxygen uptake
- PCL: World Health Organisation
- PCL: Wnt family member 1

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2. Background

2.1 PREVALENCE AND INCIDENCE OF OI

Osteogenesis imperfecta (OI), or brittle bone disease, is a phenotypically heterogeneous, rare connective tissue disorder that affects 10.6 per 100,000 persons in Denmark (5). Patients with OI usually present with blue sclera, varying degree of bone deformities, and frequent fractures. OI is most often due to dominantly inherited variants to the COL1A1 and COL1A2 genes (6). The disease may be classified according to Sillence’s classification into four groups: type I (clinical severity: mild), type II (lethal), type III (severe) and type IV (moderate) (7). The clinical heterogeneity is illustrated in Figure 1. During the recent decades several recessive forms, X-linked forms, and an autosomal dominant form of OI due to mutations in a non-collagen gene have been reported (8). These newly discovered forms of OI follow the same nomenclature and are called OI type V-XVI (moderate to severe) (9).

![Figure 1. Clinical heterogeneity of OI Shows whole-body DXA scan pictures of patients with different subtypes of OI illustrating the clinical heterogeneity of OI. Inspired by Reeder and Orwoll (10).](image)

Based on data on all newborn (live births and stillbirths after the 28th week of gestation) in the Danish county of Funen from 1970 to 1984, Andersen and Hauge (5) estimated the incidence of OI to be 21.8 per 100,000. In a Brazilian population over the same time period, the incidence of OI was estimated to be 4.3 per 100,000 births (11). This is in line with other authors from the US and Finland, where the prevalence of OI is reported as 4.0-6.7 per 100,000 births (9, 12, 13). Andersen et al. (14) used a chart review of orthopaedic-, paediatric- and obstetric- patient charts to identify all patients with OI born during the period of observations, the authors furthermore evaluated all radiograph-diagnosis to identify patients with typical OI findings on the radiographs. Clinical information was then sought on every identified patient (14). The higher incidence found in Denmark could be due to the authors included information on all births on Funen, which had not previously been done (14). Lastly the increased incidence found by Andersen et al. (14) could be due to a high geographical concentration of families with many children. Based on all patients registered with an OI diagnosis in the Danish National Patient Register (NPR) from 1977 to 2013, we have estimated the incidence of OI in Denmark to be 15 [range 5-24] per 100,000 births (1) (Figure 2). According to our data, the population prevalence of OI in Denmark was 10.3 per 100,000, with 575 patients registered with an OI diagnosis in the NPR and alive at the end of 2012 and a total population of 5,602,628 persons at the end of 2012 (1, 15).

![Figure 2. Incidence of OI in Denmark The figure shows the incidence of patients with OI born in Denmark from 1977-2013 per 100,000 births. These numbers will be influenced by the time to diagnosis, which may well vary in OI. The full line indicates the trend in incidence over time. Using a linear regression model, this trend was not significant (β-coefficient 0.17 [95% CI: -0.66-1.01], p=0.674). We used data supplied from Statistics Denmark, Division of Research, as described in detail in the attached publications, Paper I (1), II (2), and IV and in Chapter 8.1 of this thesis.](image)

In 1983 the population prevalence was estimated at 10.6 per 100,000 inhabitants (48 patients in a population of 453,921 persons) and about 85% of patients had non-lethal forms of OI (5). As a result of early prenatal diagnostics (and pregnancy termination leading to fewer cases) the non-lethal forms of OI now constitute more than 95% of children born with OI in the US (16).

2.2 DIAGNOSIS AND CLINICAL PRESENTATION OF OI

Figure 3 shows the time from birth until an OI diagnosis is registered in the Danish National Patient Register (NPR) for patients born in Denmark since 1977. The median time to diagnosis from birth was 2.8 (range 0.33) years.

![Figure 3. The diagnostic delay from birth of the OI diagnosis The figure shows the time to diagnosis in patients registered with OI in Denmark as of 2012 divided into age at diagnosis and number of years of age.](image)
The NPR, born after 1977. Due to low numbers of individuals diagnosed over the age of 16, we have grouped these together to ensure sufficient patient confidentiality. The median time from birth to first registration in the NPR (indicating the time of diagnosis) was 2.8 years (interquartile range, IQR: 0.5–10.7 years), and 90% of all patients with OI were diagnosed by 16.6 years of age. We acknowledge that some patients may not yet have been diagnosed with OI, but more than half of the patients born after 2009 will have been diagnosed prior to the end of 2012 (the latest available updated version of the NPR at the time of data extraction i.e. 27th July 2014), and more than a quarter of the patients born in January 2012 will have been diagnosed at the time of data extraction from the NPR. The Y-axis shows the total number of patients (N), and the X-axis shows the age at OI diagnosis for each individual. The dashed lines show the 25% and 75% IQR, the median and the 90% percentile.

2.1.2 Diagnosis and care of patients with OI

Molecular tests, such as analysis of the structure and quantity of type I collagen synthesised in vitro by cultured dermal fibroblasts or DNA sequence analysis, can give valuable information about phenotype and clinical severity and even provide the diagnosis in most cases (12, 16, 17). Abnormalities in type I collagen are found in 98% of individuals with type II OI, about 90% of patients with type I OI, and 84% of patients with type III and IV when analysing cultured dermal fibroblasts (18). The best practice guidelines for the laboratory diagnosis of OI published in 2012 by the European Molecular Genetics Quality Network working group (16) state that patients with suspected OI should have sequence analysis and quantitative analysis of COL1A1 and COL1A2 genes on genomic DNA (16). Such genetic testing will identify over 90% of correctly known recessive genes that cause OI (16).

The diagnosis of OI is, however, often based on clinical traits such as blue sclera, family history, and frequent fractures resulting from little or no precipitating trauma.

The spectrum of disease severity varies between OI centres due to different indications for hospital follow-up and varying population case mix. The diagnosis of specific OI types is somewhat subjective and can depend on the amount of available clinical information and the patient age as the diagnosis of OI type VI requires histological analysis of bone tissue, and hyperplastic callus diagnostic for OI type V may not be present in children (19).

Care of adult patients with OI in Denmark is centred in four highly specialised centres (Aarhus University Hospital, Odense University Hospital, Copenhagen University Hospital in Hvidovre, and Rigshospitalet). Care of paediatric patients with OI is centred in two highly specialised units for rare diseases at Aarhus University Hospital and Rigshospitalet. There may be differences in how patients are diagnosed with OI at these centres, but the diagnosis is often made clinically and supported by either collagen analysis and/or DNA tests.

2.2.2 Clinical phenotypes and genetic variants

Mutations causing haplotype insufficiency more often resulted in a quantitative collagen type 1 deficiency than did helical mutations arising from missense mutations that resulted in qualitative collagen type 1 defects (20). Collagen type 1 is a heterotrimer that contains two α1-chains and one α2-chain. Procollagen undergoes a variety of post-translation modification and folding processes, closely regulated by key proteins that facilitate the folding and chaperone the procollagen molecules into the collagen fibrils (12). Mutations to any of the genes involved in this process may cause specific forms of OI. The clinical phenotypes and genetic variants of the different types of OI are described in Table 1. The OI subtypes can be grouped into five clinical syndromes, based on the patient’s clinical features irrespective of the genotype (6).

The table shows the OI type using Silfven’s Classification (originally 1979, updated 2014), the mode of inheritance, the affected genes, the clinical phenotype characteristics and the clinical syndromes of OI. AD, Autosomal dominant, AR, Autosomal recessive. Adapted from Forlino and Marini (2015) (9) (12) and van Dijk and Silfven (2014) (6).

The non-deforming OI with blue sclera, or Silfven OI type I, is regarded as the mildest phenotype of OI (18). It is the most common form and accounted for 71% of OI cases on Funen in 1983 (5). The patients have relatively low fracture rates and few severe bone deformities. The bone deformities rarely progress over time.

Common variable OI with normal sclera is a moderately severe phenotype and comprises the autosomal dominant OI type IV, the autosomal recessive forms VII, IX, and XII as well as the X-linked PLS3-mutated form of OI (6). The patients will have recurrent fractures and variable degrees of deformity of long bones and spine.

<table>
<thead>
<tr>
<th>OI Type</th>
<th>Mode of Inheritance</th>
<th>Affected Gene</th>
<th>Clinical Phenotype</th>
<th>Clinical Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>AR</td>
<td>COL1A1 / COL1A2</td>
<td>Perinatal lethal</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COL1A1 / COL1A2</td>
<td>Progressive defor-</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COL1A1 / COL1A2</td>
<td>mation</td>
<td>Moderate</td>
</tr>
<tr>
<td>IV</td>
<td>AR</td>
<td>COL1A1 / COL1A2</td>
<td>Common variable</td>
<td>Perinatal lethal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COL1A1 / COL1A2</td>
<td>OI with normal sc-</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COL1A1 / COL1A2</td>
<td>lera</td>
<td>Moderate</td>
</tr>
<tr>
<td>V</td>
<td>AR</td>
<td>PLS3</td>
<td>Common variable</td>
<td>Perinatal lethal</td>
</tr>
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<td></td>
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<td>PLS3</td>
<td>OI with normal sc-</td>
<td>Moderate</td>
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<td></td>
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<td>PLS3</td>
<td>lera</td>
<td>Moderate</td>
</tr>
<tr>
<td>VI</td>
<td>AR</td>
<td>TMEM38B</td>
<td>Common variable</td>
<td>Lethal</td>
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<td></td>
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<td>TMEM38B</td>
<td>OI with normal sc-</td>
<td>Lethal</td>
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<tr>
<td></td>
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<td>TMEM38B</td>
<td>lera</td>
<td>Lethal</td>
</tr>
</tbody>
</table>

The table shows the OI type using Silfven’s Classification (originally 1979, updated 2014), the mode of inheritance, the affected genes, the clinical phenotype characteristics and the clinical syndromes of OI. AD, Autosomal dominant, AR, Autosomal recessive. Adapted from Forlino and Marini (2015) (9) (12) and van Dijk and Silfven (2014) (6).
sion (22). OI type IV accounted for 6% of all OI cases on Funen in 1983 (5). No data are available on the distribution of the recessive forms of OI in Denmark, but the recessive and X-linked forms of OI account for less than 10% of all OI cases.

**OI with calcification in the interosseous membranes (OI type V)** is a moderate to severe phenotype with an autosomal dominant pattern of inheritance and white sclera. The most pronounced features are hyperplastic callus, progressive calcification of the interosseous membranes and coarse mesh-like lamellation of bone in bone biopsies (6, 12).

The **progressively deforming OI subgroup** comprises the autosomal dominant type III OI that accounted for 12% of all cases of OI on Funen in 1983 (5) and the recessive forms OI type VI-VIII, IX-XI, XIII-XVI, and Bruck Syndrome (OI with joint contractions) (6). Patients are born with multiple fractures and will continue to suffer fractures, thus developing progressive bone deformities and severe length growth retardation (6). Most patients become immobile and wheelchair-bound.

The **perinatal lethal OI** subtypes comprise OI type II that accounted for 12% of all identified cases on Funen in 1983 (5). Perinatal lethal forms of the autosomal recessive forms OI type VII, VIII and IX have also been reported (12).

In a Canadian study including 598 patients (from 487 families) who were clinically diagnosed with either mild OI (OI type I) or moderate to severe OI (OI type III, IV, V, VI, VII or Cole Carpenter Syndrome), a disease-causing gene variant could be found in 585 patients (98%) (19). In the mild OI group, 86% had mutations to the COL1A1 gene, 11% had mutations to the COL1A2 gene, and no mutations could be found in 3% of patients (19). In the moderate to severe OI group, 39% had mutations to the COL1A1 gene, 38% had mutations to the COL1A2 gene, and 12% had mutations to recessive OI genes (of which mutations to SERPINF1 (4%) and CRTAP (2.9%) were the most common (19). Moderate to severe phenotypes of OI can thus more often be associated with other mutations than COL1A1 and COL1A2, possibly as much as 20% of all cases, at least in the children followed through the Shriners Hospital for Children in Montreal.

In a Danish cross-sectional study of 85 patients with OI, including 58 patients clinically diagnosed with OI type I, 12 patients clinically diagnosed with OI type III and 15 patients clinically diagnosed with OI type IV, identified 68 OI causing mutations (20). In 4 patients no mutations to either COL1A1, COL1A2 or 11 other genes associated with OI (20). The mutations causing OI type I were more often located to COL1A1 than COL1A2 (46 out of 55 mutations) (20). In both patients with OI type III and OI type IV the mutations were evenly distributed across the two genes (20).

### 2.2.3 Skeletal manifestations and fractures in OI

Skeletal fragility is a hallmark of OI and results in frequent fractures. Moreover, many patients have bowing deformities of the long bones and, depending on clinical severity, have growth deficiency (12). In a study of 95 adults with OI types I, III and IV, 47% had upper extremity deformities, 63% had lower extremity deformities, and 21% had upper and lower extremity deformities, scoliosis and/or kyphosis (23). Other skeletal features depend on both age and clinical severity and include macrocephaly, flat midface and triangular facies, basilar impression, and chest-wall deformities such as pectus excavatum, pectus carinatum, or barrel chest (12). Regardless of clinical phenotype, the incidence of scoliosis has been reported as 39-80% (24, 25). Wormian bones (intra sutural bone) are common in OI, and approximately 35% of patients with OI type I, 78% of patients with OI type IV, and 96% of patients with OI type III present with wormian bones (26).

### 2.2.4 Non-skeletal manifestations of OI

Protein account for 20% of the human body mass, 30% of the proteins found in the human body is collagen and 90% of all collagen is collagen type 1 (27). The skeleton accounts for 14% of the total body weight. In the bone 10-30% is organic material, and of this 90-95% is collagen and mostly collagen type 1 – however less then 20% of the total body collagen type 1 is found in the bone tissue.

Among 88 patients with OI, 26% had dentinogenesis imperfecta (DI) where the teeth are yellow and appear transparent, and become prone to increased wear and breakage (28).

Pre-senile hearing loss due to otosclerosis is frequently reported in OI (29). In a Danish study comprising 173 patients with OI, 50% had hearing loss with 3% anacusis, 8% sensorineural, 12% conductive, and 27% mixed causes of hearing loss (29). Thirty-two (19%) of the patients had undergone stapedectomy due to otosclerosis, with excessive growth of the bones of the middle ear, or fractures and callus formation to the bones of the inner ear (18, 29).

The central corneal thickness has been found to be decreased in children with OI aged 10.1±2.5 years of age, compared with 15 healthy age- and gender-matched controls (30). Similarly, Hald et al. showed that corneal thickness is lower than expected in adult patients with OI, and most profoundly in patients with type I OI (31). Also, OI is associated with low ocular rigidity (32). Finally, OI may be associated with open angle glaucoma may also be (32).

In a Finnish retrospective patient-record study of 47 patients with OI (64% type I, 21% type IV or VI, and 15% type III) aged 1-19 years, 70% of patients had joint hypermobility (33). Adults with OI often report hypermobility, but the prevalence and severity are unknown.

Two-thirds of patients with OI report easy bruising (34). Studies of thrombocyte aggregations have shown abnormal platelet functions (34, 35). Exposed collagen from the blood vessel wall after vessel damage will normally attract platelets and further platelet aggregation. It has been speculated that the defective collagen seen in OI can result in defects in the platelet plug formation, causing increased bleeding time and bruising (36). Further research, however, is needed to elucidate this.

### 2.2.5 Cardiovascular manifestations in OI

Collagen type 1 is an important constituent of various parts of the cardiovascular system, including the heart valves, chordae tendineae, fibrous rings of the heart, interventricular septum, aorta, and most other arteries (37, 38). In two murine models (Aga2-mouse and OIM-mouse), the mutations introduced to the
COL1A1 or COL1A2 genes have caused the homozygote mice to present skeletal, cardiovascular, and pulmonary phenotypes (39-41). There seems to be increased prevalence of cardiovascular disease in OI, but the literature is mostly cross-sectional studies or case series and case reports (42).

2.2.6 Causes of Death and life expectancy in OI
Prior to the studies comprised in this PhD thesis, little was known about the life expectancy and causes of death in patients with OI. One study has shown that in patients with more severe phenotypes the main cause of death was due to respiratory tract infections (43).

2.3 CURRENT TREATMENT OPTIONS IN OI
Treatment of OI focuses on three elements: prevention of fractures, correction of bone deformities, and treatment of other complications such as hearing loss, dental problems, and pain management.

2.3.1 Prevention of fractures
Many different drugs, such as e.g. calcitonin (44), fluoride (45) and strontium ranelate (8), aimed at preventing fractures in osteoporosis have been tested for fracture prophylaxis in patients with OI without success. Since the late 1990s, patients with OI have frequently been treated with bisphosphonates (8). This may lower the risk of fracture although the effect has been questioned (46, 47). Where most of the treatment trials report positive effects on BMD, it must be kept in mind that these effects do not automatically translate into a reduced fracture risk.

In the latest Cochrane review of bisphosphonate treatment in patients with OI, twelve trials enrolled 709 children, and two trials enrolled 110 adults (47). All trials assessing BMD reported statistically significant increases with oral or iv bisphosphonates (47). In six of the trials evaluating the effect of oral bisphosphonates compared to placebo there was no difference in the number of patients that reported at least one fracture during follow-up, but the fracture rates and risks were lower in the bisphosphonate treated groups at 1 and 2 years of intervention (47). The three studies that evaluated the effects of iv bisphosphonates versus placebo could not detect any effects on the number of patients suffering a fracture nor the fracture rates or fracture risk between the treated and non-treated participants (47).

A recent systematic review that included six randomised controlled trials (RCTs) evaluating the effect of bisphosphonates compared with placebo in patients with OI included a meta-analysis of the fracture prevention of bisphosphonates in OI (46). The study reported a trend towards a reduction in fracture rates in bisphosphonate-treated patients (risk ratio = 0.79 [95% CI: 0.62-1.01], p=0.07, I²=0%) (46). Similarly, bisphosphonates tended to reduce the proportion of patients who experienced fractures (risk ratio = 0.84 [95% CI: 0.69-1.01], p= 0.06, I²=0%) (46).

In our study (Paper II) we had no data on bisphosphonate use in the patients with OI, but we saw no period effect when we entered calendar year into our Poisson model, as would be expected had the prognosis of fractures suddenly improved with the more widespread prophylactic use of bisphosphonates in the late 1990s. In the Norwegian study of 97 adults with OI, there was no significant difference in the number of reported fractures between bisphosphonate users and non-users (48). In the Canadian study of 86 children with OI type I, 48 children were not treated with bisphosphonates at the study start, but 25 of these patients subsequently started on intravenous bisphosphonates, and the average number of annual fractures decreased from 0.77 per patient to 0.44 fractures per patient (49). This reduction in fracture rate was, however, non-significant (p=0.2) (49). The effect of bisphosphonates as fracture prevention in patients with OI is questionable.

A single RCT, including adult patients with type I, III or IV OI, has tested the effect of teriparatide (biosynthetic human parathyroid hormone 1-34) versus placebo on fracture risk and BMD (50). The study showed significant BMD increase in both hip and spine in all phenotypes, but the largest effect was seen in the milder phenotype (50). There was no effect on self-reported fracture rates between the treated patients and placebo group (50).

A Danish investigator-initiated clinical trial is currently ongoing, and patients are being recruited to the study. The TREAT-OI study is a double-blinded placebo controlled RCT testing the effect of treatment with teriparatide (PTH) and zoledronic acid in adult patients with OI (51). One-third of the patients will have zoledronic acid and placebo PTH injections for two years, followed by one year of zoledronic acid; one-third will have PTH and placebo zoledronic acid injections for two years, followed by one year of zoledronic acid; and one-third will have placebo PTH and placebo zoledronic acid injections for three years. The study will evaluate changes in bone mass, bone geometry, and bone microarchitecture using DXA, HRpQCT, and in a subset of patients transiliac bone biopsies. Other studies evaluating the effects on fracture risk and fracture rates of PTH injections followed by zoledronic acid compared to standard care are currently being planned. One study with adult UK patients will be the first with sufficient power to evaluate whether combined PTH and zoledronic acid can prevent fractures in OI.

Denosumab, a RANKL antibody, resulted in a significant increase in BMD after 48 weeks of treatment in a single-arm safety trial including 10 children with OI (52). Data on long-term follow-up, treatment in adults, or fracture prevention are not currently available. According to ClinicalTrials.gov, there are no current studies on denosumab in OI (August 2016).

Preclinical studies in the Brl+/ mouse, a murine model for OI, have tested sclerostin antibody treatment and showed increased cortical bone formation, long bone mass, and long bone strength without increasing the brittleness in growing mice (53). Romosozumab, a monoclonal antibody that binds sclerostin, has a dual effect on bone by increasing bone formation and reducing bone resorption and thus has favorable effects in both aspects of bone volume regulation in patients with osteoporosis, and have been tested in phase II trials and are currently undergoing phase III investigation as a fracture preventing drug for the treatment of osteoporosis (54). Preparations are underway for a industry-initiated, international, multicentre double-blinded RCT using recombinant humanised monoclonal antibody against human sclerostin in patients with OI type I, III or IV.
2.3.2 Surgical treatment

Non-surgical fracture management is generally preferred in OI, but is not always appropriate. The main reasons for choosing surgical treatment are to treat fractures, to prevent fractures and to correct bone deformities. When surgery is considered, the risk of non-union and the need for subsequent revision procedures must be kept in mind (55). Furthermore, anaesthesia and peri-operative management can be challenging in patients with OI. Careful positioning is needed to avoid fractures, and airway management may be difficult as overextension of the cervical spine can lead to odonto-axial dislocation or fractures. Bleeding and bruising can be difficult as overextension of the cervical spine can lead to odonto-axial dislocation or fractures. Bleeding and bruising tendencies are also well described in OI, and clotting abnormalities usually exist at the stage of platelet plug formation and may cause excessive bleeding during surgery. Lastly, patients with OI have increased risk of metabolic acidosis, hypercapnia, muscle rigidity, and progressively increasing body temperature during anaesthesia (36). The cause of the “malignant hyperthermia-like” syndrome seen in some patients with OI during anaesthesia may be due to other mechanisms than those seen in inherited malignant hyperthermia (56). In a single-centre chart-review study from Italy, Chiarrello et al. (57) had treated 29 patients with OI with a reported mean age of 8.0±8.3 [sic!] years. Of the 245 procedures performed, 166 were for 110 fractures, and 79 were for correction of deformity (57). Common surgical complications regarding fracture care were non-union or delayed union (11.4%), malunion (5.7%), and implant loosening (6.1%) (57). Surgically implanted intramedullary rodding of the lower limb long bones has been shown to improve function and ambulation in children with OI (58, 59). The correction of forearm deformities with rodding has also improved functional ability in children with OI evaluated by the Pediatric Evaluation of Disability Inventory (60). Telescopic rods are often used in pediatric patients allowing for vertical height gain, but non-telescoping rods have been used with success. In a Danish trial including 9 children, who underwent a total of 16 surgical procedures with intra medullar rods, the stabilisation of deformities in the lower extremities decreased the rate of fractures and allowed most formerly non-ambulatory patients to walk (61).

2.3.3 Pain management in OI

In children, bisphosphonate treatment has increased the lumbar spine BMD and improved vertebral height (8, 62). It has been claimed that bisphosphonate treatment could reduce bone pain. A systematic review by Rijks et al. (63) identified five studies where evaluation of bone pain was an outcome. In one of these, bone pain improved significantly with bisphosphonate therapy (63). In another study, bone pain was significantly less after 24 months of treatment compared to baseline, but the same change was seen in the placebo group. This was not reproduced in the other studies included in the review (63). There is little evidence to support the claim that bisphosphonate treatment will reduce bone pain in all patients with OI, but it may have an effect in some patients.

Non-pharmacological treatment in OI often includes physiotherapy and occupational therapy and aims at reducing pain and improving ambulation. A Dutch study evaluating the effect of training and physical therapy on fatigue and health-related quality of life (HRQoL) included 34 (22 girls) children with type I or IV OI, who were randomised to either 30 sessions of 45 minutes exercise or no intervention over a period of 12 weeks (64). The study used the subscale “subjective fatigue” of the self-reported Checklist Individual Strength-20 questionnaire and assessed HRQoL using the Child Health Questionnaire Parent-Form 50 (64). After 12 weeks of supervised training, subjective levels of fatigue were reduced, but no effect was seen on HRQoL (64).

2.4 GAPS IN CURRENT KNOWLEDGE REGARDING OI

While the bone phenotypes and genotypes in OI are well described and fast evolving, less is known about other aspects of the disease that may be associated with the quantitative or qualitative defect of collagen type 1 seen in OI. Only a few very studies have focused on life expectancy and causes of death in patients with OI and population based studies are lacking. Causes of death can be viewed as a pseudo-marker of the burden of disease during life. We need more knowledge about which diseases are associated with OI, and how the burden of these diseases may influence patients’ quality of life and longevity. The important role of collagen type 1 plays in the function and structure of the heart makes it biologically plausible that patients with OI will have increased risk of cardiovascular diseases. It is difficult to draw any conclusions on causality from cross-sectional studies but a longitudinal population based study with statistical power to correct for confounders for cardiovascular diseases could elaborate on the relationship between OI and the risk of cardiovascular diseases. Where the increased fracture rates seen in patients are well described, and are close to a diagnostic criterion of the disease, less is known about the exact differences in fracture rates between patients with OI and the general population. Furthermore, little is known about the changes in the fracture rates over time. Whole-bone strength depends on the amount of bone, the spatial distribution of the bone mass (geometry), microarchitecture of the bone, and the intrinsic properties of the materials that form the bone (65). HRpQCT is designed for in vivo assessment of volumetric bone mineral density, bone geometry, and bone microarchitecture at the distal radius and tibia. At the time of the planning of this thesis no other studies were available using HRpQCT in the evaluation of patients with OI.

3. HYPOTHESIS, STUDY OBJECTIVES AND SEARCH STRATEGIES

3.1 HYPOTHESES

Figure 4 summarises the main hypotheses of this thesis.

We hypothesise that OI will, through different mechanisms, influence the risk of death in patients with OI and will result in a shortened lifespan compared to the general population. Studies have shown excess death in relation to osteoporotic fractures, for both major osteoporotic fractures such as hip and vertebral fractures but also for non-hip and non-vertebral fractures (66). We hypothesise that patients with OI will have increased risk of death due to fractures compared to the general population. Collagen type 1 is an important constituent of different parts of the cardiovascular system (37, 38). Cardiovascular disease is a leading cause of death, and increased prevalence of cardiovascular disease will increase the risk of premature death. We hypothesise that patients with OI will have increased risk of death due to cardiovascular diseases compared to the general population as a consequence of the quantitative or qualitative defect of collagen type 1 in OI.
The prevalence of scoliosis can increase the risk of restrictive pulmonary disease, and thus the risk of pulmonary infections. We hypothesise that patients with OI will have increased risk of death due to respiratory diseases.

Bone strength is determined by bone geometry, bone microstructure, bone mass, and the quality of the bone matrix (67). Several studies in children and adults have shown that BMD is lower in patients with OI than in healthy controls (3). The bones are ‘brittle’ and absorb less energy before fracturing (68). This results in increased risk of fractures in patients with OI. We hypothesise that patients with OI will have increased fracture rates compared to the general population, but that the relative risk of fractures will decrease with increasing age. We further hypothesise that the fracture pattern seen in OI (i.e., which bones fracture at which stages of life) will mimic that of the general population. Lastly, we hypothesise that patients with OI will have altered bone microarchitecture, lower bone mass, and different bone geometry to healthy non-OI individuals that may explain some of the increased fracture risk seen in patients with OI.

The heart valves, chordae tendineae, annuli fibrosis, interventricular septum, aorta and most other arteries contain collagen type 1 (37, 38). The collagen fibres in the ventricular myocardium contribute to the tensile stiffness and maintain the architecture of the myocytes (40). In the OIM mouse model, the collagen area fraction and fibre density in the heart was 35-38% lower in the homozygote mice compared to the wild-type mice (41), indicating that collagen type 1 plays an important role in heart function.

We hypothesise that patients with OI will have increased risk of aorta and mitral valve insufficiency, increased risk of heart failure (due to enlargement of the ventricles that dilate due to the quantitative or qualitative defect of collagen type 1), increased risk of atrial fibrillation (due to dilatation of the atria caused by the quantitative or qualitative defect of collagen type 1) and increase the risk of vessel dissection and aneurisms, even after correction for non-collagen related causes of these conditions.

**Figure 4. Main hypotheses** The figure is theory-driven and shows the multifactorial causes of premature death in OI. We hypothesise that the decreased amount of collagen type 1 seen in OI increases the risk of cardiovascular diseases that in turn will increase the risk of premature death. OI also alters the bone matrix quality, increasing the risk of fractures that may be associated with increased risk of death, but may also cause immobilisation. The increased fracture risk may also lead to lower physical activity as a fracture prevention technique. Immobilisation and reduced physical activity increase the risk of cardiovascular disease through ischaemic cardiovascular disease. Fractures of the spine may cause scoliosis, while hyperlaxity in spinal ligaments causes scoliosis and/or kyphosis that increase the risk of restrictive and obstructive pulmonary diseases. This increases the risk of respiratory tract infections that also increase the risk of premature death in OI. Restrictive pulmonary disease can also increase the risk of cardiovascular disease. There may also be other, as yet unknown factors leading to premature death in OI such as clinical severity and phenotype, gender and ageing, but these are not shown for the sake of simplicity. Full lines indicate a direct relationship; dashed lines indicate an indirect relationship. OI: Osteogenesis imperfecta, CVD: cardiovascular disease, Rest. pulm.: restrictive pulmonary, Obst. pulm.: obstructive pulmonary disease.

### 3.2 STUDY OBJECTIVES

The study objectives were:

1) to evaluate the risk of death and causes of death in patients with OI and to calculate median survival time in OI compared to the general population (Paper I) (1)

2) to evaluate fracture rates and the pattern of fractures throughout life in patients with OI and compared to the general population (Paper II) (2)

3) to evaluate the bone mineral density, bone geometry, and bone microarchitecture in patients with OI type I compared to a non-OI matched reference group, and to explore the causes of the increased fracture rates in OI (Paper III) (3)

4) to evaluate the risk of cardiovascular disease in patients with OI compared to the general population (Paper IV)

### 3.3 SYSTEMATIC SEARCH AND NARRATIVE REVIEW

#### 3.3.1 Search strategy

A series of systematic searches and reviews of current literature was conducted in PubMed, Embase classic, Embase, and the Cochrane Library for the four main themes of this thesis (1: Risk of death, cause of death and life expectancy in OI; 2: Fracture risk and fracture rates in OI; 3: BMD, bone geometry, and bone microarchitecture in OI; 4: Cardiovascular diseases in OI). All included publications were hand-searched for any relevant references that were missed with the search strategy.

The search strategy, specific search strings, dates of the searches, and the search results are shown in Appendix 1.1 – 1.4.
3.3.2 Eligibility criteria for studies
Randomised controlled trials, cohort, case-control or cross-sectional studies, and case series including patients with OI were included in this review. The search was limited to literature in Danish, Norwegian, and English.

Reviews, case reports or case series with under 10 patients, commentaries, publications based on secondary data, conference papers, non-peer reviewed publications, publications only including foetal, neonatal or autopsy data or pregnancy outcome, non-OI publications only identified through the author names of Lobstein or Bruck (these are also names of OI subtypes), publications with only non-human data, studies reporting novel OI mutations (unless they included more than 10 patients and covered one of the main themes of the thesis), and publications that evaluated pharmacological or surgical treatment and did not have a non-OI reference group were excluded.

3.3.3 Data extraction
One author (LF) extracted data from the included studies on study design and size, participant age, gender, and phenotype, and characteristics of the reference group or material if present or used in the studies. Information on phenotype was extracted from the publications and presented as described by the authors if present. Values and data were presented as in the original publications.

4. CAUSES OF DEATH AND LIFE EXPECTANCY IN OI
This chapter evaluates the current literature on causes of death and life expectancy in OI. Little is currently known about the risk of death in patients with OI.

We performed a register-based cohort study using data extracted from Statistics Denmark Division of Research, which administers the health registers used in the study. Statistics Denmark is a state institution under the Ministry of Social Affairs and the Interior. We included all patients with OI registered in the NPR and compared their risk of death, primary causes of death, and median survival to a reference population, as described in Paper I (1).

We defined patients as all persons registered with an OI diagnosis in the National Patient Register (NPR) from 1977 (the start of the register) and until the latest updated version of the NPR at the time of data extraction (summer 2014). For the general population reference group, we randomly selected five individuals matched by gender, birth year, and birth month to each identified OI patient. To minimise the risk of misclassification, the controls could not be patients or first or second-degree relatives to any of the identified patients. The reference population was generated from the Danish Civil Registration Register (which administers the unique identification number (CPR) given to all inhabitants of Denmark and Danish citizens) and was supplied by Statistics Denmark Division of Research without any involvement of the study team in choosing or determining the eligibility of the chosen participants.

We extracted data from the NPR on surgery and discharge diagnosis from hospital stays, outpatient clinics, and emergency departments. We extracted data on dispensed prescriptions from the Danish National Prescription Register (DNPR), information on migration from the CPR, and data on time of death, place of death, and cause of death from the Causes of Death Register.

While case series and cross-sectional studies can provide detailed clinical information about each participant, a nationwide cohort study using register data is (in Denmark) representative of the entire population with no loss to follow-up. The nationwide coverage ensures a larger patient sample for conditions with low incidence such as OI, and the cohort design increases generalisability to the OI population. The limitations to this design are described in detail in Paper I (1) and in section 8.1 of this thesis.

4.1 LITERATURE SEARCH
The literature search was conducted as described in section 3.3 (inclusion and exclusion criteria) and Appendix 1.1 (search dates, search strings and search results) and aimed to identify all studies evaluating the risk of death, causes of death, and life expectancy in OI. A flow diagram of the study selection can be seen in Figure 5. Of the 435 publications initially identified, 242 were from Embase, 129 from PubMed and 64 from the Cochrane Library. After removal of 46 duplicate records, 389 titles and abstracts were screened. The 19 articles that fulfilled at least one inclusion criterion and no exclusion criteria were then screened by full text, and five publications were found to be eligible for data extraction. The extracted data are presented in Table 2.

![Figure 5. Flow diagram of study selection for studies covering risk and causes of death in OI](image-url)
### Table 2. Studies on causes and risk of death in OI included in this review

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Study Design</th>
<th>Total Patients (Women) [Risk of death]</th>
<th>Patient years of observation</th>
<th>Reference population (Women)</th>
<th>Observation period</th>
<th>Mild Phenotype (Women) [Risk of death]</th>
<th>Moderate Phenotype (Women) [Risk of death]</th>
<th>Severe Phenotype (Women) [Risk of death]</th>
<th>Most Severe Phenotype (Women) [Risk of death]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folkestad, (2004) (1)</td>
<td>Registry-based Cohort study</td>
<td>687 (379)</td>
<td>25,615</td>
<td>3415 (1895)</td>
<td>1977-2013</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Singer (2001) (69)</td>
<td>National Survey study</td>
<td>743 (412)</td>
<td>6970</td>
<td>Life tables for England and Wales in 2002</td>
<td>1980-1993</td>
<td>183 (275)</td>
<td>237 (130)</td>
<td>123 (70)</td>
<td>-</td>
</tr>
<tr>
<td>Paterson (1996) (70)</td>
<td>National Survey study</td>
<td>743 (412)*</td>
<td>6970</td>
<td>Life tables for England and Wales in 2002</td>
<td>1980-1993</td>
<td>* [MR 1.95, 95% CI: 0.64-3.13]</td>
<td>* [MR 1.95, 95% CI: 0.64-3.13]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Shapiro (1985) (71)</td>
<td>Case series from one centre</td>
<td>85 (40)</td>
<td>-</td>
<td>-</td>
<td>1938-1983</td>
<td>21* [0 of 21 patients died during median follow-up of 17.5 years]</td>
<td>21* [0 of 21 patients died during median follow-up of 17.5 years]</td>
<td>27 [2 of 27 patients died during median follow-up of 18.9 years]</td>
<td>16 [15 of 16 patients died during median follow-up of 6 weeks]</td>
</tr>
<tr>
<td>Singer (1982) (72)</td>
<td>Cross-sectional study</td>
<td>47 (1)*</td>
<td>-</td>
<td>-</td>
<td>15 years</td>
<td>21* [0 of 21 patients died during median follow-up of 18 months]</td>
<td>-</td>
<td>-</td>
<td>17* [15 of 17 patients died during median follow-up of 2 weeks]</td>
</tr>
</tbody>
</table>

*Same population as reported in Singer et al. (69). 1) Including patients with clinical type I and IV, 2) Including patients with first fracture in childhood after they began to walk, 3) Including patients with first fracture in childhood before they could walk, 4) Neonates with clinical severity score under 2.0, 5) Neonates with clinical severity score of 2.0-2.6, 6) Neonates with clinical severity score above 2.7. HR: Hazard Ratio, CI: Confidence interval, EDR: Excess Death Rate, MR: Mortality Ratio

### 4.2 RISK OF DEATH IN OI

The absolute risk of death is the same in all individuals, obviously. When using the term risk of death in this segment it refers to the differences between patients with OI and non-OI individuals during the observation period on a group level. This may be based on the all cause HR, the cause specific SHR, the Excess Death Rate or Mortality Ratio between the different groups included in the different studies.

In our study (Paper I) we identified 687 patients with OI, of whom 112 died during the observation period. The all-cause mortality hazard ratio for death (HR) was 2.9 [95% CI: 2.3-3.6] in patients with OI compared to the reference population (1). For men, the HR was 3.7 [95% CI: 2.6-5.2] and for women 2.4 [95% CI: 1.8-3.3] when compared to the reference population (1). Children with OI had increased all cause mortality, where 15 patients died before the age of 1, and a total of 19 patients with OI died prior to the age of 6 years and in total 26 patients with OI died prior to age 18 (fewer than 3 participants in the reference population died prior to age 18) (1). The low number of childhood deaths in the reference population resulted in an HR of 66.1 [95% CI: 15.7-278.7] for patients with OI aged less than 18 years (1).

Singer et al. (69) and Paterson et al. (70) reporting on the same study population included 743 patients with OI (383 with type I OI without dentinogenesis imperfecta (DI), 77 with type I OI with DI, 123 with type III OI, and 160 with type IV) from England and Wales in a study of life expectancy in OI. The observation period comprised 13 years (from 1980 to 1993). During 6940 patient years of observation, 57 patients died (69). The authors estimated the mortality ratio (MR) between the OI patients and the predicted mortality using data from the Office of Population Censuses and Surveys, Mortality Statistics and Review and the 1981 Life tables for England and Wales. An MR of 100% would indicate no difference between the observed and predicted mortality, while an MR above 100% would indicate increased mortality in patients with OI. The MR for patients with OI type I without DI was 140% in men and 85% in women. The MR for a combined group of OI type I with DI AND OI type IV was 295% in men and 133% in women. The MR for patients with type III OI was 113% in men and 2400% in women (69). The authors found increased childhood mortality for all groups i.e. MR of 128% for patients with type I OI without DI, 335% in the combined group of OI type I with DI and type IV OI, and 26,000% in patients with type III OI (69). Singer et al. (69) and Paterson et al. (70) used data from a survey of patients in England and Wales (69, 70) who were recruited through the British OI patient society. The authors did not provide information about the number of patients with OI living in England and Wales or about the response rate and selection bias was possible.

Shapiro (71) reviewed the charts of 85 patients who had been diagnosed with OI at a children’s hospital in Boston from 1938 to 1983. The patients were divided into four groups according to clinical severity: OI congenita A (the most severe phenotype, diagnosed at birth), OI congenita B (moderate to severe phenotype diagnosed at birth), OI tarda A (milder phenotype, not diagnosed at birth, without DI), and OI tarda B (milder phenotype, not diagnosed...
at birth, with DI) (71). All but one of the 16 patients with the most severe phenotype had died, with a median survival time of 6 months (range: stillborn – 3 years and 9 months) (71). Two patients with the moderate to severe phenotype had died at the time of the publication, both due to respiratory problems prior to their 6th month of life (71). None of the patients with the milder phenotypes had died during a median follow-up of 17.6 years (71). The study is a single-centre case series, and may be subject to selection bias, thus, the results may only hold true for the specific population. The results illustrate a clear difference in mortality risk between different phenotypes of OI, but do not evaluate the risk of death in the general OI population compared to non-OI individuals.

Spanger et al. (72) aimed to generate a clinical severity score based on radiographic findings in newborns with OI and used data from a bone dysplasia register at the Children’s Hospital, University of Mainz and the Red Cross Children’s Hospital, University of Cape Town. The authors evaluated clinical severity in 47 neonates with OI diagnosed at birth using a scoring system of skeletal pathologies to evaluate the risk of death in OI (72). Fifteen of the 17 patients with the highest clinical severity scores died within two weeks of birth; one of the 9 patients with medium high clinical severity scores died within one month of birth; and three of the 21 patients with the lowest clinical severity scores had died at the time of publication, younger than 6 months (72). It is unknown how the patients were selected for entry into the register and for evaluation, and selection bias may limit the results. The authors concluded, in concordance with other studies, that 88% of patients with the most severe phenotypes (i.e. with the highest clinical severity score) would die shortly after birth, but that 90% of patients with lower severity scores would survive (72).

Our own study and the literature confirm our hypothesis that the risk of death is increased in OI. The risk of death in OI is associated with male gender and young age. Clinical severity is reported in many studies as associated with an increased risk of death in the most severe phenotypes compared to patients with milder disease. This is however a circular argument as to some degree the patients were grouped according to their risk of their outcome i.e. patients with OI type II will by definition be lethal and thus have a much higher risk of death than patients with OI type I.

4.3 LIFE EXPECTANCY IN OI

As shown in Figure 6, in our study (Paper I) we found a median survival for men with OI to be 72.4 years [95% CI: 68.8-77.7] vs. 81.9 years [95% CI: 79.3-84.3] for men in the reference population (p<0.001) (1). The median survival for Danish women with OI was 77.4 years [95% CI: 74.6-79.8] vs. 84.5 years [95% CI: 83.0-86.2] in the reference population (1).

Paterson et al. (70) used data from the same database as Singer et al. (69) to calculate life expectancy in OI. The life expectancy in patients with type I OI without DI was equal to that of the general population (78.4 years for women and 73.6 years for men). In the combined group of patients with OI type I with DI AND patients with OI type IV, life expectancy was 72.0 [95% CI: 67.2-76.8] years in women and 68.8 [95% CI: 62.4-75.6] years in men. The confidence intervals indicate that the life expectancy was shorter than expected in women. In patients with type III OI, life expectancy was markedly reduced. Twenty-six patients had died during the observation period, and 19 of them prior to their 10th birthday. Life expectancy at birth was 28.8 [95% CI: 16.0-41.6] years for females and 43.2 [95% CI: 22.4-64.0] years for males. Due to the increased neonatal mortality, the remaining life expectancy for patients after their 1st birthday was 33.6 [95% CI: 23.2-44.0] years for females and 48.0 [95% CI: 33.6-62.4] years for males.

**Figure 6. Kaplan Meier estimates of survival in OI** The figure shows the KM plots in men and women with OI compared to the reference population. Y-axis shows the present remaining in the populations and the X-axis the population age. There is increased neonatal mortality in patients with OI, especially in men. The dashed lines indicate the reference population, the solid line the OI population.

The starting point for calculating life expectancy is the age-specific death rates of a population. Paterson et al. (70) estimated life expectancy based on 6970 patient years and 57 deaths. For small populations with few deaths, it is possible to adjust a known life table derived from a larger population by multiplying this life table by the age and gender specific standardized mortality rates for the population (e.g. patient population) of interest. Paterson et al. (70) used this approach to calculate life expectancy in patients with OI as they had calculated the mortality ratios for each group of interest, but this pragmatic approach may be prone to bias as it assumes that the mortality ratios are constant between the groups. In smaller populations, the estimation of life expectancy is prone to overestimation, and a population size of at least 5000 individuals has been recommended to estimate a population life expectancy with accuracy (73). For a low-mortality population, a total population exposure time should supersede 15,000 patient years at risk to allow accurate calculation of life expectancy (74). According to the American Centre for Disease Control, a reliable life table cannot be generated if the number of deaths in the total population (e.g. small municipalities or small ethnic groups) is under 700, and thus life expectancy cannot be estimated with adequate statistical certainty (75). Neither approach was suitable for our data, as we included births from 1899 to 2013 and has left-truncated the data as a patient had to be alive at least until 1977 to appear in the registers, and patients with OI could have died prior to entering the registers – thus introducing survival bias to our data. This is illustrated by the relatively higher than expected median survival time in the reference population. We acknowledge that our estimates of survival time are probably too high compared to that expected in the Danish population covering individuals born from 1899, but the comparison with the OI group should still illustrate the reduced median survival time seen in OI.
Our study (Paper I) and the literature confirm our hypothesis that life expectancy in patients with OI is lower than in the general population.

### 4.4 CAUSES AND RISK FACTORS FOR DEATH IN OI

#### 4.4.1 Clinical severity
Silence’s classification of OI is based on both clinical features and mode of inheritance (6, 7). Due to the lack of clinical information and information on genotype in the Danish health registers, it is not possible to identify the different phenotypes of the disease through these data sources (1). Severe phenotypes of OI have increased risk of death, and the risk of neonatal death is especially associated with clinical severity (43, 69-72). This is, however, a circular argument as the grouping of patients is done according to clinical severity. This ‘bias by indication’ must be kept in mind when evaluating the between group differences. It could be argued that a genotype to mortality risk correlation study would reduce ‘the clinical (often subjective) grouping of patients’ bias into account.

#### 4.4.2 Gender
Female gender favours longevity in patients with OI. In our study, we found that the HR for early death was higher in men than in women (1). In British patients with OI the mortality ratio for patients with type I OI without DI was 140% in men and 85% in women (69). Similarly, the mortality ratio for patients with type I OI with DI AND patients with type IV OI was 295% in men and 133% in women (69). Among those with OI type III, however, female patients had shorter life expectancy and higher mortality ratio than male patients (69). The Paterson et al. (70) and Singer et al. (69) studies included data on the same 123 (70 women) patients with OI type III, of whom 26 (19 women) died during the observation period. The expected number of deaths was 0.6 among the men and 0.4 among the women (69). This could explain the difference in mortality ratio between the genders in the most severe phenotypes, as the mortality ratio is defined as the relative difference between the observed and expected number of deaths.

#### 4.4.3 Trauma
In our study, 4.5% of deaths were due to external causes of morbidity and mortality (i.e. trauma), which was more frequent than the 1.9% in the reference population (1). This is in line with the findings of McAllion and Paterson (43), who reported that 13.2% of patients with type III OI died due to trauma, compared to 2.7% of the general population. These authors (43) evaluated the causes of death in patients with OI who had participated in a survey in 1980-1995 that was distributed through the Brittle Bones Society, and 68 of the 1297 participants had died during the observation period. There was no information about the response rate or the degree of coverage of the British OI population (43), so selection bias of participants cannot be ruled out. The authors had clinically evaluated 37 of the patients, and gathered clinical data on the remaining participants from questionnaires sent to the patients while they were still alive or a chart review (43), which may give some information bias to the clinical data. Whereas all specific causes of death were gathered through the Danish Causes of Death register in our cohort study, McAllion and Paterson identified the primary cause of death using a variety of sources such as death certificates, autopsy reports, medical records, hospital consultants, relatives, and the Brittle Bone Society (43). This approach is more prone to recall bias than our register-based approach.

We included national data that included all patients with an ICD-8 or ICD-10 diagnosis for OI and assume that the phenotype distribution is the same as that previously reported for the Danish OI population, where the mild phenotypes outnumber the more severe phenotypes (5). In the McAllion and Peterson study (43), 50% of patients had OI type III or IV.

Short-term mortality is increased after osteoporotic fractures (66), and we speculate that death associated with fractures or trauma might be underreported in our study. If a patient dies shortly after suffering a fracture, the physician is likely to register the cause of death as fracture-related, but if the patient dies from complications associated with the fracture, e.g. a pulmonary embolism, several weeks after the fracture event, then the primary cause of death might not illustrate the underlying cause (the fracture) (1). As patients with OI have more fractures than non-OI individuals (2), the risk of fracture-related death is higher in the OI population as a result of frequent exposure.

#### 4.4.4 Pulmonary diseases
In our study (Paper I) we found that patients with OI had an increased risk of death due to respiratory diseases with an SHR of 3.1 [95% CI: 1.4-6.9] (1).

Even in mild to moderate cases, scoliosis can cause pulmonary dysfunction including reduced vital capacity and exercise capacity, and recurrent respiratory infections (76). Kyphoscoliosis is frequently seen in OI, and six studies have evaluated pulmonary function in patients with OI using spirometry (24, 25, 39, 77-79). The studies evaluated total lung capacity, forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), and the FEV1/FVC ratio in patients with type I, III or IV OI and compared these results to predicted values according to age, gender, and height. The studies found that patients with OI type I often reach the predicted values of lung function, but the results for severe phenotypes were more diverse (24, 25, 39, 77-79).

The FEV1/FVC ratio is used to distinguish between an obstructive and a restrictive pattern of pulmonary function. An FEV1/FVC ratio below 70% is interpreted as an obstructive pattern of pulmonary function (80), while an FEV1/FVC ratio above 80% is interpreted as a restrictive pattern (80). The FEV1/FVC ratio can be falsely elevated in OI due to low values of both FEV1 and FVC (24). Studies have consistently shown elevated HR for mortality associated with both restrictive and obstructive pulmonary function (81). In a Norwegian cross-sectional study, Wekere et al. (25) included 97 adult patients with either OI type I, IV or III and found two patients with an obstructive pulmonary function pattern, four patients with a restrictive pattern when using actual height to predict the pulmonary function values, and two further patients when using arm-span as a proxy for vertical height. Scoliosis was negatively correlated to pulmonary function in six studies (24, 25, 39, 77-79). The degree of scoliosis varied by clinical severity and age, thus in the Norwegian study 46% of adult patients were scoliotic – 36% of those with OI type I and 100% of those with OI type III had significant scoliosis (25). In a longitudinal study of German patients with type III and IV OI, 78.3% of patients developed scoliosis during the observation period.
period from birth to young adulthood, and patients with lower airway obstruction had more severe scoliosis than those without lower airway obstruction (39). Pulmonary function declined significantly with age for all patients – from nearly normal at age 4 to about half of the predicted values by age 20, even in patients with only mild scoliosis (39). Kyphosis showed no significant correlation with FVC, FEV1 or TLC in adults with OI (24).

Arterial pO2 and pCO2 was reported within normal ranges in children with OI regardless of phenotype (79), but this study was small and included 11 patients with OI recruited through a single OI treatment centre. The pO2 and pCO2 values were compared to reference range values, and comparison to a matched reference group of non-OI individuals would have enabled the authors to evaluate whether pO2 and pCO2 were significantly different from non-OI individuals (even within normal reference ranges). Another study found that patients with OI type III had a drop in oxygen saturation (SpO2) at night, the average SpO2 being 91.9±12.2% with a nadir of 85.4±8.1%, and SpO2 was below 95% for 18% of the night (77). In patients with type IV OI, the average SpO2 was 96.8±2.4% with a nadir of 87.7±7.3%, and SpO2 was below 95% for 8% of the night (77). These results do not seem to be normally distributed based on the standard distribution, and it may have been more appropriate to report median (range) values. The authors used a two-way Analysis of Variance (ANOVA) to evaluate the difference between groups. There is no requirement for the data to be normally distributed using this model, but the residuals, are assumed to have a normal distribution – there was no indication if the authors evaluated this in their analysis. SpO2 is not expected to drop in non-OI individuals during sleep. When compared to healthy age- and gender-matched reference participants, patients with OI type III and IV had significantly lower tidal volume in both supine and seated positions, and their respiration rate was significantly higher (77).

The main causes of death in OI in the McAllion and Paterson study were pulmonary disease and respiratory tract infections (43). However, the study was not population-based and did not include the expected distribution of clinical phenotypes in an OI population, as half of their patients had more severe OI (43). Based on the prevalence of different phenotypes reported by Andersen and Hauge (5), we would expect that under 30% of the OI population would have type III or IV OI. Clinical severity is an important predictor of reduced pulmonary function. Severe phenotypes are associated with more respiratory morbidity and thus higher risk of death due to respiratory diseases.

### 4.4.5 Neurological diseases

The absolute risk of death due to neurological diseases, as given in death certificates and registered in the causes of death register, was low in our study, with 4 out of 112 deaths registered as caused by neurological diseases, and the relative risk compared to the reference population was non-significant with a SHR of 2.1 [95% CI: 0.6-6.6] (1).

Basilar impression or invagination (infolding of the occipital condyles and elevation of the clivus and posterior cranial fossa) results in the cervical spine migrating upwards into the foramen magnum (82). The prevalence of basilar impression or invagination is 25% regardless of OI phenotype, but only a few of the patients with basilar impression show neurological symptoms (22). In the McAllion and Paterson study (43), three patients died due to compression of the brain caused by basilar invagination. Deaths related to neurological diseases accounted for 12.2% (5 of 38) of deaths in OI type I and IV patients, while only 2.6% of deaths in the general population were due to neurological diseases during the same time period (43).

### 4.4.6 Gastrointestinal diseases

We found an increased risk of death from diseases of the gastrointestinal system in our study, with 9 (1.3%) deaths compared to 8 (0.2%) deaths in the reference population, resulting in a SHR of 4.2 [95% CI: 1.6-10.8] (1). The level of detail in the Danish Causes of Death Register limits the information on specific gastrointestinal diseases, and the precise causes of this increased risk are unclear. One hypothesis is increased NSAID use due to pain in patients with OI, who had higher use of prescription NSAIDs compared to the reference population (57% vs. 47%, p<0.001). Low-dose NSAID is sold over the counter (ibuprofen 200mg tablets) in Denmark and are thus not included in the Danish Prescription registries at individual level. There is also a risk of underestimating the NSAID use in the reference population – they may buy more over-the-counter drugs than the OI population, who are likely to be followed more closely by medical staff and thus have a larger proportion of their drugs prescribed and thus registered in the Danish prescription database.

The causes of death in OI are summarised in Figure 7.

**Figure 7. Causes of death in OI** The figure shows the factors that influence the risk of early death in patients with OI, based on data from the studies included in this thesis. OI most often causes qualitative or quantitative collagen type 1 defects, and the lack of available collagen alters the bone matrix quality that in turn increases the risk of bone fractures even with minor trauma. Trauma can also cause fractures that increase the risk of death. Fractures can also lead to scoliosis (as can the collagen type 1 defect). Scoliosis can lead to respiratory tract infections and thus increased risk of death. The collagen defect influence the risk of developing basillaris invagination that can cause neurological involvement.
conditions that increase the risk of death. Through an unexplained pathway, patients with OI have higher mortality from gastrointestinal disorders. Men have higher risk of death than women, at least for milder phenotypes. Cardiovascular diseases and malignancies were frequent causes of death in patients with OI, but the risk of death was similar to that of the general population and is therefore not featured in this figure.

Our study (Paper I) and the literature confirm our hypotheses that patients with OI have increased risk of death from respiratory disease and trauma causing fractures. We also found that patients with OI had increased risk of death due to gastrointestinal and neurological diseases. We could not confirm the hypothesis that patients with OI had increased risk of death from cardiovascular diseases compared to the general population, even though the absolute risk of death due to these diseases was high in our cohort study. (1).

4.5 CONCLUSION
Based on current knowledge, we conclude that patients with OI have increased risk of death compared to the general population. Their increased risk of death from trauma, respiratory diseases, neurological diseases and gastrointestinal diseases seems related to clinical severity and male gender. Studies are needed to evaluate the causes and severity of respiratory tract diseases in OI compared to the general population and to evaluate the causes of the increased risk of gastrointestinal death. Population-based longitudinal studies with information about genotype and clinical characteristics would greatly add to present knowledge.

5. FRACTURES IN OI
This chapter evaluates the current literature on several aspects of fractures in OI: the risk of fractures in patients with OI compared to non-OI individuals, fracture rates in patients with OI, typical fracture sites, and the pattern (i.e. changes in fracture rates and sites) of fractures over the lifespan of a patient with OI.

The bone fragility seen in OI leads to increased fracture risk, and most patients with OI will experience multiple fractures throughout life. Although fracture rates are not part of the diagnostic criteria for the phenotypes in Sillence’s classification, clinical severity is partly based on the number of fractures experienced. There is some clinical overlap between Sillence’s subtypes, and the phenotypes are heterogeneous. Patients with OI often report more frequent fractures during childhood and early adulthood, fewer fractures in adulthood, and increasing fracture rates in old age. Patients furthermore describe that the causal trauma differs throughout life – at younger ages, the trauma causing a fracture is often very minor and the fracture comes as a surprise, whereas fractures in later life are caused by more severe trauma and are more anticipated. Most of the treatment of OI is focused on fracture prevention. Little is however known about which patients to treat, for what duration and how to monitor patients during treatment. The effect of bisphosphonates as primary fracture prevention in patients with OI (of all ages) is questionable.

We performed a register-based cohort study using the same cohort as in Papers I (1) and IV (i.e. including all patients registered with an OI diagnosis in the NPR) and compared the fracture rates and sites to those in a reference population of non-OI individuals randomly selected from the central persons register and matched 5:1 for age and gender to the patients with OI. We counted all fractures registered in the NPR from 1995 until the end of 2012. We acknowledge that by using register data we only identify fractures that need hospital attention, and that patients with OI may to some extent treat smaller fractures at home with immobilisation and analgesia. Our data may thus reflect the fracture burden to the health care system and not necessarily that experienced by the patient. Our data also reflect current standards of care for OI and would probably be different if the condition remained untreated. The design of the study is described in detail in Paper II (2) and discussed in section 8.1 of this thesis.

5.1 LITERATURE SEARCH
The literature search was conducted as described in section 3.3 (inclusion and exclusion criteria) and Appendix 1.2 (search dates, search strings and search results) and aimed to identify all studies evaluating fracture risk and fracture burden in patients with OI. A flow diagram of the study selection can be seen in Figure 8. A total of 1423 publications were found via Embase, 1232 via PubMed, and 102 via Cochrane Library. After excluding 574 duplicates, 2183 titles and/or abstracts were screened for eligibility and resulted in 35 publications for full-text screening. One article was identified through a manual search of the references included in this chapter, leading to 19 publications being included in the review. Data from Paper II (2) are also included in the review. The study characteristics can be seen in Table 3 and 4.

![Figure 8. Flow diagram of study selection for studies on fractures in OI](image-url)
The table shows the included studies, the number of participants and the distribution of different phenotypes in each publication. We identified 20 publication through our literature search excluding our own publication (Paper II), the review includes 21 publications in total. The reported fracture outcomes are: The total number of fractures (N previous), the risk of fractures in patients with OI (IRR), age at first fracture (age at 1stFx), fracture rates per person year (Fx/year) and the pattern of fracture rates over time (Pattern). 1) Age at the start of the fracture register used to calculate fracture rates and incidence rate ratios. Total person-time at risk 10137.2 years. 2) Age at the start of the fracture register used to calculate fracture rates and incidence rate ratios. Total person-time at risk 53328.2 years. 3) Group comprising OI type V (16 [11]), type VI (11 [5]) and type VII (5 [5]) and unclassified and others (21 [12]). 4) Indicating the number or rate of vertebral fractures. 5) Spine X-ray only available in 92 of the 97 participants. 6) OI type II 7) Including information about previous fractures and number of fractures suffered after the age of 18 years of age. 8) Patients followed from birth until 10 years old. 9) Reference population matched to 23 of the patients, 10) patients with OI type II, both died early in life, 11) OI tarda II (mild), 12) OI congenital (severe), 13) OI tarda I (moderate)

<table>
<thead>
<tr>
<th>Author [Year]</th>
<th>Total OI population (women)</th>
<th>Age (range or n)</th>
<th>Reference population</th>
<th>OI Type I</th>
<th>OI Type II</th>
<th>OI Type IV</th>
<th>Other OI types</th>
<th>Reported fracture outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolf (2016)</td>
<td>85 (49)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patel (2015)</td>
<td>544 (104)</td>
<td>12.6 (0-67)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>244 (131)</td>
<td>15.6 (0-67)</td>
<td>-</td>
</tr>
<tr>
<td>Lindahl (2015)</td>
<td>222 (103)</td>
<td>-</td>
<td>-</td>
<td>151 (65)</td>
<td>17.15</td>
<td>29 (21)</td>
<td>17.72 (14.08)</td>
<td>-</td>
</tr>
<tr>
<td>Skov (2015)</td>
<td>58 (25)</td>
<td>7.0 (1-18)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>44 (7)</td>
<td>4 (7)</td>
<td>-</td>
</tr>
<tr>
<td>Britze (2014)</td>
<td>82 (32)</td>
<td>8 (2-14)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>31 (15)</td>
<td>9 (5)</td>
<td>-</td>
</tr>
<tr>
<td>Wever (2014)</td>
<td>97 (56)</td>
<td>44±12</td>
<td>-</td>
<td>75 (47)</td>
<td>45±11</td>
<td>9 (3)</td>
<td>35±7</td>
<td>-</td>
</tr>
<tr>
<td>Brix (2013)</td>
<td>86 (56)</td>
<td>13±9</td>
<td>-</td>
<td>86 (56)</td>
<td>13±9</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Aglin (2012)</td>
<td>43 (26)</td>
<td>5.7 (0-20.05)</td>
<td>-</td>
<td>11 (9)</td>
<td>6.6 (13.3-12.5)</td>
<td>22 (11)</td>
<td>5.5 (1.4-17.6)</td>
<td>-</td>
</tr>
<tr>
<td>Wever (2011)</td>
<td>97 (56)</td>
<td>44±12</td>
<td>-</td>
<td>75 (47)</td>
<td>45±11</td>
<td>9 (3)</td>
<td>35±7</td>
<td>-</td>
</tr>
<tr>
<td>Möllers  (2001)</td>
<td>111 (78)</td>
<td>20-70</td>
<td>-</td>
<td>111 (78)</td>
<td>20-70</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moore (1998)</td>
<td>32 (2)</td>
<td>7.35 (4.5-16.9)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Davies (1994)</td>
<td>9 (6)</td>
<td>11±3</td>
<td>132 (75)</td>
<td>5-13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vester (1992)</td>
<td>127 (7)</td>
<td>0-10±7</td>
<td>-</td>
<td>40 (7)</td>
<td>0-10±7</td>
<td>19 (7)</td>
<td>0-10±7</td>
<td>-</td>
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<tr>
<td>Paterson (1987)</td>
<td>78 (7)</td>
<td>20.7±16.13</td>
<td>104 (7)</td>
<td>9.32 (2.13)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Andersen (1988)</td>
<td>17 (9)</td>
<td>0.12</td>
<td>-</td>
<td>12 (7)</td>
<td>2-12</td>
<td>2 (1)</td>
<td>3-12</td>
<td>-</td>
</tr>
<tr>
<td>Paterson (1984)</td>
<td>65 (45)</td>
<td>M:59.9 F:59.9</td>
<td>-</td>
<td>53 (40)</td>
<td>2 (1)</td>
<td>11 (5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Paterson (1981)</td>
<td>166 (7)</td>
<td>0.40</td>
<td>-</td>
<td>166 (7)</td>
<td>0.40</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sills (1979)</td>
<td>74 (6)</td>
<td>-</td>
<td>127 (7)</td>
<td>-</td>
<td>21 (7)</td>
<td>3 (1)</td>
<td>14 (7)</td>
<td>-</td>
</tr>
<tr>
<td>Bazar (1975)</td>
<td>82 (32)</td>
<td>2.65</td>
<td>-</td>
<td>20 (14)</td>
<td>3.65</td>
<td>17 (12)</td>
<td>3 (2)</td>
<td>-</td>
</tr>
<tr>
<td>Fisk (1974)</td>
<td>90 (54)</td>
<td>0.2-75</td>
<td>-</td>
<td>35 (22)</td>
<td>1.65</td>
<td>12 (7)</td>
<td>0.2-18</td>
<td>-</td>
</tr>
</tbody>
</table>

The table includes studies on fractures in OI. The table shows the included studies, the number of participants and the distribution of different phenotypes in each publication. We identified 20 publication through our literature search excluding our own publication (Paper II), the review includes 21 publications in total. The reported fracture outcomes are: The total number of fractures (N previous), the risk of fractures in patients with OI (IRR), age at first fracture (age at 1stFx), fracture rates per person year (Fx/year) and the pattern of fracture rates over time (Pattern). 1) Age at the start of the fracture register used to calculate fracture rates and incidence rate ratios. Total person-time at risk 10137.2 years. 2) Age at the start of the fracture register used to calculate fracture rates and incidence rate ratios. Total person-time at risk 53328.2 years. 3) Group comprising OI type V (16 [11]), type VI (11 [5]) and type VII (5 [5]) and unclassified and others (21 [12]). 4) Indicating the number or rate of vertebral fractures. 5) Spine X-ray only available in 92 of the 97 participants. 6) OI type II 7) Including information about previous fractures and number of fractures suffered after the age of 18 years of age. 8) Patients followed from birth until 10 years old. 9) Reference population matched to 23 of the patients, 10) patients with OI type II, both died early in life, 11) OI tarda II (mild), 12) OI congenital (severe), 13) OI tarda I (moderate)
5.2 RISK OF FRACTURES IN PATIENTS WITH OI

By including a reference population, we were able to calculate the incidence rate ratio (IRR) for fractures between the OI and reference populations (2). The overall IRR was 8.1 [95% CI: 7.5-8.8]. Figure 9 shows the IRR between patients and the reference population for different fracture sites.

Only one other study has compared fracture rates in patients with OI and healthy non-OI participants. This study included 23 patients with OI type IV without DI aged 5-11 years and a reference population of otherwise healthy schoolchildren who were randomly recruited though local schools (92). The patients had experienced an average of 16.7 fractures per person and the reference individuals 0.22 fractures per person. While 34.8% of the OI patients had had a skull fracture, only 1% of the reference individuals had experienced this (92). As the participants’ parents were interviewed at home about the fracture history, and information about fractures was based on a questionnaire, the results may be subject to recall bias. Selection bias may also be present, as the patients with OI may not have been representative of the British OI population.

The lifetime number of fractures per individual in an otherwise healthy general population is unknown. In a British epidemiological study of childhood fractures, one-third of boys and girls had at least one fracture before 17 years of age (97). The combined risk of having a common osteoporotic fracture for a women aged 50 years during the remainder of her life is 30-40% (98). The incidence rate for any fracture in Denmark in 2011 was 191 (95% CI: 190-120) per 10,000 person years in people older than 20 years (99). In children, the peak fracture incidence is 1.5% in girls aged 11, and 3% in boys aged 14 years (97). It is thus likely that the average number of fractures throughout life in an otherwise healthy general population is below one fracture per individual. The number of fractures in the OI population is much higher, as summarised in Table 4.

Table 4. Number of fractures reported in OI studies

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Number of participants (women)</th>
<th>Age of participants (range)</th>
<th>Clinical Severity (OI type)</th>
<th>Number of fractures per individual reported by Author (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wekre (2011)</td>
<td>74 (47)</td>
<td>-</td>
<td>I</td>
<td>(1-150)</td>
</tr>
<tr>
<td>Wekre (2011)</td>
<td>9 (6)</td>
<td>35 (7)</td>
<td>III</td>
<td>(25-250)</td>
</tr>
<tr>
<td>Wekre (2011)</td>
<td>11 (2)</td>
<td>47 (7)</td>
<td>IV</td>
<td>(4-900)</td>
</tr>
<tr>
<td>McKernan</td>
<td>111 (78)</td>
<td>41 (range: 20-70)</td>
<td>I, II, IV</td>
<td>31</td>
</tr>
<tr>
<td>Dave (1986)</td>
<td>12 (7)</td>
<td>7 (IQR: 6-15)</td>
<td>-</td>
<td>8 (IQR: 4-12)</td>
</tr>
<tr>
<td>Andersen (1986)</td>
<td>12 (7)</td>
<td>11(5)</td>
<td>I</td>
<td>5 (IQR: 5-8)</td>
</tr>
<tr>
<td>Andersen (1986)</td>
<td>2 (1)</td>
<td>(range: 2-12)</td>
<td>II</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Andersen (1986)</td>
<td>2 (1)</td>
<td>(range: 3-12)</td>
<td>III</td>
<td>(range: 16-34)</td>
</tr>
<tr>
<td>Andersen (1986)</td>
<td>1 (1)</td>
<td>6</td>
<td>IV</td>
<td>0</td>
</tr>
<tr>
<td>Rosin (1978)</td>
<td>5 (2)</td>
<td>20 (range: 4-45)</td>
<td>Moderate</td>
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<tr>
<td>Rosin (1978)</td>
<td>3 (2)</td>
<td>24 (range: 2-56)</td>
<td>Severe</td>
<td>43 (range: 0-100)</td>
</tr>
<tr>
<td>Falvo (1974)</td>
<td>43 (25)</td>
<td>5 (range: 0-75)</td>
<td>OI tarda II</td>
<td>7 (range: 1-23)</td>
</tr>
<tr>
<td>Falvo (1974)</td>
<td>12 (7)</td>
<td>(range: 0.3-1.8)</td>
<td>OI Congenita</td>
<td>70 (range: 0-325)</td>
</tr>
</tbody>
</table>

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*Data are presented as mean±SD, but the standard deviation indicates that the data are NOT normally distributed, and median (range) would have been more appropriate for presenting the data. Although the number of fractures experienced by patients with OI varies in these studies, nearly all patients with OI had experienced a fracture prior to study enrolment regardless of age, and the number of reported fractures far supersedes that expected in a non-OI individual.

Andersen et al. (14) recorded the fracture history in the children identified through their epidemiological study of OI incidence and reported that patients with OI type I had between 1-13 fractures during the first 12 years of life, the two patients with OI type III had suffered 18 and 34 fractures whereas the one patient with OI type IV had not experienced any fractures at 6 years of age. The study was population based, but there were few patients with the more severe phenotypes included in the study. Two other studies on fractures in children reported a median of 5 and 8 fractures among children with median age of 11 and 8 years, respectively (89, 90). Both studies were very small, and the results may not apply to all children with OI. Moore et al. (89) included patients referred for evaluation due to multiple fractures, thus biasing the results by indication. Davie et al. (90) included patients with OI and a positive family history of OI, and it can be argued that hospital-recruited participants represent the most severely affected patients. Even with these caveats, we must assume a higher fracture risk in children with OI compared to the general population.

In a group of 33 men aged 20-70 with mild (67%), moderate (30%), or severe (3%) OI, a median of 36 fractures was reported prior to study participation (88). The 78 women in the study had experienced on average 28.5 fractures per participant (88). A Norwegian study of 97 adults with OI types I, III or IV (20) recorded the fracture history in the children treated for OI in Victoria, Australia. In 21 patients described as having a progressively deforming OI with normal sclera or the phenotype Silence later described as OI type IV, 66.7% had fractures at birth and 28.6% had a fracture in their first year of life (7). In patients with OI type I, Silence found that 7.7% had fractures at birth, 23.1% had a fracture in their first year of life, and most fractures occurred in preschool or school years; five patients aged 21 months to 29 years with the mildest phenotype had no previous fracture at study inclusion (7). The studies by Bauze et al. (95) and the Silence et al. (7) aimed to describe and classify patients according to common clinical traits. The studies included larger populations of patients with known OI recruited through hospital units. Selection bias cannot be ruled out. In another study of 78 patients classified as OI type IV without signs of DI, the median age at first fracture was under 1 year (range 0-11), and 25 patients had experienced a fracture at birth (92). This study was part of a larger survey and the limitations to the design have been described previously. In an relative small Egyptian cohort of 43 children with OI evaluated at mean age of 5.7 years (range 0-20), all those with OI type III had a fracture in infancy (40% at birth), 77% of those with OI type IV had fractures at birth or in infancy, while over 50% of those with OI type IV had their first fracture in early childhood (87). Fracture assessment was based on a structured interview, and even if the mean age was 5.7 years (range 0-20), this method is prone to recall bias. This is a typical limitation to studies on fracture history in OI. In a Brazilian study of 62 patients with OI type I, type III or IV with median age of 8 years (IQR: 2-14), fractures at birth had occurred in 6 of 31 (19%) patients with OI type I, 8 of 9 (89%) patients with OI type III, and 9 of 22 (41%) patients with OI type IV (86). Three of the patients with OI type I and one patient with OI type IV had no fractures prior to 60 years old, while 50% of patients with OI type I, 59% of patients with OI type IV, and 100% of patients with OI type III had experienced fractures prior to 6 years old (86). The study...
was primarily aimed to evaluate clinical and functional features of children and adolescents with OI, and recruited patients from a secondary treatment centre for OI. Even though the distribution of patients with different phenotypes are close to what would be expected, it is likely that the participants were recruited from a more severely affected group of patients than what would be expected in the general OI population. This selection bias is hard to overcome in studies of OI.

5.4 Fracture Rates in OI

Obviously, the total (cumulative) number of fractures increases with age if a patient continues to experience fractures. This can make it difficult to compare fracture risk between patient groups, and between patients and the general population, if the groups are not matched for age. Annual fracture rates are comparable between age groups and are likely to vary at different stages of life. The fracture rates, found in our study, over time are shown in Figure 10. In our study, the fracture rate was highest in patients aged 0-19 years (233.9 [95% CI: 219.9-247.8] per 1000 patient years) and lowest in patients aged 20-54 years (84.8 [95% CI: 75.5-93.8] per 1000 patient years). Patients aged over 55 years, the fracture rate (97.4 [95% CI: 81.8-113.0] per 1000 patient years) was higher than for patients aged 20-54. Although the relative risk in patients with OI decreased with age, the absolute lifetime risk of fracture was far higher in the OI cohort than in the reference population.

A questionnaire study involving 111 adults with mild forms of OI and mean and of 40.8 years showed that most fractures occurred prior to the patient’s 18th birthday, but 47.3% of spine fractures, 39.0% of hip fractures, and 34.7% of ankle fractures occurred after 18 years (88). Most patients had previous wrist fracture (58.6%) and/or forearm fracture (68.5%), and only 10.4% of wrist fractures and 20.4% of forearm fractures occurred during adult life (88). Davie et al. (90) evaluated 9 children with OI and found a median annual fracture rate of 0.69 (range: 0.28-0.91), close to that reported by Moore et al. (89) in 12 participants (median annual fracture rate 0.6; range: 0.4-3.4). The limitations to these studies have been described earlier (section 5.2). In a Canadian study of 86 children and adolescents with OI type I, the annual fracture rate of long bones in 48 patients not treated with bisphosphonates was 0.62 (in a cumulative follow-up time of 83.9 years). Long bone fracture was determined by radiography, and the authors only counted fractures occurring within 24 months of the primary visit and only included patients with at least 6 months follow-up. This approach to fracture data collection strengthens the study, but the relatively short cumulative follow-up time limits the results. In 43 Egyptian children, the annual fracture rate was much higher as patients with OI type III had 8.5 fractures per year, patients with OI type IV had 5.8 fractures per year, and patients with OI type I had 3.9 fractures per year (87). The between-group differences in fracture rates were not statistically significant (87). Falvo et al. (96) calculated the fracture rates by dividing the total of fractures reported by each patient to the observation time for the patient, this approach where also used by others and does not illustrate the actual annual fracture rates but a median/mean fracture rate in patients with OI. Falvo et al. (96) included 90 patients followed at their hospital centre and used patient reported fractures to calculate the fracture rates in patients aged 1-16 and found that patients diagnosed at birth had mean 10.6 (range: 2.3-29.5) fractures per year, patients with a more moderate phenotype (OI tarda I) had mean 2.0 (range: 0.06-10) fractures per year and patients with the mildest phenotype had mean 0.4 (range: 0.06-1.27) fractures per year. This is much as would be expected as the diagnosis of OI is made partly based on the prevalence of fractures, and that fracture during birth or in utero will lead to a diagnosis of a more severe phenotype and thus more fractures is given. A direct comparison between phenotypes, based on clinical traits, will be biased by the fact that the outcome (fracture rates) is used to group the patients and any between group comparisons will thus be bias by indication. This is discussed in detail in section 8.1.3.

In an American study of 544 patients with a median age of 12.6 (range 0-67), the annual fracture rate for patients with OI type III (1.6±2.2) was significantly higher than for patients with OI type I (0.5±0.7) or type IV (0.8±1.4) (83). Based on the standard deviations in the study, these data are unlikely to be normally distributed and median values would have displayed the between-group differences better. The fracture rates were calculated by asking the patients to recall any fractures in the 12 months prior to study entry. This possibly limits the recall bias compared to a longer recall period. In a Swedish study of 222 patients, those with OI type III had a significantly higher annual fracture rate (3.8±9.3) than patients with OI type IV (1.3±1.4) or OI type I (0.6±0.7) (84). Again, the standard deviations suggest that median (range) values would have been more appropriate. Fracture data were based on the total number of fractures recorded in patient medical charts at database lock, without information about when the fracture occurred (84). Digit and rib fractures were not counted, and the annual fracture rate was calculated by dividing the total number of fractures by the participant age (84). Medical charts are primarily a tool for physicians to document and communicate key findings of the patient’s medical history rather than a research tool, and their use may result in information bias.

The annual fracture rates reported in patients with OI type I in the American, Swedish, and Egyptian studies were higher than those for patients with type I in a Norwegian study of adults with OI that reported an annual fracture rate of 0.12 (48). Fracture history was based on a structured interview, and recall bias cannot be ruled out although participants were only asked to recall fractures or orthopaedic surgery in the previous 12 months. The authors could not demonstrate any correlation between age and total number of self-reported fractures in women aged under 50 years, women over 50 years, or in men (48). This contrasts with the findings of Paterson et al. in their study of 45 women and 20 men over 50 years old, where fracture rates increased from just above 0.1 fractures per year in premenopausal women to nearly 0.5 fractures per year in women 20-25 years after menopause (93). Both studies collected fracture data using structured interviews or questionnaires about experienced fractures and are prone to recall bias. The lack of an association between age and fractures in the Norwegian study could be because the youngest participants reported more fractures (range 1-170) than the oldest participants (range 4-100). Whether this was due to recall bias or lower fracture risk in patients with milder phenotypes is unclear. The authors of the Swedish study compared fracture rates in children and adults with OI
types I, III and IV and found a non-significant higher annual fracture rate for children with all OI types (84). The standard deviations did indicate that the fracture rate data was not normally distributed. The authors used student’s t-test to evaluate the differences in fracture rates between adults and children with OI. This test assumes a normal distribution of the data. No information was given about a transformation of the data to try to achieve a normal distribution and using a non-parametric statistical analysis would have been a more correct analysis of the between group differences. Paterson et al. (93) reported a peak fracture rate of 1.6 fractures per year in males with OI at the age of 5-10 years and a rapid decline to around 0.2 fractures per year in males older than 25 (93). Decreasing fracture rates were also found by Vetter et al. (91) who followed 127 patients with OI type I (n=40), OI type III (n=39) or OI type IV (n=48) for 10 years. Patients with OI type I had 0-1 fractures per year with a peak at 5 years of age, patients with OI type IV had a median fracture rate of 1 per year and decreasing fractures rates from the age of 2, while patients with OI type III had a median fracture rate of 3 per year from birth, which slowly decreased over the observation period (91). Vetter et al. (91) evaluated all their patients every year for the first 10 years of life, and all fractures were confirmed by the authors using radiographs (91). This study design is the least prone to bias when evaluating fracture rate, site and number in patients with OI.

In the American study by Patel et al. (83), 16 patients were categorised as OI type V (OI with calcification in intraosseous membranes) and had an annual fracture rate of 1.6±2.7. 11 patients were categorised as OI type VI (a progressively deforming phenotype) and had an annual fracture rate of 1.8±1.6, five patients were categorised as OI type VII (a common variable OI with normal sclera) and had an annual fracture rate of 0.4±0.8, and 21 patients were categorised as ‘unclassified and other’ and had an annual fracture rate of 11.8±14.5 (83). Due to the relatively large standard deviations, it is unlikely that the fracture rate data were normally distributed, and medians (range) would have been more appropriate. No data is available about changes in fracture rates over time in these more rare forms of OI.

As would expected fracture rates are associated with OI type, as patients with OI type I appear to have under 1 fracture per year, whereas more severe phenotypes have more than 1 fracture per year. It must be remembered that the clinical grouping of patients according to severity will bias the results of any between OI type differences in typical fracture rates, as the most severe patients will have more frequent fractures than the mildly affected patients by definition. Only one study has evaluated the correlation between fracture rates and the genetic variants. The Swedish study did not, however, find any differences in the fracture rates between patients with helical mutations in the α1- vs. α2- chains or between patients with any qualitative mutations in the α1- vs. α2- chains (84). Fractures are more common in childhood than adulthood, and adult fracture rates appear to be highest in postmenopausal women and older males. Our data (Paper II) and the literature confirm the hypotheses that fracture rates in OI follow the same pattern as in the general population, but with higher absolute risk (and thus higher rates).

5.5 Fracture sites in OI

In our study (Paper II), the sites with highest fracture incidence were the forearm and lower leg and ankle. The incidence rate ratios for all sites (except for spine fractures in women over 55 and hip fractures in men over 55) were significantly higher in the OI cohort than in the reference population (2). Again it must be kept in mind that the clinical grouping of patients according to severity will bias the results of any between OI type differences in frequent fracture sites, as the most severe patients will have more frequent fractures than the mildly affected patients by definition. A study of genotype and fracture site correlation is not available.

5.5.1 Long bone fractures in OI

Fractures associated with falls, such as forearm and lower limb fractures, are frequent in young children. Among 62 patients aged 0-18 years, 21 (78%) patients with OI type I had experienced an upper limb fracture, and 21 (78%) a lower limb fracture, while all 9 (100%) patients with OI type III had experienced both upper and lower limb fractures. Among patients with OI type IV, 13 (59%) had experienced suffered an upper limb fracture and 22 (100%) a lower limb fracture (86). The study aimed at evaluating muscle strength, joint range of motion, and gait in children with OI, and they corrected their analysis of the primary endpoints for differences in fracture history (which was gathered via clinical evaluation and structured interviews) (86). Lower limb fractures have been reported as being more frequent than upper limb fractures in smaller study populations (87, 90).

5.5.2 Vertebral fractures in OI

Vertebral fractures are frequent in both children and adults with OI. In a Canadian study of 58 children with type I OI evaluated with lateral spine radiographs, fractures were found in 41 patients (49). In the total patient cohort, 179 vertebrae from Th3 to L4 were evaluated, and the most frequent fractures were in the Th7 or Th8 vertebrae (49). The median number of fractures was higher in girls than to boys (median 4; range 0-14 vs. median 1; range 0-8, p=0.03) (49). In an Italian study including 44 children with OI type I, 4 children with OI type III and 10 children with OI type IV, Diacinti et al. (85) visualised 738 vertebrae from Th4 to L4 on lateral x-rays.
Twenty-two of the 58 patients (38%) had no signs of vertebral fractures, ten patients had one fracture, and 26 (45%) patients had more than one vertebral fracture (85). There seems to be a correlation, as would be expected, between OI type and prevalence of vertebral fractures in children – vertebral fractures were seen in 47 of 72 (65%) Swedish children with OI type I, 13 of 16 (82%) children with OI type IV, and all 15 children with OI type III (84).

Vertebral fractures were more prevalent in adults. In the Swedish cohort, 62 of 79 (78%) adults with OI type I, 25 of 26 (95%) adults with OI type IV, and all 14 (100%) adults with OI type III had one or more vertebral fractures (84). In a Norwegian study of 92 adults with OI types I, III or IV, the most frequently deformed vertebra was Th7 with 50% of all patients showing deformity of this vertebra (25). The authors evaluated a spinal deformity index by semi-quantitatively evaluating each visible vertebra and grading the deformity from normal to severe; they found the index to be higher in patients with OI type IV than in patients with OI type I (25).

5.5.3 Other fractures frequently reported in OI
In a group of 166 patients with OI type I, 49 (29.5%) patients reported at least one previous rib fracture, and 14 (8.4%) patients reported a previous skull fracture (94). In a group of 78 patients with OI type IV without DI, 31 (39.7%) patients had experienced a rib fracture, and 22 (28.2%) had had a skull fracture (92). Among 111 participants in a questionnaire study evaluating the musculoskeletal manifestations of mild OI in adults, 67% reported finger fractures, 49% foot fractures, and 63% toe fractures (88). Among the Norwegian study participants, 54% reported fractures to the ribs, clavicle, mandible or maxilla, nose, pelvis, or scapula (48).

In most of the reviewed literature, more patients had experienced a lower limb fracture than an upper limb fracture. In British children, however, the most frequent fracture in both sexes was lower arm or wrist fractures (97). The reason for this difference is unclear. The Swedish lifetime risk of a fracture was 13.3% for shoulder, 21.5% for forearm, 23.3% for hip, and 15.4% for spine in otherwise healthy women aged 45 (98). The lifetime risk of fracture in otherwise healthy men aged 45 was 4.4% for shoulder, 5.2% for forearm, 11.2% for hip, and 8.6% for spine (98). The lifetime risk for these fractures in OI patients is close to 100% for all OI types.

5.6 CONCLUSION
Our study (Paper II) and the literature confirm our hypothesis that patients with OI have higher fractures rates than the general population, and that the relative fracture risk decreases with increasing age. Furthermore, our study (Paper II) and the literature confirm our hypothesis that the fracture pattern in OI (i.e. which bones fracture at which stages of life) mimics that in the general population. To better understand the mechanisms and causes of fractures in OI longitudinal studies of OI patient cohorts are needed, preferably powered to be able to evaluate the genotype to fracture rate correlation. These studies should also include a sizeable group of treatment naïve patients, patients who start fracture prophylactic treatment and the possibility to evaluate the effect of treatment over time. These studies should also include routine evaluation of the spine to capture clinically silent vertebral compression fractures as well as close follow-up to minimise the risk of recall bias and non-registration bias of ‘home-treated’ fractures.

6. BONE -MINERAL DENSITY, -GEOMETRY AND -MICROARCHITECTURE IN OI
This chapter evaluates the current literature on bone mineral density (BMD), bone geometry, and bone microarchitecture in OI. Whole-bone strength depends on the amount of bone (or bone mass, often measured as BMC), the spatial distribution of the bone mass (geometry), microarchitecture of the bone (often evaluated by e.g. pQCT, HRpQCT or histomorphometric evaluations of bone biopsies), and the intrinsic properties of the materials that form the bone (65). In otherwise healthy individuals, BMD decreases with age more rapidly in women in the peri-menopausal years than in men, meaning that more women than men have areal BMD (aBMD) in the osteoporotic range (100). Human bone is about 60% mineralised to provide optimal flexibility without becoming brittle (101). Bone stiffness is increased in OI; the bones are more “brittle” and absorb less energy before fracturing (68). A change in collagen properties may alter the amount and disposition of the mineral, which would by itself affect the bone properties and strength (102). We know that collagen type I is quantitatively or qualitatively altered in most forms of OI (9). Defects in ossification and mineralisation have been described in more rare forms of OI, such as OI type V and VI where the primary defects are in endochondral bone ossification or mineralisation (9). In recessive OI types XV, XVI and XII, the osteoblast differentiation is impaired (9), further underlining the multifactorial causes of bone fragility in OI.

Bone adapts to sustain the loads it is exposed to. With the age-related bone loss that is seen in otherwise healthy individuals, trabecular thinning leads to trabecular loss and decreased bone strength, and the continued bone remodelling results in trabecularisation of the endosteal surface of cortical bone (101). The cortical thinning and increased porosity is compensated by increased bone area through the deposition of new bone on the periosteal surface of the bone (101). The increase in outer diameter helps to maintain the resistance to bending and torsional loads (65). Whether these mechanisms hold true for human OI bone is unknown. In OI mouse models, compensatory periosteal apposition has been found (103). More knowledge about the skeletal pathology in OI may improve our understanding of the mechanisms behind the increased fracture rates in OI.

We performed a cross-sectional study including 39 patients with OI type I and evaluated the three main components of whole-bone strength (BMD, bone geometry, and bone microarchitecture) using HRpQCT and DXA. We compared patients with OI to an age- and gender-matched reference population selected from a random sample of the general population, as described in Paper III (3). HRpQCT is designed for in vivo assessment of volumetric bone mineral density, bone geometry, and bone microarchitecture at the distal radius and tibia. The analysis is quick, taking approximately 3 minutes, and gives less than 5 μSv of radioactive exposure per scan, covering a 0.9 cm volume of interest at the ultradistal radius and tibia. The voxel resolution is 82 μm, as the scan is made up of 110 parallel scan slices. This allows for segmentation of cortical and trabecular bone volumes. Trabecular bone volume per tissue volume
(BV/TV) is derived from the mean trabecular volume density, assuming a mineral density of fully mineralised bone of 1.2 g HA/cm³ (104). Three-dimensional assessments of trabecular thickness and spacing are derived from BV/TV by the formula: Tb.Th= BV/TV / Tb.N and Tb.Sp= (1-BV/TV)/Tb.N, as used for standard histomorphometric evaluation of bone biopsies (105). The trabecular number (Tb.N) is extracted directly. The standard structure extraction and morphometric evaluation is performed as described by Laib et al. (106). Burghardt et al. (107) have developed a fully automatic segmentation method that identifies the periosteal and endosteal boundaries of the bone, thus isolating the cortical bone compartment and allowing evaluation of the cortical volumetric density, cortical thickness, and cortical porosity. A fixed offset of 9.5 mm in radius and 22.5 mm in tibia from the reference line, typically 1/3 to 1/2 of the endplate midline in the horizontal direction, forms the start of the scan region, which is 9.02 mm in the axial length of the radius and tibia. This may result in some bias to cross-sectional studies, as scanning patients with shorter limbs and comparing them to persons with longer limbs may result in a comparison of relatively more distal parts of the radius and tibia to more proximal parts of the bones (108). This may have introduced some bias to our study, as we did not take extremity length into account.

6.1 LITERATURE SEARCH

The literature search was conducted as described in section 3.3 (inclusion and exclusion-criteria) and Appendix 1.3 (search dates, search strings and search results) and aimed to identify all studies evaluating BMD, bone geometry, and bone microarchitecture in OI. A flow diagram of the study selection can be seen in Figure 11. A total of 2155 publications were found via Embase, 1470 via PubMed, and 102 via Cochrane Library. After excluding 920 duplicates, 2807 titles and/or abstracts were screened for eligibility and resulted in 77 publications for full-text screening. After excluding 44 articles not covering the subject matter, including less than 10 patients with OI or no reference group of non-OI individuals (though accepting fewer participants for histomorphometric studies on bone biopsies), describing mineralisation rates, reviews or without patient data, a total of 33 (including Paper III (3))

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Figure 11. Flow diagram of study selection for studies on bone mass, geometry, and microarchitecture in OI
6.2 BONE MINERAL DENSITY IN OI

Measurements of areal BMD (aBMD, in gHA/cm²) in the spine and hip are used as in vivo surrogate measures for bone fragility. aBMD is, at least in the general population and patients with osteoporosis, correlated to the risk of fractures (133). The risk of vertebral fractures is doubled for every standard deviation decrease in lumbar aBMD (134). The correlation between aBMD and fracture risk in OI is not well described, and the effect of a decrease in aBMD on the OI fracture risk is unknown. Eventhough studies regarding the fracture predictability using aBMD is lacking, aBMD is often used clinically to evaluate the need for fracture prevention treatment in patients with OI and for research as an endpoint in most treatment trials. aBMD, as measured by DXA, is a measurement of the combined cortical and trabecular BMD and is confounded by bone size. Due to the mode of measuring the BMC by DXA will overestimate the true BMD in larger bones. It is possible to measure the volumetric BMD (vBMD) of the cortex and cancellous compartment alone using pQCT or HRpQCT (135). This measurement of vBMD, in gHA/cm³, is independent of bone size (135). The differences in aBMD and vBMD is shown in figure 12.

The table shows the number of participants and distribution of phenotypes in each publication. The different modalities used to evaluate BMD, bone geometry or bone microstructure were A) DXA, B) HRpQCT, C) Bone biopsy, D) pQCT, E) Quantitative ultrasonometry of the calcaneus, and F) CT scan. 1) Including patients with types III and IV. 2) Group comprising 16 (11 women) OI type V, 11 (5) type VI, 5 (5) type VII, and 21 (12) unclassified/other. 3) Type II OI. 4) Group comprising OI types I and IV. 5) Patients with OI Type V. 6) Same population as described in Jones et al. (1999). 7) OI tarda. 8) Phenotype not state

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<td>5.0-7.0</td>
<td>5.0-5.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Riley</td>
<td>1994</td>
<td>12</td>
<td>2</td>
<td>A</td>
<td>5.0-6.5</td>
<td>5.0-7.0</td>
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<td>-</td>
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</tr>
</tbody>
</table>

Figure 12. Bone size and aBMD The bone size will influence the aBMD due to the lack of the third dimension during measurement. The figure shows how the aBMD is overestimated in large bones due to bone area alone. The DXA scanner sends two beams (with different energy) of small dose ionizing radiation from the x-ray source above the patient aimed at the patient’s hip or lumbar spine. The detector underneath the patient can then be used to calculate the BMD. Adapted from Binkley et al. (135) and Boudreaux et al. (136)
6.2.1 aBMD in OI

In the cross-sectional study (Paper III) of patients with OI type I, we found significantly lower aBMD in the lumbar spine and the total hip than in age- and gender-matched otherwise healthy individuals, randomly selected from the general population (3). Table 6 summarises the findings for aBMD from the studies that reported a T- or Z-score included in this review.

Table 6. Areal bone mineral density in patients with OI

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Site</th>
<th>Age group of (range)</th>
<th>OI type I</th>
<th>OI type II</th>
<th>OI type III</th>
<th>OI type IV</th>
<th>Other OI Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knips (2015)</td>
<td>Hip</td>
<td>Adult (10-109)</td>
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<tr>
<td>Lindahl (2015)</td>
<td>Spine</td>
<td>Children (19-91)</td>
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<td>-3.2</td>
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</tr>
<tr>
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<td>Spine</td>
<td>Adults (19-91)</td>
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<td>-3.0±1.5</td>
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</tr>
<tr>
<td>Duositi (2015)</td>
<td>Spine</td>
<td>Children (1-10)</td>
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<td>-2.5±1.3</td>
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</tr>
<tr>
<td>Patel (2015)</td>
<td>Spine</td>
<td>Children (10-11)</td>
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<tr>
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<td>Spine</td>
<td>Children (12-18)</td>
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<tr>
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<td>Spine</td>
<td>Adults (10-11)</td>
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</tr>
<tr>
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<td>Spine</td>
<td>Adults (1-10)</td>
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<td>-3.1±0.3</td>
<td>-3.1±0.3</td>
<td>-3.1±0.3</td>
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</tr>
<tr>
<td>Santeren (2012)</td>
<td>Spine</td>
<td>Adult</td>
<td>-3.0±1.4</td>
<td>-3.0±1.4</td>
<td>-3.0±1.4</td>
<td>-3.0±1.4</td>
<td>-3.0±1.4</td>
</tr>
<tr>
<td>Ben Amor (2015)</td>
<td>Spine</td>
<td>(0.5-54)</td>
<td>-3.0±1.2</td>
<td>-3.0±1.2</td>
<td>-3.0±1.2</td>
<td>-3.0±1.2</td>
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</tr>
<tr>
<td>Argin (2012)</td>
<td>Spine</td>
<td>(0-30)</td>
<td>-3.4</td>
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<td>-2.7</td>
<td>-2.7</td>
<td>-2.7</td>
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<tr>
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<td>Adult (84)</td>
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<td>-3.2±0.7</td>
<td>-3.2±0.7</td>
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</tr>
<tr>
<td>Author (year)</td>
<td>Site Age group (range)</td>
<td>OI type I</td>
<td>OI type II</td>
<td>OI type III</td>
<td>OI type IV</td>
<td>Other OI Types</td>
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</tr>
<tr>
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<td>Hip</td>
<td>Adult</td>
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<td>-1.3±1.3</td>
<td>-1.3±1.3</td>
<td>-1.3±1.3</td>
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<td>-</td>
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<td>Spine</td>
<td>Adult</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Rezech (2005)</td>
<td>Spine</td>
<td>Children (1-10)</td>
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<tr>
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<td>Adult</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>-</td>
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<td>Corteo (1999)</td>
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<td>Adult</td>
<td>-</td>
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<td>Lord (1999)</td>
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<td>Mcew (1998)</td>
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<td>Mcew (1998)</td>
<td>WB</td>
<td>Children (10-35)</td>
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<td>McCarthy (1997)</td>
<td>Fem</td>
<td>Hecat Adult</td>
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</tr>
</tbody>
</table>

The table shows the Z- or T-scores in patients with OI. 1) T-scores reported. 2) Including patients with OI type III AND IV. 3) Including patients with OI type I, III and IV. 4) Patients with OI type V. 5) Patients with OI type VI. 6) Patients with OI type VII. 7) Including patients with OI type I AND IV. 8) Patients with OI type I and quantitative collagen defects. 9) Patients with OI type I and qualitative collagen defects. 10) Includes patients with OI type I, III AND IV and indicates the Δ% difference between patients and healthy age- and gender-matched controls. 11) OI type not clearly stated. 12) Percent of BMD in a young healthy individual. 13) Phenotype not stated, shows the % of forearm BMC compared to age and gender normal values. * The between-group difference is significant. WB: whole body.

All the included studies were cross-sectional in nature, and only a few included a non-OI reference group. Most studies reported the participants’ mean or median Z-score for aBMD. Z-scores indicate the number of standard deviations a measured aBMD differs from a normative database matched for age, gender, ethnicity, and sometimes weight (137). The median number of participants in the studies was 56 (range: 8-544). Study participants were usually recruited through hospital wards, and selection bias favouring more severely affected patients cannot be ruled out.

In patients with OI the aBMD was lower than the reference material or reference group at both the total hip, lumbar spine, whole body and as was aBMC at the forearm compared to demographically similar populations from normative database data (3, 48, 49, 83-85, 87, 89, 109, 112, 114-118, 120, 121, 124-126, 130). When different OI types were compared, patients with the most severe types had the lowest aBMD at the hip, spine and whole-body (20, 48, 83, 84, 109, 121). Among children aged 0-8 years, Patel et al. (83) found no difference between OI types in lumbar spine aBMD, but from age 9, the patients with OI type I had a significantly higher aBMD than patients with OI type IV and OI type III (83). The ratio of aBMD to body weight was higher in healthy children than in children with OI, meaning that the OI bone was more heavily loaded than healthy bone (117). In patients, the aBMD to weight ratio was lower in the more severe types III and IV than in OI type I. The study included 30 children with OI type I and 24 children with OI type III or IV, and compared their measured values to aBMD of the general population and weight reference material (117). Four studies reported aBMD T-score in adults and found that patients with OI type I had T-scores of -2.5 at the spine, -1.3 at the hip, and -2.2 for whole-body BMD. T-scores were lower in more severe types (-3.6 to -3.9 for spine, -2.2 for hip, and -2.2 to -5.2 for whole-body aBMD) (109, 114, 120, 121). It should be noted that these studies had few patients with severe OI types, and the inter-individual variation was large.

An increase in aBMD would be expected in puberty, as children grow and achieve peak bone mass in early adulthood (138). In an American cross-sectional multicentre study, Patel et al. (83) compared the aBMD of young OI children to that of young OI adults to investigate for a pattern of increasing aBMD with age. For OI type I and IV, they found a higher aBMD in children aged 12-18 than in the age group of (range).
children aged 9-11, but this was not seen in OI type III (83). While the Z-score in children with OI type I and IV came closer to 0.0 with increasing age, this was not seen in patients with OI type III (83). This study was not longitudinal and therefore a direct comparison between the age groups cannot be made. For reasons not known, the patients aged 12-18 in the Patel et al. study (83) may have been more severely affected by their disease than the younger patients. While 100 children with OI type III were enrolled in the study, the number of participants in each age group is unknown, and a low statistical power cannot be ruled out when comparing the two age groups.

The aBMD seen in patients with OI overlaps the range seen in otherwise healthy individuals (125). In patients with OI type I, 62% had a distal radius aBMD above -2 standard deviations, and 30% had a distal radius aBMD above -1 standard deviation (125). The proportion of patients with distal or proximal radius aBMD above -2 standard deviations was similar in OI type IV (125). There are currently no longitudinal studies powered to evaluate whether aBMD can predict fractures in OI (as it does in osteoporosis). Patel et al. (83) performed a logistic-regression analysis to evaluate if aBMD could predict fractures, and found no significant correlation between the lumbar spine aBMD (by DXA) and fractures in the previous 12 months. The clinical heterogeneity observed in their participants limited the analysis, as patients with near-normal aBMD had had fractures in the previous year while patients with very low aBMD had not (83). In patients with OI type I, whole-body BMD and male gender were predictors of more prevalent fractures in the Norwegian OI population (48). While neither of these studies was designed to evaluate the predictive value of aBMD for fractures in OI, there seems to be a poor correlation between aBMD and fracture risk in OI. Typical skeletal findings are shown in figure 13.

6.2.2 vBMD in OI

6.2.2.1 Total vBMD in OI

Using HRpQCT we compared 39 patients with OI type I to 39 healthy age- and gender-matched reference participants (Paper III), and found the total vBMD of the ultradistal radius was non-significantly lower in the OI group (285±75 vs. 316±83 mgHA/cm³, p=0.13) (3). Similar results were reported in an Austrian population of 30 patients with OI types I, III or IV using HRpQCT to evaluate the total vBMD of the ultradistal radius (109). In contrast the total vBMD in the ultradistal radius was significantly lower (-19%), however, in patients with OI types I, III and IV compared to a matched non-OI reference group when evaluated by pQCT (118). We did find that the total vBMD of the ultradistal tibia was significantly lower in patients with OI than in the reference group (3), and this has been confirmed in later studies in adults with OI type I or type III and IV using HRpQCT (109) and in children with OI type I using pQCT (111).

Total vBMD in the radius was lower in children with type I OI than in healthy individuals in the metaphysis but not the diaphysis (116). Children with OI type I have higher total vBMD in diaphyseal bone than expected for their age, gender, and height, explained by the relatively large cortical area and an elevated cortical vBMD (116). In contrast to aBMD, total vBMD in radius and tibia was highest in more severe phenotypes (OI type IV or III) than in OI type I (20, 109). Patients are seated for the HRpQCT evaluation, and the arm or leg is placed in a carbon-fibre cast that slides into the HRpQCT gantry. In some patients with severe OI, it may be difficult to evaluate the bone using HRpQCT due to bone deformities and short limbs. This may introduce some selection bias by excluding the most severely affected patients.

6.2.2.2 Cortical vBMD in OI

We found no difference in cortical vBMD in the ultradistal radius or tibia between patients with OI type I and a healthy reference group (Paper III) (3). In severe phenotypes, cortical vBMD in the radius was equal in adult patients to than in a healthy reference group, but trended towards higher values of vBMD (109, 118). In the diaphyseal radius in children with OI type I, the cortical vBMD was 6% higher than expected according to age, gender, and height (116). In tibia, no difference in vBMD was found compared to non-OI reference individuals using plain CT of the distal radius and tibia, but this study was small and type I error (findings being by chance) cannot be ruled out. Furthermore, the study included participants from two families that may share genetic background, and thus selection bias cannot be ruled out. In other long bones, the cortical vBMD was significantly lower in patients with OI compared to a reference population (110), although these data were based on 6 bone samples extracted during orthopaedic surgery in patients with OI, and 3 samples from age-matched children without OI. The reliability of the reference material can be questioned, and patient selection may be biased by indication for surgery. There were no significant differences in cortical vBMD in the radius or tibia between patients with OI type I and more severe phenotypes (20, 109). This is summarized in figure 13.

Figure 13. Typical bone findings in OI. The figure summarises the densitometric, geometric, and microarchitectural findings in OI. Cort. = Cortical, aBMD= areal BMD, vBMD= volumetric BMD, Trab. = trabecular, Tb.N= trabecular number, Tb.Th = trabecular thickness, Tb.1/N.SD = the inhomogeneity of the trabecular network, BV/TV= bone volume per tissue volume.
6.2.2.3 Trabecular vBMD in OI
In patients with OI type I, we found trabecular vBMD to be significantly lower than in a healthy reference group (3). This has been consistently reported in both radius and tibia regardless of phenotype and age (109, 111, 116, 118, 122, 127). Danish patients with OI type III had lower trabecular vBMD in the radius when compared to patients with OI type I or OI type IV (20). Austrian patients with OI type I had higher trabecular vBMD in both radius and the tibia when compared to patients with more severe phenotypes (109).

Our data (Paper III) and the literature confirm the hypothesis that bone mass is lower in patients with OI than in non-OI individuals. This is summarized in figure 13.

6.3 BONE GEOMETRY IN OI
Bone size is an important predictor of bone strength, as larger bone is stronger than smaller bone. Laboratory testing of bone strength using human cadaveric vertebrae, distal radii and proximal femora have shown that larger bones have higher compression and bending strength, independent of aBMD (65). The geometrical properties of the hip, as evaluated with hip structure analysis based on DXA, are more strongly correlated with hip strength than aBMD (139). Whether this is true for patients with OI is unknown, but using the commercially available software for hip structure analysis we were able to illustrate that patients with OI had lower aBMD of the hip and shorter hip axis length, as well as narrower cross-sectional area and lower cross-sectional moment of inertia and sectional modulus (both indicators of bone strength) in the femoral neck, intertrochanteric region, and the femoral shaft (140). Age-related changes in bone geometry occur and to some extent attenuate the impact of the age-related decrease in BMD and bone strength. In the appendicular skeleton, endosteal resorption and periosteal apposition lead to an age-related increase in total bone area but decreased cortical thickness (65). Men have larger bone than women, but also load the skeleton with more weight (65).

6.4 Bone microarchitecture in OI
The bone microarchitecture, or structure, plays an important role in maximising the bending strength and flexibility for energy absorption when the bone is compressed (101). While the surface area of trabecular bone is far greater than that of cortical bone, 80% of the skeleton is cortical bone (141). Cortical thickness, cortical porosity, trabecular number, trabecular thickness, trabecular spacing, and the trabecular distribution are all important for bone strength (101). The trabecular microarchitecture was correlated to the failure load in an ex vivo study of human vertebrae (142). The degree to which the different parameters contribute to total bone strength differs between bone types (141). Findings regarding bone microarchitecture summarised in Figure 12.

6.4.1 Cortical microarchitecture in OI
We found the bone cortex to be thinner in patients with OI type I in the tibia. This has been confirmed in patients with OI type I, IV and III in tibia evaluated by pQCT or HRpQCT (3, 109, 111). Comparison of bone biopsies with predicted normal values or a reference population had also shown a thinner cortex in 193 patients with OI type I, III and IV (115, 119, 123, 124, 128, 132). In radius, we could not show any differences in cortical thickness between patients with OI type I and the reference group (3). When evaluating patients different types of OI including more severe phenotypes (OI type I, type III, and IV), no differences were found in cortical thickness in the radius compared to a non-OI reference group (109). This is in contrast to Gatti et al. (118) who found that cortical thickness in adults with OI type I, III or IV was 15% thinner than in a non-OI reference group. Whether this was due to a more distal volume of interest, and thus a cross-section of radius with theoretically less cortical bone than a more proximal site, is unknown. Gatti et al. (118) included patients who had previously participated in a treatment trial and were thus known to the authors. This method of recruitment is prone to selection bias, as the trial’s in-
clusion and exclusion criteria may not have selected typical patients with OI. When evaluating the clinical characteristics of real-life alendronate users for the treatment of osteoporosis to the inclusion and exclusion criteria for a large RCT for an anti-osteoporotic drug, one in two alendronate users would have been excluded from participating in the trial (143), further underlining the selection bias when including patients for RCTs.

Rauch et al. (116) showed that the cortex in bone biopsies was 25% ± 16% thinner in 70 children with OI type I compared to age- and gender-matched reference children who were recruited from patients admitted for various orthopaedic procedures. It is unlikely that the reference population was representative of the general population, however the authors did note that participants were excluded if they had a metabolic bone disease.

Hald et al. (20) found that patients with OI type IV had wider cortices in the radius and tibia than patients with type I and type III. This was in contrast to Rauch et al. (119) in iliac bone biopsies and Kocijan et al. (109) in HRpQCT of the ultradistal tibia and radius, where patients with OI type I had wider cortices than more severe phenotypes. Some of this difference may be because Rauch et al. (119) only included children in their study, while the Austrian study of Kocijan et al. (109) compared cortical thickness between patients with OI type I and patients with either OI type III or type IV.

While the study by Kocijan et al. (109) recruited patients from a single hospital centre, the authors noted that they see most of the adult patients with OI in the eastern part of Austria, and thus their patient population is likely to be representative of the general OI population. The study included 30 patients, but the size of their OI population was not given, and selection bias cannot be ruled out. Patients were matched for age and gender with controls from a population-based HRpQCT register recruiting individuals from the general Austrian population, who were excluded if they had metabolic bone disease (109). The authors tried to avoid confounders by excluding patients with known diseases that could influence bone microarchitecture, geometry or mass. The small study size was a major limitation, and patients with OI type III and IV, who had shorter arm and legs than the reference population, were also included. Using a fixed offset for the volume, it is likely that a relatively more proximal region of the tibia and radius were scanned than in the reference group, and the scanned bone may have included more cortical bone than in a more distal cross-section.

We could not demonstrate any differences in cortical porosity between patients with OI type I and a non-OI reference group when evaluated by HRpQCT (3). In an Austrian study of patients with OI type I, III or IV, there were no detectable differences in cortical porosity when compared to a healthy reference population even for the most severe phenotypes (109). In bone biopsies, cortical porosity was higher in patients with OI type I, but both these studies included only 6 patients (110, 123). While Imbert et al. (110) included three controls, McCarthy et al. compared patients to previously published reference material from age-matched healthy controls (123). Selection bias of patients cannot be ruled out in the Imbert et al. (110) study, where patient biopsies were acquired during surgery. The most likely reason that we could not illustrate increased cortical porosity in patients with OI was that the voxel resolution of the HRpQCT technique is 82 micrometres and thus will only detect pores larger than that.

### 6.4.2 Trabecular microarchitecture

In patients with OI type I, we found that the trabecular microarchitecture evaluated by HRpQCT was compromised when compared to a healthy reference population (3). In the radius, patients with OI type I have lower bone volume to tissue volume (BV/TV), a lower number of trabeculae (Tb.N) that are also more widely spaced (Tb.Sp), and the trabecular network is more inhomogeneous (Tb.1/N.SD) compared to a non-OI reference group (3). This was also found in Austrian patients with more severe phenotypes (109). The radius trabeculae seem to be thicker in patients with OI type I, although the differences between patients with OI type I and the reference groups were non-significant (3, 109). In tibia, there were no differences in trabecular thickness between patients with OI type I and the reference populations (3, 109).

When evaluating the trabecular microarchitecture using transiliac bone biopsies, the findings were similar to those found using non-invasive techniques. BV/TV was lower in patients with OI type I, IV or III than in healthy reference individuals (115, 119, 129), with lower trabecular number and thinner trabeculae (119). Jones et al. (124) found that in children with OI Type III and IV the trabecular bone was markedly sparse compared to a reference group of age matched otherwise healthy children.

In children, the trabecular number was highest in patients with OI type I and lowest in patients with OI type III, but no difference was found between phenotypes regarding trabecular thickness (119). In adults evaluated by HRpQCT, Hald et al. (20) showed that patients with OI type IV had larger BV/TV, higher trabecular number, and a less inhomogeneous trabecular network in the radius when compared to patients with OI type I. Patients with OI type III had the lowest values for BV/TV and trabecular number, and the most inhomogeneous trabecular network (20). Similar patterns were seen in the tibia, but the differences between phenotypes were non-significant (20). Kocijan et al. found that patients with OI type I had higher BV/TV, trabecular number and thickness than patients with OI type III or IV (109). These diverging results from the Danish patients could be due to different analysis strategies. Hald et al. (20) analysed between-group differences among patients with OI type I, type III or type IV, while Kocijan et al. (109) compared patients with OI type I to patients with OI types III AND IV. Hald et al. (20) showed that bone microarchitecture parameters where closest to that of non-OI individuals in patients with OI type IV and furthest from what would be expected in patients with OI type III, so combining these two groups may have evened out any between-group differences when comparing to patients with OI type I, as in Kocijan et al. (109).

### 6.5 CONCLUSION

Our study and the current literature shows that patients with OI have lower areal BMD and trabecular volume BMD, but not lower cortical volume BMD. In children with OI type I, however, the diaphyseal site of the radius had higher than expected total volume BMD due to a relatively large cortical area with elevated cortical volume BMD. The bones are smaller in patients with OI, as the total, cortical, and trabecular bone areas are lower in patients with
OI than in non-OI individuals. Bone microarchitecture is also altered in patients with OI, who have lower cortical thickness, higher cortical porosity, and fewer trabeculae of similar thickness to that in non-OI individuals but further apart in a more inhomogeneous network. This result in lower BV/TV of OI cancellous bone compared to non-OI-bone. In other words, OI bone has lower bone mass, is smaller (altered geometry), and has altered bone microarchitecture compared to the non-OI population. This could explain why patients with OI have increased fracture rates compared to non-OI individuals, as the findings would in otherwise healthy individuals be associated with higher fracture risk.

Our study (Paper III) and the literature confirm our hypothesis that patients with OI have altered bone microarchitecture, lower bone mass and different bone geometry compared to healthy non-OI individuals.

7 CARDIOVASCULAR DISEASES IN OSTEOGENESIS IMPERFECTA

Collagen type I is an important constituent of various parts of the cardiovascular system, including the heart valves, chordae tendineae, fibrous rings of the heart, interventricular septum, aorta, and most other arteries (37, 38). Collagen fibres in the ventricular myocardium contribute to tensile stiffness and maintain the architecture of the myocytes (40). The aorta and most arteries are rich in type III and type I collagen (38). In the Aga2 OI mouse model, Thiele et al. (39) showed that both severe (postnatally fatal) phenotypes and mild phenotypes (animals surviving to adulthood) had cardiovascular disease. Severely affected mice had enlarged septum, right ventricular hypertrophy, and significantly lowered ejection fraction (EF) compared to wild type mice (39). The type I collagen was disoriented, and there were fewer and thinner collagen fibrils in the cardiac tissue (39). The OIM mouse model has both a bone and a cardiovascular phenotype (38, 40, 144). Weis et al. (40) found that homozygote OIM mice had decreased collagen area fraction with lower fibre number density compared to wild-type mice. The homozygote OIM mice had thicker septal and posterior walls, but normal systolic function as evaluated by echocardiography (40). It is feasible that patients with OI will have cardiovascular disease.

We performed a register-based cohort study including all patients with OI registered in the NPR to compare the risk of cardiovascular disease in patients with OI to that in reference individuals randomly selected from the CPR matched 5:1 on age and gender to each identified patient with OI, as described in Paper IV and section 8.3 of this thesis. While case series and cross-sectional studies may provide more detailed clinical information about each participant, a nationwide cohort study using register data is (in Denmark) representative of the entire OI population with little or no loss to follow-up. Nationwide coverage also ensures a large sample for conditions with low base rates. The register-based approach enabled us to correct for confounders or risk factors for cardiovascular disease in OI and the general population. This made it possible to evaluate the effect of the general collagenopathy suspected in patients with OI on the risk of developing a cardiovascular disease. The different pathways that could theoretically influence the risk of cardiovascular disease in patients with OI are summarised in Figure 1 of Paper IV.

7.1 Literature search

The literature search was conducted as described in section 3.3 (inclusion and exclusion-criteria) and Appendix 1.4 (search dates, search strings and search results) and aimed to identify all studies that included evaluation of cardiovascular function in patients with OI and the prevalence or risk of cardiovascular disease in OI. A flow diagram of the study selection can be seen in Figure 14. We identified 588 publications via Embase, 306 via PubMed, and 102 via Cochrane Library. After excluding 163 duplicates, 833 titles and/or abstracts were screened for eligibility and resulted in 26 publications for full-text screening. After excluding 13 articles that were conference papers, did not cover the subject matter, or included only few patients with OI, 13 publications were included in the review. Information on participants and study design is summarised in Table 7. The extracted data are presented in Tables 8-11.

Table 7. Studies on cardiovascular disease in OI

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Study design</th>
<th>Methods</th>
<th>Total patients N(W)</th>
<th>Age (years)</th>
<th>Ref pop N(W)</th>
<th>Age (years)</th>
<th>Mild N(W)</th>
<th>Age (years)</th>
<th>Moderate N(W)</th>
<th>Age (years)</th>
<th>Severe N(W)</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folkersen (2016) (Paper IV)</td>
<td>Cohort study</td>
<td>A</td>
<td>687 (370)</td>
<td>43.9±12.3</td>
<td>343 (180)</td>
<td>43.7±12.3</td>
<td>63 (30)</td>
<td>43.7±12.3</td>
<td>97 (48)</td>
<td>43.3±12.3</td>
<td>11 (2)</td>
<td>47±17.3</td>
</tr>
<tr>
<td>Rebarroie (2015, 2016, 2017) (145-147)</td>
<td>Cross-sectional</td>
<td>B, C, D</td>
<td>99 (57)</td>
<td>43.9±12.3</td>
<td>52 (50)</td>
<td>43.7±12.3</td>
<td>98 (66)</td>
<td>43.5±12.3</td>
<td>22 (11)</td>
<td>43.3±12.3</td>
<td>11 (2)</td>
<td>47±17.3</td>
</tr>
<tr>
<td>Ar-Seradj (2015) (148)</td>
<td>Cross-sectional</td>
<td>B, C</td>
<td>6 (5)</td>
<td>34.3±4.5</td>
<td>24 (13)</td>
<td>44±12.3</td>
<td>-</td>
<td>-</td>
<td>1 (7)</td>
<td>-</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Ekenkoer (2013) (149)</td>
<td>Cross-sectional</td>
<td>B</td>
<td>24 (7)</td>
<td>43.9±12.3</td>
<td>24 (7)</td>
<td>4.6±3.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Figure 14. Flow diagram of study selection for cardiovascular disease in OI
were significantly more smokers or previous smokers among the
Radunovic study must be questioned. In a Norwegian cross-sectional study by
small study thus both the power and the generalisability of the
IV aged 43.9±12.3 and a reference group of 52 non-OI individuals
and a comparison was not made (146). In a cross-sectional study of
but the prevalence in the reference population was not reported,
aged 43.7±13.9, 37.4% of the patients with OI had hypertension,
patient had a diastolic blood pressure above 100 mmHg, meaning
above 140 mmHg (median 151 mmHg, range 140-190), and one
There diabetes was found in 5.0% of the Norwegian OI patients, similar to
patients with OI than in the control group (41% vs 21%, p<0.01). In
our cross-sectional study (Paper III) of patients with OI type I and a
healthy age- and gender-matched reference group, there were no
significant difference in smoking habits (3). However, as the study
included only 39 patients and only with OI Type I, it is questionable
if the results can be generalised to the Danish OI cohort.

Patients with OI experience more fractures, and may be burdened
by bone deformation and dystrophy and frequently depend on
walking aids or wheelchairs, than non-OI individuals (2). This could
lead to a less physically active lifestyle, although few data are available
to support this claim. In a study of 14 Canadian children and adolescents with OI, none of the patients reached the daily recommendation for physical activity (157). The forearm cross-sectional area (CSA) of subcutaneous fat is closely correlated to the total body percentage fat mass when comparing pQCT and whole-body DXA (158). In a study of 266 patients with OI (126 patients with OI type I, 37 patients with OI type III, and 103 patients with OI type IV) aged 5-20 who were compared to a reference group of 255 age- and gender-matched healthy controls, patients with OI had lower muscle mass but the cross-sectional area fat was similar in different types of OI and the controls, even after correcting for differences in arm length (158). It has been shown that mice with more severe OI phenotypes have low fat mass (159). Furthermore, children with OI may have a higher basal metabolism than expected, which would lead to lower body fat stores (160). Two studies included 51 children with OI type I or type IV and evaluated cardiopulmonary fitness and the effect of training (64, 78) by comparing patient VO2max and VO2peak values with predicted age- and gender-specific VO2max and VO2peak values (78). Tekken et al. (78) found a

<table>
<thead>
<tr>
<th>Type</th>
<th>Case series</th>
<th>B.C.</th>
<th>40 (27)</th>
<th>12±3 (range 3-23)</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>33 (16)</th>
<th>12±1 (range: 4-23)</th>
<th>33 (22)</th>
<th>11.9 (range: 3-23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mikkelsen (2007) (150)</td>
<td>Cross-sectional</td>
<td>B</td>
<td>40 (21)</td>
<td>40 (20)</td>
<td>41</td>
<td>35</td>
<td>3</td>
<td>2 (7)</td>
<td>-</td>
<td>3 (7)</td>
<td></td>
</tr>
<tr>
<td>Van Brussel (2008) (84)</td>
<td>BCT</td>
<td>L</td>
<td>17 (7)</td>
<td>7.9±3.6</td>
<td>17 (7)</td>
<td>13.2±3.6</td>
<td>27</td>
<td>-</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Takken (2006) (78)</td>
<td>Case series</td>
<td>B, C, S</td>
<td>17 (15)</td>
<td>23.5±3.9</td>
<td>7</td>
<td>-</td>
<td>17 (15)</td>
<td>13.2±3.6</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vetter (1988) (151)</td>
<td>Case series</td>
<td>B</td>
<td>58 (31)</td>
<td>1-16</td>
<td>-</td>
<td>-</td>
<td>18 (9)</td>
<td>2-16</td>
<td>-</td>
<td>14 (9)</td>
<td>1-15</td>
</tr>
<tr>
<td>Krafft (1987) (152)</td>
<td>Cross-sectional</td>
<td>B, C, D</td>
<td>31 (7)</td>
<td>7</td>
<td>34 (11)</td>
<td>7</td>
<td>22 (7)</td>
<td>7</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horta (1985) (153)</td>
<td>Case-series</td>
<td>B, C, D</td>
<td>109 (53)</td>
<td>1-75</td>
<td>17</td>
<td>-</td>
<td>83 (5)</td>
<td>7</td>
<td>30 (7)</td>
<td>7</td>
<td>16 (7)</td>
</tr>
<tr>
<td>White (1982) (154)</td>
<td>Case-series</td>
<td>B, C, D</td>
<td>20 (15)</td>
<td>61 (23-48)</td>
<td>-</td>
<td>-</td>
<td>17 (11)</td>
<td>65 (10-180)</td>
<td>1 (1)</td>
<td>51 (29-48)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

1) The patient time at risk for the primary outcomes was 23,588 [range: 23,497-23,645]. 2) Median age at start of observation. 3) Median person time at risk for the primary outcomes was 120,005 [range: 119,931-120,104]. 4) RCT comparing supervised training 30-45 min sessions over 12 weeks with no supervised training. 4) 15 patients could not be classified on Silence’s classification, age range 1-10 years. ?) Information not given in original work. IQR: interquartile range. W: women R1) Predicted age- and sex-specific VO2 peak values R2) Predicted mean values from age- and weight-matched control

7.2 CARDIOVASCULAR RISK FACTORS IN OI

In our study (Paper IV), more patients with OI were diagnosed with hypertension and were more frequently treated with antihypertensive drugs than the reference population (28.1% vs. 21.6%, p<0.001) (Paper IV). Danish patients with OI did not have increased prevalence of diabetes (5.5% vs. 4.3%, p=0.15) or dyslipidaemia (10.6% vs. 10.4%, p=0.84) (Paper IV). More patients with OI used non-steroidal anti-inflammatory drugs (NSAIDs) compared to the reference population (56.6% vs. 47.2%, p<0.001). Several observational studies using Danish health registers have shown increased risk of adverse cardiovascular effects associated with NSAID use in the Danish general population (155).

In a small case series (n=20) of patients with mild OI, White et al. (154) found that 11 of 20 patients had systolic blood pressure above 140 mmHg (median 151 mmHg, range 140-190), and one patient had a diastolic blood pressure above 100 mmHg, meaning that 60% of the patients were hypertensive (154). This was a very small study thus both the power and the generalisability of the study must be questioned. In a Norwegian cross-sectional study by Radunovic et al. (145-147) including 99 adults with OI type I, III or IV aged 43.9±12.3 and a reference group of 52 non-OI individuals aged 43.7±13.9, 37.4% of the patients with OI had hypertension, but the prevalence in the reference population was not reported, and a comparison was not made (146). In a cross-sectional study of 30,577 Norwegians aged 20-75 years not treated with antihypertensive drugs, the prevalence of hypertension was 30.9% (156). Diabetes was found in 5.0% of the Norwegian OI patients, similar to that expected in the general population in Norway (146). There were significantly more smokers or previous smokers among the patients with OI than in the control group (41% vs 21%, p<0.01). In our cross-sectional study (Paper III) of patients with OI type I and a healthy age- and gender-matched reference group, there were no significant difference in smoking habits (3). However, as the study included only 39 patients and only with OI Type I, it is questionable if the results can be generalised to the Danish OI cohort.

Patients with OI experience more fractures, and may be burdened by bone deformation and dystrophy and frequently depend on walking aids or wheelchairs, than non-OI individuals (2). This could lead to a less physically active lifestyle, although few data are available to support this claim. In a study of 14 Canadian children and adolescents with OI, none of the patients reached the daily recommendation for physical activity (157). The forearm cross-sectional area (CSA) of subcutaneous fat is closely correlated to the total body percentage fat mass when comparing pQCT and whole-body DXA (158). In a study of 266 patients with OI (126 patients with OI type I, 37 patients with OI type III, and 103 patients with OI type IV) aged 5-20 who were compared to a reference group of 255 age- and gender-matched healthy controls, patients with OI had lower muscle mass but the cross-sectional area fat was similar in different types of OI and the controls, even after correcting for differences in arm length (158). It has been shown that mice with more severe OI phenotypes have low fat mass (159). Furthermore, children with OI may have a higher basal metabolism than expected, which would lead to lower body fat stores (160). Two studies included 51 children with OI type I or type IV and evaluated cardiopulmonary fitness and the effect of training (64, 78) by comparing patient VO2max and VO2peak values with predicted age- and gender-specific VO2max and VO2peak values (78). Tekken et al. (78) found a
VO_{2peak} (mL/kg/min) Z-score of -1.41±1.52 but no evidence of circulatory abnormalities as a cause of this reduced cardiopulmonary fitness. Van Brussel et al. (64) randomised children with OI to 12 weeks of supervised 45-minute training sessions to evaluate the effect of training on cardiopulmonary fitness and found an 18% increase in the VO_{2peak} measurements from baseline to the end of the intervention (64). This increase in fitness was more than that expected in healthy children after a comparable training period, but no direct comparison was made between patients with OI and non- OI individuals (64). The authors suggested that the relative hypoactivity and lower initial levels of VO_{2peak}/kg in the patients with OI could explain the relatively large improvement in fitness during the intervention.

The increased prevalence of scoliosis, kyphosis, and vertebral deformities in patients with OI affects pulmonary function and increases the risk of restrictive pulmonary disease (24, 25, 77). This can reduce cardiopulmonary fitness and increase the risk of cardiopulmonary failure (76, 78). In a Swedish study on the causes of death in otherwise healthy patients with untreated scoliosis, 17 of 115 patients had died due to cardiovascular causes (161). The predicted number of cardiovascular deaths in the same cohort was 10 (161), indicating that scoliosis, which is frequent in patients with OI, may on its own increase the risk of cardiovascular disease due to a higher prevalence of restrictive pulmonary impairment, which has been associated with hypertension and other cardiovascular diseases (81). In 1039 patients with a restrictive pulmonary impairment and no cardiovascular disease at baseline, the HR compared to 7865 persons with normal pulmonary function and no cardiovascular disease at baseline was 1.6 (95% CI: 1.2-2.2) for a major cardiovascular event (AMI or stroke) over 15 years of observation (162).

In a non-OI population, the risk of heart failure is closely associated with ischaemic heart disease and with increasing age (163). Even if patients with OI may have a higher risk of cardiovascular disease, it did not lead to increased risk of myocardial ischaemic infarction in our study (11.0% vs. 10.7%, p=0.79) (Paper IV)

### 7.3 Valvulopathies in OI

Prevalence data on valvulopathies in OI are summarised in Table 10 for mitral valve regurgitation and Table 11 for aortic valve regurgitation. In our population-based cohort study (Paper IV), significantly more patients with OI were registered in the NPR with a mitral regurgitation diagnosis or surgery code compared to the reference population (1.6% vs 0.2%, SHR 6.3 [95% CI: 2.5-15.5]). After adjusting for ischaemic heart disease (a risk factor for mitral valve regurgitation (164)), the SHR was still significantly higher in the OI cohort (6.7 [95% CI: 2.8-16.2]) (Paper IV). In the same population, significantly more patients than reference individuals were registered in the NPR with an aortic regurgitation diagnosis or surgery code (0.9% vs. 0.2%, SHR 4.5 [95% CI: 1.4-13.9]). The cumulative sub-hazard increased from the age of 40 in the OI group, meaning that patients were more likely to experience the event (mitral regurgitation diagnosis in the NPR) before the given time compared to the reference population. Table 8 and 9 summarises the prevalence and risk of valvulopathies in patients with OI.

<table>
<thead>
<tr>
<th>Table 8. Mitral regurgitation in OI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author (year)</td>
</tr>
<tr>
<td>Folkestad (2010) (Paper IV)</td>
</tr>
<tr>
<td>Al-Senaidi (2015) (148)</td>
</tr>
<tr>
<td>Karasfar (2013) (Paper IV)</td>
</tr>
<tr>
<td>Thielse (2012) (39)</td>
</tr>
<tr>
<td>Neulamo (2013) (146)</td>
</tr>
<tr>
<td>Migliaccio (2007) (150)</td>
</tr>
<tr>
<td>White (1985) (126)</td>
</tr>
</tbody>
</table>

Ref.pop. = reference population or reference group, HR = Hazard ratio, SHR = Sub-hazard ratio, - no available data

<table>
<thead>
<tr>
<th>Table 9. Aortic valve regurgitation in OI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author (year)</td>
</tr>
<tr>
<td>Folkestad (2010) (Paper IV)</td>
</tr>
<tr>
<td>Al-Senaidi (2015) (148)</td>
</tr>
<tr>
<td>Karasfar (2013) (149)</td>
</tr>
<tr>
<td>Thielse (2012) (39)</td>
</tr>
<tr>
<td>Neulamo (2013) (146)</td>
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<tr>
<td>Migliaccio (2011) (150)</td>
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<tr>
<td>White (1985) (126)</td>
</tr>
</tbody>
</table>

Ref.pop. = reference population or reference group, HR = Hazard ratio, SHR = Sub-hazard ratio, - no available data

Al-Senaidi et al. (148) found no evidence of aortic or mitral valve regurgitation in children with OI type III or IV and an age-matched reference population. However, the statistical power of this study must be questioned, however, as the patient sample was a small cohort of 8 children. Furthermore the study will be prone to selection bias as the patient sample was a cohort from a single hospital clinic. The healthy controls were children referred for echocardiography for a heart murmur that was later found to be physiological or absent. In contrast, Thielse et al. (39) included 46 children with OI type III or IV who were participating in a longitudinal natural history study and evaluated at a NIH clinical centre every 1-2 years.
They found that 4 of 23 patients with OI type III had mild to moderate mitral regurgitation, 7 of 23 patients with OI type IV had mild mitral regurgitation, and one patient with OI type IV had mitral valve prolapse. Only 3 patients with OI type IV and none of the patients with OI type III had aortic regurgitation (39). Of the 58 German children included in a study by Vetter et al. (151), two patients with type III OI had congenital mitral valve prolapse, and two other patients with OI type III developed mitral valve prolapse at 8 and 11 years of age, respectively. None of the 18 patients with OI type I or the 15 patients who did not fit a Sillence classification developed valvulopathy during the study (151). No information about participant recruitment was provided in this study. In a group of Iranian children with OI, 2 of 24 patients had aortic regurgitation and no mitral regurgitations were reported, while no valvulopathies were reported among the 24 non-OI children (149). No information was given about the clinical OI phenotype of the patients with valvulopathies in this study (149). It should be noted that the study included children referred for intravenous bisphosphonate treatment, and possible selection bias must be kept in mind.

No significant difference was found in the proportion of mild mitral regurgitation between adult Norwegian patients with OI and a non-OI reference group (57.5% vs. 61.5%, p=0.64, Pearson’s Chi-squared test) (146). However, significantly more patients had moderate mitral regurgitation (7.1% vs 0.0%, p=0.05) (146). Furthermore, significantly more patients had mild or moderate aortic valve regurgitation (20.2% vs 0%, p<0.001) (146). Radunovic et al. (145-147) included patients from a population-based study (23), but only 99 of 154 patients had echocardiography, so volunteer bias favouring patients with symptoms of cardiovascular disease cannot be ruled out. Migliaccio et al. (150) included 40 patients with OI type I, III or IV and a mean age of 40 years, and excluded all patients with symptoms of cardiovascular disease. They found that 38 of 40 patients and 1 of 40 age- and gender-matched cardiovascular-healthy controls had mitral regurgitation (95% vs 1%, p<0.001) controls (150). Furthermore, 40% of patients with OI had aortic regurgitation, but none of the reference group had aortic valve pathologies (150). As this study design would only identify patients with subclinical disease (seeing that all patients with cardiovascular symptoms were excluded), the clinical importance of the findings can be questioned.

In the 20 patients with OI included by White et al. (154), the aortic valve movement appeared normal but the cusps in 13 patients showed systolic fluttering, and were described as thin in 17 patients. Mitral valve prolapse was found in a 20-year-old woman with OI type I, while in 10 other patients the mitral valve leaflets were considered thin (154). The median age of the participants in this study was 41 years (range: 13-68), and it is unknown whether the thin and fluttering cusps later caused valvulopathies.

Blinding of echocardiographic procedures is difficult and was only done by Radunovic et al. (145-147), who performed a post hoc analysis of the images recorded through three heart cycles for each participant for every standard projection, thus blinding the evaluator to diagnosis of OI. In the other studies, echocardiograms were interpreted by the investigator during the procedure and may in theory have been influenced by expectations about patients with severe OI. In the study by Migliaccio et al. (150), in which two independent investigators performed the echocardiography, intra- and intra-observer coefficients of variance were 2%.

Mitral and aortic valvulopathies are more common in patients with OI than in the general population, and echocardiographic signs of valvulopathy seem more prevalent in adults with OI than children with OI. While up to 95% of adult patients had mitral valve regurgitation and up to 40% had aortic valve regurgitation, up to 35% of paediatric patients had mitral regurgitation and 13% had mitral regurgitation. It has to be kept in mind that the included cross-sectional studies in this review were often very small and generally prone to selection bias. In the Danish health registers, we could only identify 1.6% of the OI population with a mitral regurgitation diagnosis and 0.9% with an aortic valve diagnosis in the NPR.

7.4 CARDIAC VENTRICULAR FUNCTION IN OI

In our study (Paper IV), more patients than reference individuals were registered in the NPR with a heart failure diagnosis (1.0% vs 0.2% for males, SHR 2.4 [95% CI: 1.2-5.0] and 4.0% vs 1.7% for females, SHR 2.5 [95% CI: 1.4-4.6]) (Paper IV), even after adjusting for risk factors for heart failure, such as atrial arrhythmias and ischaemic heart disease (165, 166). The median age at diagnosis was 58 years (IQR: 44-69) in the OI cohort and 76 years (IQR: 62-83) in the reference population. The cumulative incidence from age 40 increased more in the OI cohort than in the reference population.

Children with OI did not show decreased ejection fraction (EF) compared to reference groups of healthy non-OI controls (148, 149). The limitations to these studies have been described earlier (section 7.3). In adults, however, the EF was significantly lower in the Norwegian OI group compared to the reference group, but ventricular function was within normal ranges (147). There were no between-group differences in left ventricular systolic or diastolic function after exclusion of patients with established coronary disease, hypertension, cardiomyopathy (n=1) or treated with β-blockers (n=8) (147). Migliaccio et al. (150) only included patients without subjective symptoms of cardiovascular disease and in NYHA class I (i.e. no limitation of physical activity, and ordinary physical activity does not cause undue fatigue, palpitations or dyspnoea). The authors found no difference in EF between adults with OI and non-OI healthy individuals, although 95% of the patients with OI and 4% of the reference population had diastolic dysfunction (150). Diastole involves the isovolumetric relaxation and filling phase of the cardiac cycle and has both active and passive components; the filling of the left ventricle is biphasic, with rapid filling in early diastole and late filling by atrial contraction (167). In diastolic dysfunction, the ventricle cannot accept blood at low pressures, and ventricular filling is slow or incomplete unless atrial pressure rises (167).

The heart chamber dimensions may be affected in OI as the lack of collagen can cause the chambers to dilate, and furthermore the chamber dimensions will change according to the demands of the heart (168). Myocardial function is affected in heart failure due to changes in contractility, preload (left ventricular end-diastolic pressure) and afterload (systemic vascular resistance). A reduction in
contractility is initially compensated by activation of the sympathetic nervous system (compensatory increase in heart rate and contractility) as well as dilatation of the left ventricle (168). Measurements of heart chamber dimensions may indicate compensatory mechanisms to maintain normal ventricular function in OI. Studies investigating chamber dimensions in patients with OI are summarised in Table 10.

<table>
<thead>
<tr>
<th>Table 10. Heart chamber dimensions in OI</th>
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<tbody>
<tr>
<td><strong>Author</strong></td>
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<tr>
<td><strong>(year)</strong></td>
</tr>
<tr>
<td>Al-Senaid (2012,153)</td>
</tr>
<tr>
<td>Thiele (2012)</td>
</tr>
<tr>
<td>Migliaccio (2009,150)</td>
</tr>
<tr>
<td>Vetter (2009)</td>
</tr>
<tr>
<td>Hortop (2013)</td>
</tr>
<tr>
<td><strong>Right ventricle end-diastolic area</strong></td>
</tr>
<tr>
<td>Radunovic (2010,145)</td>
</tr>
<tr>
<td>Migliaccio (2009,150)</td>
</tr>
<tr>
<td><strong>Left ventricle end-diastolic diameter</strong></td>
</tr>
<tr>
<td>Al-Senaid (2012,149)</td>
</tr>
<tr>
<td>Kazemian (2013)</td>
</tr>
<tr>
<td>Thiele (2012)</td>
</tr>
<tr>
<td>Radunovic (2010,145)</td>
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<tr>
<td>Migliaccio (2009,150)</td>
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<tr>
<td>Vetter (2009)</td>
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<tr>
<td>Hortop (2013)</td>
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</table>

= no significant between-group difference, > OI chamber dimensions larger than in reference population (or predicted values), < OI chamber dimensions smaller than in reference population (or predicted values), - measured in study but no comparisons made, // indicates that two analysis were done. 1) 7 patients with OI type III and 1 patient with OI type IV Radunovic et al. (145) and Migliaccio et al. (150) evaluated the right ventricle end-diastolic area. Radunovic et al. (145) found that patients with OI had larger right ventricle end-diastolic area than controls, after correcting for the patient’s smaller body surface area. They also found that the right ventricular end-diastolic area was larger in patients with OI type III than in patients with OI type I or type IV (145).

While Migliaccio et al. (150) found no differences between patients with OI and their reference group, adult Norwegian patients with OI and Iranian and Omani children with OI had larger left ventricular end-diastolic diameter than their reference groups after correction for body surface area (146, 148, 149). Radunovic et al. (146, 147) also found that left ventricular end-diastolic diameter (corrected for body surface area) was greater in patients with OI type III than in patients with OI type I or IV, but no differences were found in EF. However, neither Migliaccio et al. (150) nor Vetter et al. (151) found any differences in left ventricular end-diastolic diameter comparing patients with OI to healthy non-OI individuals or to predicted age- and gender-matched mean values. Migliaccio et al. (150) did not take differences in body surface area between the two groups into account. Thiele et al. (39) found normal left ventricle dimensions in all 46 participants. Both Vetter et al. (151) and Thiele et al. (39) only included children, and it is unknown whether the differences in chamber dimensions are congenital or develop over time. Ventricular chamber dimensions are larger in patients with OI compared to non-OI individuals, after correction for the smaller body size seen in patients with OI.

Enlarged atria are associated with increased risk of atrial fibrillation. The Framingham Heart Study reported a 39% increased risk of new onset atrial fibrillation for each 5 mm increase in the left atrium diameter in patients evaluated by echocardiography (169). In a case series of 109 individuals from 66 families referred for genetic counselling, the atrial size found by Hortop et al. (153) was smaller than expected in patients with OI compared to the expected gender- and age-matched predicted values. This could not be reproduced in Omani, Egyptian or German children, where atrial size was the same as that in the reference population (148, 150, 151, 153). Thiele et al. (39) found that 3 of 23 patients with OI type IV and 2 of 23 patients with OI type III had dilated left atrium, while 3 patients with OI type III had a left to right shunt. The study did not include a reference population, so it is unknown whether the proportion of dilated atria in children was greater than expected. In adult patients, Migliaccio et al. (150) could not find any difference in chamber dimensions but did not correct for differences in body size between the reference population and the patients with OI. There is little evidence to support that patients with OI have enlarged atria compared to the general population.

### 7.5 Aortic Root Dimensions, Aneurysms and Vascular Dissections in OI

There have been case reports of vascular aneurysms and vascular dissections in OI (42). In our register-based study (Paper IV), we identified 9 (1.3%) patients with OI and 29 (0.8%) participants in the reference population that were registered in the NPR with a vascular dissection or aneurism diagnosis. The relative risk was not significantly different between patients with OI and the reference population (SHR 1.4 [95% CI: 0.6-3.0], p=0.38). Similarly, there were no significant differences between the two groups regarding the prevalence of surgery on peripheral arteries, aorta and its main branches, or cerebral aneurysms.

The aortic annulus was consistently shown to be greater in children and adults with OI, regardless of clinical phenotype (146, 148, 149, 151). The aortic root and the ascending aorta were found enlarged in children with OI, and in adults with OI after correction for differences in body surface area (146, 150, 151, 153). Furthermore, Kalath et al. (152) found increased aortic stiffness in the circumferential direction associated with decreased aortic pumping efficiency. These authors included 7 patients with OI type IV and 24 patients with OI type I and compared the aortic
root diameter to that of 50 healthy controls (152). This is summarized in Table 11.

Table 11. Aortic root dimensions in OI

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>OI vs Reference population /predicted values</th>
<th>OI type I vs Reference population /predicted values</th>
<th>OI type IV vs Reference population /predicted values</th>
<th>OI type III vs Reference population /predicted values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kartesniemi et al. (2015)</td>
<td>&gt; &lt;</td>
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<td>Al-Senaidi (2012)</td>
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<td>&gt;</td>
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<tr>
<td>Rastekarovic (2011)</td>
<td>&gt; &lt;</td>
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<td>Rastekarovic (2011)</td>
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<tr>
<td>Angelini (2007)</td>
<td>&gt; &lt;</td>
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<td>&gt;</td>
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<tr>
<td>Vetter (1999)</td>
<td>&gt; &lt;</td>
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<td>&gt;</td>
</tr>
<tr>
<td>Horta (1980)</td>
<td>&gt; &lt;</td>
<td>&gt;</td>
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</table>

= no significant difference between the groups, > OI aorta measurements larger than in the reference group. 1) Measuring the aorta annulus. 2) Including 7 patients with OI type III and one patient with OI type IV. 3) Measuring the ascending aorta at 3 cm from valve. 4) Measuring the aortic root.

There is little evidence to support any increased risk of vascular dissection or aneurism in OI, but we acknowledge that absence of evidence is not evidence of absence, and further studies are needed.

7.6 ECG CHANGES IN OI

In our study (Paper IV), patients with OI had increased risk of atrial fibrillation or atrial flutter compared to the reference population (SHR 1.7 [95% CI: 1.1-2.8]). The median age at diagnosis was 64 years (IQR: 50-72) in the OI cohort and 76 years (IQR: 62-83) in the reference population. Correcting for ischaemic heart disease did not influence the between-group relative risk of atrial fibrillation or atrial flutter (Paper IV). There was no difference in the risk of developing an atrial arrhythmia between women with OI and women in the reference population. The sensitivity of the NPR for cardiovascular diseases (the likelihood of being in the NPR when having a given cardiovascular disease) has been questioned, but the positive predictive value (registered with a given cardiovascular disease diagnosis and actually having his disease) remains high (170). This could result in underestimating the prevalence of some cardiovascular diseases in our study – in both the OI population and the reference population – but the relative risk difference between the two groups should not be affected. Furthermore, the patients we identify as having a given cardiovascular disease are likely to have that disease, due to the high positive predictive value of the NPR regarding cardiovascular diseases. In a British study on the epidemiology of arterial fibrillation, 2.0% [95% CI: 1.6-2.4] of participants randomly selected from the general population were diagnosed with atrial fibrillation (171). This is comparable to the prevalence of atrial fibrillation or flutter of 1.8% in our reference population that was randomly selected from the Danish general population. As patients with OI are followed at two paediatric centres and four adult highly specialised adult centres (according to Danish national guidelines), our study is subject to surveillances bias. Only hospital contact diagnoses are registered in the NPR, and patients with OI are more likely to come into contact with the hospital system than participants in the reference population. Furthermore, as patients could potentially undergo more frequent surgery than the general population due to differences in fracture rates, patients might more often be evaluated with ECG and auscultation (as part of the pre-surgical routines). This will increase the likelihood of other investigations such as echocardiography being done in patients with OI.

In children with OI type IV or III, Thiele et al. (39) found ECG changes in 7 of 46 patients. Sinus tachycardia, sinus arrhythmia and premature atrial contractions were the most frequently reported ECG findings (39). The mean heart rate per minute tended towards being higher in children with severe OI than in the non-OI healthy reference group (105.5±22.0 vs. 85.9±17.0, p=0.054) (148). In adults, 39 of 99 patients with OI and 10 of 52 of the reference population had one or more ECG abnormalities (39.5% vs 19%, p=0.01) (147). The most frequent ECG abnormalities were ST-T segment changes and negative T-waves, while the prevalence of tachycardia was not reported (147). Three patients with OI were suspected to have paroxysmal supraventricular arrhythmias, but no information about the prevalence of atrial fibrillation or flutter was given (147). However, palpitations were reported more frequently by the patients with OI than by the non-OI reference group (47.5% vs. 0%, p<0.001).

7.7 CONCLUSION

Our study (Paper IV) and the current literature confirm the hypothesis that patients with OI will have increased risk of aorta and mitral valve insufficiency, increased risk of heart failure and increased risk of atrial fibrillation, but we cannot confirm the hypothesis that patients with OI have increased risk of vascular dissections and aneurysms.

8. DISCUSSION AND CONCLUSIONS

We aimed to describe key elements of the natural history of OI such as mortality rate, life expectancy, causes of death in patients with OI and furthermore explore the risk of fractures, the cause of reduced bone strength in patients with OI and the risk of cardiovascular disease as we hypothesized that these factors would to some extent influence the risk of premature death and morbidity in patients with OI.

8.1 LIMITATIONS AND DISCUSSION – STUDY DESIGN

This PhD study includes two different study designs. Papers I, II, and IV are nationwide population- and register-based epidemiological studies that include all patients registered with an OI diagnosis and a reference population of matched individuals randomly selected from the general population. In contrast, Paper III is a
cross-sectional study of adults with OI type I and a matched refer-
ence group. The studies were powered to allow us to control for confounders (factors that might be associated with the outcome, but also with the exposure) and we also tried to limit bias by match-
ing and/or using a population-based design whenever possible.
The different approaches used are described in detail in the at-
tached publications. However, we cannot conclusively rule out any residual confounding or systematic error that may influence our results. As an example, we found that patients with OI had lower BMD measured by DXA. A large portion of the patients and none of the reference individuals were treated with bisphosphonates, which are antiresorptive drugs known to alter bone loss in patients with OI, and in some cases even result in increased BMD (8). This will conservatively bias a study of reduced BMD as it narrows the true biological gap between cases and controls. In spite of this treatment difference between the two groups, BMD was signifi-
cantly lower in the patients with OI. Any differences found be-
tween patients with OI and the general population can be regarded as real despite the bias introduced by therapy.

LF alone did the literature searches, screening of eligible publica-
tions, and data extraction for this thesis. It would have strength-
ened the review design if a second reviewer had participated in the study selection, but this was found to be outside the constraints of a PhD thesis.

8.1.1 Advantages and disadvantages of cohort studies

Cohort studies in general

The identifying features of a cohort study design is that individuals who are either exposed or not exposed to a specific factor are followed over time, and the subsequent development of the out-
comes of interest is evaluated (172). This is exemplified by Study 1 in this thesis, i.e. comparing individuals with OI (exposed) to a refer-
ence population (unexposed) regarding the risk of premature death (outcome). This differs from a case-control study in which the past history of exposure is compared for individuals who have the outcome and individuals without the outcome (172). The ad-
vantages of the cohort design are that we can identify new cases of the outcome and can look at disease progression and the natural history following exposure (e.g. OI and the risk of fractures). More-
over, case-control studies are often prone to recall bias and cannot be used to calculate the absolute or relative risk. In contrast, cohort studies can also yield incidence rates and relative risks, and may even be able - to some extent - to assess causality due to the temporal nature of the study design (172).

Cohort studies in this PhD study

There are limitations to our epidemiological cohort study design. First, we had no information about how the OI diagnosis was made in the individual cases, i.e. whether it was based on DNA analysis, collagen analysis, and/or clinical presentation. We assume that the diagnosis was correct and was based on classic symptoms of OI since that is the contemporary common medical routine in Den-
mark. Second, clinical information about the individuals with OI were limited. Thus, we could not stratify patients according to clinical severity, e.g. Sillence’s classification, as we only had access to ICD-8 or ICD-10 diagnoses for a given disorder. Third, we cannot rule out underreporting of the different outcomes that were investi-
gated in the studies. Epidemiological studies reflect the burden to the health care provider rather than to the individual patient. The studies capture the morbidity and mortality in patients with OI in Denmark given the current standard of care, and the results may or may not be different had the OI disease been allowed to take its natural course in all patients. Finally, we have no information on risk factors for e.g. cardiovascular disease such as smoking, exercise or diet.

The Danish health registers are valued sources of epidemiological research, as they have high quality data with near-complete follow-up. The nationwide population-based design ensured that even with exposures and outcomes at low basal rates, the number of events would be sufficient to calculate rates and ensure statisti-
cal power to evaluate between-group differences for even rare dis-
eases. In a cross-sectional design, we would risk selection bias (in-
cluding individuals with, relative to the general patient population, too high or too low prevalence of the outcome) and may over- or underestimate the prevalence of certain outcomes. The cohort de-
sign that includes all patients with a known disease is less prone to this bias.

The NPR was established in 1977 and at first included information on somatic hospital contacts in relation to surgery and hospital stays in surgical wards, and data on psychiatric hospital stays were added shortly after (173). Since 1995, diagnoses relating to outpa-
tient clinic and emergency room visits have also been included in the NPR (173). The NPR data can be divided into two groups. The administrative data include the patient identification number, pa-
tient’s municipality, identification of hospital ward, date and time of activity, and information on accidents leading to the hospital contact (173). The NPR clinical data are based on patient records where the treating physicians have registered diagnostic and surgical procedure codes. It is mandatory by Danish law to register this information in the NPR, as the register is used for management and financial control of the tax-financed Danish health care system (173). The Danish health registers are valued research tools, as record linkage is easy due to the unique personal identification num-
ber (174). The registers have high coverage (above 99%) of all hos-
pital contacts and less than 5% missing data on surgical procedures (173). The overall positive predictive value of the NPR is above 95%, meaning that if you are registered with a given diagnosis, the likelihood of you actually having this disease is 95% (174). Statistics Denmark offers remote access to all individual-level data in the health registers – from education level, housing and family condi-
tions to health-related data on diagnosis and dispensed medical prescriptions (174). However, only aggregated results and statisti-
cal analyses can be published to ensure the anonymity of study participants. The access to information on important exposures, confounders such as education, income and ethnicity, and to vari-
ous health-related outcomes offers great possibilities for doing ep-
idemiological research on the association and causal network be-
tween disease incidence, mortality, social issues, occupational exposures, clinical indicators, and rehabilitation (175).

No information was available on the positive or negative predictive value of having an OI diagnosis registered in the NPR. The positive predictive value indicates the likelihood of actually having OI if you are registered with an OI diagnosis in the NPR, while the negative
predictive value indicates the likelihood of not having an OI diagnosis if you are not registered in the NPR. In a Danish study of 91 adults with suspected OI aged 19-78 years, who were recruited through the patient society and clinical hospital databases, six patients were excluded after clinical evaluation as the authors considered it unlikely they had OI—even though they were registered with OI in a hospital treatment database (20). Three patients had idiopathic osteoporosis, and three were first-degree relatives to patients with OI but did not display enough typical clinical features of OI for the diagnosis to be accepted (20). The hospital databases used should represent what is normally entered into the NPR. We identified 687 patients with OI. The population prevalence of OI in Denmark has previously been reported to be 10.6 per 100,000 (5), which would in a population of approximately 5.5 million inhabitants lead to 583 patients with OI. This is very close to the number of patients we found through the NPR who were still alive at the end of the observation period. According to current national guidelines, children with OI are now treated at two highly specialised paediatric centres, and adults with OI are treated at four highly specialised university hospital centres. These patients thus see physicians with clinical experience in evaluating patients with OI, and it is highly likely that a patient with (suspected) OI will be seen by an OI specialist. We cannot rule out that patients with the mildest forms of OI will not seek medical attention and thus never be recorded in the NPR. These cases will not be included in epidemiological studies using register based data and this limits our data. The patients included in our studies are the patients diagnosed with OI and represents the patients seen in everyday OI clinics in Denmark.

Misclassification of patients featured in the NPR cannot be ruled out, but it is expected that most patients with an OI diagnosis are registered, and that the number of misclassified patients is low. Thus we firmly believe that we have identified all patients with OI in Denmark through the algorithm explained in Paper I, II, and IV and in section 4 of this thesis.

8.1.2 Cross-sectional study using HRpQCT and DXA
A cross-sectional study is an observational study in which exposure and outcome are determined simultaneously for each subject, often described as taking a “snapshot” of a group of individuals (172). The cross-sectional design is limited by the simultaneous assessment of both exposure and outcome, and a temporal relationship between exposure and outcome is thus difficult to establish (172). Seeing that OI (or exposure) is a congenital disease, any association between patients and an outcome is more plausible than if the exposure was temporal. A cross-sectional study will measure prevalent rather than incident outcomes, leaving the study more vulnerable to selection bias.

HRpQCT offers assessment of bone geometry, density, and microarchitecture in the distal tibia and radius in vivo. The technique is limited by the image resolution, segmentation, and structure extraction that may affect some of the parameters such as cortical porosity and trabecular thickness. The results from HRpQCT scans have shown high reproducibility and low coefficients of variance between measurements. Our study in patients with OI type I may be biased by the fact that we used a fixed offset for the volume of interest in both radius and tibia and compared patients with possibly shorter limbs to healthy non-OI reference individuals. The volume of interest may thus have been relatively more proximal in patients with OI than in the reference population. This would result in relatively thicker cortical bone and relatively less trabecular bone compared to more distal cross-sections. In otherwise healthy individuals, changing from the fixed offset for the volume of interest to a 4% tibial length and 7% radial length offset (a proximal shift of 1 mm at the radius and up to 2 mm at the tibia, and a distal shift of up to 3 mm at the radius and 8 mm at the tibia—from the standard offset of 9.5 and 22.5 mm) resulted in large morphological changes at both the radius (up to 34%) and the tibia (36%) (108). Whether this is also true in patients with OI is not known, and currently no studies have evaluated the effects of differences in extremity length on bone geometry and microarchitecture in individuals with and without OI.

Altered bone properties due to defective or decreased amounts of collagen are present in all patients with OI. Our study is further limited by the fact that we did not measure bone quality and did not undertake collagen analysis. However, we did participate in a study (outside the scope of this PhD), where we could confirm that the mutations leading to haplotype insufficiency seen in patients with OI type I lead to lower vBMD measures compared to patients with OI type IV (20). More patients with OI type III had missense mutations in COL1A1 or COL1A2, leading to a qualitatively defective collagen type 1, and had severely reduced aBMD and vBMD compared to patients with less severe OI (20). The quantitative defects in collagen type 1 lowered the trabecular number compared to patients with OI type IV, in whom the structural bone parameters were more similar to previous observations in healthy bone (20).

The study was the first to evaluate the bone phenotype in OI using HRpQCT and was strengthened by the age- and gender-matched reference group, which had participated in a large population-based study of bone geometry and bone microarchitecture. The selection of participants in that study is described in detail in Hansen et al. (176). The study was powered to correct for confounders of bone mineral density, bone geometry, and bone microarchitecture such as weight and smoking habits.

8.1.3 Bias of clinical severity when comparing prevalence/risk of outcomes between different phenotypical groups
The grouping of patients with OI according to Sillence’s classification is based on clinical features, and mode of inheritance in the individual patient. The assessment of the clinical severity may be based on objective findings, when evaluating the patient for diagnosis, but the clinical severity is contingent on subjective interpretation of these findings. In a newly proposed severity grading scale of OI the clinical features is further specified (6). This approach will aide clinicians and researchers to gather data that can be compared across different clinics and clinical routines. The grouping of patients according to clinical severity does however introduce a bias to studies aimed at evaluating clinical outcomes between groups of patients with different clinical severity. Exemplified in OI when comparing the fracture risk in patients with severe OI to patients with mild OI. The grouping of the patients into severe, mod-
erate and mild OI groups using fracture rates as one of the parameters which the patients are grouped according to, introduce a systematic error into the analysis as the patients are grouped according to their risk of fractures. In other words the outcome is used to group the patients – any differences in outcome is therefore given. Any conclusions drawn from such a study will be subject to circular reasoning.

Confounding by indication is a commonly used term that refers to an extraneous determinant of the outcome parameter that is present if a perceived high risk or poor prognosis is an indication for intervention (177). It can be argued that the risks of certain clinical outcomes are confounded by indication (i.e. clinical severity / Silence’s type) in OI. A confounder is associated with the exposure and the outcome, but cannot be an intermediate in the causal pathway between the exposure and the outcome. It can be discussed if the clinical severity is not a part in the causal pathway between the exposure (the mutation) and the risk of outcomes (e.g. fractures). Studies correlating genotype, or collagen defect, to clinical outcomes would not be biased by the classification according to clinical features. Many of the included studies in this review, however, base their classification of the different types of OI on the clinical features of the disease.

This classification-bias is hard to overcome. When describing the natural history of OI and thus describing the differences between the phenotypes of the disease we must acknowledge that by definition the most severe individuals are defined as the most severely affected individuals when they were grouped. Any between group differences will be given. The clinical grouping of the patients does however make it possible to compare patients within the same clinical syndromes e.g. treatment effect.

8.2 DISCUSSION OF THEMES INCLUDED IN THIS THESIS

8.2.1 Causes of death in OI

We found that patients with OI had increased risk of all-cause mortality compared to a reference population [HR: 2.90 [95% CI: 2.3-3.6], women HR 2.4 [95% CI: 1.8-3.3], men HR 3.7 [95% CI: 2.6-5.2]] (1). This resulted in a lower median survival time of 9.5 years in men with OI and 7.1 years in women with OI compared to a reference population. There was also an increased relative risk of death due to respiratory disease (SHR 3.1 [95% CI: 1.4-6.9]), gastrointestinal disease (SHR 4.2 [95% CI: 1.6-10.8]), and external causes of mortality and morbidity (including trauma and fractures) (SHR 4.7 [95% CI: 1.4-16.3]) when comparing patients with OI to the reference population. Death due to cardiovascular disease was frequently reported in OI, but there was no increased relative risk compared to the reference population of randomly selected individuals from the general population.

The studies included in this review included patients born over the last century, and this may bias the results. Firstly, much has changed in neonatal treatment options and survival of severely affected patients has increased during the last decades. Second, mortality rates following and treatment options for common illnesses such pneumonia may have changed over the time course of the different studies. In studies including a matched reference group this would not alter the relative risk between the groups for all cause mortality as the gains would positively affect the outcome in both groups, but when comparing the results across studies this may influence the results and generalisability to the current OI population.

Any evaluation of causes of death will be subject to competing risk bias. This can be illustrated by an example of a fictive disease that only occurs in persons over 75 years of age. If the exposed group has markedly reduced survival compared to the unexposed group, their risk of developing the disease is lower simply because many will die before reaching the age where they will be at risk of the disease. We took this into account in our analysis of the causes of death in OI by using a competing risk regression model to evaluate the relative risk for the different causes of death in patients with OI compared to the reference population. McAllion and Peterson (43) did not take this into account when they evaluated the causes of death in OI. They made no direct comparisons to the general population but relied on the proportion of people dying from a given disease in the general population (from official mortality statistics) and compared this to the mortality observed in patients with OI.

8.2.2 Fractures in OI

We found that patients with OI have increased risk of fractures throughout their life compared to the reference population of randomly selected individuals from the general population (IRR 8.1 [95% CI: 7.5-8.8]). The relative risk of fractures declined with age. We showed that fractures occurred with the same pattern as seen in the general population, with a peak during the toddler and adolescent years (IR 233.9 [95% CI: 219.9-247.8] per 1000 person years), fewer fractures during adulthood (IR 84.5 [95% CI: 75.5-93.8] per 1000 person years), and increased fracture rates in the elderly (IR 111.9 [95% CI: 81.8-113.0] per 1000 person years in women over 55).

Children with OI are likely to be more susceptible to long bone fractures when they start to walk and become more active, in part due to falls. In a British study of childhood fractures, the peak fracture rates were at age 11-15 years (97), which was remarkably close to the age at which the discordance between height gain and accrual of vBMD is most pronounced (97, 178). Rauch et al. (116) found that vBMD in the shaft of the radius was increased in children with OI type I due to increased cortical area and vBMD. This should result in a protective mechanism for long bone fractures in OI in this age group, so we must therefore consider other theories to explain the higher fracture rate. The growing skeleton adapts to the needs of increased bone strength and body weight, but there seems to be a lag in bone strength during periods of accelerated growth as seen during the pubertal growth spurt, meaning that weight-bearing bone can only sustain smaller traumas before breaking (179). We speculate that the inapt gains in bone strength during growth could explain some of the increased fracture rates seen in patients with OI during the first two decades of life.

It is likely that adults with OI have learned to live with their disease and make choices in daily activity that will lower their fracture risk.
This can explain some of the decrease in fracture rates in the adult life, however there are few data to support this claim.

With increasing age comes increasing risk and incidence of falls (180). Falls and bone loss could increase the fracture rates in elderly patients with OI. Furthermore, we should keep in mind that most patients with OI will reach a peak bone mass that is lower than non-OI individuals, leaving them even more exposed to fractures. Only two studies evaluated fracture rates with increasing age and found increasing risk of fractures in women after menopause compared to pre-menopausal women with OI (2, 93). While several fracture types showed increased IRR among women with OI aged 55 years and over, IRR was lower in men with OI in this age group compared to the reference population in our study. However, this may be due to the low number of older men in our cohort as a result of higher mortality (1). Patients with the most severe phenotypes of OI will have increased risk of premature death (43) and this could lead to positive selection of patients with milder phenotypes, and thus fewer fractures, in older age groups.

Fracture rates and the risk of fractures varied between OI phenotypes but also within each phenotype. Patients with OI were more likely to fracture lower limbs than upper limbs, and the absolute risk of an upper or lower limb fracture was close to 100% in patients with OI.

8.2.3 BMD, bone geometry and bone microarchitecture in OI
Patients with OI type I had altered bone geometry (lower total bone area in the radius), altered bone microarchitecture (decreased trabecular number, increased trabecular spacing, and greater trabecular inhomogeneity), and lower bone mass (decreased areal and volumetric BMD) compared to healthy reference individuals when assessed by HRpQCT and DXA. Our results suggest that the increased risk of fractures in patients with OI is a combined result of low bone mass, and altered bone microstructure and geometry.

Patients with OI had lower total vBMD compared to the non-OI individuals. This could be explained by the low trabecular vBMD found in patients with OI. The total vBMD did not follow the same pattern as for aBMD, which decreased with increasing clinical severity, as patients with the moderate phenotypes had the highest total vBMD. It is difficult to conclude how the cortical vBMD in patients with OI would typically differ from non-OI individuals based on the current literature. Cortical vBMD seems to be dependent on skeletal site, as cortical vBMD was higher in more proximal parts of the radius. One cause of the lower bone mass seen in patients with OI could be the altered osteoblast function, with osteoblasts secreting reduced amounts of biologically normal collagen type I, thus preventing accumulation of bone mass early in life and leading to a lower peak bone mass that will be associated with lower bone mass later in life (116). Rauch et al. (116) found differences in bone size (and thus bone mass) at various skeletal sites compared to non-OI children, as osteoblasts throughout the skeleton would be equally affected in children with OI type I. Another explanation could be related to the mechanostat theory that bone adapts to the bone strength needed to prevent fractures. The dendritic network of osteocytes in the skeleton enables systematic signalling of needs for bone strength gain and may be activated at a set time. This activation may occur later in patients with OI, and the adaptations in bone mass, geometry and microstructure result in bone strength below the actual mechanical needs (116). It could be argued that the governing of bone strength and the adaptation to changes in bone strength requirements works as they would in non-OI individuals, but are overruled by other factors such as increased bone turnover, remodelling or an unknown factor (119).

The literature consistently reported altered bone microarchitecture in patients with OI. Patients with OI type IV (i.e. with abnormal collagen type I in normal quantities) had larger bone, higher bone mass, and ‘better’ microarchitecture than patients with type I OI (i.e. with normal collagen type I in low quantities) (20). In contrast, patients with OI type IV experienced more fractures than patients with OI type I, suggesting that the collagen defect plays an important role in the bone strength of patients with OI. It can be argued that the increased bone turnover in OI results in trabecular perforations, loss of thin trabeculae, and increased mean thickness of remaining trabeculae (possibly explaining the normal trabecular thickness found in OI). In contrast, a non-OI individual would have a higher number of trabeculae but with a lower mean thickness, and a wider range of trabecular thickness.

All of the included studies were case series or cross-sectional in nature, and only a few included a non-OI reference group. Most studies used age, gender, and height-specific normal values to calculate mean Z-scores for the parameters measured. This is a limitation to the quality of evidence. None of the studies were longitudinal or powered to evaluate whether the densitometric, geometric or microstructural parameters could predict fractures in patients with OI.

8.2.4 Cardiovascular disease in OI
We found that patients with OI had increased risk for heart failure (SHR 2.3 [95% CI: 1.4-3.7]), mitral valve regurgitation (SHR 6.3 [95% CI: 2.5-15.5]), aortic valve regurgitation (SHR 4.5 [95% CI: 1.4-13.9]), and atrial fibrillation or flutter (SHR 1.7 [95% CI: 1.1-2.8]) compared to the reference population. However, we did not find any increased relative risk for vascular dissections or aneurisms. The sub-hazard ratio remained higher in the OI cohort after adjusting for diseases that may cause valvulopathy, atrial arrhythmia, and heart failure. We found no increased risk of arterial dissection or aneurisms in patients with OI. The development of cardiovascular diseases in OI is multifactorial, as described in Figure 1 of Paper 4.

It could be argued that the increased prevalence of fractures and bone deformities seen in the more severe OI phenotypes lead to immobilisation and a less cardiovascular-healthy lifestyle. This could result in increased cardiovascular morbidity. In the Norwegian study, 18% of the patients and none of the non-OI reference participants described dyspnoea during exertion (147). This could indicate more ischaemic heart disease in patients with OI, but also a lower fitness level in the OI cohort that results in more dyspnoea upon physical activity. We could not find an increased risk of ischaemic heart disease in our cohort. There were more ECG
changes in the Norwegian patients with OI compared to the reference individuals, but the authors found no increase in ischaemic heart disease in patients with OI (147). Hypertension was more prevalent in patients with OI, possibly due to changes in the vascular construction, as the collagen deficiency could alter the elasticity of the vascular system. Elasticity of the arterial tree is associated with hypertension (181). The increased prevalence of hypertension in OI could also be due to Berkson’s bias i.e. when a person is followed for one disorder, the odds of finding another disorder is increased, as evaluations will find subclinical disease. Patients with OI are closely followed at hospital centres, and systematic screening for other diseases is part of the normal work-up. This surveillance bias is not present in the reference population, who will have to contact their own physician with symptoms of a disease. Treatment for other disorders may thus be started earlier in patients with OI than in the reference population, resulting in a lower cardiovascular risk profile for patients with OI.

Differences between patients with OI and a reference group in the prevalence of valvulopathies, heart failure and atrial arrhythmias, even after adjusting for known risk factors for mitral regurgitation, atrial fibrillation or flutter, and heart failure were found. This suggests that the decreased collagen type 1 seen in OI could have a role in the development of cardiovascular disease in OI.

8.3 CONTRIBUTION TO THE OI FIELD
Our studies have provided new knowledge on the natural history of OI and the risks of skeletal and non-skeletal morbidity in OI. The epidemiological studies have long-term follow-up of a large number of patients and a representative reference population, allowing detailed analysis of risks and disease rates in patients with OI compared to the general population, even for outcomes with low basal rates. Large, population-based longitudinal studies are generally lacking in the OI field due to the low incidence of the disease. Findings from studies on bone structure and geometry using traditional histomorphometric analysis of bone biopsies in adults with OI need to be corroborated by studies using more advanced stereological techniques such as HR-pQCT. We were the first to evaluate bone mineral density, bone geometry, and bone microarchitecture in OI using HRpQCT. The new insight into bone phenotypes in OI increases the knowledge of the natural history of OI and may promote shed light on the development of bone fragility in patients with OI.

8.4 CONCLUSIONS
We confirm that patients with OI have increased all-cause mortality, with a higher risk of death due to respiratory diseases, gastrointestinal diseases, and trauma (bone fractures). The shorter survival of patients with OI may be due to increased risk of neonatal death associated with more severe OI phenotypes, and to increased risk of death throughout life (Paper I) (1).

We confirm that patients with OI have increased risk of fractures throughout their life compared to the general population. Though the relative risk declines with age, fractures as a whole appear to have the same pattern of absolute rates as in the general population, with a peak fracture rate during the toddler and adolescent years (IR 233.9 per 1000 person years), fewer fractures during adulthood (IR 84.5 per 1000 person years), and increased fracture rates in older women (IR 111.9 per 1000 person years) (Paper II) (2).

We confirm that patients with OI type I had altered bone geometry (lower total bone area in the radius), altered bone microstructure (decreased trabecular number, increased trabecular spacing, and greater trabecular inhomogeneity), and lower bone mass (decreased areal and volumetric BMD) compared to healthy controls. Our results suggest that the increased risk of fractures in patients with OI type I is a combined result of the known altered bone matrix quality, low bone mass, and altered bone microarchitecture and geometry (Paper III) (3).

We confirm that patients with OI have increased risk of cardiovascular disease. From the age of 50 years there was a significant increase in cumulative incidence of heart failure, mitral valve regurgitation, aortic valve regurgitation, and atrial fibrillation or flutter in the OI cohort. The sub-hazard ratio remained higher in the OI cohort after adjusting for diseases that can cause valvulopathy, atrial arrhythmia, and heart failure, which is in line with the idea that the decreased collagen type 1 found in OI can cause these diseases. We found no increased risk of vascular dissection, but larger systematic cross-sectional studies including a reference population are needed to further evaluate the risk of vascular abnormalities in patients with OI (Paper IV).

9 FUTURE PERSPECTIVES AND RESEARCH
The studies included in this thesis have generated new questions that will need to be answered to better understand the systemic implications of a genetic variants causing altered biosynthesis of collagen type 1, as seen in OI. It appears that OI is a generalised connective tissue disorder that influences most organ systems, but also affects the patient’s life choices due to fracture risk, pain, and hypermobility. Both clinical and epidemiological studies are planned for the future.

9.1 FRACTURE PREDICTION IN OI
Longitudinal data on the changes in bone mass, bone geometry, and bone microarchitecture are needed. We are currently re-evaluating the participants in Paper 3 to evaluate changes over time. This study is not powered to fracture prediction, but collaboration with other authors using baseline HRpQCT data to evaluate fracture risk is being sought. There are no studies on the predictive value of DXA on fractures in OI, and there is no knowledge about the additive effect of HRpQCT derived parameters on fracture prediction. These studies would help in identifying patients with OI who would benefit most from fracture prevention. Further studies on who to treat and when to initiate treatment and furthermore studies on how to monitor patients during fracture prophylactic treatment are needed.

9.2 NON-SKELETAL MORBIDITY IN OI
Cardiovascular, respiratory, and gastrointestinal morbidity may reduce longevity in patients with OI. The Danish health registers will enable us to evaluate the prevalence and relative risk of respiratory diseases in OI, such as asthma and COPD, as well as acute admissions due to respiratory tract infections.
Patients with Ehlers-Danlos Syndrome, another connective tissue disorder, have significantly more frequent respiratory symptoms and reduced exercise tolerance compared to healthy non-Ehlers-Danlos Syndrome individuals (OR: 3.1 [95% CI: 1.8-5.2] p=0.001) (182). During forced expiration in pulmonary function tests, approximately 60% of patients had lower airway collapse demonstrated by flow volume loops (182). We would hypothesise that the same would be true in patients with OI, and a clinical trial including patients with different OI types and a matched reference population, powered to allow correction for confounders of pulmonary function (such as smoking habits, differences in height and physical activity, scoliosis, and vertebral fractures) would enable us to test this hypothesis. Most of the current literature is cross-sectional, and a longitudinal design would strengthen the study. It would also allow us to evaluate the rate of change in pulmonary function over time.

Using the Danish health registers to evaluate the risk of gastrointestinal ulcerative diseases would enable us to confirm the hypothesis that the increased risk of death due to gastrointestinal diseases is based on increased incidence of gastrointestinal ulcers. The increased bleeding time and easy bruising seen in OI may worsen the outcome of any gastrointestinal bleeding. It may be that the reduced collagen in the arterial walls of an OI patient does not promote platelet activation to form a platelet plug in the damaged artery to the same degree as in non-OI individuals. Evaluation of platelet function and activation in patients with OI are also needed.

There are no studies on the arterial tree and pulse wave velocity in patients with OI. We found increased prevalence of hypertension in the OI cohort, but have not explored what would normally be regarded as end-organ-damage in hypertensive patients. This would require a more detailed evaluation of the patients than is available through the Danish health registers. Evaluation of pulse wave velocity, 24-hour blood pressure, nephropathy, and retinopathy would enable us to describe the degree of stiffness in to the arterial tree in patients with OI, and thus the effect of the collagen defect on the vascular system.

In our population-based cohort study, we did not find any differences between the OI group and the reference population in prevalence of vascular dissection or aneurysms. The extent of underreporting or under-diagnosis of these conditions in OI is unknown. A systematic screening for aorta aneurysms in the general population of Southern Denmark is currently underway, and evaluating a group of patients with OI using the same protocol would identify any differences between patients with OI and the general population.

9.3 THE SOCIOECONOMIC CONSEQUENCES OF HAVING OI

Hald et al. (183) compared the health-related quality of life (HRQoL) of 85 adults with mild to severe OI to that of the general population using the SF-36 questionnaire and normal reference material for Danish inhabitants. The study showed that patients with OI, regardless of severity, had significantly lower mean scores in domains describing physical HRQoL and lower mean physical component scores. In contrast, mental health scores did not generally differ between OI sub-types or compared to the general population. A mixed-methods systematic review, aimed at describing HRQoL in patients with OI, found that physical HRQoL in children and adults was poorer than that of the general population, whereas mental and psychosocial HRQoL were similar or better in patients with OI compared to the general population (184).

In a Norwegian population-based study on activities of daily living (ADL), patients with OI scored well on the Sunnaas ADL Index (23). Most patients in the study were married, had children, were highly educated, and lived independently without organised assistance for the activities tested for in the study (23).

In an interview study of 55 adults with OI type III and IV, the author concluded that patients with OI have high emotional endurance and cope well with adversity (185). In a mixed-methods systematic review, aimed at describing the psychosocial experiences of living with OI, the authors found that the patients demonstrated a strong drive for achievement in their academic, extracurricular, professional, and community involvements (186).

The psychological resilience often seen in patients with OI suggests that the group as a whole is able to cope with the disorder in a way that may compensate for some of the limitations they are faced with. Further research on adherence to the labour market, education choices, and family life is needed to understand the socioeconomic consequences of OI. The Danish health registers would be a valuable tool for projects evaluating socioeconomic differences between patients with OI and the general population.

10. LITERATURE SEARCH STRATEGIES

1.1 Causes of Death and life expectancy in OI

We searched PubMed from 1962 until 1st May 2016, Embase classic and Embase from 1947 to 1st May 2016, and all available literature in the Cochrane Library until the 1st May 2016.

Appendix 1.1.1 – PubMed Search Strategy and results

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Appendix 1.1.2 – Embase Search Strategy and results

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Appendix 1.1.3 – Cochrane Library Search Strategy and results

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1.2 Fracture rates in OI

We searched PubMed from 1962 until 1st May 2016, Embase classic and Embase from 1947 to 17th June 2016, and all available literature in the Cochrane Library until 1st May 2016.

Appendix 1.2.1 – PubMed Search Strategy and results

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### Appendix 1.2.3 – Cochrane Library Search Strategy and results

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1.3 Bone mineral density, bone geometry and bone microarchitecture in OI

We searched PubMed from 1962 until 20th June 2016, Embase classic and Embase from 1947 to 17th June 2016, and all available literature in the Cochrane Library until 1st May 2016.

Appendix 1.3.1 – PubMed Search Strategy and results

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### Appendix 1.3.2 – Embase Search Strategy and results

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### 1.4 Cardiovascular diseases in OI

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2 Cardiovascular Disease [MeSH Term]

8 #1 AND #2

9 Limit to Danish, Norwegian or English

### Appendix 1.4.2 – Embase Search Strategy and results

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### Appendix 1.4.3 – Cochrane Library Search Strategy and results

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### 11. SUMMARY

#### 11.1 ENGLISH SUMMARY

Osteogenesis Imperfecta (OI) is a hereditary disease of the connective tissue caused by mutations to, mainly, the genes that are involved in the biosynthesis of collagen type 1. Patients are grouped according to clinical severity and mode of inheritance according to Sillence’s classification (originally 1979, updated 2014). According to our data, the population prevalence of OI in Denmark was 10.3 per 100,000, with 575 patients registered with an OI diagnosis in the NPR and alive at the end of 2012 out of a total population of 5,602,628 persons. Hallmarks of the disease are multiple fractures, blue sclera and varying degrees of bone deformities. Collagen type 1 is the most abundant collagen in the body and is an important part of the structure and function of the heart and lungs, the skeleton and many other organs. We hypothesize that patients with OI will have increased prevalence and risk of fractures throughout life, lower bone mineral density (BMD), impaired bone microstructure and bone geometry and increased risk of cardiovascular diseases - thus increased risk of all cause mortality compared to the general population.

This thesis is a systematic search and narrative review covering the four main areas of interest of the PhD scholarship (risk and causes of death, fracture rates, bone mineral density, -geometry and -microstructure and cardiovascular diseases in OI). In addition to the review the thesis include the following four studies:

1) Study 1 aimed to investigate the main causes of death and the risk of premature death in patients with OI in Denmark. We used a nationwide, registry-based, cohort study design, and included all patients registered in the National Patient Register with an OI diagnosis and a matched reference population randomly selected from the Danish Civil Service Register (matched 5:1, on gender and month and year of birth for each OI patient). We identified 687 patients with OI (25,615 person years at risk) and a reference population of 3435 (132,131 person years at risk). One hundred and twelve patients with OI and 257 persons in the reference population died during the observation period from 1977 to 2013. The all-cause mortality hazard ratio between the OI cohort and the reference population was 2.90. The median survival time for men with OI was 72.4 years, compared to 81.9 in the reference population. The median survival time for women with OI was 77.4 years, compared to 84.5 years in the reference population. Patients with OI had a higher risk of death from respiratory diseases, gastrointestinal diseases and trauma.

**Conclusion:** The all-cause hazard ratio for premature death in OI was 2.9 compared to the reference population. There was an increased risk of death due to respiratory diseases, gastrointestinal diseases and death following trauma.

2) Study 2 aimed to compare the fracture rates across the lifespan of patients with OI with that of the general population. Using a nationwide, registry-based, cohort study design, we counted all fractures registered from 1995 in the National Patient Register. The study included the same population as in study 1, but patients who died before 1995 were excluded. We identified 644 patients...
(55.6% females) in the OI cohort through the Danish National Patient Register and 3,361 persons (55.2% females), randomly selected from the Civil Registry System. A total of 416 patients with OI experienced a total of 1,566 fractures during the observation period of median 17.9 years [IQ-range: 12.4-18.0], adding up to 10,137 person years. In comparison, 709 persons in the reference population experienced a total of 1,018 fractures during follow-up. Both male and female patients with OI had an increased fracture rate throughout their life. The fracture rate ratio for participants aged 0-19 years was 10.7, for participants aged 20-54 years 17.2, and for participants aged 55 years and over 4.1 when compared to the reference population. The highest fracture rate was seen in males with OI aged 0-19 years (257 fractures per 1000 person years). The fractures appear to follow the same pattern as in the general population, with a peak during the toddler and adolescent years (IR incidence rates) 233.9 per 1000 person years), fewer fractures during adulthood (IR 84.5 per 1000 person years), and increased fracture rate in older women (IR 111.9 per 1000 person years).

**Conclusion:** Patients with OI have increased risk of fractures throughout life compared to the general population. The relative risk of fractures generally declines with age, however, increases in older women.

3) Study 3 aimed to evaluate the bone mineral density (BMD) and bone geometry and -microarchitecture in patients with OI type I using a cross-sectional study design and evaluating the participants using HRpQCT. The study included 39 patients with OI type I, and 39 healthy age and gender matched non-OI individuals. The patients were shorter than the reference group (159 ± 10 cm versus 170 ± 9 cm, p < 0.001), but had similar body weight. In patients with OI, areal bone mineral density (aBMD) was 8% lower at the hip (p < 0.05) and 13% lower at the spine (p < 0.001) compared with the reference group. The trabecular volumetric bone mineral density (vBMD) was 28% lower in radius (p < 0.001) and 38% lower in tibia (p < 0.001) in patients with OI compared with the reference group. At radius, total bone area was 5% lower in OI patients than in controls (p < 0.05). In the tibia, cortical bone area was 18% lower in patients with OI (p < 0.001). In both radius and tibia the number of trabeculae was lower in patients compared to the reference group (35% and 38%, respectively, p < 0.001 at both sites). Furthermore, trabecular spacing was 55% higher in OI patients in both tibia and radius (p < 0.001 at both sites) when compared with reference group.

**Conclusion:** Patients with type I OI have lower aBMD, vBMD, bone area, and trabecular number when compared with healthy age- and gender-matched individuals.

4) Study 4 aimed to evaluate the risk of valvulopathies, atrial arrhythmias, heart failure and vascular dissections in patients with OI using a nationwide, registry-based, cohort study design. The study included the same population as in study 1. As patients with OI have increased risk of premature death, the risk of cardiovascular diseases is biased by the competing risk of death. We corrected for this increased risk by using a competing risk regression model. We found that the OI population had increased relative risk of mitral valve regurgitation (Sub Hazard Ratio (SHR) 6.3), aortic valve regurgitation (SHR 4.5), atrial fibrillation/flutters (SHR 1.7) and heart failure (SHR 2.3) compared to the reference population. There was no difference in the risk of arterial aneurisms or arterial dissections.

**Conclusion:** Patients with OI have increased risk of valvulopathies, atrial arrhythmias and heart failure when compared to the reference population, even after adjusting for risk factors for these cardiovascular diseases - indicating that the quantitative or qualitative defects of collagen type I synthesis seen in OI influence the risk of these cardiovascular diseases in patients with OI.

**12. BIBLIOGRAPHY**


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