Understanding Osteoarthritis Utilising Magnetic Resonance Imaging

By

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy, University of Tasmania

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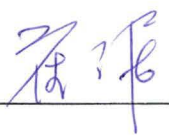
(Signed) 

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LIST OF PUBLICATIONS

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4. Poster presentation of "Factors associated with femoral head cartilage volume" at the 45th annual scientific meeting of Australian Rheumatology Association, Sydney, 2003.

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LIST OF ABBREVIATIONS

3D	3-dimensional
BMD	Bone Mineral Density
BMI	Body Mass Index
CE	Coefficient of Error
CI	Confidence Interval
COL1A1	Type I Collagen Gene
COL2A1	Type II Collagen Gene
COX-2	Cyclo-oxygenase-2
CTX-II	C-terminal Crosslinking Telopeptide of Type II Collagen
CV	Coefficient of Variation
DIP	Distal Interphalangeal
ERT	Estrogen Replacement Therapy
ESR1	Estrogen Receptor α Gene
FDA	Food and Drug Administration
FLASH	Fast Low-Angle Shot
GREES	Group for the Respect of Ethics and Excellence in Science
ICC	Intraclass Correlation Coefficient
IGF-I	Insulin-like Growth Factor 1
JSN	Joint Space Narrowing
JSW	Joint Space Width
KCV	Knee Cartilage Volume Study
LD	Linkage Disequilibrium
MRI	Magnetic Resonance Imaging
NHANES	National Health and Nutrition Examination Survey
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OA	Osteoarthritis
OR	Odds Ratio
PGs	Prostaglandins
PIP	Proximal Interphalangeal
ROA	Radiographic Osteoarthritis

ROS	Reactive Oxygen Species
SD	Standard Deviation
SNR	Signal-to-Noise Ratio
SPGR	Spoiled Gradient Recalled Acquisition In The Steady State
TASOAC	Tasmania Older Adult Cohort Study
TGFB1	Transforming Growth Factor β Gene
VDR	Vitamin D Receptor Gene
WHO	World Health Organization
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index

SYNOPSIS

Osteoarthritis (OA), whose aetiology remains elusive, is the most common form of musculoskeletal diseases. Its high prevalence, particularly in the elderly, and the resultant physical disability make OA one of the ten most disabling diseases in developed countries. Conventional radiography has been used in the assessment of joint structural change and has provided the basis for much of our understanding in OA. However, its two dimensional nature, indirect measure of the structure of the joint, and poor association with symptoms limit its value. Magnetic resonance imaging (MRI), a non-invasive imaging technique with multiplanar capabilities and unparalleled soft tissue contrast and lack of ionising radiation, has an important role in the evaluation, diagnosis, and monitoring of OA.

Based on MRI measurements of the hip and knee, this thesis examines a number of questions relevant to pathogenesis of OA as well as feasibility of MRI methodology in large epidemiological studies.

Chapter 1 consists of the literature review in two parts. The first part gives a broad overview of OA while the second part reviews the available literature to date which covers MRI evaluation of articular cartilage morphology. Based on this review, the questions that will be addressed in this thesis are raised.

Chapter 2 describes the research questions.

Chapter 3 describes the research methodology.

Chapter 4 examines the genetic contribution to muscle strength, knee pain, cartilage volume, bone size, and radiographic osteoarthritis (ROA), and assesses whether the heritability of the knee structural components is independent of ROA. A sib pair design was utilised. A sagittal T1-weighted fat-suppressed MRI scan of the right knee was performed to determine cartilage volume and bone size. A standing semi-flexed radiograph of the same knee was performed to assess the presence of ROA. Knee pain was assessed by questionnaire and muscle strength by dynamometry. Heritability was estimated using the genetic analysis program SOLAR. A total of 128 subjects (61 males and 67 females with mean age 45 years) from 51 families representing 115 sib pairs took part. Lower limb muscle strength was found to have high heritability (42%, p=0.02) as did knee pain (44%, p=0.07). Heritability estimates for cartilage volume were 65% for medial tibial, 77% for lateral tibial and 84% for patellar and for bone size were 85% for medial tibial bone area, 57% for lateral tibial bone area and 70% for patella bone volume (all $p<0.01$). For ROA, heritability was 56% for presence with a large standard error (p=0.23) and 63% for severity (p=0.01). The estimates for tibial bone areas only were markedly reduced after adjustment for body size while all estimates with the exception of knee pain were independent of ROA. Cartilage and, to a lesser extent, bone sites were largely under independent genetic control with a lesser-shared genetic component. These results suggests that with the exception of prevalent ROA all knee modalities assessed had high heritability most likely reflecting a strong genetic component. Cartilage volume, bone size and muscle strength all have potential for quantitative trait linkage analyses but their exact relevance for osteoarthritis remains uncertain at this time.

Chapter 5 presents estimates of the heritability of longitudinal changes in knee cartilage volume, chondral defects, subchondral bone size, and lower limb muscle strength. A sibpair design was utilized. Longitudinal changes in lateral and medial tibial cartilage volume and bone size as well as progression of chondral defects were determined on serial T1 weighted fat suppressed MRI images. X-ray was performed and scored for individual features of ROA at baseline. Lower limb muscle strength was measured by dynamometry. Heritability was estimated using SOLAR. A total of 128 subjects (61 males, 67 females, mean age 45 years) from 51 families representing 115 sib pairs were followed for a mean of 2.4 years. The adjusted heritability estimates for changes in cartilage volume were 73% for the medial ($P<0.01$) and 40% for the lateral ($P=0.10$); the adjusted heritability estimates for changes in bone size were 62% for the lateral ($P=0.03$) and 20% for the medial ($P=0.22$); the adjusted heritability estimate for changes in muscle strength was 64% ($P=0.01$). The heritability estimates for progression of chondral defects were 80% for the lateral compartment ($P=0.06$) and 98% for the medial compartment ($P=0.03$). These changed little after adjustment for each other and the predominantly mild ROA, with the exception of lateral compartment chondral defects. These results suggests that early longitudinal changes in knee structures of relevance to later OA such as medial tibial cartilage volume, lateral tibial bone size, progression of chondral defects as well as muscle strength have a high heritability, most likely reflecting a strong genetic component and suggesting their potential to be studied in quantitative trait linkage and association analysis.

Chapter 6 describes clinical, structural and biochemical factors associated with knee pain in younger subjects. A cross-sectional convenience sample of 372 male and

female subjects (mean age 45 years, range 26-61) was studied. Knee pain was assessed by questionnaire. Chondral defects, cartilage volume, and bone area of the right knee were determined using T1-weighted fat saturation MRI. X-ray was performed on the same knee for the assessment of radiographic features of OA. The urinary C-terminal crosslinking telopeptide of type II collagen (CTX-II) was measured by ELISA. Height and weight were measured by standard protocols and body mass index (BMI) was calculated. The prevalence of knee pain was 35% in this sample. Chondral defect scores (particularly femoral and patellar but not tibial) were significantly associated with knee pain in a dose response fashion (all $p < 0.01$). Cartilage volume and bone area were not associated with knee pain in multivariable analysis in this sample. Urinary CTX-II was higher in subjects with knee pain ($p = 0.04$), but this became non-significant after adjustment for BMI and osteophytes (both of which were significant) suggesting potential mechanisms of effect. These results suggest that knee pain is significantly associated with non-full thickness chondral defects (particularly femoral and patellar), osteophytes, CTX-II, and obesity but not other factors. MRI and biochemical measures can add to radiographs in defining unexplained knee pain in younger subjects.

Chapter 7 describes the association between chondral defects, bone marrow lesions, knee and hip ROA and knee pain in older adults. Knee pain was assessed by Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). T1 and T2 weighted fat saturation MRI scans were performed on the right knee to assess chondral defects and subchondral bone marrow lesions. X-ray was performed on the right knee and hip and scored for ROA. BMI and knee extension strength were measured. A total of 500 randomly selected male and female subjects (mean age 63

years, range 50–79) took part. The prevalence of knee pain was 48%. In multivariable analysis, prevalent knee pain was significantly associated with bone marrow lesions (Odds ratio (OR) 1.44/compartment, 95% confidence interval (CI) 1.04 - 2.00), medial tibial chondral defects (OR grade 3 vs.<3 2.32, 95% CI 1.02 - 5.28; OR grade 4 vs.<3 4.93, 95% CI 1.07 - 22.7), hip joint space narrowing (JSN)(OR 1.36/unit, 95% CI 1.07 – 1.73), BMI (OR 1.08/kg/m^2, 95% CI 1.03 - 1.13), and knee extension strength (OR 0.96/kg, 95% CI 0.94 - 0.98) but not knee ROA. These variables were also associated with more severe knee pain. In addition, there was a dose response association between knee pain and number of sites having grade 3 or 4 chondral defects (OR 1.39/site, 95% CI 1.12-1.73) with 100% subjects having knee pain if all five sites had these defects. In conclusion, knee pain in older adults is independently associated with both full and non-full thickness medial tibial chondral defects, bone marrow lesions, BMI, and knee extension strength but not knee ROA. The association between hip ROA and knee pain indicates that referred pain from the hip needs to be considered in unexplained knee pain.

Chapter 8 compares associations between anthropometric and lifestyle factors and femoral head cartilage volume/thickness and radiographic features of OA and provides evidence of construct validity for MRI assessment of femoral cartilage volume and thickness. A cross sectional sample of 151 randomly selected subjects (79 male, 72 female, mean age 63 years) from the Tasmanian Older Adult Cohort Study took part. A sagittal T1-weighted fat saturation MRI scan of the right hip was performed to determine femoral head cartilage volume, thickness, and size. A weight bearing anterior-posterior pelvic radiograph was performed and scored for ROA in the same joint. Other factors measured were height, weight, leg strength, serum

vitamin D levels and bone mineral density. Hip cartilage volume was significantly associated with female sex (regression coefficient β= -0.44 ml, 95% CI -0.87, -0.01), BMI (β= -0.05 ml/kg/m^2, 95% CI -0.08, -0.02), and femoral head size (β = 0.17ml/cm^2, 95% CI 0.10, 0.25) while hip cartilage thickness was only significantly associated with femoral head size (β=-0.03ml/cm^2, 95% CI –0.05, -0.01). Female sex was significantly associated with total ROA score (β=0.95, 95% CI 0.2, 1.7) and JSN (β=0.69, 95% CI 0.04, 1.34) but not with osteophytes. Hip radiographic JSN especially axial JSN but not osteophytes was significantly associated with hip cartilage volume (β = -0.24, p<0.01) and thickness (β = -0.34, p<0.001). In conclusion, femoral head cartilage volume and thickness have modest but significant construct validity when correlated with radiographs. Furthermore, the generally stronger associations with volume compared to ROA suggest that MRI may be superior at identifying risk factors for hip OA.

Chapter 9 examines the optimal sampling of 1.5 mm thick slices of MRI scan to estimate knee cartilage volume in males and females for cross-sectional and longitudinal studies. A total of 150 subjects had a sagittal T1-weighted fat-suppressed MRI scan of the right knee at a partition thickness of 1.5 mm to determine their cartilage volume. Fifty subjects had both baseline and 2-year follow up MRI scans. Lateral, medial tibial and patellar cartilage volumes were calculated with different samples from 1.5 mm thick slices by extracting one in two, one in three, and one in four to compare to cartilage volume and its rate of change. Measurement reliability was assessed by means of the intraclass correlation coefficient (ICC) and Bland & Altman plots. Compared to the whole sample of 1.5mm thick slices, measuring every second to fourth slice led to very little under or

over estimation in cartilage volume and its annual change. At all sites and subgroups, measuring every second slice had less than 1% mean difference in cartilage volume and its annual rate of change with all ICCs ≥ 0.98. In conclusion, sampling alternate 1.5 mm thick MRI slices is sufficient for knee cartilage volume measurement in cross-sectional and longitudinal epidemiological studies with little increase in measurement error. This approach will lead to a substantial decrease in post-scan processing time.

Chapter 10 summaries the findings of this thesis and describes the future direction of the research.

CHAPTER ONE: LITERATURE REVIEW

1.1 An overview of osteoarthritis

1.1.1 Preface

OA is the most common form of musculoskeletal diseases. Its high prevalence, particularly in the elderly, and the resultant physical disability make OA one of the ten most disabling diseases in developed countries [1]. In Australia, the prevalence of self-reported OA is 29% in people over 65 years of age, the overall financial cost of arthritis is approaching AU$9 billion (1.4% of gross domestic product in 2000), and OA accounts for most of this [2-4]. Both the prevalence of OA and the resultant economic burden will increase as the population ages. This section will give an overview of OA regarding its definition, history, pathology, clinical presentations, diagnostic criteria, epidemiology, and aetiology and risk factors and raise questions to be addressed in this thesis.

1.1.2 Definition

The term *"osteoarthritis"* was introduced by John K. Spender in reference to rheumatoid arthritis in 1886 [5] and was not originally used for the disease or diseases to which it is now applied. Joel E. Goldthwait [6] in 1904 made an important contribution in attempting to distinguish OA from rheumatoid arthritis based on radiographic findings of the striking overgrowth of marginal and subchondral bone. A clear distinction of OA was made by Edward H. Nichols and Frank L. Richardson [7] in 1909 based on pathologic examination. They described that the earliest and primary change in the joints was a degeneration of the hyaline cartilage of the articular surfaces. For many years, OA was erroneously regarded as a simple, degenerative, "wear and tear" phenomenon, an inevitable disease of aging. Expanded research has demonstrated significant differences between the aging process and OA

[8]. In 1986, the subcommittee on *Osteoarthritis of the American College of Rheumatology Diagnostic and Therapeutic Criteria Committee* proposed the following definition of OA [9]:

> A heterogeneous group of conditions that lead to joint symptoms and signs, which are associated with the defective integrity of articular cartilage, in addition to related changes in the underlying bone at the joint margins.

Over recent years, there has been increasing acceptance that OA may represent not one specific disease but rather a set of disease subtypes that lead to similar clinical and pathologic alterations. The current definition, which was developed in 1994 at a workshop entitled "*New Horizons in Osteoarthritis*" sponsored by the *American Academy of Orthopedic Surgeons, the National Institute of Arthritis, Musculoskeletal and Skin diseases, the National Institute on Aging, the Arthritis Foundation, and the Orthopaedic Research and Education Foundation*, underscores this concept [10]:

> OA is a group of overlapping distinct diseases, which may have different aetiologies but with similar biologic, morphologic, and clinical outcomes. The disease processes not only affect the articular cartilage, but also involve the entire joint, including the subchondral bone, ligaments, capsule, synovial membrane, and periarticular muscles. Ultimately, the articular cartilage degenerates with fibrillation, fissures, ulceration, and full thickness loss of the joint surface.... OA diseases are a result of both mechanical and biologic events that destabilize the normal coupling of degradation and synthesis of articular cartilage chondrocytes and extracellular matrix, and subchondral bone. Although they may be initiated by multiple factors, including genetic, developmental, metabolic, and traumatic, OA diseases are manifested by

morphologic, biochemical, molecular, and biomechanical changes of both cells and matrix which lead to a softening, fibrillation, ulceration, loss of articular cartilage, sclerosis and eburnation of subchondral bone, osteophytes, and subchondral cysts. When clinically evident, OA diseases are characterized by joint pain, tenderness, limitation of movement, crepitus, occasional effusion, and variable degrees of inflammation without systemic effects.

1.1.3 History

OA is the most common form of arthritis in humans and almost 80% of the population will have radiographic evidence of OA in at least one joint by the age of 60 [11]. However, OA is not a modern disease. It appears to have been a constant companion of people throughout antiquity. Radiographic evidence of OA has been found in skeletons from prehistoric Old World sites and in remains of New World societies [12]. A skeleton of a Neanderthal man revealed severe arthritis of the knees and spine consistent with OA. Skeletons of Java and Lansing man from 500,000 years ago and skeletons of modern man from Neolithic Europe also revealed changes suggestive of OA. Based on mummified remains, Egyptians appear to have been affected by the disease as long ago as 8000 BC [13].

The first written description of OA dates to the eighteenth century. William Heberden, an English physician born in London in 1710, developed a special interest in joint diseases. In his *Commentaries* [14] published posthumously in 1802, he gave the following account of "digitorum nodi" which are now known as Heberden's nodes:

> "What are those little hard knobs, about the size of a small pea, which are frequently seen upon the fingers, particularly a little below the top, near the joint? They have no connection with the gout, being found in persons who never had it; they continue for life; and being hardly ever attended with pain, or disposed to become sores, are rather unsightly, than inconvenient, though they must be some little hindrance to the free use of the fingers."

This was the first description of OA and the first recognition that these nodes differed from gouty tophi. In 1884, Charles J. Bouchard described nodes adjacent to the proximal interphalangeal joints that are identical to those Heberden had described distally, which are now known as Bouchard's nodes [15]. In 1941, Stecher [16] observed that Heberden's nodes were three times as common in the sisters of 64 affected subjects as in the general population. Further, he concluded that these lesions were inherited as a single autosomal dominant gene with a strong female predominance and he made a most valuable contribution by separating the post-traumatic type of node from the idiopathic inherited variety [16 17].

The recognition that OA could be a polyarticular disease occurred in the eighteenth century. In 1805, John Haygarth [18] first described 34 cases of multiple arthritis associated with Heberden's nodes that he classified separately under the heading of "nodosity of joints". He remarked that the disease was more common in women, occurred after menopause and had a prevalence of 1 in 310 in his patient population. Similar descriptions were made by Cecil and Archer in 1926 [19]. They observed that 145 of 182 cases of degenerative arthritis attending their rheumatism clinic were polyarticular and usually associated with Heberden's nodes and linked this form of

arthritis with menopause [19]. In 1952 Kellgreen and Moore provided the classical description of this disease by studying 391 cases of OA attending their rheumatic clinic and suggested the name of primary generalized OA for this distinct clinical entity [20]. They remarked that the condition occurred most often in middle-aged women, and was characterized by a distinct pattern of joint involvement, by a course in which each affected joint passes through an initial painful and more or less acute arthritic phase, and by other distinctive clinical and radiological features [20]. This helped the differentiation of two main types of OA: first a 'secondary' form in which trauma or some other joint insult leads to OA of one or more joint sites; then a 'primary' form of the disease, mainly affecting women, in which multiple joints were affected, including the hands [21].

Subsequently, Kellgren and Lawrence described a classification for grading knee radiographs for OA [22], providing further important landmarks in the history of OA. A five-point scoring system for grading radiographs [23] was subsequently adopted by the World Health Organization (WHO) and became the gold-standard in the everyday assessment of OA although its flaws have since led current researchers to devise new grading systems based on individual features of the disease [24-26]. Lawrence also conducted the first systematic epidemiological study of OA and contributed to the crucial observation that there can be a poor relationship between radiographic features and symptoms [11]. It still remains unclear what factors control symptom development in OA-affected joints.

In 1986, the *American Rheumatism Association* (now the *American College of Rheumatology*) published its first set of criteria for diagnosing knee OA clinically

without the use of radiographs based on a study of 130 cases with symptomatic OA of the knee and 107 controls with knee pain due to rheumatoid arthritis or other aetiology [9]. The same approach was employed for developing criteria for diagnosing OA of hand and hip thereafter [27 28]. Because the major inclusion criteria are joint pain on most days of the prior month, these diagnosing criteria identify patients with clinically important OA, in contrast to the criteria based on radiographic features alone. Further, Altman modified the criteria sets into algorithms, facilitating their use in clinical research and population-based studies [29].

Over the last few decades, much has changed in our understanding of OA. It has been clearly distinguished from rheumatoid arthritis, gout, ankylosing spondylitis, although the predisposition of patients with such inflammatory arthritis to the subsequent development of OA is appreciated [13]. Progress in our fundamental understanding of OA is occurring at a rapid pace. It is now recognized that OA is a syndrome with many complex aetiologies rather than a single disease entity [10]. With advanced studies, clear definitions of etiopathogenesis and pathophysiology of OA will lead more specific modalities of therapy.

1.1.4 Pathology

Although the aetiology of OA remains elusive, the pathology of OA has been extensively studied. By definition as described in **1.1.2**, OA is now considered as a group of diarthrodial joint diseases with different aetiologies but the same pathological process of eventually non-reversible architectural and compositional joint tissue changes that progress toward the functional failure of the joint. Nicholas and Richardson first distinguished pathologic changes of OA from rheumatoid

arthritis in 1909 [7]. Since then, significantly expanded research efforts and the introduction of new methodologies have led to major advances in understanding the pathology of OA. Among significant findings is the distinction in joint tissue changes between OA and aging, highlighting that OA is neither a disease of aging nor an inevitable consequence of aging of the joint, although age is strongly associated with the development of OA [30-32]. OA can also be distinct pathologically from joint injury that results in complete tissue restitution and from changes that result from pure mechanical injury, primary synovial inflammation [33]. Table 1.1.1 is reproduced from Pritzker [33] and illustrates the selected differences among the histopathologic features of OA, aging, material failure of joint tissues, and inflammatory arthritis.

Table 1.1.1 OA: Comparative histopathologic features

Feature	OA	Reversible injury	Aging	Inflammatory arthritis	Mechanical (loading to failure)
Cartilage mass	Hypertrophy, erosion	Hypertrophy	No change	Resorption, atrophy	No change
Cartilage topographic distribution	Focal, heterogenous	Focal	General, all layers	Joint margins and superficial zone most affected	Focal: at site of forces
Cartilage water	Oedema	Oedema	Dehydration	Dehydration	No change
Cartilage collagen	Pericellular degradation, interterritorial matrix degradation	Reversible deformation	↑ Advanced glycation endproducts	Degradation maximal at joint margin and superficial zone	Fiber fracture
Cartilage proteoglycan	PG depletion, not reversible	PG depletion, reversible	↓ PG synthesis	PG depletion not reversible	No change
Cartilage matrix degeneration products	Accumulative, collagen, PG, etc.	Resorption	Accumulative: oxidation, glycation, amyloid	Accumulative, collagen	No change
Cell activity	↑ cell activity, ↑ cell proliferation	↑ cell activity, reversible	↓chondrocyte activity	↑ synovial cell activity, ↓ chondrocyte activity	Chondrocyte death
Synovium	Mild, focal superficial inflammation	Mild focal superficial inflammation	Atrophy	Intense, general inflammation	Haemorrhage
Bone	Subchondral remodelling	No change	Osteopenia	Subchondral resorption	Microfracture

1.1.5 Clinical presentations

By definition as described in **1.1.2,** OA is a complex, heterogeneous condition. It is, therefore, not surprising that OA has a variable clinical presentation and a variety of patterns of expression in terms of timing of onset, pattern of involvement, and severity. Prognosis and outcomes in different patients and at different joint sites are similarly variable [34 35]. Table 1.1.2 summaries the clinical manifestations and signs of OA that most affected joints have in common.

Table 1.1.2. The common symptoms and signs of OA

Symptoms	Signs
Pain	Crepitus
Stiffness	Restricted movement
Functional impairment	Tenderness - Joint line - Periarticular
Sensation of insecurity or instability	Deformity

Symptoms of OA

Pain is undoubtedly the most important clinical symptom of OA and the usual reason for seeking medical advice. The onset is gradual or insidious, and the pain is usually mild in intensity, but worsens by using the involved joint(s), and improves or is relieved with rest. Initially, the pain may be intermittent and self-limited; pain at rest or during the night is a feature of severe disease [36].

The mechanism of pain remains unclear but is believed to be multifactorial. There is a discrepancy between degree of joint structure changes assessed on radiographs and reporting of pain in OA. Despite the poor relationship between pain and radiographic changes [37-40], the correlation between pain and radiographic features of OA is closest at the hip, then the knee, and is worst for hand and spinal apophyseal joints [11]. This may be due partly to the fact that use-related pain is the most frequently described pain in OA [35]. On the other hand, the fact that radiographs are a semi-quantitative measure that only permit limited assessment of the joint structure and poorly characterize the soft tissues may also contribute to this poor relationship between pain and radiographic changes.

As mentioned before, pain in OA is multifactorial and most likely originates from multiple sources such as the synovial membrane, joint capsule, periarticular

ligaments or muscle, periosteum, and subchondral bone as nociceptive fibres are present in these structures [41]. Recent studies on knee OA using MRI, which allows us to visualize the soft tissues of the joints, provide evidence that there is a significant association between knee pain and knee effusions, popliteal cysts, synovial thickening, and bone marrow oedema [42 43]. This has expanded our understanding of causes of pain in OA. Normal hyaline cartilage does not possess pain fibers, suggesting that articular cartilage cannot be the origin of knee pain. However, substance P nociceptive fibres have been found in abnormal cartilage such as erosion channels in horse OA [44], and superinduction of cyclo-oxygenase – 2 (COX-2) and prostaglandins (PGs) has been observed in OA-affected cartilage explants [45], suggesting that articular cartilage may directly produce pain. In the longitudinal evaluation of chondropathy arthroscopically in 41 patients with knee OA, Ayral et al [46] found that changes in cartilage breakdown over one year were significantly correlated with changes in Lequesne's functional index ($r=0.34$; $p=0.03$) which includes the presence of pain [47]. In a study of 120 middle aged women, Sowers et al [48] reported that women with radiographic OA, full-thickness articular cartilage defects, and adjacent subchondral cortical bone defects were three times more likely to have painful knee OA than other groups. In the study of 133 postmenopausal females, Hunter et al [49] linked lower patellar cartilage volume to knee pain assessed by the WOMAC. In a longitudinal study of 132 subjects with symptomatic, early (mild to moderate) knee OA, Wluka et al [50] reported that increased tibial cartilage volume loss measured by MRI was significantly associated with worsening of pain as assessed by the WOMAC. However, it remains unclear whether involvement of underlying bone is necessary for pain or whether lesser degrees of chondral damage can directly lead to pain. The thesis will test this hypothesis as one of its objectives

and examine the association between knee pain and MRI based measurements of the joint structural abnormalities.

In addition, pain in OA can also be related to other factors. Women may be more likely to report pain although the strength of this relationship varies between studies and between joints [11 51 52]. Psychological factors such as anxiety and depression have been correlated with pain in OA [53 54].

Stiffness is reported in most OA patients. It may vary in meaning from slowness of joint movement to pain on initial movement such as getting up from a chair [34]. Morning stiffness is commonly reported, but the most characteristic feature of joint stiffness in OA is the phenomenon of gelling after inactivity. This appears to be a problem of getting the joint to move after a period of rest. Stiffness is generally short-lived, compared to the more prolonged, often generalized stiffness of inflammatory arthropathy [34]. The duration is often less than 30 minutes and it is usually confined to a small number of affected joints [35].

Functional impairment in OA patients contributes an enormous health burden to our community [55]. Disability may include poor mobility, difficulty with activities of daily living, social isolation, and loss of work opportunities with consequent financial consequences [34]. The causes of functional impairment vary in different patients. Pain can be a major cause of reduced function [56], but other factors may also be important. In a study of disability in knee OA, quadriceps muscle weakness appears to be more strongly correlated with functional problems than pain or the

degree of radiographic change [57]. Reduced range of joint movement may also be a principal feature or a contributor to overall disability in OA [34].

A sensation of insecurity or instability in affected joints is also a complaint in patients with OA. This symptom is not necessarily accompanied by any objective evidence of ligamentous instability or significant joint destruction. However, muscle weakness is usually apparent, and it seems likely that this symptom is due more to diminished strength and functioning of the muscles than to mechanical abnormalities of the joints [35].

Signs of OA

On physical examination, findings are usually localized to symptomatic joints and vary with the severity of disease. Table 1.1.2 lists the common signs of OA. Some of them are incorporated into classification or diagnostic criteria for individual joints.

Coarse crepitus is typically palpable over a wide area of the joint and is felt throughout the range of movement, and stands out as one of the best signs in the clinical differentiation of OA from other diseases [9]. It is present in more than 90% of patients with OA of the knee [36] and probably due to the roughening of the joint surface and outgrowths at the rim of the joint interfering with the normally smooth movement between the joint surfaces. Cavitation or the formation of gas bubbles within the synovial fluid may also contribute [35].

Limitation of motion in the affected joints is extremely common in patients with OA. The likely explanation is that the chondrophytic and osteophytic lipping and

remodelling of the joint, combined with the capsular thickening, is preventing a free range of movement [35].

Tenderness to palpation along the joint-line suggests a capsular/intracapsular origin of pain. Point tenderness away from the joint-line suggests a periarticular lesion; pain on resisted active movements and /or stress tests may further localize the involved periarticular structure. Periarticular lesions such as bursitis and enthesopathy commonly accompany large joint (knee, hip) OA. They may be the principal cause of pain and are often readily amenable to local treatment [34].

Deformity is a sign of advanced OA, with severe cartilage loss, osteophyte, remodelling, and bone attrition. Damage confined to the medial tibial compartment may lead to a varus angulation of the knee joint. Bone destruction may lead to leg shortening in hip diseases [35]. Although deformities at individual sites may be highly characteristic of OA, none are specific [34].

1.1.6 Diagnostic criteria

Radiographic criteria

Radiographs have been used for distinguishing OA from other diseases such as rheumatoid arthritis since the beginning of the 20th century [6]. The first criteria for definition of radiographic OA were developed in 1957 [22]. By studying randomly selected 85 subjects aged between 55 and 64 years, Kellgren and Lawrence developed an ordinal 5-point grading system by amalgamating the following radiographic features as evidence of OA: (1) The formation of osteophytes on the joint margins or, in the case of the knee joint, on the tibial spines; (2) Periarticular

ossicles; (these were found chiefly in relation to the distal and proximal interphalangeal joints); (3) Narrowing of joint cartilage associated with sclerosis of subchondral bone; (4) Small pseudocystic areas with sclerotic walls situated usually in the subchondral bone; (5) Altered shape of the bone ends, particularly in the head of femur.

The grading system was later accepted as standard criteria by the WHO at a symposium held in Milan in 1961 [58]. It is fairly simple, not time consuming, and has been shown to be reproducible in several studies [59]. It has been extensively used in epidemiological studies and provided the basis for much of our understanding of OA. However, there are several limitations. These include inconsistencies in the descriptions of radiographic features of OA, the prominence awarded to the osteophyte at all sites, the unproven assumption that the grades correspond to stages in the development of disease, and failure to correspond directly with symptoms and disability [26].

Attempting to address these limitations, several research groups developed alternative scoring systems that mostly focus on the individual radiographic features that represent various aspects of cartilage loss and subchondral bone reaction in OA.

Spector et al [60] introduced a scoring system for individual features of knee OA based on features in both the tibiofemoral and the patellofemoral joints. These included tibiofemoral joint osteophytes, JSN, sclerosis, cortical collapse, patellofemoral joint space narrowing, and osteophytes. This is the first radiographic scoring system to

include evaluation of the patellofemoral joint, the importance of which had been probably underestimated previously.

Altman et al [24] developed a method of scoring knee OA according to the following individual radiographic features: JSN, osteophytes, sclerosis, alignment, and bony attrition. This method represents a first attempt to compare the methodological properties of the various features of OA. The comparison favours a combined score of several features, but in contrast to the Kellgren & Lawrence grading system it gives them equal weight [61].

Altman et al [24] also applied individual radiographic features to evaluate radiographic OA of the hand. The radiographic features considered most important in the hand included osteophytes, JSN, and periarticular subchondral erosions. Additional features include periarticular subchondral sclerosis and joint malalignment without subluxation.

Kallman et al [62] evaluated similar grading scales for individual features of hand OA using the five distal interphalangeal (DIP) joints, four proximal interphalangeal (PIP) joints, the first metacarpalphalengeal joint, and the trapezoscaphoid joint of both hands. The features included joint space narrowing, osteophytes, sclerosis, lateral deformity, and cortical collapse.

For hip OA, several alternatives to the Kellgren & Lawrence grading system have been proposed. Danielsson [63] proposed that radiographic classification of hip OA should be based on the presence of JSN or structural changes (subchondral sclerosis

or cysts), or both, but not on osteophytes alone. Croft et al [25] proposed that a single measurement of minimal joint space (the shortest distance between the femoral head margin and acetabulum) is the best radiographic criterion for use in epidemiological studies. However, one study suggests that narrowing and osteophytes are independent predictors of hip pain [64], arguing for a disease definition based on more than just a measure of joint space. Altman et al [24] introduced a method of scoring hip OA with individual features which include JSN, subchondral lucencies, marginal osteophytes, subchondral sclerosis, and femoral buttressing.

Other grading systems have also been published [65-68]. The results of reliability studies have been reviewed by Lane et al [69] and Sun et al [70]. A single experienced reader in a standardized setting can reliably classify subjects as having radiographic OA at individual joints. While the Kellgren and Lawrence grading system remains important to determine prevalence of OA in specific joints, particularly in prevalence comparison studies, the evaluation of OA by individual radiographic features allows for the characterization of the variation in OA [69].

Clinical criteria

Certainly, the presence of OA carries a definite predisposition to symptoms in the affected joints and this predisposition is related to the extent of radiographic features. However, there are potential limitations to the use of only radiographic criteria for case definition, especially in clinical research studies of OA, because most people with the disease have no symptoms although a significant correlation does exist between radiographic features and symptoms of OA in the affected joints [11].

In 1981, the *Subcommittee on Osteoarthritis of the American College of Rheumatology Diagnostic and Therapeutic Criteria Committee* was established to develop clinical criteria for the classification of OA and subsequently published sets of classification criteria for OA of the knee, hand, and hip. Altman modified the criteria sets into algorithms, facilitating their use in clinical research and population-based studies [10]. The shortcoming is that these criteria were based on the data derived from comparison between hospital patients with a diagnosis of OA and those who had inflammatory joint disease. Therefore, they have limited applicability to community-based epidemiological studies [71].

1.1.7. Epidemiology

Prevalence

Prevalence refers to a proportion of the population that has a disease at a specific point in time. It reflects both the incidence rate and the probability of surviving with disease, and represents the disease burden in a population. Prevalence of OA has been extensively studied.

The earliest population survey of OA was conducted in UK [11]. In this study, 2296 males and females aged 15 years or over were randomly selected from Leigh and Wensleydale areas, North England, and had x-rays taken of their hands, feet, knee, hip, and spine. OA at a specific joint was defined as Kellgren & Lawrence score ≥ 2. In this sample, 52% of males and 51% of females had at least one joint affected with ROA. The prevalence was slightly greater among males than females at ages less than 55 years, but the sex difference was reversed for older subjects. Females were more likely affected in multiple joints than males 25% of females but 17% of males

had at least three joints affected with ROA, while 12% of females but 7% of males had at least five joints affected. Using the same case definition, prevalence of OA of 22 joints including hand, knee, hip, feet, shoulder, and spine was surveyed in a random population sample of 6585 inhabitants of Zoetermeer in the Netherlands [72]. The prevalence of OA in all joints increased strikingly with age. It was uncommon in people under age 40, but extremely common in those above age 60, and 75% of females aged 60-70 years had OA of their DIP joints. Apart from the hands, spine, knees, and hips were more likely affected than other joints. A similar pattern was reported by other population-based studies [73-75] conducted in Europe although a different case definition was used in those studies and the prevalence estimates were slightly different.

The prevalence of OA in the U.S population is reported to be comparable to that in Europe. In the Health Examination Survey [76], 37% of 6672 subjects aged between 18 and 79 years had ROA of the hand or feet; of them, 23% were in the moderate or severe stages. The prevalence increased steadily with advancing age from 4% among young adults to 85% in the oldest age group. Comparison with other surveys conducted in the U.S. suggests that prevalence of hand OA is substantially higher in Blackfoot and Pima Indians, but lower in Eskimos, than in the general US population [76].

Similarly, in the first National Health and Nutrition Examination Survey (NHANES) [77], prevalence of knee OA increased gradually with advancing age from 1.6% in people under 44 years to 13.8% in people aged 65 years or over. The prevalence estimates were even higher in other studies [59 78 79]. Women had a higher prevalence

than men among those aged 55 years or older, while black American women had a higher prevalence of knee OA than white Americans [78] [80]. This was reported in another study [81] in which blacks had higher prevalence of knee OA than whites in England. In contrast, consistently low prevalence of hip OA among black populations from Jamaica, South Africa, Nigeria, and Liberia (1-4%) has been reported [82]. Recent studies using the same study protocol have revealed that Chinese people in China have a substantially lower prevalence of OA of the hip and hand than whites in US, while only Chinese women have higher prevalence of knee OA than white women [83-85]. Similar results were found when comparing the prevalence of ROA of the hand and knee between Japanese and Caucasians [86].

In Australia, the National Health Survey was conducted in 2001 [2]; Approximately 26,900 people from all states and territories and across all age groups were included. Prevalence of self-reported OA was 6% in people aged 15-64 years and 29% in people aged 65 years old or over. In Australians older than 65 years, women had a higher self-reported OA than men (35% vs. 21%), and indigenous Australians were more than twice as likely to report having OA than non-indigenous Australians [2]. However, there have been no population-based studies that have systematically evaluated the prevalence of symptomatic and radiographic OA in Australia.

Genetic variation and lifestyle may be implicated by the differences in the prevalence of OA between different ethnic groups in the same population and populations of different races. Inconsistencies in study design, sampling procedure, and case definition make the interpretation difficult, however. In a recent survey of 4151 subjects conducted in Copenhagen, Denmark [87], use of three definitions of hip OA

produced different results. Prevalence of hip OA was higher in men than women if defined by Kellgren/Lawrence score or Croft global score, but equalized if defined by minimum joint space width (JSW) <=2mm, suggesting that it is necessary to use a same case definition when comparing prevalence of the disease across populations.

Notwithstanding these varying estimates of the prevalence of OA across populations, OA is already one of the ten most disabling diseases in developed countries. Worldwide estimates are that 9.6% of men and 18.0% of women aged over 60 years have symptomatic OA, 80% of those with OA have limitations in movement, and 25% cannot perform their major daily activities [1].

Incidence

Incidence refers to the number of new occurrences of the disease in a population over a period of time. Cumulative incidence provides an estimate of probability that a person will develop the disease during a given period of time. In contrast to the prevalence studies, there are only few studies reporting incidence of OA.

In the Framingham Osteoarthritis Study, 751 subjects with a mean age of 55 years at baseline were included in a study of hand radiographic OA defined by Kellgren/Lawrence score ≥ 2 [88]. The cohort was followed across a 24-year period and the crude cumulative incidence was 83% for at least one right hand joint developing ROA. Women were more often affected than men (87% vs. 76%). The most frequently affected joint was the distal interphalangeal joint, followed by the base of the thumb, proximal interphalangeal, and metacarpophalangeal joints. In the same study cohort, 869 subjects with a mean age of 70 years at baseline were

included for an incidence study of knee OA and this cohort was followed up for a mean period of 8.1 years. The cumulative incidence of ROA (defined by Kellgren/Lawrence score ≥ 2) of the knee was 15.6%, and women had higher incidence than men (18.1% vs. 11.1%). Similarly, the cumulative incidence of symptomatic knee OA was higher in women (8.1%) than in men (4.3%). Similar results were also reported in the Chingford study [89].

In the Rotterdam Study [90], 875 subjects aged 55 years or over were followed up for a mean period of 6.6 years period. The cumulative incidence of ROA of the hip, defined as a decrease of joint space width of the hip (>/=1.0 mm) at follow-up, was 9.3%.

In a study describing the incidence of symptomatic hand, hip, and knee OA from a Massachusetts health maintenance organization [91], the age- and sex-standardized incidence rate for symptomatic hand, hip, and knee OA was measured. It and all increased with age but with some decrease in survivors aged 80 years or over (Figure 1.1.1). Women had higher incidence for each joint than men after the age of 50. Between 70-89, the knee OA rates among women reached a maximum incidence of 1% per year.

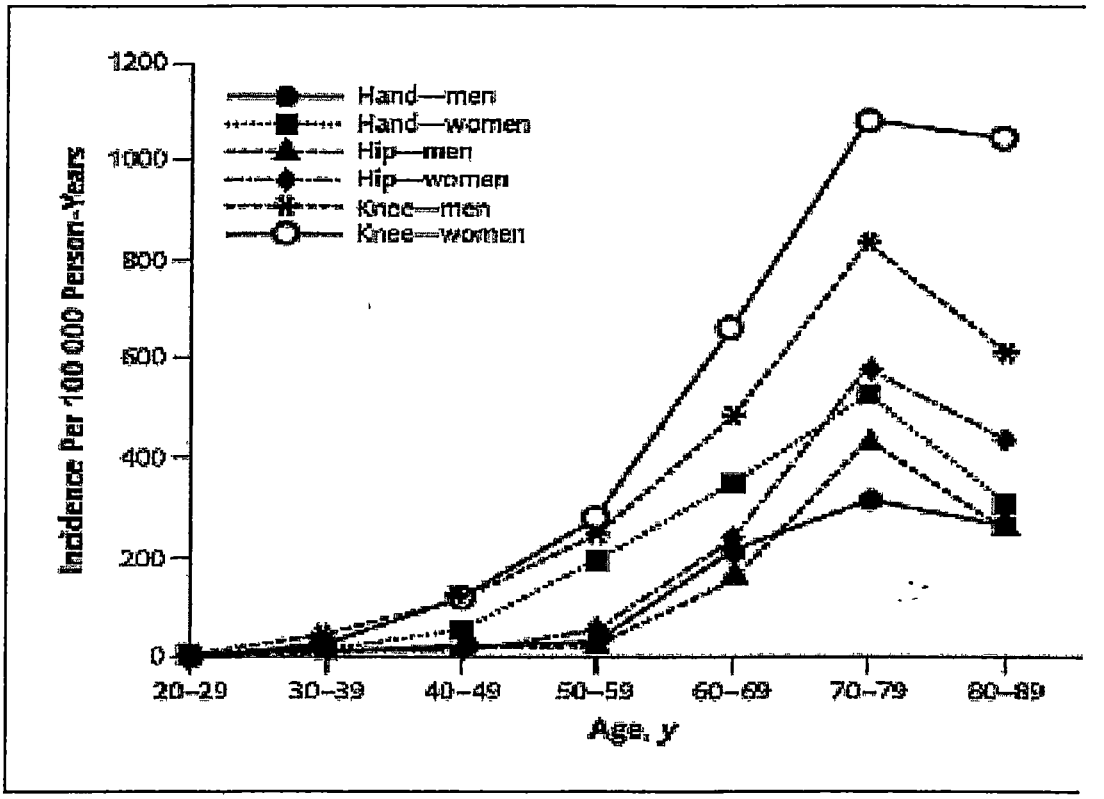


Figure 1.1.1. Incidence of symptomatic osteoarthritis of the hand, hip, and knee in members of the Fallon Community Health Plan, 1991-1992, by age and sex. (Adapted from Oliveria SA et al, 1995)

1.1.8 Aetiology and risk factors

Although its aetiology remains elusive, OA has been recognized as a multifactorial and complex disease. Evidence is growing for the role of systemic factors and of local biomechanical factors in the development of OA.

Age: Age is the strongest risk factor in the development of OA regardless of the joint sites. Both prevalence and incidence of OA in all joints increase strikingly with advancing age as described in the previous section. By the age of 60, almost 80% of the population will have radiographic evidence of OA in at least one joint [11]. This has led to an impression that OA is an inevitable disease of aging and it is only a matter of time and anyone who lives long enough will eventually have the disease.

However, research available to date does not support this common view. Although it is still unclear, some individuals develop OA early in life whereas others maintain normal cartilage morphology and function up to advanced age. Significant variability exists in the degree of involvement in different joints. While OA is common in the hands, spine, hips, and knees, it is relatively rare in wrists, shoulders, and ankles [30]. Furthermore, a study of articular cartilage demonstrated that denatured type II collagen is more predominant in OA-affected cartilage than in normal aging cartilage; OA-affected and normal aging cartilage differ in the amount of water content and in the ratio of chondroitin sulfate to keratan sulfate constitutes. Degradative enzyme activity is increased in OA, but not in normal aging cartilage [92] [93].

While aging alone may not directly cause OA, it facilitates and predisposes people to the development of OA together with other risk factors (eg. overweight, joint injury, genetic factors, and so on) present [30]. This is probably related to associated biologic changes, including the decreased responsiveness of chondrocytes to growth factors that stimulate repair, an increase in the laxity of ligaments around the joints making older joints relatively unstable and more susceptible to injury, and a failure of major shock absorbers or protectors of the joint with age [94].

Gender: Among subjects younger than 50 years, the prevalence and incidence of OA in most joints is higher in men than women. Among older subjects, women are more often affected with OA of the hand, foot, and knee than men [91 95 96].

OA in women occurs not only more frequently but also with more severity. A meta-analysis of 34 population-based OA studies recently conducted by us [97] demonstrated that knee OA is significantly more severe in women than in men among those aged 55 years or over. There is no evidence that women have more severe hip and hand OA than men, however.

Multiple joint involvement of OA is also more prevalent in women than in men. In a survey of 2296 males and females aged 15 years old or over, Lawrence et al [11] reported that 25% of females had at least three joints affected with ROA compared to 17% of males, and that 12% of females had at least five joints affected compared to 7% of males.

This susceptibility of older women to the development of OA suggests that estrogen deficiency plays a role in causing the disease. Indeed, both cross-sectional [98] and longitudinal [99] studies with relatively large samples have demonstrated that estrogen replacement therapy (ERT) is associated with significantly reduced risk of either hip or knee OA. This risk reduction was more pronounced in those on ERT 10 years or longer, suggesting that ERT have potential protective effect on the development of OA. However, other studies[100] [101] reported ERT was associated with an increased relative risk of OA, questioning the protective effect of hormone therapy.

Genetics: Familial clustering of OA was documented more than half a century ago. In 1941, Stecher [16] reported that sisters of 64 females with Heberden's nodes in the distal and proximal interphalangeal joints, and carpometacarpal joints of the hand, were three times more likely than the general population to exhibit nodal OA

beginning in the fifth decade of life. Further, he concluded that these lesions were inherited as a single autosomal dominant gene with a strong female preponderance [102]. More recently, Livshits et al [103] conducted a segregation analysis of ethnically homogeneous pedigrees in the Russian Federation and the results were supportive of the hypothesis of a major gene effect plus multifactorial component. The estimates obtained using the standard three-factor variance decomposition analysis suggested that age (72.8%) and major gene (14.5%) were the main sources of interindividual differences in the development of hand OA. The contribution of the putative major gene on age- and sex-adjusted OA phenotype variation was 55%.

In a study that compared 181 first-degree relatives of 20 males and 32 females with definite OA in six or more groups of joints with a random sample of the population examined in the same way and at the same time, Kellgren et al [104] reported that the frequency of multiple joint OA was nearly twice as high in these relatives than in the general population. Further, they concluded that the more severe forms of the generalised OA appeared to be more closely associated with Heberden's nodes. The genetic contribution to generalized OA has been confirmed by classic twin studies and other family based studies. The most frequently examined combination of joints was hand and knee, and the heritability estimates (defined as the proportion of the variance in the development of OA that is due to the genetic factors) ranged from 30 to 78% [105-108]. The segregation analysis in the Framingham Study suggested that the most likely pattern of inheritance of generalized ROA was that of a major Mendelian recessive gene with a residual multifactorial component [108].

Genetic factors also play a role in the development of hip OA. In the study of 135 monozygotic and dizygotic 277 healthy female twin pairs, MacGregor et al [109] reported significant heritability estimates of 58% for overall hip OA and 64% for JSN. This significant genetic contribution to the development of hip OA was confirmed by sibling studies. The relative risk for siblings of subjects with hip OA ranged from 3.9-6.4 [110 111].

With regard to knee OA, although significant genetic contribution has been reported for knee OA in combination with hand OA [104 106-108], the results for isolated knee OA are conflicting. In a study of 307 female twins, Spector et al [107] first reported a significant genetic control in the development of knee OA with a heritability estimate of 39% for the quantity of disease. In a sibling study, Neame et al [112] reported a heritability of 62%. Similar results were reported by two other studies [113 114] although a different case definition was used. However, these results have not been replicated in other studies. Data from the Baltimore Longitudinal Study on Aging found no significant sib-sib correlation for knee OA [106]. In the study of 257 siblings of 118 probands with multiple OA-affected joints, Bijkerk et al [105] found ROA of the knee was not statistically significantly correlated in family members. In another study, Riyazi et al [111] reported that siblings of probands with OA in the knee did not have an increased likelihood of knee OA. Interpreting these conflicting results is difficult because of the different case definitions used. Using total knee replacement may not be informative for the aetiology of less severe knee OA whereas using Kellgren/Lawrence radiographic score gives weight to osteophytes and may underestimate the importance of genetic influences on articular cartilage. In addition, the knee is a complex joint and the development of the knee OA involves all the joint

tissues. Genetic factors may play a particular role in specific tissues in the pathogenesis of knee OA. To date, there has been no data on the genetic influence on individual structures in the development of knee OA, partly because there was no non-invasive method directly to assess individual knee tissues until the recent advent of MRI, which allows direct visualisation of individual joint tissues, especially articular cartilage. Based on MRI assessment, this thesis will examine the genetic contribution to individual structures of knee and their change over time as one of its objectives.

Once a substantial genetic basis for a disease is established, identification of the responsible genes becomes the logical next step. Two approaches are currently being applied to the search for disease genes in OA: genome-wide linkage studies and candidate gene association studies. In the near future genome-wide association studies with hundreds of thousands of markers may also be feasible [115].

A number of genome-wide linkage scans in various OA populations have been conducted [116-121]. Several regions have been identified as harbouring susceptibility genes for OA, particularly for hand, hip or generalized OA. Some of these susceptibility regions are replicated in other studies, but most are not. Consequently, it is expected that a number of genes could contribute to OA [122]. However, the specific underlying genetic factors and mechanisms in the development of OA remain to be elucidated. Association studies of candidate genes have been carried out for type II collagen (COL2A1) [123-128], vitamin D receptor (VDR) [129-133], type I collagen (COL1A1) [131 134], estrogen receptor α (ESR1) [131 135 136], insulin-like growth factor 1 (IGF-I) [137 138], transforming growth factor β (TGFB1) [139], and aggrecan [140].

Some of these studies demonstrated positive associations between genetic variation at these loci and OA-related phenotypes, while others failed to replicate the initial reports, as frequently occurs in candidate gene studies of complex disorders [141]. Interpretation of these results is difficult because lack of replication can arise from a variety of sources including the presence of hidden stratification in some populations, different allele frequency distributions or linkage disequilibrium (LD) structures across different populations, allelic/locus heterogeneity, differences in study design, publication bias, as well as the tendency of investigators to perform preliminary studies or replication studies that are statistically underpowdered [142 143]. Using the recent proposed gene-based approach [143], in which all common variation within a candidate gene is considered jointly, may be less susceptible to these potential problems and replication may become feasible. However, it requires detailed knowledge of genetic variation in coding sequences as well as regulatory and other regions affecting gene function, which is not generally available at present. Study of functional alleles in the candidate genes, once identified, may speed understanding of OA.

Obesity: Obesity is perhaps the strongest modifiable risk factor for the development of OA. There is a great deal of evidence substantiating the association between obesity and the incidence and progression of knee OA. The data from the first NHANES [80], the Chingford study (both cross-sectional and longitudinal data) [144 145], and the longitudinal Framingham study [146], all demonstrated that obesity was significantly associated with an increased risk for the knee OA. The relative risk ranged from 2.07 to 4.8. This association was stronger for women than for men [146]. Moreover, Felson et al [147] reported in the longitudinal Framingham study that weight

change significantly affected the risk for the development of knee OA in women. A decrease in BMI of 2 kg/m^2 or more (weight loss, approximately 5.1 kg) over the 10 years before the examination decreased the odds for developing OA by over 50% (odds ratio, 0.46; 95% CI, 0.24 to 0.86; P = 0.02). Among those women with a high risk for OA due to elevated baseline body mass index (greater than or equal to 25), weight loss also decreased the risk (for 2 kg/m^2 of BMI, odds ratio, 0.41; P = 0.02).

The influence of obesity on the development of hip OA was systematically reviewed by Lievense et al [148]. Five longitudinal and seven cross-sectional studies were included in their review. The associations between obesity and hip OA were stronger in studies in which the diagnosis of hip OA was based not only on radiological criteria but also on clinical symptoms. Overall, moderate evidence was found for a positive association between obesity and the occurrence of hip OA, with an odds ratio of approximately 2.

With regard to the association between obesity and hand OA, the study results are inconsistent. In the Tecumseh Community Health Longitudinal Study of 1276 participants aged 50-74 years at the follow-up, Carmen et al [149] reported that baseline obesity, as measured by an index of relative weight, was found to be significantly associated with the 23-year incidence of OA of the hands among subjects disease-free at baseline. Greater baseline relative weight was also associated with greater subsequent severity of OA of the hands. In the study of incident symptomatic OA in 134 matched case-control pairs of women aged 20-89 years, Oliveria et al [150] reported that body weight was a predictor of incident OA of the hand with an odds ratio of 3.0 for women in the upper tertiles of weight compared with women in the

lowest tertile. In the Chingford study, Hart et al [144] reported a modest association between obesity and distal interphalangeal joint OA and carpometacarpal joint OA (OR 1.51, 1.71, respectively). However, data from the Ulm Osteoarthritis Study [151], the Baltimore Longitudinal Study of Aging [152], and the first NHANES [153], do not support the significant association between obesity and hand OA.

The significant association between obesity and OA of the knee and hip but not hand suggests a mechanical effect of obesity on the development of OA, rather than a metabolic effect. A force of three to six times the body weight is exerted on each knee, alternately, while walking; therefore, any increase in weight may be multiplied by this factor to reveal the excess force an overweight person exerts. A less strong association between obesity and hip OA compared to knee OA is possibly due to the different multiplier effects of body weight across the two joint sites or differences in distribution of load across the hip and knee during weight-bearing [154].

Bone mineral density (BMD): An inverse association between osteoporosis and OA has been noted clinically for many years. Foss and Byers [155] first reported the association between these two diseases. Since then, numerous studies have been conducted to examine the relationship between OA and BMD. Cross-sectional studies added more evidence to support the observation that subjects with clinical or radiographic OA have higher adjusted levels of bone mass than those without OA, particularly with OA of the hip and knee and in women [156-161].

However, longitudinal studies revealed a more complex relationship between the two diseases. In the 23-year longitudinal Tecumseh Community Health Study, Sowers et

al [162] reported that women who had more cortical area , indicating greater bone mass at baseline, were more likely to develop hand OA. These women also experienced a significantly greater widening of the medullary cavity over time, an indicator of increased bone resorption. In addition, women with increasing levels of OA involvement also had an increased likelihood of greater cortical area loss.

In the Rotterdam Study, Burger et al [163] followed 1723 persons from the general elderly population for two years and demonstrated that knee and hip radiographic OA was associated with significantly increased BMD at the femoral neck (3-8%) at baseline, with the exception of knee radiographic OA in men. BMD increased significantly in direct relation to the number of affected sites and higher Kellgren score. Both men and women showed a significant trend towards increasing BMD with increased number of affected OA sites. More interestingly, radiographic OA was associated with significantly elevated bone loss with age (in men, only for radiographic OA of the hip).

In the Framingham Study, Zhang et al [164] followed 473 women (ages 63 to 91) for 8 years. The risk of incident radiographic knee OA increased from 5.6% among women in the lowest age-specific quartile of BMD to 14.2, 10.3, and 11.8% among women in the 2nd, 3rd, and highest quartiles, respectively. Multivariable adjusted OR of incident OA for each higher quarter of BMD were 2.5, 2.0, and 2.3, respectively ($p = 0.222$ for trend). This was mainly reflected in an increased risk of osteophyte development. However, the risk of progressive OA decreased from 34.4 to 22.0, 20.3, and 18.9% for subjects in successfully higher quarters of BMD. Compared to those in the lowest quartile of BMD, adjusted OR for progressive

disease were 0.3, 0.2, and 0.1 among women in the 2nd, 3rd, and highest quartiles (p <0.001 for trend), respectively, mainly due to its effect of lowering the risk of joint space loss. Compared to those who lost more than 0.04 g/cm^2 of BMD over the followup period, women who gained BMD were at increased risk of incident but at a significantly decreased risk of progressive knee OA. BMD change was not associated with osteophyte development, but a gain in BMD lowered the risk of joint space loss.

In the Chingford Study, Hart et al [165] followed 830 middle-aged women for 48 months. 95 women with incident knee osteophytes had significantly higher baseline spine BMD (1.01 gm/cm^2 versus 0.95 gm/cm^2, or 6.3%; P = 0.002) and significantly higher hip BMD (0.79 gm/cm^2 versus 0.76 gm/cm^2, or 3.9%; P = 0.02) than those without incident disease. No difference in spine BMD was seen for the 33 women whose osteophytes progressed compared with nonprogressors, but hip BMD was modestly reduced by 2.5%. The 81 women with incident JSN had nonsignificantly higher baseline spine BMD (3.0%), while no difference was seen for the 30 women whose JSN had progressed. For hip BMD, a nonsignificant increase of 1.3% was seen in those with incident JSN, and a nonsignificant reduction of 2.7% was seen in those whose JSN progressed. Peripheral fractures, mainly in the distal forearm (27.6%) and vertebrae (28.3%), were sustained by 145 women. Women with a peripheral fracture had a reduced risk of subsequently developing incident knee OA (OR 0.30, 95% CI 0.11-0.84). Although numbers were smaller, nonsignificant reductions in odds of incident OA were seen for those with distal forearm (OR 0.40, 95% CI 0.11-1.49) and vertebral (OR 0.20, 95% CI 0.07-1.61) fractures.

In the Baltimore Longitudinal Study of Aging, Hochberg et al [166] followed two groups of subjects. One group of 298 Caucasian men and 139 Caucasian women aged 20 years or above had radiographs of the hands and knees read for features of OA and two or more measurements of BMD at the forearm at least 4 years apart. The second group of 179 Caucasian men and 110 Caucasian women aged 20 years or over had longitudinal knee radiographs on average 10 years apart, a subgroup of whom had baseline measurement of lumbar spine and/or femoral neck BMD. They found that women with radiographic OA of the hand had a significantly greater adjusted rate of bone loss at the radius than women with normal hand radiographs; no such differences were noted in men for hand OA. There were no significant differences in adjusted rate of bone loss at the radius in men or women by presence of radiographic knee OA. Higher BMD at the lumbar spine but not at the femoral neck was associated with an increased risk of developing incident radiographic knee OA after adjustment for age, gender, and body mass index.

In summary, the relationship between OA and osteoporosis is more complex than expected. High BMD and bone loss at a greater rate is associated with subsequent incident OA mainly if defined as osteophyte development. People with established OA may undergo greater bone loss than those without the disease, and this is also associated with progression of the disease. Gain in BMD may protect from progression of OA in those with the established disease.

Nutritional factors: A variety of reactive oxygen species (ROS) are formed continuously in tissues by endogenous and exogenous mechanisms. ROS mediated damage accumulates with age and contributes to many common age-related diseases,

including OA [167]. There is some evidence that antioxidants from diet or other sources may prevent the development or delay the progression of OA.

In the longitudinal Framingham Osteoarthritis Cohort Study, McAlindon et al [168] reported that a moderate intake of vitamin C (120-200 mg/day) led to a 3-fold reduction in risk of OA progression. This related predominantly to a reduced risk of cartilage loss (adjusted OR = 0.3, 95% CI 0.1-0.8). Those with high vitamin C intake also had reduced odds of developing knee pain (adjusted OR = 0.3, 95% CI 0.1-0.8). A reduction in odds of OA progression was seen for beta carotene (adjusted OR = 0.4, 95% CI 0.2-0.9) and vitamin E intake (adjusted OR = 0.7, 95% CI 0.3-1.6), but was less consistent. No significant association was found between incident OA and any nutrients. In the same cohort, McAlindon et al [169] reported that low intake and serum levels of vitamin D was linked to threefold increased risk of progression of the knee OA. Low serum levels of vitamin D also predicted loss of cartilage, as assessed by loss of joint space (OR 2.3; 95% CI 0.9 - 5.5) and osteophyte growth (OR 3.1; 95% CI 1.3 - 7.5). Incident OA of the knee occurring after baseline was not consistently related to either intake or serum levels of vitamin D.

In another longitudinal study, Lane et al [170] followed 237 subjects for average 8 years. The risk of incident hip OA defined as the development of definite joint space narrowing was increased for subjects who were in the middle (OR 3.21, 95% CI 1.06-9.68) and lowest (OR 3.34, 95% CI 1.13-9.86) tertiles for serum 25-vitamin D compared with subjects in the highest tertile. Vitamin D levels were not associated with incident hip OA defined as the development of definite osteophytes or new

disease according to the summary grade. No association between serum 1,25-vitamin D and changes in radiographic hip OA was found.

Few randomised controlled trials have been conducted to investigate the effect of antioxidants on OA. Jensen et al [171] demonstrated that 1g calcium ascorbate (containing 898mg vitamin C) daily reduced pain significantly compared to placebo in 133 patients with radiographically verified symptomatic OA of the hip and /or knee joints. But the demonstrated effect was less than half as pronounced as commonly reported for non-steroidal anti-inflammatory drugs (NSAIDs). Similarly, a short-term effect of vitamin E in relieving pain in patients with established OA has been documented [172-174], but long-term clinical trials did not demonstrate the same effect [175 176].

In summary, these results support the hypothesis that antioxidant micronutrients may benefit people with established OA, and vitamin D may influence the development of OA through cartilage loss rather than subchondral bone remodelling. However, more research is needed to evaluate the importance of nutrition in the aetiology and progression, and possibly the treatment, of OA.

Physical activities and occupational factors: Physical activity has been recommended as an intervention for many health conditions. A potential side effect with this recommendation is that an increased level of physical activity may lead to an increased risk for OA.

In the cohort of 5818 elderly women from the Study of Osteoporotic Fractures, Lane et al [177] examined the cross sectional association of radiographic OA of the hip and past recreational and sports related physical activity. The odds of moderate to severe radiographic hip OA in elderly women was modestly increased in elderly women who were in the highest quartile for all physical activities performed as a teenager (OR 1.7, 95% CI 1.1-2.4), at age 50 (OR 1.4, 95% CI 1.0-1.9) and weight bearing activities at age 30 (OR 1.4, 95% CI 1.0-1.9) compared to women in the lowest quartile of activity. The odds of symptomatic hip OA (grade > or =2 hip OA + hip pain) was modestly increased in women who were in the highest quartile for all physical activities as a teenager (OR 2.0, 95% CI 1.2-3.4), at age 50 (OR 1.6, 95% CI 1.0-2.4), and weight bearing activities at age 30 (OR 1.6, 95% CI 1.0-2.4) compared to women in the lowest quartile of activity. These data suggested that recreational physical activities performed by women before menopause may increase the risk of radiographic and symptomatic hip OA. However, given the nature of the study design, the result needs to be replicated in a cohort study.

An analysis of the Cooper Clinical Data showed there was no association for men between hip/knee OA and low joint stress from physical activities that after adjustment for age, body mass index, years of follow-up, and history of hip/knee joint injury. Moderate/high joint stress was associated with reduced risk of hip/knee OA (adjusted OR 0.62, 95% CI 0.43-0.89). Among women, both levels of joint stress were associated with reduced risk of hip/knee OA (OR 0.58, 95% CI 0.34-0.99 for low and OR 0.24, 95% CI 0.11-0.52 for moderate/high) [178].

An analysis of data from the Allied Dunbar National Fitness Survey also produced little evidence to suggest that increased levels of regular physical activity throughout life lead to an increased risk of knee OA. But these data suggested that previous knee injury was associated with an increased risk for knee OA, and most injuries were caused through participation in physical activities [179].

Evidence has been accumulating that OA is more common in people who have performed heavy physical work through their life, particularly in those whose jobs have involved repetitious tasks that overload the joints and fatigue muscles that protect the joints. Several occupational groups have been shown to be at increased risk of developing OA. Cotton workers have higher risk for hand OA [180]. Coal miners have higher risk for hip, knee, and shoulder OA compared to more sedentary occupations [181]. Dockers were found to have more knee OA than civil servants in sedentary occupations [182]. Data from the Framingham Study suggested that men whose jobs required knee bending and at least medium physical demands had higher odds of later radiographic knee OA (at least definite osteophytes) than men whose jobs required neither (43.4 vs 26.8%; OR of OA 2.22, 95% CI 1.38-3.58). Odds of severe radiographic OA (osteophytes and JSN) and of bilateral radiographic OA were also significantly increased in these men [183]. Data from a register-based cohort study demonstrated that male farmers, construction workers, firefighters and some food processing workers had an excess risk of hospitalization due to OA of the hip. Male farmers, construction workers and firefighters also had increased risks of OA of the knee. Female mail carriers had an excess risk of OA of the hip, and female cleaners had excess risk of OA of the knee [184].

1.1.9 Summary

OA is a group of diseases with similar biologic, morphologic, and clinical outcomes, mainly affecting hands, knee, hip, and spine. Its high prevalence, particularly in the elderly, makes OA one of the ten most disabling diseases in developed countries. The aetiology of OA remains elusive but appears to be multifactorial with both genetic and environmental factors playing a role in the development of the diseases. Radiographs have been used in epidemiological studies to identify causes and risk factors of OA, but their two dimensional nature and semi-quantitative grading scales as well as the inability to characterize soft tissues limit their value. Magnetic resonance imaging (MRI), which allows direct visualization of joint structures and provides accurate and reproducible quantitative estimates of joint structures including cartilage volume and bone area, has the potential to enhance our understanding of OA and will be reviewed in the next section of this chapter.

1.2 MRI evaluation of articular cartilage morphology

1.2.1 Preface

Non-invasive assessment of the structural change of the joint is of the utmost importance due to the high prevalence and the socio-economic cost of OA and the availability of variable management strategies [185]. Until recently, conventional radiography was the only available non-invasive method used in the assessment of the structural change of the joint. However, its two-dimensional nature, indirect measurement, and poor association with symptoms limit its value. MRI, a non-invasive imaging technique with multiplanar capabilities and unparalleled soft tissue contrast and lack of ionising radiation, has an ever-increasing role in the evaluation, diagnosis, and monitoring of OA.

By reviewing available literature to date, the aims of this section is to describe optimal MR pulse sequences for imaging articular cartilage, assess the reliability of MRI-based measurements and compare their performance to that of x-ray, and to raise the questions that this thesis will address.

1.2.2 Optimal MR pulse sequence and image analysis method for articular cartilage quantification

Pulse sequence refers to the complex sequence of events occurring during MR data acquisition by switching on radiofrequency and magnetic gradient fields. The spin echo pulse sequence is the most commonly used pulse sequence for most clinical applications of MR imaging. By employing a 180° radio-frequency pulse to rephase the protons following the original 90° excitation pulse, it has the advantage of correcting for fixed magnetic heterogeneities and minimizing susceptibility effects.

Gradient echo pulse sequence employs partial flip-angles (< 90°) and collects echoes by gradient reversal rather than 180° radiofrequency pulses. It is faster than spin echo but more vulnerable to magnetic susceptibility effects. However, the rapid speed with which images can be obtained makes the use of 3-dimensional acquisition feasible. Three-dimensional imaging allows thinner slice thicknesses and improves signal-to-noise ration, but takes longer and is more vulnerable to motion artefacts. The pulse sequence timing can be adjusted to give T1-weighted, Proton or spin density, and T2-weighted images for different clinical purposes.

The goal of imaging articular cartilage is to depict accurately cartilage structure and abnormalities. However, articular cartilage is extremely thin, has complex geometrical morphology, relatively short transverse relaxation time (T2), and complex biochemical composition. This presents a real challenge to MRI. In OA-affected cartilage, MRI faces an even greater challenge because the cartilage surface becomes more difficult to define due to focal signal changes, fibrillation, and tissue thinning, as well as the appearance of repair tissue. The use of pulse sequences optimised for articular cartilage allows segmentation, volume calculation, three-dimensional display, as well as permitting surface irregularities and focal defects to be detected with a high degree of accuracy and reproducibility.

Many studies have compared the accuracy of different pulse sequences for evaluation of articular cartilage structure and abnormalities [186-189]. T_1 weighted conventional spin echo sequences have good a signal-to-noise ratio (SNR) and good spatial resolution, but poor contrast between cartilage, joint fluid, and adipose tissues [190]. This inhibits accurate delineation of the cartilage. Both proton density and T_2

weighted conventional spin echo sequences have the advantage of the T_2 effect of joint fluid acting as a surrogate contrast agent within the joint, producing good contrast between cartilage and joint fluid. On the MR images obtained with these sequences in combination with fast spin-echo technique, which provides both good SNR and high spatial resolution, normal cartilage appears as a dark image, in contrast to the adjacent high signal joint fluid, intermediate signal intensity fat, and low signal intensity cortical bone, and the focal cartilage surface defects is readily detected [191-193]. Without fat suppression, a technique that improves contrast between cartilage and surrounding structures, the other soft tissues such as menisci, tendons, and ligaments are also reasonably displayed, allowing simultaneous evaluation of these structures, in contrast to fat suppressed images that are better for detection of bone marrow oedema. However, the deepest cartilage layers are not well displayed, and overestimation of the depth of a cartilage lesion or underestimation of cartilage quantification (e.g. cartilage volume) may occur [194]. Moreover, both T1- and T2-weighted spin echo images are limited by a minimum practical slice thickness of 2-3 mm [195]. Therefore, high-resolution 3 dimensional techniques are necessary.

Gradient echo sequences allow volume (3D) acquisition within reasonable imaging time. Volume acquisition makes it possible to acquire very thin slices, thus improving the spatial resolution and permitting images to be reformatted into multiple planes. SNR is also better than with spin echo sequences for a given slice thickness [196]. Spoiled gradient echo sequences such as spoiled gradient recalled acquisition in the steady state (SPGR) or fast low-angle shot (FLASH) produce T_1 weighted images with sufficient contrast between cartilage (hyperintense) and intra-articular fluid (hypointense). When fat suppression or water-excitation, a technique

that increases contrast between lipid-containing and non-lipid-containing tissues, is combined with a 3D SPGR sequence, cartilage is the only bright articular structure while other structures are in hypointense (Figure 1.2.1), providing the best visualization and the highest image resolution of articular cartilage [188 197-199]. Sufficient contrast and spatial resolution allows for the detection of cartilage defects with high sensitivity and specificity [200-202]. Moreover, the thin-section volume acquisitions allow segmentation and accurate volume calculation of articular cartilage. Over recent years, these pulse sequences have been used for cartilage quantification by most investigators [197 203-208].

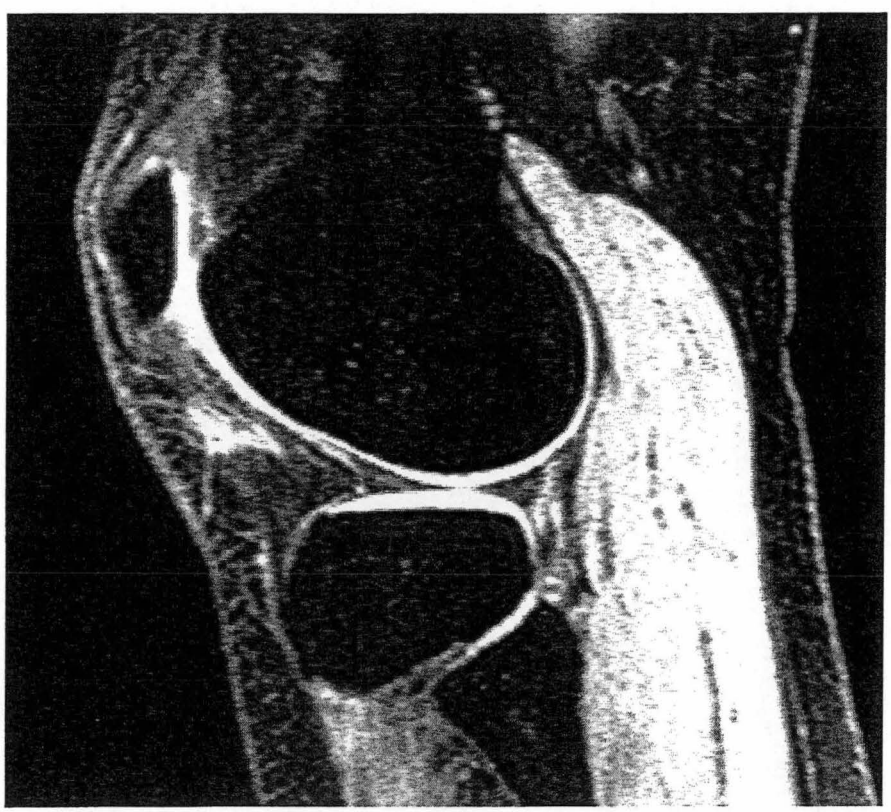


Figure 1.2.1. Single T1 weighted fat saturation sagittal image of a knee with use of 3D gradient recalled acquisition in the steady state. Articular cartilage is hyperintense and sufficiently discriminated from the surrounding structures of the knee.

However, cartilage volume determination requires not only high contrast between articular tissues and high spatial resolution, but also accurate segmentation of the cartilage from its neighbourhood in consecutive MR images. Segmentation is the process by which appropriate image points (voxels) are assigned to a specific anatomic structure, such as a cartilage plate. Due to the relatively low contrast in some areas of the joint surface (e.g. joint contact areas, vicinity synovial folds, tendons and ligaments, damaged and repaired tissue, and so on), reliable fully automated segmentation of cartilage has not yet been developed. Various semi-automated segmentation techniques have been developed to date. Region growing techniques are sensitive to irregularities at the cartilage surface but often fail in regions where contrast is low [209 210]. Other techniques such as B-spline snake (deformable contour) algorithm [195 211], active shape models [212], edge detection methods [213], and active contours [214] have also been developed, but each of these methods requires verification and manual editing by an experienced reader on a section by section basis and this becomes more important for injured or damaged cartilage and the time saved by these techniques would be cancelled out. Moreover, cartilage volume obtained by semi-automatic segmentation techniques such as B-spline snake algorithm tends to be less accurate [215 216] than manual segmentation [197], which has been used for cartilage quantification for both cross-sectional and longitudinal studies by the same group [50 197 203 217-222]. However, manual segmentation is very time-consuming and requires one to several hours per subject. For these reasons, it has been difficult to apply the method to large studies. Therefore, as one of its objectives, this thesis will test the hypothesis that selective sampling of 1.5 mm thick slices of MR images with 0.3 mm in-plane resolution obtained with fat suppressed SPGR sequence, which is most recommended optimal

image resolution and MR pulse sequence [223-225], can be used to estimate knee cartilage volume in both male and female subjects of cross-sectional and longitudinal studies with little increase in measurement error but substantial reduction in post scan processing time.

1.2.3. Validity and reliability of quantitative and semi-quantitative measurement of articular cartilage

Validity is concerned with whether the method is actually measuring what it purports to be measuring. Reliability is concerned with whether the method will produce the same result when administered repeatedly to an individual. Poor validity degrades the precision of a single measurement, and reduces the ability to characterize relationships between variables. Poor reliability also degrades the precision of a single measurement and reduces the ability to track changes in measurements. This is of critical importance in longitudinal studies of articular cartilage where a low precision will require a larger sample to make up for errors in measurement.

Cartilage volume: Most MRI-based quantitative measurement of human cartilage has been focused on the knee joint because it displays the largest cartilage volume and is one of the most frequently OA-affected joints. Accuracy of MRI-based knee cartilage volume measurement has been evaluated by comparative analysis in unselected cadaver joints [197 199 209 226], in amputated joints [197 208], and in knee joints of patients prior to total knee replacement [197 208]. These studies demonstrate that knee cartilage volume can be accurately measured by MRI with an error of < 10% compared to the volume estimated by means of water displacement. All these studies used similar MR pulse sequences, but different cartilage segmentation techniques

may influence the accuracy of the cartilage quantification. Using manual segmentation, Cicuttini et al [197] demonstrated the average error in estimation of the cartilage volume by MRI was 8.3% for patellar cartilage and 9.2% for both femoral and tibial cartilage. Peterfy et al [208] showed 5.9-8.2% for the total knee cartilage volume using region growing segmentation. Using B-spline snake algorithm semi-automatic segmentation technique, Burgkart et al [215] and Graichen et al [216] found a high error (6.6 to -27%) in estimation of cartilage volume. This may be due to the fact that the cartilage measured was severely OA-affected. The B-spline snake algorithms are not as robust for complex objects with large deformations or topological changes such as focal cartilage defects (fissuring) thus insufficiently delineate the cartilage boundaries accurately, especially in severe OA-affected cartilage.

Reliability (reproducibility) has been studied in healthy and OA patients by repeating measurements on the same sets of MR images by the same or different observers [197 207 208], after joint repositioning and reshimming of the magnet [204 207 227], and different scanners [228]. Regardless of the health status of the patients (either healthy or OA-affected), the coefficient of variation (CV) is less than 5% for intra-observer reproducibility [197 207] but up to 7.8% for inter-observer reproducibility [207 208]. The variability of knee cartilage volume measurement is relatively small when comparing two data sets in which joint repositioning and reshimming of the magnet was involved [207 227].

Waterton et al [229] assessed diurnal variation in the femoral articular cartilage of the knee in young adults. Six volunteers were each scanned early in the morning and at

the end of a working day spent mainly standing, and this protocol was repeated on 3 successive weeks. Analysis of variance showed no significant diurnal variation in cartilage volume measurement. The reproducibility (CV) for overall volume was 1.6%, suggesting that diurnal variation is not an issue in measuring knee cartilage volume.

Eckstein et al [227] assessed long-term and resegmentation precision of knee cartilage volume in 12 healthy volunteers under short-term imaging conditions (acquisitions taken immediately after each other with joint repositioning), long-term imaging conditions (acquisitions taken roughly over 9 months, but postprocessed immediately after each other), and resegmentation (postprocessing) of the same data sets spaced over 12 months. Error under long-term imaging condition (CV 1.4% to 5.6%) was not significantly larger than that under short-term acquisition conditions (CV 1.7% to 5.3%). No systematic drift was observed in this data, suggesting that scanner drift as well as variation in imaging (temperature, humidity) and patient conditions (physical activity pattern prior to imaging) do not represent a critical problem in knee cartilage volume measurement. However, resegmentation precision error was somewhat higher (CV 2.5% to 6.0%) compared with either long-term or short-term precision errors, suggesting digital postprocessing in longitudinal studies should preferably be performed in one session.

Morgan et al [228] evaluated the reliability of different scanners for knee cartilage volume measurement. Five healthy female volunteers were recruited at Macclesfield and had both knees scanned using three different scanners in three cities in the UK to provide data for inter-scanner variability. The machines used were Siemens, GE, and

Philips scanners. The results showed that there was a small systematic difference between the scanners. The between-volunteer variability when scans were taken using different scanners was higher (CV 9-18.7%) compared with the variability within a scanner (CV 8.7-18.3%), suggesting that the same brand scanner should be used in studies of knee cartilage volume.

These studies demonstrated that knee cartilage volume can be accurately and reproducibly measured by the same scanner and a single experienced reader. Recent reports suggested that cartilage volume of other joints such as shoulder and hip can also be measured accurately and reproducibly by MRI.

Graichen et al [205] studied cadaver shoulder specimens from eight healthy subjects by MRI using T1 weighted 3D gradient echo sequence (FLASH, fast low angle shot) with selective water excitation. The glenoid and humeral head cartilage volume derived from MRI was compared with that obtained by means of water displacement. The systematic difference ranged from ±1% to ±3%, and the absolute difference ranged from 4 to 7%, suggesting the cartilage volume of the shoulder can also be accurately measured by MRI.

Cicuttini et al [230] assessed the feasibility of MRI for measuring hip cartilage volume. Ten femoral head specimens were obtained from 10 patients undergoing total hip replacement and scanned by 1.5-T whole body magnetic resonance unit with T1-weighted fat suppressed 3D fast SPGR sequence. The femoral head cartilage volume derived from MRI was compared with that obtained by means of water displacement. The average over- or under-estimation of the hip cartilage volume by MRI

quantification was 0.6 (12%) ± 0.6 ml. In addition, they assessed the reproducibility by the same reader reading the same images of hips of six randomly selected patients who underwent MRI for clinical indications. The overall CV as a measure of intra-observer reproducibility was 6.6%, with individual subject values ranging from 1.2% to 10.2%. The ICC was 0.94. This demonstrated that hip cartilage volume can also be measured with good accuracy and reproducibly from optimised MR pulse sequence. However, there is very little literature regarding studies of hip OA using this method. It also remains uncertain whether a MRI based method such as cartilage volume measurement is superior to x-ray and can be used to identify risk factors and early stage of the hip OA. Therefore, as one of its objectives, this thesis will compare associations between anthropometric and lifestyle factors and femoral head cartilage volume/thickness and radiographic features of OA and assess evidence of construct validity for MRI assessment of femoral cartilage volume and thickness.

Cartilage thickness: MRI may be used to assess articular cartilage thickness. Kladny et al [231] assessed the accuracy of cartilage thickness measurements by comparing data obtained by cartilage thickness measurements in MRI with corresponding histological sections of 14 human proximal tibial articular surfaces. Each was cut into five medial and lateral slices and each of these slices was divided into three sectors providing 420 sectors, 406 of which were evaluated in their study. They found that there were no significant differences in cartilage thickness measurements in different grades of OA. But the mean percentage difference between cartilage thickness in MRI and histology was about 10%. Cartilage thickness measurements in MRI were more accurate in cartilage thicker than 2 mm ($r = 0.94$) than in thinner cartilage layers ($r = 0.73$). This could be due partly to the fact

that the measurement in thin cartilage is relatively small compared with a routine pixel size, suggesting that the thickness measurement by MRI in thin cartilage is less reliable.

In humeral head cartilage, where the thickness is about 1.2 mm, Hodler et al [232] examined the accuracy of MRI measurements with various pulse sequences including fat suppressed SPGR. They found the mean MR-anatomic difference in the cartilage thickness was 0.37-0.49 mm, suggesting MRI with currently used MR pulse sequences cannot accurately measure the cartilage thickness of the humeral head. Similarly, Graichen et al [205] evaluated the accuracy of the cartilage thickness of the human shoulder between MRI and A-mode ultrasound. The absolute difference in the cartilage thickness of the shoulder between these two methods was 15.6% and 20.7% for humeral head and glenoid cavity, respectively.

In the femoral head where the cartilage thickness is about 1.8 mm, Hodler et al [233] examined the accuracy of hip cartilage thickness measured by MRI in 10 cadaveric hips. They found that the Pearson correlation coefficient between MR and anatomic measurements of hip cartilage thickness ranged from 0.25 to 0.58, suggesting that measurement of hip cartilage thickness in MRI is not sufficiently accurate. Similarly, McGibbon et al [234] showed that the acetabulum cartilage thickness was over- or under-estimated by 15-20% by MRI compared with light microscopy.

Although thickness values of 4 mm and 3.7 mm in the medial and lateral femorotibial joints have been quoted [235] and several studies [204 215 227 236] have shown that the cartilage thickness of the knee could be measured by MRI with acceptable

reproducibility, thickness measurements are prone to inter-observer and intra-observer error due to several factors. The deepest, basal layers of hyaline cartilage blend with the zone of provisional calcification (tide mark), and its hypointense visualization on MR images has been attributed to subchondral bone, the deep calcified layer of the cartilage, differences in water content, susceptibility differences between hyaline cartilage and subchondral bone, differences between the T2 relaxation times of different cartilage layers, and chemical shift artefacts [233]. Cartilage thickness at specific sites within a joint may also vary as a function of weight bearing. Waterton et al [229] observed that cartilage thickness of the femoral articular cartilage of the knee in six healthy young volunteers decreased in load bearing regions after a period of weight-bearing, while overall volume measurements remained constant. Longitudinal measurements of articular cartilage thickness are thus liable to error due to the difficulty in fixing the position of the point of measurement and changes in thickness due to normal daily activity.

Chondral defects: The MR semi-quantitative scoring system of chondral defects, described by Yulish et al [237], is based on the arthroscopic classification of Outerbridge [238]. Grade 0 indicates intact cartilage. Grade 1 corresponds to thickening and softening, without morphologic defect. Grade 2 involves superficial fissuring or fibrillation of the articular surface, or shallow ulceration or erosion composing less than 50% of the total thickness of the cartilage. Grade 3 is a partial-thickness defect of more than 50%, but less than 100%, of the cartilage thickness. A grade 3 lesion does not extend to the underlying bone, whereas a grade 4 lesion is a high-grade lesion with full-thickness cartilage defect extending to underlying bone. There are other MR classification systems of chondral defects described in the literature

including Recht's modified Noyes classification [202] [239], Drape's modified classification [240] [241], and Boegard's approach [242] [243]. They are all similar and based mainly on the thickness of the cartilage defect.

As described in **1.2.2**, 3D SPGR is one of the most accurate sequences for detecting cartilage lesions and is used by most investigators, although other MR sequences are also used [193]. Accuracy and reproducibility for detection of chondral defects of the knee by MRI has been evaluated by comparative analysis in cadaveric joints [200] and in vivo with arthroscopy as the gold standard [201] [202] [240] [244]. These studies demonstrated that MRI could be used to detect chondral defects with a sensitivity of 81-93%, specificity of 94-97%, and accuracy of 95-97%. The majority of false positive results occur in grade 1 chondral defects, indicating that MRI overgrades intracartilaginous lesions relative to arthroscopy [201] [240]. This discrepancy can be attributed to the fact that lesions without surface irregularities are inherently difficult to diagnose arthroscopically. Grade 1 chondral defects in MRI may represent actual articular cartilage derangement and may serve as predictors of future articular cartilage degeneration. It is hoped that further development of high resolution MR imaging techniques such as T2 mapping, sodium MR imaging, diffusion-weighted imaging, and contrast-enhanced imaging will be sensitive to subtle structural and biochemical changes and help to elucidate the nature of the grade 1 cartilage lesions [245] [246]. These new methods under development promise to further refine and enhance our ability to characterize both the morphology and biochemical content of articular cartilage.

1.2.4 Comparison between MRI and x-ray measurements

Cartilage volume and chondral defects can be accurately and reproducibly measured by MRI as described in the previous section, but the question is whether MRI measurement is superior to x-ray measurements and is sensitive enough to detect early stage OA and OA progression.

Cicuttini et al [220] compared tibial cartilage volume as measured by MRI with radiologic assessment of the tibiofemoral joint in 252 subjects aged 40 years or over. They found that JSN, seen on both medial and lateral radiographs of the tibiofemoral joint, was inversely associated with the respective tibial cartilage volume. This inverse relationship was strengthened with adjustment for age, sex, BMI, and bone size. Similarly, they also found a strong association between patellar cartilage volume and JSN as measured on skyline and lateral patellofemoral radiographs in another study [218]. These results demonstrate the complementarity of both imaging techniques.

Jones et al [247] studied the cross-sectional association between early radiographic OA of the knee and the cartilage volume in 372 male and female subjects aged 26 years or more. They found that grade one medial JSN was associated with substantial reductions in cartilage volume at both the medial and lateral tibial and patellar sites within the knee (adjusted mean difference 11-13%), suggesting that MRI is superior at detecting early OA of the knee.

However, all these studies were cross-sectional in nature, and the x-ray measurement was semi-quantitative. Gandy et al [206] conducted a longitudinal study to investigate

whether knee cartilage volume as assessed by MRI is able to detect change over time in patients with OA. They observed that the average decrease in medial tibiofemoral joint space width in weight-bearing extended radiographs was 0.21 ± 0.37 mm over 3 years follow-up, but there was no significant MRI volume change in any of the knee cartilage compartments. The loss in total knee cartilage volume as measured by MRI was only 1.6% over the 3 years. They argued that radiographs might be more sensitive than analysis of total cartilage plates by MRI, because radiographic measurements were obtained in the central aspect of the joint surface, where most of the change may occur. However, the cohort was relatively small (only 16 OA patients), and the MRI scanner was a 1.0 T magnet rather than the more commonly used 1.5 T. In-plane pixel resolution was 0.55 mm rather than 0.3 mm which is mostly recommended for knee cartilage volume measurement [223], and the reported precision errors were high.

In contrast, in the study of evaluating the change in knee cartilage volume over a two-year period with the use of MRI and correlating the MRI changes with radiologic changes in 32 patients with symptomatic knee OA, Raynauld et al [248] reported that progression of cartilage loss at all followup points was statistically significant ($P < 0.0001$), with a mean +/- SD of 3.8 +/- 5.1% for global cartilage loss and 4.3 +/- 6.5% for medial compartment cartilage loss at 6 months, 3.6 +/- 5.1% and 4.2 +/- 7.5% at 12 months, and 6.1 +/- 7.2% and 7.6 +/- 8.6% at 24 months. No significant change in weight-bearing semiflexed positioned radiographs was observed. While 27 of the 31 patients had a loss of medial cartilage over 2 years detected by MRI, only 50% of the patients with a JSW measurement at both baseline

and year 2 showed a decrease in the minimum JSW. Also, no statistical correlation between loss of cartilage volume and radiographic changes was observed.

Similarly, Pessis et al [241] studied 20 patients with symptomatic knee OA of the medial compartment prospectively. After one year, significant worsening of chondropathy was found with MRI using the SFA-MR score, but no statistically significant changes with plain radiographs and arthroscopy.

Based on these results, MRI appears to be better than radiography at detecting change in articular cartilage morphology. The Food and Drug Administration (FDA) of the USA expects that MRI-based measurement of cartilage volume may be able to replace x-ray measurement of JSN in clinical trials, and the Group for the Respect of Ethics and Excellence in Science (GREES) has suggested that MRI measurement may be used as an outcome in phase II trials in OA [249]. So far, one randomised controlled trial of supplementary vitamin E in knee OA has used MRI-based cartilage volume measurement [176]. However, published data to date are limited, and all these studies were of knees. Larger longitudinal studies with measurement of other joints are needed to clarify the clinical relevance of MRI measurement in OA disease progression.

1.2.5 Current status of OA research by MRI

As described in previous sections, MRI can assess articular cartilage morphology accurately and reproducibly and appears to be superior to radiography in detecting early changes. In recent years, there has been a growing interest in utilising MRI for epidemiological studies of OA. Although published data are limited and focused on

knees, and the studies have had a small sample size due partly to the cost and substantial post-scan processing time required, significant findings have been reported.

Bone size: Cartilage volume is dependent on bone size (e.g. joint surface area). A large bone needs more cartilage to be covered. Several studies have demonstrated that knee cartilage volume is strongly associated with joint surface area [197 222 250 251], and there was a substantial difference in bone size between males and females (male vs. female mean differences 21% to 43%) in reports by Faber et al [251] and Ding et al [252], suggesting cartilage volume as an outcome measurement needs to be adjusted for bone size or normalized to individual bone size to produce meaningful results, particularly in cross-sectional studies [223]. Indeed, Burgkart et al [253] reported that normalization of cartilage volume to the original joint surface area increased the discriminatory power, when making clear distinctions between patients and healthy adults, relative to cartilage volume alone or to normalization of cartilage volume to body weight and body height when applying T and Z score system to MRI quantitative assessment of OA.

More recently, Wluka et al [254] reported in a study of a relatively large sample (149 subjects aged 40 years or over) that women with knee OA had larger medial and lateral tibial plateau bone area (mean ± SD, 1850 ± 240 mm^2 and 1279 ± 220 mm^2, respectively) than healthy women (1670 ± 200 mm^2 and 1050 ± 130 mm^2, $p < 00001$ for both differences). In an analysis of a larger sample (372 subjects aged 26 or over), Jones et al [247] reported substantial increases in both lateral and medial tibial joint surface area in subjects with grade one osteophytosis (adjusted mean difference

10-16%, all p<0.001). In an analysis of longitudinal study of 123 subjects with established knee OA, Wluka et al [255] reported that total tibial articular cartilage decreased by 5.3 ± 5.2% (95% CI 4.4% - 6.2%) per year. The annual percentages of loss of medial and lateral tibial cartilage were 4.7 +/- 6.5% (95% CI 3.6%, 5.9%) and 5.3 +/- 7.2% (95% CI 4.1%, 6.6%), respectively. This cartilage loss was not associated with bone size. The annual percentage loss of patella cartilage was 4.5 +/- 4.3% [256]. Given the limited data available, it is unclear whether there is a gain in bone size longitudinally in OA-affected subjects and whether it is associated with cartilage loss. Nevertheless, these results suggest that the association between knee cartilage volume and bone size is more complex than expected. Further longitudinal studies in healthy and OA-affected population are needed to shed more light on this complex relationship.

Female sex: Jones et al [203] studied 92 children between 9 and 18 years old, and found that males had significantly more knee cartilage than females. Sex accounted for 6-36% of the variation in cartilage volume and thickness. In a follow up of subjects 1.6 years later on average, they found that most children gained articular cartilage during growth, but males gained it faster than females at all sites [257].

In a study of 18 young healthy, non-athletic female and male individuals, Faber et al [251] reported that the knee cartilage volume in all cartilage plates was higher in men . The gender-specific differences ranged from 19.9% in the patella to 46.6% in the medial tibia. These differences were statistically significant in the femur and tibia but not in the patella. The gender specific differences in the mean and maximal cartilage thickness were less pronounced than the differences in volume, and were not

statistically significant in any of the joint surfaces. In a larger sample (n = 95), the gender differences of cartilage thickness become significant, but when matching men and women with identical body weight or height, cartilage thickness values showed a trend to be larger in the men than in the women, but the differences did not reach statistical significance [258].

Cicuttini et al [197] examined sex difference in knee cartilage volume among 28 subjects with knee pain. The volume of the femoral and patella cartilage, but not the tibia cartilage, was found to be significantly larger in men than women. This difference was independent of other potential confounders including age, weight, height, and femoral condylar bone volume.

In a large sample (n = 372), Ding et al [252] found that males had 33-42% higher cartilage volume at all knee sites. This difference decreased to 8-18% after adjustment for body height, weight, and bone size, but remained significant (all $p < 0.05$). Moreover, they found that the sex differences in cartilage volume were greater in those over 50 years of age compared with younger subjects. These differences were independent of ROA.

In the longitudinal study of 110 subjects with OA, Cicuttini et al [256] reported that the rate of patella cartilage loss was greater in women (5.3% per annum) than men (3.5% per annum) independently of age, BMI, and pain. However, Wluka et al [255] reported no sex difference in the rate of tibial cartilage loss over 2 years in 123 subjects with knee OA.

These results indicate that females have less knee cartilage than males throughout life and that the size of the differences may be site specific. The reason is unclear, but sex hormones may be implicated for both cartilage development and loss at later life. In the study of 45 healthy males with mean age of 52.5 ± 13.2 years, Cicuttini et al [219] reported a positive association between serum free testosterone levels and tibial cartilage volume. Serum testosterone explained up to 8% of the variation in the knee cartilage volume. Similarly, Wluka et al [259] reported that ERT users among 81 postmenopausal women aged 50 years or over had higher tibial cartilage volume than non-users independent of bone size. Total tibial cartilage volume was 7.7% greater in the group of ERT users than in the untreated group and this difference persisted after exclusion of women with knee OA.

However, no differences in the amount of patella cartilage was found in women on ERT compared to those on not on ERT [221]. In the longitudinal study of 81 postmenopausal women previously studied [259], Wluka et al [260] found no association between ERT and the rate of reduction in knee cartilage volume. More recently, Hanna et al reported [261] in the longitudinal study of 28 healthy men previously studied [219] that tibial cartilage loss was associated with serum free testosterone level independent of age, BMI, baseline tibial cartilage volume, bone size, and total bone mineral content. Overall, testosterone accounted for 14.5% of the variation in change in tibial cartilage volume. Interpretation of these results is difficult because the sample size was relatively small and cross-sectional and longitudinal results were not consistent although they were from the same cohort. Independent longitudinal studies with a larger sample size are required.

Obesity: Although obesity has been recognised as a risk factor for OA, particularly knee OA, available MRI based studies yielded conflicting results. Some cross-sectional studies reported a significant association between BMI and chondral defects and bone size [262] and knee cartilage volume in healthy adults [219 259], while others reported no association [263]. In children, Jones et al [203 257] observed no association between BMI and knee cartilage volume both cross-sectionally and longitudinally. Longitudinal studies available to date reported that there was no association between BMI and longitudinal change in tibial cartilage volume in healthy subjects[260 261] or subjects with OA [255], but there is a significant association with patella cartilage volume in subjects with OA [256]. These results, although limited, suggest that BMI may not directly influence knee cartilage volume. Indeed, Hudelmaier et al [264] demonstrated in 59 asymptomatic individuals that muscle cross-sectional area was more highly correlated with knee cartilage morphology including volume and thickness than with body height and weight. A more recent study by Cicuttini et al [265] examined the relationship between body composition and knee cartilage volume in 86 healthy, middle-aged subjects. The study demonstrated that muscle mass including total body muscle mass, muscle mass in legs and limbs but not body fat was associated with knee cartilage volume, and reduced muscle mass was significantly associated with loss of tibial cartilage volume in the medial and lateral compartments.

Menisci abnormality: Meniscal abnormalities are thought to be a risk factor for knee OA. However, without direct assessment of cartilage, it is difficult to judge the causal relationship between meniscal abnormalities and OA as the menisci also

contributes to joint space measured by radiography [266]. MRI has the advantage of revealing the relationship between meniscal abnormalities and cartilage loss.

Cicuttini et al [267] reported that there was substantial loss of tibial cartilage 29 months after partial meniscectomy compared with controls with normal knee radiographs. The difference in tibial cartilage volume loss between cases and controls was 6.9% (95% CI 3.4-10.3%) after adjustment for age, BMI, and sex. More recently, Berthiaume et al [268] studied 32 patients with symptomatic knee OA for two years. Of the 32, 24 (75%) had mild to moderate or severe meniscal damage (tear or extrusion) at baseline. A highly significant difference in global cartilage volume loss was observed between subjects with severe medial meniscal tear and those with absence of tear (mean (SD): - 10,1 (2.1)% v –5.1(2.4)%, p = 0.002). An even greater difference was found between the medial meniscal changes and medical compartment cartilage volume loss (-14.3(3.0)% in the presence of severe tear but -6.3(2.7)% in the absence of tear; $p < 0.0001$). Similarly, a major difference was found between the presence of a medial meniscal extrusion and loss of medial compartment cartilage volume (-15.4(4.1)% in the presence of extrusion but -4.5(1.7)% with no extrusion; $p < 0.001$). In the average 1.8 year follow up study of 43 patients, Biswal et al [269] reported that patients who had sustained meniscal tears showed a higher average rate of progression of cartilage loss (22%) than those who had intact menisci (14.9%) ($P <= 0.018$).

Symptoms: It is known that radiographic measurements are modestly but significantly associated with OA symptoms, and it is expected that MRI-based measurements will help to link the joint structural changes with symptoms. However,

the available data are limited. Only three studies to date have studied the association between MRI based measurements and symptoms.

In the cross-sectional study of 133 postmenopausal females, Hunter et al [49] found that patella cartilage volume was inversely associated with pain, function, and global score of the WOMAC domains independently of BMI, physical activity, and leg extensor power (all $p = 0.01$).

In a 2-year longitudinal study of 132 subjects with symptomatic, early (mild to moderate) knee OA, Wluka et al [50] reported a weak association between tibial cartilage volume and symptoms at baseline. They also observed significant association between increased cartilage loss and worsening of symptoms of OA: pain (Spearman rank correlation $r_s = 0.28$, $p = 0.002$), stiffness ($r_s = 0.17$, $p = 0.07$), and deterioration in function ($r_s = 0.21$, $p = 0.02$).

However, in a 2-year longitudinal study of 32 patients with symptomatic knee OA, Raynauld et al [248] reported that there was no association between changes in cartilage volume and changes in clinical variables such as the patient's and physician's global assessments, the 3 dimensions of the WOMAC (pain, stiffness, and function), and the physical components of the Short Form 36 health survey.

Overall, available data are limited, but they suggest that cartilage volume and its loss are of importance in OA. Cicuttini et al [270] demonstrated that articular cartilage volume loss in a knee was an independent risk factor for subsequent replacement of that knee. For every 1% increase in rate of tibial cartilage loss there was a 20%

increase in the risk of undergoing a knee replacement at 4 years (95% CI 10% - 30%).

In addition, other MRI based measurements of the joint have been reported to be associated with pain of the knee OA. Hill et al [43] reported in 458 subjects with mean age of 67 that moderate or large effusions and synovial thickening assessed on MRI were more frequent among those with knee pain than those without pain, suggesting these features are associated with the pain of knee OA. Felson et al [42] reported in 401 subjects with mean age of 66.8 that bone marrow lesions on MRI were strongly associated with the presence of pain in knee OA. However, it is unclear whether this association is independent of cartilage damage. As mentioned in **1.1.5,** this thesis will examine the association between knee pain and MRI-based measurements including knee chondral defects, cartilage volume, bone size, and bone marrow lesions and assess whether the association is independent of each other.

1.2.6 Summary

With optimal MR pulse sequences such as T1 weighted SPGR with fat saturation, articular cartilage can be imaged and quantified accurately and reproducibly by reliable image analysis techniques. While available epidemiological data are limited, MRI-based measurements promise to enhance our ability to unravel the complex multifactorial nature of OA. This thesis, based on MRI measurements of the hip and knee, will address several issues regarding the pathogenesis of OA. An outline of the research objectives is given in the next chapter.

CHAPTER TWO: RESEARCH OBJECTIVES

The research questions of this thesis have been raised and their background and rationale have been described in sections **1.5, 1.8, 2.2, and 2.3 of Chapter 1.** They can be summarised as follows:

1. To examine the genetic contribution to knee cartilage volume, bone size, knee pain, low limb muscle strength, and radiographic osteoarthritis in a sibpair study (Chapter 4).

2. To examine the genetic contribution to longitudinal changes in knee cartilage volume, bone size, low limb muscle strength as well as progression of chondral defects in a sibpair study (Chapter 5).

3. To describe the association between knee pain and cartilage volume, bone size, chondral defects, biochemical maker in younger subjects (Chapter 6).

4. To describe the association between knee pain and chondral defects, subchondral bone marrow lesions, and knee and hip radiographic osteoarthritis in older adults (Chapter 7)

5. To compare the associations between anthropometric and lifestyle factors and femoral head cartilage volume/thickness and radiographic features of osteoarthritis of the hip to provide evidence of construct validity for MRI assessment of hip cartilage and thickness (Chapter 8).

6. To determine the optimal sampling of MRI slices for assessment of knee cartilage volume in cross-sectional and longitudinal studies (Chapter 9).

CHAPTER THREE: METHODOLOGY

This thesis is conducted as parts of two studies conducted at the Menzies Research Institute: the Knee Cartilage Volume study (KCV) and the Tasmania Older Adult Cohort study (TASOAC). This chapter will describe the methodology of these two studies.

3.1 Subjects

Source population: The population studied consisted of people living in the Southern Tasmania (latitude 42° south, population 229,000), a geographically defined region of Tasmania, Australia, that includes the state capital (Hobart). Those aged 26 years or over in this population were the source population for the KCV, while those aged between 50 and 79 years in this population were the source population for the TASOAC.

Subject recruitment:

KCV: Subjects were selected from two sources. Half of the subjects were the adult children of subjects who had a knee replacement performed for primary knee OA at any hospital in Hobart during the years 1996-2000. The diagnosis was confirmed by reference to the medical records of the orthopaedic surgeon and the original radiograph where possible. The other half were randomly selected by computer generated random numbers from the most recent version (2000) of the electoral roll of persons registered to vote in elections. Subjects from either group were excluded on the basis of contraindication to MRI (including metal sutures, presence of shrapnel, iron filing in eye and claustrophobia). No women were on hormone replacement therapy at the time of the study.

TASOAC: Subjects aged between 50 and 79 years were selected randomly from the electoral roll, with an equal number of males and females. Institutionalised adults were excluded. Subjects were also excluded if they had contraindication for MRI (e.g. metal sutures, presence of shrapnel, iron filling in eye, and claustrophobia).

The Chapters that follow report the results of the studies from different subsamples of these two studies. The subsample for each will be described in the relevant chapter.

3.2 Main measures

MRI of the knee: All subjects in the two studies underwent MRI scan on their right knee on the same scanner at Royal Hobart Hospital. Knees were imaged in the sagittal plane on a 1.5-T whole body magnetic resonance unit (Picker, Cleveland, OH) with use of a commercial transmit-receive extremity coil. The following image sequences were used: A T1-weighted fat saturation 3D gradient recall acquisition in the steady state; flip angle 55 degrees; repetition time 58 msecs; echo time 12 msec; field of view 16 cm; 60 partitions; 512 x 512 pixel matrix; acquisition time 11 min 56 sec; one acquisition. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31 x 0.31 mm (512 x 512 pixels). The image data were then transferred to a workstation.

Knee cartilage volume was determined by means of image processing on an independent workstation using the free software program Osiris as previously described [203]. The volume of lateral and medial tibial and patellar cartilage plates was isolated from the total volume by manually drawing disarticulation contours around

the cartilage boundaries on a section-by-section basis (Figure 3.1). These data were then resampled by means of bilinear and cubic interpolation (area of 312 and 312 μm and 1.5 mm thickness, continuous sections) for the final 3D rendering. The volume of the particular cartilage plate was then determined by summing all the pertinent voxels within the resultant binary volume. Using this method we had high reproducibility. The intra-observer reproducibility (done by CD) expressed as coefficient of variation (CV) for cartilage volume measures was 2.1% for medial tibial, 2.2% for lateral tibial and 2.6% for patella, which is very similar to the reported [255].

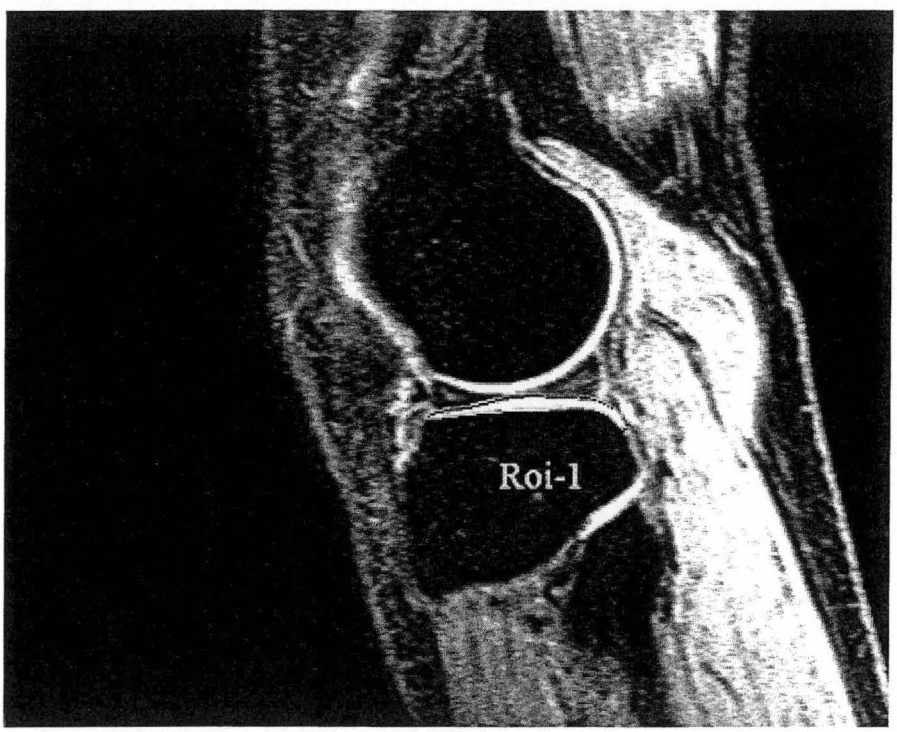
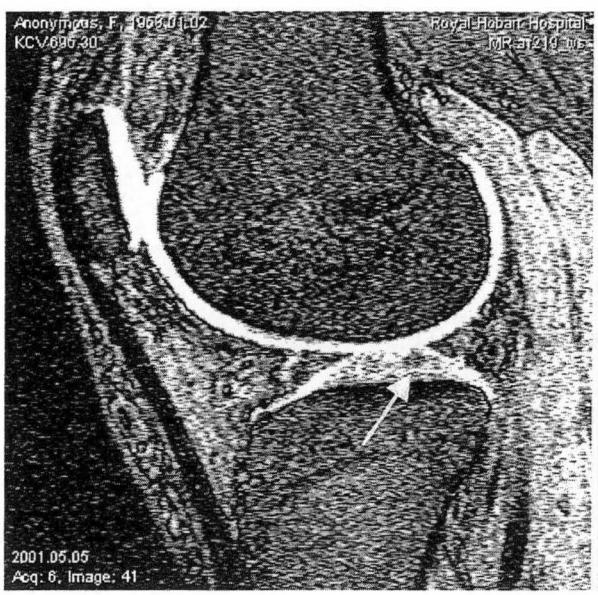


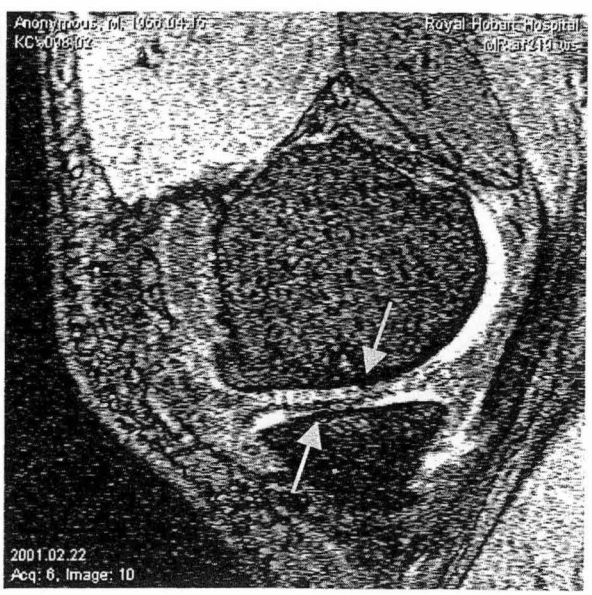
Figure 3.1. Single T1 weighted fat saturation sagittal image of a study subject's knee with lateral tibial cartilage outlined during segmentation on the workstation.

The cartilage defects were graded on the same serial T1 weighted MR images with a modification of a previous classification system [240] at medial tibial, medial femoral, lateral tibial, lateral femoral, and patellar (Figure 3.2) as previously described [271]:

grade 0, normal cartilage; grade 1, focal blistering and intracartilaginous low-signal intensity area with an intact surface or bottom; grade 2, irregularities on the surface or bottom and loss of thickness of less than 50%; grade 3, deep ulceration with loss of thickness of more than 50%; grade 4, full-thickness chondral wear with exposure of subchondral bone. A cartilage defect had to be present on at least two consecutive slices. The highest score was used if more than one defect were present on the same site. The cartilage was considered to be normal if the band of intermediate signal intensity had a uniform thickness. The method had high inter- and intra-observer reproducibility. Intraobserver reliability (done by CD) expressed as ICC was 0.90 for the medial tibiofemoral compartment, 0.89 for the lateral tibiofemoral compartment and 0.94 for the patellar compartment and this was assessed on the whole sample of the KCV. Interobserver reliability (done by CD & CH) was assessed in a series of MR images for 50 subjects and yielded an ICC of 0.90 for the medial tibiofemoral compartment, 0.85 for the lateral tibiofemoral compartment and 0.93 for the patellar compartment.



a.



b.

Figure 3.2. Chondral defects appeared on T1 weighted MR image of the knee. a. grade1 lateral tibial chondral defect; b. grade 3 and 4 medial tibial and femoral chondral defects, respectively.

The same serial T1 weighted MRI images were converted into isotropic volumes and reformatted in the axial plane. Medial and lateral tibial plateau area was determined from the three input images closest to the joint. The areas of the medial and lateral tibial plateau were directly measured from these images (Figure 3.3). The total patella bone volume was calculated using the same method as for cartilage volume. The method had high reproducibility. The CVs (done by CD) were 2.2% for the patellar bone volume, 2.3% for the medial tibial plateau area, and 2.4% for the lateral tibial plateau area.

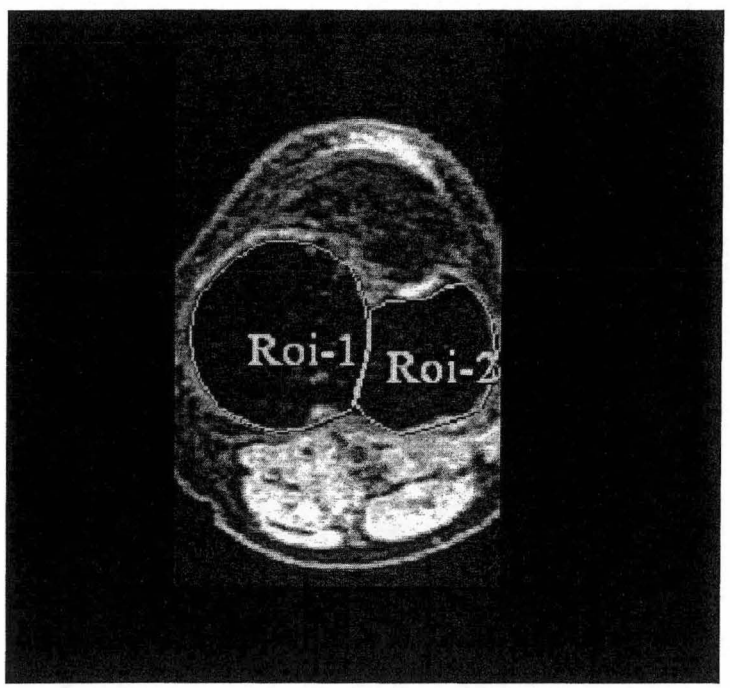
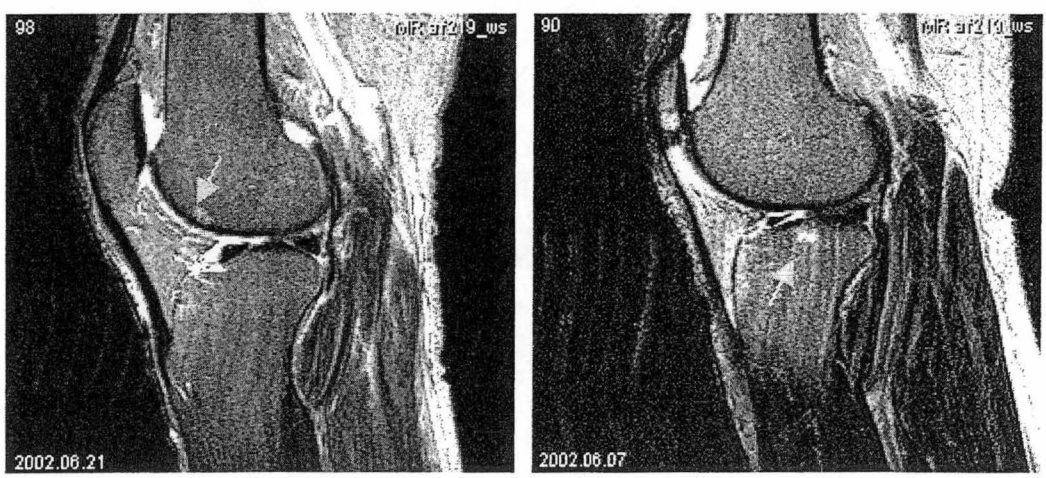


Figure 3.3 Axial T1 weighted fat saturation MR image of the knee showing the method of measuring the tibial plateau bone area. Roi-1 = medial tibial plateau area. Roi – 2 = lateral tibial plateau area.

In addition, MRI with T2-weighted fat saturation 2D fast spin echo was also performed on the right knee of the TASOAC subjects. The follow sequences was used: flip angle 90 degrees; repetition time 3067 msce; echo time 112 msce; field of view 16 cm/ 15 partitions; 228 x 256 matrix; Sagittal images were obtained at a partition thickness of 4 mm with between-slices gap 0.5-1.0 mm. Subchondral bone marrow lesions were assessed on this series of T2 weighted MR images and defined as discrete areas of increased signal adjacent to the subcortical bone at lateral, medial femur and/or tibia. Each bone marrow lesion was scored on the basis of lesion size. A lesion was scored as grade 1 if it was only present on one slice, grade 2 if on two consecutive slices, or grade 3 if on three or more consecutive slices. The highest score was used if more than one lesion were present on the same site. Prevalent bone marrow lesions were defined as total score ≥ 1 (Figure 3.4). The intra-observer reproducibility (done by GZ) was assessed in 50 subjects with at least one-week interval between two readings. The ICCs were 0.89, 0.96, 0.94, 1.00 for lateral tibia and femur, and medial tibia and femur, respectively.



a. b.

Figure 3.4. Subchondral bone marrow lesions (a. grade 1, b. grade 2) appeared on T2 weighted fat saturation 2D MR image of the knee.

MRI of the hip: A total of 151 subjects from TASOAC underwent MRI scanning on their right hip. A detailed description of the MR sequences used and their reproducibility is contained in Chapter 8.

X-ray: A standing AP semiflexed radiograph of the right knee was performed on all subjects. Radiographs were then assessed utilizing the Altman atlas[24]. Each of the following was assessed: medial JSN (0-3), lateral JSN (0-3), medial femoral osteophytes (0-3), medial tibial osteophytes (0-3), lateral femoral osteophytes (0-3), lateral tibial osteophytes (0-3), medial femoral sclerosis (0-3), medial tibial sclerosis (0-3), lateral femoral sclerosis (0-3), and lateral tibial sclerosis (0-3). Intra-observer repeatability (done by VS & CH) was assessed in 40 subjects from the TASOAC study with an ICC of 0.65-0.85, and in 50 subjects from the KCV study (done by GJ & FS) with an ICC of 0.98-0.99. The high ICC in the KCV study may represent an overestimate of the actual agreement due to the high proportion of normal radiographs.

Weight bearing anterior-posterior pelvic radiographs with both feet in 10° internal rotation were also obtained. Radiographic features of axial and superior JSN, and osteophytes of the right hip were graded using the Altman atlas [24] on a 4-point scale (0-3), where 0 = no disease and 3 = most severe disease. Intra-observer repeatability was assessed in 40 subjects with ICC's of 0.60 – 0.87 in the TASOAC sample.

3.3 Assessment of knee pain

Knee pain was assessed by standard questionnaire in both studies. The TASOAC study used the WOMAC questionnaire (see appendix 1), whereas the KCV study used the following single question: Have you had knee pain for more than 24 hours in the last 12 months or daily pain on greater than 30 days in the last year? (see appendix 2)

3.4 Other study factors

Age at baseline; recorded on the day of measurement.

Weight was measured to the nearest 0.1kg (with shoes, socks and bulky clothing removed) using a single pair of electronic scales (Seca Delta Model 707) which were calibrated using a known weight at the beginning of each clinic.

Height was measured to the nearest 0.1cm (with shoes, socks, and headgears removed) using a stadiometer (The Leicester Height Measure).

Leg strength was measured by dynamometry (TTM Muscular Meter, Tokyo) with both legs involved simultaneously. The muscles measured with this technique are predominantly quadriceps and hip flexors. Subjects were instructed in each technique prior to testing and each measure was performed twice.

Knee extension strength in the right leg was measured by a pocket balance (Stamina, Germany). Subjects were instructed in the technique prior to the testing. There were two attempts and the greatest force was recorded.

Urine CTX-II was measured. The detailed description is contained in Chapter 6.

BMD and serum Vitamin D levels were measured. The detailed description is contained in Chapter 8.

3.5 Ethical issues

Both studies were approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee and all subjects provided informed written consent.

3.6 Sample size

Formal sample size calculations were not undertaken as part of the plan for this thesis, because both the KCV and the TASOAC were underway by the time this thesis commenced. The subject numbers were constrained by the numbers recruited in the KCV and the TASOAC at the time each study in this thesis was undertaken. It proved that the sample size was more than adequate to answer the research questions of the thesis. Sample size varies with each research project and will be discussed, where relevant, in the individual chapters.

3.7 Statistics

These vary considerably and those used in each analysis will be discussed in detail in the relevant chapter.

CHAPTER FOUR: GENETIC CONTRIBUTION TO MUSCLE STRENGTH, KNEE PAIN, CARTILAGE VOLUME, BONE AREA, AND RADIOGRAPHIC OSTEOARTHRITIS: A SIBPAIR STUDY

4.1 Introduction

OA is the most common form of arthritis and a leading cause of musculoskeletal disability in most developed countries [55]. The knee is one of the most frequently affected joints with a prevalence of 30% in people older that 65 years [59] and high resultant disability [272]. While its aetiology and pathogenesis remains poorly understood, knee OA has been strongly associated with several environmental factors including obesity [146 153 273-276], previous injury [277 278], vitamin D [169] and meniscectomy [267 279 280]. In addition, a modest but significant genetic effect for knee ROA has been reported in most studies [106-108 113 114]. However, radiographs only provide a broad-brush view of joint pathology due to their semi quantitative grading scales. MRI can allow direct visualization of joint structures and provide accurate and reproducible quantitative estimates of cartilage volume and bone area/volume [203 208 219 256] and thus has the potential to be analysed as quantitative traits in linkage analysis. In addition, other knee features such as muscles and pain are important in knee joint function [281 282]. The aim of the study, therefore, was to estimate the heritability of muscle strength, knee pain, cartilage volume, bone size, and ROA, and to assess whether the heritability of the knee structural components is independent of ROA.

4.2 Materials and methods

Study subjects were derived from KCV. Briefly, subjects were the adult children of patients who had a knee replacement performed for idiopathic knee OA at any Hobart hospital during the years 1996-2000. The diagnosis was confirmed by reference to the medical records of the orthopaedic surgeon and the original radiograph where possible. No specific selection criteria were applied for the knee replacement subjects. Offspring were excluded on the basis of contraindication to MRI (including metal sutures, presence of shrapnel, iron filing in eye and claustrophobia). Subjects with knee pain and knee injuries were not excluded. The Southern Tasmanian Health and Medical Human Research Ethics Committee approved the study and written informed consent was obtained from all participants.

Anthropometrics. The weight and height measurements were described in section 3 of Chapter 3. BMI was calculated as weight in kilograms divided by the square of height in metres. Knee pain was assessed by the questionnaire (*Appendix 2*) and was defined as pain for >24 hours in the last 12 months or daily pain on more than 30 days in the last year. Low limb muscle strength was measured as described in section 3 of Chapter 3 and the repeatability estimates (Cronbach's α) were 0.91. The device was calibrated by suspending known weights at regular intervals.

MRI. T1 weighted fat saturation 3D MRI scan with SPGR was performed on the right knee and cartilage volume at lateral, medial tibial and patellar site as well as lateral, medial tibial plateau area and patellar bone volume were measured. The details of the method were described in section 2 of Chapter 3. Femoral cartilage volume was not assessed as cartilage volume at the two tibial sites and the patella site

correlate strongly with femoral cartilage volume, which thus add little extra information [283]. Using this method we had high reproducibility. The details of the reproducibility were described in section 2 of Chapter 3.

X-ray. A standing AP semiflexed radiograph of the right knee was performed on all subjects and scored for individual features of radiographic knee OA utilising the Altman atlas [24]. The details of the method and the reproducibility were described in section 2 of Chapter 3. ROA was defined in two ways: presence of disease (score >0) and total score (0-12) as indication of the disease severity.

Statistical methods

A variance components analysis was performed to estimate heritabilities of various traits. Using the software package SOLAR [284], trait variance was modeled as a mixture of genetic variance (attributed to many genes with small, additive effects) and random variance (due to random environmental variations not correlated between subjects within families). Then the estimated heritability was defined as the proportion of genetic variance in the model with the maximum likelihood. Heritability estimates are high when intra-family variation in trait scores is low compared to inter-family variation. By analysing the covariance in trait scores between all pairs of relatives in a family simultaneously, SOLAR can be used to estimate heritabilities and standard errors in families of arbitrary complexity, including the families in our study with more than two siblings. While this variance-components analysis assumes a normally-distributed trait, the method has been shown to be equivalent for discrete traits and reasonable for dichotomous traits [285].

To assess whether the estimated heritabilities differed from zero, a null model with only the random variance term was also fitted. All models were fitted after first adjusting trait scores within SOLAR for various combinations of covariates (i) age and sex; (ii) age, sex, weight, and height; and (iii) age, sex, weight, height, and ROA score. For MRI traits, further analyses were performed to assess whether there was shared or independent genetic effects for bone and cartilage separately. Goodness of fit was calculated for all models (with the exception of the step four models) and listed as R^2 values (both continuous and Kullback-Leibler for dichotomous traits). To test whether the models and standard errors were affected by lack of independence, we randomly selected one sib pair from each family where there was more than one sib pair and repeated the age and sex adjusted analyses. A p value of less than 0.05 was regarded as statistically significant.

4.3 Results

A total of 128 subjects representing 115 sib pairs with an average age of 45 years took part in the present study (response rate 71%). The structure of the families studied is presented in Table 4.1. The general characteristics and study traits are presented in Table 4.2. The distributions of all of the MRI measures closely approximated a normal distribution. Overall, knee pain was common while ROA was relatively uncommon in this group and was predominantly grade 1.

Table 4.3 presents the age and sex adjusted heritability estimates for independent samples only (51 pairs) versus the whole sample (115 pairs). Results were generally comparable with very similar standard errors although there was a trend to higher estimates in the independent sample. Table 4.4 presents the heritability and goodness of fit estimates both before and after adjustment for age, sex, body size, ROA, other cartilage sites if cartilage and other bone sites if bone. The estimates for cartilage volume changed little after adjustment for body size. However, tibial bone areas decreased markedly while knee pain and muscle strength increased. Further adjustment for ROA severity resulted in only small changes in heritability estimates for all variables with the exception of knee pain that became of borderline significance. In particular, the cartilage volume estimates decreased by 1-7% but all remained statistically significant. In general, the cartilage estimates decreased by 5-25% after adjustment for other cartilage sites but remained statistically significant while the bone estimates decreased by 1-11% after adjustment for other bone sites with parallel decreases in statistical significance. Goodness of fit for continuous variables was excellent (39-75%) and modest for ordinal and dichotomous variables (1-9%).

Table 4.1. Structure of the families studied

	No. of families (no. of offspring)	No. of sib pairs
Family size		
2 children	34(68)	34
3 children	10(30)	30
4 children	6(24)	36
6 children	1(6)	15
Total	51(128)	115

Table 4.2: Characteristics of subjects (N=128)*

Factor	**Mean (SD) or %**
Age, years	44.8 (7.0)
No. male/no. female	61/67
Height, cm	169.3 (8.4)
Weight, kg	78.6 (15.4)
Muscle strength, kg	130 (50)
% With knee pain	50%
Radiographic measures	
% With any knee ROA	16%
Total ROA score, range 0-12	0.3 (0.8)
MRI measures	
Medial tibial cartilage volume, ml	2.3 (0.6)
Lateral tibial cartilage volume, ml	2.7 (0.7)
Patella cartilage volume, ml	3.6 (0.9)
Medial tibial bone area, mm^2	17.7 (2.7)
Lateral tibial bone area, mm^2	12.1 (2.1)
Patella volume, ml	13.8 (3.3)

*Except where indicated otherwise, values are the mean ± SD. ROA = radiographic osteoarhtiris; MRI = magnetic resonance imaging.

Table 4.3: Heritability estimates (H^2, %) for knee ROA, strength, and structure in independent samples versus whole sample*

	Independent sample (51 pairs)		Whole sample (115 pairs)	
	H^2 (SE)	P	H^2 (SE)	P
Knee pain	55 (41)	0.04	44 (30)	0.07
Muscle strength	59 (28)	0.15	42 (21)	0.02
Radiographic measures				
Any knee ROA	90 (63)	0.13	61 (53)	0.16
Total ROA score (0-12)	57 (27)	0.03	61 (25)	0.02
MRI measures				
Medial tibial cartilage volume	95 (23)	<0.001	65 (22)	0.001
Lateral tibial cartilage volume	100 (NA)	<0.001	77 (20)	<0.001
Patella cartilage volume	79 (24)	<0.001	84 (21)	<0.001
Medial tibial bone area	79 (25)	0.003	85 (20)	<0.001
Lateral tibial bone area	29 (29)	0.18	57 (22)	0.004
Patella bone volume	80 (24)	0.002	70 (21)	<0.001

*Adjusted for age and sex in each pair member prior to estimation of heritability. NA = not applicable (see Table 2 for other definitions).

Table 4.4: Heritability estimates (H^2, %) for knee ROA, strength, and structure: effect of body size, ROA, and other MRI measures*.

	Adjusted step 1			Adjusted step 2			Adjusted step 3			Adjusted step 4		
	H^2 (SE)	P	R^2	H^2 (SE)	P	R^2	H^2 (SE)	P	R^2	H^2 (SE)	P	R^2
Knee pain	44 (30)	0.07	1	58 (33)	0.04	2	53 (34)	0.06	2	NA	NA	NA
Muscle strength	42 (21)	0.02	58	59 (22)	0.002	61	60 (22)	<0.001	61	NA	NA	NA
Radiographic measures												
Any knee ROA	61 (53)	0.16	5	56 (67)	0.23	9	NA	NA	NA	NA	NA	NA
Total ROA score (0-12)	61 (25)	0.02	6	63 (26)	0.01	8	NA	NA	NA	NA	NA	NA
MRI measures												
Medial tibial cartilage volume	65 (22)	0.001	39	60 (22)	0.003	43	59 (23)	0.004	43	54 (25)	0.014	NA
Lateral tibial cartilage volume	77 (20)	<0.001	40	74 (20)	<0.001	45	69 (20)	<0.001	48	44 (21)	0.011	NA
Patella cartilage volume	84 (21)	<0.001	39	88 (20)	<0.001	48	81 (21)	<0.001	51	75 (22)	<0.001	NA
Medial tibial bone area	85 (20)	<0.001	53	32 (22)	0.07	74	40 (23)	0.04	75	29 (23)	0.10	NA
Lateral tibial bone area	57 (22)	0.004	44	14 (22)	0.26	56	17 (20)	0.18	66	18 (20)	0.16	NA
Patella bone volume	70 (21)	<0.001	46	63 (23)	0.003	63	63 (23)	0.003	63	54 (25)	0.016	NA

*Adjusted in each pair member prior to estimation of heritability in step 1 for age and sex, step 2 for age, sex, weight and height, step 3 for all previous factors and radiographic osteoarthritis score, step 4 for all previous factors and other cartilage sites if cartilage and other bone sites if bone.

4.4 Discussion

This is the first study to estimate the heritability of knee structures assessed by MRI. It documents a high heritability of cartilage volume and bone size at tibial and patellar compartments of the knee, muscle strength, and knee pain. It also confirms a significant genetic contribution to severity but not presence of knee ROA. With the exception of bone size, the estimates were independent of age, gender, height, and weight. Interestingly, with the exception of knee pain, all estimates were largely unchanged after further adjustment for familial resemblance in ROA. MRI cartilage and, to a lesser extent, bone sites were largely under independent genetic control with a lesser-shared genetic component.

Recent studies have demonstrated that quantitative assessment of knee cartilage volume is both reliable and reproducible, being associated with OA risk factors such as gender, age, BMI, physical activity [197 203 219]. It is also significantly correlated with radiographic features of the knee OA, especially JSN [220], which has been employed as a surrogate measure of articular cartilage. Using radiographic assessment, a previous twin study suggested that knee JSN had a heritability estimate of up to 41% [107]. The twin study may underestimate the heritability due to the semi-quantitative methods or may overestimate it due to the assumption of equal shared environments in the twin model. Family studies have generally suggested a lower heritability supporting the latter hypothesis [105 106 108]. However, the current study demonstrates consistent and higher heritability estimates for cartilage volume at all sites, supporting the former hypothesis. The current study suggests that cartilage volume would be a suitable candidate for quantitative trait analysis. However, there are some caveats to this. Firstly, while the heritability estimates for cartilage volume were consistently higher than estimates for ROA (both from the published literature and

the current study), they were not significantly higher as the confidence limits overlapped. Secondly, while cartilage loss is the hallmark of OA, there is debate about the contribution of cartilage volume to the development of disease. The heritability of knee cartilage volume in this study decreased by 1-7% but remained significant after adjusting for familial resemblance in ROA. This observation suggests either that cartilage volume is under genetic control, but is of uncertain relevance to the onset of the knee OA or that radiographic assessment is a poor measure of OA joint pathology. Further longitudinal studies will be required to assess the genetic contribution to cartilage loss.

Comparisons with the other studies are made more difficult due to differences in sex, age and type of subject studied. However, the heritability estimates for ROA from the current study are somewhat higher than formerly reported in the literature [107 113 114]. While the heritability estimates were both around 60%, they only achieved statistical significance for ROA severity. This was due to very large standard errors for prevalent ROA possibly due to its relative rarity in this young sample, but also implies that genetic factors more likely predispose people to more severe disease as previously reported for total knee replacement [113].

It is well recognized that subchondral bone changes such as osteophytes, sclerosis are associated with OA, both radiographically and pathologically. It has been proposed that subchondral bone plays a role in the initiation and progression of cartilage damage [286]. Recently, subchondral bone oedema has been linked to knee pain [42 287] while abnormalities on bone scintigraphy have been linked to progression of disease [288]. Greater bone size in the proximal femur in hip OA subjects has also been observed [289]. We have observed a higher medial tibial plateau area in the offspring of

subjects who have had a total knee replacement for knee OA as compared to controls [217].

The present findings indicate that bone size of the knee is under strong genetic control and that most of the family resemblance in tibial area is mediated by body size (primarily height), suggesting a structural gene(s). The significance of these results requires further investigation. Mechanical mechanisms may be implicated. Similar observations apply to muscle strength and/or knee pain. These are under strong genetic control and are both altered in the offspring of subjects who have had a total knee replacement for knee OA as compared to controls [217]. In particular, the analysis suggests that knee pain is of direct relevance to the inheritance of knee ROA. It is possible that the relative rarity of ROA combined with the young age of the sample may have not allowed full adjustment for ROA risk in later life and further studies with both a higher prevalence of and more severe ROA are desirable. MRI cartilage and, to a lesser extent, bone sites had a largely independent genetic component with a less important shared genetic component in variance components analysis suggesting that the different sites are primarily under the control of different genes.

The current study has a number of potential limitations. There is controversy about the ideal study to estimate heritability of disease. The twin model is often used but has been criticised as overestimating heritability due to the assumption of similar shared environments between monozygotic and dizygotic twins. This has been documented for bone mineral density [290] but not for OA. Family studies such as this study may be more likely to represent the true heritability but make it more difficult

to assess the contribution of shared environment. Using multiple sib pairs from the same family may bias heritability estimates upwards and falsely decrease standard errors. However, this did not occur in our dataset with very comparable results and standard errors in the independent sample and the whole sample and even a trend to higher heritability estimates in the independent sample. While the variance-components analysis assumes a normally distributed trait, the method has been shown to be equivalent for discrete traits and perform reasonably for dichotomous traits [285]. Our data would support this with the greatest standard errors and resultant least robustness for dichotomous traits such as pain and any ROA. The choice of subjects who at all are at higher risk of disease may bias the heritability estimates. It is most likely that this bias will act to decrease estimates by decreasing genetic heterogeneity in comparison to an unselected sample. However, our data do not support this with heritability estimates for ROA that are higher than previous reports [113 114] and very high estimates for knee structures. In addition, the response rate was 71% suggesting non-response bias was not of major concern in this study and the variance estimates for the MRI measures were very similar to a control population even though there was more knee pain [217]. Nevertheless, these results need to be confirmed in less selected samples. Measurement error in the assessment of both MRI and ROA may have reduced the estimates. However, both assessment techniques have high reproducibility in our hands suggesting this is not of major concern and are offset by the blinded reading of MRIs and radiographs by different observers.

In conclusion, with the exception of prevalent ROA, all knee modalities assessed had high heritability most likely reflecting a strong genetic component. Cartilage volume,

bone size and muscle strength all have potential for quantitative trait linkage analyses but their exact relevance for OA remains uncertain at this time.

4.5 Postscript

This chapter documented significant genetic influence on knee cartilage volume, bone size, and muscle strength. Whether genetic factors play a role in longitudinal changes of these variables will be examined in the next chapter.

CHAPTER FIVE: THE GENETIC CONTRIBUTION TO LONGITUDINAL CHANGES IN KNEE STRUCTURE AND MUSCLE STRENGTH: A SIBPAIR STUDY

5.1 Introduction

OA is the most common form of arthritis and a leading cause of musculoskeletal disability in most developed countries [55]. The knee is one of the most frequently affected joints with a prevalence of 30% in people older than 65 years [59] and high resultant disability [272]. Apart from the importance of environmental factors, a modest but significant genetic effect on knee OA has been demonstrated in most studies [106-108 113 114]. However, most of the studies use radiographs as outcome measure, which provides only a broad-brush view of joint pathology due to their two dimensional nature and semi quantitative grading scales. MRI can allow direct visualization of joint structures and provide accurate and reproducible quantitative estimates of cartilage volume and bone area [203 208 219 256] and thus has the potential to be used in quantitative traits for linkage and association analysis.

In the previous cross-sectional studies including Chapter 4, we reported high heritability for knee cartilage volume, chondral defects, bone size, and lower limb muscle strength which were largely independent of ROA suggesting they are under strong genetic control but of uncertain relevance to ROA [291 292]. However, all these measures appear to have relevance to symptoms, OA progression and/or arthroplasty. Cartilage volume is associated with knee pain[49] and its rate of loss is an independent predictor of worsening of pain in people with OA [50] and of subsequent knee arthroplasty [270]. Chondral defects are also associated with knee pain [293] and more rapid cartilage loss [294]. Muscle weakness is well recognized as a risk factor for the development of OA [295] and appears to be more strongly correlated with decreased function in persons with OA than pain or the degree of radiographic change [57]. Bone size may also be implicated in the pathogenesis of OA [217]. Thus, it appears that

adjustment for ROA may not be the best method of assessing relevance to OA especially in early disease as mild ROA is associated with substantial reductions in cartilage volume and increases in joint surface area [247] suggesting that much has happened at a structural level prior to the onset of ROA.

With regard to genetic studies, an independent twin study has confirmed the cartilage volume estimates [296]. However, in cross-sectional studies, the genetic contribution may reflect both the effect of growth and subsequent loss. Certainly, cartilage volume loss is high in those with well-established OA [255] but the factors underlying this are uncertain. Longitudinal studies are required to estimate the genetic contribution to change in all the above factors. The aim of the study, therefore, was to utilise a sib pair design to estimate the heritability of longitudinal changes in knee cartilage volume, chondral defects, subchondral bone size and lower limb muscle strength and to assess whether these estimates are independent of ROA.

5.2 Materials and methods

Study subjects were derived from the KCV study. Briefly, subjects were the adult children of patients who had a knee replacement performed for idiopathic knee OA at any Hobart hospital during the years 1996-2000. The diagnosis was confirmed by reference to the medical records of the orthopaedic surgeon and the original radiograph where possible. No specific selection criteria were applied for the knee replacement subjects. Offspring were excluded on the basis of contraindication to MRI (including metal sutures, presence of shrapnel, iron filing in eye and claustrophobia). All study factors were measured at baseline and approximately 2 years later. The Southern Tasmanian Health and Medical Human Research Ethics Committee approved the study and written informed consent was obtained from all participants.

Anthropometrics. The weight and height measurements at baseline were described in section 3 of Chapter 3. Muscle strength was measured at baseline and follow up by the same dynamometer at the lower limb (involving both legs simultaneously) using the same standard protocol. The details of the method were described in section 3 of Chapter 3. Repeatability estimates (Cronbach's alpha) were 0.91. The device was calibrated by suspending known weights at regular intervals. The longitudinal change in muscle strength was expressed as percentage per year and computed by difference in muscle strength between follow up and baseline divided by the muscle strength at baseline and the follow up time interval. Medical history such as knee pain and knee injury was collected by questionnaire at baseline.

MRI. A MRI scan of the right knee was performed at baseline and follow up by the same machine and using the same protocol. Lateral and medial tibial cartilage volume was determined. The details of the method were described in section 2 of Chapter 3. Global cartilage volume was computed as the sum of lateral and medial tibial cartilage volume. The measurement was done by a single observer (CD) for both baseline and follow up. The intra-observer reproducibility was high and described in details in section 2 of Chapter 3. The longitudinal changes in lateral and medial tibial and global cartilage volume were expressed as percentage per year and computed by difference in cartilage volume between follow up and baseline divided by the baseline cartilage volume and interval between scans.

The cartilage defects were graded on the same serial MR images. The details of the method were described in section 2 of Chapter 3. A single observer (CD) was utilized to score chondral defects for both baseline and follow up. The details of the reproducibility were described in section 2 of Chapter 3. The score at the lateral tibial and femoral sites was added up to create a lateral compartment chondral defect score. A similar approach was applied for the medial compartment. The difference in chondral defects score between baseline and follow up was computed by subtracting the baseline score from the follow up score with progression of chondral defects defined as any difference ≥ 1.

The same serial MRI images were converted into isotropic volumes and reformatted in the axial plane. Medial and lateral tibial plateau area was determined from the three input images closest to the joint. The details of the methods were described in section 2 of Chapter 3. Global bone area was computed as the sum of lateral and

medial tibial plateau areas. This also was done by a single observer (CD) for both baseline and follow up. The reproducibility was described in section 2 of Chapter 3. The longitudinal changes in lateral and medial tibial and global plateau area were expressed as percentage change per year and computed by difference in plateau area between follow up and baseline divided by the baseline plateau area and interval between scans.

X-ray. A standing AP semiflexed radiograph of the right knee was performed on all subjects at baseline. Individual radiographic features of OA were scored. The details of the method and the reproducibility were described in section 2 of Chapter 3.

Statistical methods

The same method as in Chapter 4 was used in the analysis. Briefly, trait variance was modeled as a mixture of genetic variance (attributed to many genes with small, additive effects) and random variance (due to random environmental variations not correlated between subjects within families). Then the estimated heritability was defined as the proportion of genetic variance in the model with the maximum likelihood. Heritability estimates are high when intra-family variation in trait scores is low compared to inter-family variation.

To assess whether the estimated heritabilities differed from zero, a null model with only the random variance term was also fitted. All models were fitted after first adjusting trait scores within SOLAR for various combinations of covariates (i) age, sex, weight, and height; (ii) previous covariates, knee pain, previous knee injury, and longitudinal changes in muscle strength; (iii) all previous covariates and longitudinal

changes in cartilage volume (for studies of bone size) /bone size (for studies of cartilage volume and chondral defects); (iii) all previous covariates and ROA score. To test whether the models and standard errors were affected by lack of independence, we randomly selected one sib pair from each family where there was more than one sib pair and repeated the age, sex, height, and weight adjusted analyses. A p value of less than 0.05 was regarded as statistically significant.

5.3 Results

A total of 128 subjects (61 males and 67 females) representing 115 sib pairs with an average age of 45 years took part at baseline. Ten subjects were lost to follow up (follow-up rate 92%) and three families were excluded because only one sibling was left. The average follow up time was 2.4 years (range 1.7-3.3 years). The structure of the families studied is presented in Table 5.1, and the general characteristics and study traits are shown in Table 5.2. The distribution of longitudinal changes in cartilage volume, bone size, and muscle strength approximated a normal distribution. Overall, change in cartilage volume and bone size at the medial tibial site were larger than that in the lateral tibial site. Knee pain was common while ROA was relatively uncommon and mild at baseline in this group.

Table 5.3 presents the heritability estimates for the independent sample only versus the whole sample. Results were generally comparable, with slightly high standard error and a trend toward higher estimates for muscle strength and chondral defects in the independent sample.

Table 5.4 presents the heritability estimates for the study traits. After adjustment for age, sex, height, and weight in step 1, changes in global cartilage volume, lateral bone size, and muscle strength all had significant heritability. After adjustment for knee pain, previous knee injury, and change in muscle strength in step 2, the heritability estimates increased by 8-50% with the largest increase for global cartilage volume. In addition, the heritability estimates for change in medial tibial cartilage volume and progression of chondral defects at medial compartment became statistically significant. Further adjustment for bone size or cartilage volume (where

appropriate) and ROA in step 3 and 4 led to small reductions in the heritability estimates for all study traits with exception for chondral defects at lateral compartment where there was a 75% decrease.

Table 5.1. Structure of the families studied

	Follow up	
Family size	No. of families (no. of offspring)	No. of sibpairs
2 children	35(70)	35
3 children	9(27)	27
4 children	3(12)	18
6 children	1(6)	15
Total	48(115)	95

Table 5.2. Characteristics of the subjects (n=115)*

Age at baseline	44.8 ± 7.0
Female (%)	52
Height (cm) at baseline	169.3 ± 8.4
Weight (kg) at baseline	78.6 ± 15.4
Knee pain at baseline (%)	50
Knee injury history at baseline (%)	19
Any knee ROA at baseline (%)	16
Total ROA score (0-12) at baseline	0.3 ± 0.8
Changes in muscle strength (% per year)	-2.8 ± 8.6
Changes in cartilage volume (% per year)	
Lateral tibial	-2.0 ± 3.2
Medial tibial	-3.7 ± 4.4
Global	-2.8 ± 3.2
Changes in bone size (% per year)	
Lateral tibial	-0.02 ± 3.1
Medial tibial	0.7 ± 2.1
Global	0.5 ± 1.9
Progression of chondral defects (%)	
Lateral compartment	33
Medial compartment	38

* Except where indicated otherwise, values are the mean ± SD. ROA = radiographic osteoarthritis

Table 5.3. Heritability estimates (H^2, %) for study traits in the independent sample versus the whole sample*

	Independent sample (48 pairs)		Whole sample (95 pairs)	
	H^2 (SE)	P	H^2 (SE)	P
Changes in cartilage volume				
Lateral tibial	24(31)	0.22	26(25)	0.14
Medial tibial	41(29)	0.08	33(24)	0.07
Global	37(29)	0.11	47(23)	0.02
Changes in bone size				
Lateral tibial	47(29)	0.06	54(25)	0.01
Medial tibial	24(30)	0.21	23(24)	0.16
Global	32(30)	0.15	32(23)	0.07
Changes in muscle strength	86(29)	<0.01	54(28)	0.03
Progression of chondral defect				
Lateral compartment	63(57)	0.16	21(46)	0.32
Medial compartment	45(56)	0.23	25(42)	0.27

*Adjusted for sex, age, height, and weight prior to estimation of heritability.

Table 5.4. Heritability estimates for longitudinal changes in knee cartilage volume, bone size, lower limb muscle strength, and progression of knee chondral defects*

	Step 1		Step 2		Step 3		Step 4	
	H^2 (SE)	P	H^2 (SE)	P	H^2 (SE)	P	H^2 (SE)	P
Changes in cartilage volume								
Lateral tibial	26(25)	0.14	40(31)	0.10	41(30)	0.09	37(31)	0.12
Medial tibial	33(24)	0.07	73(25)	<0.01	62(27)	0.01	63(27)	0.01
Global	47(23)	0.02	97(23)	<0.001	89(25)	<0.001	86(26)	<0.01
Changes in bone size								
Lateral tibial	54(25)	0.01	62(31)	0.03	63(33)	0.04	63(33)	0.04
Medial tibial	23(24)	0.16	20(26)	0.22	20(26)	0.22	20(26)	0.21
Global	33(23)	0.07	32(28)	0.11	25(29)	0.20	26(30)	0.19
Changes in muscle strength	54(28)	0.03	64(28)	0.01	74(29)	<0.01	74(29)	<0.01
Progression of chondral defects								
Lateral compartment	21(46)	0.32	80(71)	0.06	45(58)	0.22	5(59)	0.46
Medial compartment	25(42)	0.27	98(NA)	0.03	100(NA)	0.04	100(NA)	0.04

*Prior to estimation of heritability, adjustments were made for age, sex, height, and weight (step 1), for all previous covariates and knee pain, previous knee injury, and changes in muscle strength (step 2), for all previous covariates, and changes in bone size/cartilage volume where appropriate (step 3), and for all previous covariates and total ROA score (step 4).

5.4 Discussion

In the first longitudinal evaluation of the genetic contribution to knee structure and lower limb muscle strength, we have documented significant and high heritability estimates particularly for longitudinal changes in global cartilage volume, medial tibial cartilage volume, lateral tibial plateau size, muscle strength, as well as progression of chondral defects. These heritability estimates are higher than, but largely independent of, ROA. Furthermore, the heritability estimates of the study traits remained largely unchanged after adjustment for each other, suggesting that they are under independent genetic control, with at most a small-shared genetic component.

In previous cross-sectional studies, we (Chapter 4) and others [296] demonstrated a high heritability for both lateral and medial tibial cartilage volume. This longitudinal study is consistent with the previous results, highlighting the strong genetic component to both knee cartilage volume and its rate of change. In contrast to previous studies, which suggested both lateral and medial tibial cartilage volume had a high and significant heritability [291], we demonstrated a stronger genetic influence on the medial than on the lateral tibial cartilage volume. This is surprising given the cross sectional results and needs to be confirmed in further studies but most likely reflects the relatively greater effect of measurement error in longitudinal studies. However, it is a possible explanation for why OA targets the medial compartment more commonly than the lateral compartment [297]. Alternatively, cohort effects may bias the results in the cross-sectional study. Similarly, we found that lateral tibial plateau size had a higher and significant heritability than medial although the longitudinal changes over two years in medial tibial plateau size was larger than for

the lateral. This also contrasts with the cross-sectional findings [291] in which both lateral and medial tibial plateau size had low heritability after adjusting for body size. The observed significant increase in the medial but not the lateral tibial plateau probably reflects either the OA disease process and/or subchondral bone remodelling. Indeed, people with knee ROA have larger tibial plateau size than those without ROA, and this is more pronounced in the medial than the lateral tibial plateau [254]. Adjustment for ROA led to no changes in the heritability estimates of both cartilage volume and bone size, which casts doubt on the relevance of these MRI measures to OA. However, these measures all have relevance to various facets of knee OA and there are a number of reasons as mentioned in the introduction to question the value of adjusting for ROA in younger samples as cartilage loss and bone expansion need to be substantial before ROA is evident. Nevertheless, these results need to be confirmed in independent samples with different races/ethnicities and a higher prevalence of both radiographic and symptomatic OA.

The heritability estimates for cartilage volume and bone size remained largely unchanged after adjustment for each other, suggesting they are largely under independent genetic control, with a lesser-shared genetic component. However, adjustment for knee pain and previous knee injury surprisingly led to an increase in the heritability estimates for both cartilage volume and bone size with the maximum increase of 40% for medial tibial cartilage volume. This implies negative confounding that seems unlikely given the variables in question or may represent better estimates due to less environmental noise.

Similar to our previous cross sectional report [292], we demonstrated a high heritability for the progression of chondral defects. The heritability increased by 59-83% after adjustment for knee pain and previous knee injury. Again, this implies negative confounding or the effect of less environmental noise. However, a 75% reduction of the heritability for progression of chondral defects at the lateral compartment after adjustment for ROA supports direct relevance to OA. This was not the case for medial compartment whose heritability remained unchanged even after adjustment for ROA. The reason for this discrepancy remains unclear, but the higher standard error for the heritability estimates indicates that the results are not robust possibly reflecting relative limitations of the program we used for dichotomous traits as compared to continuous traits [285]. It is likely that the true heritability is substantially lower than 98% for the medial compartment.

Consistent with our cross-sectional study in the Chapter 4, we demonstrated in this longitudinal study a strong genetic component to loss of lower limb muscle strength over time. Muscle weakness is well recognized as a risk factor for the development of OA [295].The current study suggests that change in muscle strength is under strong genetic control. Identification of susceptibility gene(s) for muscle strength may help to provide a new approach in the prevention of OA.

The current study has a number of potential limitations. Firstly, There is controversy about the ideal study design for estimating heritability of disease. The twin model is often used but has been criticized as overestimating heritability due to the assumption of similar shared environments between monozygotic and dizygotic twins. This has been documented for bone mineral density [298] but not for OA. Family

studies such as the present one may be more likely to represent true heritability but make it more difficult to assess the contribution of shared environment. Using multiple sibpairs from the same family may bias heritability estimates upward. However, the heritability estimates from an independent sample (one pair from each family) were very comparable to that from the whole sample, indicating this is not an issue in the current study and consistent with our previous report [291]. Secondly, the choice of subjects who are at all at higher risk of disease may bias the heritability estimates and limit the generalizability of the results to the general population. However, it is most likely that this bias will act to decrease estimates by decreasing genetic heterogeneity in comparison with an unselected sample. Our data may partly support this with some inconsistency in estimates between sites. Thirdly, measurement error in the assessment of both MRI results and muscle strength may have reduced the estimates. However, both assessment techniques have high reproducibility at our institution, suggesting that this is not of major concern. Fourthly, we did not assess meniscal degeneration or extrusion, which has been reported to be associated with loss of cartilage volume [268], but it remains totally uncertain whether these influence heritability results or will be heritable themselves. Lastly, the follow up rate was 92%, suggesting that lost to follow up was not of major concern in this study. However, the follow up period is relatively short and longer studies may be required to accurately associate the clinical significance of the MRI changes.

In conclusion, early longitudinal changes in knee structures of relevance to later OA such as medial tibial cartilage volume, lateral tibial bone size, progression of chondral defects as well as muscle strength have a high heritability, most likely

reflecting a strong genetic component and suggesting their potential to be studied in quantitative trait linkage and association analysis.

5.5 Postscript

This chapter documented a significant genetic contribution to longitudinal changes in knee medial cartilage volume, lateral tibial bone size, muscle strength as well as progression of chondral defects, consistent with Chapter 4, providing evidence that all these variables examined have potential to be studied in quantitative trait linkage and association analysis. The next chapter will examine the correlates of knee pain in younger subjects.

CHAPTER SIX: CORRELATES OF KNEE PAIN IN YOUNGER SUBJECTS

6.1 Introduction

Knee pain and resultant disability is the most important clinical feature of knee OA [299 300]. However, knee pain correlates poorly with radiographic features [11] with only 50% of subjects with radiographic knee OA having pain [53 301 302]. This is in part due to the fact that radiographs only permit limited assessment of knee structure and poorly characterize the soft tissues. It is more likely that knee pain originates from multiple sources such as the synovial membrane, joint capsule, periarticular ligaments or muscle, periosteum, and subchondral bone as nociceptive fibres are present in these structures [41]. This is evident from recent reports of significant association between knee pain and knee effusions, popliteal cysts, synovial thickening, and bone marrow edema identified by MRI [42 43].

Cartilage loss, which is the central component in the development of OA, can occur without knee pain, as cartilage does not contain nociceptive nerve fibres. However, substance P nociceptive fibres have been found in abnormal cartilage such as erosion channels in horse OA [44] and superinduction of COX-2 and PGs has been observed in OA-affected cartilage explants [45], suggesting that articular cartilage may indirectly produce pain. Changes in the severity of cartilage loss on arthroscopy correlate significantly with pain and disability [46]. In particular, subjects with full-thickness articular cartilage defects accompanied by adjacent subchondral cortical bone defects are more likely to have pain in the presence of knee OA [48]. Lower patellar cartilage volume has been linked to knee pain [49], and tibial cartilage volume loss has been associated with knee pain [50]. It remains unclear whether involvement of underlying bone is necessary for pain or whether lesser degrees of chondral damage can lead to pain. Biomarkers such as urinary CTX-II, a specific markers of type II collagen

breakdown, have been reported as an important predictor of progression of joint damage [303], but there is no published data relating it to pain.

The aim of the study, therefore, was to describe clinical, structural and biochemical factors associated with knee pain in younger subjects.

6.2 Materials and methods

Subjects were all participants in the KCV study. The details were described in section 1 of Chapter 3. The Southern Tasmanian Health and Medical Human Research Ethics Committee approved the study and all subjects provided informed written consent.

Knee pain. Knee pain was determined by self-administered questionnaire (*Appendix 2*) if subjects answered yes to the following question: Have you had knee pain for more than 24 hours in the last 12 months or daily pain on greater than 30 days in the last year? Severity assessment was not available. Subjects were also asked the following questions in the assessment of previous knee injury and their occupation involving significant knee bending: Have you had a previous knee injury requiring non-weight bearing treatment for more than 24 hours or surgery? And if employed, does your occupation involve significant knee bending and carrying heavy objects? (*Appendix 2*)

Anthropometry. The weight and height measurements were described in section 3 of Chapter 3. BMI was calculated as weight in kilograms divided by the square of height in metres. Overweight was defined as a BMI more than 25 kg/m^2 while obesity was defined as a BMI more than 30 kg/m^2.

Knee cartilage volume and chondral defects. T1 weighted fat saturation 3D MRI scan was performed on the right knee. Lateral and medial tibial and patellar cartilage volumes were determined on the series of the sagittal MR images. The details of the method and the reproducibility were described in section 2 of Chapter 3. Femoral

cartilage volume was not assessed as cartilage volume at two tibial sites and the patella site correlate strongly with femoral cartilage volume, which thus add little extra information [283].

Chondral defects at medial tibial, medial femoral, lateral tibial, lateral femoral and patellar sites were assessed on these series of the sagittal MR images. The details of the method and the reproducibility were described in section 2 of Chapter 3. A prevalent cartilage defect within any compartment was defined as a cartilage defect score of ≥2. None of the subjects had two or more cartilage lesions at one site.

Knee bone size measurement. Knee tibial plateau bone area and patellar bone volume were determined by MRI. The details of the method and the reproducibility were described in section 2 of Chapter 3.

Urinary CTXII. Urinary CTX-II was measured by a competitive ELISA (Cartilaps, Nordic Bioscience, Herlev, Denmark) based on a mouse monoclonal antibody raised against the EKGPDP sequence of human type II collagen C-telopeptide [304]. This sequence is found exclusively in type II collagen and not in the other collagens including type I or other structural proteins. Intra and inter assay CVs are lower than 8 % and 10% respectively.

X ray. A standing AP semiflexed radiograph of the right knee was performed on all subjects. Individual radiographic features of OA were scored. The details of the method and the reproducibility were described in section 2 of Chapter 3. The presence of radiographic OA was defined as a total score ≥1.

Statistics. Descriptive statistics of characteristics of the sample were tabulated. Comparison between people with and without knee pain was made by unpaired t-test, or Chi-Square test wherever appropriate. Associations between knee pain and individual factors studied were assessed by logistic regression modeling with adjustment for age, sex, previous knee injury, and case-control status. To examine potential mechanisms further adjustment for BMI and osteophytes was performed. A p value less than 0.05 (two-tailed) or a 95% CI not including the null point was considered statistically significant. All statistical analyses were performed on SPSS version 10.0 for Windows (Chicago, IL).

6.3 Results

A total of 372 subjects took part in this study. One subject had missing information on knee pain. The analysis included 371 subjects (male 158, female 213) age between 26 and 61 (mean age 45). Characteristics of the study sample are present in Table 6.1. The prevalence of knee pain was 35%. There was no difference in age and height between people with or without knee pain, but people with knee pain were heavier than subjects without knee pain, and males had a higher prevalence of knee pain than females. The prevalence of femoral and patellar chondral defects, osteophytes, previous knee injury as well as occupations involving knee bending were higher in people with knee pain than those without knee pain. In unadjusted analysis, there was a significant difference in BMI, CTX-II, lateral tibial cartilage volume, lateral bone area, and medial bone area between people with and without knee pain.

Table 6.2 presents the results of the multivariable analysis of the association between prevalence of knee pain and individual study factors. Total chondral defect score was significantly associated with knee pain and this significance persisted after adjustment for BMI and osteophytes. There appeared to be site specificity for pain with significant associations for femoral and patellar chondral defect scores but not tibial chondral defects. The significant association between knee pain and femoral and patellar chondral defects was independent of each other ($p = 0.01$ and 0.03 for femoral and patellar respectively in multivariate analysis). The prevalence of knee pain increased from 31-33% to 38-47% for people with less than 50% thickness chondral defects and to 56-65% for people with more than 50% thickness defects at distal femoral and patellar sites (Figure 6.1). There was a non-significant trend to

increasing prevalence of knee pain with increased tibial chondral defects (Figure 6.1). No significant association was found between knee pain and cartilage volume or bone area in multivariate analysis in this sample.

CTX-II was significantly higher in those with knee pain, but this association became non significant after further adjustment for BMI and osteophytes (Table 6.2). Figure 6.2 demonstrates the prevalence of knee pain increased from 26% to 30% for second tertile, and to 44% for third tertile. CTX-II was also significantly correlated with BMI ($r = 0.13$, $p = 0.02$) and chondral defects (Spearman's rho = 0.20, $p < 0.001$) but not osteophyte score ($r = 0.07$, $p = 0.23$).

After adjustment for age, sex, previous knee injury, occupation, and case-control status, BMI was significantly associated with knee pain, and this significance persisted even after adjustment for chondral defects and osteophytes (Table 6.2). When categorized, the prevalence of knee pain increased from 24% to 38% for overweight people and to 46% for obese people (p for trend < 0.01). Osteophytes but not joint space narrowing were also significantly associated with knee pain, even after adjustment for BMI and chondral defects (Table 6.2). The prevalence of knee pain increased from 33% to 63% for people with an osteophyte score of one and to 78% for people with score 2 or higher (p for trend < 0.01). Both previous knee injury and occupation involving knee bending were significantly associated with prevalence of knee pain in multivariable analysis ($p < 0.01$ for both), but age and sex were not.

Table 6.1. Characteristics of the study population*

	No knee pain N = 242	Knee pain N = 129	P value
Sex (female %)	**61.6**	**49.6**	**0.03†**
Age (yr)	45.1(7.2)	45.2(6.3)	0.94
Height (cm)	168.9(8.6)	169.7(8.4)	0.37
Weight (kg)	**75.7(16.7)**	**82.1(15.1)**	**<0.001**
BMI (kg/m^2)	**26.4(5.0)**	**28.5(5.0)**	**<0.001**
Previous knee injury (%)	**15**	**28**	**<0.01**
Occupation involving knee bending (%)	**31**	**56**	**<0.001**
Lateral tibial cartilage volume (ml)	**2.6(0.7)**	**2.7(0.7)**	**0.05**
Medial tibial cartilage volume (ml)	2.2(0.6)	2.3(0.6)	0.11
Patellar cartilage volume (ml)	3.4(1.0)	3.5(1.0)	0.62
Lateral bone area (cm^2)	**11.8(1.9)**	**12.3(2.2)**	**0.02**
Medial bone area (cm^2)	**17.2(2.7)**	**17.8(2.8)**	**0.03**
Patellar bone volume (ml)	13.7(3.3)	14.0(3.3)	0.34
Femoral chondral defects (%)	**11**	**20**	**<0.01†**
Tibial chondral defects (%)	25	34	0.09†
Patellar chondral defects (%)	**26**	**39**	**<0.01†**
Radiographic OA (%)	15	20	0.22†
Osteophytes (%)	**3**	**15**	**<0.001†**
Joint space narrowing (%)	14	15	0.82†
CTXII (ng/mmol Cr)	**137.6(108.9)**	**162.1(95.5)**	**0.04**

*Values are the mean (SD) for continuous variables; BMI = body mass index, CTXII = collagen type II C-telopeptide fragments in urine corrected by creatinine. †Chi-Square test, otherwise Unpaired t-test.

Table 6.2. Association between knee pain and study factors*

	Step 1			Step 2			Step 3		
	Odds ratio	95% CI	P value	Odds ratio	95% CI	P value	Odds ratio	95% CI	P value
BMI (per kg/m^2)	**1.08**	**1.03, 1.13**	**0.002**	**1.06**	**1.01, 1.11**	**0.02†**	**1.06**	**1.01, 1.12**	**0.02†**
Cartilage volume (per ml)									
Lateral tibial	1.07	0.69, 1.66	0.75	1.06	0.68, 1.66	0.78	1.17	0.73, 1.85	0.52
Medial tibial	0.95	0.58, 1.54	0.84	0.94	0.57, 1.53	0.80	0.98	0.59, 1.62	0.93
Patellar	0.86	0.64, 1.17	0.34	0.89	0.68, 1.16	0.38	0.91	0.67, 1.25	0.57
Bone area (per cm^2)									
Lateral tibial	1.10	0.93, 1.29	0.27	1.07	0.90, 1.26	0.45	1.01	0.83, 1.21	0.95
Medial tibial	0.90	0.66, 1.24	0.52	0.98	0.86, 1.12	0.78	0.95	0.83, 1.09	0.46
Patellar (per ml)	0.94	0.71, 1.25	0.69	0.91	0.68, 1.21	0.50	0.97	0.87, 1.06	0.47
Chondral defects (per unit)									
Distal femoral cartilage	**1.69**	**1.23, 2.33**	**0.001**	**1.60**	**1.16, 2.20**	**0.004**	**1.50**	**1.07, 2.10**	**0.02**
Tibial cartilage	1.14	0.82, 1.59	0.44	1.06	0.76, 1.49	0.72	0.82	0.55, 1.22	0.32
Patellar cartilage	**1.47**	**1.16, 1.87**	**0.002**	**1.43**	**1.12, 1.82**	**0.004**	**1.36**	**1.06, 1.74**	**0.02**
Total knee cartilage	**1.27**	**1.11, 1.45**	**0.001**	**1.23**	**1.08, 1.41**	**0.003**	**1.17**	**1.02, 1.36**	**0.03**
CTXII (per SD)	**1.28**	**1.01, 1.61**	**0.04**	1.22	0.97, 1.55	0.10	1.18	0.92, 1.50	0.16
Radiographic feature (per unit)									
Total osteophytes score	**2.32**	**1.16, 4.62**	**0.02**	**2.17**	**1.10, 4.27**	**0.03**	**2.51**	**1.05, 5.98**	**0.04‡**
Total JSN score	0.99	0.58, 1.69	0.96	0.86	0.49, 1.53	0.61	0.63	0.32, 1.24	0.19

* Logistic regression modelling, step 1 adjusted for age, sex, previous knee injury, occupation involving knee bending, and case-control status; step 2 adjusted for BMI and variables adjusted in step 1; step 3 adjusted for osteophytes and variables adjusted in step 2. †Adjusted for total chondral defects. ‡Adjusted for total chondral defects. Age and sex were not statistically significant but previous knee injury, occupation involving knee bending, and case control status was in the multivariable model.

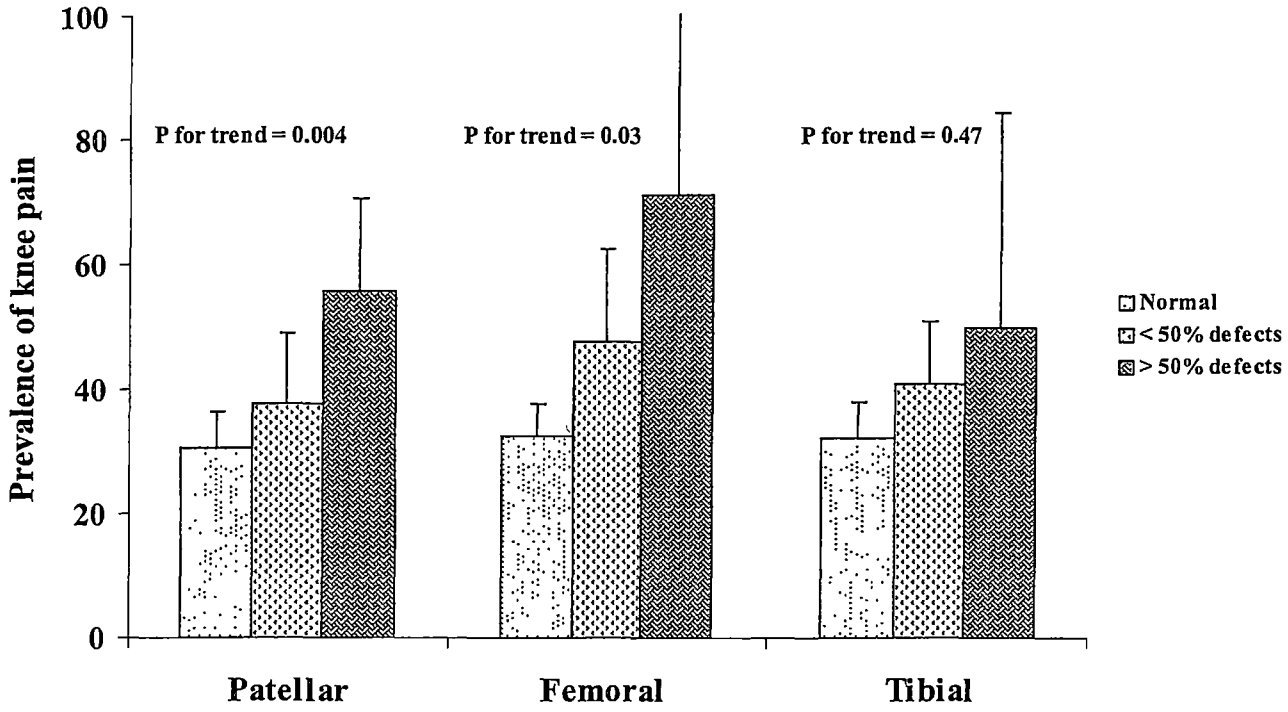


Figure 6.1. Prevalence of knee pain for people with normal cartilage, less than 50% defects, and more than 50% thickness defects at patellar, femoral, and tibial sites. The bars stand for 95% CI for the prevalence. P values were adjusted for sex, age, previous knee injury, occupation involving knee bending, and case-control status.

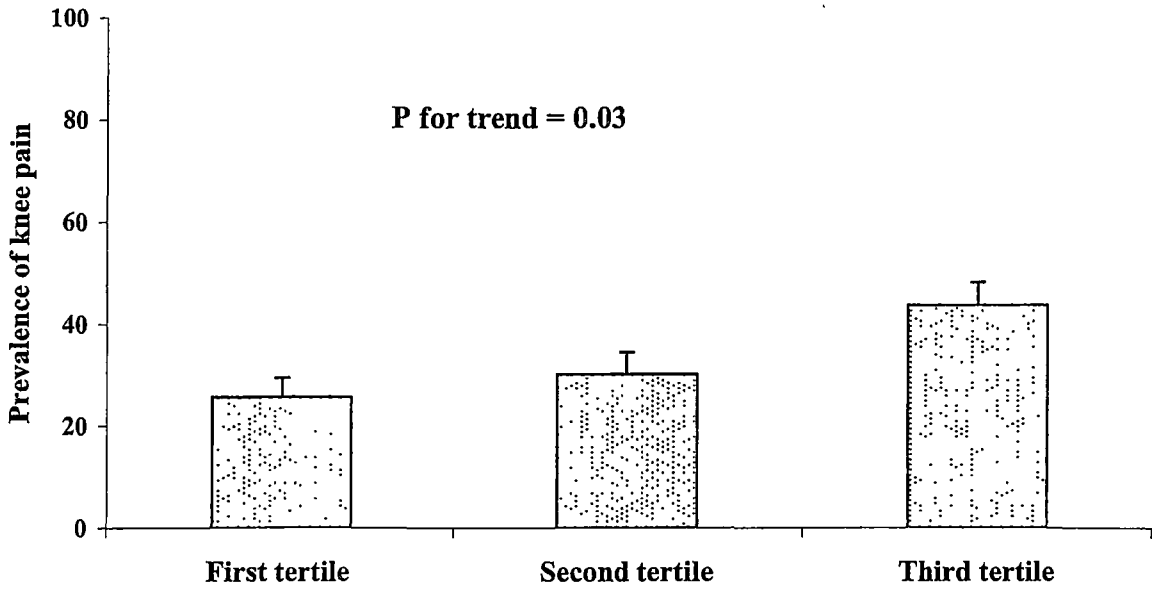


Figure 6.2. Prevalence of knee pain for people grouped by tertiles according to their urinary CTX-II (ng/mmol Cr) (1st: < 93, 2nd: 93 – 153. and 3rd: >153). The bars stand for 95% CI for the prevalence. P value was adjusted for sex, age, previous knee injury, occupation involving knee bending, and case-control status.

6.4 Discussion

This study suggests that chondral defects, particularly distal femoral and patellar chondral defects, osteophytes, and obesity are the significant determinants of knee pain in younger subjects. Also, CTXII, a biomarker of type II cartilage breakdown, may act as a marker of the obesity and chondral defect associations with pain. Joint space narrowing, cartilage volume and bone size were not associated with knee pain in this sample.

Although the articular cartilage does not contain nociceptive fibers, Substance P nociceptive fibres have been found in abnormal cartilage such as erosion channels in horse OA [44]. Changes in the severity of cartilage loss on arthroscopy does correlate with pain and disability in knee OA subjects [46]. Using MRI, which is considered an accurate means of detecting and grading moderate and advanced cartilage lesions in the knee joint [208], subjects with full-thickness articular cartilage defects accompanied by adjacent subchondral cortical bone defects are more likely to have pain in the presence of knee OA [48]. In the current study, we demonstrate that the total chondral defect score is strongly associated with knee pain with a dose response relationship. This is consistent with a previous study of full thickness defects [48], but also suggests that lesser degrees of chondral defect can also lead to pain. The apparent discrepancy between our results and the previous study may be due to sample size considerations[48]. The association between chondral defects and CTX-II [271] suggests that chondral defects are associated with increased cartilage breakdown and is consistent with a recent report that CTX-II is associated with progression of joint damage in OA[303 305].

The association between knee pain and chondral defects appears to have site specificity. The prevalence of knee pain increases significantly with an increased chondral defects score at femoral and patellar sites, suggesting that these compartments may be most important for pain. This site specificity might imply the patellofemoral articulation is important for pain, as we could not distinguish the location of the defects. However, after adjustment for each other in a multivariable model, both remained significant, indicating they are independently associated with knee pain. Interestingly, we could not find a significant association between knee pain and tibial chondral defects even though the prevalence of tibial chondral defects was twice as high as that of distal femoral chondral defects, and nearly the same as that of patellar chondral defects. The reason for this remains elusive. A possible explanation is that tibial cartilage uncovered by menisci may be more likely to be degraded due to underload or disuse rather than a disease process [306]. This may be true as most tibial chondral defects were less than 50%, which was less severe than patellar chondral defects where 12% people had more than 50% defects. This finding is also consistent with previous reports in which symptomatic knee OA has been found to be most commonly related to patellofemoral disease [301]. The mechanism for the observed chondral defect-pain association remains unclear. Chondral defects may result in the transmission of abnormal pressures to the underlying subchondral bone. Indeed, the odds ratio for chondral defects decreased after adjustment for osteophytes. The fact that the significance persists even after this adjustment suggests other underlying pathological processes.

In contrast to previous reports [49 50], we found no significant association between cartilage volume and knee pain. The sample size of the previous studies [49 50] was

relatively small and the significant association between cartilage volume and knee pain was weak. Thus, it is possible that cartilage volume may indirectly reflect other joint measures such as chondral defect severity.

We confirmed the results of others [38] [307] that osteophytes rather than JSN are the major radiographic correlate of knee pain. Also, we confirmed that BMI is strongly associated with knee pain as previously reported [308]. A significant association of BMI with both CTX-II and chondral defects in this sample [262] supports the hypothesis that cartilage damage is on the pathway between obesity and knee pain. However, the persistence of the association between knee pain and BMI even after adjustment for these factors implies other explanations such as systemic or metabolic factors behind obesity.

In addition, both previous knee injury and occupation involving knee bending are significantly associated with knee pain independently of chondral defects, radiographic OA, and BMI, but the mechanism remains unclear.

There are potential limitations to the current study. Firstly, the study was primarily designed to look at genetic mechanisms of knee OA and utilized a matched design. The matching was broken for the current study but adjustment for case control status did not alter the results even though pain was more common in the offspring [309]. Indeed, while there was a reduction in power, the results otherwise did not differ if examined in offspring and controls separately. While the sample is a convenience sample, Miettinen [310] states that for these associations to be generalisable to other populations three key criteria need to be met regarding selection, sample size and

adequate distribution of study factors all of which are met by this study. Secondly, a number of studies have reported the prevalence of knee pain and the estimate varies with case definitions, the composition of the study samples, and the methods used [39] [311-313]. Nevertheless, the prevalence of knee pain in the current study was surprisingly high. The pain definition, while straightforward, was more liberal than other studies and contained two subgroups of pain, which we could not separate for the analysis. Also we did not have severity data, as we did not expect such a high prevalence at the time of study planning. Nevertheless, the resultant high prevalence of knee pain had no bearing on the associations apart from increasing the study power. However, while these results need confirmation with more extensive pain assessment, most would accept that this definition does represent significant pain. Thirdly, categories of chondral defects were somewhat broad due to our semi-quantitative method, which does not allow exact measurement of the defect size. While this may weaken associations we still observed strong dose response associations suggesting this is not of major concern. Measurement error in the assessment of MRI may have weakened the association. However, the assessment techniques have high reproducibility in our hands suggesting this is not of major concern and further offset by the blinded reading. Fourthly, our MRI views do not allow us to assess other abnormalities such as bone marrow lesions and knee effusion, which also have associations with knee pain[42] [43]. It is possible that these abnormalities mediate the associations between our study factors and knee pain in our sample. However, in older subjects, this was not the case (Chapter 7). Lastly, the study was cross-sectional in design and cannot comment on causal directions, thus longitudinal data is required to confirm these results.

In conclusion, knee pain is significantly associated with non-full thickness chondral defects, particularly femoral and patellar chondral defects, osteophytes, CTX-II, and obesity but not other factors. MRI and biochemical measures can add to radiographs in defining unexplained knee pain in younger subjects.

6.5 Postscript

This chapter demonstrated that knee pain is associated with non-full thickness chondral defects, osteophytes, CTX-II, and obesity in younger subjects. Whether these associations exist in older adults will be examined in the next chapter.

CHAPTER SEVEN: CORRELATES OF KNEE PAIN IN OLDER ADULTS

7.1 Introduction

Knee pain is an important clinical symptom and the major determinant of knee arthroplasty[313]. The reported prevalence of knee pain varies according to case definition and age profile of subjects, but clearly increases with age [308 311 312 314] and will inevitably grow as the proportion of older people in the population increases[308]. However, the causes of knee pain remain uncertain. The correlation between ROA and pain is significant but modest [11 53 302 315] with osteophytes being most consistently associated with knee pain[37-39] but inconsistent reports for JSN [37 38 40]. However, JSN only indirectly assesses cartilage morphology, and may underestimate the importance of cartilage damage. Furthermore, the radiographic joint space consists not only of articular cartilage, but also other soft tissues such as menisci [266]. Normal hyaline cartilage does not possess pain fibers, suggesting that articular cartilage cannot be the origin of knee pain. However, substance P nociceptive fibres have been found in abnormal cartilage such as erosion channels in horse OA[44], and superinduction of COX-2 and PGs has been observed in OA-affected cartilage explants[45], suggesting that articular cartilage may indirectly produce pain.

In a study using MRI, researchers found that subjects with full-thickness articular cartilage defects accompanied by adjacent subchondral cortical bone defects are more likely to have pain in the presence of knee OA[48]. In the previous chapter, we reported non-full thickness chondral defects at distal femoral and patellar sites were significantly associated with self-reported knee pain in younger subjectsy. To date, there have been no data reported for older groups.

In addition, knee pain can also originate from other sources such as the synovial membrane, joint capsule, periarticular ligaments or muscle, periosteum, and subchondral bone as nociceptive fibres are present in these structures[41]. This is evident from recent reports of significant association between knee pain and knee effusions, popliteal cysts, and synovial thickening[43]. Subchondral bone marrow lesions have been reported to have an association with knee pain in people with knee ROA[42]. However, it is unclear whether this association is independent of cartilage damage, and whether it is relevant in a non-OA population. Also, over half of people who report hip pain also report knee pain[311], implying either pathology at both sites or that unexplained knee pain may be referred from hip OA. This has not been formally evaluated.

The aim of this cross-sectional study was to investigate the association between knee pain and chondral defects, subchondral bone marrow lesions and knee and hip ROA in older male and female subjects.

7.2 Materials and methods

Subjects were derived from TASOAC, an ongoing prospective population-based study aimed at identifying the environmental, genetic and biochemical factors associated with the development and progression of OA at multiple sites (hand, knee, hip, and spine). The details were described in section 1 of Chapter 3. Briefly, subjects aged between 50 and 79 years were selected randomly from the roll of electors in Southern Tasmania (population 229,000), a comprehensive population listing, with an equal number of males and females. Subjects were excluded if they had contraindication for MRI (e.g. metal sutures, presence of shrapnel, iron filling in eye, and claustrophobia). Institutionalised persons were also excluded. The study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee and written informed consent was obtained from all participants. The current study consisted of the first 500 participants to complete the interview, MRI scan and x-ray by October 2003.

Knee pain. Knee pain was assessed by self-administered questionnaire using the WOMAC [316] (*Appendix 1*). Five categories of pain (walking on flat surface, going up/down stirs, at night, sitting /lying, and standing upright) were assessed separately with a 10-point scale from 0 (no pain) to 9 (most severe pain). Each score was then summed to create a total pain score (range 0-45). With no a priori reason to categorise pain, prevalent knee pain was defined as a total score ≥ 1.

Anthropometry. The height and weight measurements were described in section 3 of Chapter 3. BMI was calculated as weight in kilograms divided by the square of

height in metres. Knee extension strength in the right leg was measured by a pocket balance. The details of the method were described in section 3 of Chapter 3.

MRI. T1 and T2 weighted fat suppressed MRI scans were performed on the right knee. The details of the method were described in section 2 of Chapter 3.

Chondral defects were assessed on the T1-weighted MR images and scored with a modification of a previous classification system [240] at medial tibial, medial femoral, lateral tibial, lateral femoral and patellar sites. One observer (GZ) scored the MRI, blinded to knee pain score. The details of the method and the reproducibility were described in section 2 of Chapter 3.

Subchondral bone marrow lesions were assessed on the T2-weighted MR images and defined as discrete areas of increased signal adjacent to the subcortical bone at lateral, medial femur and/or tibia. One observer (GZ) scored the bone marrow lesions, blinded to knee pain score. Prevalent bone marrow lesions were defined as total score ≥ 1. The details of the method and the reproducibility were described in section 2 of Chapter 3.

X-ray. A standing AP semiflexed radiograph of the right knee was performed on all subjects and scored for individual radiographic features of the knee OA. The details of the method and the reproducibility were described in section 2 of Chapter 3.

Weight bearing anterior-posterior pelvic radiographs with both feet in 10° internal rotation were obtained and also scored for individual radiographic features of the hip

OA in the same manner. The details of the method and the reproducibility were described in section 2 of Chapter 3.

Data analysis. Comparisons between subjects with and without knee pain were made by unpaired t-test, Mann-Whitney U-test or Chi-Square test (as appropriate). Preliminary analysis suggested there was no difference in prevalence of knee pain between subjects with lower chondral defect scores (grade 0, 1, and 2), therefore these were combined into one group for further analysis. No dose response association between bone marrow lesions at each site and prevalent knee pain was detected, and the number of the sites with any bone marrow lesions was used for the prevalent pain analysis. With the WOMAC pain score dichotomized as 0 (score 0) or 1 (score $\geq$1), logistic regression modeling was utilized to estimate the prevalence odds of reported knee pain and study factors. For the analysis of the association between pain severity and study factors, two approaches were utilized. Subjects with more severe pain were identified and defined as a the WOMAC pain score $\geq$4 which was the median of the total WOMAC pain score in subjects with a score $\geq$1. The comparison was then made between people with more severe pain and those without pain by logistic regression modeling. In addition, with the subjects with pain score=0 excluded, linear regression modeling was used to estimate the associations between the zero-skewness logarithmic transformation of the total pain score and the same study factors. Excluding the 52% (261/500) of subjects without reported pain (score=0) was necessary because the residuals were heavily skewed. Stata's fracpoly procedure was utilized in each type of modeling to check the appropriate scale of covariates. The predictor for a study factor was expressed on a linear scale only if no non-linear transform significantly improved model fit.

A p value less than 0.05 (two-tailed) or a 95% confidence interval (CI) not including the null point was considered statistically significant. All statistical analyses were performed on Intercooled Stata 8.2 for windows (StataCorp LP).

7.3 Results

A total of 500 subjects (male: 248, female: 252) with a mean age of 63 years were included in this study. Table 7.1 presents the characteristics of the study population. The prevalence of knee pain was 48%. Most subjects reporting knee pain reported mild pain, with 88% having a WOMAC total pain score of less than 8 (score range 0-45). Females were more likely to report knee pain than males. There was a significant difference in weight, BMI, and knee extension strength between subjects with and without knee pain. The prevalence of grade two or higher chondral defects was higher at all sites except for medial tibia in subjects with knee pain compared to those without, but this difference was small and not statistically significant. However, the difference was more pronounced for severe chondral defects (defined as grade ≥ 3) and statistically significant except for the lateral femoral site. Prevalence of bone marrow lesions, knee JSN and osteophytes, and hip JSN was also significantly higher in subjects with knee pain.

There was a significant increase in the prevalence of knee pain with increasing chondral defects from grade ≤ 2 up to grade 4 at all knee sites with the exception for lateral tibial site (Figure 7.1). Table 7.2 presents the results of multivariable analysis of association between prevalence odds of knee pain and study factors. Knee pain was statistically significantly and independently associated with BMI, knee extension strength, number of the sites with bone marrow lesions, medial tibial chondral defects, and hip JSN. These significant associations persisted after further adjustment for knee osteophytes, which was not statistically significant in the final model (P = 0.51). Age was borderline significant and negatively associated with prevalent knee pain. Knee JSN was not significantly associated with prevalent knee pain (P=0.07)

after adjustment for age, sex, BMI, knee extension strength, bone marrow lesions, hip JSN, and knee osteophytes.

Figure 7.2 documents a significant association between prevalent knee pain and the number of compartments with grade ≥3 chondral defects. The prevalence of knee pain increased with increasing numbers of compartments with defects, with 100% of subjects having pain if all five compartments had these defects. Similarly, prevalence of knee pain increased markedly with increasing hip JSN total score (Figure 7.3).

Table 7.3 presents the results of multivariable analysis of association between the study factors and more severe knee pain. Similar to the prevalent knee pain, more severe knee pain was statistically significantly and independently associated with BMI, knee extension strength, number of the sites with bone marrow lesions, medial tibial chondral defects, and hip JSN. These significant associations persisted even after further adjustment for knee osteophytes with the exception being medial tibial chondral defects whose association with more severe knee pain became borderline ($p = 0.09$). Knee osteophytes was not statistically significant in the final model ($P = 0.24$)

In linear regression analyses with subjects with the WOMAC pain score=0 excluded, severity of knee pain was significantly and independently associated with BMI and hip JSN, with 5.2% and 2.5% respectively of the variation in the WOMAC pain score explained by BMI and hip JSN. The associations for knee extension strength and medial tibial chondral defects were in the direction expected from the prevalence

odds analysis, but with 52% of subjects excluded from this analysis, none of these associations were statistically significant (Table 7.4).

Table 7.1. Characteristics of the study population*

	No knee pain N = 261	**Knee pain** N = 239	P
Sex, female (%)	46	56	0.03
Age (yr)	63.0(7.4)	62.7(7.1)	0.70
Height (cm)	167.8(9.0)	166.5(9.0)	0.10
Weight (kg)	75.9(13.6)	79.8(16.1)	<0.01
BMI (kg/m^2)	26.9(4.1)	28.7(5.3)	<0.001
Knee extension strength (kg)	32.4(10.6)	28.4(11.6)	<0.001
Total chondral defect score (range 1-20)	8.49(3.1)	9.36(3.9)	0.01
Any lateral femoral chondral defect (%)†	43	45	0.61
Any medial femoral chondral defect (%)†	73	74	0.91
Any lateral tibial chondral defect (%)†	61	68	0.09
Any medial tibial chondral defect (%)†	83	83	0.87
Any patellar chondral defect (%)†	58	62	0.35
Severe lateral femoral chondral defect (%)‡	4	7	0.11
Severe medial femoral chondral defect (%)‡	12	21	<0.01
Severe lateral tibial chondral defect (%)‡	30	42	<0.01
Severe medial tibial chondral defect (%)‡	7	18	<0.001
Severe patellar chondral defect (%)‡	37	50	<0.01
Any bone marrow lesion (%)¶	28	41	<0.01
Total knee ROA score (range 0-14)	0.9(1.3)	1.7(2.3)	<0.001
Any knee JSN (%)¶	53	62	0.05
Any knee osteophyte (%)¶	6	13	<0.01
Any knee sclerosis (%)¶	6	7	0.49
Total hip ROA score (range 0-11)	0.8(1.3)	1.2(1.8)	<0.01
Any hip JSN (%)¶	28	42	0.001
Any hip osteophyte (%)¶	17	18	0.73
Any hip sclerosis (%)¶	2	2	0.65

* Unpaired t-test / Mann-Whitney U-test or Chi-square test were used where appropriate. The results reported are percentage for binary variables, and the mean (standard deviation) for continuous variables. †Defined as grade ≥2. ‡Defined as grade ≥3. ¶ Defined as grade ≥1.

Table 7.2. Multivariable analysis of association between prevalent knee pain and study factors

	Step 1*	Step 2†
	OR (95% CI)	OR (95% CI)
Age (yr)	0.97 (0.94, 1.00)	0.97 (0.94, 1.00)
Sex (f vs. m)	0.82 (0.49, 1.38)	0.84 (0.50, 1.42)
BMI (kg/m^2)	**1.08 (1.03, 1.14)**	**1.08 (1.03, 1.13)**
Knee extension strength (kg)	**0.96 (0.94, 0.98)**	**0.96 (0.94, 0.98)**
Bone marrow lesion (per site)	**1.45 (1.05, 2.01)**	**1.44 (1.04, 2.00)**
Lateral femoral chondral defects		
Grade 3 versus grade 2 or less	0.92 (0.31, 2.72)	0.90 (0.30, 2.69)
Grade 4 versus grade 2 or less	1.81 (0.25, 13.32)	1.42 (0.16, 12.60)
Medial femoral chondral defects		
Grade 3 versus grade 2 or less	1.27 (0.67, 2.39)	1.24 (0.66, 2.36)
Grade 4 versus grade 2 or less	0.60 (0.14, 2.53)	0.56 (0.13, 2.39)
Lateral tibial chondral defects		
Grade 3 versus grade 2 or less	1.61 (0.96, 2.73)	1.64 (0.97, 2.76)
Grade 4 versus grade 2 or less	0.87 (0.46, 1.62)	0.84 (0.45, 1.59)
Medial tibial chondral defects		
Grade 3 versus grade 2 or less	**2.36 (1.05, 5.34)**	**2.32 (1.02, 5.28)**
Grade 4 versus grade 2 or less	**5.45 (1.22, 24.34)**	**4.93 (1.07, 22.74)**
Patellar chondral defects		
Grade 3 versus grade 2 or less	1.24 (0.69, 2.25)	1.25 (0.69, 2.27)
Grade 4 versus grade 2 or less	1.52 (0.94, 2.44)	1.53 (0.95, 2.46)
Hip JSN (per grade)	**1.35 (1.06, 1.71)**	**1.36 (1.07, 1.73)**

The results reported are odds ratio (95% confidence intervals). *Adjusted for all other factors listed. †Further adjusted for knee osteophytes, which was not significant in the final model. Bone marrow lesion expressed as numbers of compartments (eg. lateral femoral and tibial, medial femoral and tibial) with presence of lesions. Hip JSN is the sum of the JSN score (eg. hip axial and superior).

Table 7.3. Association between more severe knee pain and study factors*

	Step 1	Step 2
	OR (95% CI)	OR (95% CI)
Age (yr)	0.97 (0.94, 1.01)	0.97 (0.94, 1.01)
Sex (f vs m)	0.62 (0.33, 1.15)	0.65 (0.35, 1.22)
BMI (kg/m^2)	**1.14 (1.08, 1.20)**	**1.14 (1.08, 1.20)**
Knee extension strength (kg)	**0.95 (0.92,0.97)**	**0.95 (0.92, 0.98)**
Bone marrow lesion (compartment)	**1.72 (1.17,2.52)**	**1.66 (1.12, 2.45)**
Lateral femoral chondral defects (grade)	1.41 (0.62, 3.21)	1.14 (0.45, 2.90)
Medial femoral chondral defects (grade)	0.97 (0.52, 1.80)	0.95 (0.51, 1.77)
Lateral tibial chondral defects (grade)	0.94 (0.66, 1.32)	0.91 (0.64, 1.30)
Medial tibial chondral defects (grade)	**2.04 (1.06, 3.95)**	1.82 (0.91, 3.65)
Patellar chondral defects (grade)	1.11 (0.83, 1.48)	1.12 (0.84, 1.49)
Hip JSN (grade)	**1.36 (1.08, 1.71)**	**1.38 (1.09, 1.74)**

*Logistic regression model was used and the analysis was done with subjects with the WOMAC pain score=0 and ≥4. The results reported are odds ratio (95% confidence intervals). The model included all the variables listed in the table in step 1 and further adjustment for knee osteophytes was made in step 2 which was not statistically significant.

Table 7.4. Association between severity of knee pain and study factors*

	Univariable analysis	Multivariable analysis†	
	ß (95% CI)	ß (95% CI)	Partial R^2 (%)
Age (yr)	0.001 (-0.02, 0.02)	-0.01 (-0.03, 0.01)	0.4
Sex (f vs m)	-0.02(-0.29, 0.26)	-0.26(-0.60, 0.08)	0.9
BMI (kg/m^2)	**0.05 (0.02, 0.07)**	**0.05 (0.02, 0.07)**	**5.2**
Knee extension strength (kg)	-0.01 (-0.02, 0.003)	-0.01 (-0.03, 0.001)	1.4
Bone marrow lesion (compartment)	-0.08 (-0.26, 0.11)	-0.06 (-0.26, 0.14)	0.1
Lateral femoral chondral defects (grade)	0.32 (-0.05, 0.68)	0.18 (-0.25, 0.61)	0.3
Medial femoral chondral defects (grade)	-0.06 (-0.30, 0.19)	-0.20 (-0.53, 0.13)	0.6
Lateral tibial chondral defects (grade)	0.06 (-0.12, 0.24)	0.01 (-0.20, 0.22)	0.0
Medial tibial chondral defects (grade)	0.02 (-0.21, 0.26)	0.03 (-0.30, 0.36)	0.0
Patellar chondral defects (grade)	-0.08 (-0.23, 0.07)	-0.15 (-0.31, 0.02)	1.3
Knee osteophyte (grade)	0.13 (-0.06, 0.32)	0.14 (-0.12, 0.39)	0.1
Hip JSN (grade)	**0.18 (0.03, 0.32)**	**0.16 (0.01, 0.30)**	**2.5**

*Linear regression model was used and the analysis was done with subjects with the WOMAC pain score=0 excluded. The results reported are regression coefficients (ß) (95% confidence intervals) expressed as change in Ln(WOMAC pain score –0.2688059) per unit increase of the study factors. †Adjusted for all other factors listed.

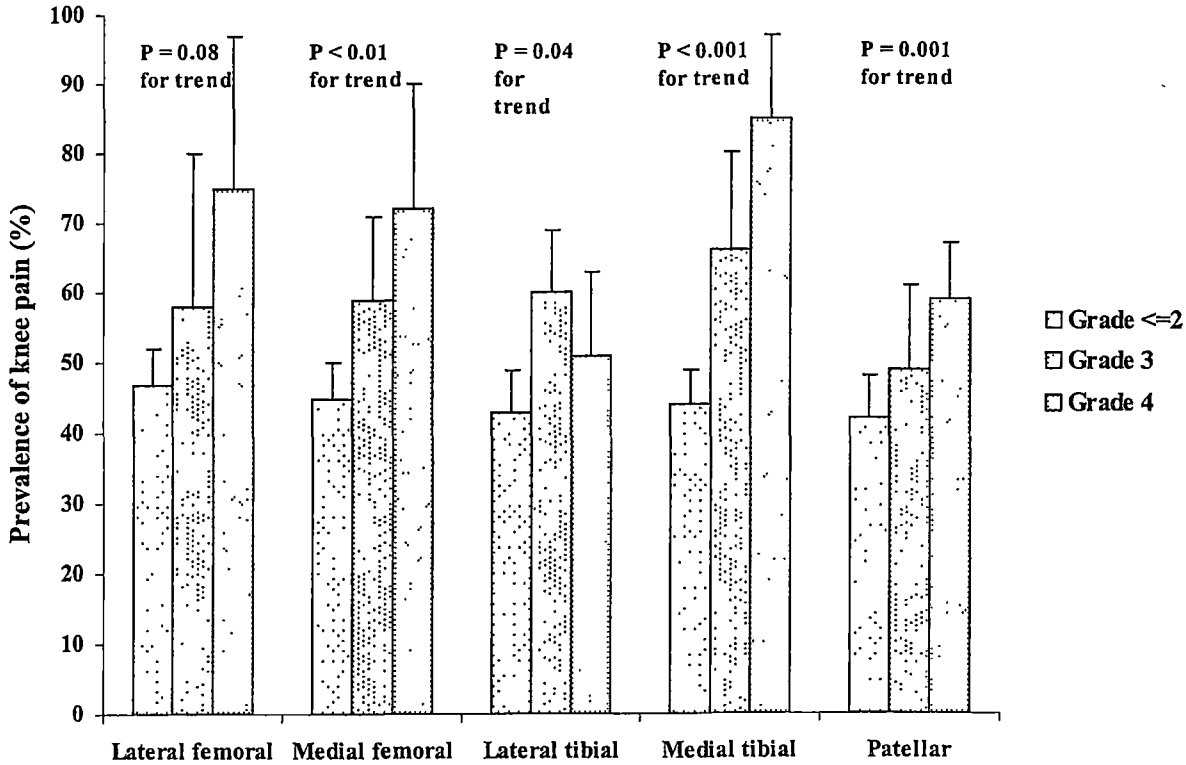


Figure 7.1. Association between prevalent knee pain and chondral defects.

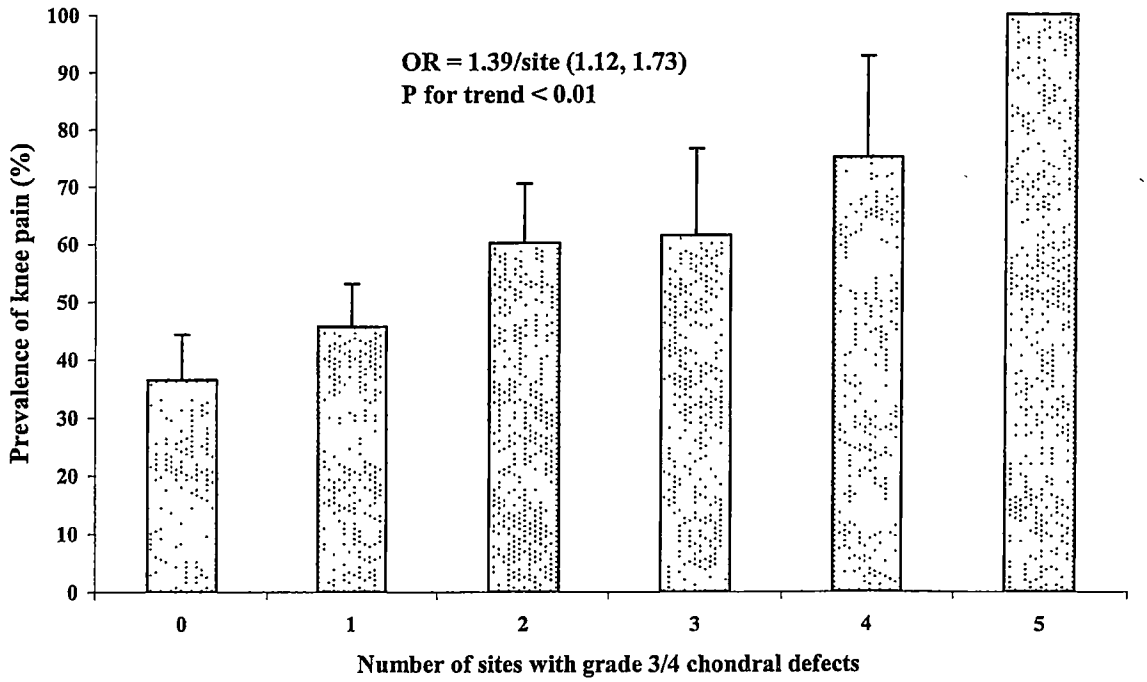


Figure 7.2. Additive association between prevalent knee pain and number of sites with chondral defects grade ≥3. The odds ratio (OR) (95% confidence intervals) for trend was adjusted for age, sex, BMI, knee extension strength, bone marrow lesions, knee JSN and osteophyte, and hip JSN.

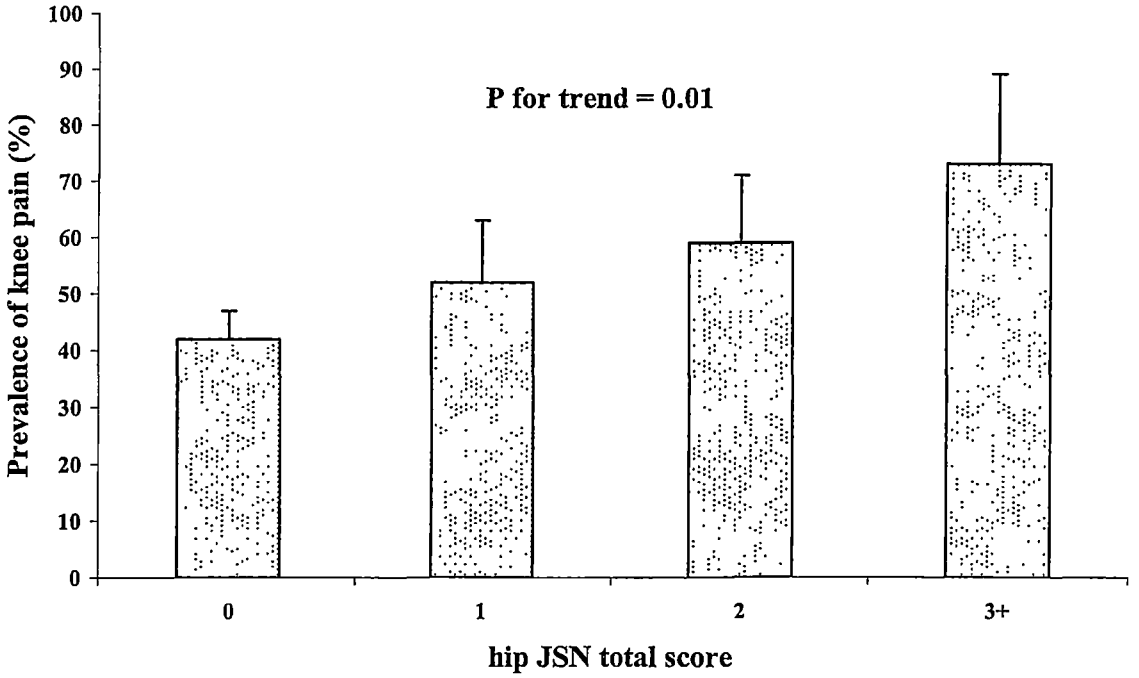


Figure 7.3. Association between prevalence of knee pain and hip JSN total score. P for trend was adjusted for age, sex, BMI, knee extension strength, bone marrow lesions, chondral defects, and knee osteophytes.

7.4 Discussion

This study suggests that both prevalent and more severe knee pain in older adults is independently associated with non-full and full thickness chondral defects at the medial tibial plateau, bone marrow lesions, hip JSN, BMI, and knee extension strength but not osteophytes.

Consistent with the findings in Chapter 6, we found a significant and independent association between prevalent knee pain and chondral defects in this older adult sample. However, in contrast to the results of Chapter 6 in which we demonstrated that grade 2 defects were associated with increasing prevalence of knee pain, we only detected the association for more severe chondral defects. This may be due to the low prevalence of grade zero and one chondral defects in this sample compared to younger age groups. Indeed, the prevalence of grade 2 chondral defects was higher at all sites in subjects with knee pain than those without although the difference was not statistically significant. This association was most marked at the medial tibial plateau, which again contrasts with our findings of femoral and patellar sites in younger subjects, suggesting possible site specificity between younger and older age groups. Furthermore, we demonstrated that medial tibial chondral defects were also significantly associated with more severe pain although this significance became weak after adjustment for knee osteophytes. This is most likely due to the sample size reduction in the analysis as the odds ratios were similar in magnitude in both forms of analysis. Alternatively, a possible threshold effect of chondral defects on knee pain may occur. Importantly, we also demonstrated an additive association between the number of sites with chondral defects and knee pain, indicating the importance of chondral defects at all sites independent of knee ROA and other

factors we measured. These findings extend previous reports in which only subjects with full-thickness articular cartilage defects accompanied by adjacent subchondral cortical bone defects were more likely to have pain in the presence of knee OA [48]. The apparent discrepancy between our results and those of that study may be due to sample size considerations [48], However, the variation in results between studies indicates the need for further studies.

The mechanism for an association between knee pain and chondral defects remains unknown. Loss of articular cartilage leads to a decrease in the protection of the underlying bone and the increase in physical stresses transmitted to the subchondral bone resulting in subchondral bone structure changes such as subchondral bone sclerosis and bone marrow lesions, which may cause knee pain. However, the association was independent of knee ROA and bone marrow lesions, suggesting that damaged articular cartilage can directly lead to pain [44 45].

Bone marrow lesions were common in this random sample and comparable with previous studies, which is surprising given the much lower prevalence of radiographic OA [42 48]. The presence of bone marrow lesions was strongly associated with prevalent knee pain as well as more severe pain, consistent with and expanding those reports [42 48]. In addition, we documented an additive effect of knee compartments with presence of bone marrow lesions on knee pain, indicating the importance of bone marrow lesions in all compartments. Furthermore, we demonstrated that the strong association between bone marrow lesions and knee pain was independent of chondral defects and knee ROA, expanding the findings of those reports in which the association was confined in subjects affected with OA [42] and

accompanied with full-thickness chondral defects [48] and directly linking bone marrow lesions to pain even though the underlying histopathology remains uncertain.

A modest but significant correlation between knee ROA and symptoms has been reported previously [11 53 302 315]. Interestingly, in the current study, the significant association between knee pain and knee ROA including JSN and osteophytes became non-significant after adjustment for other factors including chondral defects, bone marrow lesions, and hip ROA, suggesting the correlation is mediated by other factors. Thus, these factors may be more important for knee pain.

A recent report [311] of a strong coexistence of knee and hip pain suggests either pathology at both sites or that unexplained knee pain may be referred from hip OA. In this study, we demonstrated a strong association between prevalence and severity of knee pain and hip ROA, particularly JSN which is postulated as the best index for the presence of hip ROA [25]. Given the cross-sectional nature of our data, we cannot comment on a causal relationship between hip ROA and knee pain. However, it is biologically plausible and it is unlikely that the association is mediated by unmeasured factors in the knee such as effusions or synovitis. Furthermore, the significance persisted after adjustment for other factors including knee ROA and was of a dose response nature, suggesting that a substantial component of unexplained knee pain is referred from hip OA as has long been recognized in clinical practice.

In common with other reports [317 318], we also demonstrated a strong association between BMI and knee pain. The prevalence and severity of knee pain increases with increasing BMI. The reason for this association remains elusive, but it is most likely

due to repetitive application of increased axial loading at the knee joint [319]. In this study, the association was independent of other factors. Similarly, we demonstrated a strong negative association between knee pain and knee extension strength, consistent with other studies [57 320 321].

There are a number of potential limitations to the current study. Firstly, the reported prevalence of knee pain varies with case definitions, the composition of the study samples, and the methods used [39 311-313]. We chose a conservative definition of knee pain, and this contributed to the high prevalence of knee pain in this sample. There are no other comparative Australian prevalence studies with which to compare our results, but we also had a high prevalence in a younger sample (Chapter 6). Secondly, misclassification in the assessment of MRI indices is possible, but we had high reproducibility of the assessment techniques, suggesting that this is not a major concern. Thirdly, as chondral defects and bone marrow lesions were assessed on different MRI images with different slice thicknesses, it is difficult to assess whether those bone marrow lesions were adjacent to the chondral defects. Against this was the observation that the significant associations between both these factors and pain were independent. Fourthly, the reproducibility for x-rays was good rather than excellent, which may contribute to a weakening of associations. Fifthly, administration of the WOMAC was not knee specific thus results may be misclassified and actually be stronger than we report. This may be less important as people reporting knee pain are more likely to have bilateral knee pain [311]. Lastly, the study is cross sectional in design and any causal relationship should be corroborated in future longitudinal studies.

In conclusion, our results suggest that knee pain is independently associated with both full and non-full thickness medial tibial chondral defects, bone marrow lesions, BMI, and knee extension strength but not knee ROA, expanding our understanding of knee pain in older adults. Furthermore, a strong association between knee pain and hip JSN indicates that referred pain from hip needs to be considered in unexplained knee pain.

7.5 Postscript

This chapter demonstrated that knee pain is independently associated with both full and non-full thickness medial tibial chondral defects, bone marrow lesions, BMI, knee extension strength, and hip JSN but not knee ROA, expanding our understanding of knee pain in older adults. The next chapter will examine factors associated with hip cartilage volume.

CHAPTER EIGHT: FACTORS ASSOCIATED WITH HIP CARTILAGE VOLUME MEASURED BY MRI

8.1 Introduction

OA is the most common form of arthritis and results in substantial morbidity and disability in the elderly [95 322]. Hip OA affects around 4% of the Caucasian population over the age of 55 years [96] and 76% of total hip replacements in women from the Nurses' Health Study [323] were due to primary osteoarthritis. Defects in cartilage are widely considered to be the initial problem in OA [324] although this viewpoint is not shared by all authors[325]. Cartilage loss can be detected indirectly by radiographic JSN only at a relatively advanced stage of the disease. Recently, there is an increasing interest in the use of MRI that allows direct and non-invasive visualization of joint structures such as cartilage, bone and synovium [326]. MRI has been shown to be a valid and reproducible method of knee cartilage measures (both thickness and volume) [204 208 215 222 252 327] and we have reported significant associations between knee cartilage volume and JSN[218 220].

However, in comparison to the knee, there is little information on hip cartilage measures by MRI. Radiographic JSW has been considered to be a surrogate marker of hip cartilage thickness [328]. The relation between hip JSW and demographic and anthropometric factors has been studied, but the results are inconsistent [329 330], possibly due to the indirect assessment of cartilage and the effect of positioning. Recent evidence suggests that MRI can also be used in the assessment of hip cartilage morphology. In a validation study [230] of ten patients who underwent total hip replacement, femoral head cartilage volume measured by 3D MRI with T1-weighted fat suppression was compared to the volume measured by means of water displacement, with average overestimation of cartilage volume by MRI quantification of 0.6 ± 0.6 ml. In addition, the reproducibility was assessed in six

randomly selected patients who underwent MRI for clinical indications with an ICC of 0.94, indicating cartilage volume at the hip can be measured by MRI with good accuracy and reproducibility. Also, significant correlations between hip cartilage thickness as measured on MRI and anatomical measurement have been reported [233] [331]. To date, there have been no published studies of factors related to quantitation of hip cartilage by MRI or associations between MRI based measures and radiographs [332]. The aim of this study, therefore, was to compare associations between anthropometric and lifestyle factors and femoral head cartilage volume/thickness and radiographic features of OA and to provide evidence of construct validity for MRI assessment of femoral cartilage volume and thickness.

8.2 Materials and methods

Subjects were participants of the TASOAC study. The details were described in section 1 of Chapter 3. The current study consisted of a consecutive subsample derived from the TASOAC. Subjects were excluded if they had had total hip replacement and/or contraindication for MRI (e.g. metal sutures, presence of shrapnel, iron filling in eye, and claustrophobia). The Southern Tasmanian Health and Medical Human Research Ethics Committee approved the study and written informed consent was obtained from all participants.

Measurements. The height and weight measurements were described in section 3 of Chapter 3. BMI was calculated as weight in kilograms divided by the square of height in metres. Leg strength measurement was described in section 3 of Chapter 3. Repeatability estimates (Cronbach's α) were 0.91. The devices were calibrated by suspending known weights at regular intervals. Blood specimen were obtained and stored by standard protocols and serum 25-hydroxy vitamin D was measured by using the IDS Gamma-B 25-Hydroxy vitamin D kit. BMD measurements (g/cm^2) of the neck of the femur and spine were performed by dual energy X-ray absorptionmetry (DXA) using a Hologic Delphi densitometer (Hologic, Waltham, MA).

MRI measurements. A MRI scan of the right hip was performed. The hip was imaged in the sagittal plane on a 1.5-T whole body magnetic resonance unit (Picker, Cleveland, OH) with the use of a phased array flex coil. The following image sequence was used: a T1-weighted fat saturation 3D gradient recall acquisition in the steady state; flip angle 55 degrees; repetition time 58 msecs, echo time 12 msec; field

of view 16 cm; 60 partitions; 512 x 512 - pixel matrix; acquisition time 11 min 56 seconds, and one acquisition. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.39 x 0.39 mm (512 x 512 pixels).

Femoral head cartilage volume, thickness, and bone size were measured by one reader and determined by means of image processing on an independent workstation using the software program Osiris (Version 3.5, Geneva University Hospital) as previously described [230]. The image data were transferred to the workstation and an isotropic voxel size was then obtained by a trilinear interpolation routine. The volume of the femoral head cartilage was isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on each section (Figure 8.1a). These data were then resampled by bilinear and cubic interpolation for the final 3D rendering. The volume of the femoral head cartilage was determined by summing all the pertinent voxels within the resultant binary volume. Intra-observer (done by GZ) repeatability was assessed in 100 subjects on the same images with at least a one-week interval between measures and the CV was 2.5%. Inter-observer (done by GZ & CD) reproducibility was assessed in 20 subjects with a CV of 4.4%.

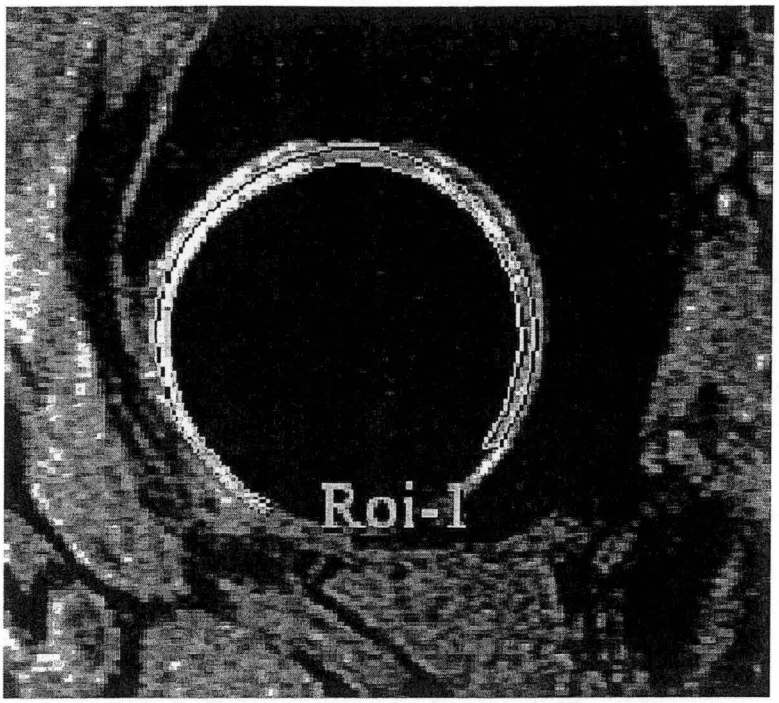
The sagittal image that was closest to the centre of the femoral head was determined by studying the MR images. The measurements of the femoral head cartilage bone size were determined on this image. The bone size was measured by drawing contours around the femoral head bone and the area was calculated automatically by Osiris programme as an indicator of bone size (Figure 8.1b). Intra-observer (done by GZ) repeatability was assessed in 30 subjects at least one-week interval on the same images between measures and the CV was 1.1%. The thickness of femoral head

cartilage was measured on the same image as the femoral head size. Marks were placed every 45°, with the midpoint of the femoral head used as a reference point, with a total of four points marked on the image. Cartilage thickness was measured on the workstation with a digital calliper provided within the Osiris programme to the closest 0.1 mm, and average and maximum thickness was used in the analysis. Intra-observer reproducibility (done by GZ) was assessed in 30 subjects at an at least one-week interval between measures and the CV was 6.9% and 5.8% for the average and maximum thickness, respectively.

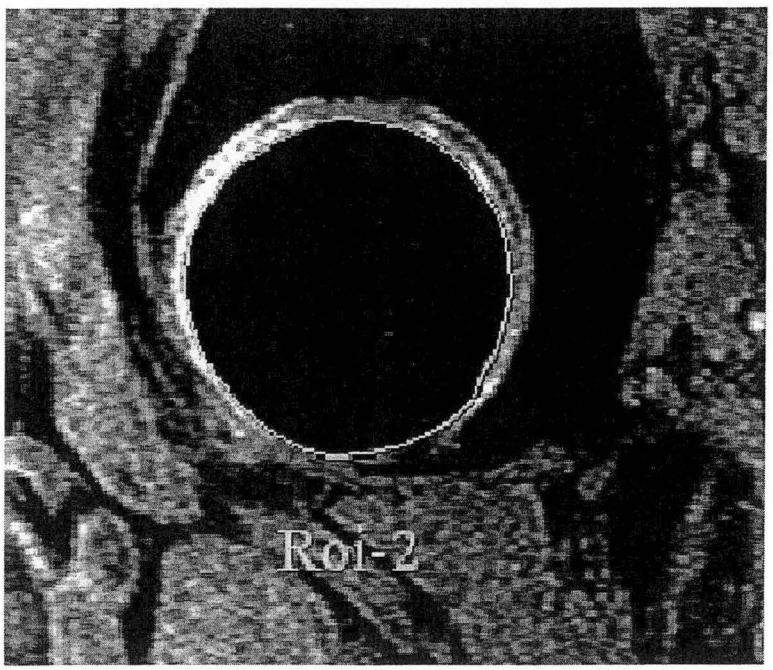
Radiographs. Weight bearing anterior-posterior pelvic radiographs with both feet in 10° internal rotation were obtained and scored for individual features of the hip ROA. The details of the method and the reproducibility were described in section 2 of Chapter 3. The total ROA score was computed by summing the osteophyte and JSN scores, which was then used as an indicator of hip ROA severity. The presence of the hip ROA was defined as the total ROA score ≥ 1.

Statistics. Preliminary analysis revealed that there was a significant difference in serum vitamin D levels, spine and hip BMD, and femoral head size between males and females leading to the possibility of confounding by sex. Thus, all initial linear regression models were sex adjusted. Then, multivariable linear regression modelling was performed with the final model only containing statistically significant variables and age, which was considered an important explanatory variable. The association between radiographic features of hip OA and study factors was also examined using linear regression model for the sake of comparability with MRI measures. Boxplots were used to examine the correlation between femoral head cartilage volume /

thickness and hip radiographic JSN. A p value of less than 0.05 (two-tailed) or a 95% confidence interval not including the null point were considered statistically significant. All statistical analyses were performed using the SPSS-package v.12.0.1 for Windows (SPSS Inc. Chicago, IL).



a.



b.

Figure 8.1. MRI image of the hip

a. With femoral head cartilage outlined

b. With femoral head cross sectional area outlined

8.3 Results

A total of 151 subjects (male 79, female 72) aged between 50 and 79 took part in this study. The characteristics of the study population and comparison between males and females are presented in Table 8.1. The mean age was 63 and there was no difference in age and BMI between males and females. However, there were significant differences in height, weight, leg strength, hip and spine BMD, femoral head cartilage volume and femoral head size. Females had slightly higher average cartilage thickness than males, but this was not statistically significant.

Table 8.2 presents the results of the univariable analysis of the association between hip cartilage volume and thickness and the study factors after adjustment for sex. Hip cartilage volume was positively and significantly associated with age and femoral head size, and negatively with BMI, hip BMD, self-reported hip OA, hip ROA total score, hip superior and axial JSN score but not osteophytes. The thickness of femoral head cartilage was also negatively significantly associated with hip ROA score, hip axial and superior JSN, but not osteophytes. In this sample, femoral head size was significantly negatively associated with average thickness of femoral head cartilage (Table 8.2) and borderline significantly with age ($r = 0.16$, $p = 0.05$), while BMI was significantly negatively associated with maximum thickness (Table 8.2) and with age ($r = - 0.17$, $p = 0.04$).

In the multivariable analysis, age, leg strength, and hip BMD become non-significant in the final model. Sex, BMI and femoral head size were significantly and independently associated with hip cartilage volume (Table 8.3). The results were similar when the analysis was done in males and females separately (data not

shown). Only femoral head size was significantly and negatively associated with average thickness of femoral head cartilage.

Femoral head cartilage volume was significantly correlated with total hip radiographic JSN (Spearman's rho = -0.24, P <0.01), superior JSN (Spearman's rho = -0.18, P = 0.03) and axial JSN (Spearman's rho = -0.23, P <0.01). There was a significant negative association between cartilage volume and increasing grades of JSN, particularly with axial JSN (Figure 8.2) with a 13% reduction in hip cartilage volume per grade. Similarly, there was a significant negative association between femoral head cartilage thickness and increasing JSN (Spearman's rho = -0.34, P <0.001) (Figure 8.3). On average, there was a 9% reduction in thickness of femoral head cartilage per grade of hip axial JSN.

In relation to hip ROA, self-reported hip OA was significantly associated with total ROA score and JSN score but not osteophyte score (Table 8.2). The association between femoral head size and total ROA score became non-significant in multivariable analysis (Table 8.2 & 8.3). Only female sex was significantly associated with total ROA score and JSN score but not osteophytes in the multivariable analysis (Table 8.3).

Table 8.1. Characteristics of the study population*

	Males N = 79	Females N = 72	P value
Age (yr)	64(8.1)	62(7.7)	0.17
Height (cm)	**173.8(6.2)**	**160.5(6.1)**	**<0.001**
Weight (kg)	**83.0(13.01)**	**70.2(12.82)**	**<0.001**
BMI (kg/m^2)	27.4(3.8)	27.3(4.9)	0.86
Leg strength (kg)	**125.5(43.3)**	**58.3(27.4)**	**<0.001**
Hip BMD (g/cm^2)	**1.0(0.2)**	**0.9(0.1)**	**<0.001**
Spine BMD (g/cm^2)	**1.1(0.2)**	**1.0(0.1)**	**<0.001**
Vitamin D (nmol/l)	**66.2(17.6)**	**58.7(18.2)**	**0.01**
Femoral head cartilage volume (ml)	**5.9(1.0)**	**4.7(0.8)**	**<0.001**
Average cartilage thickness (mm)	1.6(0.2)	1.7(0.2)	0.42
Maximum cartilage thickness (mm)	2.0(0.3)	2.0(0.3)	0.45
Femoral head size (cm^2)	**18.6(2.0)**	**14.1(1.5)**	**<0.001**
Self reported hip OA (%)†	7	16	0.08
Hip ROA total score (range 0-6)	0.9(1.3)	1.3(1.6)	0.23
Any hip ROA (%)†	46	56	0.22
Hip JSN total score	0.6(1.1)	0.9(1.4)	0.15
Any hip JSN (%)†	34	44	0.20
Hip osteophyte total score	0.4(0.7)	0.3(0.8)	0.73
Any hip osteophyte (%)†	25	25	0.96

* Unpaired t-test/Mann-Whitney U-test or Chi-Square test was used wherever relevant. Values are mean (SD) except for indicated. BMI: body mass index. BMD: bone mineral density. OA: osteoarthritis. ROA: radiographic osteoarthritis. JSN: joint space narrowing.

† Percentage

Table 8.2. Univariable analysis of association between cartilage volume, thickness, radiographic features of hip OA and study factors with adjustment for sex*

	Cartilage volume		Average thickness		Maximum thickness		Total ROA score		JSN score	
	ß	P	ß	P	ß	P	ß	P	ß	P
Age (per year)	**0.02**	**0.04**	0.003	0.20	0.004	0.18	0.01	0.39	0.02	0.26
BMI (per unit)	**-0.05**	**<0.01**	-0.01	0.10	**-0.01**	**0.04**	0.03	0.35	0.04	0.15
Vitamin D (per nmol/l)	0.004	0.32	0.00	0.74	-0.001	0.32	-0.004	0.58	-0.01	0.28
Femoral head size (per cm^2)	**0.17**	**<0.001**	**-0.03**	**<0.01**	-0.01	0.36	**0.14**	**0.04**	0.08	0.16
Hip BMD (per g/cm^2)	**-0.90**	**0.05**	-0.10	0.39	-0.04	0.76	-1.03	0.16	-0.64	0.30
Spine BMD (per g/cm^2)	-0.10	0.81	-0.12	0.25	0.10	0.39	0.57	0.39	0.11	0.85
Leg strength (per kg)	0.00	0.92	0.00	0.60	0.00	0.77	-0.001	0.67	-0.004	0.19
Self reported hip OA (y/n)	-0.44	0.07	-0.11	0.07	-0.11	0.13	**1.10**	**< 0.01**	**0.88**	**< 0.01**
Hip ROA total score (per grade)	**-0.14**	**<0.01**	**-0.06**	**<0.001**	**-0.05**	**<0.001**	-	-	-	-
Hip superior JSN (per grade)	**-0.28**	**0.01**	**-0.11**	**<0.001**	**-0.13**	**<0.001**	-	-	-	-
Hip axial JSN (per grade)	**-0.35**	**0.001**	**-0.12**	**<0.001**	**-0.11**	**<0.001**	-	-	-	-
Hip osteophyte (per grade)	0.02	0.87	-0.04	0.08	-0.001	0.97	-	-	-	-

* Linear regression model was used. OA: osteoarthritis. ROA: radiographic osteoarthritis. JSN: joint space narrowing. BMI: body mass index. BMD: bone mineral density.

Table 8.3. Multivariable analysis of association between cartilage volume/radiographic features of hip OA and study factors*

	Cartilage volume (ml)		Total ROA score		Total JSN score		Total osteophyte score	
	ß	95% CI	ß	95% CI	ß	95% CI	ß	95% CI
Age (per year)	0.01	-0.01, 0.03	0.01	-0.02, 0.04	0.02	-0.01, 0.04	-0.004	-0.02, 0.01
Sex (f vs. m)	**-0.44**	**-0.87, -0.01**	**0.95**	**0.20, 1.70**	**0.69**	**0.04, 1.34**	0.26	-0.13, 0.66
BMI (per kg/m^2)	**-0.05**	**-0.08, -0.02**	0.03	-0.03, 0.08	0.04	-0.01, 0.09	-0.01	-0.04, 0.02
Femoral head size (per cm^2)	**0.17**	**0.10, 0.25**	0.13	-0.001, 0.26	0.07	-0.05, 0.18	0.06	-0.01, 0.13

* Linear regression model was used. OA: osteoarthritis. ROA: radiographic osteoarthritis. JSN: joint space narrowing. BMI: body mass index.

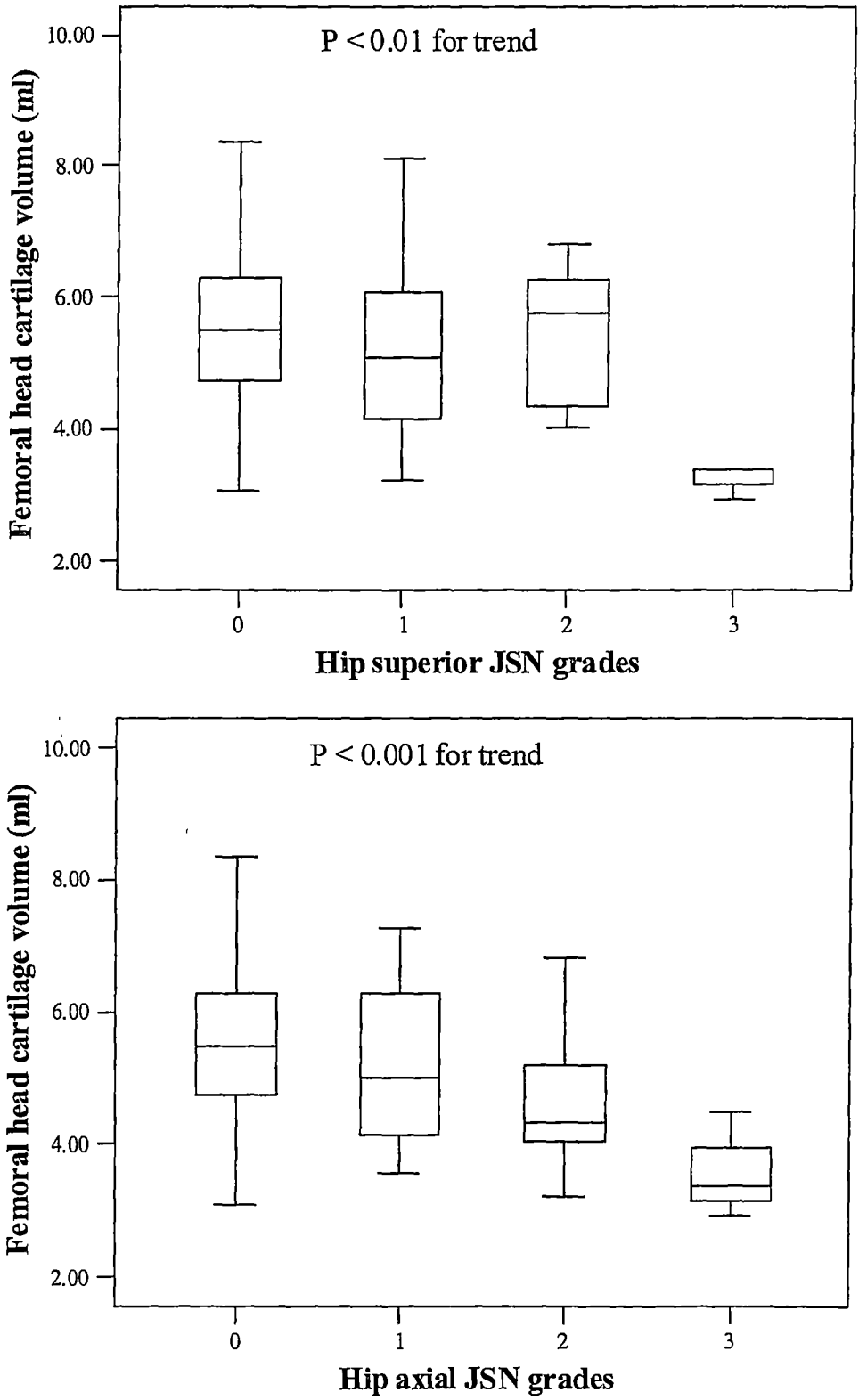


Figure 8.2. Boxplot of femoral head cartilage volume (ml) versus hip radiographic joint space narrowing. Boxes represent 25^{th}-75^{th} percentiles (interquartile range (IQR)); horizontal lines within boxes represent medians; vertical bars represent 1.5 times the IQR.

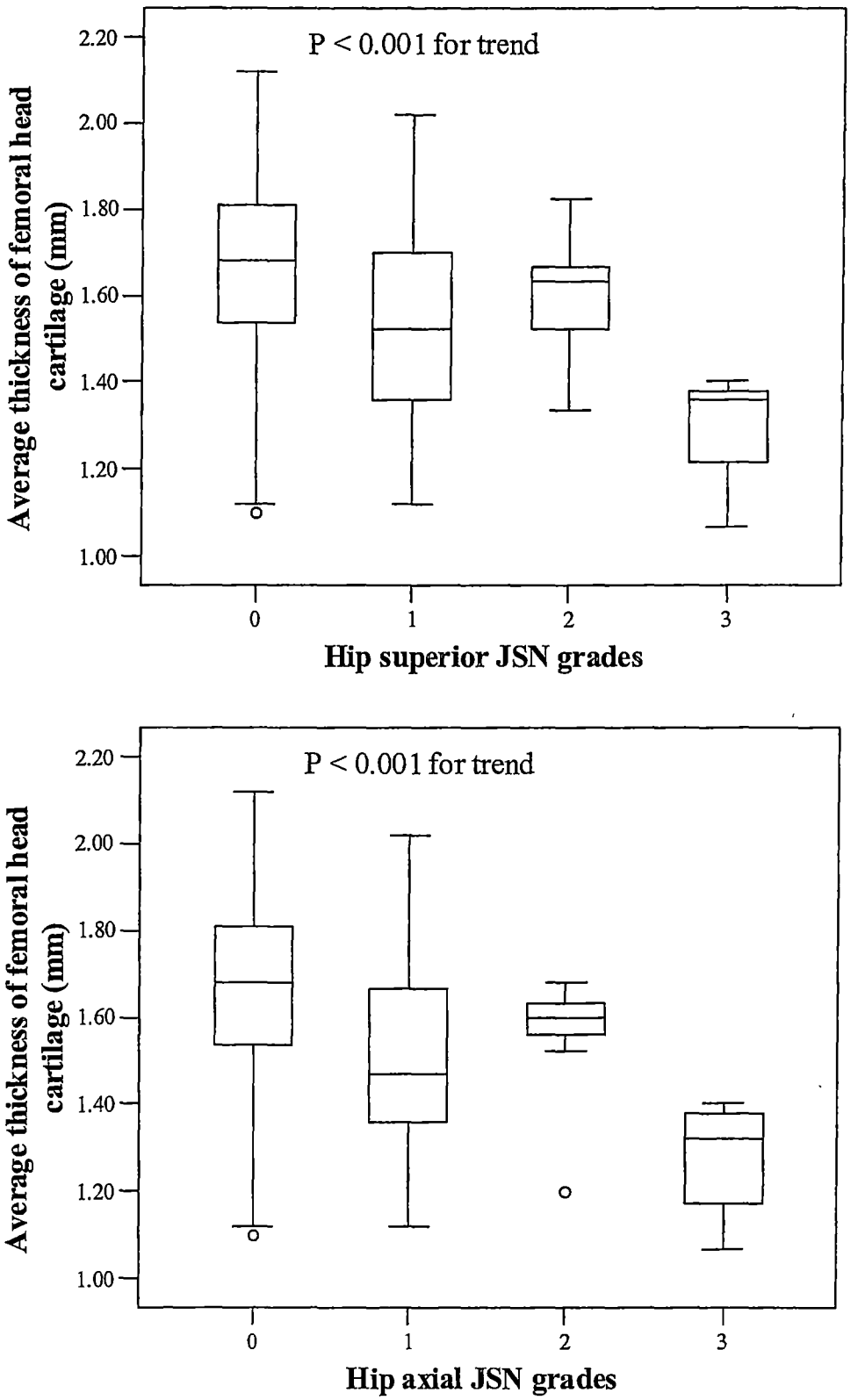


Figure 8.3. Boxplots of average thickness of femoral head cartilage versus hip radiographic joint space narrowing. Boxes represent 25^{th}-75^{th} percentiles (interquartile range (IQR)); horizontal lines within boxes represent medians; vertical

bars represent 1.5 times the IQR; circles represent values below the 1.5 times IQR value.

8.4 Discussion

This is the first study that compares associations between anthropometric and lifestyle factors and femoral head cartilage volume/thickness and radiographic features of OA.

Radiographic hip JSN but not osteophytes was significantly associated with hip cartilage volume, particularly axial JSN with a 13% reduction per grade. In addition, hip JSN was significantly correlated with hip cartilage thickness with a 9% reduction per grade. This provides evidence for both face and construct validity of measuring hip cartilage morphology by MRI particularly for volume as thickness had poorer reproducibility. However, given that radiographic hip JSN is the current gold standard, the correlation with femoral head cartilage thickness is modest in the current study most likely due to the fact that joint space consists of not only femoral head but also acetabular cartilage. This may also reflect the semi-quantitative nature of and measurement error inherent in the radiographic scoring system, as the decrements in cartilage volume per category were large.

In the current study we demonstrate a substantial sex difference in hip cartilage volume. On average, the hip cartilage volume is 1.2 ml smaller in women compared with men. The difference reduced after adjustment for other factors including femoral head size and BMI, but remained significant. This is similar to the knee joint in which women have a significantly lower cartilage volume than in men [197 203 222]. Previous reports suggest that there is no sex difference in the prevalence of hip OA possibly due to utilizing the Kellgren-Lawrence score, a composite score of JSN and osteophytes, to define hip OA [97]. More recent work has suggested that JSW is likely

to be the most robust and useful radiographic feature of defining hip OA [25]. Based on this definition of hip OA, there was a significant sex difference with hip JSN being more common in women[330 333]. Indeed, female sex was significantly associated with hip JSN but not osteophytes in the multivariable analysis in this sample.

Although the impact of obesity on the occurrence of hip OA has been well studied, the results are inconsistent [72 319 334]. There is a modest influence of obesity on the development of clinically assessed hip OA which includes pain and ROA[148]. In the current study we demonstrate that a higher BMI was independently associated with lower hip cartilage volume. However, there was no association between BMI and radiographic measures, e.g. hip JSN, osteophytes, or total hip ROA score, suggesting that radiographic based assessment of hip OA may be inferior at identifying potential determinants of hip OA. The reason why obesity is associated with lower hip cartilage volume is unclear. One possible explanation is that obesity increases the force across the joint and causes cartilage damage hence lower cartilage volume.

Femoral head size was the major factor associated with femoral head cartilage volume. This is not surprising since a larger femoral head will need more cartilage coverage. In the current study we demonstrate a negative association between femoral head size and hip cartilage thickness, indicating that cartilage may attenuate to some degree even though it has a larger overall volume. This finding contrasts to a previous report [335] where the thickness of femoral head cartilage was not related to femoral head diameter. This is most likely to be due to the small sample of the previous study. Furthermore, X-ray based studies have suggested a positive

correlation between femoral head diameter and hip joint space width. [329]. However, this result was not adjusted for possible confounders particularly sex.

In contrast to a previous report of similar size [336] in which knee cartilage volume was positively associated with total body BMD accounting for 13% variation in tibial knee cartilage volume, we did not detect any significant association between hip cartilage volume/thickness and BMD in the multivariable analysis. The relationship of BMD to ROA has been well studied with most studies reporting a positive association between BMD and ROA when defined in terms of osteophytes [157-159]. These results suggest that the influence of BMD on hip cartilage volume may differ to knee cartilage volume. Similarly, we did not demonstrate any association between hip cartilage volume/thickness and serum vitamin D levels. However, vitamin D may only be related to progression of OA[169 337]. Given our sample size, we had 80% power to detect an R^2 of 5% in hip cartilage volume explained by either BMD or Vitamin D. Thus longitudinal studies in larger samples may be required to rule out a smaller effect.

The underlying advantage of the present study is the direct 3D visualization of the cartilage by MRI, thus more accurate and precise measurement of the cartilage morphology compared to radiographs, with the exception of cartilage thickness measurement, which is two-dimensional. However, there are a number of potential limitations. Firstly, discrimination of femoral head cartilage from acetabular cartilage may introduce error and distraction of the hip may be more helpful in separating the femoral head cartilage from acetabular cartilage [338 339]. The accurate delineation of articular cartilage depends on high contrast relative to adjacent tissues. The method

we used in the study has been shown to be useful in providing sufficient spatial resolution and image contrast to allow good accuracy and reproducibility in the quantification of femoral head cartilage volume [230]. The intra-observer repeatability for volume in our study was 2.5% which is similar to that of the knee cartilage measurements using the same MRI technique [203], and the interobserver variation is acceptable but somewhat higher at 4.4%. Secondly, scans were performed throughout the day and it is possible that there is diurnal variation in hip cartilage volume due to the compression of cartilage over the course of the day; however, this has not been shown to be the case for knee cartilage volume [227]. Thirdly, the thickness of the cartilage has been proposed as a marker in the studies of hip cartilage morphology [233 331 338 339]. Given cartilage thickness was measured only on the central sagittal section in the current study and the thickness distribution may be inhomogenous in patients with OA [339], this may contribute to the lack of association between the cartilage thickness and female sex and BMI in the current study especially when combined with its lower reproducibility. Furthermore, the major potential limitation of measuring joint cartilage thickness is the difficulty in reselecting identical section locations in follow-up MRI studies[204]. The measurement of cartilage volume can minimize this limitation. Fourthly, we had a high prevalence of ROA in this sample. There are no other comparative Australian prevalence studies to determine generalisability. However, this increased the power to look at associations between ROA and hip cartilage measures of MRI. Lastly, the design was cross sectional, thus any causal relationships should be corroborated in longitudinal studies.

In conclusion, femoral head cartilage volume and thickness have modest but significant construct validity when correlated with radiographs. Furthermore, femoral head cartilage volume was significantly associated with female sex, BMI, and femoral head size while only female sex were associated with hip total ROA score and hip JSN, suggesting that MRI may be superior at identifying risk factors for hip OA.

8.5 Postscript

This chapter provided evidence that MRI-based measurements of the femoral head cartilage, particularly cartilage volume, may be superior at identifying risk factors for hip OA. The next chapter will examine the optimal sampling of 1.5 mm thick MRI slices for the assessment of knee cartilage volume for cross-sectional and longitudinal studies.

CHAPTER NINE: OPTIMAL SAMPLING OF MRI SLICES FOR THE ASSESSMENT OF KNEE CARTILAGE VOLUME FOR CROSS-SECTIONAL AND LONGITUDINAL STUDIES

9.1 Introduction

OA is the most common form of arthritis and a leading cause of musculoskeletal disability in most developed countries [55]. The knee is one of the most frequently affected joints with a prevalence of 30% in people older than 65 years [59] and high resultant disability [272]. Defects in cartilage are widely considered to be the initial problem in OA [340 341], although this viewpoint is not shared by all investigators [325]. Detection of cartilage morphological change is critical in the evaluation, diagnosis, and monitoring of OA. Conventional radiography is used in evaluating the progression of OA but is limited by its inability to directly visualise cartilage. MRI offers the distinct advantage of detecting morphologic changes in articular cartilage and is a sensitive and accurate test for evaluating articular cartilage non-invasively [202 208 227-229]. The correlation coefficient is 0.99 between knee cartilage volumes measured by MRI and the true volumes by means of water displacement [208]. This method uses 1.5mm thick MRI slices and has high reproducibility with coefficients of variation of 2-3% [203] and has been used in both cross sectional and longitudinal studies of OA [203 222 252 256 257]. However, the method is difficult to apply to large studies as most techniques used in measuring knee cartilage volumes require substantial post-image processing [203] and the process has not yet been automated. One possible solution is to select a sample from within the 1.5 mm thick slices to reduce the post-image processing time, as has been reported for the estimation of brain compartment volume [342] and fetal volume[343]. The aim of the study, therefore, was to determine the optimal sampling of 1.5 mm thick MRI slices required to estimate the volumes of and rate of change in lateral, medial tibial and patellar cartilage with minimal increase in measurement error.

9.2 Materials and methods

Subjects. This study consisted of two datasets; one was part of the TASOAC, Another dataset was a younger adult sample from the KCV. The details were described in section 1 of Chapter 3. Both studies were approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee and all subjects provided informed written consent.

MRI. An MRI scan of the right knee was performed on all subjects. Knee cartilage volume was determined by means of image processing on an independent workstation. The details of the method were described in section 2 of Chapter 3. Briefly, the image data were transferred to the workstation. The volumes of individual cartilage plates (medial tibial, lateral tibial and patella) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a slice-by-slice basis. All individual slice areas for each cartilage site and each subject were subsequently transferred to and recorded on a spreadsheet. The total area of each individual cartilage was then multiplied by the slice thickness to produce a volume estimate. This "all slice" estimate of cartilage volume (based on slice thickness of 1.5mm) was used as the gold standard for other comparisons.

Then, the volumes of all individual cartilage plates were recalculated based on different sampling intervals from 1.5 mm thick slices by extracting one in two, one in three, and one in four slice areas from the individual data file. These were then summed and the total was multiplied by the corresponding slice distance.

Femoral cartilage volume was not assessed in this study as it is strongly correlated with tibial cartilage volume and thus adds little extra information [283], tibial cartilage

volume is the parameter that is most frequently examined in the literature [203 206 216 291 336 344], and femoral cartilage volume has worse reproducibility than tibial cartilage volume [227].

Other measurements. The weight and height measurements were described in section 3 of Chapter 3. BMI was calculated as weight in kilograms divided by the square of height in metres.

X-ray was performed on the right knee and scored for individual features of the knee OA. The total score could vary from 0-12. Any knee ROA was defined as total score ≥1.The details of the method and the reproducibility were described in section 2 of Chapter 3.

Statistics

Descriptive statistics of the characteristics of the study subjects were tabulated. The annual change in knee cartilage volume was calculated as percent change by means of dividing absolute volume change by baseline cartilage volume. Intraclass correlation coefficient (ICC) was utilized to assess the measurement agreement. The difference in cartilage volume measured with different samples extracting one in two, one in three, and one in four 1.5 mm thick slices of MR image compared to that measured using 1.5 mm thickness was calculated and expressed as percent absolute difference. Desirable agreement was defined as an ICC ≥0.98 with ≤1% difference between two measurements. In addition, Bland & Altman plots [345] were also utilized. Desirable agreement was defined as the mean difference between two measurements close to zero with 95% of individual differences being within 2SD. All analyses were performed using the SPSS statistical package (version 12.1, SPSS, Chicago, IL).

9.3 Results

A total of 150 subjects took part in this study: 100 subjects with cross-sectional data (female: 48, male: 52) were from the TASOAC study and 50 subjects with longitudinal data (female: 31, male: 19) were from the KCV study. Each subject had approximately 60 MRI slices of 1.5mm thick, which took about one hour to be measured for the cartilage volume. Characteristics of the study sample are presented in Table 9.1. Subjects from the TASOAC were older, heavier and had a higher prevalence of ROA than those from the KCV. Most of participants with ROA were mild with a total ROA score ≤ 3 out of 12. Lateral and medial tibial cartilage volumes were lower in subjects from the KCV than those from the TASOAC.

In cross-sectional analysis, compared to the cartilage volume measured using 1.5 mm thickness, decreasing the number of the slices by extracting one in two to one in four led to a very little underestimation in the magnitude of the average cartilage volume at lateral, medial tibial and patellar sites with ICCs of 0.98-1.00 (Table 9.2). The maximum underestimation was 3.3% at the medial tibial site with one in four slices (Table 9.2). Similar results were obtained when the analysis was done separately for people with and without ROA (Table 9.3) although the differences tended to be larger in the ROA group. The difference also tended to be larger for medial tibial cartilage in the TASOAC sample and lateral tibial cartilage for the KCV sample (Table 9.2). At all sites and subgroups, cartilage volume measured with one in two slices had less than 1% difference compared to that measured with all 1.5mm slices with an ICC of 1.0 (Table 9.2 & 9.3). Bland & Altman plots showed that the mean difference was zero for lateral tibial cartilage and -0.01 ml for medial tibial and

patellar cartilage with 95% of individual differences within ± 2SD. The variability was random and uniform throughout the range of cartilage volume (Figure 9.1).

Similarly, in longitudinal analysis, compared to the cartilage volume change using 1.5 mm thick slices, decreasing the number of the slices by extracting one in two to one in four slices led to very little over or under estimation of the mean changes in cartilage volume at lateral, medial tibial and patellar sites (Table 9.4). The mean difference ranged from -0.05% to 0.14% with the maximum difference at the patellar site. ICCs ranged from 0.85 to 0.99 (Table 9.4). The difference became larger but all were $\leq$1% in subjects with and without ROA (Table 9.4). At all sites, the annual change in cartilage volume measured with one in two slices had an ICC $\geq$0.98 with less than 0.3% difference compared to that measured using all the slices. Bland & Altman plots showed that 95% of the individual differences were within ± 2SD and the variability was random and uniform throughout the range of cartilage volume (Figure 9.2).

Table 9.1. Characteristics of the study population*

	TASOAC dataset N=100	KCV dataset N=50
Age (year)	62.3(7.6)	42.8(6.1)
Sex (female %)†	48	62
Height (cm)	167.4(8.7)	168.6(7.9)
Weight (kg)	76.0(15.0)	73.9(13.7)
BMI (kg/m^2)	27.1(4.3)	25.9(4.1)
Any knee ROA (%)†	62	18
Knee ROA total score (0-12)	1.3 (1.7)	0.2(0.7)
Lateral tibial cartilage volume (ml)‡	3.0(0.7)	2.6(0.5)
Medial tibial cartilage volume (ml)‡	2.7(0.5)	2.2(0.5)
Patellar tibial cartilage volume (ml)‡	3.5(1.0)	3.5(0.9)
Lateral tibial cartilage volume change (%) per year‡	-	-1.2(3.4)
Medial tibial cartilage volume change (%) per year‡	-	-2.9(3.9)
Patellar cartilage volume change (%) per year‡	-	-3.8(3.4)

*Values are mean (SD) except for indicated. BMI: body mass index. ROA: radiographic osteoarthritis. †Percentage. ‡ Measured with the whole sample of 1.5mm thick MRI slices.

	Whole sample (n=150)		TASOAC sample (n=100)		KCV sample (n=50)	
	%Difference (SD)	ICC†	%Difference (SD)	ICC†	%Difference (SD)	ICC†
Lateral tibial cartilage						
The whole sample	Reference	Reference	Reference	Reference	Reference	Reference
1/2 whole sample‡	-0.04(1.5)	1.00	0.35(1.4)	1.00	-0.84(1.4)	1.00
1/3 whole sample‡	-0.61(2.3)	1.00	0.11(2.1)	1.00	-2.09(1.8)	1.00
1/4 whole sample‡	-1.12(3.4)	1.00	-0.11(3.0)	1.00	-3.18(3.3)	0.99
Medial tibial cartilage						
The whole sample	Reference	Reference	Reference	Reference	Reference	Reference
1/2 whole sample‡	-0.50(1.7)	1.00	-0.98(1.4)	1.00	0.46(1.7)	1.00
1/3 whole sample‡	-1.70(3.3)	0.99	-2.97(2.8)	0.99	0.83(2.9)	1.00
1/4 whole sample‡	-3.27(5.0)	0.98	-5.09(3.9)	0.97	0.38(4.9)	0.99
Patellar cartilage						
The whole sample	Reference	Reference	Reference	Reference	Reference	Reference
1/2 whole sample‡	-0.36(1.2)	1.00	-0.40(1.2)	1.00	-0.29(1.3)	1.00
1/3 whole sample‡	-0.91(2.0)	1.00	-0.93(2.0)	1.00	-0.86(1.9)	1.00
1/4 whole sample‡	-2.24(3.0)	1.00	-2.12(2.9)	1.00	-2.50(3.3)	1.00

Table 9.2. Agreement analysis of knee cartilage volume measured with different samples of 1.5mm thick MI slices*

* SD: standard deviation. ICC: intraclass correlation coefficient. †All P <0.001. ‡Derived by extracting one in two one in three, or one in four of the 1.5mm thick MRI slices.

	ROA absent (n=76)		ROA present (n=68)	
	Difference (SD)	ICC†	Difference (SD)	ICC†
Lateral tibial cartilage				
The whole sample	Reference	Reference	Reference	Reference
1/2 whole sample‡	-0.30(1.4)	1.00	0.24(1.6)	1.00
1/3 whole sample‡	-1.14(2.3)	1.00	-0.01(2.1)	1.00
1/4 whole sample‡	-1.85(3.4)	0.99	-0.29(3.4)	1.00
Medial tibial cartilage				
The whole sample	Reference	Reference	Reference	Reference
1/2 whole sample‡	-0.39(1.7)	1.00	-0.77(2.2)	1.00
1/3 whole sample‡	-1.20(3.3)	0.99	-2.13(3.4)	0.99
1/4 whole sample‡	-2.56(5.3)	0.98	-3.77(4.5)	0.98
Patellar cartilage				
The whole sample	Reference	Reference	Reference	Reference
1/2 whole sample‡	-0.38(1.2)	1.00	-0.40(1.2)	1.00
1/3 whole sample‡	-0.87(1.9)	1.00	-1.10(2.0)	1.00
1/4 whole sample‡	-2.02(2.9)	1.00	-2.50(3.2)	1.00

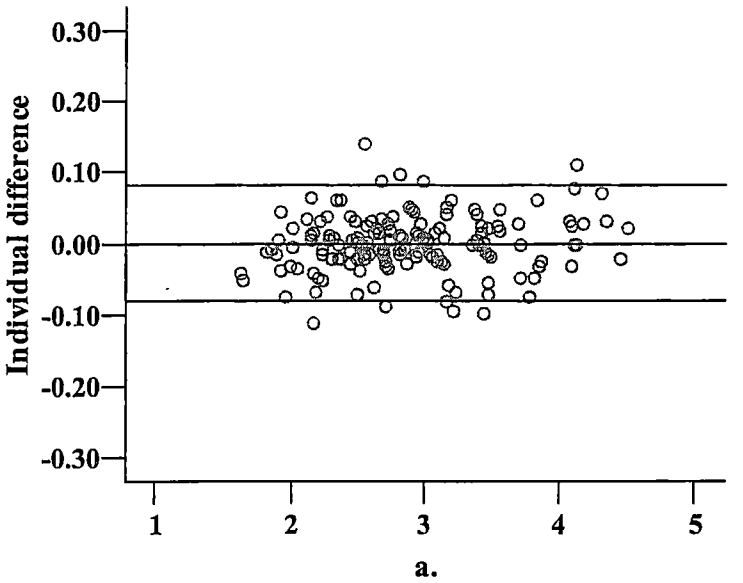
Table 9.3. Agreement analysis of cartilage volume measured with different samples of 1.5mm thick MRI slices in people with and without ROA*

*Six subjects had missing values for ROA. Difference in cartilage volume measured with different thick slices of MR images is expressed as percentage. ICC: intraclass correlation coefficient. ROA: radiographic osteoarthritis. SD: standard deviation. † All P<0.001. ‡Derived by extracting one in two, one in three, or one in four 1.5mm thick MRI slices.

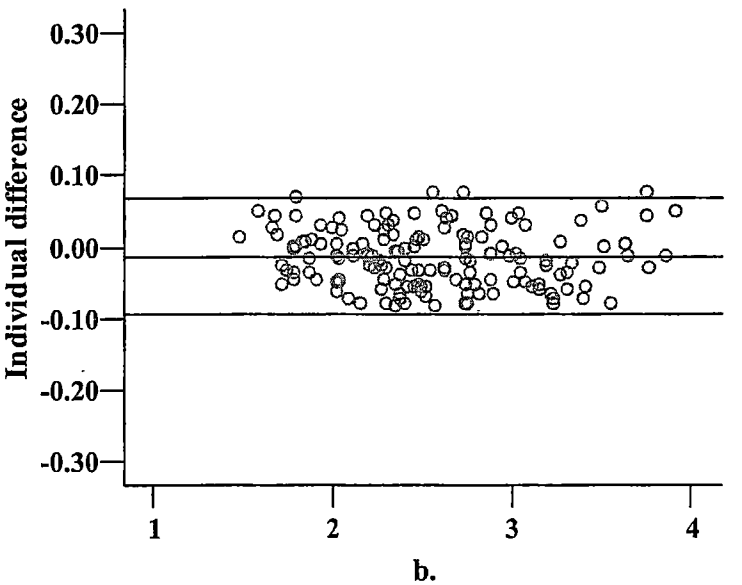
Table 9.4. Agreement analysis of the annual change in knee cartilage volume measured with different samples of 1.5mm thick MRI slices*

	Whole sample (n=50)		ROA present (n=9)		ROA absent (n=41)	
	Difference (SD)	ICC†	Difference (SD)	ICC†	Difference (SD)	ICC†
Lateral tibial cartilage						
The whole sample	Reference	Reference	Reference	Reference	Reference	Reference
1/2 whole sample‡	0.06(0.9)	0.99	0.23(1.1)	0.99	0.02(0.9)	0.98
1/3 whole sample‡	0.05(1.5)	0.96	-0.65(1.4)	0.98	0.20(1.5)	0.95
1/4 whole sample‡	-0.03(2.2)	0.92	-0.04(2.4)	0.95	-0.02(2.2)	0.91
Medial tibial cartilage						
The whole sample	Reference	Reference	Reference	Reference	Reference	Reference
1/2 whole sample‡	-0.05(1.1)	0.98	-0.29(1.0)	0.99	0.00(1.1)	0.98
1/3 whole sample‡	-0.03(1.8)	0.95	0.24(1.8)	0.97	-0.10(1.8)	0.95
1/4 whole sample‡	0.02(3.0)	0.85	-1.04(2.7)	0.92	0.25(3.1)	0.83
Patellar cartilage						
The whole sample	Reference	Reference	Reference	Reference	Reference	Reference
1/2 whole sample‡	0.10(0.8)	0.99	-0.07(0.7)	1.00	0.13(0.8)	0.99
1/3 whole sample‡	0.10(1.5)	0.96	-0.18(1.4)	0.98	0.16(1.5)	0.95
1/4 whole sample‡	0.14(1.8)	0.93	0.61(1.5)	0.97	0.03(1.9)	0.92

* Difference in the annual change in cartilage volume was expressed in percentage. SD: standard deviation. ROA: radiographic osteoarthritis. ICC: intraclass correlation coefficient. †All $P < 0.001$. ‡Derived by extracting one in two, one in three, or one in four 1.5mm thick MRI slices.


Individual difference
0.30
0.20
0.10
0.00
-0.10
-0.20
-0.30
1
2
3
4
5

a.


Individual difference
0.30
0.20
0.10
0.00
-0.10
-0.20
-0.30
1
2
3
4

b.

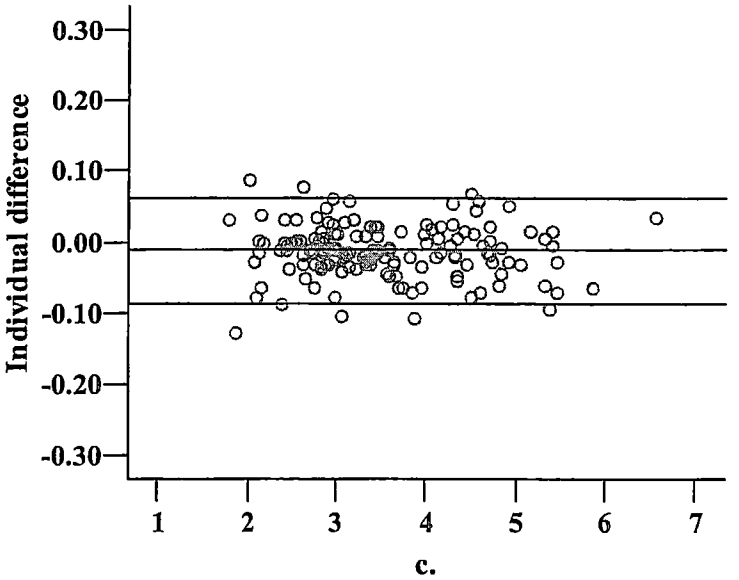
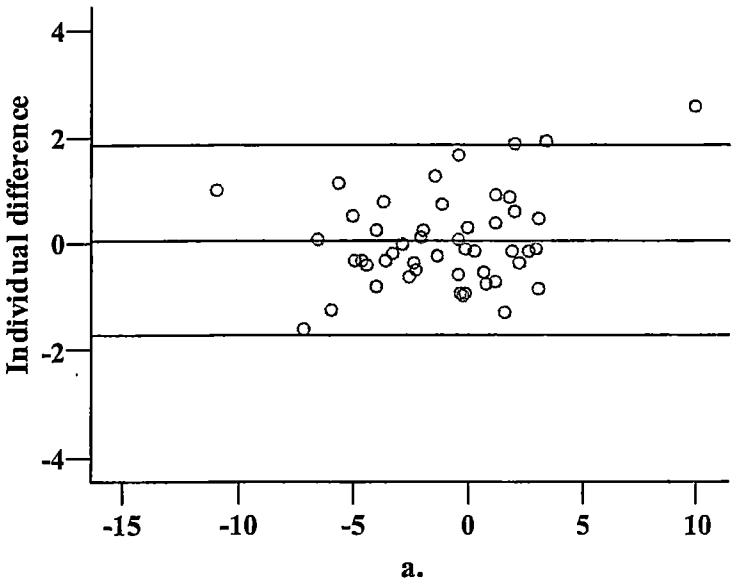
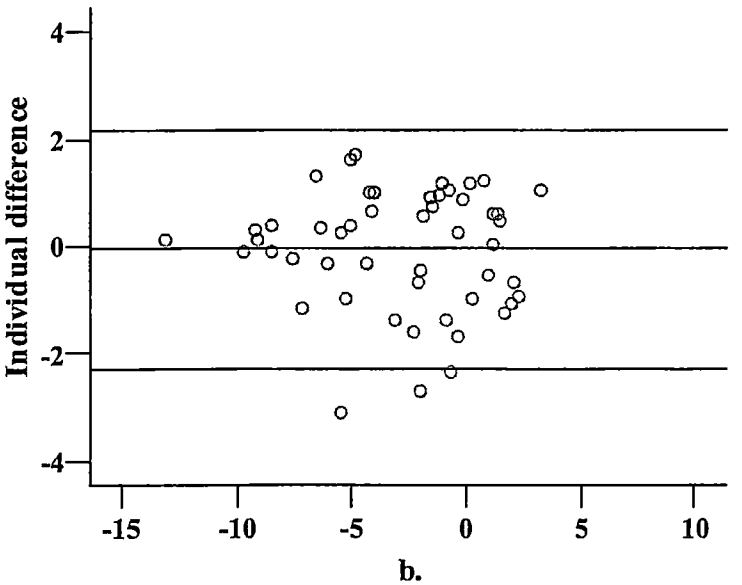


Figure 9.1. Bland & Altman plots of cartilage volume measured by every second 1.5mm thick MRI slice compared to that measured by the total sample at lateral (a), medial tibial (b), and patellar (c) sites. The x-axis represents average values of two measurements while the y-axis represents the individual difference between two measurements, and the three horizontal lines stand for mean individual difference ± 2SD.


Individual difference
4
2
0
-2
-4
-15
-10
-5
0
5
10

a.


Individual difference
4
2
0
-2
-4
-15
-10
-5
0
5
10

b.

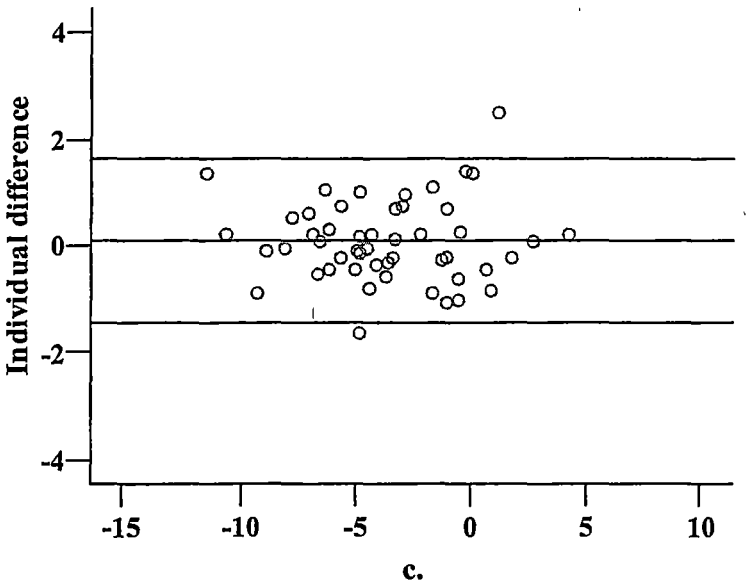


Figure 9.2. Bland & Altman plots of the annual change in cartilage volume measured by every second 1.5mm thick MRI slice compared to that measured by the total sample at lateral (a), medial tibial (b), and patellar (c) sites. The annual change in cartilage volume was expressed as a percentage. The x-axis represents average values of two measurements while the y-axis represents the individual difference between two measurements, and the three horizontal lines stand for mean individual difference ± 2SD.

9.4 Discussion

This study suggests that lateral, medial tibial and patellar cartilage volumes measured from up to one in four 1.5 mm thick slices are quite comparable to those obtained from 1.5 mm thick slices. If the agreement is defined at high levels expected to lead to minimal measurement error, then knee cartilage volume can be measured sufficiently and accurately with one in two slices both cross-sectionally and longitudinally regardless of ROA status and/or reader. This approach will lead to a substantial decrease in post-scan processing time (approximately half an hour from one hour if measuring every slice) and make large-scale studies of knee cartilage volume more feasible.

Currently, there is no reported information on the number of the slices of MRI scans to measure cartilage volume apart from a recent paper from our own group which had similar findings to this study with different readers and geographic location [346]. In a study estimating fetal volume by MRI, Roberts et al reported that using the same thickness of MRI slices (10 mm), volume measured from the low sampling intensity (the distance between scan section midplanes T= 4.5cm) was virtually identical to those obtained with the high sampling intensity (T=1.5cm) with a coefficient of error (CE) < 5% [343]. In the study estimating brain compartment volume from MR Cavalieri slices [342], irrespective of slice thickness, a minimum of 3, 5, and 10 slices provided estimates of the true total volume of grey matter and white matter in the cerebrum with CEs of 10, 5, and 3%. For a given number of slices CE decreases rapidly when the slices are thicker than the gaps between them; when the slices are thinner than the gaps, then CE is similar to that in the situation when the slice thickness is zero. The current study demonstrates similar results for knee cartilage. Decreasing the number

of slices by extracting up to one in four 1.5mm slices resulted in a very little underestimation in average volume of lateral, medial tibial and patellar cartilage. The maximum mean difference in cartilage volume obtained from one in four slices to that obtained from all slices was 3.3%, which is substantially smaller than the difference of 9% between cartilage volume obtained from 1.5 mm thick slices of MR image and that measured by means of water displacement [197 208 215 216]. The difference increased slightly when we analysed the data separately for people with and without ROA, but the results were similar for both groups, suggesting ROA within the range we report has very limited effect on the cartilage volume measured with subsamples of MRI slices. If we arbitrarily define an ICC $\geq$0.98 with $\leq$1% difference as optimal as it is expected to minimise the measurement error and only slightly increase the variance, then cartilage volume and its rate of change can be measured accurately with one in two 1.5 mm thick slices for lateral, medial tibial and patellar cartilage. Bland & Altman plots confirmed this with a random scatter about zero as would be expected if there is no difference between two measurements and uniform variability throughout the range of measurements. Of note, for longitudinal data even decreasing the number of slices by extracting up to one in four resulted in a maximum difference of 0.14% in mean annual change in cartilage volume which is very small when compared to the 5% cartilage loss annually we have reported in patients with OA [255]. Thus, a subsample of MRI slices could also be utilised with marked decreases in processing time allowing greater numbers of subjects to be studied offsetting the accompanying increase in measurement error.

Ideally, the more slices used, the more accurate the estimation of the object's volume, as they may contain more information. However, for a completely regular

structure, such as a cylinder, the area of a single slice with length gives an exact volume. It is therefore reassuring but not surprising that the current study demonstrates a minimum reduction in the knee cartilage volume and volume change over time as tibial and patellar cartilages have a relatively regular structure. A different interpretation may apply to femoral cartilage and we do not have data on this imaging site.

The current study simply examined the effect of decreasing the number of slices on the estimation of knee cartilage volume and volume change while all other variables were kept constant. We did not re-scan the study subjects but simply estimated the cartilage volume by using one in two, one in three, or one in four slices. This has an advantage of allowing us to examine the single effect of sampling intensity in the situation where all other variables such as re-positioning the subject and measurement were kept constant. The effect of these errors on measurement have been well-documented [204] [208]. For longitudinal analysis, all the MR images were processed by a single observer. For cross sectional analysis, two observers processed the MR images, one for the TASOAC data, and another for the KCV study. However, the difference was even smaller in the whole sample than in the two separate samples providing reassurance that our results may be generalisable to different observers as documented with different readers and machines in Melbourne [346].

The current study has a number of potential limitations. Firstly, which sampling intensity should be used in the MRI scan of knee cartilage depends on the purpose of the measurement. Our results cannot be applied to individual cartilage volume,

particularly for an individual's longitudinal loss of cartilage, but only for mean cartilage volume in groups as the individual difference in cartilage volume increases with decreasing sampling intensity. Secondly, decreasing sampling intensity will increase measurement error as the remaining slices focus on different portions of the irregularly shaped cartilage. Depending on what particular surfaces remain, however, the overall volume may be increased or decreased. If this is random, then the mean will remain the same as demonstrated in the current study. Thirdly, the ICC can be influenced by traits in the sample in which it is assessed. Age, sex and BMI have been reported to be associated with knee cartilage volume [219]. These may result in a higher ICC in the current study, as between-subject variance would become larger. However, subgroup analyses by sex, BMI (< 25, >= 25), and age (<50, >=50yr) did not change the results. Further analysis using the Bland & Altman method confirmed the good agreement and interchangability between thick and thin slices, indicating that the result of the current study should be applicable to other populations regardless of the demographic factors related to cartilage volume. Fourthly, the participants in the study had only mild ROA, and these conclusions may not apply to subjects with more advanced OA. Lastly, the annual change in cartilage volume in our sample can not be generalized to other populations as half of our longitudinal study sample had a higher genetic susceptibility to OA [217 291].

In conclusion, knee cartilage volume and its rate of change can be accurately measured with every second 1.5mm thick MR slice. This approach will lead to a substantial decrease in post-scan processing time and make large-scale studies of knee cartilage volume more feasible.

9.5 Postscript

This chapter demonstrated that knee cartilage volume can be measured with every second 1.5mm thick MR slice with very little increase in measurement error, making MRI-based measurements of the knee cartilage in large-scale epidemiological studies of OA feasible.

CHAPTER TEN: SUMMARY AND FUTURE DIRECTIONS

OA is the most common form of musculoskeletal diseases. Certainly, its resultant burden on our society will continue to grow as the population ages. Although the aetiology is still unclear, it is now realized that OA is a group of overlapping distinct diseases with multiple pathogenetic mechanisms implicated in its development and progression. Based on MRI measurements of the knee and hip, the work contained in this thesis has made a number of important novel contributions to and expanded our understanding of the disease.

10.1 Summary of the main findings

Genetics. The role of genetic factors in the development of OA has been described for many decades, but the results for the isolated knee OA are conflicting. The study was the first to examine the genetic contribution to individual knee structures cross-sectionally and longitudinally. Our data demonstrated that knee cartilage volume, bone size, muscle strength and their rate of change over time as well as progression of chondral defects had high heritability estimates, ranging from 42 to 98% depending on the variable of interest, most likely reflecting a strong genetic component and suggesting their potential to be studied in quantitative trait linkage and association analysis.

Knee pain. The cause of knee pain remains elusive but appears to be multifactorial. The study demonstrated that knee pain was significantly associated with both full and non-full thickness chondral defects, subchondral bone marrow lesions, BMI, knee extension strength, CTX-II, and obesity, suggesting MRI and biochemical measures can add to radiographs in defining unexplained knee pain. Furthermore, a strong association between hip ROA and knee pain indicates that referred pain from

the hip needs to be considered in unexplained knee pain and supports the long recognised clinical association.

Hip cartilage volume. This is the first study to compare associations between anthropometric and lifestyle factors and femoral head cartilage volume/thickness and radiographic features of OA. Our data demonstrated that radiographic hip JSN but not osteophytes was significantly associated with hip cartilage volume, particularly axial JSN with a 13% reduction per grade. In addition, hip JSN was significantly correlated with hip cartilage thickness with a 9% reduction per grade. This provides evidence for both face and construct validity of measuring hip cartilage morphology by MRI particularly for volume as thickness had poorer reproducibility. Further, femoral head cartilage volume was significantly associated with female sex, BMI, and femoral head size while only female sex were associated with hip total ROA score and hip JSN, suggesting that MRI may be superior at identifying risk factors for hip OA.

Optimal sampling of MRI slices. MRI slices of 1.5 mm thickness have been used in both cross sectional and longitudinal studies of OA, but is difficult to apply to large studies, as most techniques used in measuring knee cartilage volumes require substantial post-image processing. The study demonstrated that knee cartilage volume and its rate of change can be accurately measured with every second 1.5mm thick MRI slice with little increase in measurement error. This approach will lead to a substantial decrease in post-scan processing time and make large-scale studies of knee cartilage volume more feasible.

10.2 Future directions

Genetics. There is currently considerable work in progress with regard to identification of specific genes involved in OA. But the current major endpoint for association and linkage studies is radiographic OA. This may be less powerful due to the fact that radiography is two-dimensional nature and semi-quantitative, but OA is a complex disease with multiple genes involved. This thesis demonstrated that knee cartilage volume, bone size, muscle strength, and their rate of change over time as well as progression of chondral defects all had significant heritability. This information will be critical in helping researchers to establish endpoints that are both relevant and productive for these gene-searching techniques, as these variables are tissue specific and the measurements are quantitative. Therefore, the next step is to conduct larger family or population based association studies to clarify which genes are important in the determination of these specific tissues such as cartilage volume and bone size and their relevance to OA susceptibility.

OA symptoms. Symptoms such as pain are the major reason for people with OA to seek medical advices. The study linked knee pain to both full and non-full thickness loss of the articular cartilage, bone marrow lesions, expanding our understanding of the causes of knee pain and providing critical information in facilitating clinical trials for specific cause of knee pain. However, the study was cross-sectional in nature. Future efforts are to examine the association between natural history of the development of knee pain and the progression of chondral defects and bone marrow lesions. The TASOAC study is an ongoing prospective study, and has a great opportunity to answer this question.

Hip OA. This thesis first documented that femoral head cartilage volume and thickness have modest but significant construct validity when correlated with radiographs. Furthermore, the generally stronger associations with volume compared to ROA suggest that MRI may be superior at identifying risk factors for hip OA. However, the study was a pilot with small sample. More work is needed. Future work is to confirm these results in an independent sample and describe longitudinal change in hip volume. Furthermore, the association between hip cartilage volume and other risk factors of hip OA such as developmental abnormalities of hip will be examined. The link between hip cartilage volume and symptoms of the hip OA such as pain should also be investigated.

Other research directions. It has been demonstrated in the literature that MRI-based measurements of the structure of the joint are accurate and reproducible as described in section 2 of Chapter 1 of this thesis. Linking knee pain to specific structural abnormalities of the joint such as chondral defects, bone marrow lesions, and low knee extension strength in this thesis provides critical information and make it possible to investigate interventions targeting specific causes. Using MRI-based measurements such as chondral defects and bone marrow lesions as the endpoint, studies to evaluate cartilage repair therapies or interventions targeting at reducing bone marrow lesions and their role in relieving knee symptoms will provide important information on these therapies.

Bone marrow lesions are easily identified on T2 weighted MR images and linked to knee pain. To better understand their role in the development of OA, further studies are needed. Studies on correlation between bone marrow lesions and biomarkers of

bone and cartilage turnover, the relationship between bone marrow lesions and the cartilage loss over time, and the pathophysiology of MRI-based bone marrow lesions will provide critical insight.

Further, the structural determinants of mechanical dysfunction and pain in OA are not well understood, but probably involve a multitude of interactive pathways as the result of the whole joint organ involvement in the development of the disease. This thesis demonstrated the significant association between knee pain, chondral defects, and bone marrow lesions. Studies on the relationship between knee pain and other joint tissues such as meniscal abnormalities, synovial thickening are needed. Moreover, studies to evaluate the relationship between meniscal abnormalities, synovial thickening and cartilage loss over time will provide new insight into pathology of OA.

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APPENDICES

Appendix 1

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Menzies Centre for
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University of Tasmania

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Phone: (03) 6226 7700
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ID Number

Dr Graeme Jones

TASOAC
General Questionnaire

Date Questionnaire Completed

/ /

Instructions for completing the questionnaire:

Please answer all questions to the best of your ability (leave blank if unknown).

Please write in block letters using the boxes where provided

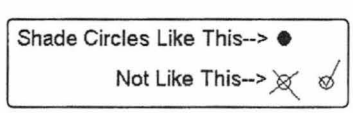
Use a black/blue pen

Cross out any mistakes & write correct answer just below the relevant boxes

Indicate your response by filling in the circle next to the most appropriate answer or by writing clearly in the boxes or space provided.

Your answers will be completely confidential.

Example:



For optimum accuracy, please print in
capital letters and avoid contact with
the edge of the box.
The following will serve as an example:

0750108180

Name and Address

Surname

Given Names

Title

Maiden Name (if applicable)

Address

Suburb

State

Post Code

Home Phone Number

Business Phone Number

Mobile Phone Number

1. **How long have you lived at this address?** Years

Date of Birth

/ /

Place of Birth

City/Town

State/Country

8998108182

5 Rate the following today

This section assesses pain, stiffness and functional deficit on a scale from 1 - 10

Example	none									severe
Example of no pain	● 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
Example of severe pain	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	● 10

1. Referring to your knees only how much <u>pain</u> do you experience when

	none									severe
a. Walking on a flat surface	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
b. Going up and down stairs	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
c. At night while in bed	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
d. Sitting or lying	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
e. Standing upright	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10

2. Referring to your knees only how much <u>stiffness</u> do you experience

	none									severe
a. After first awakening	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
b. Later in the day	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10

3. Referring to your knees only how much <u>functional deficit</u> do you experience when

	none									severe
a Descending stairs	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
b. Ascending stairs	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
c Rising from bed	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
d. Rising from sitting	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
e. Putting on socks	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
f. Taking off socks	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
g. Bending to the floor	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
h. Lying in bed	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10

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Question 3 continued	none									severe
i. Walking on flat surface	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
j. Getting in/out of the bath	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
k. Standing	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
l. Sitting	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
m Getting in/out of the car	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
n. Getting on/off the toilet	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
o. Heavy domestic chores	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
p. Light domestic chores	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10
q. Shopping	O 1	O 2	O 3	O 4	O 5	O 6	O 7	O 8	O 9	O 10

4. Do you have pain at any of these sites?

a. Neck	Yes	O 1	No	O 2
b. Back	Yes	O 1	No	O 2
c Hands	Yes	O 1	No	O 2
d. Shoulders	Yes	O 1	No	O 2
e. Hips	Yes	O 1	No	O 2
f Knees	Yes	O 1	No	O 2
g Feet	Yes	O 1	No	O 2

Appendix 2

Menzies Centre for
Population Health Research

University of Tasmania

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ID Number ☐☐☐ : ☐☐

Dr Graeme Jones
Dr Flavia Cicuttini

Tasmanian Knee Cartilage Volume Study

Appointment Questionnaire
Control

Name and Address

Surname

Maiden Name (if applicable)

Given Names

Address

Suburb State Post Code

Home Phone Number Business Phone Number Mobile Phone Number

Appointment Date

☐☐ / ☐☐ / ☐☐☐☐

Dynomometer Calibrated ○

Bike Calibrated ○

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1 Date of Birth

☐☐ / ☐☐ / ☐☐☐☐

Gender
Male ○ **Female** ○

2 Smoking

Have you ever smoked cigarettes on a regular basis? **Yes** ○ **No** ○

If yes, what age did you start smoking regularly? ☐☐

Do you currently smoke cigarettes? **Yes** ○ **No** ○

If you have given up smoking, what age were you when you gave up? ☐☐

Please state the number of cigarettes that you smoke each day (or used to smoke each day if you have given up) ☐☐

3 Family History of Osteoarthtitis

a. Does/did your mother suffer from Osteoarthritis of the knee? **Yes** ○ **No** ○ **Don't Know** ○

b. If yes, Has/did you mother had/have a Total Knee Replacement? **Yes** ○ **No** ○ **Don't Know** ○

c. Does/did your father suffer from Osteoarthritis of the knee? **Yes** ○ **No** ○ **Don't know** ○

d. If yes, has/did your father had/have a Total Knee Replacement? **Yes** ○ **No** ○ **Don'tKnow** ○

e. Does/did your Mother suffer from knee pain for more than 24 hours in the last 12 months or daily pain for more than 30 days in the last year? **Yes** ○ **No** ○ **Don't know** ○

f. Does/did your Father suffer from knee pain for more than 24 hours in the last 12 months or daily pain for more than 30 days in the last year? **Yes** ○ **No** ○ **Don't know** ○

4 History of Knee pain

a. If employed, does your occupation involve significant knee bending and carrying heavy objects? eg.Delivery work. **Yes** ○ **No** ○

b. Have you had knee pain for more than 24 hours in the last 12 months or daily pain on greater than 30 days in the last year? **Yes** ○ **No** ○

c. Have you had a previous knee injury requiring non-weight bearing treatment for more than 24 hours or surgery? **Yes** ○ **No** ○

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