# a journey full of pain
## through the general population

marjolein de kruijf

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## Een reis vol met pijn door de algemene populatie

**Proefschrift**

Ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam

op gezag van de rector magnificus

**Prof. Dr. H.A.P. Pols**

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**Marjolein de Kruijf**
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# Contents

# CHAPTER 1

General introduction

# What is pain?

### Normal pain anatomy and physiology

Pain is a useful and even necessary warning system to protect the body from further harm. In the normal situation, a painful stimulus, either by tissue damage or inflammatory, is detected by nociceptors in the skin or other tissues. From there, mainly C- and A-delta-fibers transport the signal to the spinal cord which carries it towards the brain.

In the brain, the sensory cortex localizes the pain and the limbic system is responsible for the emotional processing. Descending inhibitory tracts can facilitate, modulate and suppress the incoming pain signal. (Figure 1)
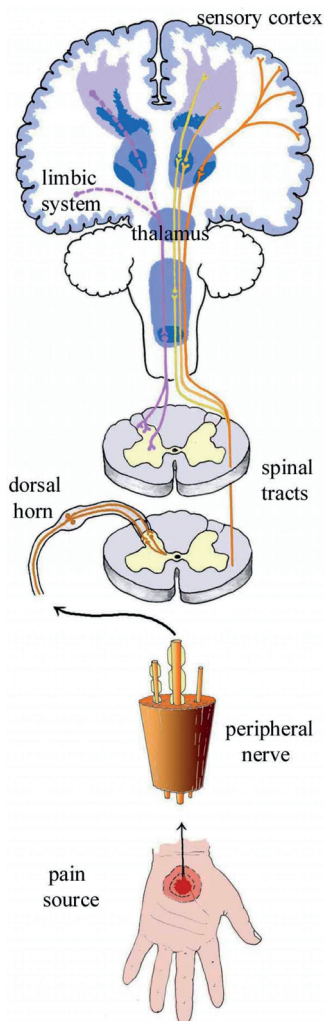


Figure 1. Diagram of pain pathways. Painful stimuli travel from the pain source up to the sensory cortex in the brain. Descending inhibitory tracts, originating from the limbic system, modulate the stimulus on the spinal level. By dr. David Nelson, adapted with permission.

### Classification of pain

Pain can be classified based on the neurophysiology into nociceptive and non-nociceptive pain (Figure 2).

Nociceptive pain is caused by activation or sensitization of free nerve endings (nociceptors) and is usually caused by (the risk of) tissue damage. It can be sub-divided into somatic pain (originating from bone, soft tissue, joints and muscles) and visceral pain (originating from organs). Joint pain (most often caused by osteoarthritis in an elderly population) and back pain are examples of nociceptive pain conditions.

Non-nociceptive pain can be neuropathic pain, in which there is a lesion or illness of the nervous system, or idiopathic, in which there is no apparent cause of the experienced pain. Fibromyalgia and irritable bowel syndrome are examples of chronic non-nociceptive pain.

In some pain conditions, such as migraine and in some cases of chronic back pain, it is thought that both nociceptive and non-nociceptive mechanisms play a role.
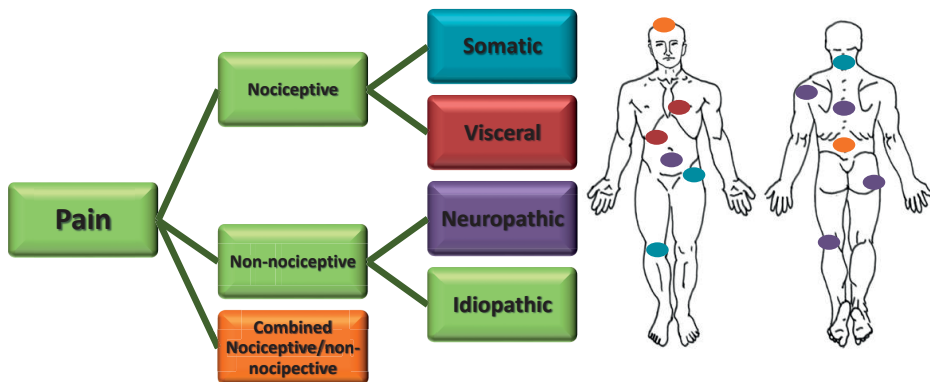
**Figure 2.** Classification of pain by neurophysiological mechanism with in colors some examples on where in the body the pain can arise.

### Pathophysiology of chronic pain

In chronic pain, the normal pain processing is altered by central sensitization. The nervous system is able to change its structure and physiology when exposed to repeated stimuli, which is called plasticity. Under a continuous stimulating condition, peripheral nerves can become more sensitive to noxious stimuli and the pain processing in the central nervous system is altered, causing the descending inhibitory systems to function improperly and making it more susceptible for the development of chronic pain elsewhere in the body.

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### Definition of chronic pain

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage[1].

Chronic pain is pain that persists past the normal time of healing, which sometimes may be less than a month and in other cases more than six months[2]. In most research however, pain persisting after three months is considered chronic. Since different causes of pain may have different durations of normal healing, a generalized definition of pain lasting for more than three months may not be sufficient to properly identify individuals with chronic pain. With the inclusion of more objective measures, the definition of chronic pain may be more robust.

## Chronic musculoskeletal pain in the general population

Chronic musculoskeletal pain is a common disabling condition with a great impact on daily functioning [3, 4]. In the Netherlands, 19% of all individuals aged 21 years and older experience chronic pain and in elderly this is more than half. This means that more than 2 million Dutch people experience pain on a daily basis, which is a higher incidence than most common diseases like diabetes and coronary heart disease[5].

The quality of life of chronic pain patients is decreased and psychological problems occur often, even more than in cancer patients [6-8]. Although 85% of the chronic pain syndromes have an underlying cause, such as osteoarthritis, there are many individuals in which the chronic pain they experience remains unexplained. The lower quality of life is among others caused by a lack of social participation, consequences for work and sleep disturbances. Especially the unexplained part of chronic pain in combination with limited understanding from the society is likely to underlie the lower quality of life in these subjects.

Chronic pain accounts for an annual socioeconomic burden of 20 billion euros in the Netherlands alone, mainly due to sickness absence and social security funds [9]. Therefore, it is not surprising that the European Federation of 'International Association for the Study of Pain (IASP)' chapters considers chronic pain as a major health care problem and advises to see chronic pain not only as a symptom but as a disease in its own right [10].

## Causes and consequences of chronic pain and central sensitization

Chronic pain is a complex trait with a multifactorial etiology. In the remaining of this chapter, the topics studied in this thesis will be introduced. First, the genetic and hormonal influences on the development of chronic pain are discussed. From there, we look further into the structural changes of the brain in chronic pain and functional changes in the nervous system, represented by an altered heat pain sensitivity. At the end, the consequences of chronic pain and osteoarthritis, as one of the major causes of chronic pain in the elderly, on gait and mobility are described.

### Genetics of pain sensitivity

Evidence from birth-cohort and twin studies suggest that chronic pain and the sensitivity to painful stimuli and pain tolerability have a rather large heritability up to 60% [11-13]. This suggests a genetic vulnerability. However, the exact genes involved are unknown until now.

It is unlikely, that there is a single unique pain gene since it is generally accepted that susceptibility to chronic pain is very similar in its genetic architecture to other complex traits. Therefore, multiple genes might be involved and each individual DNA variant probably only contributes a little to the overall variability. For other complex traits, such as diabetes or osteoporosis, large-scale association studies have been able to identify consistent associations and new candidate genes [14, 15]. Therefore, performing genome-wide association studies (GWAS) on pain phenotypes such as experimental heat pain sensitivity might be helpful to further elucidate the genetics of pain sensitivity. Experimental pain sensitivity can also serve as an endophenotype for the susceptibility for developing chronic pain. Therefore, studying the genetics behind this endophenotype may also help in the search for pain genes.

Until now, there are no successful large genetic studies on (heat) pain sensitivity. Most research focused on a number of candidate genes in relation to pain and pain perception. One of the most studied genes is the catechol-O-methyltranferase (COMT) gene which influences pain perception through enzymatic breakdown of neurotransmitters. Despite the large number of studies on the relation between amino acid variations in this gene and various pain outcomes, results are still inconsistent [16-18].

### Hormonal influences on chronic pain

The prevalence of chronic pain is higher in women and it is known that pain experience and coping strategies differ between men and women [19-21]. Hormonal influences have been considered to underlie a least part of the differences in chronic

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pain between men and women. There is some limited evidence that estrogens play a role in the apparent gender specificity in pain experience[22]. Estrogen and testosterone are the two most studied sex hormones in relation to pain.

Testosterone is thought to be important during the development of the nervous system and is hypothesized to create a set point for pain sensitivity. [23]

Estrogen is thought to be more of influence later in life. More or longer estrogen exposure during life, for example measured by the number of menstrual cycles, has been found to be associated with more migraine later in life [24], whereas lower current estrogen levels, like in postmenopausal women, is associated with a higher prevalence of chronic musculoskeletal pain[22, 25].

**Imaging the brain in chronic pain**

Since plasticity of the nervous system underlies part of the pathophysiology of chronic pain, structural changes are likely to occur in the brain. Studying these changes can help us understand the pathophysiology of chronic pain better which might help in developing treatment strategies.

Previous studies that examined structural brain alterations in chronic pain, focused on a variety of pain phenotypes, such as migraine, back pain, osteoarthritis and fibromyalgia [26-35]. Regions that are part of the limbic system (Figure 3) and the signaling pathway were among the identified pain-associated brain areas. Despite the possible identification of structural brain alterations in these selected
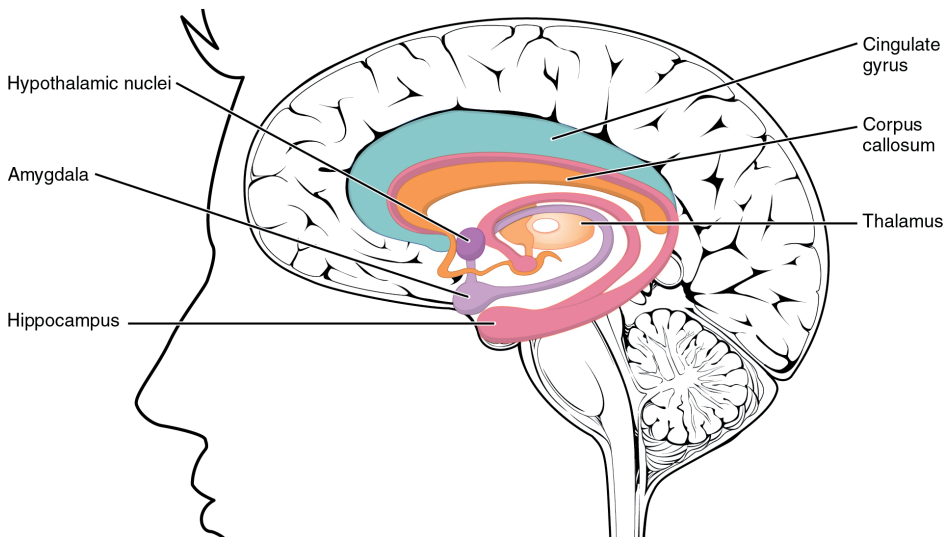


**Figure 3.** Visualization of the major parts of the anatomy of the limbic system in the brain, among others the hippocampus, hypothalamus, amygdala and thalamus.
By OpenStax College [CC BY 3.0 (http://creativecommons.org/licenses/by/3.0)]

clinical cases, it remains unclear which brain regions are morphologically altered in chronic pain and experimental pain in the general population.

Besides structural MRI research, diffusion tensor imaging (DTI) can provide additional information on the microstructural organization of the white matter in the brain. Previous studies investigating chronic pain and white matter microstructural organization have focused on specific chronic pain disorders, such as irritable bowel syndrome, fibromyalgia, migraine and chronic pancreatitis [36-40], but the results have been inconsistent and lacked power to determine the common and specific white matter tracts involved in chronic pain.

## Quantitative sensory testing

In daily clinical practice, quantitative sensory testing (QST) is often used as a tool to diagnose central and peripheral sensitization in chronic pain patients. However, it can also be used to monitor effectiveness of pain treatment [41-45]. QST quantifies the sensitivity for experimental stimuli and there are many different modalities which can be used. Commonly used stimuli are mechanical (pin-prick), pressure and thermal stimuli.

In previous articles, specific pain phenotypes, originating from clinical study case groups, have been studied, such as neuropathic pain, fibromyalgia and osteoarthritis related pain, and changes in QST measurements were observed [41, 43, 45, 46]. Efforts were made to define reference thresholds for clinical use, but there is still limited information on QST normal values measurements in aging individuals and whether there are additional determinants influencing this measurement [47].

## Osteoarthritis and mobility

Chronic pain in the lower body is often a cause for a decreased mobility and a lower quality of life. Especially in older individuals, this decreased mobility is also related to a higher mortality [48-52].

Gait is an accurate health indicator and poor gait is strongly associated with a higher risk of falls and mortality [50, 53-57]. Gait is a complex concept that can be assessed using many parameters. It has been shown that these parameters can be summarized into seven gait domains (Figure 4), comprehensively capturing the gait pattern: (1) Rhythm, reflecting cadence and single support time; (2) Variability, reflecting variability in step length and time; (3) Phases, reflecting double support time and single support time as a percentage of the total stride time; (4) Pace, reflecting step length and velocity; (5) Tandem, reflecting errors in tandem walking; (6) Turning, reflecting the number of steps and time needed to turn; and (7) Base of Support, reflecting stride width and stride width variability [58, 59]
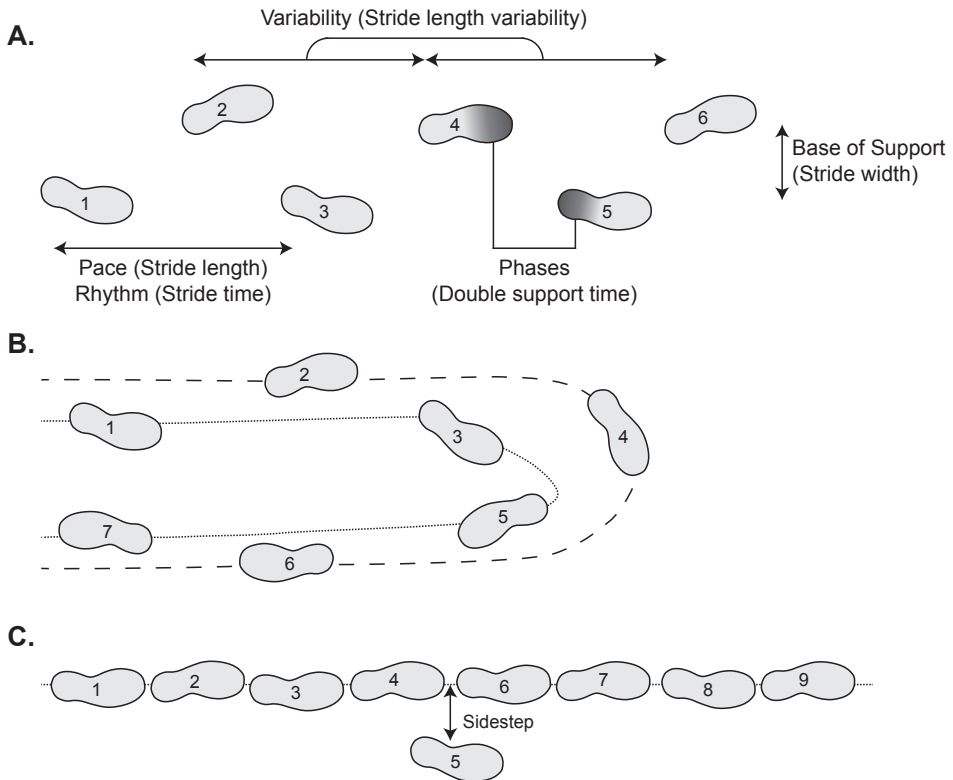
**Figure 4.** Illustration of the 7 gait domains. A: Normal walk (Rhythm, Variability, Phases, Pace and Base of support), B: Turn, C: Tandem walk

Previous studies on the association between pain and gait parameters mainly focused on osteoarthritis (OA), the most common cause of pain in the lower body of elderly people[60-66]. The experienced pain does not always reflect the joint damage. Therefore, studying the association between gait and chronic pain in the lower body independent of the presence of OA will inform more about the independent effect of lower body pain on gait.

Better understanding of the relationship between lower body pain, OA and gait may allow for new interventions to decrease pain and gait problems, and hence related morbidity and mortality.

## The Rotterdam Study

The studies presented in this thesis are performed within the large population based prospective cohort study, the Rotterdam Study (RS). In the Netherlands and among

the participants, it is also known as 'Erasmus Rotterdam Gezondheid Onderzoek' (ERGO). This ongoing study started in 1990 and examines determinants of chronic disabling disease in the elderly[67]. Up to know, more than 15,000 participants of 45 years and older are included in the three sub-populations, RS-I, RS-II (initiated in 1999) and RS-III (initiated in 2006).

All participants were examined extensively at baseline with a home interview and an extensive set of approximately 1,500 examinations at the research center. For example, blood and urine was collected and x-rays were taken of the major joints. Many of these examinations were repeated every 3-5 years in the last 25 years. Therefore, 5 follow-up visits are available for RS-I. In figure 5, the visits from all 3 cohorts up to 2013 are shown.
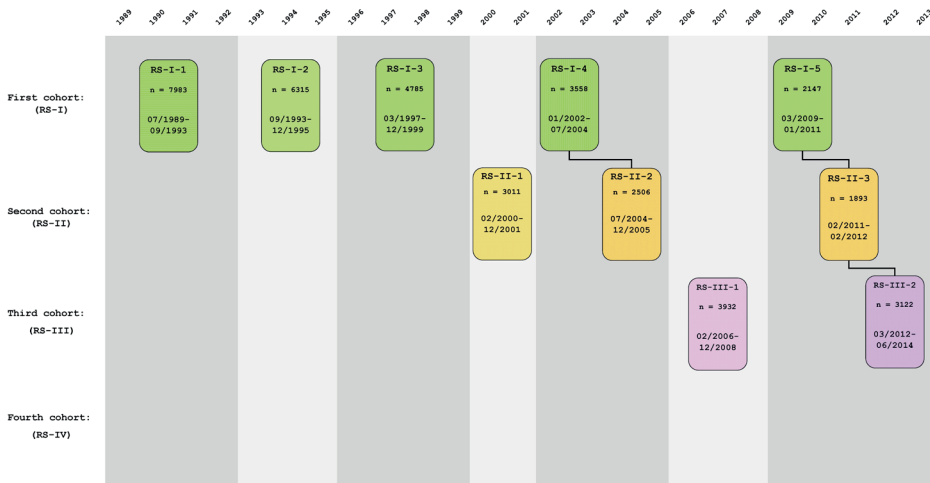


**Figure 5.** Diagram of the examination cycles of the Rotterdam Study (RS). RS-I-1 refers to the baseline examination of the original cohort (pilot phase 07/1989–12/1989; cohort recruitment 01/1990–09/1993). RS-I- 2, RS-I-3, RS-I-4, and RS-I-5 refer to re-examination of the original cohort members. RS-II-1 refers to the extension of the cohort with persons in the study district that became 55 years since the start of the study or those of 55 years or over that migrated into the study district. RS-II-2 refers to re-examination of the extension cohort. RS-III-1 refers to the baseline examination of all persons aged 45 years and over living in the study district that had not been examined (i.e., mainly comprising those aged 45–60 years). RS-II-3 and RS-III-2 refer to ongoing and future re-examinations. Examination RS-I-4 and RS-II-2 were conducted as one project and feature an identical research program. Similarly, examinations RS-I-5, RS-II-3, and RS-III-2 will share the same program items. Adapted from: Albert Hofman, et al. The Rotterdam Study: 2016 objectives and design update. Eur J Epidemiol. 2015;30:661-708.

**Aim of this thesis**

The overall objective of this thesis is the identification and characterization of causal and consequential determinants of chronic musculoskeletal pain in the general population. In chapter 2, the genetic determinants influencing heat pain sensitivity thresholds are studied. Next, the influence of hormones during development and during the course of life is studied in chapter 3. In chapter 4, quantitative and qualitative determinants of brain structure in chronic pain are presented. Epidemiological considerations are raised in chapter 5 regarding thermal quantitative sensory testing, a widely used tool for measuring pain sensitivity and sensitization in chronic pain. The influence of osteoarthritis and chronic pain on gait is studied in chapter 6.

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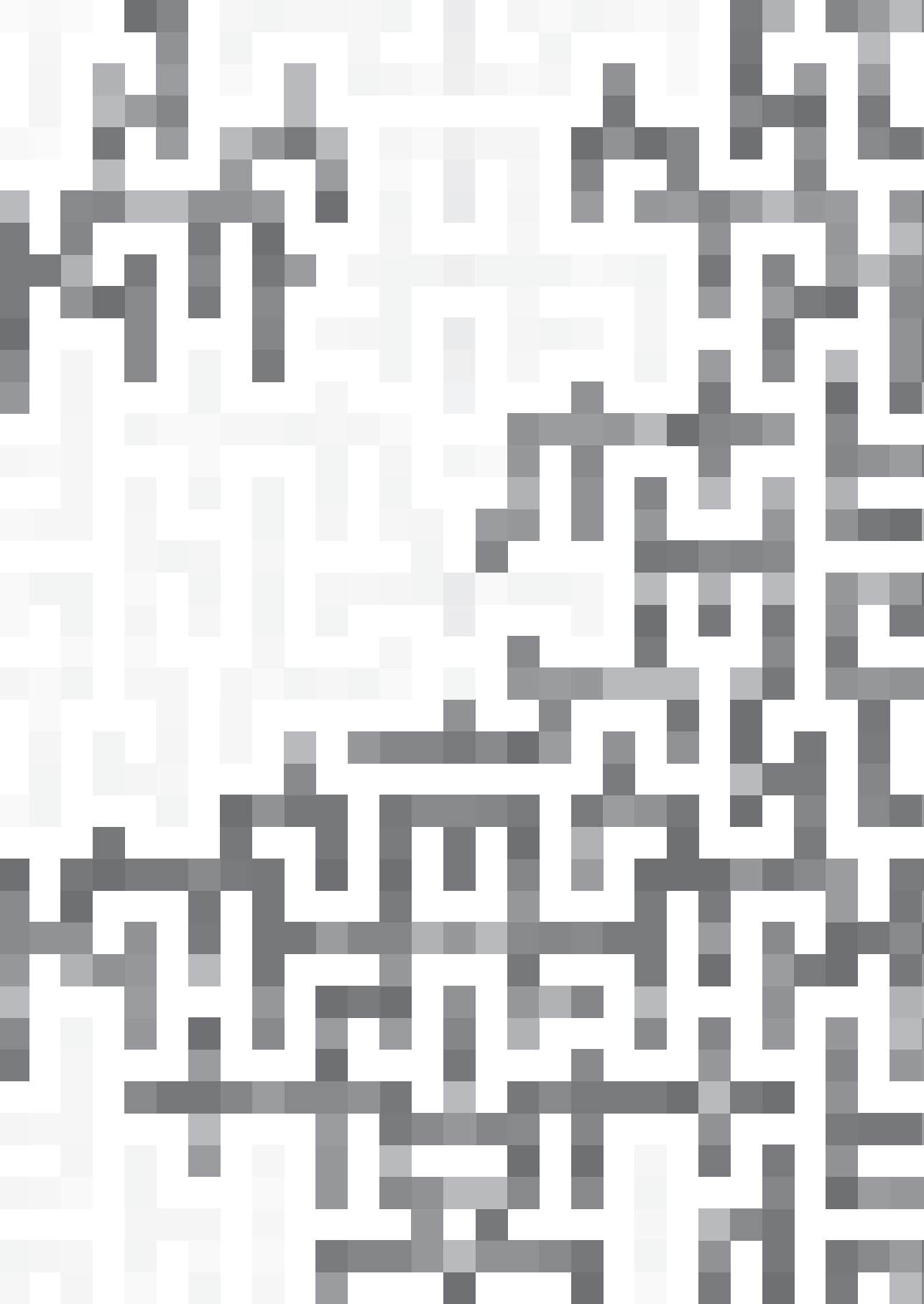
# CHAPTER 2

Genetics of pain threshold
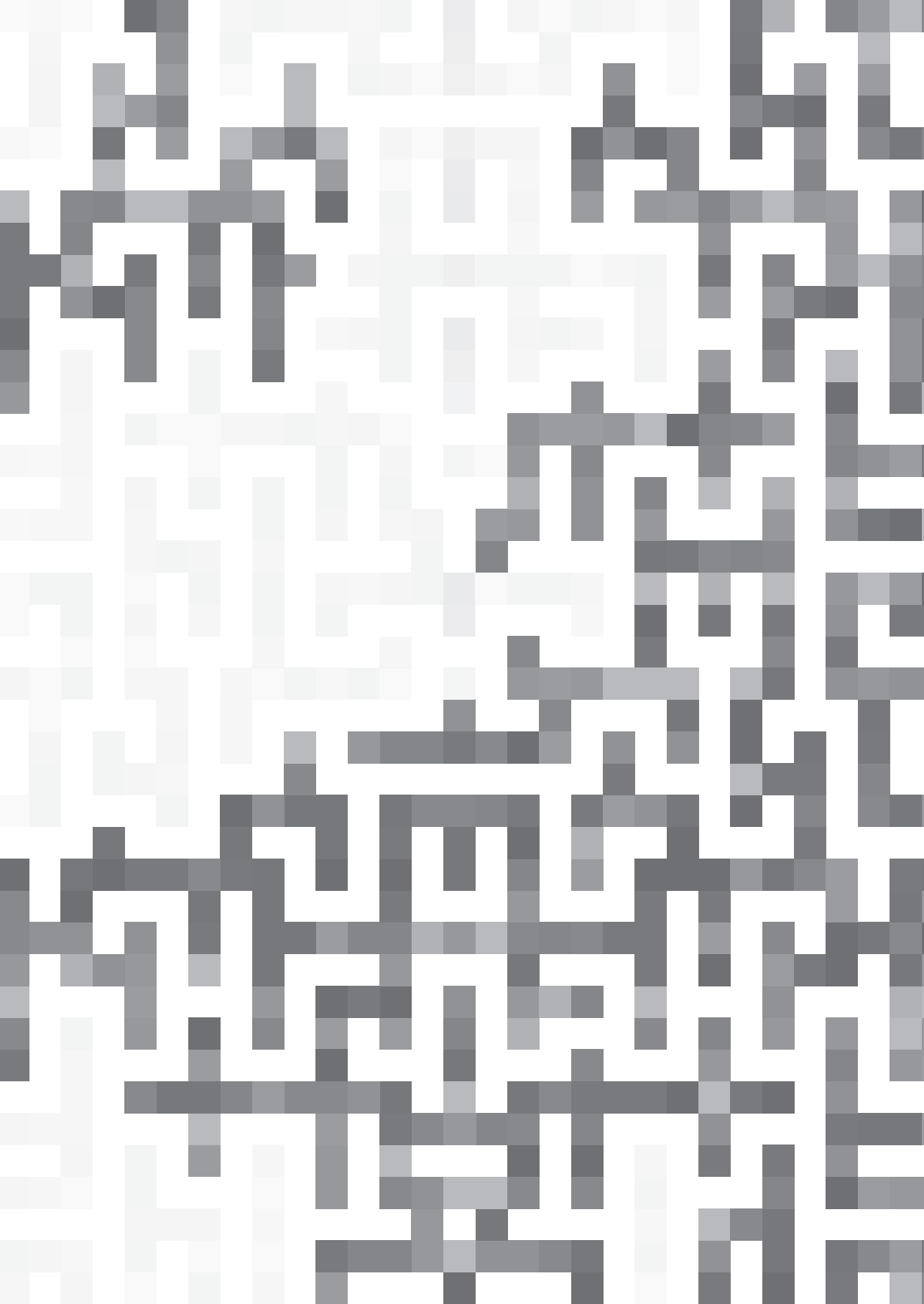
# CHAPTER 2.1

## Genetics of the heat pain threshold in the general population

Marjolein de Kruijf, Marjolein J. Peters, Cindy G. Boer, Carolina M. Gomez, Fernando Rivadeneira, Frank J.P.M. Huygen, André G. Uitterlinden, Joyce B. J. van Meurs

## Abstract

*Introduction:* Chronic pain and pain sensitivity are complex traits with a variety of potential determinants. Although not yet fully elucidated, pain sensitivity and the risk for chronic pain are thought to be partly genetic. In our study, we attempt to further elucidate the genetic predisposition of pain sensitivity.

*Methods:* In a total number of 3,795 participants from the Rotterdam study (a large prospective population based cohort) heat pain thresholds (HPT) were determined. We estimated the total additive genetic influence on HPT measurements due to common genetic variation using GCTA, and we performed a genome wide association study (GWAS) to identify new loci associated with HPT in the general population. Finally, we reviewed the literature for previously reported DNA variants associated with experimental pain thresholds and tried to replicate these findings in our dataset.

*Results:* The overall heritability estimate of HPT was 19%. In individuals without chronic pain, this estimate was 32% compared to 9% in individuals with chronic pain. In addition, the heritability was higher in women compared to men. Our GWAS revealed one genome-wide significant signal (1:176688345:D) which is located in the twelfth intron of the *PAPPA2* gene (p= $2.48*10^{-8}$). Additionally, we found six suggestive signals (P<$1.0*10^{-6}$). Genetic variants previously associated with pain sensitivity were not replicated in our study.

*Conclusion:* A significant proportion of the variability of HPT is explained by genetics. The extent to which HPT is genetically determined is higher when individuals do not experience chronic pain. Future genetic studies on pain sensitivity should take the presence of chronic pain into account since it influences the phenotype substantially. This largest genetic screen for pain sensitivity up to date provides new potential genetic loci for further research.

## Introduction

Chronic pain and pain sensitivity are complex traits with a variety of potential determinants. The development of chronic pain and an increased sensitivity by sensitization of the nervous system are unintended consequences after tissue damage. In this scenario, the pain is prolonged or more severe compared to what might be expected during a normal healing process [1].

   A wide variety of risk factors have been described for the development of chronic pain. One of them is an intrinsic high pain sensitivity, which can be assessed by experimental pain sensitivity measurements [2]. In theory, experimental pain sensitivity is less sensitive to bias due to disease or tissue damage, compared to more subjective pain phenotypes such as pain severity scores [3]. There are many different measurements to determine pain sensitivity, such as pain thresholds and tolerance for different stimuli. The heat pain threshold (HPT) is one of the most studied measurements for pain sensitivity: the HPT is noninvasive and can be used for measuring pain sensitivity and pain thresholds. Measurements can be done over multiple body points, and the temperature and the duration of the pain stimulus can be highly controlled [3]. Finally, there is good reproducibility between two sessions [4].

   The proportion of genetic influence on pain has been under debate. In previous studies, the heritability of pain sensitivity and chronic pain has been estimated in classical twin studies. A review by Nielsen *et al*. [5] showed that the heritability estimates of specific pain phenotypes differ: for example, back and neck pain have heritability estimates ranging from 0% to 68% [6-13], osteoarthritis has heritability estimates ranging from 0% to 53% [14,15], and irritable bowel syndrome has heritability estimates ranging from 0% to 48% [16-20]. The heritability of experimental pain sensitivity has been studied scarcely, with only three previous study reports. All three reports had a twins design and used various experimental designs, such as cold pressor tests and heat pain thresholds [21-23], and relatively small sample sizes were used. Consequently, heritability estimates ranged between 0% and 60%. Other studies investigating the genetic background of pain sensitivity focused on candidate genes previously described to play a role in pain, or studied the genetic variants in modestly sized pain patient populations [24-36].

   In a previous study by our group, we investigated the genetic background of chronic widespread pain [37]. Although we identified a DNA variant to be associated with CWP, we also identified significant heterogeneity among phenotype definitions among the cohorts. As for other complex traits, it would be helpful to dissect the pain phenotype into quantitative underlying endophenotypes, such as intrinsic pain sensitivity, which can be measured by experimental pain thresholds.

The present study therefore focuses on experimental HPT as an endophenotype underlying the development of chronic pain.

The aim of the current study was to further elucidate the genetic predisposition of pain sensitivity, defined as the HPT. In the Rotterdam Study, a large prospective population based cohort, we estimated the heritability of the HPT and the influence of gender and the presence of chronic pain on the heritability of the trait. We performed a genome wide association study (GWAS) to search for potential new genetic markers associated with HPT in a general population. And finally, we reviewed the literature for significantly pain sensitivity associated variants and we tried to replicate those findings in our population.

## Methods

### Study population

This study is performed within the Rotterdam Study (RS), a large prospective population-based cohort study of men and women aged 45 years and over. The study design and rationale are described elsewhere in detail [38]. In summary, determinants, incidence and progression of chronic disabling diseases in the elderly are studied. The first cohort (RS-I) within the Rotterdam study started in 1990 and included 7,983 individuals ages 55 years and older. In 1999, an additional 3,011 subjects were included in Rotterdam study II (RS-II). The third cohort (RS-III) was invited in 2005, adding 3,932 individuals aged 45 years and over. All participants were examined in detail at baseline and at subsequent follow up visits, which took place approximately every six years. In summary, a home interview and extensive set of examinations at the research center was performed. For the present study, we used data from 3,795 participants for whom data on experimental pain sensitivity, data on the presence of chronic pain and genetic information were available. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the *"Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)"*. All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

### Genotyping

Genotyping was done using Illumina Infinium HumanHap550 Beadchips (RS-II), or the Illumina Infinium HumanHap610 Beadchips (RS-III). Details about genotyping and Quality Control have been described previously [37]. In short, a total of 2,612 subjects were genotyped in RS-II (Illumina 550 duo) and a total of 3,523 subjects in

RS-III (Illumina 610 quad). Exclusion criteria were a call rate <98%, Hardy-Weinberg p-value <10-6 and minor allele frequency < 0.01%, autosomal heterozygosity, sex mismatch and outlying identity-by-state clustering estimates. A total of 2,157 for RS-II and 3,048 for RS-III passed genotyping quality control. Data was imputed with the 1000-Genomes reference panel (phase 1, version 3) using MACH version 1.0.15/1.0.16 [39]. A total number of 30,072,738 SNPs were available for association analysis.

**2.1**

**Experimental pain sensitivity assessment: Heat pain threshold measurement**

In the 3,795 participants of the Rotterdam study included in this study, quantitative sensory testing was conducted. We used a commercially available thermo-sensory analyzer, the TSA II (Medoc Advanced Medical Systems, Durham, NC). The measurement probe had a surface of 30x30mm, and was placed on the inner site of the non-dominant forearm.

During the HPT measurement, the starting temperature of the probe was 32 degrees Celsius. Then, the probe would increase in temperature with 1.5 degrees per second until the participant ended the test or the maximum temperature of 50 degrees Celsius was reached. The participant was asked to push a large red 'quiz button' and therewith end the measurement at the moment the stimulus started to feel unpleasant or painful. After each measurement, the temperature returned to 32 degrees Celsius before the next measurement started. The HPT measurement was repeated five times in a row. For the analysis, the average temperature of the last three measurements was used.

**Heritability estimation**

To quantify the proportion of HPT variance explained by genetic variants, we used the restricted maximum likelihood (REML) method. This method is able to quantify heritability estimates attributable to all genetic variants and is implemented in the Genome-wide Complex Trait Analysis (GCTA) package. [40] We created one genetic relationship matrix (GRM) file for the unrelated participants in our RS-II and RS-III populations, and included all genotyped SNPs. This resulted in a GRM file of 495,775 SNPs for 3,795 samples. No pairs of individuals exceeded the GCTA standard cutoff coefficient of 0.025 for genetic relatedness. A p-value<0.05 was considered to be statistically significant in this analysis.

The mean HPT (as described before) was used as phenotype and adjustments were made for age and gender.

Additional analyses were performed in which we stratified both for gender and the presence of chronic musculoskeletal pain.

**Genome Wide Association Study (GWAS) and meta-analysis**

We performed two GWAS for HPT: one in RS-II and one in RS-III. We used MACH2QTL via GRIMP [41], which uses the genotype dosage values (0-2 as a continuous variables) as the predictor in a linear regression framework. HPT was used as the outcome measurement and adjustments were made for age, gender and the presence of chronic pain. In addition, the GWAS was repeated in participants without chronic pain.

Quality control was done with EasyQC [42]. The effective allele count was calculated for all SNPs by 2*minor allele frequency*$R^2$(correlatedness of the data)*sample size. SNPs with an effective allele count >5 were included in the meta-analysis. An effective allele count of >5 represents minor alleles appearing at least 5 times in the study population.

The summary statistics of the results of the GWAS in RS-II and RS-III were meta-analyzed using METAL (*www.sph.umich.edu/csg/abecasis/metal*) after genomic control correction to the standard errors and p-values. METAL applies an inverse-variance methodology assuming fixed effects with Cochran's Q and $I^2$ metrics to quantify between-study heterogeneity.

For the GWAS, the statistical significant threshold was set on $5.0*10^{-8}$. SNPs with a p-value $< 1.0*10^{-6}$ were called suggestive signals.

**Systemic review of genetic variants previously described**

We systematically searched the literature for previous associations with experimental pain thresholds. We used the Human Genome Epidemiology (HuGe) Navigator Phenopedia database for this. [43] This database provides a comprehensive archive of studies assessing the associations between phenotypes and genetic variants and this database is continuously updated.

The phenopedia tool provides a list of genes previously associated with your phenotype of interest, and includes links to the articles in which these associations were published. We used the search term 'pain threshold' on 16 September 2014. All publications were manually screened for the phenotype studied and the SNPs identified. We only included the studies which investigated the association of genetic variants with measures of quantitative sensory testing. The SNPs selected for the analysis were those described to be significantly associated with the pain threshold phenotype. Additionally, an rs-id needed to be available.

For all reported SNPs, we examined their association with HPT in our GWAS meta-analysis results. The significance threshold was set at p-value<0.05.

## Results

### Population characteristics

For this study, 1,326 individuals from the third follow up visit of RS-II and 2,469 individuals from the second follow up visit of RS-III were included in whom HPT measurements and genotype information was available. Characteristics are shown in Table 1. The participants from RS-II were significantly older and had a lower percentage of women. The prevalence of chronic pain was significantly higher in RS-II and mean HPT measures were slightly lower in RS-II compared to those in RS-III.

**Table 1.** Study population characteristics

|  | Total | RS-II | RS-III |
|---|---|---|---|
| N= | 3,795 | 1,326 | 2,469 |
| Age, mean (SD) | 65.9 (7.5) | 72.6 (5.2) | 62.3 (6.0) |
| Women, % (n) | 56% (2,125) | 54% (716) | 57% (1,407) |
| Chronic pain present, % (n) | 44% (1,670) | 47% (623) | 42% (1,037) |
| Heat pain threshold, mean (SD) in degrees Celsius | 47.5 (3.0) | 47.3 (3.2) | 47.7 (2.8) |

*RS-II = Rotterdam Study II; RS-III is Rotterdam Study III; SD = standard deviation; n = sample size.*

### Heritability estimation

In the complete population, the GCTA estimate of genetic influence due to the additive effect of common SNPs was 19% (SE 0.09; p-value=0.02). Since gender is one of the major factors determining HPT, we subsequently stratified the population according to gender. We oberved the heritability estimate in women to be 35% (SE 0.20; p-value 0.04), while it was 9% in men (SE 0.28; p-value 0.38). Chronic pain is known to influence HPTs significantly through central sensitization. Therefore, we studied the heritability of HPT separately in individuals with and without chronic pain. For individuals with chronic pain, the heritability was estimated to be 8% (SE 0.20; p-value=0.35). For individuals without chronic pain, this was higher and statistically significant with 32% (SE 0.17; p-value=0.03).

### GWAS meta-analysis

A total of 30,072,738 markers were tested for the association with HPT in our population of in total 3,795 individuals. Genomic control inflation factors for the p-values in RS-II and RS-III were low ($\lambda$=1.01 and 1.001 respectively). The Quantile-Quantile plot indicated no substantial population stratification due to cryptic relatedness, population substructure or other biases (Figure 1). The results of the GWAS meta-analysis are summarized in a Manhattan Plot of the p-values (Figure 2).
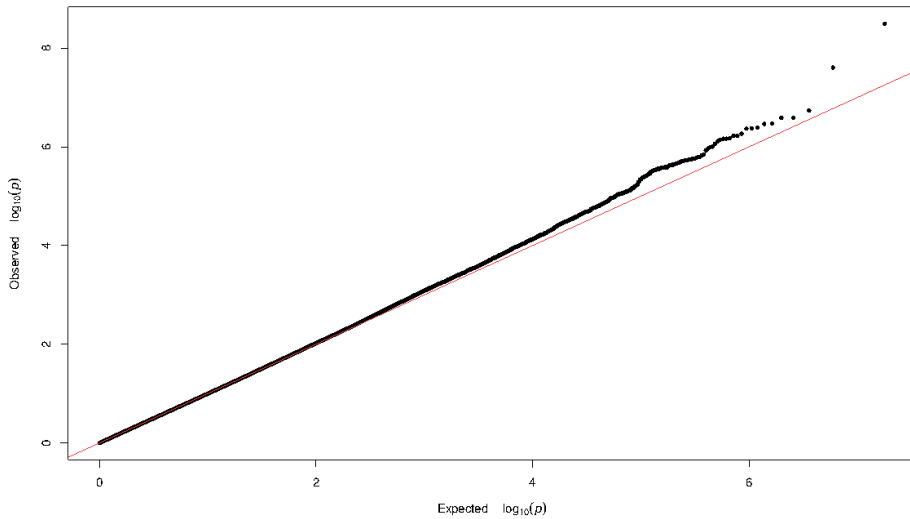
**Figure 1.** Quantile-quantile plot (Q-Q plot) for the GWAS meta-analysis with HPT.
This plot compares additive model statistics to those expected under the null distribution using fixed-effects for all analyzed 1000G imputed SNPs passing the quality control. Analysis adjusted for the presence of chronic pain.
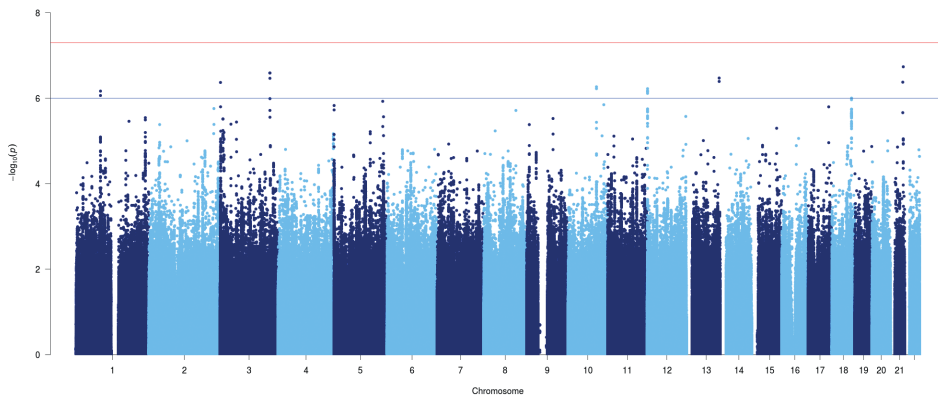


**Figure 2.** Manhattan plot of the P-values of the GWAS meta-analysis of HPT in RS-II and RS-III. Analysis adjusted for the presence of chronic pain. The red line represents the line for genome wide significance (p-value $5.0*10^{-8}$), the blue line represents the line for suggestive signals (p-value $1.0*10^{-6}$).

We identified one SNP on chromosome 1 to be genome-wide significant (p-value <$5.0* *10^{-8}$). This SNP represents a deletion located on position 176,688,345 on chromosome 1 (p-value=$2.48 *10^{-8}$). It is a relatively rare deletion (minor allele frequency = 0.02), and it is located in the twelfth intron of the *PAPPA2* gene. The *PAPPA2* gene encodes for a protein which is thought to be a local regulator of insulin-like growth factor (*IGF*) bioavailability. *IGF* is implicated in nociceptive (pain)

**Table 2.** Genome Wide Association Analysis 'Heat Pain Threshold' adjusted for presence of chronic pain, tophits signals p-value < $1.0*10^{-6}$

| Marker | Chr | Pos | Coded allele | Other allele | AF | Beta | StdErr | P-value (all partici-pants) | P-value (no pain cases) | Position | Gene |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1:176688345:D | 1 | 176688345 | D | I | 0.02 | −2.09 | 0.38 | $2.48*10^{-8}$ | $1.11*10^{-5}$ | intron 12 | PAPPA2 |
| rs13049646 | 21 | 43651846 | T | G | 0.98 | 1.52 | 0.29 | $1.84*10^{-7}$ | $1.24*10^{-5}$ | intron 2 | ABCG1 |
| rs187924640 | 3 | 169948817 | T | G | 0.02 | −1.57 | 0.31 | $2.56*10^{-7}$ | $8.23*10^{-7}$ | intron 1 | PRKCI |
| rs512766 | 10 | 97228338 | C | G | 0.10 | 0.60 | 0.12 | $5.41*10^{-7}$ | $2.96*10^{-4}$ | intron 1 | SORBS1 |
| rs74371079 | 12 | 1084044 | A | G | 0.06 | −0.81 | 0.16 | $5.98*10^{-7}$ | $2.00*10^{-4}$ | intron 1 | RAD52 |
| rs141493091 | 1 | 84375770 | A | C | 0.04 | −1.09 | 0.22 | $6.81*10^{-7}$ | $7.60*10^{-4}$ | intron 15 | TTLL7 |
| rs7239184 | 18 | 67129023 | T | G | 0.96 | 0.91 | 0.19 | $9.94*10^{-7}$ | $3.52*10^{-3}$ | intron 1 | DOK6 |

*Chr = chromosome; Pos = position; Coded allele = effect allele; AF = allele frequency of coded allele; Beta = effect size of effect allele; StdErr = standard error of the effect.*

sensitivity of primary afferent neurons [44]. Additionally, the deletion is located 140kb downstream of the *ASTN1* gene. *ASTN1* (or astrotactin 1) is a neuronal adhesion molecule required for migration of young postmitotic neuroblasts in cortical regions of developing brain, including cerebrum, hippocampus, cerebellum, and olfactory bulb [45].

Next to the genome-wide significant hit, we found six other suggestive signals with a p-value <$1.0*10^{-6}$ (Table 2). Five of six top SNPs have relative low allele frequencies (MAF<0.05).

We found one locus to be suggestive in both the analyses: in the original GWAS (adjusting for chronic pain), the intronic SNP rs187924640 (MAF=0.015) was a suggestive hit (p=$2.56*10^{-7}$), and in the sensitivity analysis (excluding chronic pain cases) this SNP was also close to significance (p-value=$8.23*10^{-7}$). The PRKC1 gene encodes for the protein kinase C iota type, which is implicated in the regulation of neuronal growth and specification [46-48].

**Systematic review of genetic variants previously described**

In the HuGe navigator, the search term 'Pain threshold' provided a total of 60 publications describing 44 different genes. After selection for pain threshold phenotypes and SNPs having an rs-id, we were left with fifteen publications. In these articles, nine SNPs in six different genes (*COMT*, *DRD3*, *OPRK*, *OPRM1*, *SLA6A4* and *HTR1A*) were previously reported to be significantly associated with pain threshold phenotypes. The selected SNPs, the direction of the effect in the previous articles and the results in our GWAS study are shown in Table 3. None of the nine SNPs were significantly associated with HPT in our GWAS meta-analysis results.

**Table 3.** Associations of HPT with the candidate gene SNPs.

| | Coded allele | Other allele | AF | Beta | P-value (all participants) | Effect direction in literature* | References |
|---|---|---|---|---|---|---|---|
| COMT | | | | | | | |
| rs4680 | A | G | 0.55 | −0.09 | 0.18 | −, −, −, −, − | [25-27,31,34] |
| DRD3 | | | | | | | |
| rs6280 | T | C | 0.69 | 0.02 | 0.77 | − | [35] |
| OPRK | | | | | | | |
| rs6473799 | A | G | 0.77 | 0.09 | 0.27 | − | [36] |
| rs7016778 | A | T | 0.88 | 0.15 | 0.17 | + | [36] |
| rs7824175 | C | G | 0.92 | −0.01 | 0.94 | − | [36] |
| rs9479757 | A | G | 0.10 | −0.05 | 0.69 | + | [30] |
| OPRM1 | | | | | | | |
| rs1799971 | A | G | 0.89 | 0.02 | 0.82 | +,+ | [24,28] |
| SLC6A4 | | | | | | | |
| rs25531 | T | C | 0.93 | 0.025 | 0.89 | +, + | [29,33] |
| HTR1A | | | | | | | |
| rs6295 | C | G | 0.50 | 0.04 | 0.63 | + | [32] |

*A negative direction (−) means a higher sensitivity for QST coinciding with a lower HPT; A positive direction (+) means a lower sensitivity for QST coinciding with a higher HPT.*
*Coded allele = effect allele; AF = allele frequency of coded allele; Beta = effect size of effect allele.*

## Discussion

In this population based study, we aimed to identify the genetic background of an experimental measure of pain sensitivity, the heat pain threshold (HPT). We observed an overall heritability estimate of 19% which was dependent on gender and the presence of chronic pain. We performed a genome wide association study (GWAS) to search for potential new loci and found seven interesting new loci. In a candidate SNP approach, we were not able to replicate the earlier associated SNPs with the HPT in our study.

Although not yet fully elucidated, a significant proportion of the variability of HPT is explained by genetics. The method we used to measure heritability is different from twin and pedigree analysis. Our method uses only common DNA variants in linkage with the genotyped SNPs (on the Illumina SNP arrays) to estimate heritability, while family-based studies use all genetic variants, including rare variants [40,49]. Since there are less SNPs included in our analysis, the heritability of the trait will be underestimated. The GCTA method has been applied to other complex traits like height, and in this study a heritability of 55% for height was observed [50]. This is much lower than the heritability estimates based on twin studies, in which

89-93% of the height variance can be explained by genetics [51]. Therefore, we expect the heritability estimate of the HPT to be higher than the 19% we identified. The advantage of GCTA is that this method is able to estimate the heritability in a large sample of unrelated individuals, which makes it more generalizable to a general population [52].

Interestingly, we found an evident difference in the heritability estimate of HPT between genders and between individuals with and without chronic pain. In women and in individuals without chronic pain, the phenotypic variance is explained genetically for one third. In men and in individuals with chronic pain, the heritability estimate was not significant. In our study sample, almost 20% of all men reached the maximum threshold of 50 degrees Celsius for the HPT. As a consequence, part of the variability of the HPT-measurement is lost, which results in lower power to measure heritability in this part of the population. Another explanation could be that the HPT in men is influenced by other, not yet identified, factors. Our results also showed that heritability of HPT is much higher in individuals without chronic pain compared to those that have pain. It is known that experimental pain sensitivity (like HPT) is influenced by the presence of chronic pain, caused by central sensitization of the nervous system [1]. We hypothesize that the presence of chronic pain overrides the subtle genetic effects observed in the general population. This may be one of the reasons why former studies were not able to find consistently influencing genes for pain sensitivity phenotypes. Therefore, the presence of chronic pain should be taken into account when performing genetic analysis on HPT and potentially for other pain sensitivity thresholds in future studies.

To the best of our knowledge, we here present results from the largest genetic study on experimental pain performed up to date. In the GWAS for the HPT adjusted for chronic pain, there was one deletion (1:176688345:D) on chromosome 1 which reached genome wide significance (p= $2.48*10^{-8}$). This deletion is located in the twelfth intron of the *PAPPA2* gene, of which the encoded protein is thought to be a local regulator of insulin-like growth factor (IGF) bioavailability. IGF is implicated to play a role in the nociceptive (pain) sensitivity of primary afferent neurons. Neurotrophy, neurogenesis and metabolic functions are shown to be influenced by *IGF* in the adult brain [53]. In vitro, upregulation of *IGF* showed a higher sensitivity of primary afferent neurons [54,55]. Additionally, the deletion is located 140kb downstream of the *ASTN1* gene. *ASTN1* (or astrotactin 1) is a neuronal adhesion molecule required for migration of young postmitotic neuroblasts in cortical regions of developing brain, including cerebrum, hippocampus, cerebellum, and olfactory bulb [45]. Trafficking of the *ASTN1* protein is regulated by the *ASTN2* gene [56]. Interestingly, an SNP within *ASTN2* (rs4836732) was found to be associated with the pain-related phenotype total hip replacement in women (p=6.11E-11) [57].

One suggestive hit, located within the PRKCI gene, was associated with HPT in both the overall analysis (including all participants) and the sensitivity analysis (without chronic pain cases). The PRKCI gene encodes the protein kinase C iota gene, which has been found to regulate neuronal growth in the hippocampus in embryonic rats, specification of neurons during development in cerebellar purkinje cells in zebrafish and inhibiton of spinal cord precursors, also in zebrafish [46-48]. These functional associations indicate that the PRKCI gene might in fact be influencing neuronal functioning.

Although very interesting, our GWAS findings need to be replicated in an independent cohort, before definite conclusions can be drawn. Since the power to detect SNPs associated with our phenotype was relatively low (n=3,795), there might be some false positive hits. Additionally, there might be interesting signals among the suggestive SNPs and replication should demonstrate the true signals. After replication of our findings, functional testing of candidate genes would help to give more insight into the biology of HPT.

In the study of candidate genes previously reported to be associated with pain sensitivity measurements, we showed that none of the SNPs was significantly associated with HPT in our GWAS meta-analysis, although our sample size was at least 10 times larger. This can be explained by the fact that many of the previous reported loci were investigated in small populations of pain patients. This could indicate that the associations found are more associated to the pain syndrome than the pain sensitivity itself. The lack of reproducibility of SNPs in candidate genes in large GWAS meta-analyses has been shown before for other phenotypes such as BMD [58].

In conclusion, our study reports a heritability estimate for HPT of 19%. We identified significant influences of gender and chronic pain on the heritability estimates of HPT. Therefore, future genetic studies on pain sensitivity should be adjusted for gender and the presence of chronic pain, or individuals with chronic pain should be excluded from the analysis. This will result in a more homogenous pain phenotype and this will increase the chances of finding new genetic loci involved. The exact genes influencing HPT remain not fully elucidated, but this study provides new potential genes for further research.

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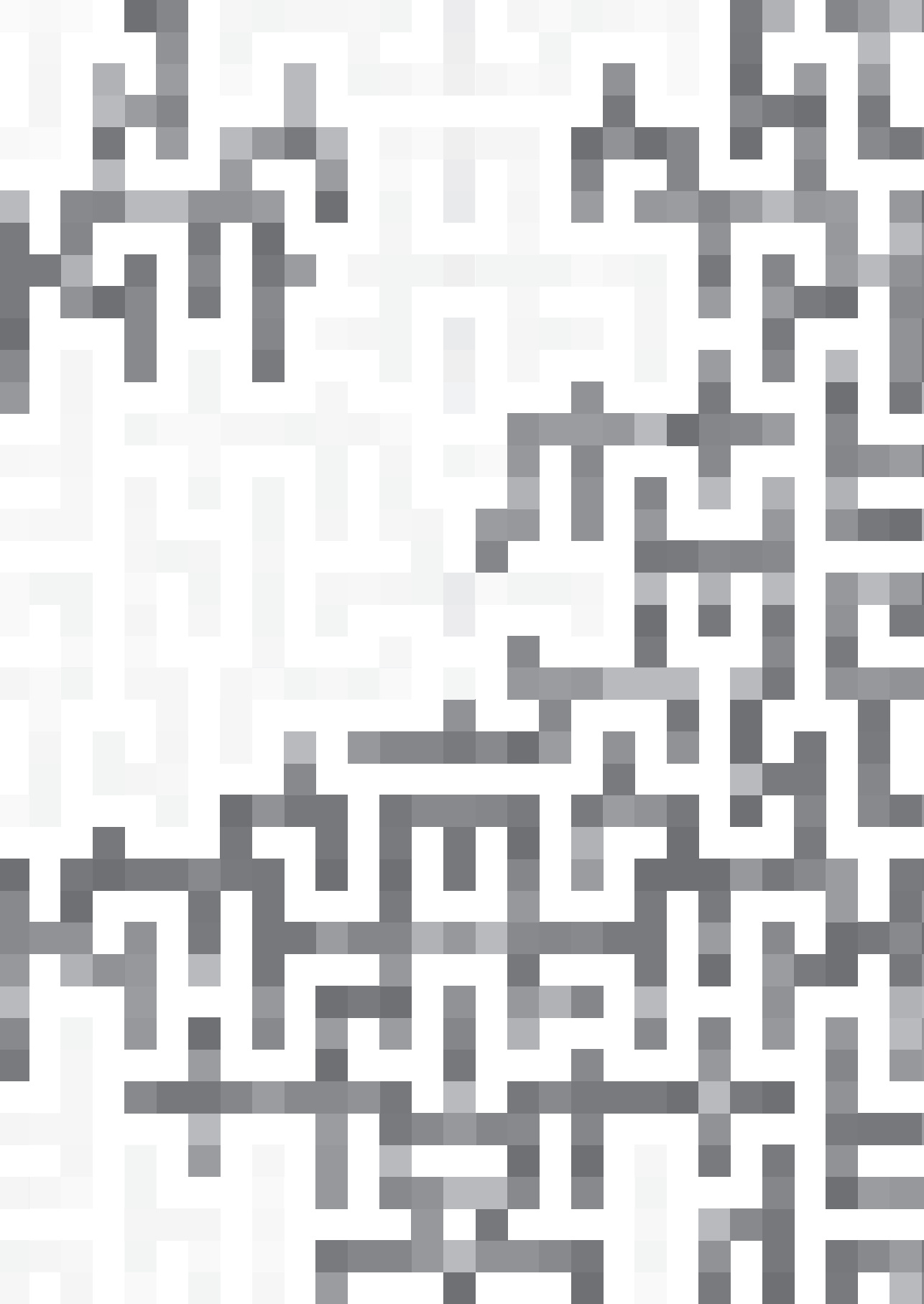
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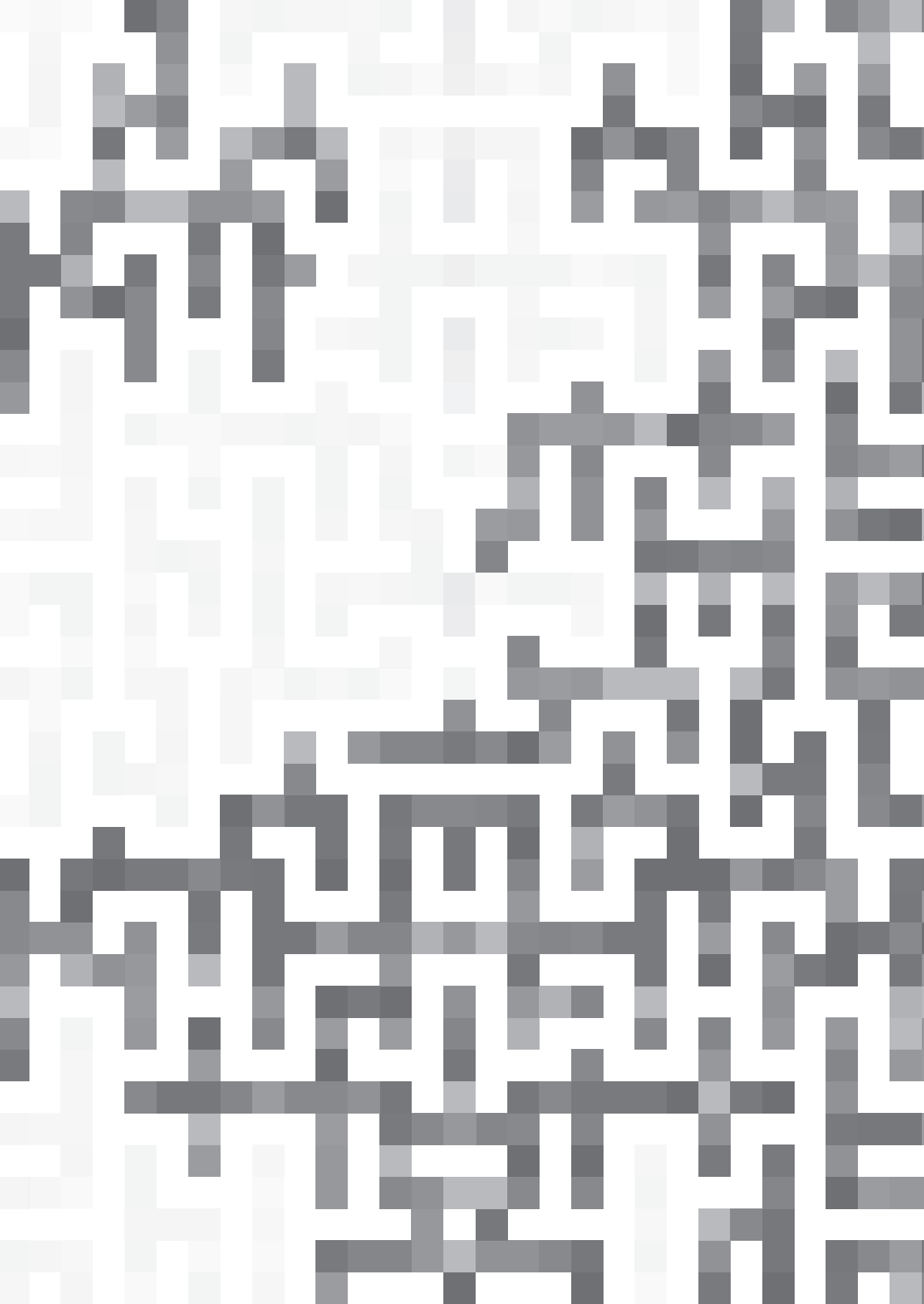
# CHAPTER 3

Hormonal influences
on chronic pain

# CHAPTER 3.1

Finger length pattern as a biomarker for osteoarthritis and chronic joint pain: A population-based study and meta-analysis after systematic review

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## Abstract

*Objective:* Type 3 finger length pattern (longer fourth digit than second digit) is influenced by prenatal androgens and has been studied previously as a biomarker for sexually dimorphic traits. Because osteoarthritis (OA) and chronic pain are known to be sexually dimorphic traits, we evaluated the association between finger length pattern and OA and chronic joint pain.

*Methods:* This study was part of the Rotterdam Study, a prospective population-based cohort study. We examined 4,784 participants. Associations between type 3 finger length and radiologic knee, hip, and hand OA and chronic joint pain were analyzed using a logistic regression model. Our results for OA were combined with previously published data in a meta-analysis.

*Results:* Participants with type 3 finger length pattern had an odds ratio of 1.64 for hand OA (P = $1.06*10^{-7}$). No associations with radiologic knee or hip OA were observed in the Rotterdam Study. The meta-analysis of previously published data and our novel data showed a significant association between type 3 finger length pattern and clinical symptomatic knee OA, but no association was found with radiologic knee OA. In addition, within the Rotterdam Study, we observed an odds ratio of 1.41 for individuals having joint pain at multiple sites (P=$1.4*10^{-3}$).

*Conclusion:* Type 3 finger length pattern, as an indicator of prenatal androgen ex-posure, was associated with having symptomatic knee OA, chronic pain, and hand OA. Therefore, it may be applicable as an easy measurable biomarker to identify susceptible subjects for these traits.

## Introduction

Finger length pattern, or digit ratio, is the ratio between the second digit (2D; index finger) and fourth digit (4D; ring finger) and is thought to be influenced by the balance between androgens and estrogens in a narrow window during embryogenesis in the second trimester. During this period, sex hormones play an important role in the development of several organ systems, such as the reproductive system and the brain. Relatively high androgen levels during this period are associated with a longer 4D compared with the 2D, resulting in a lower ratio of 2D to 4D length (2D:4D) [1]. This association has been shown in an experimental murine study in which elevation of androgen levels during development resulted in a decreased 2D:4D [2]. This result indicates that a lower 2D:4D is a crude measure for relatively high levels of prenatal androgen exposure.

To determine finger length pattern, 2 distinct methods have been described, both using hand radiographs [3]. The first method is a simple and fast visual classification method in which the soft tissue outline of the 2D and 4D is classified into 3 groups: the ring finger is shorter than (type 1), equal to (type 2), or longer than (type 3) the index finger. The second method measures the length of the 2D and 4D and computes a ratio (2D:4D). A 2D:4D < 1 represents the same phenotype as a type 3 finger length pattern, a 2D:4D = 1 is similar to type 2, and a 2D:4D > 1 corresponds to type 1.

Finger length pattern has been studied in relation to a number of diseases and physiologic and psychological traits, and the majority of these traits are associated with sex or sex hormones. Associations have been described with coronary heart disease [4], autism [5], homosexuality [6], fertility [7], and age at menarche [8,9]. In these associations, type 3 finger length pattern (relatively higher androgen levels during development) has been described as a biomarker for increased risk of developing several different sex-related traits. This makes finger length pattern an interesting phenotype to study in relation to other sex- or sex hormone–related traits, such as osteoarthritis (OA) and chronic joint pain.

OA is known to be a sex-related phenotype, with a higher incidence in women, specifically after menopause, indicating a sex hormone component in the etiology [10]. Four previous studies have examined the association between finger length type and OA risk, but these studies had inconsistent findings. The first study, using a case control design, showed that type 3 finger length pattern was positively associated with having symptomatic knee or hip OA [11]. This finding was confirmed in a nested case control study by Ferraro et al. [12] for knee OA; however, Haugen et al. [13] failed to replicate this association with radiographic knee OA in a population-based cohort. The final study showed a positive association between

3.1

type 3 finger length pattern and total knee replacement in the general population [14]. The inconsistencies between these studies could be due to a difference in the definition of OA. The studies that showed an association compared clinical symptomatic OA cases to pain-free controls. The population-based cohort study used radiologic OA in the knee as the definition and did not find an association. Therefore, we hypothesized that the previously found associations might have been driven by pain rather than joint damage.

Like finger length pattern, embryogenic brain development is influenced by prenatal androgen exposure, specifically in the development of sex differences in the brain [15]. Chronic pain is also sex associated; therefore, prenatal androgen exposure, as measured by finger length pattern, might be associated with the risk for chronic pain by this shared association with sex and thus androgens.

In this study, we aimed to further elucidate the association of type 3 finger length pattern with OA of the knee, hip, or hand. We systematically reviewed the literature for previous studies examining the association between finger length pattern and OA and meta-analyzed the results, including our own novel data. Subsequently, we aimed to evaluate the association between type 3 finger length pattern and chronic joint pain.

## Patients and methods

### Study population

The Rotterdam Study is a large prospective population-based cohort study of men and women aged ≥ 45 years. The study design and rationale have been described elsewhere in detail [16]. In summary, the objective of the Rotterdam Study was to investigate the determinants, incidence, and progression of chronic disabling diseases in the elderly. The first cohort, Rotterdam Study I, consisted of 7,983 people aged ≥ 55 years in 1989. This study population was extended in 1999, adding 3,011 participants in Rotterdam Study II (RS-II) and adding 3,932 subjects ≥ 45 years and older in Rotterdam Study III (RS- III) in 2006. All participants were examined at baseline in detail. A home interview was conducted and subjects had an extensive set of examinations at the research center, including radiographs taken from the hip, knee, and hand. The Medical and Ethical Review Committee of Erasmus University Medical School approved the study and written informed consent was obtained from each participant. The data used in this study are the baseline data of a subset of the participants in RS-II and RS-III. In these subjects, radiographs of the hands were available and finger length pattern was determined.

### Finger length pattern

The radiograph of the right hand at the baseline visit was used to determine finger length pattern. Finger length pattern was visually scored as type 1 (ring finger shorter than index finger), type 2 (ring finger same as index finger), or type 3 (ring finger longer than index finger). Interreader correlation was high ($\kappa = 0.85$).

### Definition of outcome measures

Knee and hip OA: OA of the hip and the knee was scored by the Kellgren/Lawrence (K/L) grading system. The definition of having knee or hip OA was a K/L score of $\geq$ 2, as described previously [17]. Total joint replacements (TJRs) due to primary OA visible on radiographs were considered as OA. TJRs due to fractures and other diseases were excluded.

   Hand OA: Radiographs of the hand were scored for radiographic features of OA at the following joint levels: distal interphalangeal (DIP) joints, the interphalangeal joint of the thumb, proximal interphalangeal (PIP) joints, metacarpophalangeal joints, first carpometacarpal joint (CMC1), and trapezioscaphoid (TS) joint. An overall grade for OA was scored by the K/L grading system. Hand OA was defined as having a K/L score $\geq$ 2 in 2 of 3 joint groups (DIP, PIP, and CMC1/TS), as previously described [17].

   Chronic joint pain: All participants completed a pain homunculus to report the chronic painful sites in the body. The pain homunculus showed a picture of the front and the back of the human body. Participants were asked the following question: "Did you have pain anywhere in your body, for at least half of the days, during the last 6 weeks?" Circles were drawn around the painful areas by the participant. The homunculi were scored using a template assigning 14 different joint pain regions (e.g., neck, shoulders, elbows, hands, low back, hips, knees, and feet). Chronic joint pain was defined as subjects having $\geq$ 1 painful sites. Subjects having > 2 painful joints were identified as a more severe phenotype.

   Symptomatic OA: Symptomatic OA was defined as having both radiologic evidence of OA and chronic pain in the same joint. Subjects with asymptomatic OA showed radiologic evidence for OA in the knee but did not experience chronic pain in the affected joint. The data from the pain homunculus, as described above, were used to assess pain in the affected joint. In the analysis, symptomatic OA was compared with asymptomatic OA.

### Statistical analysis

Binary logistic regression was used to test the association between type 3 finger length pattern and the different outcome measures. All analyses were adjusted for

age, sex, and body mass index (BMI). SPSS, version 17.0, was used for the association analysis. A P value less than 0.05 was considered statistically significant.

Association analysis of OA: For radiographic knee OA, hip OA, hand OA, and symptomatic OA, odds ratios (ORs) were assessed. The subjects derived from the RS-II and RS- III were combined for these analyses. Separate analyses of the 2 subcohorts were also performed to detect possible cohort effects.

Association analysis of chronic joint pain: The association between type 3 finger length pattern and joint pain at any site was evaluated in the same manner, by binary logistic regression. Subsequently, we studied the association with chronic joint pain at > 2 sites of the body compared to no joint pain. Analyses were performed for the 2 different cohorts of the Rotterdam Study separately to detect possible cohort effects and combined to increase the power.

Systematic review of the literature: We identified and included all articles describing the association of finger length pattern and OA by a systematic search using the PubMed database on January 31, 2013, with the keywords ("Osteoarthritis" or "Arthrosis" or "OA") and ("Finger length pattern" or "digit ratio"). Subsequently, we extended the search by screening the references of the studies included.

Meta-analysis: The results retrieved from previous published studies and our novel data from the Rotterdam Study were used for the meta-analysis. We used Comprehensive Meta-Analysis software, version 2. If heterogeneity existed ($I^2 > 25\%$), a random-effects model (Dersimonian-Laird) was used instead of a fixed-effects model (inverse-variance method) for the analysis.

## Results

### Baseline characteristics

Finger length pattern was determined in 2,205 participants in the RS-II and 2,579 participants in the RS-III. The baseline characteristics are shown in Table 1. In concordance with previous studies [2,3,18 –20], type 3 finger length pattern was more prevalent in men than women (35% versus 18%; P < 0.001). Radiographic OA in the knee, hip, or hand was more frequently present in women compared with men (36% versus 25%; P < 0.001). This distribution was similar for symptomatic OA (35% in women versus 27% in men; P= 0.002). In addition, a higher incidence of chronic joint pain was observed in women than in men (50% versus 37%; P < 0.001).

### Association analysis of OA in the Rotterdam Study

Table 2 shows the results of the association analysis of type 3 finger length pattern and the different types of radiographic OA in the Rotterdam Study. We did not find

**Table 1.** Characteristics of the study participants*

| | RS-II | | | RS-III | | |
|---|---|---|---|---|---|---|
| | Total (n=2205) | Men (n=985) | Women (n=1220) | Total (n=2579) | Men (n=1100) | Women (n=1483) |
| Age, mean ± SD years | 64.5 ± 7.7 | 64.1±7.3 | 64.8 ± 7.9 | 56.7 ± 6.9 | 56.6 ± 6.6 | 56.8 ± 7.0 |
| Body Mass Index, mean ± SD kg/m2 | 27.2 ± 4.2 | 27.0 ± 3.9 | 27.4 ± 4.4 | 27.9 ± 4.8 | 28.1 ± 4.2 | 27.7 ± 5.2 |
| Finger length pattern: | | | | | | |
| Type 1 | 353 (16) | 100 (10) | 253 (21) | 467 (18) | 119 (11) | 348 (24) |
| Type 2 | 1,221 (55) | 515 (52) | 706 (58) | 1,514 (59) | 612 (56) | 902 (61) |
| Type 3 | 631 (29) | 370 (38) | 261 (21) | 602 (23) | 369 (34) | 233 (16) |
| Radiographic osteoarthritis: | | | | | | |
| Knee | 384/2,102 (18) | 137/940 (15) | 247/1,162 (21) | 270/2,562 (11) | 106/1,096 (10) | 164/1,466 (11) |
| Hip | 161/2,109 (8) | 64/946 (7) | 97/1,163 (8) | 71/2,459 (3) | 26/1,047 (3) | 45/1,412 (3) |
| Hand | 692/2,149 (32) | 194/964 (20) | 498/1,185 (42) | 275/2,341 (12) | 71/996 (7) | 204/1,345 (15) |
| Symptomatic osteoarthritis: | | | | | | |
| Knee | 123/323 (38) | 37/115 (32) | 86/208 (41) | 92/268 (34) | 30/106 (28) | 62/162 (38) |
| Hip | 73/135 (54) | 22/61 (36) | 51/74 (69) | 14/70 (20) | 5/26 (19) | 9/44 (21) |
| Hand | 139/570 (24) | 24/152 (16) | 115/418 (28) | 57/275 (11) | 9/71 (13) | 48/204 (24) |
| Chronic joint pain | 864/1,835 (47) | 316/798 (40) | 548/1,037 (53) | 1,110/2,566 (43) | 393/1,095 (36) | 717/1,471 (49) |
| Chronic joint pain > 2 sites | 318/1835 (17) | 91/798 (11) | 227/1037 (22) | 342/2566 (13) | 84/1095 (8) | 258/1471 (18) |

*Values the number of cases/total number (%), unless stated otherwise. RS-II = Rotterdam Study II; RS-III = Rotterdam Study III

an association between type 3 finger length pattern and knee or hip OA. However, we observed that participants with type 3 finger length pattern had an OR of 1.64 for hand OA (P= 1.061*10$^{-7}$). The association was of similar magnitude in both sexes (OR 1.61, P= 9.3*10$^{-5}$ for men versus OR 1.66, P= 3.4*10$^{-4}$ for women).

Severe OA could lead to shortening of the digits and an overestimation of the presence of type 3 finger length pattern. As a sensitivity analysis, we therefore excluded participants with severe OA of the 2D. Severe OA was defined as a K/L score of ≥ 3 and comprising joint space narrowing. The association between finger length pattern and hand OA was attenuated to an OR of 1.43. Nevertheless, the association remained highly significant (P= 3.26*10$^{-4}$).

Additionally, a sensitivity analysis was performed to study the association of type 3 finger length pattern and the radiographic severity of knee OA, as described by the K/L score. No significant association was found (OR 1.05, P= 0.474).

**Table 2.** Association analysis finger length pattern and OA*

| | No. of cases/no. total (%) | | | |
| --- | --- | --- | --- | --- |
| | Type 1/2 | Type 3 | OR (95% CI) | P |
| Combined RS-II and RS-III | | | | |
|    Knee OA | 465/3459 (13) | 189/1205 (16) | 1.01 (0.83-1.24) | 0.901 |
|    Hip OA | 152/3288 (4) | 80/1180 (7) | 1.25 (0.92-1.69) | 0.149 |
|    Hand OA | 643/3322 (19) | 324/1167 (28) | 1.64 (1.36-1.96) | < 0.0001† |
| Stratified analysis | | | | |
|    RS-II | | | | |
|       Knee OA | 258/1494 (17) | 126/608 (21) | 1.08 (0.83-1.40) | 0.584 |
|       Hip OA | 101/1498 (7) | 60/611 (10) | 1.21 (0.84-1.73) | 0.300 |
|       Hand OA | 455/1526 (30) | 237/623 (38) | 1.52 (1.22-1.91) | < 0.001† |
|    RS-III | | | | |
|       Knee OA | 207/1965 (11) | 63/597 (11) | 0.91 (0.66-1.25) | 0.559 |
|       Hip OA | 51/1890 (3) | 20/569 (4) | 1.34 (0.77-2.33) | 0.309 |
|       Hand OA | 188/1797 (11) | 87/544 (16) | 1.99 (1.45-2.72) | < 0.0001† |

*All analyses are adjusted for age, gender and body mass index. OA = osteoarthritis; OR = odds ratio; 95% CI = 95% confidence interval; RS-II = Rotterdam Study II; RS-III = Rotterdam Study III.
† Significant

### Systematic review of the literature

The literature search in PubMed and reference screening resulted in 4 studies that examined the association between type 3 finger length pattern and OA. We contacted Haugen et al [13] to calculate the OR for the association between the visually classified type 3 finger length pattern, knee OA, and hand OA, because these data were not shown in their study. The characteristics of the included studies are shown in Table 3.

### Meta-analysis

In the previous studies, the association with knee OA especially had conflicting results. Because the phenotype definition was also comparable between the studies, knee OA was used in the meta-analysis. In Figure 1, the results of the meta-analysis of the association between visually classified type 3 finger length pattern and the risk of having knee OA are shown. In the fixed-effects model, large heterogeneity was found between the studies ($I^2$ = 77%). Therefore, a random-effects model was used that showed an overall borderline significant association between type 3 finger length pattern and knee OA (P= 0.035). We subsequently stratified the meta-analyses based on the definition of cases and controls because the phenotype definition was very diverse across the studies, and this could have been the source of the observed heterogeneity.

**Table 3.** Included studies in the meta-analysis for the association of type 3 finger length pattern and knee osteoarthritis*

| Author, year [ref] | Study type | Method for determining Finger length pattern | OA case definition | Control definition | Joints | No. of cases | No. of controls | Result for type 3 finger length pattern† |
|---|---|---|---|---|---|---|---|---|
| Zhang et al. 2008 [11] | cases-control | Visual and ratio | Clinically symptomatic OA | No radiological hip OA and no hip or knee pain | Knee<br>Hip | 1,013<br>995 | 836<br>1,050 | 1.94 (1.54-2.44)<br>1.37 (1.13-1.67) |
| Ferraro et al. 2010 [12] | nested case-control | Visual and ratio | Radiological OA | No radiological hip/knee/hand/foot or spine OA, no joint pain anywhere | Knee | 236 | 242 | 2.59 (1.54-4.37) |
| Sigurjonsdottir et al. 2013 [14] | Population based cohort | Visual on photographs | OA related TKR or THR; Severe handOA on photograph | No TKR or THR; No severe handOA on photograph | Knee<br>Hip<br>Hand | 223<br>316<br>673 | 4,947<br>4,854<br>4,497 | 1.65 (1.24-2.20)<br>1.14 (0.90-1.44)<br>1.19 (0.99-1.41) |
| Haugen et al. 2011 [13] | Population based cohort | Visual and ratio | Radiological OA | No radiological OA | Knee<br>Hand | 224<br>666 | 782<br>354 | 0.93 (0.61-1.43)<br>1.28 (0.86-1.90) |
| De Kruijf et al. (present study) | Population based cohort | Visual | Radiological OA | No radiological OA | Knee<br>Hip<br>Hand | 654<br>232<br>967 | 4,010<br>4,336<br>3,523 | 1.01 (0.83-1.24)<br>1.25 (0.92-1.69)<br>1.64 (1.36-1.96) |

*OA = Osteoarthritis; TKR = Total Knee Replacement; THR = Total Hip replacement.
†Values are the odds ratio (95% confidence interval).

3.1

| Study name | Statistics for each study | | | | Odds ratio and 95%CI |
|---|---|---|---|---|---|
| | Odds ratio | Lower limit | Upper limit | p-Value | |
| Zhang et al. 2008 | 1.94 | 1.25 | 3.01 | 0.003 | |
| Ferraro et al. 2010 | 2.59 | 1.54 | 4.36 | < 0.001 | |
| Haugen et al. 2011 | 0.93 | 0.61 | 1.42 | 0.736 | |
| Sigurjonsdottir et al. 2013 | 1.65 | 1.24 | 2.20 | 0.001 | |
| de Kruijf et al. (this study) | 1.01 | 0.83 | 1.23 | 0.923 | |
| Overall effect | 1.46 | 1.03 | 2.08 | 0.035 | |
| (Random effects model, I²=81%) | | | | | |
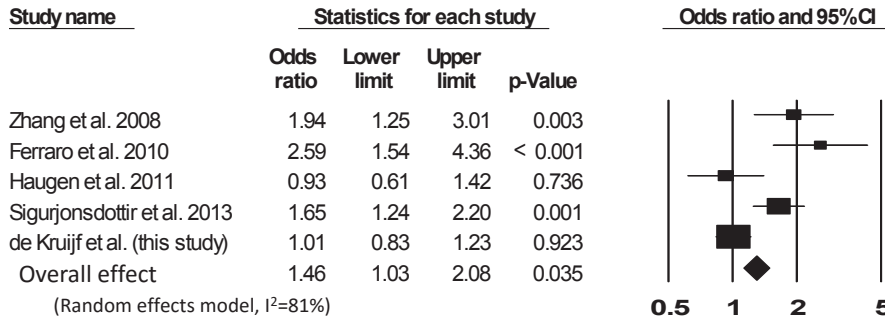
**Figure 1.** Forest plot meta-analysis of the association between type 3 finger length pattern and knee osteoarthritis (Included both clinical and radiological knee osteoarthritis). 95% CI = 95% confidence interval.

The first analysis included the studies with a clinical, symptomatic definition for the cases and/or controls. In addition, we included data from the Rotterdam Study examining symptomatic knee OA cases versus asymptomatic cases. This meta-analysis showed a low heterogeneity ($I^2$ = 43%) and a significant association with type 3 finger length pattern (OR 1.70, P= $7.0*10^{-8}$) (Figure 2A).

The second meta-analysis performed included the studies examining the association of type 3 finger length pattern with radiologic OA in the general population. This meta-analysis showed no evidence of an association between type 3 finger length pattern and radiologic OA (OR 0.99, P= 0.957) (Figure 2B).

Additionally, we performed meta-analyses on hip OA (summary effect OR 1.27, P= 0.001 in the fixed-effects model) and hand OA (summary effect OR 1.37, P= 0.009 in the random-effects model [$I^2$ = 68%]). However, there were only 3 studies (including our own) available for this analysis and the phenotype definition was diverse. Specifically, the 2 previous studies for hip OA had a clinical OA phenotype (including pain in their definition), while our study was based on a radiographic OA definition.

| Study name | Statistics for each study | | | | Odds ratio and 95%CI |
|---|---|---|---|---|---|
| | Odds ratio | Lower limit | Upper limit | p-Value | |
| Zhang et al. 2008 | 1.94 | 1.25 | 3.01 | 0.003 | |
| Ferraro et al. 2010 | 2.59 | 1.54 | 4.36 | < 0.001 | |
| Sigurjonsdottir et a;. 2013 | 1.65 | 1.24 | 2.20 | 0.001 | |
| de Kruijf et al. (this study) | 1.22 | 0.80 | 1.86 | 0.356 | |
| Overall effect | 1.70 | 1.40 | 2.06 | < 0.001 | |
| (Fixed effects model, I²=43%) | | | | | |

**Figure 2A.** Forest plot meta-analysis of the association between type 3 finger length pattern and symptomatic knee osteoarthritis (Included only clinical or symptomatic knee osteoarthritis). 95% CI = 95% confidence interval.
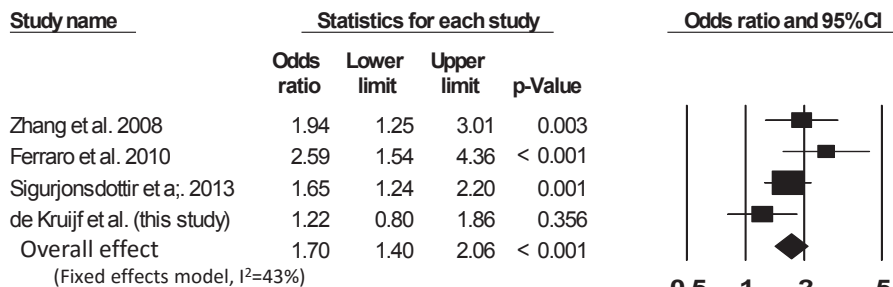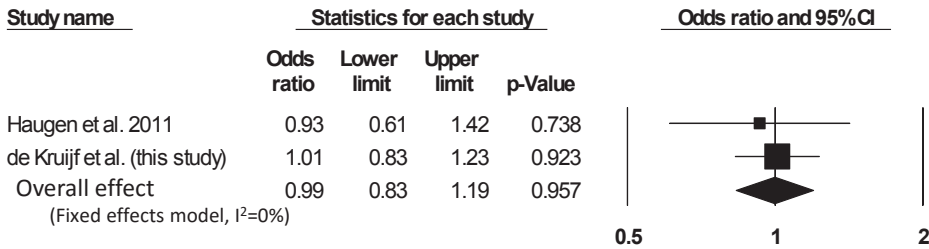
**Figure 2B.** Forest plot meta-analysis of the association between type 3 finger length pattern and radiological knee osteoarthritis (Included only radiological knee osteoarthritis). 95% CI = 95% confidence interval.

## Association analysis of symptomatic OA and joint pain in the Rotterdam Study

First, we analyzed the association between type 3 finger length pattern and symptomatic versus asymptomatic OA, but found no significant association for symptomatic knee OA (OR 1.22, P= 0.352), symptomatic hip OA (OR 0.74, P= 0.410), or symptomatic hand OA (OR 1.35, P= 0.093). Next, we investigated the association between chronic joint pain, independent from the underlying cause, and type 3 finger length pattern; the results are shown in Table 4. Type 3 finger length pattern was associated with joint pain (OR 1.18, P= 0.022). In the subsequent analysis for more severe pain cases (joint pain at > 2 sites), this effect became stronger, with an OR of 1.41 (P= $1.4*10^{-3}$).

**Table 4.** Association analysis finger length pattern and chronic joint pain*

| | No. of cases/total no. (%) | | OR (95% CI) | P |
|---|---|---|---|---|
| | Type 1/2 | Type 3 | | |
| Combined RS-II and RS-III | | | | |
|    Chronic joint pain | 1,463/3,297 (44) | 511/1,104 (46) | 1.18 (1.02-1.37) | 0.022 |
|    >2 different sites | 478/2,318 (21) | 182/777 (23) | 1.41 (1.14-1.74) | < 0.001† |
| Stratified analysis | | | | |
|   RS-II | | | | |
|     Chronic joint pain | 616/1,331 (46) | 248/504 (49) | 1.27 (1.02-1.58) | 0.032† |
|     >2 different sites | 227/944 (24) | 91/348 (26) | 1.37 (1.01-1.86) | 0.043† |
|   RS-III | | | | |
|     Chronic joint pain | 847/1,966 (43) | 263/600 (44) | 1.12 (0.92-1.36) | 0.247 |
|     >2 different sites | 251/1,374 (18) | 91/429 (21) | 1.47 (1.10-1.98) | 0.010† |

*All analyses are adjusted for age, sex and body mass index. OR = odds ratio; 95% CI = 95% confidence interval; RS-II = Rotterdam Study II; RS-III = Rotterdam Study III.
† Significant

As described previously, the prevalence of type 3 finger length pattern can be overestimated by severe hand OA in the 2D, leading to misclassification. In a sensitivity analysis, we excluded subjects with severe OA in the 2D because severe hand OA might be correlated with OA and pain at other sites of the body. The analysis showed similar ORs for joint pain anywhere in the body (OR 1.18, P= 0.033) and for joint pain at > 2 different sites of the body (OR 1.40, P= $3.63*10^{-3}$).

## Discussion

In this large population-based cohort study, we found no evidence of an association between type 3 finger length pattern and radiographic knee or hip OA. A meta-analysis of these results with previous publications showed high heterogeneity, possibly caused by a mixture of radiographic and symptomatic definitions of cases and controls. When the meta-analysis was restricted to the symptomatic definition for knee OA, a highly significant association was found. For hip OA, the meta-analysis was also significant, but because of the limited data, especially for radiographic hip OA, we were not able to investigate the role of the difference between radiographic and symptomatic OA. In addition, we observed in the Rotterdam Study that a longer ring finger compared to the index finger was associated with having chronic joint pain. All of these associations were independent of well-known risk factors of OA and pain, such as age, sex, and BMI.

Previously, 3 case-control studies [11,12,14] focused mainly on OA of the knee. The definition of the OA cases was heterogeneous, ranging from radiographic OA to severe clinically relevant OA or OA-related total knee replacement. More importantly, the controls were allowed to have neither radiographic OA nor joint pain at several sites of the body. In these settings, an association was found between type 3 finger length pattern and knee OA. The study by Haugen et al. was a population-based study that used a radiographic knee OA definition, while the controls were subjects who did not have radiographic knee OA. This was similar to our case definition, and the 2 population-based cohorts did not replicate the association between knee OA and type 3 finger length pattern [13]. This suggests that it was not the OA in these studies but the chronic pain that might explain the association found. Indeed, we found a consistent positive association of type 3 finger length pattern and chronic joint pain in the Rotterdam Study. The association became even stronger when we selected a more severe phenotype (chronic joint pain in > 2 sites), suggesting a robust association. These findings were not influenced by the presence of severe hand OA, which could have been a confounder in this association.

Keogh et al. previously examined the relationship between finger length pattern and pain by studying experimental pain in 50 healthy adults [21]. The authors observed that women with type 3 finger length ratio were more sensitive to experimental pain. Although these results need further replication, these findings are consistent with our results.

A possible mechanism behind this association is the influence of embryogenic sex hormone exposure on brain development [15,22]. We speculate that higher androgen levels might influence the regulation and coping of pain stimuli by the brain, thereby increasing the susceptibility for developing chronic joint pain. A supposed mechanism for this phenomenon can be the effect of androgens on the setting of the hypothalamic–pituitary–adrenal stress axis [23].

We observed a highly significant association between type 3 finger length pattern and OA of the hand. Additionally, the meta-analysis of the results from hand OA combined with the previous studies showed similar results. OA of the fingers can lead to shortening because of narrowing of the joint space due to cartilage breakdown, and can therefore confound the finger length pattern determination and lead to misclassification. We therefore performed a sensitivity analysis excluding all subjects with joint space narrowing in the 2D. This attenuated the association toward a smaller effect size (OR 1.43 for type 3 finger pattern), but the association remained highly significant. This association has been previously described by Haugen et al. [13]. When the authors excluded subjects with joint space narrowing in the index finger, the effect was attenuated to a lower effect size, but still had an OR of 1.35, similar to our results. This might suggest that a consistent association with hand OA was apparent in both of the population studies, even after adjustment for severe hand OA. However, we cannot completely exclude some residual confounding.

Androgen levels are known to be involved in the development of the skeleton, which could explain the relationship with OA. For example, androgens have been shown to influence HOX gene expression during development of chondrocytes in the fingers [24]. This might not only result in differences in the development of finger length, but also a cartilage more susceptible to OA later in life.

In summary, we observed a consistent association between type 3 finger length pattern and radiographic hand OA, but found no evidence for an association with radiographic knee or hip OA. However, a meta-analysis suggested that symptomatic knee OA is significantly associated with type 3 finger length pattern. In addition, we found an association between type 3 finger length pattern and chronic joint pain. Future prospective studies are needed to explore the possibilities of type 3 finger length pattern as a noninvasive biomarker for chronic joint pain.

3.1

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3.1

# CHAPTER 3.2

Lower sex hormone levels are associated with more chronic musculoskeletal pain in community dwelling elderly women

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## Abstract

*Background:* Chronic pain is more prevalent in women than men, with increasing differences between sexes in advanced age. This could be caused by differences in sex hormone levels. We therefore studied the relation between sex hormones and the prevalence and incidence of chronic pain.

*Methods:* The association between sex hormone levels and chronic pain was examined in 9,717 participants aged 45 years and older from the Rotterdam Study, a population-based study. Chronic pain was defined as pain in the lower back, hands, knees and/or hips for at least three months. Sex hormone levels included estrogen, testosterone, androstenedione and 17-hydroxyprogesterone. Relations between hormones and prevalent and new onset chronic pain were analyzed using linear and logistic regression, stratified by gender.

*Results:* Women with androstenedione or estradiol levels in the lowest tertile had more chronic pain (odds ratio 1.20; 95% CI 1.03-1.39 and odds ratio 1.27; 95% CI 1.10-1.48 respectively). Mean estradiol levels were lower among men with chronic pain (mean difference −3.88 pmol/L; P= 0.005). Lowest tertile 17-hydroxyprogesterone in women was associated with 38% more new onset pain. All these associations were independent from age, BMI, health and lifestyle factors and osteoarthritis.

*Conclusions:* Lower sex hormones levels are associated with chronic musculoskeletal pain, independent from lifestyle and health related factors, in community dwelling elderly women. These results suggest that sex hormones play a role in chronic pain and should be taken into account when a patient presents with chronic pain. Therefore, sex hormones may be a potential treatment target for these patients.

## Introduction

Chronic pain is sexually dimorphic, with women having a higher prevalence of chronic pain conditions, such as fibromyalgia and chronic widespread pain, when compared to men. [2, 17, 26]

The differences between women and men with respect to their risk for chronic pain are multifaceted and complex. Previous studies have suggested that sex hormones play a role in the development of chronic pain and also in the sensitivity to painful stimuli. [6, 12, 22] Especially in women, the changes in sex hormones over time seem to influence their sensitivity for pain. For example, chronic pain disorders such as musculoskeletal pain and fibromyalgia are increasing in prevalence after menopause and differences in pain sensitivity have been observed during the menstrual cycle.[1, 3, 20, 21]

Estrogen and testosterone are the two most frequently studied sex hormones in relation to pain. Testosterone is thought to be important during the development of the nervous system and is hypothesized to influence pain sensitivity later in life. In rats, higher testosterone levels are shown to increase pain sensitivity later in life. [13]

Estrogen is thought to influence pain sensitivity and incidence of pain later in life. More or longer estrogen exposure during life has been found to be associated with a higher rate of migraine later in life [4], whereas lower current estrogen levels, like in postmenopausal women, has been linked to chronic musculoskeletal pain [7, 19]. However, no large studies have investigated the relationship between hormone levels in women and the occurrence of chronic pain in the general population. In men, testosterone is suggested to have a putative protective role against the development of musculoskeletal pain [5], although a recent large population-based study in men was unable to find this relationship [25], and instead observed that elevated levels of gonadothropins were associated with chronic pain.

One of the major pathologies in elderly individuals causing chronic musculoskeletal pain is osteoarthritis. This disease is also known to be sexually dimorphic and its incidence rises as well after menopause [16].

To further elucidate the impact of sex hormonal aspects on chronic musculoskeletal pain, we investigated the association of levels of the main sex hormones, estrogen and testosterone, and its precursors, with the prevalence and incidence of chronic musculoskeletal pain adjusted for the main confounders.

3.2

## Methods

### Study population

This study was embedded in the Rotterdam study, a large prospective population-based cohort study which included men and women of 45 years and over. The design and rationale of this study are described in detail elsewhere [14].

In summary, the Rotterdam Study aims to investigate the determinants, incidence and progression of chronic disabling diseases in the elderly. The first cohort, Rotterdam Study I (RS-I) was initiated in 1989 including 7983 persons aged 55 years and older and was extended in 1999 with 3011 additional participants in Rotterdam Study II (RS-II). In 2005, Rotterdam Study III (RS-III) added another 3932 individuals aged 45 years and older. For all three inclusion cycles together, the participation rate at baseline was 72%.

All participants were examined in detail at baseline (N= 14,926) and follow-up visits every ±5-10 years [14]. In summary, a home interview was conducted and the subjects underwent an extensive set of examinations at the research center.

The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. A written informed consent was obtained from all participants.

### Chronic musculoskeletal pain

During the home interview, participants were asked about current musculoskeletal pain in hands, hips, knees and lower back using a dichotomous variable (yes/no). If present, the duration of this pain was recorded. Chronic musculoskeletal pain was defined as pain in the hands, hips, knees and/or lower back for more than three months.

To investigate whether sex hormone levels may be associated with the development of chronic musculoskeletal pain, we studied the relation between hormone levels and new onset chronic pain. For this purpose, we selected the individuals without chronic pain at the moment of sex hormone measurement. New onset pain was defined as not having pain at this first visit and presence of chronic pain at the next follow up visit (5-10 years later). Controls in this analysis were participants without chronic pain at the first visit and without chronic pain at the follow up visit. A flow chart of the included individuals per cohort is provided in Figure 1.

### Sex hormone measurements

Sample collection: Blood samples were collected in non-fasting individuals between 8:30 and 16:00, for determination of steroid levels, at the third visit of RS-I (1997-1999,
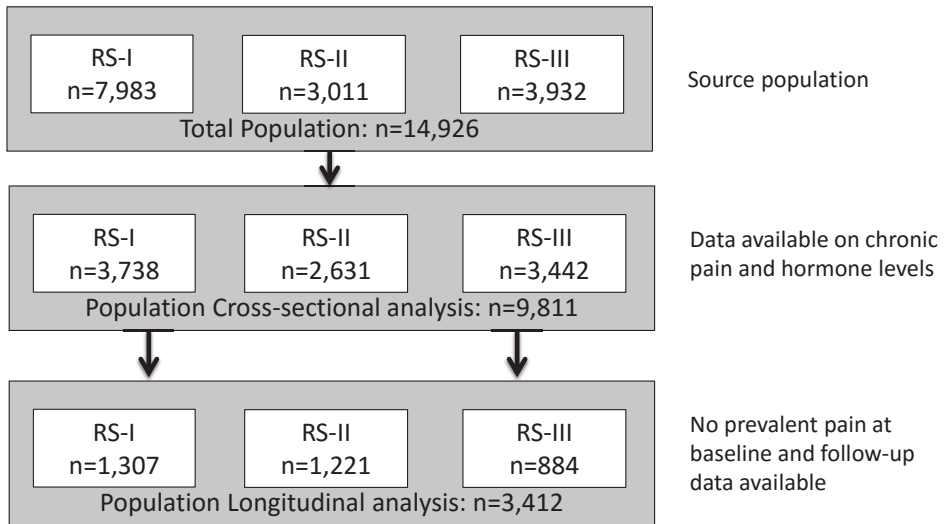
**Figure 1.** Flow chart of included individuals from the Rotterdam study

3920 participants), the baseline visit of RS-II (2000-2001, 2750 participants) and the baseline visit of RS-III (2006-2008, 3465 participants).

Analytical determinations: Estradiol was measured using an electrochemilumi-nescence immunoassay on a Cobas 8000 Modular Analyzer (Roche Diagnostics GmbH, Mannheim, Germany) (lower limit of detection of 18.4 pmol/L). Testoster-one, androstenedione and 17-hydroxyprogesterone were measured simultaneously with a LC-MS/MS method using the CHS™ MSMS Steroids Kit (Perkin Elmer, Turku, Finland). The Steroids Kit uses a combined solvent extraction and protein precipi-tation method with acetonitrile containing the deuterated internal standards $^2H_5$-testosterone, $^2H_5$-androstenedione and $^2H_8$-17α-hydroxyprogesterone. The internal standard underwent processing identical to the analytes. The chromatographic separation was performed on a Waters® (Milford, MA, USA) Acquity™ UPLC HSS T3 1.8 μm column (diameter 1 mm, length 10 cm) and in-line filter frit 0.2 μm with an acetonitrile/MeOH gradient. A Waters XEVO-TQ-S system equipped with an ESI source operating in the electrospray positive mode was used for quantitation. The lower limits of quantitation for testosterone, androstenedione and 17-hydroxypro-gesterone were 0.07, 0.20 and 0.10 nmol/L, respectively. The intraassay coefficients of variation for testosterone, androstenedione and 17-hydroxyprogesterone were <5.7%, <7.4% and <6.1%, respectively.

**Health and lifestyle factors**

Alcohol consumption: The alcohol consumption use was investigated by interview data and defined by number of alcoholic beverages consumed per day. Six catego-

ries were made for the analysis (no alcohol use, 1-2 beverages, 3-4 beverages, 5-6 beverages, 7-9 beverages, >10 beverages).

Smoking: From the interview data we formed 3 categories for smoking to be used in the analysis (never smoked, ever smoked and current smoker).

Depression: For the definition of depression, we used the Center for Epidemiological Studies Depression scale (CES-D). [18] This self-reporting scale of depressive symptoms gives a score between 0-80. Depression was defined as a CES-D score of 16 or above.

Medication use: As a proxy for overall health, we used the amount of different medications used as derived from the interview data. Used medications were categorized using the Anatomical Therapeutic Chemical Classification System (ATC) (http://www.whocc.no/atc/structure_and_principles/). Two categories were made for medication use: <5 different medications and ≥5 different medications.

**Osteoarthritis definition**

Radiographs were scored for the presence of a total hip replacement and radiographic osteoarthritis (ROA) of the hip, knee and hand according to the Kellgren and Lawrence (K/L) score [15]. Knee and hip ROA were defined as a K/L score≥2 of one or both joints or a total joint replacement (TJR). Hand ROA was defined as presence of a K/L score ≥2 in 2 out of 3 hand joint groups (DIPs, PIPs, CMC1/TS) of one or both hands. The overall presence of osteoarthritis (OA) was then defined as having OA in the hip, knee or hand.

**Statistical analysis**

The distribution of the various hormone levels followed a normal distribution, except for estradiol for which many subjects had undetectablelevels. A total of 1786 women and 27 men had estradiol level below the detection limit. We therefore refrained from analyzing estradiol as a continuous variable in women. Associations between hormone levels and chronic musculoskeletal pain, were analyzed using multivariate regression models adjusted for age and Body Mass Index (BMI), health and lifestyle factors (alcohol use, smoking, depression, medication use) and years since menopause (women only).

The relation between the risk of chronic pain (both prevalent and new onset) and various hormone levels was then evaluated with a tertile-based analysis. The tertiles were defined in a sex-specific manner and all analyses were stratified for sex.

Logistic regression analysis was used to estimate the risk of chronic pain. Risks were calculated in the tertile based analyses, with the highest tertile of hormone level as the reference. All estimated risks for chronic pain were adjusted for age,

Body Mass Index (BMI), health and lifestyle factors (alcohol use, smoking, depression and medication use) and years since menopause (women only) and stratified for gender. In the analyses for new onset chronic pain, additional adjustment was done for follow-up time.

In further analyses, we studied the associations between hormone levels and specific locations of chronic joint pain: the knee, hip, lower back and the hand.

We used an additional model for all the association analyses between hormone levels and chronic musculoskeletal pain, with adjustment for the presence of OA.

SPSS version 21.0 (SPSS INC., Chicago, USA) was used for the analyses and a P <0.05 was considered to be statistically significant. Since the different analyses were performed in a sequel to elucidate the associations found, we did not adjust the significance level for multiple comparisons.

3.2

## Results

In this study, a total of 9.811 participants of the Rotterdam Study were included with a mean age of 64.9 years. The population consisted of 5,545 women and 4,266 men. The population characteristics are shown in Table 1. Chronic musculoskeletal pain, in accordance with the used definition, was present in 62% of the women compared to 45% of the men. For the individuals without pain at the moment of hormone measurement, 1558 developed chronic pain before the next follow-up measurement (mean follow up time = 5.6 years ± 2.3). Mean hormone levels were higher in men compared to women. Since most of the women (89%) were postmenopausal, estradiol levels were low in a large amount of the women. For 1786 women (32%), estradiol levels were below the detection level. We therefore only evaluated the association between estradiol levels and chronic pain in women using the tertile-based analysis, in which the women with undetectable levels were part of the lowest tertile. The distribution of the other hormones followed a skewed normal distribution.

### Sex hormone levels and presence of chronic musculoskeletal pain

Table 2 shows that in a linear regression model, women with chronic pain had lower mean 17-hydroxyprogesterone (HP) levels as compared to women without pain (mean difference 0.06 mmol/L).

A minority of women (11%) were pre- or peri-menopausal, which could be of influence in this analysis. We therefore repeated the analysis excluding the pre- and peri-menopausal women. This did not influence the results significantly. For

**Table 1.** Population characteristics

| | Total | | Females | | Males | |
|---|---|---|---|---|---|---|
| | N= | Mean (SD) or percentage | N= | Mean (SD) or percentage | N= | Mean (SD) or percentage |
| Age | 9811 | 64.9 (9.8) | 5545 | 65.2 (10.1) | 4266 | 64.4 (9.4) |
| BMI | 9753 | 27.3 (4.3) | 5505 | 27.5 (4.7) | 4248 | 27.1 (3.7) |
| Alcohol (mean consumptions/day) | 9234 | 1.1 (0.7) | 5233 | 0.9 (0.6) | 4001 | 1.3 (0.8) |
| Smoking (% present/ past) | 9800 | 47.9/19.3 | 5538 | 38.4/18.9 | 4262 | 60.3/19.8 |
| Depression (% positive CES-D) | 1761/9382 | 18.8 | 1248/5297 | 23.6 | 513/4085 | 12.6 |
| Medication use (% 5 of more medications) | 1403/9533 | 14.7 | 869/5384 | 16.1 | 534/4149 | 12.9 |
| | | | | | | |
| Knee osteoarthritis | 1390/8168 | 14.2 | 928/4561 | 20.3 | 462/3607 | 12.8 |
| Hip osteoarthritis | 633/8232 | 6.5 | 393/4613 | 8.5 | 240/3619 | 6.6 |
| Hand osteoarthritis | 1849/7957 | 18.8 | 1307/4459 | 29.3 | 542/3498 | 15.5 |
| | | | | | | |
| Estradiol (pmol/L) | 9653 | 87.65 (141.3) | 5473 | 75.13 (183.3) | 4180 | 104.03 (40.6) |
| Testosterone (nmol/L) | 9724 | 7.99 (9.0) | 5495 | 0.95 (0.8) | 4229 | 17.14 (6.0) |
| Androstenedione (nmol/L) | 9660 | 2.92 (1.4) | 5465 | 2.67 (1.4) | 4195 | 3.25 (1.5) |
| 17-hydroxyprogesterone (mmol/L) | 9717 | 1.96 (1.5) | 5493 | 1.18 (1.0) | 4224 | 2.96 (1.3) |
| | | | | | | |
| Prevalent chronic pain | 5362/9811 | 54.7 | 3461/5545 | 62.4 | 1901/4266 | 44.6 |
| new onset chronic pain | 1558/3412 | 15.9 | 857/1637 | 52.4 | 701/1775 | 39.5 |
| | | | | | | |
| Knee pain | 2568/9811 | 26.2 | 1763/5545 | 31.8 | 805/4266 | 18.9 |
| Hip pain | 1515/9811 | 15.4 | 1100/5545 | 19.8 | 415/4266 | 9.7 |
| Back pain | 3285/9811 | 33.5 | 2135/5545 | 38.5 | 1150/4266 | 27.0 |
| Hand pain | 2243/9811 | 22.9 | 1695/5545 | 30.6 | 548/4266 | 12.8 |

men, a significantly lower estradiol level was found in individuals with chronic pain. No associations were found with the other 3 hormone levels.

The subjects were grouped into sex-specific tertiles for the hormone levels, the cutoff points for each of the hormone levels are given in table 3. We subsequently analyzed the hormone levels by taking the highest tertile as the reference (Table 3). All analyses were adjusted for potential confounders, including age, BMI, health and lifestyle factors, and years since menopause (women only).

**Table 2.** Cross sectional analysis of the differences in sex steroid levels between chronic pain cases vs subjects without chronic pain

| | Women | | Men | |
|---|---|---|---|---|
| | Chronic pain vs no chronic pain | new onset chronic pain | Chronic pain vs no chronic pain | new onset chronic pain |
| | N= 4781 | N= 1526 | N= 3687 | N= 1387 |
| | MD (SE) P-value | MD (SE) P-value | MD (SE) P-value | MD (SE) P-value |
| Androstenedione | −0.07 (0.04) 0.93 | **−0.18 (0.07) 0.01** | −0.05 (0.05) 0.26 | −0.03 (0.08) 0.67 |
| 17-Hydroxyproges-terone | **−0.06 (0.03) 0.05** | **−0.14 (0.06) 0.03** | 0.016 (0.044) 0.717 | −0.04 (0.07) 0.56 |
| Testosterone | −0.03 (0.02) 0.15 | 0.01 (0.04) 0.96 | 0.25 (0.19) 0.20 | −0.03 (0.30) 0.91 |
| Estradiol* | NA | NA | **−3.88 (1.37) 0.005** | 1.72 (2.02) 0.40 |

MD: Mean Difference; SE: Standard Error. Age, BMI, health and lifestyle factors (alcohol use, smoking, depression and medication use) and years since menopause adjusted univariate general linear models were used. *For Estradiol, women were not analyzed (NA) because 33% of the women had estradiol levels below detection limit, for men only individuals with values above the detection limit were included in the analysis (4179/4206 males). Hormone levels were in nmol/L for androstenedione and testosterone, in mmol/L for 17-hydroxyprogesterone and pmol/L for estradiol.

3.2

We observed a consistent higher risk (20-27% higher risk) of chronic pain for the women having estradiol and androstenedione levels in the lowest tertile. For men, no significant associations between hormone levels and the presence of chronic pain was found (supplemental table 1).

We next investigated whether the associations between hormone levels and chronic pain in women could be explained by the presence of OA, as a common musculoskeletal pain disorder. In total, we had 7764 individuals with data on OA (for hip, hand and knee), data on hormone levels and data on chronic pain available. Table 3 shows that additional adjustment for the presence of osteoarthritis in 'multivariate 2' did not affect the risk estimate significantly.

**Sex hormone levels and chronic pain among the different joint sites**

To further investigate whether sex hormone levels were consistently associated with pain at specific joint sites, the association analyses were repeated for pain in the knee, hand, hip and lower back separately, as shown in table 4 for women. In earlier analysis, we observed that women in the lowest tertile had an increase in the risk of chronic pain compared to the other two tertiles. We therefore show the odds ratios for the lowest tertile as compared to the highest tertile.

**Table 3.** Cross sectional multivariate analyses of the relationship between hormone levels and risk for chronic pain in women

| | Multivariate 1 N= 4782 | Multivariate 2 N= 4364 |
|---|---|---|
| | OR (95% CI) P-value | OR (95% CI) P-value |
| **Androstenedione** | | |
| 1st tertile  <1.97 | **1.20 (1.03-1.39)** **0.02** | **1.23 (1.05-1.44)** **0.01** |
| 2nd tertile  1.97-2.94 | 1.07 (0.92-1.23) 0.40 | 1.11 (0.95-1.29) 0.19 |
| 3th tertile  >2.94 | Reference | Reference |
| **17-hydroxyprogesterone** | | |
| 1st tertile  <0.71 | 1.10 (0.95-1.28) 0.22 | 1.15 (0.98-1.34) 0.09 |
| 2nd tertile  0.71-1.16 | 1.03 (0.89-1.19) 0.70 | 1.06 (0.91-1.23) 0.47 |
| 3th tertile  >1.16 | Reference | Reference |
| **Testosterone** | | |
| 1st tertile  <0.66 | 1.11 (0.96-1.29) 0.17 | 1.11 (0.95-1.30) 0.18 |
| 2nd tertile  0.66-1.00 | 1.02 (0.88-1.18) 0.78 | 1.01 (0.87-1.18) 0.86 |
| 3th tertile  >1.00 | Reference | Reference |
| **Estradiol** | | |
| 1st tertile  <19.12 | **1.27 (1.10-1.48)** **0.002** | **1.32 (1.13-1.55)** **0.001** |
| 2nd tertile  19.12-51.75 | **1.25 (1.07-1.45)** **0.004** | **1.28 (1.09-1.50)** **0.003** |
| 3th tertile  >51.75 | **Reference** | **Reference** |

Results are from multivariate logistic regression analysis. OR denotes Odds Ratio, CI denotes Confidence interval. Cutoff points for tertile are given in nmol/L for androstenedione and testosterone, in mmol/L for 17-hydroxyprogesterone and pmol/L for estradiol.
Multivariate 1: This analysis included adjustments for age, BMI, health and lifestyle factors (alcohol use, smoking, depression and medication use) and years since menopause for women.
Mutivariate 2: Multivariate analysis 1 and additional adjustment for presence of osteoarthritis.

Interestingly, although the trends were similar for all associations, knee and hip pain were especially associated with estradiol levels (OR 1.31; 95%CI 1.12-1.53 and OR 1.22; 95%CI 1.02-1.46 respectively). For lower back pain, only androstenedione level was also found to be associated (OR 1.18; 95%CI 1.01-1.37). Chronic hand pain was associated with androstenedione, testosterone as well as estradiol levels (OR 1.23; 95%CI 1.06-1.44, OR 1.19; 95%CI 1.02-1.39 and OR 1.22; 95%CI 1.05-1.43 respectively).

**Table 4.** Cross sectional multivariate analyses of the relationship between hormone levels and risk for chronic pain at specific sites in women

| | Chronic knee pain | Chronic hip pain | Chronic low back pain | Chronic hand pain |
|---|---|---|---|---|
| | OR (95% CI) p-value | OR (95% CI) p-value | OR (95% CI) p-value | OR (95% CI) p-value |
| **Androstenedione** | | | | |
| Multivariate 1 | 1.13 (0.97-1.33) 0.11 | 1.14 (0.95-1.37) 0.16 | **1.18 (1.01-1.37) 0.04** | **1.23 (1.06-1.44) 0.01** |
| Multivariate 2 | 1.10 (0.93-1.32) 0.27 | 1.18 (0.96-1.44) 0.11 | NA | **1.23 (1.03-1.46) 0.022** |
| **17-hydroxyprogesterone** | | | | |
| Multivariate 1 | 1.10 (0.95-1.29) 0.21 | 1.04 (0.87-1.24) 0.67 | 1.11 (0.96-1.29) 0.16 | 1.15 (0.99-1.34) 0.07 |
| Multivariate 2 | 1.13 (0.94-1.34) 0.18 | 1.08 (0.88-1.32) 0.45 | NA | 1.13 (0.95-1.34) 0.18 |
| **Testosterone** | | | | |
| Multivariate 1 | 1.02 (0.87-1.19) 0.81 | 1.19 (0.99-1.43) 0.06 | 1.15 (0.99-1.34) 0.06 | **1.19 (1.02-1.39) 0.03** |
| Multivariate 2 | 0.96 (0.81-1.14) 0.63 | 1.12 (0.92-1.37) 0.26 | NA | **1.18 (0.99-1.400) 0.06** |
| **Estradiol** | | | | |
| Multivariate 1 | **1.31 (1.12-1.53) 0.001** | **1.22 (1.02-1.46) 0.03** | 1.15 (0.99-1.33) 0.08 | **1.22 (1.05-1.43) 0.01** |
| Multivariate 2 | **1.37 (1.15-1.64) $4.82 \times 10^{-4}$** | **1.26 (1.03-1.54) 0.02** | NA | **1.20 (1.01-1.44) 0.04** |

Multivariate logistic regression was performed using tertiles for hormone levels. The odds ratio is for subjects in the lowest tertile, as compared with subjects in the highest tertiles. Odds ratios are given with 95% confidence limits between brackets.
Multivariate 1: This analysis included adjustments for age, BMI, health and lifestyle factors (alcohol use, smoking, depression and medication use) and years since menopause. N= 4782
Multivariate 2: Multivariate analysis 1 and additional adjustment for presence of site-specific osteo-arthritis. N= 3968. These data were not available for low back.

Consistent with earlier analysis for overall chronic pain risk in men (as shown in supplemental table 2), we did not observe consistent associations between hormone levels and specific joint sites. Only androstenedione levels were significantly associated with chronic hand pain.

### Sex hormone levels and new onset chronic musculoskeletal pain

We estimated the risk for future chronic pain in those individuals that had no chronic pain at the time of hormone measurement (n=3412). A total of 1558 individuals developed chronic pain between the first visit, when hormone measurement was performed, and the follow up visit 5-10 years after.

**Table 5.** Longitudinal analysis of new onset chronic pain and the association with hormone levels

| | Women N= 1527 | Men N= 1377 |
| --- | --- | --- |
| | Odds ratio (95% CI) P-value | Odds ratio (95% CI) P-value |
| **Androstenedione** | | |
| 1st tertile  <1.97 | 1.17 (0.89-1.53) 0.26 | 0.95 (0.73-1.25) 0.72 |
| 2nd tertile  1.97-2.94 | 0.99 (0.77-1.29) 0.96 | 1.07 (0.83-1.37) 0.63 |
| 3th tertile  >2.94 | Reference | Reference |
| **17-hydroxyprogesterone** | | |
| 1st tertile  <0.71 | **1.38 (1.06-1.80) 0.02** | 0.97 (0.75-1.27) 0.65 |
| 2nd tertile  0.71-1.16 | 1.18 (0.92-1.53) 0.260 | 0.97 (0.76-1.25) 0.82 |
| 3th tertile  >1.16 | Reference | **Reference** |
| **Testosterone** | | |
| 1st tertile  <0.66 | 1.09 (0.83-1.42) 0.54 | 0.98 (0.75-1.27) 0.85 |
| 2nd tertile  0.66-1.00 | 1.05 (0.81-1.36) 0.73 | 0.98 (0.72-1.19) 0.56 |
| 3th tertile  >1.00 | Reference | Reference |
| **Estradiol** | | |
| 1st tertile  <19.12 | 0.84 (0.69-1.09) 0.19 | 0.87 (0.68-1.13) 0.30 |
| 2nd tertile  19.12-51.75 | 0.91 (0.69-1.20) 0.50 | 1.02 (0.79-1.31) 0.91 |
| 3th tertile  >51.75 | Reference | Reference |

Results are from multivariate logistic regression analysis. OR denotes Odds Ratio, CI denotes Confidence interval. Cutoff points for tertile are given in nmol/L for androstenedione and testosterone, in mmol/L for 17-hydroxyprogesterone and pmol/L for estradiol.
This analysis included adjustments for age, BMI, health and lifestyle factors (alcohol use, smoking, depression and medication use) and years since menopause for women.

We observed that women with new onset chronic pain had lower androstenedione and 17-hydroxyprogesterone levels at baseline (Table 2, mean difference −0.18 nmol/L; P= 0.01 and mean difference −0.14 mmol/L; P= 0.03). No associations between baseline hormone levels and new onset chronic pain were found in men. We then tested whether the hormone levels were associated with the onset of chronic pain in the tertile-based analysis (Table 5). Consistent with the previous analysis, lower levels 17-hydroxyprogesterone was associated with higher incidence of chronic pain in women at follow up (OR 1.38 95%CI 1.06-1.80). Women

with 17-hydroxyprogesterone levels in the lowest tertile had a 38% increased risk of new onset chronic pain when compared to women in the highest tertile.

In men, we did not find a significant association between sex hormone levels and new onset chronic musculoskeletal pain. Additional analyses, adjusting for prevalent OA, did essentially not influence the risk estimates.


## Discussion

In this large population based study cohort including more than 9,500 people aged 45 years and over, we observed that women with chronic pain had lower androstenedione, 17-hydroxyprogesterone and estradiol levels. In addition, we found that women with new onset chronic pain also had lower androstenedione or estradiol level at baseline. Women with androstenedione levels in the lowest tertile, had a 38% higher risk for new onset chronic musculoskeletal pain when compared to women with androstenedione levels in the highest tertile. All these associations were independent from possible confounders, such as age, years since menopause, BMI, health and lifestyle factors and osteoarthritis. In addition, we demonstrated that the trends of these associations were consistent among specific sites: for knee, hip, lower back and hand pain. Hand pain was associated with three of the four studied sex hormones in women. Lower levels of androstenedione, testosterone and estradiol resulted in higher risk for chronic hand pain.

Estradiol is thought to be pain modulating in a complex manner.[7] The mechanisms for this modulating effect are thought to involve neuroanatomical and neurochemical systems which regulate the nociceptive circuitry at spinal and supraspinal level. Estrogen exposure over time, but also current estrogen levels can sensitize the body for painful stimuli and therefore facilitating the development of chronic pain. The influence of estrogen levels on pain sensitivity is also shown by studies on pain sensitivity during the different phases of the menstrual cycle, during which estrogen and other sex hormones are known to fluctuate [1, 7, 12].

A large study in elderly men by Tajar et al found, for musculoskeletal pain, a significant association with gonadotrophins but not with steroid hormones [25]. This is in line with the results presented in this study, in which we did not find consistent associations between chronic musculoskeletal pain in men and the sex hormones studied. From our study, it can be noted that sex hormones are associated with chronic pain much more for women than for men. One possible explanation may include the differences in pathophysiology of chronic pain between men and women. In women, hormones may play a larger role. The exact mechanisms behind these differences remain to be investigated. In a recent study, the sexual

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dimorphism in pain sensitivity was attributed to involvement of adaptive immune cells in female mice. In this study, they demonstrated that T-cell infiltration is involved in mechanical allodynia, only in female mice. In absence of these adaptive immune cells and in testosterone treated female mice, they observed a more male, glial-dependent pathway. [23] Therefore, we hypothesize that sex hormones are involved in this observed difference between males and females, since especially estrogen is known to enhance the immune system. [8]

Besides estradiol levels, we found androstenedione and 17-hydroxyprogesterone to be significantly associated with chronic musculoskeletal pain in women. Androstenedione is an important precursor for other sex hormones. Since circulating estradiol levels are low in postmenopausal women, the precursors might have a more important role in the mechanism of action. The precursors can be metabolized into active sex hormones in the peripheral tissues. [11] In support of this hypothesis, animal studies demonstrated aromatization of androgens into estrogen in the dorsal root ganglion in the spinal cord [10]. This might imply a mechanism in which estrogen regulates pain in a paracrine manner.

Our study has a number of strengths: It has a population-based and longitudinal design. In addition, to our knowledge, the sample size is larger than any other previous study. Moreover, the assessment of lifestyle and health confounders was done using validated instruments.

A number of issues should be noted when interpreting the results of this study. First, the population we used were 45 years and over, which means that most women in the study were postmenopausal and had very low estradiol levels. In addition, a large part of the analysis was done using a cross-sectional design, which prevented us to draw firm conclusions about causality of the findings. However, longitudinal analyses, showed similar effect sizes for new onset musculoskeletal pain in women. Probably since the included number of individuals in this analysis was much lower, statistical level of significance was less decisive as compared to the cross sectional design. The fact that the hormone levels were also associated with new onset chronic pain, suggests that the differences in hormone levels are preceding the pain, and not the other way around. Sex hormones are known to fluctuate during the day. In our study, blood samples were collected between 8:30 and 16:00, which could have influenced our measurements. It is unlikely that this fluctuation influenced the associations found in this study, since the pain assessment does not vary across the day and considering the large number of individuals included in this study. Pain severity scores were not available in this study, but could have improved the definition of chronic pain.

In women, we found consistent results of sex hormones being associated with prevalent and new onset pain in all different analyses. For men, there were also

some significant, but less consistent associations found. Considering the number of different tests performed, these findings in men could be subject to type I errors and should therefore be interpreted with caution.

We also performed analyses in which we adjusted the identified associations for the presence of osteoarthritis. The main reason for this was the fact that estrogen is thought to be involved in the development of osteoarthritis, the main cause of musculoskeletal pain in the elderly. [24] Our results suggest an independent association of androstenedione and estradiol, on the prevalence of chronic musculoskeletal pain, in women. As previously described, the role of estradiol and other sex hormones in the pathophysiology of osteoarthritis is not very clear, although the hormonal changes in postmenopausal women are often thought to be involved[9]. This study supports the limited influence of sex hormones on the pathophysiology.

In summary, lower sex hormones levels are associated with an increased risk for having and developing chronic musculoskeletal pain, independent from lifestyle and health related factors, in women. These results suggest that sex hormones may play a role in chronic pain and should be taken into account when a patient presents with chronic pain. Therefore, sex hormones may be a potential treatment target for these patients.

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3.2

**Supplementary table 1.** Cross sectional multivariate analyses of the relationship between hormone levels and risk for chronic pain in men

| | Multivariate 1 N= 3688 | Multivariate 2 N= 3400 |
|---|---|---|
| | OR (95% CI) P-value | OR (95% CI) P-value |
| **Androstenedione** | | |
| 1st tertile  <1.97 | 1.08 (0.90-1.28) 0.42 | 1.12 (0.93-1.34) 0.23 |
| 2nd tertile  1.97-2.94 | 1.03 (0.88-1.22) 0.69 | 1.07 (0.90-1.28) 0.42 |
| 3th tertile  >2.94 | Reference | Reference |
| **17-hydroxyprogesterone** | | |
| 1st tertile  <0.71 | 0.97 (0.82-1.15) 0.74 | 1.00 (0.84-1.19) 0.98 |
| 2nd tertile  0.71-1.16 | 0.90 (0.76-1.06) 0.19 | 0.93 (0.78-1.10) 0.38 |
| 3th tertile  >1.16 | Reference | Reference |
| **Testosterone** | | |
| 1st tertile  <0.66 | 0.94 (0.79-1.11) 0.47 | 0.93 (0.78-1.12) 0.45 |
| 2nd tertile  0.66-1.00 | 0.89 (0.73-1.01) 0.06 | 0.86 (0.72-1.02) 0.07 |
| 3th tertile  >1.00 | Reference | Reference |
| **Estradiol** | | |
| 1st tertile  <19.12 | 1.15 (0.98-1.38) 0.09 | 1.17 (0.99-1.39) 0.07 |
| 2nd tertile  19.12-51.75 | 1.17 (0.99-1.35) 0.06 | **1.20 (1.01-1.43) 0.04** |
| 3th tertile  >51.75 | Reference | Reference |

Results are from multivariate logistic regression analysis. OR denotes Odds Ratio, CI denotes Confidence interval.

Multivariate 1: This analysis included adjustments for age, BMI, health and lifestyle factors (alcohol use, smoking, depression and medication use) and years since menopause

Mutivariate 2: Multivariate analysis 1 and additional adjustment for presence of osteoarthritis.

**upplementary table 2.** Cross sectional analysis of hormone levels and prevalent chronic knee, hip, low back and hand pain in men

| | Chronic knee pain | Chronic hip pain | Chronic low back pain | Chronic hand pain |
|---|---|---|---|---|
| | OR (95% CI) p-value | OR (95% CI) p-value | OR (95% CI) p-value | OR (95% CI) p-value |
| **Androstenedione** | | | | |
| Multivariate 1 | 1.11 (0.89-1.39) 0.34 | 0.74 (0.55-1.01) 0.06 | 1.07 (0.88-1.30) 0.49 | **1.33 (1.03-1.72) 0.03** |
| Multivariate 2 | 1.20 (0.94-1.53) 0.14 | 0.72 (0.51-1.00) 0.05 | NA | **1.40 (1.05-1.88) 0.02** |
| **17-hydroxyprogesterone** | | | | |
| Multivariate 1 | 1.08 (0.88-1.33) 0.47 | 1.11 (0.83-1.49) 0.47 | 1.00 (0.83-1.21) 0.97 | 0.97 (0.76-1.25) 0.83 |
| Multivariate 2 | 1.19 (0.94-1.49) 0.15 | 1.09 (0.79-1.49) 0.59 | NA | 1.02 (0.77-1.35) 0.89 |
| **Testosterone** | | | | |
| Multivariate 1 | 0.96 (0.78-1.19) 0.71 | 0.98 (0.74-1.31) 0.91 | 0.95 (0.79-1.15) 0.61 | 0.97 (0.76-1.24) 0.82 |
| Multivariate 2 | 0.98 (0.78-1.24) 0.89 | 0.91 (0.67-1.24) 0.55 | NA | 0.96 (0.73-1.26) 0.76 |
| **Estradiol** | | | | |
| Multivariate 1 | 1.09 (0.89-1.34) 0.38 | 0.97 (0.73-1.28) 0.82 | 1.17 (0.97-1.41) 0.10 | 1.10 (0.87-1.39) 0.44 |
| Multivariate 2 | 1.09 (0.87-1.36) 0.48 | 0.85 (0.63-1.16) 0.31 | NA | 1.05 (0.81-1.38) 0.70 |

Multivariate logistic regression was performed using tertiles for hormone levels. The odds ratio is for subjects in the lowest tertile, as compared with subjects in the highest tertiles. Odds ratios are given with 95% confidence limits between brackets.

Multivariate 1: This analysis included adjustments for age, BMI, health and lifestyle factors (alcohol use, smoking, depression and medication use) and years since menopause. N= 3688.

Multivariate 2: Multivariate analysis 1 and additional adjustment for presence of site-specific osteo-arthritis. N=3164. These data were not available for low back.

3.2

# CHAPTER 4

Imaging the brain in chronic pain

# CHAPTER 4.1

Structural brain alterations in community dwelling individuals with chronic joint pain

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## Abstract

*Background and purpose:* Central sensitization in chronic pain involves structural brain changes that influence vulnerability to pain. Identifying brain regions involved in pain processing and sensitization can provide more insight into chronic pain. This study examines structural brain changes in chronic pain and experimental pain in a large population-based study.

*Materials and methods:* For 3892 participants in the Rotterdam study, global and regional MR imaging brain volumes were automatically segmented and quantified. Chronic joint pain was defined as pain for more than half of all days during the past 6 weeks. Heat pain thresholds were measured in a subset of 1538 individuals. The association between the presence of chronic joint pain and global and lobar brain volumes was studied. Subsequently, literature was reviewed and the association of chronic pain and heat pain thresholds with 11 brain regions associated with musculoskeletal pain in previous publications was studied.

*Results:* Total gray matter volume was smaller in women with chronic pain (Beta 0.066, P= 0.016). This effect was primarily driven by lower gray matter volume in the temporal lobe (Beta 0.086, P= 0.005), the frontal lobe (Beta 0.060, P= 0.039), and the hippocampus (Beta 0.099, P= 0.002). In addition, we observed that a lower heat pain threshold was associated with smaller volumes of the hippocampus (Beta 0.017, P= 0.048), the thalamus (Beta 0.018, P= 0.009), and the anterior cingulate cortex (Beta 0.016, P= 0.037). In men, no significant associations were observed.

Conclusions: The primary identified brain areas, the temporal and frontal lobes and the hippocampus, indicated involvement of emotional processing. The volumetric differences found indicated a sex-specific neuroplasticity in chronic pain. These results emphasized sex-specific and multidisciplinary pain treatment.

## Introduction

Chronic musculoskeletal pain is very common in the general elderly population, with a prevalence up to 50%–60%. Experienced chronic joint pain does not always reflect the extent of objective pathology. [1-4] Central sensitization plays an important role in the development of chronic joint pain. Chronic pain and central sensitization result in higher vulnerability for developing chronic pain at multiple sites and higher sensitivity for painful stimuli. [5] Differences in pain processing may be expressed in functional and structural changes in the nervous system. MR imaging allows us to identify brain regions involved in this process of central sensitization, which can provide more insight into chronic pain.

Previous studies that examined structural brain alterations in chronic pain focused on a variety of pain phenotypes, such as migraine, back pain, osteoarthritis, and fibromyalgia. [6-15] Typically, the study size was small; the largest studies included approximately 100 subjects. The small sample sizes of these studies led to a modest statistical power, thereby influencing the reproducibility of the results. [16] In addition, all previous reports had a case-control design, which selected individuals who were referred to the clinic as chronic pain cases. As a result, many different areas were shown to associate with a particular pain phenotype but only a few areas of the brain showed consistent associations. For example, the thalamus was found to be positively associated with chronic low back pain by Schmidt-Wilcke et al. [17] but negatively associated with chronic low back pain by Apkarian et al. [18] Regions that are part of the limbic system and signaling pathway were among the identified pain-associated brain areas. Furthermore, each different pain phenotype showed different patterns of structural brain changes, with some overlapping regions, for example, the hippocampus. [7] Despite the possible identification of structural brain alterations in these selected clinical cases, it remains unclear which brain regions are morphologically altered in chronic pain in the general population. Therefore, in this study, after review of the existing literature, we attempted to replicate previous identified regions to find brain structures robustly associated with musculoskeletal pain.

Individuals with chronic pain are shown to be more sensitive to experimental pain stimuli. Central sensitization can be detected by lower pain thresholds. [19-21] The stimulus response curve is shifted to the left, which results in lower pain thresholds or higher reported pain intensity scores for a stimulus. The spread of central sensitization, manifested because general hyperalgesia is one of the fundamental processes in the development of chronic pain. [22-25] Lower pain thresholds, as part of central sensitization, might be associated with structural brain changes. Thus far, the relation between experimental pain and structural brain alterations

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has only been studied in 1 study of 80 healthy individuals. [26] In addition, Semino-wicz et al. [27] showed, in a rat model for long-term neuropathic pain, that thermal and mechanical hyperalgesia is associated with structural brain changes.

Given the high prevalence of chronic musculoskeletal pain in the elderly and the burden of chronic musculoskeletal pain on quality of life, more insight into the pathophysiology is necessary to understand chronic pain in the general population and improve treatment options. In this study, we examined, in a large population-based cohort study, the association of chronic musculoskeletal pain and heat pain thresholds with MR imaging– based structural brain changes. We studied changes in global and lobar brain volumes and, in addition, specific brain regions previously reported to be associated with musculoskeletal pain phenotypes.

## Materials and methods

### Study Population: The Rotterdam Study

The Rotterdam Study is a large prospective population-based cohort study of persons aged 45 years and older. The study design and rationale are described elsewhere in detail. [28] In summary, the objective of the Rotterdam Study is to investigate the determinants, incidence, and progression of chronic disabling diseases in the elderly. The first cohort, Rotterdam Study I consisted of 7983 persons aged ≥ 55 years and was initiated in 1989. This study population was extended in 2000, which added 3011 participants in Rotterdam Study II and, in 2005, added another 3932 subjects aged ≥ 45 years in Rotterdam Study III. All the participants were examined in detail at baseline. In summary, a home interview was conducted, and the subjects had an extensive set of examinations at the research center.

The participants in the study as presented here were derived from the Rotterdam Scan Study, [29] an ongoing population-based cohort study that investigated brain changes on MR imaging, which is embedded in the Rotterdam Study. The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all the participants.

### MR Imaging Acquisition and Processing

MR imaging scanning was performed on a 1.5T-scanner with an 8-channel head coil (GE Healthcare, Milwaukee, Wisconsin). An extensive description of the scan protocol is provided elsewhere. [29] In short, the protocol included a T1-weighted

sequence, a proton-attenuation weighted sequence, and a fluid-attenuated inversion recovery sequence. [29]

Automated brain tissue classification based on a k-nearest-neighbor-classifier algorithm extended with white matter lesion segmentation [30,31] was used to quantify global and lobar brain volume, gray matter volume, white matter volume, and intracranial volume (in $mL^3$). This method has been optimized and validated for the Rotterdam Scan Study and includes a standardized and validated image analysis workflow to enable objective, accurate, and reproducible extraction of brain volumes. [29] Segmentation and labeling of smaller specific brain regions was performed by FreeSurfer version 4.5 (http://surfer.nmr.mgh.harvard.edu/). [26] This procedure automatically assigns a neuroanatomic label to each voxel in an MR imaging volume based on probabilistic information obtained from a manually labeled training set. FreeSurfer was used with the default parameters, including skull-stripping and using the automatically generated brain mask.

4.1

**Review of the Literature and Selection of Candidate Replication Regions**

We systematically searched the literature by using the PubMed data base on July 7, 2014, with search terms "structural and brain and MR imaging and chronic and pain." In addition, we screened the references of included articles to extend the search. The review of literature identified 83 articles, of which 68 articles were excluded based on the following selection criteria: 1) the article represents original data, and 2) the trait of interest is musculoskeletal pain. Finally, 15 studies were included in the total review.

**Assessment of Chronic Joint Pain**

All the participants completed a pain homunculus to report chronic painful sites in the body. The pain homunculus showed a picture of the front and the back of the human body. The participants were asked the following question, "Did you have pain anywhere in your body, for at least half of the days, during the last six weeks?" Circles were drawn by the participant around the painful areas. The homunculi were scored by using a template that assigned 14 different joint pain regions (eg, neck, shoulders, elbows, hands, low back, hips, knees, feet). Chronic joint pain was defined as subjects having one or more painful sites. Furthermore, participants should have visited a medical physician at least once for this chronic joint pain. This information was derived from the questionnaire during the home interview.

In addition, because we used a more heterogenic pain phenotype compared with previous studies, we defined 3 chronic pain phenotypes to be able to compare our results better with previous literature. The phenotypes examined in the studies, also selected for the review, were fibromyalgia, chronic low back pain, and hip

osteoarthritis pain. Because we did not have data on fibromyalgia, we used chronic widespread pain as a proxy. Chronic widespread pain was defined as subjects having pain in the left side of the body, in the right side of the body, above the waist, below the waist, and in the axial skeleton (by following the Fibromyalgia Criteria of the American College of Rheumatology). [32] Hip osteoarthritis pain was defined as a Kellgren-Lawrence score of ≥ 2 and chronic pain in the same hip. Controls in these analyses were individuals without chronic pain.

### Heat Pain Threshold Measurement

For the measurement of heat pain threshold, we used a commercially available thermosensory analyzer, the TSA II (Medoc Advanced Medical Systems, Durham, North Carolina). The probe, with a surface of 2 cm by 2 cm was placed on the ventral site of the non-dominant forearm. The start temperature of the probe was 32°C. The temperature increased by 2°C per second, and the participant was asked to push a large quiz button when the temperature became painful. This measurement was repeated 5 times; the mean of the last 3 measurements was used. Because this was measured approximately 5 years after the brain MR imaging was acquired, we included only those individuals with a stable pain state. Individuals with chronic pain at both the time of brain MR imaging and chronic pain at the time of the heat pain threshold measurement were considered as cases, individuals without chronic pain at both time points were considered as controls.

### Population for Analysis

A total of 4898 participants who were part of Rotterdam Study I, Rotterdam Study II, or Rotterdam Study III were invited to undergo an MR imaging. We excluded individuals who had dementia (n= 30) or had MR imaging contraindications (n= 389). Of 4479 eligible persons, 4082 (91%) participated. Due to physical inability, imaging could not be performed in 44 individuals. Of 4038 persons with complete MR imaging examinations, 59 had to be excluded because of motion artifacts or susceptibility artifacts on their scans, which left 3979 persons with complete brain MR imaging. Pain data were not available for 87 of these persons, whereas data on the need for medical treatment for the pain medication was not available for 516 individuals, which left 3376 persons for the analyses. For the association analysis with the heat pain threshold measurements, a subset of 839 individuals with a stable pain state as described above was used.

### Statistical Analysis

Linear regression models were used to test the association between chronic musculoskeletal pain, heat pain thresholds, and brain volumes. We calculated z

scores (X – mean/SD) of the brain volumes to allow direct comparability between the various effect estimates for the analyses between pain and the different brain structures. Z score standardization is done in the common way by (brain volume – mean brain volume)/SD.

Because previous studies found differences on pain-associated regions between the sexes, we stratified for sex and adjusted for age, intracranial volume, and the presence of depression according to the self-reporting Center for Epidemiologic Studies Depression scale, defined by a score of > 16. SPSS version 21.0 (SPSS Statistics for Windows; IBM, Armonk, New York) was used for the association analysis. The null hypothesis tested was that there is no difference in brain volumes in the studied structures between individuals without chronic musculoskeletal and those with chronic musculoskeletal pain. The second hypothesis tested was that brain volumes of the studied structures are not associated with heat pain thresholds. A P value of < 0.05 was considered statistically significant.

**Association Analysis with Chronic Musculoskeletal Pain and Heat Pain Threshold**

First, we performed an association analysis without hypothesizing where to expect structural alterations in the brain in chronic joint pain. Therefore, we investigated the association of global volumes of gray and white matter with chronic joint pain. Next, we segmented the brain into the 4 main lobes (frontal, temporal, parietal, and occipital). Gray and white matter volumes in the different lobes were then studied for the association with chronic joint pain. Subsequently, we investigated the association of chronic joint pain with the volumes of the selected regions reported in the literature (Table 1).

In the effort to replicate previous findings, we examined the association of chronic widespread pain as a proxy for fibromyalgia, chronic low back pain, and hip osteoarthritis pain with brain region volumes in our sample. In addition, we investigated the association of the brain region volumes with heat pain thresholds.

**Results**

Population characteristics for the 3892 persons with brain MR imaging and chronic pain information are shown in Table 2. The prevalence of chronic pain and depression was higher in women compared with men, and the total intracranial volume was smaller in women. Heat pain thresholds were higher in men compared with women (48.0°C vs 46.6°C).

4.1

**Table 1.** Selected brain regions for analysis

| Brain region | Reference | +/- | N | Chronic pain disorder |
|---|---|---|---|---|
| Thalamus | Apkarian et al. 2004 [18] | - | 52 | CLBP |
| | Schmidt-Wilcke et al. 2006 [17] | + | 36 | CLBP |
| | Schmidt-Wilcke et al. 2007 [40] | - | 42 | Fibromyalgia |
| | Ivo et al. 2013 [9] | - | 28 | CLBP |
| S1 | Rodriguez-Racke et al. 2009 [39] | - | 32 | Hip OA |
| | Seminowicz et al 2011 [41] | - | 34 | CLBP |
| | Kong et al 2013 [36] | + | 36 | CLBP |
| Insular cortex | Kuchinad et al. 2007 [37] | - | 20 | Fibromyalgia |
| | Rodriguez-Racke et al. 2009 [39] | - | 32 | Hip OA |
| | Valet et al. 2009 [42] | - | 39 | Pain syndrome (DSM IV) |
| | Robinson et al. 2011 [11] | - | 25 | Fibromyalgia |
| | Seminowicz et al 2011 [41] | - | 34 | CLBP |
| Anterior cingulate cortex | Burgmer et al. 2009 [34] | - | 28 | Fibromyalgia |
| | Valet et al. 2009 [42] | - | 39 | Pain syndrome (DSM IV) |
| | Rodriguez-Racke et al. 2009 [39] | - | 32 | Hip OA |
| | Seminowicz et al 2011 [41] | - | 34 | CLBP |
| | Jensen et al. 2013 [35] | - | 39 | Fibromyalgia |
| Midcingulate cortex | Kuchinad et al. 2007 [37] | - | 20 | Fibromyalgia |
| | Buckalew et al. 2008 [33] | - | 16 | CLBP |
| | Wood et al. 2009 [43] | - | 14 | Fibromyalgia |
| | Robinson et al. 2011 [11] | - | 25 | Fibromyalgia |
| | Ivo et al. 2013 [9] | - | 28 | CLBP |
| Prefrontal cortex Dorsolateral | Apkarian et al. 2004 [18] | - | 52 | CLBP |
| | Seminowicz et al 2011 [41] | - | 34 | CLBP |
| | Ivo et al. 2013 [9] | - | 28 | CLBP |
| Prefrontal cortex Ventrolateral | Rodriguez-Racke et al. 2009 [39] | - | 32 | Hip OA |
| | Burgmer et al. 2009 [34] | - | 28 | Fibromyalgia |
| | Seminowicz et al 2011 [41] | - | 34 | CLBP |
| Posterior cingulate cortex | Kuchinad et al. 2007 [37] | - | 20 | Fibromyalgia |
| | Valet et al 2009 [42] | - | 39 | Pain syndrome (DSM IV) |
| | Wood et al. 2009 [43] | - | 14 | Fibromyalgia |
| | Robinson et al. 2011 [11] | - | 25 | Fibromyalgia |
| Orbitofrontal cortex | Schmidt-Wilcke et al. 2007 [40] | + | 42 | Fibromyalgia |
| | Valet et al. 2009 [42] | - | 39 | Pain syndrome (DSM IV) |
| | Rodriguez-Racke et al. 2009 [39] | - | 32 | Hip OA |
| | Seminowicz et al 2011 [41] | - | 34 | CLBP |
| Hippocampus | Lutz et al. 2008 [38] | - | 60 | Fibromyalgia |
| | Zimmerman et al. 2009 [14] | - | 20 | Chronic pain |
| Amygdala | Burgmer et al. 2009 [34] | - | 28 | Fibromyalgia |
| | Rodriguez-Racke et al. 2009 [39] | - | 32 | Hip OA |

Note: + indicates larger volume in chronic pain; - indicates smaller volume in chronic pain; CLBP, chronic low back pain; OA, oateoarthritis; DSM IV, Diagnostic and Statistical Manual of Mental Disorder, 4th edition.

**Table 2.** Baseline characteristics of the study population

|  | Total (n=3376) | Male (n=1525) | Female (n=1851) |
|---|---|---|---|
| Mean age, years ± SD | 60.3 ± 8.7 | 60.4 ± 8.7 | 60.1 ± 8.7 |
| Chronic pain, no. (%) | 1191 (35.3) | 414 (27.1) | 777 (42.0) |
| Mean intracranial volume, mL ± SD | 1126 ± 119 | 1203 ± 102 | 1062 ± 91 |
| Positive CESD, no. (%) | 328 (8.5) | 81 (4.6) | 247 (11.7) |
| Mean heat pain threshold, °C ± SD (n=1538) | 47.2 ± 3.2 | 48.0 ± 2.7 | 46.6 ± 3.4 |

Note: CESD indicates Center for Epidemiologic Studies Depression scale.
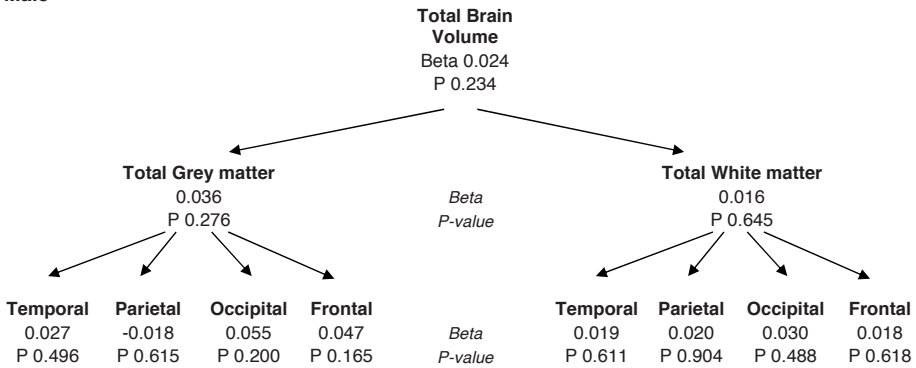
### Chronic Joint Pain and Global and Lobar Brain Volumes

The associations between chronic musculoskeletal pain and global and lobar brain volumes are shown in Figure 1. No significant association between chronic musculoskeletal pain and total brain volume was observed in the overall population. When we stratified according to sex, we observed a significant association with total gray matter in the women. Total gray matter was smaller in women with chronic pain (difference in Z score, Beta 0.066; P= 0.016). When we divided the brain into the 4 main lobes, this lower gray matter volume was found to be primarily located in the temporal lobe (Beta 0.086, P= 0.005) and the frontal lobe (Beta 0.060, P= 0.039). In the men, we did not find differences in global brain volumes between participants with and those without chronic pain. Excluding participants with depression defined by a Center for Epidemiologic Studies Depression scale score of > 16 (81 men, 247 women) in the sensitivity analysis did not alter these effects.

### Chronic Joint Pain and Predefined Brain Regions

Next, we focused our analysis on volumes of specific brain regions that were previously reported in the literature as being associated with musculoskeletal pain phenotypes (Table 1). These regions were selected on the basis of a systematic review. In total, 15 studies that assessed the relationship between brain structures and chronic pain were included in this review. [9,11,14,17,33-43] All brain regions previously reported to be significantly associated with chronic pain are shown in the supplemental table, together with the direction of the effect. We decided to include the brain regions that were reported to be associated with musculoskeletal pain at least twice. The 11 selected regions are shown in Table 1, together with the sample size of each study, which were all fewer than 100 individuals. Segmentation of the 11 brain regions was done in 4898 individuals with the use of FreeSurfer software. We observed a significantly smaller hippocampal volume in women with chronic musculoskeletal pain (Beta 0.099, P= 0.002), whereas men showed a similar trend, though this did not reach significance (Figure 2). When data for men and women were analyzed together, a highly significant association was seen (Beta 0.092, P= $4.69*10^{-4}$).

**Male**



**Female**
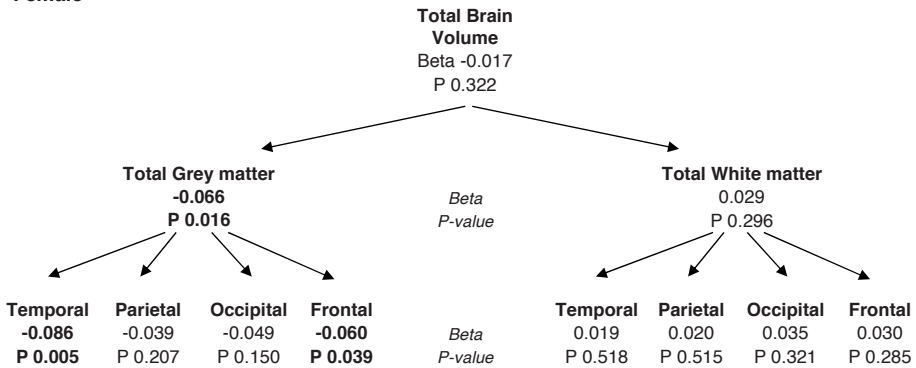


**Figure 1.** Chronic musculoskeletal pain and global brain volumes
Analyses adjusted for age, Intracranial Volume and Depression
Beta is the difference in standardized brain volume for individuals with chronic joint pain compared to those without chronic joint pain.

We next studied specific pain subtypes to mimic earlier reports. We studied chronic widespread pain, chronic low back pain, and hip osteoarthritis pain separately as determinants for brain region volumes. For the analyses of chronic widespread pain and hip osteoarthritis pain, we observed similar effect directions for hippocampal volume but only for chronic low back pain was statistical significance reached (Beta 0.115, P= 0.033).

Among the subset with heat pain threshold measurements, we observed only in the women, a positive association between heat pain thresholds and hippocampal volume, thalamic volume, and the volume of the anterior cingulate cortex (Figure 3), which indicated that lower pain sensitivity thresholds, which represent central sensitization, were indeed coinciding with smaller hippocampal, thalamic, and anterior cingulate cortex volumes.
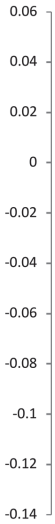
**Figure 2.** Brain volumes in regions of the limbic system and signal processing in relation to chronic musculoskeletal pain in female (A) and male (B) subjects. Plots represent beta and standard error; Beta is the difference in standardized brain volume for individuals with chronic joint pain compared to those without chronic joint pain; Analyses were adjusted for age, intracranial volume and depression. *P= 0.002. Thal indicates thalamus; Hippo, Hippocampus; Amyg, Amygdala; OFC, Orbitofrontal cortex; vlPFC, ventrolateral prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; PCC, posterior cingulate cortex; MCC, mid cingulate cortex; ACC, anterior cingulate cortex; Insula, insular cortex; S1, primary somatosensory cortex.

**A. Female subjects**

| | Thal | Hippo | Amyg | OFC | vlPFC | dlPFC | PCC | MCC | ACC | Insula | S1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Beta | 0.018 | 0.017 | 0.017 | -0.007 | 0.013 | 0.002 | -0.016 | -0.006 | -0.016 | -0.0005 | 0.002 |

**B. Male subjects**

| | Thal | Hippo | Amyg | OFC | vlPFC | dlPFC | PCC | MCC | ACC | Insula | S1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Beta | 0.002 | 0.007 | 0.005 | 0.004 | 0.013 | 0.004 | 0.001 | 0.021 | -0.0004 | 0.003 | -0.009 |
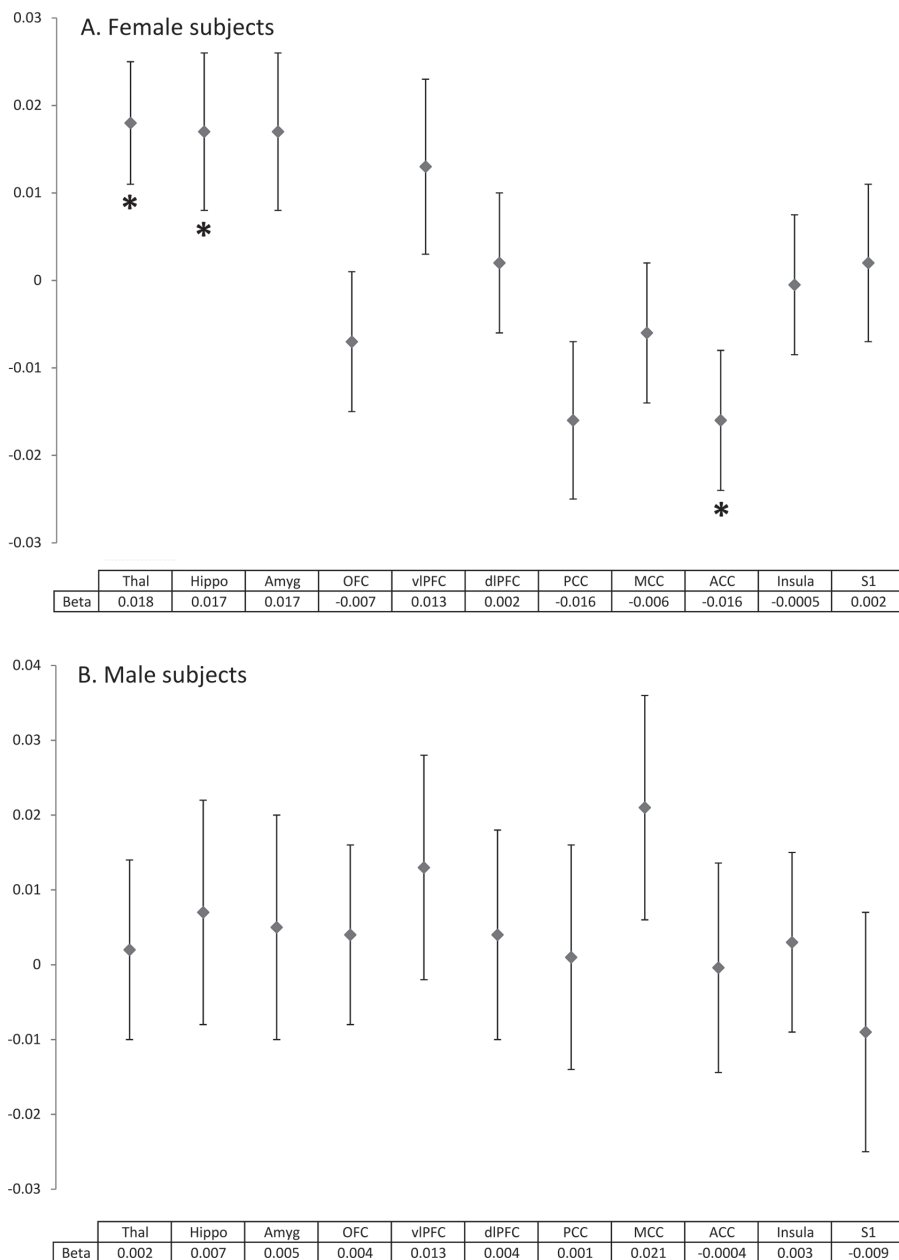
**Figure 3.** Quantitative Sensory Testing (Heat pain threshold) and structural brain alterations in female (A) and male (B) subjects. Plots represent beta and standard error; Beta is the difference in standardized brain volume per degree of temperature (Celsius); Analyses were adjusted for age, intracranial volume and depression. *P < 0.05. Thal indicates thalamus; Hippo, Hippocampus; Amyg, Amygdala; OFC, Orbitofrontal cortex; vlPFC, ventrolateral prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; PCC, posterior cingulate cortex; MCC, mid cingulate cortex; ACC, anterior cingulate cortex; Insula, insular cortex; S1, primary somatosensory cortex.

## Discussion

In this large population-based cohort of individuals with ages ≥ 45 years, we observed that chronic musculoskeletal pain was associated with a smaller global gray matter volume in the women. This smaller volume was primarily found in the temporal lobe, more specifically, in the hippocampus, part of the limbic system. In addition, again in the women, a lower heat pain threshold, which indicates higher (central) pain sensitivity, was associated with smaller volumes of the hippocampus, thalamus, and anterior cingulate cortex, regions that are involved in the limbic system and descending pain processing pathways. In the men, no significant associations between chronic joint pain or heat pain thresholds and brain volumes were observed.

4.1

To our knowledge, this is the first study that examined the association between chronic joint pain and structural brain changes in a population-based study. The number of studied patients was approximately 30 times larger than any previous study that examined the relationship between chronic pain and structural brain changes. Previous studies that examined structural brain alterations in pain consisted mostly of small and very specific clinical patient populations. [6-13,15] We used a hierarchical approach in studying brain structural differences. We first examined global brain tissue volumes and lobar volumes. Subsequently, we investigated those brain regions that were reported at least twice in the previous literature to be associated with chronic musculoskeletal pain. This strategy was chosen because previous studies showed inconsistent findings, which might be due to the different clinical pain phenotypes and low power that led to conflicting results, as highlighted previously. [16] Indeed, we were unable to replicate most of the previously implicated brain regions, which indicated that these brain regions are not consistently associated with chronic musculoskeletal pain.

The development of the brain is sex specific and influenced by sex hormones. It is shown that sex differences are also present with respect to pain processing. [44-48] Therefore, we stratified our analyses according to sex. In women, gray matter in the temporal lobe and, especially, in the hippocampus was smaller in those with chronic pain. The hippocampus has previously been suggested as one of the altered structures in the brain in several pain states. [6,7] In women, this involvement of the limbic system, therefore, could indicate a more emotional coping of pain.

Smaller volumes of the hippocampus, thalamus, and anterior cingulate cortex were also associated with lower heat pain thresholds in women in our study. The thalamus is important in the descending inhibitory signaling, which is known to be compromised in central sensitization in chronic pain, [5,7] which makes our findings more plausible. To our knowledge, this is the first study that examined the associa-

tion between heat pain thresholds and brain structure volumes. A limitation of the analysis of heat pain threshold and brain volumes was that the 2 measurements were done during 2 different visits, with several years in between. To minimize this time bias, we examined only those participants who had chronic pain at both visits versus those who had no chronic pain at both visits.

In this study, we examined both the presence of chronic musculoskeletal pain and also heat pain sensitivity thresholds and their relationship to structural brain alterations. The presence of chronic musculoskeletal pain is a very subjective phenotype because it is determined by using questionnaires, and there is no test to measure pain. Heat pain thresholds are closely related to the sensitivity for developing chronic pain and for having chronic pain, which, therefore, makes it a more objective measure for chronic pain. The combined use of questionnaire data and heat pain thresholds to find associations with structural brain alterations, therefore, strengthens the results.

A possible disadvantage of population-based studies is the more heterogeneous pain phenotype compared with the selected clinical populations. However, this reflects the situation in the general population and shows that central sensitization occurs not only in a selected patient population. In addition, chronic pain in community-dwelling subjects represents a huge problem, which affected 35.3% of our study population. However, its nature and cause is poorly understood, and often no apparent reason can be assigned to the chronic pain state. Studying pain in an unselected population without the selection bias of clinical reference could provide new insight in possible pathways involved in any chronic pain state.

The cross-sectional aspect of this study made us unable to speculate on the brain volume changes being a cause or an effect in the pathology of chronic pain. A previous study on structural brain changes in pain related to severe hip osteoarthritis showed normalization of these differences after hip replacement surgery, which indicates that the pain is causing structural brain changes, [39] but larger longitudinal studies are necessary for confirmation because sample size was small in this study (n= 10).

Depression coincides with chronic musculoskeletal pain, and, because both might affect the limbic system, [11] we adjusted the analysis for the presence of depression. In addition, excluding persons with depression from the model did not change the results, which indicated that our findings were not influenced by the presence of depression.

Because we started our study hypothesis-free and continued examining smaller regions, we performed a considerable amount of tests, which might have led to spurious findings. If we would have used a Bonferroni correction for the statistically significant P-value for the brain structures, then this would result in a P-value of

0.05/11 = 0.004; this is when assuming independency of the tests. Many results would still be considered statistically significant. However, not all of the tests were independent because the smaller regions were included in the larger lobes. Therefore, deciding which exact P-value to use would have been challenging. In addition, the performed analyses were not hypothesis-free because we tried to replicate previous published results. Moreover, especially in the women, we showed very consistent and robust findings, with increasing effect sizes when we narrowed the examined regions.

## Conclusions

In this large population-based study, we found that chronic musculoskeletal pain was associated with structural changes in parts of the limbic system in the brain. The hippocampus, especially, showed a very consistent and strong relationship with chronic joint pain and heat pain thresholds in women, which indicated a key role in the development of central sensitization and chronic pain. Structural alterations in the brain in individuals with chronic pain support the presence of central sensitization. This process of central sensitization increases the risk for a longer period of chronic pain and increases the risk for developing chronic pain at other sites. [5] These results stress the importance of a multidisciplinary and sex-specific therapeutic approach to improve successful treatment.
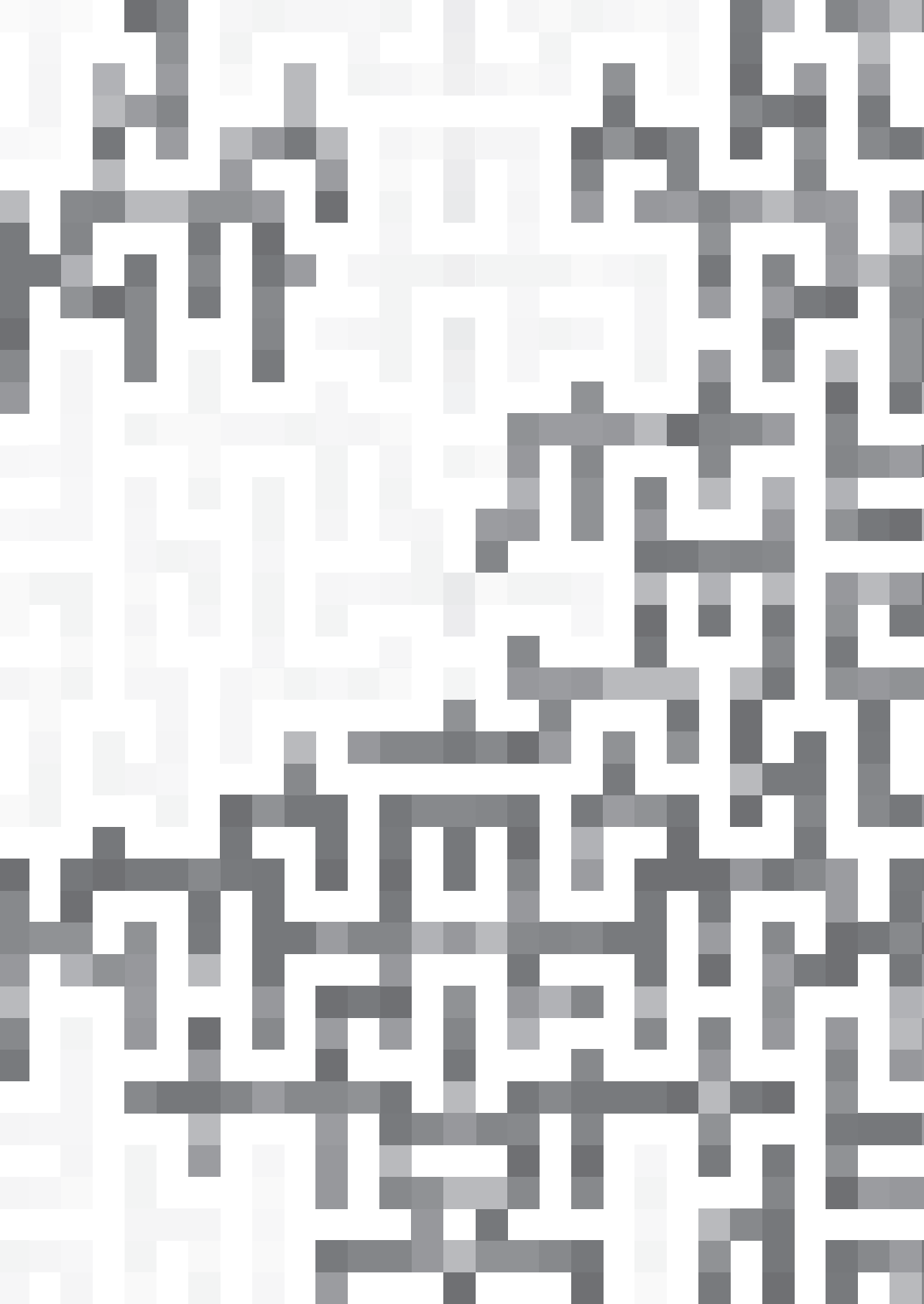
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**Supplemental table** 1. Selected articles for review of previously found associations between brain region volumes and chronic pain phenotypes

| Article | Brain regions identified | Direction +/- |
|---|---|---|
| Ivo et al. 2013 [9] | Dorsolateral prefrontal cortex | - |
| | Thalamus | - |
| | Midcingulate cortex | - |
| Robinson et al. 2011 [11] | Midcingulate cortex | - |
| | Anterior cingulate cortex | - |
| | Insular cortex | - |
| Zimmerman et al. 2009 [14] | Hippocampus | - |
| Apkarian et al. 2004 [18] | Dorsolateral prefrontal cortex | - |
| | Thalamus | - |
| Buckalew et al. 2008 [33] | Posterior parietal cortex | - |
| | Midcingulate cortex | - |
| Burgmer et al. 2009 [34] | Prefrontal cortex | - |
| | Amygdala | - |
| | Anterior cingulate cortex | - |
| Jensen et al. 2013 [35] | Anterior cingulate cortex | - |
| Kong et al. 2013 [36] | S1 | + |
| Kuchinad et al. 2007 [37] | Cingulate cortex | - |
| | Insular cortex | - |
| | Medial frontal cortex | - |
| | Parahippocampal giri | - |
| Lutz et al. 2008 [38] | Postcentral giri | - |
| | Amygdala | - |
| | Hippocampus | - |
| | Superior frontal cortex | - |
| | Anterior frontal cortex | - |
| | Anterior cingulate cortex | - |
| Rodriguez-Raecke et al. 2009 [39] | Anterior cingulate cortex | - |
| | Insular cortex | - |
| | Operculum | - |
| | Dorsolateral prefrontal cortex | - |
| | Amygdala | - |
| | Brainstem | - |
| Schmidt-Wilcke et al. 2007 [40] | Superior temporal girus | - |
| | Thalamus | - |
| | Orbitofrontal cortex | + |
| | Cerebellum | + |
| | Striatum | + |
| Seminowicz et al. 2011 [41] | S1 | - |
| | Insular cortex | - |
| | Anterior cingulate cortex | - |
| | Dorsolateral prefrontal cortex | - |
| | Ventrolateral prefrontal cortex | - |
| | Orbitofrontal cortex | - |
| Valet et al. 2009 [42] | Insular cortex | - |
| | Anterior cingulate cortex | - |
| | Posterior cingulate cortex | - |
| | Orbitofrontal cortex | - |
| Wood et al. 2009 [43] | Parahippocampal giri | - |
| | Posterior cingulate cortex | - |
| | Anterior cingulate cortex | - |

4.1

# CHAPTER 4.2

Chronic musculoskeletal pain is related to cerebral white matter microstructural integrity: a population-based study

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## Abstract

*Background:* Increasing evidence relates chronic pain to grey matter alterations of the brain, but the role of white matter remains unclear, although it is known to facilitate signal transmission. Therefore, we investigated the association of chronic pain and white matter microstructure.

*Methods:* 3509 participants from the population-based Rotterdam Study (mean age 59.5 years) underwent chronic pain assessment and diffusion-MRI scanning. First, we studied the association of chronic pain with fractional anisotropy (FA) and mean diffusivity (MD) of global and lobar white matter. Second, we performed tract-specific analyses for 25 predefined white matter tracts, including axial diffusivity (AD) and radial diffusivity (RD). Finally, we investigated whether the identified tracts associated with perseverance of chronic pain.

*Results:* Chronic pain associated with lower global MD (beta −0.05, p-value 0.04), mainly driven by lower MD in the left frontal lobe (−0.05, p-value 0.05) and left temporal lobe (−0.07, p-value 0.01). Tract-specific analyses showed an association of chronic pain with diffusion-MRI parameters in the left medial lemniscus (FA: beta 0.09, p-value 0.0002; MD: −0.07, p-value 0.01, RD: −0.09, p-value 0.002). Medial lemniscus FA was associated with chronic pain persistence after 5 years of follow-up (beta 0.13; p-value 0.01).

*Conclusions:* Chronic pain is associated with lower MD in the left frontal and temporal lobe, and with higher FA, lower MD, and lower RD in the left medial lemniscus. These findings suggest increased wiring of the cerebral white matter, which may be lateralized. Left medial lemniscus FA related to persistence of chronic pain, indicating a potential prognostic value.

## Introduction

Chronic musculoskeletal pain is very common, with a prevalence of 40-60 percent, and increases with age[9]. In a chronic pain condition, central sensitization can cause a higher sensitivity for clinical and experimental pain [18, 31]. This mechanism of central sensitization is not yet fully understood, but structural and functional changes in the brain are thought to be involved. There is growing evidence for grey matter alterations in studies comparing chronic pain patients with healthy controls [1, 11, 20, 23]. We recently showed that grey matter alterations are also present in a population-based study of elderly community-dwelling individuals with chronic pain [6]. However, little research has been performed on the involvement of cerebral white matter in chronic musculoskeletal pain.

White matter and grey matter together facilitate signal transmission to and from the spinal cord and between brain regions. Therefore, studying the relation between chronic pain and white matter can bring us closer to understanding the brain involvement in chronic pain. Diffusion-magnetic resonance imaging (MRI) is an advanced MRI-technique to investigate the microstructure of cerebral white matter. The diffusion-MRI metrics fractional anisotropy (FA) and mean diffusivity (MD) are most commonly studied. FA correlates with white matter tract coherence and is influenced by factors such as the number of axons, their geometry and the myelinisation of axons. MD, the average rate of water diffusion per voxel, is sensitive to cellularity, edema and necrosis [30]. In addition, axial and radial diffusivity may provide complementary information on mechanisms of change in white matter microstructure. Results of animal studies have indicated that decreased axonal diffusivity is related to axonal injury and that increased radial diffusivity is associated with myelin breakdown [25].

Previous studies investigating the relation between chronic pain and white matter microstructure have focused on specific chronic pain disorders, such as irritable bowel syndrome, fibromyalgia, migraine and chronic pancreatitis[8, 10, 20, 26, 27], but the results have been inconsistent and lacked power to determine the common and specific white matter tracts involved in chronic pain.

In our study, a large prospective population-based cohort of individuals aged 45 years and older, we investigated associations of chronic musculoskeletal pain and global, lobar and tract-specific diffusion-MRI parameters. Herewith, we aimed to identify robust and more generalizable associations and add to the knowledge of the pathophysiology of central sensitization in chronic musculoskeletal pain.

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## Materials and Methods

### Study population

The Rotterdam Study is a population-based cohort study among inhabitants of Ommoord, a suburb of Rotterdam, the Netherlands [13]. The original cohort (RS-I) consisted of 7983 participants, aged 55 years and older. In 2000 there was a first expansion of the cohort adding 3011 persons who had become 55 years of age or had moved into the district since the start of the study (RS-II), and in 2005 there was a second expansion with 3932 participants who had become 45 years of age or had moved into the district (RS-III).

The participants in the study as presented here were derived from the Rotterdam Scan Study, an ongoing population-based cohort study investigating brain changes on MRI, which is embedded in the Rotterdam Study since 2005 [14]. A total of 5430 eligible participants were invited from all three Rotterdam Study cohorts. After excluding individuals who were diagnosed with dementia or had MRI contraindications (including claustrophobia) 4841 participated and underwent diffusion-MRI scanning of the brain. Among them, 53 scans were excluded due to incomplete acquisitions and 112 scans with artifacts hampering automated processing were excluded. We excluded 160 individuals with MRI- defined cortical infarcts. Of the 4516 remaining participants with complete diffusion-MRI data, 3509 individuals also had pain data available, which constitutes the population for analysis in the present study.

The Rotterdam Scan Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. All participants gave written informed consent.

### Assessment of chronic musculoskeletal pain

A pain homunculus was used to report chronic painful regions in the body. The pain homunculus showed a picture of the front and the back of the human body. Participants were asked the question: "Did you have pain anywhere in your body, for at least half of the days, during the last six weeks?" Participants indicated painful areas by drawing circles around those painful areas. Trained researchers scored the homunculi using a template, to assign 14 different joint pain regions (e.g. neck, shoulders, elbows, hands, low back, hips, knees and feet). Chronic musculoskeletal pain was defined as persons having one or more painful regions. The pain homunculus was part of the exam during all baseline and follow-up visits to the research center. Since the MRI took place during a separate visit, we used the homunculi

closest to the MRI exam. Mean time interval between interview and MRI visit was 1.76 years (SD 1.43)

### MRI acquisition and processing

Brain MRI was performed on a 1.5T MRI unit (General Electric Healthcare, Milwaukee, USA, software version 11x) dedicated to research project. The imaging protocol and sequence are described in more detail elsewhere [14]. In short, a T1-weighted sequence, a proton density-weighted sequence (PD), a T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence, and a single shot, diffusion-weighted spin echo echo-planar imaging (EPI) sequence were included in the structural imaging protocol. Maximum b-value was 1000 s/mm$^2$ in 25 non-collinear directions; three volumes were acquired without diffusion weighting (b-value = 0 s/mm$^2$) [14].

A number of 1338 subjects were scanned with the phase and frequency encoding directions swapped for the diffusion acquisition due to a technical issue [5]. We treated this as a potential confounder, see statistical analysis. T1 and PD weighted scans were segmented into grey matter, white matter, cerebrospinal fluid (CSF) and background tissue, using an automated segmentation approach, based on a conventional k-nearest-neighbor classifier [3, 28]. Supratentorial intracranial volume was estimated by summing total grey and white matter volumes, and CSF volume. White matter lesions were segmented using the tissue segmentation and the FLAIR image with an automated post-processing technique [3]. An automated multi-atlas segmentation with majority voting was performed to segment the frontal, parietal, occipital and temporal lobe.

Cortical infarcts were rated visually on FLAIR, proton-density-weighted and T1-weighted sequences, and in case of involvement of grey matter, they were classified as cortical infarcts.

### Diffusion post processing and tractography

All diffusion data were pre-processed using a standardized pipeline [4]. In short, MRI data were corrected for subject motion and eddy currents. A diffusion tensor model was estimated on the corrected data to compute FA and MD [4]. The diffusion images were combined with the tissue segmentation to obtain global and lobar average diffusion-MRI measurements (FA, MD) inside the normal-appearing white matter (i.e. the white matter excluding white matter lesions). The corrected diffusion data was separately used for the diffusion tractography, described previously [5].

Inside 25 tracts, 11 of which were defined for left and right hemispheres separately, aggregated tract-specific white matter microstructural diffusion-MRI characteristics

(median FA, MD, radial, and axial diffusivity) were obtained and we standardized tract-specific diffusion-MRI measures (zero mean, unit SD). Tracts were categorized into brainstem tracts, projection tracts, association fiber tracts, limbic tracts and callosal tracts, based on anatomy [5].

Tract segmentations were used to obtain tract-specific volumes and, combined with the tissue segmentation, white matter lesion volumes. White matter lesion volumes were natural log transformed to accommodate their skewed distribution.

We could not fully incorporate the cerebellum in the field of view of the diffusion scan. Therefore, for tractography alternative seed masks were selected until reasonable coverage was achieved to account for partial coverage of the medial lemniscus at the lower border of the scan. This was treated as a potential confounder in all models including the medial lemniscus (see statistical analysis).

### Statistical analysis

Associations of chronic pain with diffusion-MRI parameters of white matter microstructure globally (FA, MD), per lobe (FA, MD), and tract specifically (FA, MD, axial and radial diffusivity) were evaluated using multiple linear regression models.

Mean differences in z-scores (95% CI) between chronic pain versus no pain groups were measured. Analyses were adjusted for age, sex, intracranial volume, white matter volume, and white matter lesion volume (using tract-specific volumes and white matter lesion volumes in the tract-specific analyses).

In all analyses, we treated the phase encoding direction of the diffusion scan as a potential confounder. In all tract specific analyses in which the medial lemniscus was studied we additionally adjusted for the variable position of the seed masks, as explained above.

Additional analyses were performed for significant findings. We additionally adjusted the analyses for the presence of depression. The presence of depression was determined using the self-reporting CES-D scale (Center for Epidemiologic Studies Depression). A score above 16 was considered positive for the presence of depression. Gender differences were explored by stratification of the analyses by gender. Since we also had information on chronic pain at follow up (approximately 5 years later), we also performed a longitudinal analysis, to elucidate whether the whiter matter alterations could predict to perseverance of chronic pain over time. In this analysis, individuals with chronic pain on both visits were compared to the individuals whom had chronic pain on the first visit and did not have chronic pain anymore on the second visit. This analysis was executed for 2305 individuals of which 301 still had chronic pain.

For the tract-specific analysis, we corrected the p-value for multiple comparisons using Šidák correction, after estimating the number of independent tests, resulting

in a threshold for significance of $3.6*10^{-3}$ (at alpha level of 0.05). Analyses were carried out using SPSS 20.0.2 for Windows or R version 2.15.0.

## Results

### Population characteristics
Characteristics of the study population are described in Table 1. In total we compared 1499 individuals with chronic musculoskeletal pain to 2010 individuals without pain. Persons with chronic pain were more often female (61% vs 50%) and the mean age was slightly higher (60 vs 59 years). The presence of depression was different between groups (respectively 4.9% and 13.1% for no chronic musculoskeletal pain and chronic pain). There was no significant difference between prevalence of pain in the left side of the body or the right side of the body (25% vs 24%).

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**Table 1.** Population characteristics

|  | Total, N=3509 | No chronic pain, n=2010 | Chronic pain, n=1499 | Difference, p-value |
|---|---|---|---|---|
| Age (years) | 59.5 (8.2) | 59.0 (8.1) | 60.3 (8.4) | $7.93*10^{-6}$ |
| Female gender | 55% | 50% | 61% | $2.05*10^{-11}$ |
| CESD positive** | 8.4% | 4.9% | 13.1% | $5.44*10^{-18}$ |
| Intracranial volume (ml) | 1128 (119) | 1135 (119) | 1117 (118) | $4.21*10^{-6}$ |
| White matter volume (ml) | 412 (60) | 415 (60) | 408 (59) | $1.77*10^{-4}$ |
| White matter lesion volume (ml)* | 4.48 (6.82) | 3.49 (4.06) | 3.85 (4.44) | 0.06 |
| Global mean FA | 0.34 (0.01) | 0.34 (0.01) | 0.34 (0.01) | 0.47 |
| Global mean MD | 0.73 (0.02) | 0.73 (0.02) | 0.74 (0.02) | 0.39 |

Values represent means (SD) or percentages. *Values represent medians (IQR). **CESD positive is defined as CESD score >16. Abbreviations: FA; fractional anisotropy, MD; mean diffusivity, $10^{-3}$ mm$^2$/sec.

### Chronic pain and global and lobar diffusion-MRI measures
The association between chronic musculoskeletal pain and global and lobar diffusion measures of white matter microstructure is shown in Table 2. We observed lower global MD in individuals with chronic musculoskeletal pain compared to those without chronic pain (mean difference in z-score −0.05, p-value 0.04). For the analysis of separate brain lobes, again a lower MD was found for presence of chronic musculoskeletal pain, in the left frontal lobe (mean difference in z-score −0.05, p-value 0.05) and in the left temporal lobe (mean difference in z-score −0.07, p-value 0.01). There was no association between chronic musculoskeletal pain and FA, neither on a global nor lobar level.

**Table 2.** Chronic musculoskeletal pain versus no pain and global diffusion-MRI measures

|  | **Fractional Anisotropy** | **Mean Diffusivity** |
|---|---|---|
| Global | 0.01 (−0.05;0.06) 0.31 | **−0.05 (−0.10;−0.003) 0.04** |
| Lobar |  |  |
| Frontal left | 0.01 (−0.04;0.06) 0.71 | **−0.05 (−0.11;−0.0002) 0.05** |
| Frontal right | −0.03 (−0.08;0.03) 0.39 | −0.02 (−0.08;0.04) 0.55 |
| Temporal left | 0.04 (−0.02;0.10) 0.17 | **−0.07 (−0.12;0.01) 0.01** |
| Temporal right | −0.03 (−0.09;0.04) 0.43 | −0.03 (−0.08;0.02) 0.18 |
| Parietal left | 0.01 (−0.05;0.07) 0.70 | −0.04 (−0.09;−0.01) 0.10 |
| Parietal right | 0.003 (−0.06;0.06) 0.92 | −0.04 (−0.09;0.01) 0.13 |
| Occipital left | 0.03 (−0.04;0.09) 0.40 | −0.02 (−0.08;0.03) 0.37 |
| Occipital right | −0.003 (−0.07;0.06) 0.92 | −0.05 (−0.11;0.01) 0.14 |

Values represent the mean differences in z-scores (95% CI) and p-values between chronic pain vs no chronic pain. Linear regression adjusted for age, sex, intracranial volume, white matter volume and log-transformed white matter lesion volume. Mean diffusivity x $10^{-3}$ mm$^2$/sec.

**Chronic pain and tract-specific diffusion-MRI measures**

The complete results of the association analysis of chronic musculoskeletal pain and diffusion measures of all 25 white matter tracts are described in detail in Table 3.

A significant association of chronic musculoskeletal pain and tract-specific diffusion-MRI measures was found in the left medial lemniscus (Figure 1). FA in this tract was higher in individuals with chronic musculoskeletal pain (mean difference in z-score 0.09, p-value $2.0*10^{-4}$), MD and radial diffusivity were lower (mean difference in z-score −0.07, p-value 0.01, and −0.09, p-value $1.50*10^{-3}$ respectively). Additional adjustments for global FA or global MD did not change these results. None of the other white matter tracts showed a significant association between chronic musculoskeletal pain and white matter diffusion measures.

**Additional analyses for the significant associations of white matter diffusion-MRI and pain**

Additional adjustment for depression for the association of global MD, left frontal lobe MD and left temporal lobe MD with chronic musculoskeletal pain, did not change the effect sizes and significance (beta −0.05; p-value 0.02, beta −0.06; p-value 0.05 and beta −0.06; p-value 0.02 respectively). For the MRI diffusion mea-
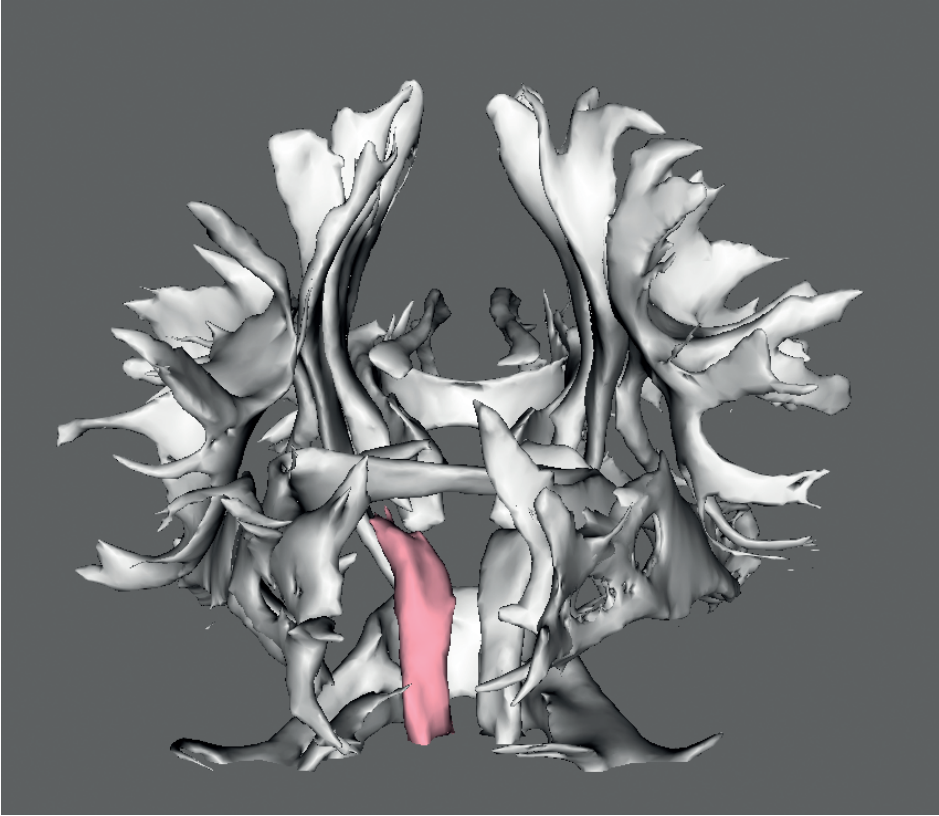
**Table 3.** Chronic musculoskeletal pain versus no pain and tract specific diffusion-MRI measures

| | Fractional anisotropy | Mean diffusivity | Axial diffusivity | Radial diffusivity |
|---|---|---|---|---|
| **Tracts in brainstem** | | | | |
| Middle cerebellar peduncle | −0.01 (−0.06;0.03) | 0.01 (−0.04;0.07) | −0.001 (−0.04;0.04) | 0.01 (−0.06;0.07) |
| Left Medial lemniscus [a] | **0.09 (0.04;0.15)\*** | **−0.07 (−0.13;−0.01)\*** | −0.02 (−0.08;0.04) | **−0.09 (−0.15;−0.04)\*** |
| Right Medial lemniscus [a] | 0.02 (−0.05;0.06) | −0.05 (−0.11;0.01) | −0.002 (−0.07;0.02) | −0.04 (−0.10;0.02) |
| **Projection fibers** | | | | |
| Left Corticospinal tract | −0.01 (−0.07;0.06) | −0.02 (−0.07;0.02) | −0.02 (−0.07;0.03) | −0.02 (−0.07;0.04) |
| Right Corticospinal tract | 0.03 (−0.08;0.04) | −0.04 (−0.07;0.05) | −0.02 (−0.09;0.03) | −0.04 (−0.07;0.05) |
| Left Anterior thalamic radiation | 0.002 (−0.05;0.05) | −0.02 (−0.05;0.02) | −0.01 (−0.04;0.03) | −0.02 (−0.05;0.02) |
| Right Anterior thalamic radiation | −0.002 (−0.05;0.05) | −0.01 (−0.05;0.03) | 0.004 (−0.03;0.04) | −0.01 (−0.05;0.03) |
| Left Superior thalamic radiation | 0.03 (−0.03;0.09) | −0.03 (−0.07;0.01) | −0.01 (−0.06;0.03) | −0.03 (−0.078;0.01) |
| Right Superior thalamic radiation | 0.04 (−0.02;0.10) | −0.02 (−0.06;0.02) | 0.01 (−0.03;0.05) | −0.04 (−0.08;0.01) |
| Left Posterior thalamic radiation | −0.05 (−0.10;0.01) | 0.01 (−0.03;0.05) | −0.002 (−0.05;0.04) | 0.02 (−0.02;0.06) |
| Right Posterior thalamic radiation | −0.02 (−0.07;0.04) | −0.01 (−0.05;0.03) | −0.01 (−0.06;0.03) | 0.003 (−0.04;0.04) |
| **Association fibers** | | | | |
| Left Superior longitudinal fasciculus | 0.02 (−0.03;0.08) | −0.02 (−0.07;0.02) | −0.02 (−0.06;0.02) | −0.02 (−0.07;0.02) |
| Right Superior longitudinal fasciculus | 0.02 (−0.04;0.07) | −0.03 (−0.07;0.02) | −0.03 (−0.08;0.01) | −0.03 (−0.07;0.02) |
| Left Inferior longitudinal fasciculus | 0.002 (−0.05;0.06) | −0.04 (−0.08;0.01) | −0.04 (−0.08;0.01) | −0.02 (−0.07;0.03) |
| Right Inferior longitudinal fasciculus | 0.001 (−0.05;0.06) | −0.02 (−0.06;0.03) | −0.03 (−0.07;0.02) | −0.02 (−0.07;0.03) |
| Left Inferior fronto-occipital fasciculus | −0.02 (−0.07;0.03) | −0.01 (−0.05;0.03) | −0.02 (−0.06;0.03) | −0.004 (−0.05;0.04) |
| Right Inferior fronto-occipital fasciculus | 0.01 (−0.04;0.06) | −0.02 (−0.07;0.02) | −0.03 (−0.08;0.02) | −0.02 (−0.06;0.02) |
| Left Uncinate fasciculus | 0.03 (−0.02;0.08) | −0.02 (−0.07;0.03) | 0.003 (−0.05;0.05) | −0.03 (−0.08;0.02) |
| Right Uncinate fasciculus | 0.03 (−0.02;0.08) | −0.03 (−0.07;0.02) | −0.01 (−0.05;0.04) | −0.04 (−0.09;0.02) |

4.2

**Table 3.** Chronic musculoskeletal pain versus no pain and tract specific diffusion-MRI measures (continued)

| | Fractional anisotropy | Mean diffusivity | Axial diffusivity | Radial diffusivity |
|---|---|---|---|---|
| *Limbic system fibers* | | | | |
| Left Cingulate gyrus part of cingulum | −0.03 (−0.09;0.03) | 0.01 (−0.05;0.07) | −0.02 (−0.08;0.05) | 0.03 (−0.03;0.08) |
| Right Cingulate gyrus part of cingulum | 0.02 (−0.04;0.09) | −0.02 (−0.08;0.04) | 0.02 (−0.04;0.08) | −0.03 (−0.09;0.03) |
| Left Parahippocampal part of cingulum | 0.02 (−0.04;0.08) | −0.03 (−0.05;0.07) | −0.03 (−0.09;0.03) | −0.02 (−0.03;0.08) |
| Right Parahippocampal part of cingulum | −0.02 (−0.08;0.04) | −0.01 (−0.07;0.05) | −0.03 (−0.09;0.03) | −0.01 (−0.07;0.05) |
| *Callosal fibers* | | | | |
| Forceps major | 0.02 (−0.03;0.07) | −0.03 (−0.08;0.04) | 0.001 (−0.05;0.05) | −0.02 (−0.09;0.03) |
| Forceps minor | −0.02 (−0.07;0.03) | −0.01 (−0.05;0.04) | −0.04 (−0.10;0.02) | 0.01 (−0.04;0.05) |

Values represent the mean differences in z-scores (95% CI) between chronic pain vs no chronic pain. Results in bold were significant after correction for multiple testing ($p<3.6*10^{-3}$). Linear regression adjusted for age, sex, intracranial volume, white matter and the log-transformed white matter lesion volumes of the investigated tract. [a] Additionally adjusted for the variable position of the seed mask. Fractional anisotropy, 0-1; Mean diffusivity, $10^{-3}$ mm²/sec.

**Figure 1.** The left medial lemniscus, a white matter tract significantly associated with chronic musculoskeletal pain, is shown in pink

sures of the left medial lemniscus, the only significantly associated white matter tract with chronic pain, the association also remained significant when additionally adjusting for the presence of depression (MD beta −0.09; p-value 0.004 and FA beta 0.09; p-value $1.0*10^{-3}$). A stratified analysis according to gender did not reveal gender differences in the associations (results not shown).

   Longitudinal analyses; To further investigate whether changes in the white matter microstructural integrity predicted the perseverance of chronic pain, we examined the association of MRI diffusion measures of the left medial lemniscus between individuals who still have chronic pain approximately 5 years later and those individuals of whom the pain had disappeared. Fractional anisotropy of the left medial lemniscus was associated with persistence of chronic pain (beta 0.13; p-value 0.01). Mean diffusivity was not significantly associated with persistence of chronic musculoskeletal pain, showing a similar effect as in the cross-sectional analysis (beta −0.043; p-value 0.446).

## Discussion

In this study, we observed lower mean diffusivity of global normal-appearing cerebral white matter in individuals with chronic musculoskeletal pain compared to individuals without pain. This association was mainly driven by lower MD in the left frontal and left temporal lobe. Additionally, in the tract-specific analysis, we found that chronic pain was associated with higher FA, a trend towards a lower MD, and lower radial diffusivity in the left medial lemniscus, but not in other tracts. Interestingly, we found that this association is present in individuals that have persistent pain especially, indicating a potential prognostic value.

To the best of our knowledge, this is the largest study to investigate the association between chronic musculoskeletal pain and diffusion-MRI measures of white matter microstructure globally, per lobe and tract-specifically. In comparison, the largest study up to now examining this relationship was a case-control study of 46 cases with 33 age-matched controls [19]. Another strength of this study is the population-based setting reducing selection bias, which might influence case control studies. This will increase the generalizability of the results to the general population with everyday pain. In addition, the fact that we examined an overall chronic musculoskeletal pain phenotype adds to the generalizability of the results to all sorts of chronic musculoskeletal pain instead of specific chronic pain states such as fibromyalgia or low back pain. Furthermore, diffusion-MRI measurements were performed with fully automated methods (observer-independent), that are publicly available [5].

Our study also has some limitations. A potential disadvantage is misclassification of the determinants, since pain questionnaires are subjective. Misclassification of individuals with or without chronic pain can occur in both ways. It is, however, not certain if misclassification will occur with greater frequency in one of the two groups or that this misclassification will be non-differential and thus will only dilute the results. In the tract-specific analyses we used median FA, MD and median axial and radial diffusivity, instead of the mean, since the median is more robust to variations in the tails of the measurement distributions. While effective to reduce the dimensionality of the analysis, this might have discarded spatial information that is retained in voxel-based techniques. An important limitation is that the cerebellum was not fully incorporated in the diffusion scan and the varying field of view makes conclusions on brain stem tracts less reliable.

In individuals with chronic pain, we found a lower global MD mainly driven by a lower MD in the left frontal and left temporal lobe. These findings point towards higher degree of microstructural integrity in individuals with chronic pain. The frontal and temporal lobe are part of the limbic system which plays an important

role in the emotional response to chronic pain [22]. More activity in these areas might have spurred further microstructural reinforcement and thereby explain these findings. In the tract-specific analysis, chronic musculoskeletal pain associated with higher FA and lower radial diffusivity in the medial lemniscus only. This is in contrast to a recent study [19] investigating tract-specific white matter involvement in chronic musculoskeletal pain patients. This study found lower values of FA and higher values of radial diffusivity in several white matter structures; corpus callosum, cingulum, internal capsule and external capsule, uncinated fasciculus, superior longitudinal fasciculus and the cerebral peduncles containing the corticospinal tracts in chronic low back pain patients compared to healthy controls. Our study differs from the study above at essential points. We used automated measurements instead of region of interest-based measurements which might lead to more objective measurements. Furthermore, unlike earlier studies, we controlled our analyses for macrostructural white matter changes ((tract-specific) white matter volume and white matter lesion volume), to filter out real microstructural changes of the white matter.

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Our findings of lower values of MD and higher values of FA in the medial lemniscus suggest a better white matter microstructural integrity of this white matter tract, in line with another study [16] that found denser white matter connections cerebellar in fibromyalgia patients compared to healthy controls. This study suggests that in fibromyalgia patients, the medial prefrontal and orbitofrontal cortex loses connections with neighboring frontal regions and in contrast to this the cerebellum gains connections. Furthermore, denser white matter connections were related to greater evoked pain hyperalgesia and clinical pain interference. In chronic musculoskeletal pain, painful stimuli are continuously being transferred and processed. Chronic painful stimuli therefore possibly result in an increased tract density or increased 'wiring' of white matter tracts, since a previous study reported on remodeling of brain tissue after periods of neuronal activation [2], leading to (or coinciding with) central sensitization. In this phenomenon the central nervous system becomes more sensitive to painful stimuli in general and the descending inhibitory system is compromised. Dorsal column-medial lemniscal (DCML) system neurons are involved in conditioned pain modulation (the diffuse noxious inhibitory control-like effect). Neurons of the DCML system send out collateral branches that synapse with association neurons in the posterior horn of the spinal cord. Action potentials travelling through the lateral spinothalamic tract can be suppressed by action potentials that originate in neurons of the DCML system. Increased activity in the DCML tends to close the gate, reducing pain action potentials transmitted [24].

In the stratified analyses for gender we did not find gender differences. Adjusting for gender is therefore sufficient in studying diffusion MRI measures and chronic musculoskeletal pain, to correct for differences in other determinants between genders, such as the higher presence of chronic pain in women.

We observed that chronic pain primarily associated with white matter microstructural integrity of the left hemisphere. It has been hypothesized that chronification of pain occurs more often on the left side of the brain, because the right cerebral hemisphere would be less efficient in processing painful stimuli [12, 21]. Existing literature is contradictory whether or not lateralization of chronic pain and sensitization in the brain exists [2, 17, 21, 29]. There was no clear difference between the prevalence of left of right-sided chronic pain. Therefore, the presented data can be interpreted as evidence for left sided lateralization of the chronic pain mechanism in the brain.

In the global and lobar analyses, we only found associations with MD but not with FA. In the tract specific analysis however, we found the strongest associations with FA. Increased wiring of tracts may lead to higher values of FA per voxel with concomitant decrease in MD. In complex regions with crossing fiber anatomy, FA may behave counter-intuitively and is less sensitive, as explained before [7, 15]. This might attenuate differences detected by FA in a global analysis, making MD more powerful to detect differences in a global analysis. Tract-specific analyses, however, may be less susceptible than global or lobar approaches to include large regions of crossing fibers in general and in particular in case of the medial lemniscus. Therefore, FA may be more sensitive to detect differences locally, than MD. Overall however, it should be noted that the different diffusion measures describe different aspects of white matter microstructure, which precludes one to one comparison in terms of sensitivity.

In conclusion, in a large population-based study of individuals of 45 years or older, we found indications for better wiring being present in the cerebral white matter in individuals with chronic musculoskeletal pain. This stronger wiring was mainly present lateralized in the left hemisphere in the frontal lobe, temporal lobe and medial lemniscus. This study adds to the existing knowledge of structural brain changes in chronic pain. The connections between cortical structures and the peripheral nervous system, as represented by the white matter, are also altered in chronic pain.
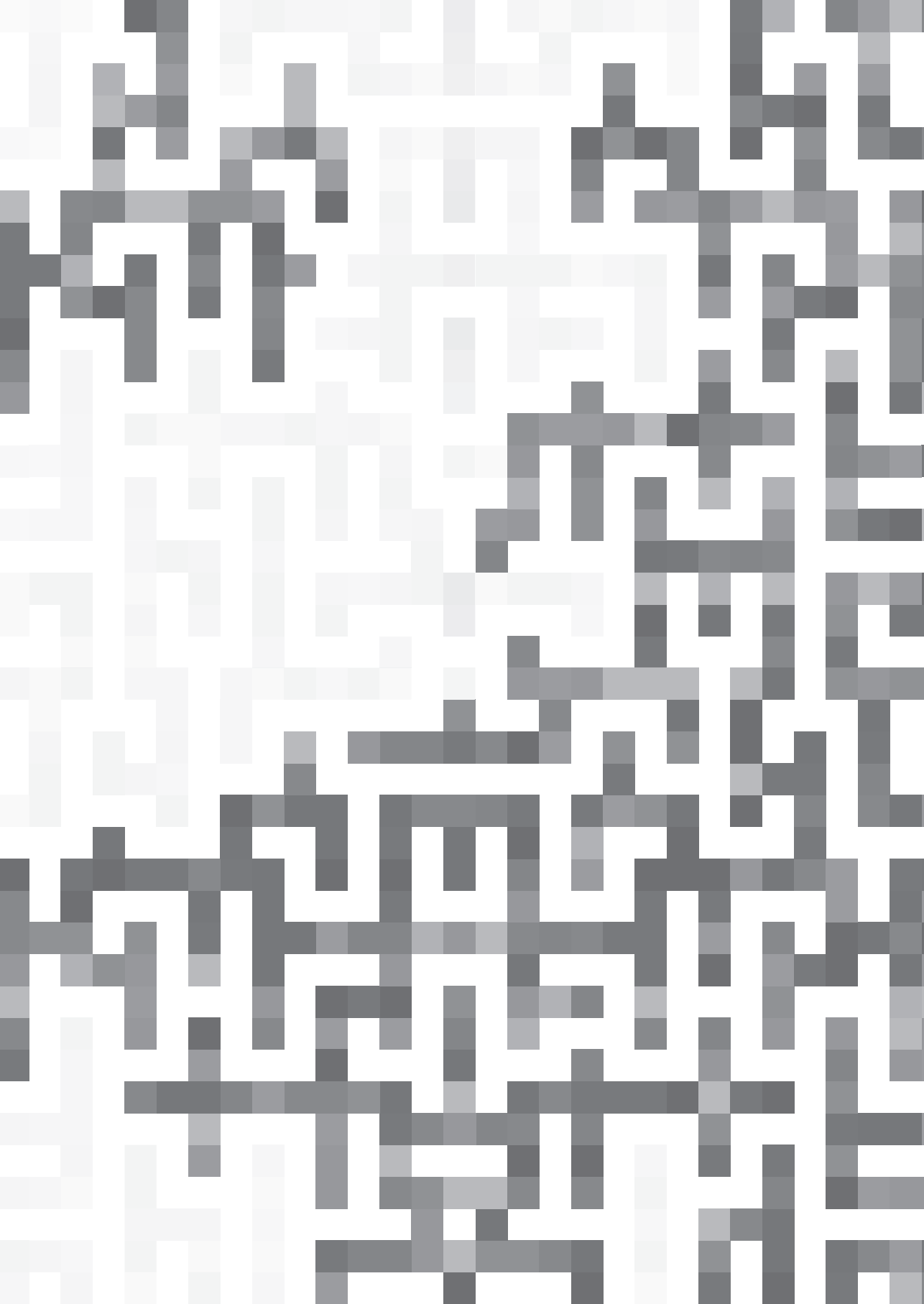
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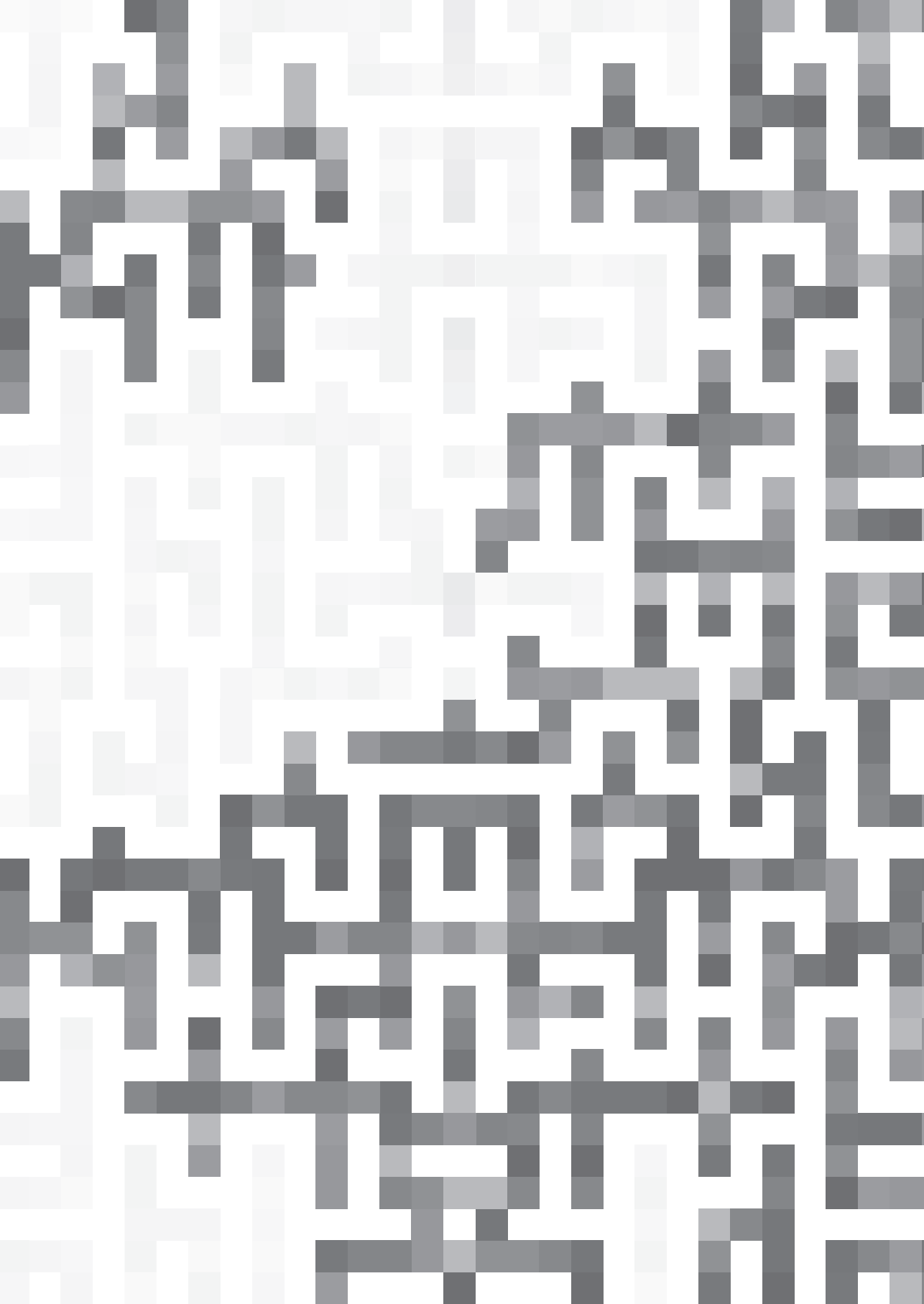# CHAPTER 5

Thermal quantitative sensory
testing (QST) and chronic pain

# CHAPTER 5.1

Determinants for quantitative sensory testing and the association with chronic musculoskeletal pain in the general elderly population

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## Abstract

*Objective:* Chronic musculoskeletal pain is accompanied by central sensitization, which can be determined with quantitative sensory testing (QST). In this study, we aim to investigate whether central sensitization, as measured by thermal QST, is detectable in community-dwelling elderly individuals suffering from self-reported chronic pain and identify determinants influencing thermal QST measurement analyses and interpretation.

*Methods:* In 3,936 participants of the Rotterdam Study, cold and warmth sensitivity and heat pain thresholds were determined using the thermo-sensory analyzer TSA II (Medoc Advanced Medical Systems, Durham, NC, U.S.A.). Using Cox regression, associations were studied with chronic pain and potential determinants (body mass index [BMI], reaction speed, systolic and diastolic blood pressure, skin color, skin temperature, seasonal influence, depression, anxiety, atopic eczema, age at menarche, years since menopause, hormone replacement therapy (HRT) use during menopause, and reproductive lifespan).

*Results:* In addition to the effect of age and gender on thermal sensitivity, darker skin color and the presence of atopic eczema were associated with higher sensitivity for heat pain. Cold sensitivity and warmth sensitivity thresholds were both influenced by BMI, reaction speed, skin temperature, season, depression, dark skin color, years since menopause, and reproductive lifespan. The presence of chronic pain was associated with 0.2 degrees lower heat pain threshold in all participants, and 0.3 degrees lower in individuals with chronic pain in more than 2 sites.

*Conclusion:* Higher sensitivity for heat pain, one feature of central sensitization, is present in community-dwelling elderly with chronic pain. Additional determinants should be considered when analyzing and interpreting QST measurements.

## Introduction

Quantitative sensory testing (QST) is a widely used method for assessing large and small nerve fiber function. It can be used to diagnose peripheral nervous system disorders but also diagnose and follow up sensitization of the central nervous system, as part of the pathophysiology of chronic pain. [1-5]

There are many different stimuli, which can be included in the protocol for quantitative sensory testing such as thermal, pressure, mechanical, electrical, and vibration stimuli. For the different stimuli, individual detection thresholds and pain thresholds can be determined. [6]

In previous articles, specific pain phenotypes originating from clinical study case groups have been studied, such as neuropathic pain, fibromyalgia, and osteoarthritis, and changes in QST measurements were observed. [1,3,5,7] The sample size of these studies was often modest. Efforts were made to define the reference thresholds for clinical use, but there is still limited information on thermal QST measurements in community-dwelling aging individuals. [6]

Age and gender were previously found to be associated with thermal QST measurements, and interaction between age and gender for some of the QST measures was suggested. [8] However, there may be additional determinants to take into account when analyzing and interpreting these measurements. For example, the body mass index (BMI) is associated with a higher incidence of chronic pain [9-10] and could therefore be a confounder in studies examining stimuli that are applied to the skin, such as thermal stimuli thresholds. Additionally, in 2 recent papers by Olsen et al., the association between blood pressure and acute pain sensitivity has been studied. They found that hypoalgesia is more prevalent in individuals with higher blood pressure which was hypothesized to be linked through chronic pain-related dysfunction in interacting cardiovascular–pain modulatory systems. [11,12]

As the QST measurements request the participants to push a "stop" button at a certain pain or sensitivity threshold, reaction speed may influence the results too, especially as the reaction time increases with age. When using thermal stimuli, it is plausible to take environmental temperature (seasons) or skin temperature into account.

Psychological factors have often been implicated to play a role in chronic pain, [13] and they might also have their influence on QST measurements, as mental state could influence performance on the test.

Another factor previously reported to be important in chronic pain is ethnicity or skin color; the prevalence of chronic pain and catastrophizing behavior has been reported to be higher in Afro-Americans compared to non-Hispanic whites. [14] Not only skin color but also skin conditions which compromise the skin barrier such

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as atopic eczema might lead to altered results in QST. Additionally, as itch stimuli and pain stimuli travel via the same nerve fibers, continuous itch could also cause sensitization. [15,16]

It is known that postmenopausal women are more prone to develop chronic pain, [17] possible by lowered sex hormones, and this might be reflected in the QST measurements as well. Therefore, we examined the influence of several hormone exposure-related phenotypes: age at menarche, years since menopause, reproductive lifespan, and hormone replacement therapy (HRT) use during menopause.

The aim of our study was to identify the determinants to be considered when interpreting thermal QST measurements, especially in the elderly population. In addition, we examined whether central sensitization is present and detectable in community-dwelling elderly individuals with chronic pain using a limited thermal QST protocol. Therefore, we studied the detection limits for cold and warmth stimuli and heat pain thresholds within the Rotterdam Study, a large prospective population-based study of individuals aged 45 years and over.

## Methods

### Study Population

The study population was embedded in the Rotterdam study, a large prospective population-based study of men and women of 45 years and older. The objective of the Rotterdam Study is to investigate determinants, incidence, and progression of chronic disabling disease in the elderly. The study design and rationale are described elsewhere in detail. [18] The first cohort, Rotterdam Study I (RS-I) started in 1989 with 7,983 participants. The study was extended in 1999 with another 3,011 individuals in Rotterdam study II (RS-II). The third cohort, Rotterdam Study III (RS-III) started in 2006 adding 3,932 participants. All participants were examined in detail at baseline and follow-up visits approximately every 6 years. In summary, a home interview was conducted and an extensive set of examinations at the research center was conducted.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)." All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

### Quantitative Sensory Testing

The quantitative sensory testing was conducted in a total of 4,039 participants. The measurements were performed between November 2010 and June 2014 during the fifth follow-up measurement of RS-I, the third follow-up measurement of RS-II, and the second follow-up measurement of RS-III.

We used a commercially available thermo-sensory analyzer, the TSA II (Medoc Advanced Medical Systems, Durham, NC, U.S.A.). The probe had a surface of 2 by 2 cm and was placed on the inner site of the non-dominant forearm.

We measured 3 different thermal sensitivity thresholds: the cold sensitivity threshold, the warmth sensitivity threshold, and the heat pain threshold. For each measurement, the starting temperature of the probe was 32°C, while a minimum of zero degrees Celsius and a maximum of 50°C were set for safety reasons. After each measurement, the temperature was reset to 32°C before starting the next measurement. The participants were asked to push a large "red quiz button" when reaching the test threshold. All 3 threshold measurements were repeated 5 times in a row with 5-seconds in between the tests. For the analysis, the mean value of the last 3 measurements was used. First, the probe decreased in temperature with 1 degree per second, to measure the cold sensitivity threshold. The participant was asked to push the button when feeling the sensation of a decrease in temperature. Second, the probe increased in temperature, with 1 degree per second, to measure the warmth sensitivity threshold. The participant was asked to push the button when feeling the sensation of an increase in temperature. The third measurement was the heat pain threshold: During this measurement, the probe temperature increased with 1.5 degrees per second. The participant was asked to push the button when the warmth stimulus started to feel unpleasant or painful.

Excluded from the population for analyses were 30 individuals with preexistent peripheral of central nerve damage, including cerebral vascular accidents and paralysis in the medical history.

### Chronic Musculoskeletal Pain

To identify individuals with chronic musculoskeletal pain, all participants completed a pain drawing. This drawing showed a drawing of the front and back of a human body, and the participant encircled the painful sites in their body where they experienced pain during at least half of the days during the last 6 weeks. In earlier analysis in this population, we observed that of all individuals indicating pain during the last 6 weeks, more than 90% of the reported pain was present for more than 3 months. Therefore, in this study, we considered the pain reported in the pain drawing as being chronic pain. The additional requirement for the individuals to have pain for at least half of the days adds a measure for clinical importance.

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The pain drawings were scored using a template with 14 different musculoskeletal joint regions (neck, lower back, 2 shoulders, 2 elbows, 2 hands, 2 hips, 2 knees, and 2 feet).

We examined 3 different pain phenotypes within this study. The first is whether the participant had pain anywhere in their body vs. participants without pain. The second phenotype is the number of different painful sites analyzed as a continuous variable: a score between 0 and 14. The third pain phenotype we defined studied a more severe pain phenotype: Cases were defined as participants having pain in more than 2 different sites of their body and controls having no pain. In this last analysis, we excluded individuals with pain at one or 2 sites.

### Body Mass Index and Blood Pressure

Height and weight were measured with the subject in a standing position with indoor clothing without shoes.

Body mass index (BMI) was calculated using the commonly used formula: $m/h^2$ (m = weight in kg; h = height in m). Blood pressure was measured twice in sitting position with an automatic device. The mean values of systolic and diastolic blood pressure were used in the analysis.

### Reaction Time

We measured reaction time in our study population using the computer program Reaction Time V4.03 (http://delphiforfun.org/programs/Reaction_times.htm). The participants were shown a big blue square on the screen of a computer and pushed a quiz button as fast as they could if the square appeared. This was repeated 7 times, and the mean of the last 5 measurements was calculated as the reaction time.

### Skin Temperature and Seasonal Influence

The skin temperature was measured using an infrared skin thermometer (Infrared clinical thermometer FTN, Medisana AG, Hilden, Germany). This measurement was performed at the same site of the QST measurement, the inner site of the non-dominant forearm, and the temperature was recorded in degrees Celsius.

Using the date of the measurement, we defined in which season the measurement took place. Seasons were defined astronomically; spring starting at March 21, summer starting at July 21, autumn at September 21, and winter at January 21.

### Psychological Factors: Depression and Anxiety

For the definition of depression, we used the Center for Epidemiological Studies Depression scale (CES-D). [19] This self-reporting scale of depressive symptoms

gives a score between 0 and 80. Depression was defined as a CES-D score of 16 or above.

We determined whether anxiety symptoms were present by a selection of questions from the Munich Composite International Diagnostic Interview. [20] Presence of anxiety symptoms was defined as ever having a sudden anxiety attack, unreasonable fear in specific circumstances and/or unusual fear, anxiety or worrying during the last 4 weeks.

### Dermatological Determinants: Skin Color and Atopic Eczema

Skin color was determined visually as described elsewhere. [21] During a full-body skin examination, the perceived skin color was graded by a trained physician into 5 categories of darkness, reflecting the observed constitutive skin color assessed at sun unexposed body sites. The perceived skin color categories included the following: (1) very white, (2) white, (3) white to olive, (4) light brown, (5) brown to black.

The presence of atopic eczema was determined by trained physicians at the research center and defined by the previously described UK diagnostic criteria of atopic dermatitis. These guidelines comprise one major criterion ("An itchy skin condition") plus 3 or more minor criteria ("history of involvement of the skin creases," "a personal history of asthma or hay fever," "a history of generally dry skin in the last year," "visible flexural eczema," and "onset under 2 years of age"). [22-24]

### Reproductive Traits: Years Since Menopause, Reproductive Lifespan

Data on menopause, menarche, and HRT use during menopause were derived from the home interview. We calculated years since menopause and reproductive lifespan. Reproductive lifespan was calculated by subtracting age at menarche from age at natural menopause. In the studied population, there were no premenopausal women included.

### Statistical Analysis

Associations of the quantitative sensory measurements with the various determinants were tested with Cox regression analyses. For safety reasons, the heat pain threshold measurement stopped at 50°C. However, approximately 15% of the participants reached this temperature without pressing the button; this means that the actual heat pain threshold of these individuals was higher than 50°C. To solve this issue, a Cox regression model was fitted and the subjects who reached the maximum temperature were censored. For the analysis, we used the time to threshold in seconds as outcome, to be able to compare the increase of temperature in the warmth sensitivity threshold and heat pain threshold and the decrease in tempera-

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ture in the cold sensitivity threshold measurement. Beta therefore represents the adjusted mean differences to the measured threshold per unit for the continuous determinants and adjusted mean differences to the measured threshold for presence of the dichotomous determinants. We also studied the association between age and the variance of the QST measurements within subjects. All analyses were adjusted for age and gender, and $P < 0.05$ was considered statistically significant.

For the association analysis between the QST measurements and chronic pain, we additionally performed analysis stratified for gender and age categories (50 to 59 years, 60 to 69 years, and 70 years and older). Also, we fitted a model with the newly found determinants as described above to study the influence of these determinants on the association of QST measurements and chronic pain.

Software package SPSS Version 21 (SPSS INC., Chicago, IL, U.S.A.) was used for all analyses.

## Results

### Population Characteristics

Within in Rotterdam study, quantitative sensory testing was conducted in a total of 4,053 participants between November 2010 and June 2014. A total of 103 participants were excluded (47 because of not being alert or very tired, 26 had problems with understanding the test, and 30 participants had other reasons for a potential unreliable test results including, for example, neurodegenerative disease such as paralysis; 14 subjects were excluded because of technical difficulties) resulting in 3,936 participants for the analysis. Population characteristics are shown in Table 1.

Mean age in this population is 66.0 years and 55.8% is female. Chronic pain is present in 45.4% of the individuals in the study and 12.9% have chronic pain in more than 2 sites of their body.

Figure 1 shows the distribution of the QST measures stratified for men and women. The warmth sensitivity measure showed a skewed normal distribution, the cold sensitivity measure distribution resembles half of a normal distribution. A considerable number of individuals did not reach their heat pain threshold before the measurement was stopped at 50°C for safety reasons, especially in men (24%).

### Potential Determinants for QST Measurements

The results of the univariate association analysis of the QST measures with the various potential influential determinants are shown in Table 2. As expected, age and gender were strong determinants for each of the QST measures. All other

**Table 1.** Population characteristics

|  | N= | Total, n=3,936 |
| --- | --- | --- |
| Age | 3,936 | 66.0 (7.6) |
| Female gender | 3,936 | 55.8% |
| BMI | 3,933 | 27.6 (4.4) |
| Blood pressure |  |  |
| Systolic, mmHg | 2,208 | 145 (22) |
| Diastolic, mmHg | 2,208 | 85 (11) |
| Reaction Speed, s | 3,828 | 0.37 (0.15) |
| Skin temperature, ˚C | 3,936 | 31.7 (0.9) |
| Season measurement | 3,936 |  |
| Spring | 953 | 24.2% |
| Summer | 623 | 15.8% |
| Autumn | 1,213 | 30.8% |
| Winter | 1,147 | 29.1% |
| Depression, CESD > 15 | 3,773 | 17.4% |
| Anxiety | 3,901 | 15.7% |
| Skin color | 2,528 |  |
| Very white | 146 | 5.8% |
| White | 1,908 | 75.5% |
| White to olive | 380 | 15.0% |
| Light brown | 45 | 1.8% |
| Brown to black | 49 | 1.9% |
| Atopic eczema (UK crit) | 3,723 | 6.0% |
| Age at menarche, years | 1,539 | 13.7 (6.0) |
| Years since Menopause | 1,440 | 18.5 (9.7) |
| HRT use during menopause | 1,540 | 9.3% |
| Reproductive Lifespan, years | 1,422 | 35.6 (7.0) |
| Chronic pain | 3,901 | 45.2% |
| Chronic painsites | 3,901 | 1.0 (1.8) |
| >2 chronic painsites vs no pain | 2,814 | 12.9% |
| Cold Sensitivity ˚C | 3,936 | 28.8 (2.5) |
| Warmth Sensitivity ˚C | 3,936 | 35.3 (2.0) |
| Heat Pain Threshold ˚C | 3,936 | 47.5 (3.0) |

Values represent means (and standard deviation) or percentages (%)

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potential determinants were tested for their association with the 3 QST thresholds, while adjusting for age and gender.

We also investigated the influence of age on the QST measurements itself. To this end, we studied the difference in variance in all 3 measurements for the age
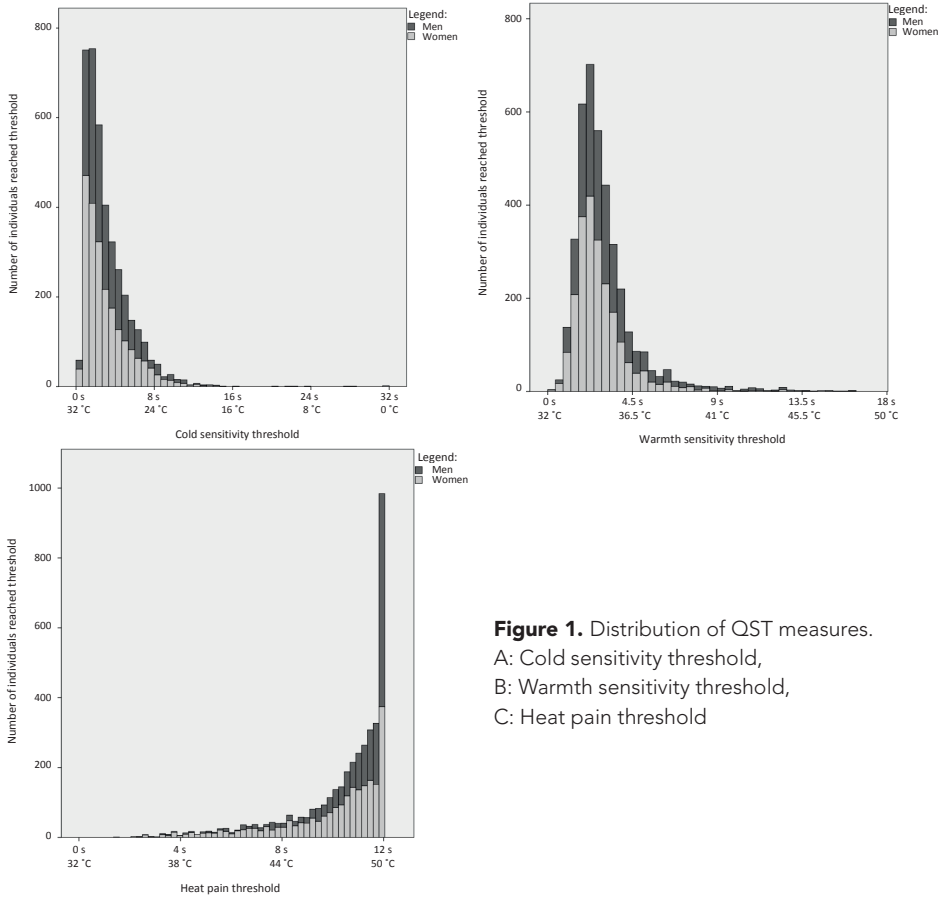
**Figure 1.** Distribution of QST measures.
A: Cold sensitivity threshold,
B: Warmth sensitivity threshold,
C: Heat pain threshold

categories 50 to 59 years, 60 to 69 years, and 70+ years of age (Table S1). As shown in Table 3, variance is increasing by age, for the heat and cold sensitivity threshold suggesting that these measurements are potentially less reliable in elderly subjects.

### Cold Sensitivity Threshold

For cold sensitivity, the reaction speed was a strong determinant, independently associated from age and gender. A slower reaction speed was highly significantly associated with a longer time to threshold (beta −0.014; P-value $9.82 \times 10^{-14}$). A BMI above the mean of 27.6 was associated with a cold sensitivity threshold (CST) that was 0.12 degrees lower than subjects with a BMI lower than 27.6, which means that these individuals were less sensitive for cold stimuli. A lower skin temperature and environmental temperature (in winter) were both associated with a lower sensitivity for cold. In winter, the cold sensitivity threshold was approximately 0.14 degrees higher than in spring. Subjects with depression or anxiety symptoms were sig-

**Table 2.** Cox regression analysis of QST-measures and the studied potential influential determinants

| | Cold sensitivity | | Warmth sensitivity | | Heat pain threshold | |
|---|---|---|---|---|---|---|
| | Beta (SE) | P-value | Beta (SE) | P-value | Beta (SE) | P-value |
| Age | **−0.021 (0.002)** | **2.42*10⁻²⁴** | **−0.029 (0.002)** | **2.92*10⁻⁴⁵** | **0.006 (0.002)** | **0.014** |
| Female gender | 0.036 (0.032) | 0.260 | **0.253 (0.032)** | **5.48*10⁻¹⁵** | **0.573 (0.036)** | **3.01*10⁻⁵⁷** |
| BMI | **−0.014 (0.004)** | **1.64*10⁻⁴** | **−0.011 (0.004)** | **0.002** | 0.006 (0.004) | 0.148 |
| Blood pressure* | | | | | | |
|     Systolic | −0.007 (0.010) | 0.490 | **0.023 (0.011)** | **0.037** | 0.003 (0.011) | 0.812 |
|     Diastolic | −0.001 (0.018) | 0.978 | 0.032 (0.019) | 0.092 | 0.014 (0.020) | 0.504 |
| Reaction Time | **−0.918 (0.123)** | **9.82*10⁻¹⁴** | **−0.642 (0.123)** | **1.86*10⁻⁷** | 0.117 (0.118) | 0.322 |
| Skin Temperature | **0.114 (0.018)** | **2.52*10⁻¹⁰** | **−0.102 (0.018)** | **1.20*10⁻⁸** | 0.003 (0.020) | 0.862 |
| Season(ref=Spring) | | | | | | |
|     Summer | −0.008 (0.052) | 0.880 | −0.040 (0.052) | 0.433 | −0.013 (0.056) | 0.818 |
|     Autumn | −0.010 (0.044) | 0.818 | **−0.126 (0.044)** | **0.004** | −0.037 (0.048) | 0.442 |
|     Winter | **−0.140 (0.044)** | **0.002** | **−0.136 (0.044)** | **0.002** | −0.056 (0.048) | 0.243 |
| Depression | **−0.096 (0.044)** | **0.028** | **−0.092 (0.044)** | **0.036** | 0.008 (0.047) | 0.859 |
| Anxiety | **−0.091 (0.045)** | **0.040** | −0.070 (0.044) | 0.117 | 0.036 (0.048) | 0.457 |
| Skin color (ref =White) | | | | | | |
|     Very white | −0.001 (0.087) | 0.994 | −0.050 (0.087) | 0.561 | 0.047 (0.091) | 0.604 |
|     White to olive | 0.038 (0.056) | 0.496 | 0.087 (0.057) | 0.125 | −0.003 (0.061) | 0.964 |
|     Light brown | −0.212 (0.152) | 0.163 | **0.426 (0.151)** | **0.005** | **0.365 (0.158)** | **0.021** |
|     Brown to black | **−0.631 (0.147)** | **1.66*10⁻⁵** | **0.329 (0.146)** | **0.024** | **0.620 (0.149)** | **3.22*10⁻⁵** |
| Atopic eczema | −0.042 (0.070) | 0.550 | 0.001 (0.070) | 0.984 | **0.186 (0.073)** | **0.011** |
| Age at menarche | 0.003 (0.004) | 0.491 | −0.0005 (0.004) | 0.911 | −0.006 (0.005) | 0.205 |
| Yrs since Menopause | **−0.011 (0.005)** | **0.021** | **−0.011 (0.005)** | **0.020** | −0.005 (0.005) | 0.340 |
| HRT use menopause | 0.037 (0.090) | 0.680 | 0.004 (0.090) | 0.962 | 0.096 (0.094) | 0.306 |
| Reproductive Lifespan | **0.010 (0.005)** | **0.023** | **0.010 (0.004)** | **0.030** | 0.005 (0.005) | 0.245 |

Beta represents adjusted mean difference in time (s) to threshold per unit of the continuous determinant or for the presence of the dichotomous determinant. Positive beta corresponds to shorter time to threshold and thus a higher sensitivity. SE: Standard error. Age analyses adjusted for gender and gender analyses adjusted for age. *Blood pressure: per 10 mmHg. All other analyses adjusted for age and gender.

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nificantly less sensitive to cold stimuli (beta −0.096 and beta −0.091, respectively). Individuals with brown to black skin color were less sensitive to cold stimuli and felt the probe getting colder at 0.6 degrees lower temperature than individuals with white skin. A longer time since menopause in women was associated with a lower cold sensitivity and a longer reproductive lifespan increased the sensitivity to cold.

**Table 3.** Association of QST measurement variance and age categories, adjusted for gender

| Age categories | Cold sensitivity threshold | | Warmth sensitivity threshold | | Heat pain threshold | |
|---|---|---|---|---|---|---|
| | Mean variance | p-value | Mean variance | p-value | Mean variance | p-value |
| 50-59 years n=941 | 0.800 | Reference | 0.585 | Reference | 3.089 | Reference |
| 60-69 years n=1936 | 1.075 | 0.016 | 0.636 | 0.550 | 3.080 | 0.958 |
| 70+ years n=1059 | 1.314 | $7.20*10^{-5}$ | 0.846 | 0.007 | 3.343 | 0.199 |

### Warmth Sensitivity Threshold

Similar to the cold sensitivity, reaction speed was also a highly associated determinant for warmth sensitivity thresholds (beta −0.642; P-value $1.86 \times 10^{-7}$). In addition, a higher BMI was associated with a higher warmth sensitivity threshold (beta −0.011; P-value 0.002). A higher systolic blood pressure was associated with a lower sensitivity for heat (beta 0.023 per 10 mm Hg; P-value 0.037). Both skin color and environmental temperature were associated with warmth sensitivity; in winter, the warmth sensitivity threshold was 0.14 degrees higher than in spring, which was mainly driven by skin temperature. Depressive symptoms were associated with higher detection thresholds, corresponding to lower sensitivity (beta −0.092; P-value 0.036).

Subjects with brown to black skin color were more sensitive to warmth stimuli than subjects with white skin color (beta 0.329; P-value 0.024). In women, the number of years since menopause was associated with a lower sensitivity for warmth stimuli (beta −0.011; P-value 0.020), and the length of the reproductive lifespan was associated with a higher sensitivity for warmth (beta 0.010; P-value 0.030).

### Heat Pain Threshold

The mean heat pain thresholds (HPT) were 0.6 degrees lower in women than in men. Participants with brown to black skin experienced the warmth stimulus as being unpleasant or painful 0.6 degrees earlier than participants with white skin. Additionally, individuals with atopic eczema were more sensitive to heat pain. No associations were found for the other potential determinants. Given the large differences in HPT measures between the sexes, we additionally stratified the analysis according to sex and found similar results.

### Chronic Musculoskeletal Pain

We studied whether the presence of chronic musculoskeletal pain was associated with altered QST measures. As shown in Table 4, neither cold sensitivity nor warmth sensitivity threshold was associated with chronic pain (Table 4). However, the heat

**Table 4.** Cox regression analysis for the relation between QST-measures and chronic pain

|  | Beta (SE) | P-value |
|---|---|---|
| Cold Sensitivity |  |  |
| Chronic pain | 0.007 (0.033) | 0.839 |
| Painsites | 0.011 (0.009) | 0.211 |
| >2 painsites vs no pain | 0.080 (0.050) | 0.106 |
| Warmth Sensitivity |  |  |
| Chronic pain | −0.060 (0.033) | 0.067 |
| Painsites | −0.002 (0.009) | 0.866 |
| >2 painsites vs no pain | −0.022 (0.050) | 0.660 |
| Heat Pain Threshold |  |  |
| Chronic pain | **0.108 (0.036)** | **0.002** |
| Painsites | **0.032 (0.009)** | **4.90*10$^{-4}$** |
| >2 painsites vs no pain | **0.195 (0.053)** | **2.43*10$^{-4}$** |

Beta represents adjusted mean difference in time (s) to threshold per unit of the continuous determinant or for the presence of the dichotomous determinant. Positive beta corresponds to shorter time to threshold and thus a higher sensitivity. SE: Standard error. All analyses adjusted for age and gender.

pain threshold (HPT) was associated with the presence of chronic musculoskeletal pain (beta 0.108; P-value 0.002), representing a higher sensitivity for heat pain in subjects with chronic pain compared to subjects with no chronic pain. Moreover, a larger effect size was observed when the more severe phenotype of chronic pain in more than 2 sites in their body was tested (beta 0.195; P-value 2.43 × 10$^{-4}$), corresponding to a 0.3 degrees lower HPT. In addition, the HPT was associated with the number of affected sites, indicating that this QST measure is related to the centralization and consequent spreading of chronic pain.

Stratification according to gender showed that the association of HPT with chronic pain was driven by women (beta 0.122; P-value 0.007 vs. beta 0.086; P-value 0.136 in men). We further examined whether age was affecting this association and examined whether the difference in HPT between individuals with and without chronic pain changed at different ages. For the individuals aged 50 to 59 years, beta was 0.177 with a P-value of 0.016; for the 60 to 69 years of age group a beta of 0.099, P-value 0.052; and for the oldest group aged 70 years and older, beta 0.049, P-value 0.471. These results suggest that central sensitization measured by HPT is less detectable in older subjects.

In a second model, we adjusted for the previously significantly associated determinants skin color and atopic eczema, and we found an attenuation of the observed association between chronic pain and HPT by skin color. After adjustment, there was no significant association between HPT and the presence of chronic pain but a

trend remained (beta 0.069; P-value 0.116). However, in this last model, much less subjects were available for the analysis (n= 2,515) because of missing values for skin color.

## Discussion

In this large prospective population-based study, we observed significant associations between chronic musculoskeletal pain and heat pain thresholds, indicating central sensitization is present in these community-dwelling individuals. Additionally, we found several determinants to be independently associated with thermal stimuli thresholds which might be important to consider when analyzing and interpreting test results. For cold sensitivity detection thresholds, the identified determinants were age, BMI, reaction time, skin temperature, winter, anxiety, depression, skin color, years since menopause, and reproductive lifespan. The determinants for the warmth sensitivity detection include age, sex, BMI, reaction time, skin temperature, autumn and winter, depression, skin color and years since menopause, and reproductive lifespan. Heat pain thresholds were independently associated with age, sex, skin color, and atopic eczema.

To our knowledge, this is the first study to examine determinants of thermal QST measurements and the association with chronic musculoskeletal pain in such a large set of individuals aged 50 years and over. A recent article by Johansen et al. studied QST measures in a large set of elderly individuals, but focused their research on persistent postoperative pain. [25] As the prevalence of chronic pain is increasing with age, this is a very relevant population to study pain sensitivity. As sensitivity to, for example, thermal, stimuli are lower in older subjects, reference values as determined in younger and healthier subjects can influence the interpretation of QST measurements. The reference values as previously determined could therefore be not very useful in an elderly population. We observed a considerable amount of the individuals in this study reaching the maximum threshold for the heat pain threshold (50°C), which was set because of safety reasons: 24% of men and 10% of women reached the threshold of 50°C. In addition, we found that in elderly subjects, the variance of the QST measures was also more pronounced, especially for cold sensitivity and warmth sensitivity thresholds, limiting the reliability of the results in these subjects. To our knowledge, this is the first study to show this limitation of the thermal QST in elderly subjects. This suggests that the heat pain threshold measurements might be difficult to interpret, especially in elderly men and other QST modalities might be more informative.

In our study, we investigated the sensitivity to thermal stimuli using 3 modalities: the cold detection threshold, the warmth detection threshold, and the heat pain threshold. In particular, the heat pain thresholds were previously identified to be associated with central and peripheral sensitization in individuals having chronic pain. Descending inhibitory pathways are compromised in chronic pain states, which increase the sensitivity to painful stimuli. However, up to now, this has only been determined in chronic pain patients in the clinic compared to healthy controls. We identified that the HPT is increased in community-dwelling elderly individuals with self-reported chronic pain and we additionally showed that the HPT is related to the number of pain sites, indicating spreading of pain. This indicates that central sensitization is present in the general population and stresses the importance of good treatment for acute pain to limit the number of subjects who develop chronic pain.

We observed a consistent association between BMI and the temperature sensitivity measurements. A higher BMI was associated with a higher prevalence of chronic pain, and potential mechanisms include higher levels of proinflammatory cytokines. These cytokines might also play a role in higher sensitivity for thermal stimuli, as they can sensitize the peripheral nerve endings. [26] Further research focusing on the influence of these proinflammatory cytokines on QST measures is necessary to elucidate this hypothesis.

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Reaction speed decreases with age and greatly influences QST results. In particular, in an older population, the reaction speed should be taken into account for interpreting the results of the cold and the warmth detection threshold. We did not find an association between the reaction speed and the heat pain thresholds. During the heat pain threshold measurement, the subject feels the temperature rising until the moment it will become unpleasant or painful, so they can estimate how much longer they must wait until pushing the button. This anticipation could be the reason for reaction speed being less influential in this measurement. In addition, the duration of the heat pain threshold measurement is longer than the sensitivity threshold measurements, and therefore, reaction speed may influence the results less.

Very limited research has been performed on the influence of skin or environmental temperature on QST measurements, although it is very plausible that the detection limits for cold and warmth are dependent on temperature of the skin and/or the environment. We were able to show that skin temperature is indeed associated with these detection limits and they should be used as covariates when analyzing these measurements. We also found an association between the winter season and the QST measurements, but this was primarily driven by skin

temperature. This means that environmental temperature influences the thermal QST measurements via the skin temperature.

The influence of psychological factors on pain and pain sensitivity, such as depression and anxiety, has been suggested before. [13] Depression and anxiety could lead to altered results of QST measurements. For example, anxiety could influence the test results: Participants may drop out early due to their fear of pain. To some extent, this effect was seen when the threshold was measured for the first time, as subjects tended to push the button earlier the first time compared to the rest of the measurements. We therefore measured each threshold 5 times and used the mean values of the last 3 measurements. We found marginally significant associations between depression, anxiety, and the thermal detection thresholds, which were no longer significant in the multivariate analysis. We therefore conclude that these psychological factors had little influence on the measured thermal thresholds in this population.

QST measures and also the prevalence of chronic pain had previously been found to be associated with ethnicity. Skin color is one of the visible differences between ethnicities. Although differences in pain sensitivity are most likely multifactorial, local factors such as skin conditions could also be part of the mechanism. Not only the amount of melanocytes in the skin is different between skin colors, but also larger fibroblasts are observed in dark skin. A dark skin is known to be more sensitive to inflammation and injury, so it is plausible that the structural differences also cause a higher sensitivity to other external stimuli such as temperature and pain. [27-29]

We observed higher pain sensitivity in individuals with atopic eczema. Atopic eczema compromises the integrity of the skin barrier and therefore, nerve endings in the skin could be more sensitive to heat pain stimuli as we showed in our study. Additionally, it is known that neuronal processing of itch and pain is closely related. This might be explained by sensitization of the nervous system by itch, resulting is a higher sensitivity to experimental thermal pain stimuli, as described previously. [15,16]

In 2 recent publications by Olsen et al., [11,12] hypertension was found to be associated with hypoalgesia in a large population-based study. We tried to replicate these results by examining the relationship between systolic and diastolic blood pressure and thermal QST measurements and found only a marginal association between a higher systolic blood pressure and a higher sensitivity for warmth but not pain. Olsen et al. used the cold pressor test and pain ratings for assessing pain, and this study was therefore more focusing on the pain intensity and pain inhibitory system rather than detection thresholds like in our study. This might suggest that high blood pressure influences pain intensity but not detection.

Postmenopausal women have more risk for developing chronic musculoskeletal pain, which can be due to hormonal changes and potential sensitization. After menopause, estrogen levels drop as ovarian production stops. In previous literature, estrogen levels were found not to be associated to thermal QST measurements although this was performed only in premenopausal women. [30] We found significant association between years after menopause and reproductive lifespan with warmth detection limits and not with the other 2 QST measures, which suggest that there is only a limited influence of estrogen exposure on thermal QST measures in postmenopausal women. It remains to be determined whether estrogen levels in postmenopausal women directly influence pain sensitivity.

Limitations of our study include the limited amount of tested modalities of QST. Due to the extent of this study and the large amount of examinations performed, there was limited time available to examine more QST modalities.

In addition, because we used a general definition of chronic musculoskeletal pain, it is possible that some of the individuals had peripheral nerve changes due to their chronic pain syndrome, which could affect the QST measurement and the identified associations. However, this would be a very small amount compared to the total amount of individuals, and individuals with known peripheral nerve damage were excluded.

In conclusion, we observed lower heat pain thresholds in individuals with chronic musculoskeletal pain, indicating that central sensitization is present in this preclinical population. Additionally, we identified determinants that are important to be taken into consideration when analyzing and interpreting QST measurements.

5.1

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5.1

# CHAPTER 6

Gait analysis in osteoarthritis
and chronic pain

# CHAPTER 6.1

## Asymptomatic radiographic hip osteoarthritis is associated with gait differences, especially in women

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## Abstract

*Objectives:* Hip and knee osteoarthritis (OA) are debilitating diseases that impair gait at severe stages. Although associations between OA and gait are established for normal walking, little is known on its relation with turning and tandem (heel-to-toe) walking. Additionally, it is unknown how asymptomatic OA associates with gait, and whether associations differ by sex. We investigated how symptomatic and asymptomatic hip and knee OA associate with gait in community-dwelling individuals.

*Methods:* In 2385 participants of the population-based Rotterdam Study, gait was assessed by electronic walkway and summarised into seven gait domains. Hip and knee radiographs were graded for radiographic OA (ROA) using the Kellgren and Lawrence (K&L) score. Linear regressions were used to investigate associations between ROA and gait. Analyses were repeated including only participants with asymptomatic ROA, defined as a K&L-score of 2 without pain.

*Results:* In total, 154 participants (6.5%) had hip ROA and 493 (16.8%) knee ROA. We found no associations of knee ROA with gait. Hip ROA associated with Rhythm (0.27 SD [95%CI: 0.11; 0.43], p<0.001), Tandem (−0.25 SD [−0.42; −0.07], p=0.005), and Turning (−0.28 SD [−0.46; −0.10], p=0.003). Associations between hip ROA and gait differed significantly between men and women. Hip ROA associated with Tandem and Turning in men, while associating with Rhythm and Base of Support in women. Asymptomatic hip ROA associated with Rhythm and Tandem.

*Conclusions:* Hip ROA, but not knee ROA, associates with gait differences in normal walking, turning, and tandem walking in community-dwelling individuals. These associations differ between the sexes, and are even present for asymptomatic ROA.

## Introduction

Osteoarthritis (OA) is a debilitating disease that limits people in daily functioning, eventually leading to loss of independence. [1, 2] Prevalence of hip and knee OA is high in the elderly (7-30% in people aged ≥65 years) and expected to increase even more due to the aging population and increasing prevalence of obesity. [2-4] Both hip and knee OA are characterised by joint pain and stiffness, which may severely impair locomotion and gait. [5-9]

Gait is an important indicator of health and poor gait associates with higher fall risk and mortality. [10-14] Gait is a complex concept that can be assessed using many parameters. These parameters, as assessed by electronic walkways, can be summarised into seven independent gait domains that comprehensively describe gait. [15, 16]

Of these gait domains, previous studies found hip and knee OA to associate with Base of Support (larger step width), Pace (slower gait velocity), Phases (shorter support on both legs), Rhythm (higher cadence), and Variability (larger gait variability among steps). [5-9] Additionally, OA in only one leg (one-sided OA) was found to associate with larger gait asymmetry. [6-8]

Yet, previous studies included participants with mainly severe and symptomatic OA. [5-9] Little is known on associations of subclinical and asymptomatic OA with gait, which requires investigating a community-dwelling population. Early identification of people with OA may allow for early, and hence expectedly more effective, intervention. [17] Another consideration is that previous studies only focused on associations of OA with gait in normal walking. [5-9] However, the ability to turn and walk tandem may deteriorate earlier with developing OA because of the complex nature of these walking conditions. Additionally, although sex-differences in associations of knee OA with gait have been reported, it is unknown whether sex influences associations of hip OA with gait. [9]

We aimed to investigate associations of radiographic hip and knee OA with gait in normal walking, turning, and tandem walking, in a community-dwelling population. Additionally, we investigated sex-differences in associations of OA with gait.

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## Methods

### Setting

This study was embedded in the Rotterdam Study, a population-based cohort study from the Netherlands. [18] In 1990 and 2000, all inhabitants of Ommoord, a suburb of Rotterdam, aged 55 years and older were invited to participate. In 2006,

the cohort was extended by inviting all inhabitants of Ommoord aged 45 years and older. At baseline and every 3-4 years of follow-up, participants undergo a home interview and extensive medical examination at the research centre. From March 2009 onwards, gait assessment was included in the study protocol. The current study includes all participants that completed gait assessment between March 2009 and December 2011. This study was approved by the medical ethical committee of the Erasmus MC. All participants gave written informed consent.

## Assessment of hip and knee OA

Weight-bearing anteroposterior radiographs of knee and hip were obtained as previously described. [19] Hip and knee OA were scored using the Kellgren and Lawrence (K&L) grading system. Radiographic OA (ROA) was defined as a K&L-score of two or higher. [20]

In a sub-population, joint pain was identified with pain homunculi, showing a picture of the front and back of the human body. Participants were asked: "Did you have pain anywhere in your body, for at least half of the days, during the last six weeks?" If answered positively, participants had to mark painful areas with circles. Subsequently, a template was used to assign these areas to 14 different joint pain regions. For the current study, only pain in hip or knee was considered.

We considered a joint to have asymptomatic ROA when having a K&L-score of 2 without pain.

## Gait assessment

Details on our gait assessment protocol have been described elsewhere. [16] In short, gait was assessed using a 5.79 meter long electronic walkway (4.88 meter active area; GAITRite Platinum; CIR systems, Sparta, NJ, USA). Participants walked in three walking conditions: normal walk, turn, and tandem walk. In normal walk, participants walked at their usual pace over the walkway. This process was performed eight times, of which the first recording was regarded as a practice walk and excluded from analyses. In turn, participants walked at their usual pace over the walkway, turned halfway, and returned. In tandem walk, participants walked heel-to-toe over a straight line present on the walkway.

Principal components analysis was used to summarise gait (means of both legs) into seven independent domains, as described previously: Base of Support, reflecting stride width and stride width variability; Pace, reflecting step length and velocity; Phases, reflecting double support time and single support phase (single support as a percentage of the gait cycle); Rhythm, reflecting cadence and single support time; Tandem, reflecting errors in tandem walking; Turning, reflecting turning time and turning step count; and Variability, reflecting step length variability

and step time variability. [16] To evaluate walking behaviour of a single leg, we used the highest correlating gait parameter from those domains that could be calculated for a single leg: step width variability for Base of Support, step length for Pace, single support phase for Phases, single support time for Rhythm, and step length variability for Variability. [16] We did not assess walking behaviour of a single leg for Tandem and Turning. Gait asymmetry was calculated as the value on gait parameters of the left leg minus the value on parameters of the right leg.

## Study population

Between March 2009 and December 2011, 3242 persons were invited for gait assessment. Of these, 108 persons did not undergo gait assessment for the following reasons: perceived physical inability (n=52), technical reasons (n=43), refusal (n=11), and other reasons (n=2). Of the remaining participants, we excluded 34 participants for performing less than 16 steps in normal walks [21], 13 participants for use of walking aids, and one person for not following instructions.

Of 3086 participants with valid gait assessments, 2512 had radiographs available of both hips and/or both knees. Of these, 127 participants were excluded for having a total hip or knee replacement. Of 2385 included participants, 2292 had gradable radiographs available of both hips and 2361 of both knees.

Participants were used in four distinct analyses (see next section). In the first analysis on gait domains, 212 participants were excluded for missing the turn or tandem walk, resulting in 2087 participants included in analyses on hip ROA and 2154 on knee ROA.

In analyses of one-sided hip ROA with gait asymmetry, gait of the osteoarthritic leg, and gait of the non-osteoarthritic leg, 49 participants were excluded for having hip ROA in both legs. Similarly, 182 participants were excluded in analyses of one-sided knee ROA for having knee ROA in both legs. Hence, 2243 participants were included in analyses on one-sided hip ROA and 2179 in analyses on one-sided knee ROA.

## Statistical analysis

We performed four distinct analyses to investigate associations of hip and knee ROA with gait.

First, we used gait domains to investigate associations between hip and knee ROA with gait in both legs.

Second, to analyse gait asymmetry, we recoded ROA of hip and knee as 1 if present in the left leg and −1 if present in the right leg. Participants without hip or knee ROA were coded as 0. Hence, positive associations between ROA and gait asymmetry imply that one-sided ROA relates to larger gait asymmetry with

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higher values of gait parameters in the osteoarthritic leg. In contrast, negative associations imply larger gait asymmetry with higher values in gait parameters of the non-osteoarthritic leg.

Third, to analyse gait in the osteoarthritic leg of participants with one-sided hip or knee ROA, we used gait parameters of the ROA leg for participants with one-sided ROA, and means of both legs for participants free of the respective ROA.

Fourth, in analyses of the non-osteoarthritic leg, we used gait parameters of the leg without ROA for participants with one-sided ROA, and means of both legs for participants without ROA.

Linear regression analyses were used to investigate associations of hip or knee ROA with gait domains, gait asymmetry, gait in the osteoarthritic leg, and gait in the non-osteoarthritic leg. Univariate ANOVAs were used to calculate mean z-scores of gait domains per K&L-score. All analyses were adjusted for age, sex, height, weight, and time interval between radiographic and gait assessment. Analyses on Tandem were additionally adjusted for mean step length and step count in the tandem walk.

Analyses were repeated after sex-stratification and with sex*ROA interaction terms included. To investigate whether associations were driven by gait velocity, all analyses were repeated while adjusting for gait velocity. [6, 7]

To investigate whether associations remained for asymptomatic ROA, analyses were repeated in the sub-population with pain data available, including only participants without ROA or with asymptomatic ROA.

All statistical analyses were performed using IBM SPSS version 21.0.0.1 for Windows.

## Results

Participants had a mean age of 65.9 years (Standard deviation [SD] 8.9) and 53.6% were women (Table 1). Of 2385 participants, 512 (21.5%) had ROA. Of these, 112 (4.7%) had ROA in hip only, 358 (15.0%) in knee only, and 42 (1.8%) in both hip and knee. Differences in prevalence of hip and knee ROA between men and women were non-significant.

Of 154 participants with hip ROA, 105 (68.2%) had one-sided hip ROA. Of 400 participants with knee ROA, 218 (54.5%) had one-sided knee ROA.

**Table 1.** Population characteristics.

| | Total (n = 2385) | Men (n = 1106) | Women (n = 1279) |
|---|---|---|---|
| Age, years | 65.9 (8.9) | 66.6 (9.2) | 65.4 (8.6) |
| Height, cm | 169.6 (9.2) | 176.5 (6.6) | 163.5 (6.4) |
| Weight, kg | 78.7 (14.3) | 85.7 (12.6) | 72.6 (12.9) |
| ROA[a], n | 512 (21.5%) | 227 (20.5%) | 266 (20.8%) |
| Hip ROA, n | 154 (6.5%) | 74 (6.7%) | 80 (6.3%) |
| One-sided hip ROA, n | 105 (4.4%) | 44 (4.0%) | 61 (4.8%) |
| Knee ROA, n | 400 (16.8%) | 176 (15.9%) | 224 (17.5%) |
| One-sided knee ROA, n | 218 (9.1%) | 102 (9.2%) | 116 (9.1%) |

Values are means (standard deviations) or numbers of participants (percentages).
[a] Radiographic hip or knee osteoarthritis.
Abbreviations: n, number of participants; cm, centimetres; kg, kilograms; ROA, radiographic osteo-arthritis.

## Associations of ROA with gait domains

Hip ROA associated with higher Rhythm (0.27 SD [95% confidence interval: 0.11; 0.43], p<0.001), lower Tandem (−0.25 SD [−0.42; −0.07], p=0.005), and lower Turning (−0.28 SD [−0.46; −0.10], p=0.003) (Table 2).

In Figure 1, higher K&L-scores of the hip are shown to associate with lower Tandem (p-trend=0.03) and Turning (p-trend=0.03), but higher Rhythm (p-trend<0.001). No significant p-trends were found across K&L scores for the other gait domains.

In sex-stratified analyses, hip ROA associated with lower Tandem (−0.32 SD [−0.55; −0.09], p=0.006) and Turning (−0.33 SD [−0.58; −0.09], p=0.008) in men, while associating with higher Base of Support (0.27 SD [0.04; 0.50], p=0.02) and Rhythm (0.30 SD [0.09; 0.52], p=0.005) in women (Table 3).

Knee ROA did not associate with any gait domain, but did demonstrate trends of an association with Tandem (−0.11 SD [−0.23; 0.01], p=0.08) and Turning (−0.11 SD [−0.23; 0.01], p=0.06) (Table 2).

In sex-stratified analyses, we found an association of knee ROA with Tandem (−0.23 SD [−0.40; −0.07], p=0.006) in women (Supplement 1). Additionally, we found this association to be significantly stronger (p=0.005) for women than men.

Adjustment for gait velocity did not change associations for hip or knee ROA.

## Associations of one-sided ROA with gait

One-sided hip ROA associated with larger gait asymmetry, with shorter single support time and phase but larger step length in the osteoarthritic compared to the non-osteoarthritic leg (Table 4).

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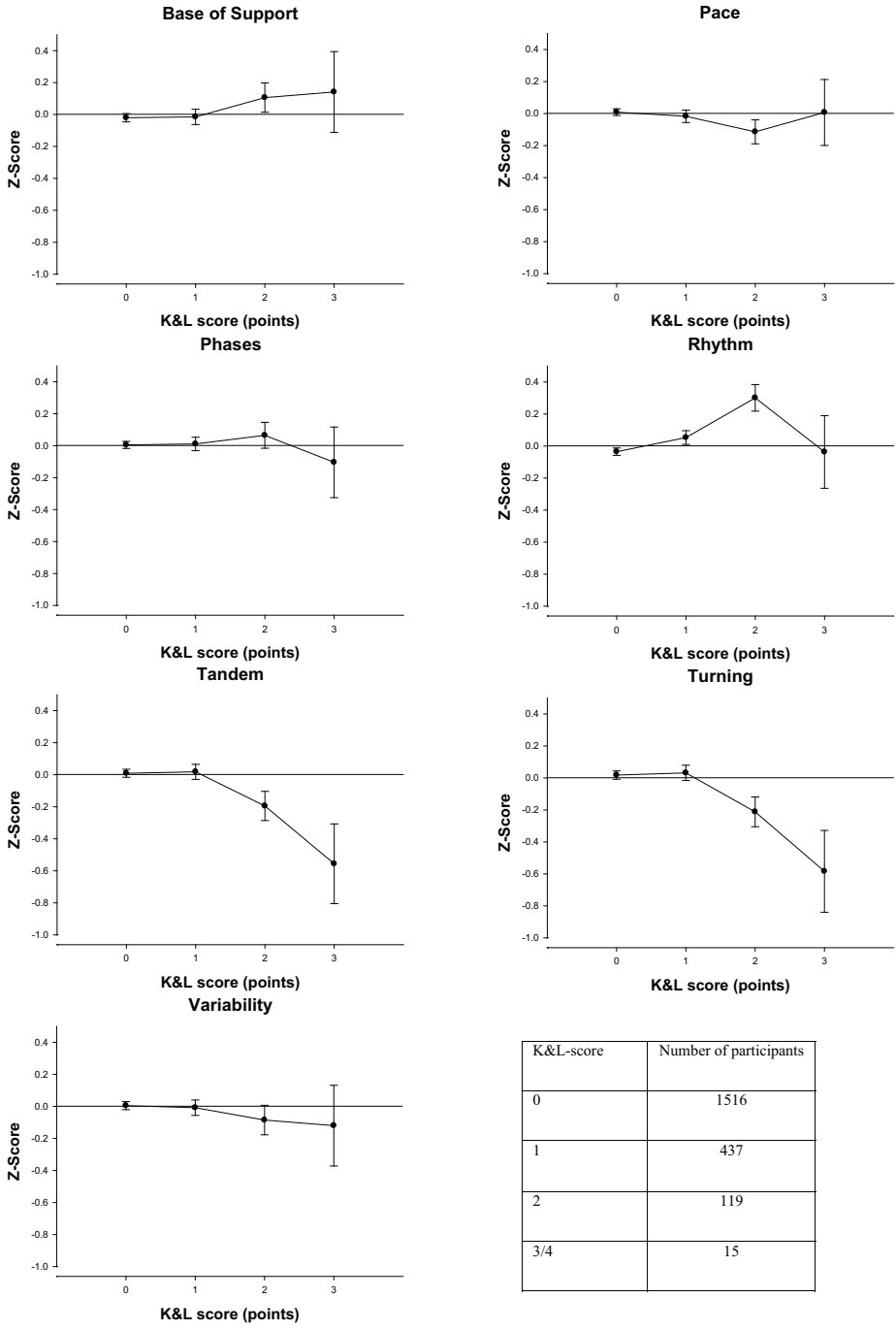
**Figure 1.** Z-scores of gait domains across Kellgren and Lawrence scores. Dots are means adjusted for age, sex, height, weight, and time interval between radiographic and gait assessment. Error bars represent standard errors of the means.

**Table 2.** Associations of radiographic hip and knee osteoarthritis with gait.

| | Base of Support | Pace | Phases | Rhythm | Tandem[a] | Turning | Variability |
|---|---|---|---|---|---|---|---|
| Hip ROA | 0.13 (−0.05; 0.31) | −0.10 (−0.24; 0.04) | 0.04 (−0.12; 0.19) | **0.27 (0.11; 0.43)** | **−0.25 (−0.42; −0.07)** | **−0.28 (−0.46; −0.10)** | −0.09 (−0.27; 0.09) |
| Knee ROA | 0.03 (−0.09; 0.14) | 0.04 (−0.06; 0.13) | −0.02 (−0.12; 0.08) | −0.03 (−0.13; 0.08) | −0.11 (−0.23; 0.01) | −0.11 (−0.23; 0.01) | 0.06 (−0.06; 0.17) |

Values represent differences in z-scores of gait (95% confidence interval) for presence of osteoarthritis in one or two legs. All analyses were adjusted for age, sex, height, weight, and the time interval between radiographic and gait assessment. Results in bold represent nominally significant associations (p<0.05).

[a] Additionally adjusted for the step count and step size within the tandem walk.

Abbreviations: ROA, radiographic osteoarthritis.

**Table 3.** Associations of radiographic hip osteoarthritis with gait, stratified for sex.

| | Base of Support | Pace | Phases | Rhythm | Tandem[a] | Turning | Variability |
|---|---|---|---|---|---|---|---|
| Men | −0.01 (−0.29; 0.26) | −0.12 (−0.34; 0.10) | 0.11 (−0.11; 0.33) | 0.23 (−0.01; 0.47) | **−0.32 (−0.55; −0.09)** | **−0.33 (−0.58; −0.09)** | 0.03 (−0.23; 0.28) |
| Women | **0.27 (0.04; 0.50)** | −0.09 (−0.28; 0.10) | −0.02 (−0.24; 0.19) | **0.30 (0.09; 0.52)** | −0.17 (−0.43; 0.09) | −0.23 (−0.49; 0.03) | −0.21 (−0.45; 0.03) |

Values represent differences in z-scores of gait (95% confidence interval) for presence of radiographic hip osteoarthritis in one or two legs. All analyses were adjusted for age, sex, height, weight, and the time interval between radiographic and gait assessment. Results in bold represent nominally significant associations (p<0.05).

[a] Additionally adjusted for the step count and step size within the tandem walk.

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**Table 4.** Associations of one-sided radiographic hip and knee osteoarthritis with gait.

| | SW Variability (cm) | SL (cm) | SS Phase (%) | SS Time (0.1s) | SL Variability (cm) |
|---|---|---|---|---|---|
| **Asymmetry** | | | | | |
| Hip ROA | −0.02 (−0.12; 0.07) | **0.68 (0.21; 1.16)** | **−0.36 (−0.62; −0.11)** | **−0.04 (−0.07; −0.02)** | −0.12 (−0.27; 0.03) |
| Knee ROA | −0.05 (−0.12; 0.02) | 0.24 (−0.09; 0.58) | −0.12 (−0.29; 0.06) | −0.01 (−0.03; 0.01) | 0.04 (−0.07; 0.14) |
| **Osteoarthritic leg** | | | | | |
| Hip ROA | −0.07 (−0.24; 0.10) | −0.71 (−1.98; 0.55) | **−0.36 (−0.65; −0.08)** | **−0.09 (−0.16; −0.03)** | 0.12 (−0.03; 0.28) |
| Knee ROA | −0.03 (−0.14; 0.09) | 0.52 (−0.37; 1.42) | −0.09 (−0.29; 0.11) | 0.00 (−0.04; 0.04) | 0.01 (−0.10; 0.13) |
| **Non-osteoarthritic leg** | | | | | |
| Hip ROA | −0.05 (−0.21; 0.12) | **−1.40 (−2.67; −0.13)** | 0.02 (−0.27; 0.30) | −0.05 (−0.11; 0.01) | **0.24 (0.08; 0.40)** |
| Knee ROA | 0.02 (−0.09; 0.14) | 0.26 (−0.64; 1.16) | 0.03 (−0.17; 0.23) | 0.01 (−0.03; 0.06) | −0.02 (−0.14; 0.09) |

Values represent differences in gait parameters of asymmetry, osteoarthritic leg, or non-osteoarthritic leg (95% confidence interval) for presence of one-sided ROA versus no ROA of the respective body part. Analyses were adjusted for age, sex, height, weight, and time interval between radiographic and gait assessment. Results in bold represent nominally significant associations (p<0.05).

Abbreviations: ROA, radiographic osteoarthritis; SW, stride width; cm, centimetres; SL, step length; SS, single support; s, seconds.

**Table 5.** Sex-stratified associations of one-sided radiographic hip osteoarthritis with gait.

| | SW Variability (cm) | SL (cm) | SS Phase (%) | SS Time (0.1s) | SL Variability (cm) |
|---|---|---|---|---|---|
| Asymmetry | | | | | |
| Men | −0.04 (−0.20; 0.11) | 0.18 (−0.64; 0.99) | −0.19 (−0.59; 0.21) | −0.03 (−0.07; 0.02) | −0.11 (−0.35; 0.13) |
| Women | −0.01 (−0.14; 0.12) | **1.03 (0.46; 1.59)** | **−0.49 (−0.82; −0.16)** | **−0.06 (−0.09; −0.02)** | −0.13 (−0.33; 0.07) |
| Osteoarthritic leg | | | | | |
| Men | −0.03 (−0.30; 0.25) | −0.43 (−2.44; 1.57) | 0.09 (−0.32; 0.49) | −0.04 (−0.14; 0.06) | −0.01 (−0.26; 0.24) |
| Women | −0.10 (−0.31; 0.10) | −1.00 (−2.63; 0.63) | **−0.66 (−1.05; −0.27)** | **−0.13 (−0.21; −0.05)** | **0.24 (0.04; 0.44)** |
| Non-osteoarthritic leg | | | | | |
| Men | 0.02 (−0.26; 0.29) | −0.62 (−2.63; 1.38) | 0.34 (−0.07; 0.74) | −0.01 (−0.11; 0.09) | 0.10 (−0.14; 0.35) |
| Women | −0.10 (−0.30; 0.11) | **−2.05 (−3.68; −0.41)** | −0.19 (−0.58; 0.20) | −0.08 (−0.16; 0.01) | **0.36 (0.15; 0.56)** |

Values represent differences in gait parameters of asymmetry, osteoarthritic leg, or non-osteoarthritic leg (95% confidence interval) for presence of one-sided radiographic hip osteoarthritis versus no radiographic hip osteoarthritis. All analyses were adjusted for age, sex, height, weight, and the time interval between radiographic and gait assessment. Results in bold represent nominally significant associations (p<0.05).

Abbreviations: SW, stride width; cm, centimetres; SL, step length; SS, single support; s, seconds.

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Additionally, one-sided hip ROA associated with shorter single support time and phase of the osteoarthritic leg and larger step length and step length variability of the non-osteoarthritic leg.

When stratifying for sex, all associations described above were present in women (Table 5). Additionally, one-sided hip ROA associated with larger step length variability of the osteoarthritic leg in women. In men, no associations were found for one-sided hip ROA.

We found significant sex-interactions, with associations of one-sided hip ROA with shorter single support phase of the osteoarthritic (p=0.004) and non-osteoarthritic leg (p=0.03) being stronger in women than men.

One-sided knee ROA did not associate with gait differences (Table 4). Similarly, we found no associations in sex-stratified analyses of one-sided knee ROA (Supplement 2). Adjustment for gait velocity did not change associations for one-sided hip or knee ROA.

### Associations of asymptomatic ROA with gait

Of 2385 participants, 1909 (80.0%) had pain data available, including 80 participants with hip ROA and 288 with knee ROA. Of these, 60 (75.0%) participants had asymptomatic hip ROA and 177 (61.5%) asymptomatic knee ROA.

After restriction to asymptomatic ROA, hip ROA remained significantly associated with Rhythm, Tandem, and single support time of the osteoarthritic leg in the overall population. In women, hip ROA remained significantly associated with Rhythm, larger gait asymmetry in single support time and phase, and single support time in both the osteoarthritic and non-osteoarthritic leg. No associations remained for asymptomatic knee ROA.

## Discussion

Our study shows that hip ROA associates with gait in a community-dwelling population. We found this relation to remain when restricting to asymptomatic hip ROA. Hip ROA associated with gait differences in Rhythm, Tandem, and Turning. One-sided hip ROA was associated with larger gait asymmetry, and differences in gait parameters of both the osteoarthritic and non–osteoarthritic leg. Associations between hip ROA and gait were mainly driven by women. Knee ROA only associated with Tandem in women.

Strengths of our study include the population-based setting, assessment of gait in three walking conditions, radiographic classification of OA, investigation of sex-differences, and restriction to participants with asymptomatic OA.

Limitations of our study include the inability to assess gait mechanics, e.g. flexion angles and rotation moments. Additionally, the cross-sectional design precluded investigation of whether gait differences are a consequence of OA, or whether a deviant gait pattern increases the probability of developing (more severe) OA. Finally, gait was assessed at the research centre. Therefore, we may have missed people with severe OA that inhibited them to come to the research centre. Additionally, people with OA that did visit the research centre may have refused gait assessment more often. Hence, our findings may only be generalizable to a relatively healthy population with less severe OA cases.

We found hip ROA to associate with gait differences in a community-dwelling population, even when restricting our osteoarthritic participants to those with asymptomatic hip ROA. Previous studies used clinic-based samples of patients with more advanced and severe hip OA. [6, 7] Our findings imply that hip OA may already impact gait at an early stage, in absence of pain symptoms. Interestingly, not only did hip ROA associate with gait differences in normal walking, but also in turning and tandem walking. This suggests that assessment of turning and tandem walking may provide additional information to identify people with early-stage hip OA. Previous research has already highlighted the importance of identifying OA at an early stage, to increase effectiveness of interventions to prevent or reduce its progress. [17] Our results suggest that gait assessment may aid in such an early identification of hip OA.

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Similar to a previous study, we found hip ROA to associate with taking quicker steps (higher Rhythm). [6] In contrast to that study, we found no association of hip ROA with larger stride width (higher Base of Support) in the overall population, but only in women. Both taking quicker steps and larger stride width have been suggested to be compensatory mechanisms to reduce pain. [6] However, our findings suggest that the quicker steps may instead result from a reduced motion range of the OA hip, because associations remained after restriction to participants with asymptomatic ROA. [6, 22] To the best of our knowledge, we are the first study to report that persons with hip ROA, especially men, have more difficulty in turning and tandem walking. These associations may be especially important, because problems in turning and tandem walking may reflect balance deficits that increase fall risk. [11, 23] Hence, decreased range of hip motion in participants with hip OA may increase fall risk and thus risk of fractures and other related morbidities.

Consistent with previous research, we found one-sided hip ROA to associate with larger gait asymmetry, with shorter single support time and phase but larger step length in the osteoarthritic compared to the non-osteoarthritic leg. [6] The asymmetry in single support time and phase came from shorter single support on the osteoarthritic leg compared to normal, while asymmetry in step length came from

shorter step length of the non-osteoarthritic leg. These associations are presumably compensatory mechanisms to avoid load on the osteoarthritic leg, which is supported by their attenuation after restriction to asymptomatic ROA. We found one-sided hip ROA to additionally associate with larger step length variability in the non-osteoarthritic leg. Larger step length variability of the non-osteoarthritic leg may reflect adaptations to unexpected limitation of movement or sudden onset of pain in the osteoarthritic leg. A previous study only found an association with step length variability in the osteoarthritic leg. [7] We found a similar association, but only in women. In general, larger gait variability suggests loss of gait control, and is clinically important through its strong association with the risk of falling. [12, 13]

We found associations of hip ROA with gait to be generally stronger in women than men. Only associations of hip ROA with Tandem and Turning were stronger in men. We found significant sex-interactions, with one-sided hip ROA associating stronger with single support phase of osteoarthritic and the non-osteoarthritic leg in women compared to men. These sex-differences suggest that women may either alter their gait pattern at an earlier stage of hip OA or change their gait pattern to a greater extent as an adaptive mechanism to reduce pain. Alternatively, a certain gait pattern, with higher prevalence in women (e.g. due to different anatomy of the pelvis), may lead to larger wear and tear of the hip joint, and thus to OA. Notwithstanding the mechanism, these findings suggest that, when using gait to identify hip OA, sex should be taken into account.

In contrast to most previous studies, we only found trends of associations for knee ROA with gait. [5, 6, 8, 9] This discrepancy may be explained by the relatively few participants with severe knee OA in our study. Possibly, knee OA only affects gait at more severe stages, or more power is needed to identify the subtle associations of subclinical knee OA with gait. Otherwise, restricted movement of the knee may be compensated by movement of the hip, while restricted movement of the hip may be harder to compensate, resulting in gait differences at an earlier stage.

## Conclusions

In a community-dwelling population, hip OA associates with gait differences in normal walking, turning, and tandem walking. These associations were also found for asymptomatic hip OA, suggesting that gait patterns already change in absence of pain symptoms. One-sided hip OA associates with larger gait asymmetry and gait differences in both osteoarthritic and non-osteoarthritic leg. Associations of hip OA with gait are generally stronger in women than men. These findings suggest that, especially in women, gait assessment could aid in early identification of persons with hip OA.

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**Supplemental table 1.** Associations of radiographic knee osteoarthritis with gait, stratified for sex.

| | Base of Support | Pace | Phases | Rhythm | Tandem[a] | Turning | Variability |
|---|---|---|---|---|---|---|---|
| Men | 0.04 (–0.15; 0.22) | –0.05 (–0.20; 0.10) | –0.10 (–0.24; 0.05) | –0.14 (–0.29; 0.02) | 0.05 (–0.10; 0.20) | –0.11 (–0.28; 0.05) | 0.09 (–0.08; 0.26) |
| Women | 0.03 (–0.11; 0.18) | 0.12 (0.00; 0.24) | 0.06 (–0.08; 0.20) | 0.08 (–0.06; 0.21) | **–0.23 (–0.40; –0.07)** | –0.11 (–0.28; 0.06) | 0.00 (–0.15; 0.16) |

Values represent differences in z-scores of gait (95% confidence interval) for presence of radiographic knee osteoarthritis in one or two legs. All analyses were adjusted for age, sex, height, weight, and the time interval between radiographic and gait assessment. Values in bold represent nominally significant associations (p<0.05).
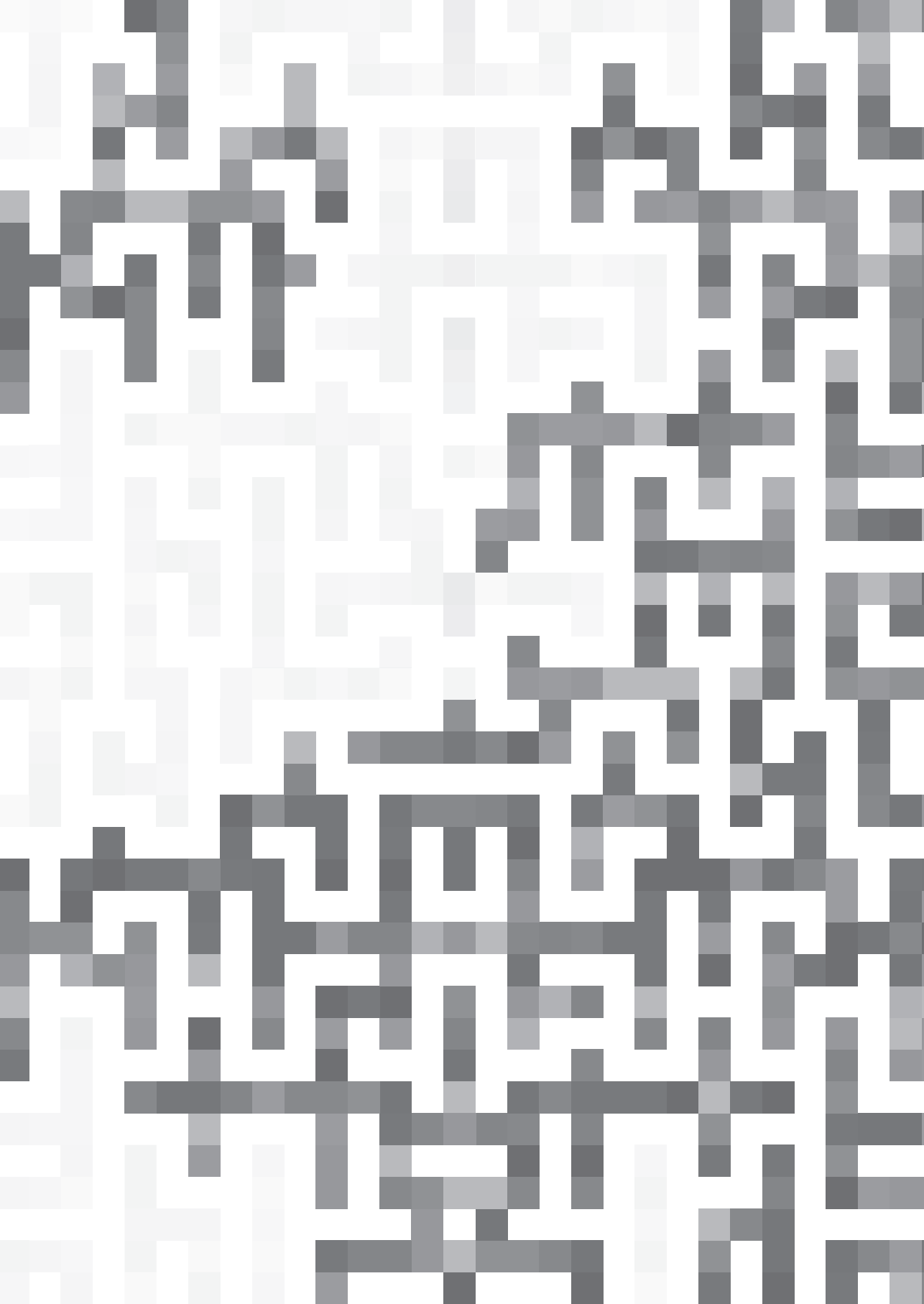
[a] Additionally adjusted for the step count and step size within the tandem walk.

**Supplemental table 2.** Sex-stratified associations of one-sided radiographic knee osteoarthritis with gait.

| | SW Variability (cm) | SL (cm) | SS Phase (%) | SS Time (0.1s) | SL Variability (cm) |
|---|---|---|---|---|---|
| **Asymmetry** | | | | | |
| Men | 0.00 (–0.10; 0.10) | 0.31 (–0.23; 0.84) | –0.06 (–0.33; 0.21) | –0.01 (–0.04; 0.02) | 0.00 (–0.17; 0.16) |
| Women | –0.09 (–0.18; 0.01) | 0.17 (–0.25; 0.59) | –0.16 (–0.40; 0.08) | –0.01 (–0.04; 0.01) | 0.07 (–0.07; 0.22) |
| **Osteoarthritic leg** | | | | | |
| Men | –0.01 (–0.20; 0.17) | 0.26 (–1.10; 1.62) | –0.10 (–0.37; 0.18) | 0.04 (–0.02; 0.11) | 0.02 (–0.15; 0.19) |
| Women | –0.04 (–0.19; 0.11) | 0.77 (–0.43; 1.96) | –0.04 (–0.32; 0.24) | –0.04 (–0.10; 0.02) | 0.03 (–0.12; 0.18) |
| **Non-osteoarthritic leg** | | | | | |
| Men | –0.01 (–0.19; 0.18) | –0.05 (–1.42; 1.31) | 0.02 (–0.25; 0.30) | 0.06 (–0.01; 0.13) | 0.02 (–0.15; 0.19) |
| Women | 0.06 (–0.09; 0.21) | 0.54 (–0.66; 1.74) | 0.08 (–0.21; 0.36) | –0.03 (–0.09; 0.03) | –0.04 (–0.19; 0.10) |

Values represent differences in gait parameters of asymmetry, osteoarthritic leg, or non-osteoarthritic leg (95% confidence interval) for presence of one-sided radiographic knee osteoarthritis versus no knee osteoarthritis. All analyses were adjusted for age, sex, height, weight, and the time interval between radiographic and gait assessment. Results in bold represent nominally significant associations (p<0.05).

Abbreviations: SW, stride width; cm, centimetres; SL, step length; SS, single support; s, seconds.

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# CHAPTER 6.2

## Chronic joint pain in the lower body is associated with gait differences independent from radiographic osteoarthritis

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## Abstract

*Background:* Gait is an important indicator of health. Chronic lower body pain may impair gait and lead to morbidity and mortality. We investigated the associations between lower body pain and gait in community-dwelling individuals, independent from osteoarthritis (OA).

*Methods:* This population based cohort study included 2304 Rotterdam Study participants who underwent electronic walkway gait assessment. Thirty different variables resulting from gait assessment were summarized into seven gait domains using principle components analysis: i.e. Rhythm, Variability, Phases, Pace, Tandem, Turning, and Base of Support. Chronic lower body pain was assessed using pain drawings. OA was defined as a Kellgren & Lawrence score of 2 or higher on radiographs of the hip and/or knee. Linear regression analysis was used to study associations.

*Results:* Participants with chronic pain in the leg and hip, had lower Rhythm, Phases, and Pace, independent from OA. Additionally, we found unilateral pain to associate with larger gait asymmetry. No associations were found between chronic pain and the other gait domains, including gait variability. However, within individuals with hip pain, gait variability was higher in individuals with radiographic OA compared to those without OA.

*Conclusions:* This is the first population based study showing chronic lower body pain associates with gait differences independent from OA. Participants with pain were found to walk with slower and smaller steps, longer double support and more asymmetry. Proper care and treatment of chronic pain could be a way of reducing gait problems and thereby fall risk and associated mortality. In addition, gait assessment may help identifying individuals with OA from those having pain due to other causes.

## Introduction

Chronic pain is very common in elderly people and affects daily functioning. Chronic lower body pain often causes decreased mobility and lower quality of life. Especially in older individuals, this decreased mobility is related to higher mortality [1-5].

Gait assessment, by electronic walkway, can provide accurate measurement of mobility. Gait is an accurate health indicator and strongly associates with falls and mortality [3,6-10].

Gait is highly complex and can be studied in many different ways, resulting in many different variables. As a consequence, the overlap across studies in variables used to study gait is limited. Ideally, gait is studied using as many variables as possible, but this would result in multiple testing as well as collinearity across variables. Over recent years, various studies have tried to solve this issue by principal components analysis (PCA) [10-13]. Using this method, the different studies investigating gait patterns will be better comparable since they use the same gait domain variables.

Previous studies on pain and gait parameters mainly focused on osteoarthritis (OA), the most common cause of lower body pain in elderly people [14-20]. The clinical definition of OA includes joint pain and joint damage on radiographs. However, joint damage does not necessarily result in joint pain. Similarly, not all individuals with chronic joint pain also have joint damage. It is therefore unclear whether previous observed gait differences in clinical OA case studies were due to OA or pain. Pain is the main reason for individuals to turn to their physician, but only part of the individuals coming to the general practitioner with joint pain also has OA. We therefore here study the relation between gait and chronic pain in the lower body. We additionally studied whether the observed differences in gait patterns were dependent on the presence of OA and whether individuals with OA could be identified with gait patterns among the individuals with pain. This study will add to the knowledge of gait in joint related complaints, since it is focused on the joint pain compared to the previous studies investigating joint damage. In addition, recognizing gait differences between OA-related and OA-unrelated pain might provide opportunities to distinguish people with OA from those having musculoskeletal pain due to other factors.

Additionally, better understanding of the relationship between lower body pain and gait may allow for new interventions to decrease pain and gait problems, and hence related morbidity and mortality. We studied associations of pain in the lower body with gait in a community dwelling population of middle-aged and elderly individuals.

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## Methods

### Study population

Between March 2009 and March 2012, 3651 persons were invited for gait assessment. We excluded 163 individuals for physical inability to perform gait assessment, 115 because of having hip or knee prosthesis and 27 due to technical problems. Thirty-four subjects were excluded because they completed less than 16 steps, reducing validity [22]. Furthermore, 12 participants refused gait assessment, 3 participants used walking aids, one did not follow instructions and 2 were excluded for other reasons.

Of the remaining 3294 participants, 2304 had pain drawings data available, because pain drawings were temporarily removed from the study protocol. These 2304 participants were used in the analysis.

### Chronic joint pain assessment

Pain drawings were presented to participants to assess chronic joint pain. The pain drawing showed a picture of the front and the back of the human body. Participants were asked the following question: "Did you have pain anywhere in your body, for at least half of the days, during the last six weeks?". Pain was recorded as 'yes' or 'no'. Painful sites were marked by the participant. The drawings were scored using a template assigning 14 joint regions, including neck, shoulders, elbows, hands, low back, hips, knees and feet. For the current study, we used pain of the hips, knees, feet and low back. Leg pain was defined as hip, knee or foot pain. We created a summary score for pain in the lower body, in which we summed the presence of pain in the left leg, right leg and low back and divided this score by three.

### Gait assessment

The full gait assessment protocol is published previously [13]. In short, gait was assessed using a 5.79 m long electronic walkway with pressure sensors (GAITRite Platinum; CIR systems, Sparta, NJ: 4.88 m active area; 120 Hz sampling rate), an accurate tool for gait assessment [23-25].

Participants followed a standardized gait protocol consisting of three walking conditions: normal walk, turning and tandem walk. In 'normal walk', participants walked eight times at their usual pace across the walkway. The first walk was considered a practice walk and was not used for gait parameter calculations. In 'turning', participants walked across the walkway, turned halfway, and returned to their starting position. In 'tandem walk', participants walked heel-to-toe over a line, visible on the walkway.

Principal components analysis summarized mean gait parameters of both legs into seven gait domains, as previously described: (1) Rhythm, reflecting cadence and single support time; (2) Variability, reflecting variability in step length and time; (3) Phases, reflecting double support time and single support time as a percentage of the total stride time; (4) Pace, reflecting step length and velocity; (5) Tandem, reflecting errors in tandem walking; (6) Turning, reflecting the number of steps and time needed to turn; and (7) Base of Support, reflecting stride width and stride width variability [13]. In the results, a lower value of the gait domains represents a worse gait.

From the principal component analysis, we can derive correlation estimates for every parameter for the amount of variance of gait explained by that particular variable. To investigate walking behavior of one leg, we used the highest correlating gait parameters from the gait domains that could be computed for a single leg: single support time for Rhythm, step length variability for Variability, single support percentage for Phases, step length for Pace, and stride width variability for Base of Support. To study gait asymmetry, values on gait parameters of the right leg were subtracted from the values on gait parameters of the left leg.

### Statistical analysis

We studied associations between lower body pain and gait domains by comparing gait patterns in people with pain to those without lower body pain.

Additionally, we investigated the effect of unilateral pain on gait asymmetry and effects in the painful or non-painful leg. Individuals with bilateral pain were excluded. We recoded pain in the left leg as 1, pain in the right leg as −1 and no pain as 0. Hence, a positive association indicates unilateral pain to result in larger asymmetry with higher values of gait parameters in the painful leg compared to the unpainful leg. To elucidate gait differences in the painful leg, we compared the painful leg of people having unilateral pain to the mean of both legs for individuals without pain. Similarly, to elucidate gait differences in the unpainful leg, we compared the unpainful leg of people with unilateral pain to the mean of both legs for individuals without pain.

We used linear regression analyses to investigate the associations of chronic pain in the lower body with the different gait domains, gait asymmetry, gait in the painful leg, and gait in the unpainful leg. All analyses were adjusted for age, sex, height, weight and time interval between pain and gait assessment. 'Tandem walk' analyses were additionally adjusted for mean step length and step count.

To investigate the influence of OA, we repeated the analysis on lower body pain and the gait domains, after excluding participants with radiographic OA in the hip or knee. Individuals in which OA was rated on radiographs as a Kellgren & Lawrence score of 2 or higher were used [26].

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Subsequently, we investigated whether gait is affected by OA in individuals with pain in hip and/or knee, by associating hip and knee OA with the seven gait domains within the individuals that have pain, representing symptomatic or clinical OA.

SPSS version 21.0 (SPSS INC., Chicago, USA) was used for all analysis.

## Results

Population characteristics are described in Table 1. Mean age of the participants was 63.5 years and 54.8% were female. Pain in the lower body was present in 35.6% of the subjects, with pain in the lower back, hip, knee or foot being present in respectively 17.1%, 8.6%, 16.4% and 7.9% participants.

**Table 1.** Population characteristics

| Characteristic | Total (n = 2304) |
| --- | --- |
| Age [years] | 63.5 (7.5) |
| Sex [females] | 1262 (54.8) |
| Height [cm] | 170.0 (9.2) |
| Weight [kg] | 79.5 (14.7) |
| Body Mass Index | 27.4 (4.1) |
| Pain in the lower body [n] | 820 (35.6) |
| Pain lower back [n] | 395 (17.1) |
| Pain Hip [n] | 198 (8.6) |
| Pain Knee [n] | 377 (16.4) |
| Pain Foot [n] | 183 (7.9) |
| Summary score lower body pain [points] | 0.18 (0.28) |

Values are means (standard deviations) or numbers (percentages).
Abbreviations: cm = centimeters, kg = kilograms, n = number.

### Associations between pain in the lower body and gait domains

The summary score of the number of painful body parts (right leg/left leg/low back) was associated with lower Rhythm (−0.19 SD; *P*= 0.005), Phases (−0.20 SD; P= 0.002) and Pace (−0.19 SD; P= 0.003), but higher Variability (0.16 SD; P= 0.046) (Table 2). We subsequently found that leg pain resulted in lower Rhythm (−0.11 SD; P= 0.014), Phases (−0.15 SD; P < 0.001) and Pace (−0.10 SD; P= 0.018), while low back pain was only associated with lower Pace (0.10 SD; P= 0.034). Further investigation showed that the majority of the gait differences due to leg pain were driven by hip pain. Additionally, foot pain foot was associated with Phases (−0.14 SD; P= 0.047), while knee pain was not associated with gait differences, despite larger power compared to hip and foot pain.

**Table 2.** Associations of pain in the lower body with the gait domains

| Domain | Rhythm | Variability | Phases | Pace | Tandem[a] | Turning | Base of Support |
|---|---|---|---|---|---|---|---|
| Summary score lower body pain[b] | **−0.19 \*\*** (**−0.33; −0.06**) | **0.16 \*** (**0.00; 0.31**) | **−0.20 \*\*** (**−0.34; −0.07**) | **−0.19 \*\*** (**−0.31; −0.07**) | 0.02 (−0.13; 0.17) | 0.01 (−0.14; 0.17) | 0.03 (−0.12; 0.18) |
| Pain lower back | −0.06 (−0.16; 0.04) | 0.05 (−0.06; 0.16) | −0.05 (−0.15; 0.05) | **−0.10 \*** (**−0.19; −0.01**) | −0.03 (−0.14; 0.08) | 0.04 (−0.07; 0.16) | −0.05 (−0.16; 0.06) |
| Pain leg | **−0.11 \*** (**−0.20; −0.02**) | 0.08 (−0.02; 0.18) | **−0.15 \*\*** (**−0.23; −0.06**) | **−0.10 \*** (**−0.18; −0.02**) | 0.03 (−0.07; 0.12) | −0.01 (−0.11; 0.09) | 0.04 (−0.06; 0.14) |
| Pain hip | −0.13 (−0.26; 0.01) | 0.03 (−0.13; 0.18) | **−0.19 \*\*** (**−0.32; −0.06**) | **−0.16 \*** (**−0.28; −0.03**) | 0.12 (−0.03; 0.27) | 0.04 (−0.11; 0.19) | 0.07 (−0.08; 0.22) |
| Pain knee | −0.09 (−0.19; 0.01) | 0.01 (−0.10; 0.12) | −0.04 (−0.14; 0.06) | −0.07 (−0.16; 0.03) | 0.04 (−0.07; 0.15) | −0.03 (−0.14; 0.08) | 0.05 (−0.06; 0.17) |
| Pain foot | −0.06 (−0.20; 0.08) | 0.08 (−0.08; 0.24) | **−0.14 \*** (**−0.28; 0.00**) | −0.08 (−0.21; 0.05) | −0.04 (−0.20; 0.12) | −0.02 (−0.17; 0.14) | 0.06 (−0.10; 0.22) |

Values represent differences in z-scores of gait (95% confidence interval) for pain in the respective body part. A lower value of gait represents worse gait. Pain was scored as 1 = yes and 0 = no. Results in bold survived thresholds of nominal significance (*p < 0.05; **p < 0.005). All analyses were adjusted for age, sex, height, weight, and time interval between pain and gait assessment. N = 2139.
a Additionally adjusted for the step count and step size within the tandem walk.
b Pain lower body = (pain in lower back + pain left leg + pain right leg) / 3.

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**Table 3.** Associations of unilateral pain in the leg, hip, knee and foot with gait asymmetry between both legs

| Domain | Rhythm | Variability | Phases | Pace | Base of Support |
|---|---|---|---|---|---|
| Gait parameter | Single Support Time (0.1 s) | Step length SD (cm) | Single Support Phase (%) | Step length (cm) | Stride width SD (cm) |
| Unilateral pain leg | | | | | |
| Asymmetry | −0.01 (−0.03; 0.00) | 0.01 (−0.08; 0.11) | −0.13 (−0.28; 0.02) | **0.42 ** (0.14; 0.70)** | 0.01 (−0.05; 0.07) |
| Unilateral pain hip | | | | | |
| Asymmetry | −0.02 (−0.05; 0.00) | −0.13 (−0.27; 0.02) | **−0.24 * (−0.47; −0.01)** | **0.50 * (0.07; 0.94)** | −0.05 (−0.15; 0.04) |
| Unilateral pain knee | | | | | |
| Asymmetry | −0.01 (−0.03; 0.01) | 0.07 (−0.06; 0.19) | −0.09 (−0.29; 0.10) | 0.05 (−0.31; 0.41) | 0.02 (−0.06; 0.10) |
| Unilateral pain foot | | | | | |
| Asymmetry | −0.01 (−0.04; 0.02) | **0.26 ** (0.09; 0.42)** | −0.11 (−0.38; 0.16) | **0.65 * (0.15; 1.15)** | 0.01 (−0.10; 0.12) |

Values represent differences in gait parameters (95% confidence interval) for presence of pain in the respective body part. Results in bold survived thresholds of nominal significance (*p < 0.05; **p < 0.005). All analysis were adjusted for age, sex, height, weight, and time interval between pain and gait assessment. Leg: n = 2012; hip: n = 2231; knee: n=2103; foot: n = 2215.
Asymmetry: difference in gait asymmetry between painful and unpainful leg compared to asymmetry between both legs of people without pain.
Abbreviations: s = seconds, SD = standard deviation, cm = centimetres, n = number of participants.

**Associations of unilateral pain with gait asymmetry**

Unilateral leg pain associated with more gait asymmetry in step length, with larger step length in the painful leg compared to the unpainful leg (0.42 cm; P= 0.003) (Table 3). Additionally, unilateral leg pain associated with smaller step length and shorter single support percentage in both the painful and the unpainful leg compared to participants without leg pain (−0.30 SD; P < 0.001 and −0.84 SD; P= 0.025) (Supplemental Table 1).

Unilateral hip pain associated with more asymmetry in step length and single support percentage, with larger step length (0.50 cm; P= 0.023) and shorter single support percentage (−0.24%; P= 0.045) in the painful leg compared to the unpainful leg (Table 3). Similar to unilateral leg pain, we found unilateral hip pain to associate with shorter single support percentage in the painful leg and smaller steps and shorter single support percentage in the unpainful leg, compared to participants without hip pain (Supplemental Table 1).

Unilateral foot pain was also associated with larger asymmetry in step length (0.56 SD; P= 0.010), with larger steps in the painful leg compared to the unpainful

**Table 4.** Relation between gait domains and OA in participants with pain in the hip or knee

| Domain | Rhythm | Variability | Phases | Pace | Tandem[a] | Turning | Base of Support |
|---|---|---|---|---|---|---|---|
| OA hip | 0.23 (−0.15; 0.62) | **−0.45 *** **(−0.85; −0.05)** | 0.03 (−0.34; 0.41) | −0.08 (−0.41; 0.24) | −0.08 (−0.48; 0.32) | −0.29 (−0.68; 0.11) | 0.00 (−0.41; 0.41) |
| OA knee | 0.15 (−0.07; 0.37) | −0.01 (−0.24; 0.22) | 0.16 (−0.05; 0.38) | 0.12 (−0.07; 0.30) | −0.12 (−0.35; 0.11) | −0.06 (−0.28; 0.17) | −0.07 (−0.30; 0.16) |

Values represent differences in z-scores of gait (95% confidence interval) for presence of osteoarthritis in the respective body part. Osteoarthritis was scored as 1 = yes and 0 = no. Results in bold survived thresholds of nominal significance (*$p < 0.05$). All analyses were adjusted for age, sex, height, weight and the interval between OA and gait assessment. (n= 419, of which 25 with hip OA and 98 with knee OA)
[a]Additionally adjusted for the step count and step size within the tandem walk

leg (Table 3). Furthermore, we found unilateral foot pain to associate with larger asymmetry in step length variability, with larger step length variability in the painful leg compared to the unpainful leg (0.26 cm; P= 0.003) (Table 3). We found no significant associations between unilateral knee pain and gait (Table 3 and Supplemental Table 1).

### Role of OA in associations between pain in the lower body and gait

To study whether the observed associations between pain and gait were driven by OA, we excluded 582 participants with radiographic hip and/or knee osteoarthritis. Interestingly, the associations remained largely unchanged (Supplemental Table 2), suggesting that associations of lower body pain with gait are at least not completely driven by hip and/or knee OA.

We next examined whether we could differentiate individuals with and without radiographic OA using gait pattern, within the subjects with hip or knee pain. In contrast to a higher variability when comparing hip pain versus no hip pain, we found painful hip OA to associate with lower Variability compared to pain without hip OA (−0.45 SD; P= 0.026). For painful knee OA, no significant associations with the gait domains were found (Table 4).

## Discussion

In this study, we show that chronic lower body pain associates with gait differences in community-dwelling individuals. Lower body pain associated with Rhythm (taking slower steps), Pace (taking smaller steps), Variability (less variability among steps)

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and Phases (longer double support time). These associations are mainly driven by leg pain, especially hip, and remained after exclusion of participants with OA. Furthermore, we found unilateral pain to associate with larger gait asymmetry and gait differences in both the painful and unpainful leg. For hip pain, gait variability was higher in individuals with radiographic OA compared to those without OA.

To our knowledge, we are the first large population-based study to investigate lower body pain in relation to gait. Few studies investigated the relation between pain and gait and focused on OA-related pain, the most common cause of joint pain in elderly. Hence, comparison of our findings with previous studies is limited. We found lower body pain to associate with slower and smaller steps with longer double support, which corresponds with previous studies investigating gait in knee OA [18-20]. In our study, these associations were especially driven by hip pain. Interestingly, the associations remained after excluding participants with radiographic OA, suggesting they were independent. Hence, lower body pain may have clinical impact outside of OA, as longer double support is a risk factor for falling and gait speed (Rhythm, Phases, and Pace combined) strongly relates to mortality [3,10].

In addition, we found unilateral leg pain to associate with gait asymmetry and differences in gait of legs. Unilateral leg, hip and foot pain showed similar patterns of associations, such as larger step length asymmetry with larger step length in the painful compared to the unpainful leg. We found shorter single support time for the painful leg, which contrasts with a previous study in OA where they found a longer single support time for the affected leg, supporting independence of our findings from OA [15]. The observed lower single support percentage in both legs corresponds to a recent review on effects of OA on gait in osteoarthritic and non-osteoarthritic leg [27]. Most likely, these associations are the result compensatory mechanisms to reduce load on the painful leg, by way of increasing its step length and increasing double support. Interestingly, we found unilateral foot pain to associate with larger asymmetry in step length variability, with larger step length variability in the painful leg. This association is especially important, because larger variability is considered a strong risk factor of falling [8-10].

Similar to a previous study, we found no associations of knee pain with gait [28]. Since directionality of associations was similar to hip and foot pain with smaller effect sizes, knee pain most likely has a less pronounced effect on gait and may therefore require more power to identify associations.

Joint pain is one of the hallmarks of OA, a common joint disease in older people that may have devastating consequences [29]. In a clinical setting, it is often difficult to distinguish OA-related pain from pain caused by other pathologies without radiographic examinations. In literature, both hip and knee OA strongly affect the gait pattern, suggesting a possible role for gait assessment in differentiating

OA-related pain from pain due to other causes [15,27]. Interestingly, we observed that individuals with hip OA pain have less variability between steps compared to individuals with hip pain due to other causes. This finding may indicate that gait assessment can aid in identifying OA in people with pain and might be helpful for the general practitioner. Before implementation of gait measurement into general practice, further research in patients presenting with hip pain in the general practice should be conducted. For example, additional value on top of other known physical tests, such as range of motion, should be explored.

Strengths of our study include the large population-based cohort design, enabling us to identify associations that may be generalizable to the general population. Additionally, the gait assessment in different walking conditions, including turning and tandem walking, gives a comprehensive description of gait differences with chronic pain. Furthermore, because we did not focus on a single pathological origin of pain, we could provide information on the relations of general pain with the gait pattern. Radiographs were taken in all individuals, independent if they were suspected to have OA or not, so there were no suspected but undiagnosed subjects in the analysis concerning OA.

Limitations of our study include that gait was assessed at the research center, which may have prevented participants with severe gait problems from participating. Generalizability of our results may therefore be limited to a relatively healthy elderly population. Additionally, due to the cross-sectional study design, we are not able to identify the temporal relationship between pain in the lower body and gait. Although it is most likely that pain leads to gait differences, it is also possible that a deviant gait pattern causes joint pain. Hence, associations may be bidirectional, implicating that both interventions targeting pain may improve the gait pattern and interventions targeting gait may decrease pain. Future studies should further investigate this possible bi-directionality in the associations between lower body pain and gait.

In conclusion, in community-dwelling individuals, chronic lower body pain associates with gait differences, independent of OA. Individuals with pain in the lower body take slower and shorter steps with longer double support. Additionally, unilateral pain associates with larger gait asymmetry, and gait differences in both painful and unpainful leg. Our results further suggest that gait patterns might aid in distinguishing between OA and other pathology in people with musculoskeletal pain. Prospective analysis would be valuable to determine whether gait analysis is predictive for progression and/or pain.

Proper care and treatment of chronic pain could be a way of reducing gait problems and thereby fall risk and associated mortality. Future studies should investigate whether treatment of lower body pain aids in improving gait, and thereby reduces gait-related morbidity and mortality.

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6.2

**Supplementary table 1.** Associations of unilateral pain in the leg, hip, knee and foot with gait in the painful and unpainful leg.

| Domain | Rhythm | Variability | Phases | Pace | Base of Support |
|---|---|---|---|---|---|
| Gait parameter | Single Support Time ( s) | Step length SD (cm) | Single Support Phase (%) | Step length (cm) | Stride width SD (cm) |
| Unilateral pain leg | | | | | |
| Painful leg | 0.02 (−0.02; 0.05) | 0.04 (−0.05; 0.14) | **−0.30 ** (−0.46; −0.13)** | **−0.84 * (−1.58; −0.11)** | 0.05 (−0.05; 0.16) |
| Unpainful leg | 0.03 (−0.01; 0.06) | 0.03 (−0.06; 0.13) | **−0.20 * (−0.36; −0.03)** | **−1.28 ** (−2.02; −0.54)** | 0.04 (−0.06; 0.15) |
| Unilateral pain hip | | | | | |
| Painful hip | 0.01 (−0.05; 0.07) | −0.06 (−0.19; 0.08) | **−0.57 ** (−0.81; −0.32)** | −0.90 (−1.98; 0.17) | −0.08 (−0.23; 0.07) |
| Unpainful hip | 0.03 (−0.02; 0.09) | 0.07 (−0.07; 0.21) | **−0.35 ** (−0.60; −0.11)** | **−1.43 * (−2.51; −0.35)** | −0.03 (−0.18; 0.12) |
| Unilateral pain knee | | | | | |
| Painful knee | 0.02 (−0.03; 0.07) | 0.10 (−0.02; 0.22) | −0.09 (−0.30; 0.12) | −0.31 (−1.23; 0.60) | 0.05 (−0.08; 0.18) |
| Unpainful knee | 0.03 (−0.02; 0.07) | 0.04 (−0.07; 0.16) | −0.04 (−0.25; 0.16) | −0.38 (−1.30; 0.53) | 0.03 (−0.10; 0.15) |
| Unilateral pain foot | | | | | |
| Painful foot | −0.01 (−0.08; 0.05) | **0.21 * (0.05; 0.36)** | **−0.30 * (−0.58; −0.02)** | **−1.32 * (−2.56; −0.09)** | 0.08 (−0.09; 0.25) |
| Unpainful foot | 0.00 (−0.07; 0.06) | −0.05 (−0.20; 0.11) | −0.21 (−0.49; 0.07) | **−1.97 ** (−3.20; −0.74)** | 0.07 (−0.10; 0.24) |

Values represent differences in gait parameters (95% confidence interval) for presence of pain in the respective body part. Results in bold survived thresholds of nominal significance (*p < 0.05; **p < 0.005). All analyses were adjusted for age, sex, height, weight, and time interval between pain and gait assessment.

Leg: n=2012; Hip: n = 2231; Knee: n = 2103; Foot: n = 2215.

Painful leg: Difference between gait parameters of painful leg for cases (subjects with unilateral pain) and mean gait parameters for controls (subjects without pain)

Unpainful leg: Difference between gait parameters of unpainful leg for cases (subjects with pain) and mean gait parameters for controls (subjects without pain)

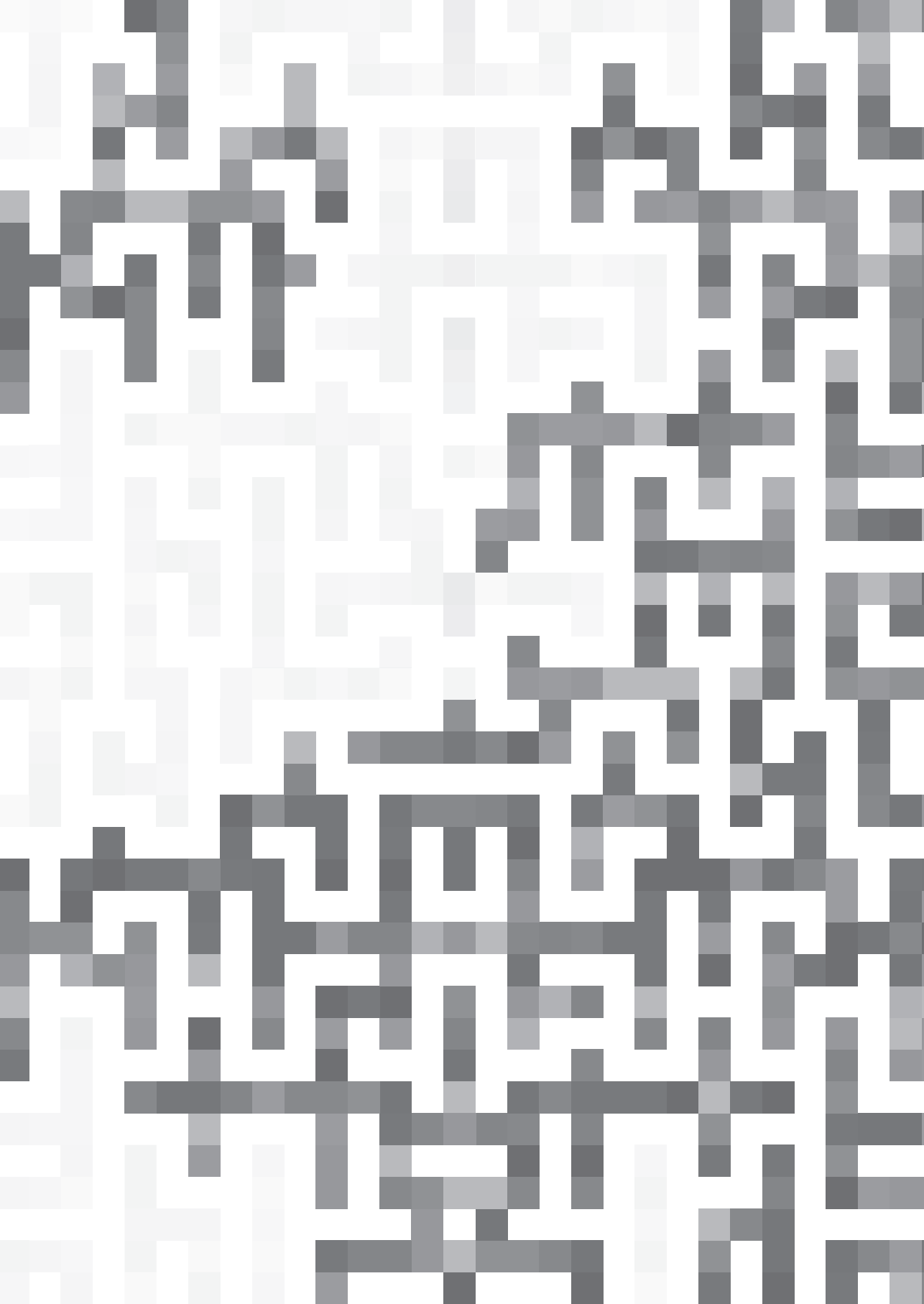Abbreviations: s = seconds, SD = standard deviation, cm = centimetres, n = number of participants.

Gait Differences in Chronic Joint Pain in the Lower Body **177**

**Supplemental table** 2. Associations of pain in the lower body with gait domains, without participants with osteoarthritis

| Domain | Rhythm | Variability | Phases | Pace | Tandem[a] | Turning | Base of Support |
|---|---|---|---|---|---|---|---|
| Summary score lower body pain[b] | **−0.20 *** **(−0.36; −0.04)** | **0.21 *** **(0.02; 0.39)** | **−0.24 **** **(−0.40; −0.08)** | **−0.18 *** **(−0.33; −0.03)** | 0.00 (−0.16; 0.16) | 0.02 (−0.16; 0.21) | 0.08 (−0.10; 0.26) |
| Pain lower back | −0.06 (−0.17; 0.06) | 0.08 (−0.05; 0.21) | −0.03 (−0.14; 0.08) | −0.10 (−0.20; 0.01) | −0.04 (−0.16; 0.07) | 0.05 (−0.08; 0.18) | −0.04 (−0.17; 0.09) |
| Pain leg | **−0.11 *** **(−0.22; 0.00)** | 0.10 (−0.02; 0.22) | **−0.19 **** **(−0.29; −0.09)** | −0.08 (−0.18; 0.02) | 0.00 (−0.11; 0.11) | 0.00 (−0.12; 0.12) | 0.05 (−0.06; 0.17) |
| Pain hip | −0.15 (−0.32; 0.01) | 0.03 (−0.16; 0.22) | **−0.26 **** **(−0.42; −0.10)** | −0.14 (−0.29; 0.02) | 0.06 (−0.10; 0.23) | 0.09 (−0.10; 0.28) | 0.14 (−0.05; 0.32) |
| Pain knee | −0.08 (−0.21; 0.05) | 0.05 (−0.10; 0.19) | −0.08 (−0.20; 0.05) | −0.07 (−0.19; 0.04) | 0.02 (−0.11; 0.15) | −0.03 (−0.17; 0.12) | 0.07 (−0.07; 0.21) |
| Pain foot | −0.03 (−0.20; 0.14) | 0.09 (−0.10; 0.28) | −0.13 (−0.29; 0.04) | −0.01 (−0.16; 0.15) | −0.12 (−0.29; 0.05) | −0.03 (−0.22; 0.16) | 0.07 (−0.12; 0.26) |

Values represent differences in z-scores of gait (95% confidence interval) for pain in the respective body part. A lower value of gait represents worse gait. Pain was scored as 1 = yes and 0 = no. Results in bold survived threshold of nominal significance (*p < 0.05; **p < 0.005). All analysis were adjusted for age, sex, height, weight, and time interval between pain and gait assessment. (n=1557)

[a] Additionally adjusted for the step count and step size within the tandem walk

[b] Pain lower body = (pain in lower back + pain left leg + pain right leg) / 3.

6.2

# CHAPTER 7

General discussion

Chronic musculoskeletal pain is not just a symptom but a complex disease in its own right, with individual and environmental factors playing important roles in the pathophysiology. The overall objective of this thesis was to unravel disease determinants of chronic musculoskeletal pain.

We identified some potential new genetic and environmental risk factors for chronic musculoskeletal pain. We explored structural and functional alterations in the nervous system in chronic musculoskeletal pain and identified the impact of osteoarthritis and chronic lower body pain on mobility. In the general discussion, some general issues concerning chronic pain research and possible new directions for further research will be debated.

## Definition of chronic pain

### Objective versus subjective pain measurement

A very challenging but interesting and important issue in chronic pain research is the definition of chronic pain.

Pain is defined by the IASP as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain is always subjective. Each individual learns the application of the word through experiences related to injury in early life. Biologists recognize that those stimuli which cause pain, are liable to damaged tissue. Accordingly, pain is that experience we associate with actual or potential tissue damage. It is unquestionably a sensation in a part or parts of the body, but it is also always unpleasant and therefore also an emotional experience" [1]. The emotions involved are influenced by many individual and environmental factors. The subjective character of pain makes it more difficult to form a consensus on the definition. Therefore, a pure objective measure to quantify the pain experienced per individual does not exist. On the other hand, there are concrete endpoints linked to chronic pain that can be measured. Some of the measures that could potentially be used are thermal sensitivity thresholds and alterations in the structure of the brain. In this thesis, these measures are studied in more detail in a population-based study.

Thermal sensitivity, especially heat pain threshold, is increased in chronic pain. This is described in more detail in chapter 5 in this thesis. These thresholds can be used to more objectively measure pain sensitivity. With the measurement of these thresholds, control on the stimulus is applied, but the experience of these thresholds remains a subjective experience of the tested individual. In this thesis, we show that these thresholds are associated with several factors that are influenced by environmental exposure, such as BMI, skin color and psychological factors such

as depression. Additionally, we found a strong indication that pain sensitivity is also heritable for a significant part. This is described in chapter 2. The findings in this thesis stress again the multifactorial complexity of not only chronic pain but also the potential objective measures underlying chronic pain and pain sensitivity.

In chapter 4, we describe a study in which we show structural differences in the brain in individuals with chronic musculoskeletal pain. A lower volume of grey matter was found in the temporal and frontal lobe and the hippocampus, which are part of the limbic system. This strengthens the consensus that emotional processing is a substantial component in the pathogenesis of chronic pain.

Other objective measures, which might be relevant to study in relation to chronic pain, could be the measurement of central sensitization or conditioned pain modulation e.g. diffuse noxious inhibitory control (DNIC). With these measurements, sensitization and neuronal plasticity of the nervous system and the descending inhibitory tracts, which are thought to be affected in chronic pain, can be studied more objectively. The descending inhibitory tracts are important in normal pain processing but can also be influenced by strong emotions to block pain.

However, subjective measurement of pain and chronic pain might reflect the burden of the disease in a better way. The influence of experienced pain on the quality of life is the major reason for the existence of pain medicine and research. Subjective pain experience account for the sickness absence and a higher health care consumption.

Since there are many factors influencing the chronification of pain, and some of them may still be unknown (Figure 1), a combination of objective and subjective measures would therefore better comprehend all aspects of chronic pain.

**Duration to chronification of pain**

Several definitions are used for the term chronic pain. Most of them are time dependent, e.g. Pain existing for more than three months or six months. A definition which gives more right to the term chronic pain is "pain that persists beyond the normal time of healing", which sometimes may be less than a month and in other cases more than six months[2]. Every injury has a different prognosis and for some conditions, especially degenerative diseases like osteoarthritis, healing may not occur at all. Therefore, a generalized definition of chronic pain being present if the pain exceeds three months of duration, as defined by the IASP, may not be sufficient to properly identify individuals with chronic pain.

In the studies presented in this thesis focusing on chronic musculoskeletal pain, we mostly used the definition 'pain anywhere in the body for more than half of the days in the last six weeks' which was recorded using a pain drawing. In this pain drawing, a drawing of a human body was shown in which the participant could
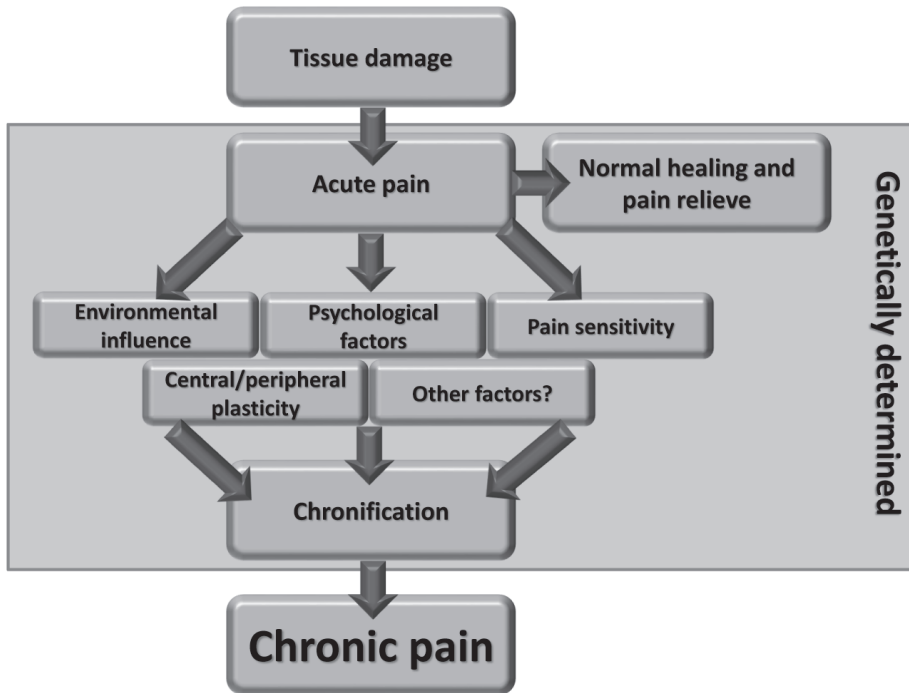
**Figure 1.** The multifactorial aspects of the development of chronic pain. Pain starts with tissue damage. When acute pain is not relieved by normal healing, chronification can occur. The course from acute to chronic pain is influenced by many different factors.

indicate the painful areas if present. We also collected questionnaire data on joint pain, in which the participants were asked if they had pain in a specific joint and for how long. Both definitions have their strengths and weaknesses.

With the pain drawings, pain in the last six weeks can be assessed, which according to the IASP definition is not yet to be called chronic pain. However, we observed that over 95% of the individuals experiencing knee or hip pain on a daily basis for the last six weeks also experience this pain for longer than 3 months (based on specific questionnaire data).

In our studies, multiple results indicated that central sensitization, as part of the pathophysiology of chronic pain, is present in the general elderly population using the pain drawing data. Heat pain thresholds were significantly lower in individuals with chronic pain according to the pain drawing and also, structural brain MRI differences were found using the same data. Chronification of pain might therefore be defined as the moment at which central sensitization is present, which may be earlier than 3 months. If we can develop reliable objective measures to identify this

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central sensitization, we will be able to make a better definition for chronic pain then to just look at duration, which will also improve the studies on chronic pain.

Although the questionnaire data is able to identify the individuals with pain longer than 3 months, the pain drawing includes the question if the pain is present for more than half of the days, which gives an idea on the burden of the disease.

Chronic pain can therefore be dissected in a number of different components. One of them is the duration of pain, while an objective measure, such as central sensitization, and a measure for severity or burden of the disease are other components that should be taken into consideration.

## Sex differences in chronic pain and pain sensitivity

Chronic musculoskeletal pain and pain sensitivity are known to be sexually dimorphic. As described in this thesis, we found additional evidence in our studies for this phenomenon. The most notable observation was that the majority of the associations found were sex specific and mostly observed in women.

There are a few hypotheses on why the associations found in this thesis were mainly found in women. In a recent study, it was shown that pain sensitization is regulated by different immune cells in male and female mice, indicating different pathways for chronification of pain between sexes [3]. In addition, the difference in hormonal exposure during development and during life may be of great influence. As we showed in chapter 3.1, testosterone exposure during development influences the development of chronic pain later in life. Additionally, in chapter 3.2, we show that lower sex hormone levels are associated with an increased prevalence of chronic pain, especially in women.

Since pain is for a part subjective and psychological aspects such as coping and emotional behavior are different between sexes, these might play a role in the differences in reporting chronic pain between men and women. This might add another challenge in studying these differences in chronic pain and pain sensitivity.

## Studying chronic pain in the general population

In this thesis, chronic pain was studied in the Rotterdam Study, a population based study of individuals aged 45 years and older. Within this population almost half of all individuals experience chronic pain, of which half is caused by osteoarthritis. Most pain research worldwide is performed within very specific chronic pain syndromes, such as migraine or complex regional pain syndrome. Although these

studies are capable of investigating determinants associated with those chronic pain syndromes, they lack the ability of generalizing their conclusions to community dwelling individuals without those clearly defined pain phenotypes. Only 23% of all individuals with chronic pain ever visit a pain specialist and for approximately 15% who do visit a medical professional no specific diagnosis can be made [4]. Thus, by studying only the patients in specialized pain clinics, many individuals who suffer from chronic pain on a daily basis, are not included. Therefore, a population based study using a more heterogeneous pain phenotype may lead to new insights into the pathophysiology of chronic pain. Since the prevalence of chronic pain increases with age, a community dwelling study population of individuals of 45 years and over is highly relevant for daily practice.

The use of the Rotterdam study for pain research also caused some limitations. Since pain research is not the primary aim of the Rotterdam study and many other research questions are being studied, time and resources are limited to extensively measure all individuals with respect to their pain phenotype. This means choices had to be made, which questions to ask and which tests to perform. For example, quantitative sensory testing has a lot of different modalities, but the choice was made to only incorporate thermal stimuli in the research protocol. Thermal QST measurements are relatively easy and quick to perform and are commonly used in other studies, also in other population based studies. Another limitation is that the statistical methods in such a large study with thousands of people are different from the methods used in smaller studies. For example, in the study of structural brain alterations, voxel based techniques are computationally very challenging in such a large study population. In small sample sizes, this method has been used many times successfully but is prone to spurious findings due to multiple testing. In the study presented in this thesis, a volume segmentation method was used, which is more common in large imaging studies and follows a clear hypothesis-driven approach. This deviating approach makes our study different from other clinical studies published before. One of the major advantages of our study is the more generalizable results when compared to very specific clinical studies. But therefore, it also introduces more heterogeneity into the study population which could prevent us from finding some of the associations.

## Genetics of pain sensitivity

The proportion of genetic influence on pain and pain sensitivity has been under debate. In previous studies, the heritability of chronic pain and pain sensitivity has been estimated in classical twin studies. A recent review showed that large dif-

ferences between heritability estimates can be found depending on the clinical phenotype [5], ranging from a heritability of around 50% for migraine to around 25% for irritable bowel syndrome. Heritability of experimental pain sensitivity has been studied scarcely, with only 3 previous study reports. All three reports studied twins and used various experimental designs, with relative modest sample sizes [6-8]. Consequently, heritability estimates fluctuated from 0 to 60%. In this thesis, the genetics of one of the experimental pain modalities, the heat pain threshold, is investigated further, using a heritability calculation, a candidate gene approach and a genome wide association study (GWAS).

## Heritability of heat pain threshold

In this population based study of individuals of 45 years and older, we found a heritability estimate of 19% for heat pain threshold. But once we stratified our population for the presence of chronic pain, the heritability for heat pain threshold was non-significant in participants with chronic pain and 32% in participants without chronic pain. We also found a significant difference of heritability estimates between women and men (35% vs 9%).

Chronic pain is known to influence the heat pain threshold, as we have also demonstrated in chapter 5 in this thesis. Up to now, genetic studies on heat pain threshold and other quantitative sensory measurements did not take the presence of chronic pain into account. Since there is this large influence on the heritability estimate, genetic analysis for quantitative sensory testing should be stratified by or at least adjusted for the presence of chronic pain.

We found that within a population of men aged 45 years and older, the heat pain threshold is not easily measured in all subjects. In our study sample, almost 20% of all men reached the maximum threshold of 50 degrees Celsius for the heat pain threshold. As a consequence, part of the variability of the measurement is lost, which results in lower power to measure heritability in this part of the population. Another explanation for the low heritability estimate for heat pain threshold in men could be that this threshold in men is influenced by other factors, for example psychological, emotional factors or stronger descending inhibitory tracts.

The findings in this heritability analysis indicates that in individuals without chronic pain, 32% of the variance in the heat pain threshold measurements is explained by genetic variance of common SNPs. Since only part of the genetic variance is included in these common SNPs, the true heritability is likely to be even larger.

To estimate the heritability of the heat pain threshold, defined as the proportion of phenotypic variance due to only additive genetic effects within a population, we

used the restricted maximum likelihood (REML) method. This method is able to quantify heritability estimates attributable to all investigated variants in genome wide association studies and is implemented in the Genome-wide Complex Trait Analysis (GCTA) package [9]. There are several ways to estimate heritability, including twin studies, pedigree analysis, etc. Heritability estimated from pedigree data is not the same as measuring the proportion of phenotypic variation explained by all SNPs in the population (which was used in this thesis) because the former includes the contribution of all causal variants, whereas the latter only includes the contribution of causal variants that are in linkage disequilibrium (LD) with genotyped common SNPs [9, 10]. This means that the true heritability estimate of heat pain threshold will probably be higher, since GCTA is only able to detect a part of the heritability. Yet, the GCTA method has been successfully applied for identifying the amount of 'missing' heritability in population based studies for other complex traits such as height [11]. In complex traits, it is unlikely that one or a few genetic variants account for the full phenotypic variance seen in the population. The heritability estimate, as determined by this GCTA method, takes into account that the combination of variants in the genetic material makes up for the susceptibility for the studied disease or condition.

**Genome wide association studies**

In our search of finding the genes underlying the phenotypical differences in heat pain thresholds in the general population, we performed genome wide association studies (GWAS). One genome wide significant hit was found, a deletion on chromosome 1 in the PAPPA2 gene. This gene encodes for a protein which is thought to regulate IGF, which is implicated to play a role in nociceptive sensitivity of primary afferent neurons.

In addition, we found a total of six other suggestive signals. Replication in independent cohorts and additional functional testing are needed to demonstrate the true signals and causal associations.

Although we have performed the GWAS in the largest population sample with heat pain thresholds available at this moment (n= 3,795), we probably are still underpowered to find all potential genetic signals. Chronic pain is a complex disease, with many genetic and non-genetic factors playing a role. In the search for genetic variations in other complex traits, such as BMI or cognition, large collaborations with a population sample of more than 50,000 individuals were needed to find more genetic variation associated with the trait. [12, 13]

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**Systematic review of genetic variants previously described**

Most other studies investigating the genetic background of pain sensitivity, focused on selected candidate genes previously described with pain and studied polymorphisms in those genes in modestly sized pain patient populations [14-26].

In a better powered setting, we studied six such candidate genes previously described to be associated with pain sensitivity measures (COMT, DRD3, OPRK, OPRM1, SLA6A4 and HTR1A) and did not replicate the previous findings. Since we used a population based study instead of specific patient populations, it is still possible that the previous associations are true for the studied phenotypes. Therefore, the genes previously described to be associated with pain sensitivity are probably not the causal genes and the search for genes influencing pain sensitivity is a long way from completed.

## Suggestions for further research

Based on the findings in this thesis and the general issues raised in this chapter, several suggestions for further research can be provided.

### Stratification according to sex

Across the chapters in this thesis and also in other literature, the sex differences in occurrence of chronic pain and pain sensitivity are clearly present. There are many factors different between the sexes influencing the sensitivity and susceptibility to pain, such as the hormonal influences, anatomy and physiology of the nervous system and also the psychological factors such as coping behavior and catastrophizing. Future epidemiological as well as genetic research should therefore stratify according to sex in order to find more robust and personalized risk factors for chronic pain.

### Experimental pain sensitivity

In the Rotterdam study, only responses to thermal stimuli were measured in order to study experimental pain sensitivity. We chose this measure because it is easily measured and noninvasive. This makes it appropriate for the use in a large population based study, such as the Rotterdam Study. Yet, there are several other experimental pain modalities, such as mechanical pressure, weighted pinprick and electrical stimuli.

As demonstrated in Figure 2, A-delta fibers and C fibers are both responsible for the transport of noxious stimuli. A-delta fibers are mostly sensitive to mechani-

cal noxious stimuli and in a lesser extend to thermal noxious stimuli. In a state of sensitization, like in a chronic pain state, these A-delta fibers become more sensitive for thermal stimuli as well. In a population based setting, many individuals are not sensitized, which could be an explanation for the large number of men in which we were not able to adequately measure the heat pain threshold. Studying other modalities of experimental pain stimuli, like mechanical pressure or electrical stimulation, may therefore be more fruitful in this population.

An interesting other objective measure for altered pain sensitivity could be conditioned pain modulation, e.g. diffuse noxious inhibitory control (DNIC). By studying the difference between the pain tolerance threshold before and after exposure to a second noxious stimulus (such as ice water), the function of the descending inhibitory tracts, which are thought to be affected in chronic pain, can be measured. Research up to now suggest DNIC is associated with chronic pain but the relevance to selection and efficacy of pain treatment is not yet elucidated. [27]

Quantitative sensory testing can be very useful in the general population to study chronic pain and pain sensitivity pathways. In chronic pain patients, it can also be very useful to determine progress during treatment. For the diagnosis of chronic pain, central sensitization or neural plasticity in an individual patient in a clinical setting, QST is less useful at this moment, since reliable normal values are not available for all subpopulations, such as the elderly. Normal values are different for each individual and are influenced by many known but also unknown factors.
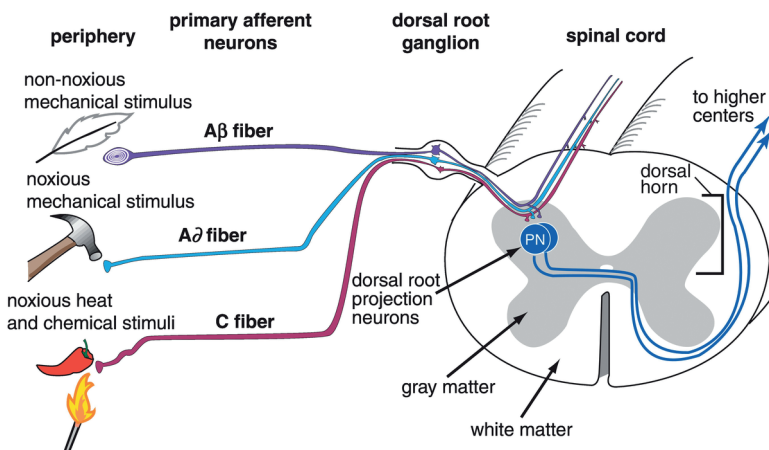


**Figure 2.** Activation of nociceptive nerve fibers. Detection of a noxious stimulus occurs at the peripheral terminals of primary afferent neurons and leads to generation of action potentials that propagate along the axon to the central terminals. Aβ fibers respond only to non-noxious stimuli, Aδ fibers respond to noxious mechanical stimuli and subnoxious thermal stimuli, and C fibers respond only to noxious mechanical, heat, and chemical stimuli. Adapted from Stahl, S.M., 2008, Essential Pharmacology Online.

If all individuals included in large QST databanks would be analyzed for cluster-ing, we potentially could define subgroups and identify different phenotypes. It is hypothesized that fulfilling the criteria of a single phenotype will help, in future, to personalize the choice of therapy and improve individual effects of treatment of chronic pain.

### Genetics of chronic pain and pain sensitivity

As described in the first part of this chapter, defining the phenotype of chronic pain and pain sensitivity is challenging and this may in part be the reason that the genetic influence remains largely uncertain. The heritability estimates of several pain phenotypes surely suggest a genetic influence, but the GWAS results suggest a complex genetic architecture with many genetic variants with a small effect. The identification of the involved genes therefore requires larger sample sizes and col-laborative networks to better understand the genetics of chronic pain. As shown in Figure 1, there are many different factors influencing the development of chronic pain, which should be incorporated in the pain definition to create a more robust phenotype.

Incorporation of pain severity scores might be an interesting addition to better define chronic pain compared to the focus on duration of pain, since it adds a measure of burden. In addition, studying individuals with joint damage with and without joint pain may further elucidate the genetic factors influencing if someone is susceptible for developing joint pain. Efforts in our study have not been success-ful in this respect, mainly because of the lack of power for this analysis. Although the Rotterdam study is considerably large, collaboration efforts will be needed with improved phenotyping to further genetic pain research.

Other genetic methodological approaches could also provide more insight in the genetic architecture of pain. For example, sequencing for rare alleles or gene-environment interactions.

Furthermore, the identified new genetic loci associated with chronic pain, espe-cially PAPPA2, should be further investigated. Functional research has the potential to demonstrate true causal pathways and may lead to not only better understand-ing of chronic pain but also new drug targets.
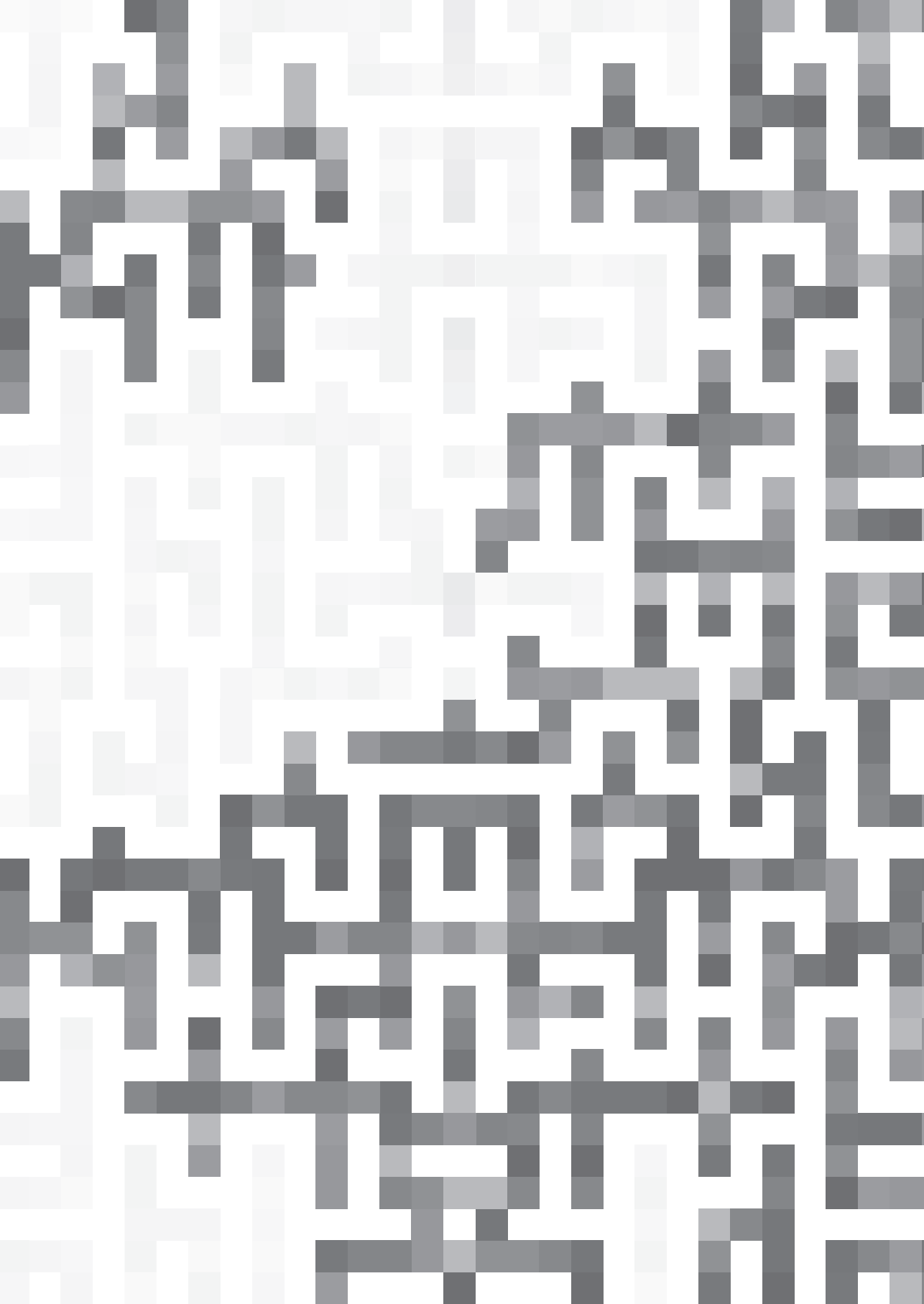
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# CHAPTER 8

Summary/Samenvatting

## Summary

Chronic musculoskeletal pain is a common disabling condition with a great impact on daily functioning. In the Netherlands, 19% of all individuals aged 21 years and older experience chronic pain and in elderly this is more than half. This means that more than 2 million Dutch people experience pain on a daily basis, which is a higher prevalence than most common diseases like diabetes and coronary heart disease.

Chronic pain and pain sensitivity are complex traits with a variety of potential determinants. The development of chronic pain and an increased sensitivity to stimuli, caused by sensitization of the nervous system is an unintended consequence after tissue damage. In this scenario, the pain is prolonged or more severe compared to what might be expected during a normal healing process.

A wide variety of risk factors have been described for the development of chronic pain and pain sensitivity. The overall objective of this thesis was to identify and characterize causal and consequential determinants of chronic musculoskeletal pain and pain sensitivity in the general population.

First, in **Chapter 2**, the results of the study on the genetic background of heat pain sensitivity are presented. In 3795 individuals, the heritability for heat pain threshold was estimated to be 19%, and within individuals without chronic pain even 32%. Chronic pain influences the heat pain threshold substantially, as part of the pathophysiology of chronic pain via sensitization of the central nervous system.

Genetic polymorphisms previously found to be associated with pain sensitivity were not replicated in this study. In the search for potential new genetic markers, using a genome-wide association study, 6 suggestive signals were found and one genome-wide significant locus in the PAPPA2 gene, which is thought to be a local regulator of insulin-like growth factor (IGF) bioavailability. IGF is implicated to play a role in the nociceptive (pain) sensitivity of primary afferent neurons.

In **Chapter 3**, hormonal influences on the development of chronic pain are described. Type 3 finger length pattern (longer 4th digit compared to the 2nd digit) is influenced by a higher prenatal androgen exposure. The results in **Chapter 3.1** show that this finger length pattern is associated with joint pain at multiple sites with an odds ratio of 1.41. In addition, the association of osteoarthritis, as one of the major causes of joint pain in the elderly population, with type 3 finger length pattern was studied in a meta-analysis with previously published data. Type 3 finger length pattern showed to be associated with hand osteoarthritis and symptomatic knee osteoarthritis.

Since chronic pain is more prevalent in women, the relation between sex hormones and the occurrence and incidence of chronic musculoskeletal pain is

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described in **Chapter 3.2**. Within 9,717 participants aged 45 years and older, the association between sex hormones (estradiol, testosterone, androstenedione and 17-hydroxyprogesterone) and chronic pain was studied. Women with estradiol or androstenedione levels in the lowest tertile had a higher prevalence of chronic pain, independent from age, BMI, health and lifestyle factors or the presence of osteoarthritis. The lowest tertile of 17-hydroxyprogesterone in women was associated with 38% more incident chronic pain.

What happens to the brain in chronic pain is studied using MR imaging in more than 3,500 individuals in **Chapter 4**. First, the results of structural alterations in the brain in chronic pain are discussed in **Chapter 4.1**. Global and regional brain volumes were automatically segmented and quantified. The total grey matter volume was smaller in women with chronic pain. This effect was primarily driven by smaller grey matter volume in the temporal lobe, the frontal lobe and the hippocampus. The identified volumetric differences in the specific brain areas, suggest gender-specific neuroplasticity in chronic pain and involvement of emotional processing.

The role of brain white matter microstructure in chronic pain is investigated in **Chapter 4.2**. Using diffusion MR imaging of the brain, the association of chronic pain and the microstructure (fractional anisotropy and mean, axial and radial diffusivity) of global, lobar and tract specific white matter was studied. The results presented suggest increased wiring of the cerebral white matter, especially in the left frontal and temporal lobe. In addition, increased wiring was also found in the left medial lemniscus in chronic pain and also in persistent chronic pain, indicating a potential prognostic value.

Central sensitization is part of the pathophysiology of chronic pain and can be determined with quantitative sensory testing. In **Chapter 5**, the thermal QST modality is studied. Higher sensitivity for heat pain, one feature of central sensitization, was found to be present in community dwelling elderly with chronic pain.

Furthermore, determinants which can influence the measurement, analyses and interpretation of the thermal QST were identified. Based on experience and previous literature, the determinants studied were body mass index (BMI), reaction speed, systolic and diastolic blood pressure, skin color, skin temperature, seasonal influence, depression, anxiety, atopic eczema, age at menarche, years since menopause, hormone replacement therapy use during menopause and reproductive lifespan. In addition to the effect of age and gender on thermal sensitivity, darker skin color and the presence of atopic eczema were associated with a higher sensitivity for heat pain. Cold sensitivity and warmth sensitivity thresholds were both influenced by BMI, reaction speed, skin temperature, season, depression, dark skin color, years since menopause and reproductive lifespan. These additional determinants should be considered when analysing and interpreting QST measurements.

Chronic pain and osteoarthritis (OA) is associated with disability and decreased mobility. Therefore, the study on chronic pain and osteoarthritis and their influence on gait is presented in **Chapter 6**. Gait is an important indicator of health and impaired gait is related to increased morbidity and mortality.

In **Chapter 6.1**, the results are shown of the study on how hip and knee OA are related to gait in community dwelling individuals. Hip OA, but not knee OA, associates with gait differences in normal walking, turning, and tandem walking. Hip OA is associated with a higher rhythm (cadence) in normal walking and with making more mistakes in tandem walk and with making more steps during turning.

To further investigate whether the observed changes in gait in OA differ from changes in gait due to other causes, the association of lower body pain and gait parameters is further elucidated in **Chapter 6.2**. The results show that pain in the leg and hip, independent from OA, is associated with a lower rhythm (cadence) which is in contrast with the higher rhythm found in hip OA. In addition, individuals with chronic lower body pain were more likely to walk with smaller steps, with a longer double support and with more asymmetry. Gait assessment may help to differentiate individuals with OA from those having pain due to other causes.

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## Samenvatting

Chronische gewrichtspijn is een veelvoorkomende invaliderende aandoening met een grote impact op het dagelijks functioneren. In Nederland heeft 19% van alle individuen boven de 21 jaar chronische pijn en voor ouderen is dit meer dan de helft. Dat zijn meer dan 2 miljoen Nederlanders die dagelijks pijn ervaren, wat betekent dat het meer voorkomt dan veelvoorkomende ziektes zoals diabetes en hart- en vaatziekten.

Chronische pijn en een verhoogde pijngevoeligheid zijn complexe aandoeningen met een groot aantal aan mogelijke oorzakelijke factoren. Het ontstaan van chronische pijn en een verhoogde pijngevoeligheid door zogenaamde sensitisatie van het zenuwstelsel is een ongewenst gevolg na weefselschade. Hierdoor houdt de pijn langer aan en is heftiger van aard dan wat verwacht mag worden tijdens een normaal helingsproces.

Een grote aantal risicofactoren zijn beschreven voor de ontwikkeling van chronische pijn en een verhoogde pijngevoeligheid. Het doel van dit proefschrift is het identificeren en karakteriseren van oorzakelijke factoren en gevolgen van chronische gewrichtspijn en een verhoogde pijngevoeligheid in de algemene populatie.

Allereerst, in **Hoofdstuk 2**, worden de resultaten van de studie naar de genetische achtergrond van pijngevoeligheid gepresenteerd. In 3795 individuen, werd de erfelijkheid van de hittepijndrempel geschat op 19% en in alleen de personen zonder chronische pijn was dit zelfs 32%. Chronische pijn beïnvloedde de hittepijndrempel substantieel, als onderdeel van het ziektemechanisme van chronische pijn, door een verhoogde gevoeligheid van het centrale zenuwstelsel.

Eerder in de literatuur beschreven associaties tussen pijngevoeligheid en variaties in het DNA werden ook in deze populatie onderzocht, maar deze associaties werden niet teruggevonden. Middels een 'genoom-wijde' associatie studie is er gezocht naar mogelijke nieuwe genetische markers voor de hittepijndrempel, waarbij er 6 suggestieve signalen en 1 genoom-wijd significante locus in het PAPPA2 gen werden gevonden. Dit gen wordt gezien als een lokale regulator van de biologische activiteit van insulin-like growth factor (IGF). IGF speelt onder andere een rol in de gevoeligheid van de zenuwen die de pijnprikkel van de receptor naar het ruggenmerg vervoert.

In **Hoofdstuk 3**, wordt de invloed van hormonen op de ontwikkeling van chronische pijn beschreven. Het type 3 vingerlengtepatroon (een langere ringvinger ten opzichte van de wijsvinger) wordt onder andere veroorzaakt door blootstelling aan geslachtshormoon tijdens de ontwikkeling in de baarmoeder. De resultaten in **Hoofdstuk 3.1** laten zien dat personen met dit type vingerlengtepatroon, 41% vaker pijn ervaren in meerdere gewrichten. Ook is de associatie van artrose, als een

van de meest voorkomende oorzaken van gewrichtspijn in de oudere populatie, met type 3 vingerlengtepatroon onderzocht. Type 3 vingerlengtepatroon was geassocieerd met artrose van de handen en symptomatische artrose van de knie.

Aangezien chronische pijn meer voorkomt bij vrouwen, is in **Hoofdstuk 3.2** bestudeerd wat de relatie is tussen geslachtshormonen en de prevalentie en incidentie van chronische gewrichtspijn. In 9717 deelnemers van 45 jaar en ouder, is de associatie tussen geslachtshormonen (estradiol, testosteron, androsteendion en 17-hydroxyprogesteron) en chronische pijn onderzocht. Vrouwen met estradiol of androsteendion hoeveelheden in het bloed in het laagste tertiel hadden vaker chronische pijn, onafhankelijk van leeftijd, BMI, gezondheids- en leefstijlfactoren of de aanwezigheid van artrose. Het laagste tertiel van 17-hydroxyprogesteron bij vrouwen was geassocieerd met 38% meer incidente chronische pijn.

Wat er gebeurd in het brein bij chronische pijn is bestudeerd middels MRI in meer dat 3500 individuen in **Hoofdstuk 4**. Als eerste zijn de resultaten van structurele veranderingen in het brein in chronische pijn bediscussieerd in **Hoofdstuk 4.1**. De totale hoeveelheid grijze stof was kleiner in vrouwen met chronische pijn. Dit was met name gedreven door een kleinere hoeveelheid grijze stof in de temporaal-kwab, de frontaalkwab en de hippocampus. De resultaten uit dit hoofdstuk sug-gereren dat er bij chronische pijn sprake is van geslacht specifieke veranderingen in het brein en dat emotionele verwerking hierbij betrokken is.

De rol van microstructuur van witte stof in het brein in chronische pijn is bestu-deerd in **Hoofdstuk 4.2**. Middels diffusie MRI van het brein, werd de associatie van chronische pijn met maten voor microstructurele integriteit van de witte stof be-studeerd. De gepresenteerde resultaten suggereren een versterkte bedrading van de witte stof in het brein, met name in de linker frontaalkwab en temporaalkwab.

Daarnaast zijn de tekenen van versterkte bedrading in de linker mediale lem-niscus niet alleen bij de aanwezigheid van chronische pijn gevonden maar ook bij langer aanhoudende chronische pijn, wat kan wijzen op een mogelijke prognosti-sche waarde.

Centrale sensitisatie is deel van de pathofysiologie van chronische pijn en kan bepaald worden middels kwantitatieve sensorische testen (QST). In **Hoofdstuk 5**, is de thermale QST-modaliteit onderzocht. Een hogere gevoeligheid voor hittepijn, een van de kenmerken van centrale sensitisatie, is aangetroffen bij ouderen met chronische pijn in de algemene populatie.

Bovendien zijn er factoren geïdentificeerd welke de meting, analyse en inter-pretatie van de thermale QST kunnen beïnvloeden. Gebaseerd op ervaringen en voorgaande literatuur, bestonden de bestudeerde factoren uit BMI, reactietijd, systolische en diastolische bloeddruk, huidskleur, temperatuur van de huid, seizoensinvloeden, depressie, angst, atopisch eczeem, leeftijd waarop de eerste

menstruatie plaatsvond, aantal jaren na de menopauze, hormoonvervangende therapie tijdens de menopauze en de duur van de reproductieve levensfase.

Naast het effect van leeftijd en geslacht op hittepijn gevoeligheid, was een donkere huidskleur en de aanwezigheid van atopisch eczeem geassocieerd met een hogere gevoeligheid voor hittepijn. Koude en warmte gevoeligheid werd beïnvloed door BMI, reactietijd, temperatuur van de huid, seizoen, depressie, een donkere huidskleur, aantal jaren sinds de menopauze en de duur van de reproductieve levensfase. Deze additionele factoren moeten in acht worden genomen worden bij de analyse en interpretatie van QST-metingen.

Chronische pijn en artrose zijn geassocieerd met invaliditeit en een verlaagde mobiliteit. Daarom zijn in **Hoofdstuk 6** de resultaten gepresenteerd van de studie naar chronische pijn, artrose en de invloed daarvan op het looppatroon. Het looppatroon is een belangrijke indicator voor gezondheid en een slechter looppatroon is geassocieerd aan een verhoogde morbiditeit en mortaliteit.

In **Hoofdstuk 6.1** is onderzocht hoe heup en knie artrose gerelateerd zijn aan het looppatroon in oudere individuen in de algemene populatie. Heup artrose is geassocieerd met looppatroon veranderingen tijdens het normale lopen, het omdraaien en tijdens het 'voetje voor voetje' (tandem) lopen. Heup artrose is geassocieerd met een hoger ritme (cadans) tijdens het normale lopen en met het maken van meer fouten tijdens het tandem lopen en het nemen van meer stappen tijdens het omdraaien.

Om verder te onderzoeken of de geobserveerde veranderingen in het looppatroon bij artrose verschilt van de veranderingen in het looppatroon bij pijn door andere oorzaken, is de associatie van pijn in het onderlichaam en het looppatroon verder belicht in **Hoofdstuk 6.2**. De resultaten laten zien dat pijn in het been en de heup, onafhankelijk van artrose, geassocieerd is met een lager ritme (cadans). Dit is in contrast met het hogere ritme dat bij heup artrose werd gevonden. Daarnaast liepen individuen met chronische pijn in het onderlichaam vaker met kleinere stappen, stonden langer op twee benen en was er meer asymmetrie tussen de twee benen. Looppatroonanalyse kan daarom helpen om te differentiëren tussen artrose en pijn door andere oorzaken.

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## About the author

Marjolein de Kruijf was born on April 18th, 1985 in Sittard. After completing secondary school at 'Bisschoppelijk college' in Echt, she started pursuing her dream in becoming a medical doctor at the University of Maastricht.

In 2009, after obtaining her medical degree, she left Limburg and started working at the Amphia hospital in Breda. There, she made her first miles as a medical doctor at the emergency room and in the surgical department. During this period, she also discovers her next career move: to become an anaesthesiologist.

Before she started her training residencies in anaesthesiology at Erasmus Medical Centre in Rotterdam in October 2014, she was given the opportunity to perform a PhD research on chronic pain at the Genetic Laboratory of Internal Medicine, of which the results are presented in this thesis.
During this period, she also obtained a health science master degree with a specialization in clinical epidemiology.

Marjolein likes to practice aikido in her spare time and lives in Gorinchem together with Sarco Bosschaart.

# PhD Portfolio

| | |
|---|---|
| **Name:** | **PhD period:** |
| Marjolein de Kruijf, MD | June 2011- September 2014 |
| **Erasmus MC Department:** | **Promotors:** |
| Internal Medicine-Genetic Laboratory, | Prof. Dr. A.G. Uitterlinden |
| in collaboration with Anesthesiology | Prof. Dr. F.J.P.M. Huygen |
| **Research School:** | **Copromotor:** |
| NIHES/MolMed | Dr. J.B.J. van Meurs |

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**PhD Training**

| | Year | Workload |
|---|---|---|
| Research master | | |
| NIHES: Master of Science in health sciences, specialization 'Clinical epidemiology'. | 2011-2013 | 70 ECTS |
| | | |
| Other courses | | |
| Course on Molecular Diagnostics VI | 2011 | 1 ECTS |
| Basic Human Genetics Course: Genetics for Dummies | 2011 | 0.5 ECTS |
| SNP Course VIII SNP's and Human disease | 2011 | 2 ECTS |
| Introduction to clinical and public health genomics | 2012 | 1.9 ECTS |
| Courses for the Quantitative Researcher | 2012 | 1.4 ECTS |
| Research management for PhD students | 2012 | 1 ECTS |
| English biomedical writing and communication | 2012 | 4 ECTS |
| | | |
| (Inter)national conferences and seminars | | |
| Pain in Europe VII, EFIC, Hamburg, Germany | 2011 | 4 days |
| Wetenschapsdagen inwendige geneeskunde, Antwerp, Belgium | 2012 | 2 days |
| 10th IASP Research symposium: pain and genetics, Miami beach, Florida, USA | 2012 | 3 days |
| 16th Molecular Medicine Day, Rotterdam | 2012 | 1 day |
| NCHA meeting, Amersfoort | 2012 | 2 days |
| 17th World congress of the Osteoarthritis Research Society International, Barcelona, Spain | 2012 | 4 days |
| Wetenschapsdagen inwendige geneeskunde, Antwerp, Belgium | 2013 | 2 days |
| NCHA meeting, The Hague | 2013 | 2 days |
| 18th World congress of the Osteoarthritis Research Society International, Philadelphia, USA | 2013 | 4 days |
| PhD day, Erasmus MC, Rotterdam | 2013 | 0.5 day |
| Wetenschapsdag Nederlandse Vereniging voor Anesthesiologie, Zeist | 2013 | 1 day |
| Pain in Europe VIII, EFIC, Florence, Italy | 2013 | 4 days |
| 11th IASP Research symposium: Brain and Pain, Arnhem | 2013 | 2 days |

| | | |
|---|---|---|
| 19th World congress of the Osteoarthritis Research Society International, Paris, France | 2014 | 4 days |

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**Presentations**

| | Year | Type |
|---|---|---|
| 'Fingerlength pattern as a biomarker for prenatal androgen exposure in the risk for osteoarthritis and pain'<br>- Wetenschapsdagen inwendige geneeskunde, Antwerp | 2013 | Oral |
| 'Structural brain alterations in individuals with chronic pain'<br>- Wetenschapsdag NVA, Zeist | 2013 | Oral |
| 'Pain sensitivity: Sex differences and Genetics',<br><br>- Wetenschapsdagen inwendige geneeskunde, Antwerp<br><br>- IASP: pain and genetics, Miami beach, Florida, USA<br><br>- NCHA meeting, the Hague<br><br>- Molmed day, Rotterdam<br><br>- OARSI, Barcelona, Spain | 2012 | Poster |
| 'Finger length pattern as biomarker for prenatal androgen exposure in the risk for osteoarthritis and pain'<br><br>- NCHA meeting, the Hague<br><br>- OARSI, Philadelphia, USA | 2013 | Poster |
| 'Structural brain alterations in community dwelling individuals with chronic joint pain',<br>- OARSI, Philadelphia, USA<br><br>- EFIC, Florence, Italy<br><br>- IASP, Nijmegen | 2013 | Poster |
| 'Gait is differently affected in subjects with radiographic osteoarthritis compared to individuals with joint pain',<br>- OARSI, Paris, France | 2014 | Poster |

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**Teaching activities**

| | Year | Workload |
|---|---|---|
| Lecturing: VO Thyroid, medical students | 2012-2013 | 1 ECTS |
| Supervising 2 master thesis students, Erasmus Medical School | 2012 | 2x5 months |
| Supervising 3 medical students as research assistants scoring x-rays of the Rotterdam Study | 2012-2014 | 2h/week |

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**Other**

| | Year | Workload |
|---|---|---|
| Weekly work discussion Genetics lab: oral presentation 4/yr | 2011-2014 | 2 ECTS |
| Endocrinology meetings: oral presentation 2/yr | 2011-2014 | 1 ECTS |
| Exit interviews with participants of the Rotterdam Study | 2011-2013 | 5 ECTS |
| Scoring 'pain mannequins' in Rotterdam Study | 2011-2014 | 5 ECTS |

## List of publications

**This Thesis**

**Marjolein de Kruijf**, Marjolein J. Peters, Cindy G. Boer, Carolina M. Gomez, Fernando Rivadeneira, Frank J.P.M. Huygen, André G. Uitterlinden, Joyce B. J. van Meurs, *Genetics of the heat pain threshold in the general population,* Manuscript in preparation

**Marjolein de Kruijf**, Hanneke J.M. Kerkhof, Marjolein J. Peters, Sita M.A. Bierma-Zeinstra, Albert Hofman, André G. Uitterlinden, Frank J.P.M. Huygen, Joyce B.J. van Meurs, *Finger length pattern as a biomarker for osteoarthritis and chronic joint pain: A population-based study and meta-analysis after systematic review,* Arthritis Care & Research, 2014 sep;66(9): 1337-43

**Marjolein de Kruijf**, Lisette Stolk, M. Carola Zillikens, Yolanda B. de Rijke, Sita M.A. Bierma-Zeinstra, Albert Hofman, Frank J.P.M. Huygen, André G. Uitterlinden, Joyce B.J. van Meurs, *Lower sex hormone levels are associated with more chronic musculoskeletal pain in community dwelling elderly women,* Submitted to PAIN (pending revisions)

**Marjolein de Kruijf**, Daniel Bos, Frank J.P.M. Huygen, Wiro J. Niessen, Henning Tiemeier, Albert Hofman, André G. Uitterlinden, Meike W. Vernooij, M. Arfan Ikram, Joyce B.J. van Meurs, *Structural brain alterations in community dwelling individuals with chronic joint pain,* American Journal of Neuroradiology, 2015;nov 5 [Epub ahead of print]

**Marjolein de Kruijf**, Lotte G.M. Cremers, Marius de Groot, Frank J.P.M. Huygen, Albert Hofman, Wiro J. Niessen, André G. Uitterlinden, M. Arfan Ikram, Joyce B.J. van Meurs, Meike W. Vernooij, *Chronic musculoskeletal pain is related to cerebral white matter microstructural integrity: a population-based study,* Submitted

**Marjolein de Kruijf**, Marjolein J. Peters, Leonie C. Jacobs, Henning Tiemeier, Tamar E.C. Nijsten, Albert Hofman, André G. Uitterlinden, Frank J.P.M. Huygen, Joyce B.J. van Meurs, *Determinants for quantitative sensory testing and the association with chronic musculoskeletal pain in the general elderly population*, Pain Practice, 2015 Jul 23 [Epub ahead of print]

Vincentius J.A. Verlinden*, **Marjolein de Kruijf***, Sita M.A. Bierma-Zeinstra, Albert Hofman, André G. Uitterlinden, M. Arfan Ikram, Joyce B.J. van Meurs, Jos N van

der Geest, *Asymptomatic radiographic hip osteoarthritis is associated with gait differences, especially in women*, *Both authors contributed equally, Submitted

**Marjolein de Kruijf***, Vincentius J.A. Verlinden*, Frank J.P.M. Huygen, Albert Hofman, Jos N van der Geest, André G. Uitterlinden, Sita M.A. Bierma-Zeinstra, M. Arfan Ikram, Joyce B.J. van Meurs, *Chronic joint pain in the lower body is associated with gait differences independent from radiographic osteoarthritis*, *Both authors contributed equally, Gait and Posture 2015 sep;42(3): 354-9

**Other publications by this author**

Peters MJ, Broer L, Willemen HL, Eiriksdottir G, Hocking LJ, Holliday KL, Horan MA, Meulenbelt I, Neogi T, Popham M, Schmidt CO, Soni A, Valdes AM, Amin N, Dennison EM, Eijkelkamp N, Harris TB, Hart DJ, Hofman A, Huygen FJ, Jameson KA, Jones GT, Launer LJ, Kerkhof HJ, **de Kruijf M**, McBeth J, Kloppenburg M, Ollier WE, Oostra B, Payton A, Rivadeneira F, Smith BH, Smith AV, Stolk L, Teumer A, Thomson W, Uitterlinden AG, Wang K, van Wingerden SH, Arden NK, Cooper C, Felson D, Gudnason V, Macfarlane GJ, Pendleton N, Slagboom PE, Spector TD, Völzke H, Kavelaars A, van Duijn CM, Williams FM, van Meurs JB, *Genome-wide association study meta-analysis of chronic widespread pain: evidence for involvement of the 5p15.2 region,* Annals of Rheumatic Disease 2013 Mar;72(3): 427-36

**de Kruijf M**, Vroemen JP, de Leur K, van der Voort EA, Vos DI, Van der Laan L, *Proximal fractures of the humerus in patients older than 75 years of age: should we consider operative treatment?,* Journal of orthopaedics and traumatology 2014 Jun;15(2): 111-5

Thuijls G, Derikx JP, **de Kruijf M**, van Waardenburg DA, van Bijnen AA, Ambergen T, van Rhijn LW, Willigers HM, Buurman WA, *Preventing enterocyte damage by maintenance of mean arterial pressure during major nonabdominal surgery in children*, Shock 2012 Jan;37(1): 22-7

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