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Aspects of brain health and pain tolerance in a general population

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Summary

Pain is a major burden both for affected individuals and for society. While it has been established that processing in the brain is key to the experience of pain, most of the research is done in healthy volunteers or patients with specific pain conditions. Less is known about how the status of the brain may relate to pain sensitivity in the general population.

While clinical pain can differ because of differences in the disease or injury causing it, experimental pain studies provide the opportunity to study the relationship between the brain and a standardized nociceptive stimulus.

The Tromsø study is a population-based study that has collected a broad selection of health data, including MRI of the brain, cognitive testing, and experimental pain assessments. This provides a unique opportunity to examine these relationships in a general population.

We examined whether total and regional gray matter volume (GMV), cognitive function and previous cerebral stroke were associated with pain tolerance to the cold pressor test (CPT). In the CPT, the participants are asked to keep their hand and wrist in a cold-water bath (3°C) for as long as they can or until a maximum time (106 or 120 seconds).

We found that larger total GMV was associated with longer endurance time to the CPT. Larger effect sizes and significant association after correction for multiple testing were found in several clusters across the brain, including in the postcentral gyri, insula, cingulate and orbitofrontal cortices, regions known to be commonly activated by painful stimuli in functional studies. Likewise, higher scores on cognitive tests were associated with longer tolerance to the CPT and to cuff pain algometry (CPA). Stroke was associated with increased risk of hand withdrawal at earlier times.

Our findings have possible clinical implications, as patients with stroke or other brain diseases affecting gray matter volume or cognitive function might be more sensitive – or have more difficulty coping with – pain. It is also possible that the status of the brain has implications with regards to risk for chronic pain.

In summary, the more you have got of a healthy brain, the more pain you are able to tolerate.

Sammendrag

Smerte er et betydelig problem både for de personene som har den og for samfunnet som helhet. Forskning har gjort det klart at prosessering i hjernen er avgjørende for opplevelse av smerte, men det meste av forskningen er gjort med friske frivillige forsøkspersoner eller personer med sykdommer og man vet mindre om hvorvidt det er sammenheng mellom ulikheter i hjernens tilstand og ulikheter i smerteføl somhet i en befolkning. Ulikheter i hvor mye smerte som oppleves ved sykdommer og skader kan ha å gjøre med alvorligheten av tilstanden, mens eksperimentelle smertetester gjør det mulig å undersøke forholdet mellom hjernen og et standardisert smertestimulus.

Tromsøundersøkelsen er en befolkningsbasert helseundersøkelse som har samlet en bred mengde data, inkludert MR av hjernen, tester av kognitiv funksjon og eksperimentelle smertetester. Dette gir en unik mulighet til å undersøke disse forholdene i en generell befolkning.

Vi har undersøkt om volum av grå substans i hjernen, resultater på tester av kognitiv funksjon, eller det å ha hatt et hjerneslag har sammenheng med smertetoleranse testet med kuldepressortest. I denne testen blir deltagerne bedt om å holde hånda i kaldt vann (3°C) så lenge de klarer eller til en makstid (106 eller 120 sekunder).

Vi fant at det å ha større volum av grå substans var forbundet med å tolerere kuldepressortesten lenger. Effekten var sterkest blant annet i områder i postsentral gyrus, insula, gyrus cinguli og orbitofrontal cortex, områder som er kjent å være viktige i smerteprosessering. På tilsvarende måte var bedre testresultat på kognitive tester forbundet med lenger toleransetid. Det å ha hatt et hjerneslag var forbundet med økt sannsynlighet for å trekke ut hånda på tidligere tidspunkt.

Disse funnene kan ha betydning i klinisk sammenheng, ettersom pasienter med hjerneslag eller andre hjernesykdommer som påvirker grå substans eller kognitiv funksjon kan være mer følsomme for smerte. Det er også mulig at hjernens tilstand kan ha betydning for risiko for å utvikle kronisk smerte.

Oppsummert; jo mer du har av en frisk hjerne, jo mer smerte er du i stand til å håndtere.

List of papers

This thesis is based on the following three papers:

I. Gray matter volume and pain tolerance in a general population: the Tromsø Study
Tonje Anita Melum, Torgil Riise Vangberg, Liv-Hege Johnsen, Ólöf A. Steingrímsdóttir, Audun Stubhaug, Ellisiv B. Mathiesen, Christopher S. Nielsen
Published in Pain, 2023

II. Associations between cognitive test scores and pain tolerance. The Tromsø Study.
Tonje Anita Melum, Ólöf A. Steingrímsdóttir, Henrik B Jacobsen, Bente Johnsen, Audun Stubhaug, Henrik Schirmer, Ellisiv B. Mathiesen, Christopher S. Nielsen
Submitted

III. Pain tolerance after stroke: The Tromsø study
Tonje Anita Melum, Anders P. Årnes, Hein Stigum, Audun Stubhaug, Ólöf Anna Steingrímsdóttir, Ellisiv B. Mathiesen, Christopher S. Nielsen
Published in European Journal of Pain, 2023

Abbreviations

ACC: Anterior cingulate cortex

BMI: Body mass index

CI: Confidence interval

CNS: Central nervous system

CPA: Cuff pain algometry

CPT: Cold pressor test

CPSP: Central post-stroke pain

CVD: Cardiovascular disease

DRG: Dorsal root ganglion

FDR: False discovery rate

GMV: Gray matter volume

HDL-cholesterol: High density lipoprotein cholesterol

HR: Hazard ratio

ICV: Intracranial volume

MRI: Magnetic resonance imaging

OFC: Orbitofrontal cortex

PAG: Periaqueductal gray

PNS: Peripheral nervous system

PSSP: Post-stroke shoulder pain

S1: Primary somatosensory cortex

S2: Secondary somatosensory cortex

SD: Standard deviation

WHO: World Health Organization

1 Introduction

1.1 Pain definition and perspectives

Pain is an experience which can represent a wide range of pathophysiological mechanisms and meanings (1). Prevalence estimates of chronic pain range from 9% to 64% in population-based studies (2). While 19 % of adult Europeans suffer from chronic pain of moderate to severe intensity (3), pain is also part of normal life and has important protective functions. Considering the large number of people seeking help for conditions causing pain, or who undergo potentially painful medical procedures, the number of people who experience pain severe enough to require attention from health professionals in a given day or year can hardly be overestimated.

Meanwhile, the pain experienced in response to the same injury, disease or procedure is considerably different across individuals (4) and is also influenced by context. Pain is by modern definition "an unpleasant sensory *and* emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (1) (italics added). Historically, it has been regarded as either one or the other. Early philosophers such as Aristotle and Plato saw pain as an emotion, among the "passions of the soul" (5, 6), and the notion of pain as an affective "quale" was prevailing into the 19th century (7). Descartes, in his "Treatise of man", published in 1664, was among the first to propose a somatosensory pathway conveying information on painful stimuli from the periphery to the brain, equating it to the pulling of a rope that rings a bell (6, 8). In parallel with impressive progress in the mapping of the somatosensory system, such as von Frey's discovery of four somatosensory modalities (cold, heat, pain and touch) in 1895, the understanding of pain as the result of transmission of pain signals from specific pain receptors directly to a specific pain center of the brain advanced and gained broad acceptance, and a focus on pain as sensation dominated the research field (7).

Throughout the early 20th century, knowledge on the role of the brain in pain processing was generally focused on the thalamus, which was understood to be the center of integration and perception of pain. This was supported by Dejerine and Roussy´s description of pain after thalamic strokes in 1906 and Head and Holmes´ description of sensory disturbances after cerebral lesions in 1911 (9). While some earlier reports did suggest a role of the cortex (9), this knowledge increased and gained momentum in the aftermaths of the second world war. In

1951, Marshall published a description of 10 patients with cortical injuries and altered pain sensibility and concluded that “the cerebral cortex is concerned with pain sensibility” (9). Beecher’s description of the surprisingly little pain that accompanied severe wounds from battle (10) has become a well-known example of the importance of emotional and contextual factors that have later been elucidated.

In 1965, Melzack and Wall proposed the gate control theory, introducing a modulatory mechanism of pain transmission from the periphery to the brain (8), which placed psychological factors such as anxiety into the pain processing physiology rather than just reactions to pain (11). From being opposing theories, this provided a model where the sensory and affective dimensions of pain could be joined. A few years later, a model of pain as the result of a “sensory, motivational and cognitive process” was described (7).

The concept of a neuromatrix was proposed by Melzack in 1990, based on observations suggesting that the explanation of phantom limb phenomena must lie in the brain. According to this theory, the substrate for the perception of the physical self was “a network of neurons that extends throughout widespread areas of the brain”, with “sub-signatures” specialized to process information related to injury or other major sensory events (12). The knowledge on neurobiological underpinnings of pain processing subsequently gained considerable momentum enabled by the advancing opportunities in functional imaging studies since the early 1990s (13). It has become clear that the brain, rather than being a passive recipient, processes and modulates pain, and that this is a substantial contribution to the pain that is experienced as well as the selection of behavior in response to it.

1.2 Pain processing in the nervous system

Pain nociception and transmission

In the case of tissue damage, pain is the result of a process of nociception, transmission and processing which is initiated when a noxious stimulus activates nociceptors. These are pseudounipolar neurons with their cell bodies in the dorsal root ganglion (DRG), sending a peripheral axon to the skin and a central axon to the dorsal horn of the spinal cord (14). The peripheral axons have free nerve endings with receptors. Receptors can be sensitive to specific nociceptive stimuli but are often polymodal. Many, including those sensitive to cold, heat and inflammatory stimuli, belong to the Transient Receptor Potential (TRP) family (15, 16). While the discovery of the PIEZO channels and their role in mechanical allodynia was a potentially important step, channels responsible for mechanical pain remain elusive (17).

When a noxious stimulus has activated a receptor on the peripheral terminal, an action potential is elicited and carried along the axon of the primary afferent nociceptor to the spinal cord, with a speed depending on the diameter of the axon and whether it is covered by a myelin sheath. Most nociceptors have axons with broadly distributed endings and hence large receptive fields, and small diameter unmyelinated axons (C-fibers) which transmits the signal rather slowly (0.14-1.4 m/s). Others have nerve endings clustered in smaller areas, providing more precise localization of the stimulus, and thicker, myelinated axons (mostly Aδ-fibers) that allow for faster transmission (from 5-30 m/s) (14).

In the spinal cord, the axon of the peripheral nociceptor terminates in the ipsilateral dorsal horn (18). Here, the primary afferent neurons synapse with secondary afferent neurons, which convey the signal to the brain and brainstem. A large proportion of secondary afferents carrying nociceptive signals cross over to the contralateral side of the spinal cord and ascend through the lateral spinothalamic tract to the thalamus. Other secondary afferent neurons travel from the dorsal horn through other spinal pathways, including the spinomesencephalic and spinoreticular, who relay information to brainstem nuclei and directly to cortical regions, in addition to through the thalamus (19). Brainstem nuclei influence spinal nociception as they exert inhibitory and facilitatory effects on spinal pain circuits (20, 21), a process known as descending pain modulation (DPM). Cortical and subcortical regions contribute to DPM both via the brainstem nuclei and through direct corticospinal pathways (21). Both at spinal

cord and brainstem level, precognitive responses to pain can be initiated, such as withdrawal reflexes, change in heart rate and autonomic responses (21).

Pain processing in the brain

In the brain, the signal is processed by a large network of regions (Figure 1). This results in a conscious pain experience (or not), while cortical and subcortical brain regions also influence spinal transmission directly and through influence on brainstem nuclei (DPM) (21).

Regions often found to be consistently activated by painful stimuli include the thalamus, somatosensory cortices (primary (S1) and secondary (S2), insula, anterior cingulate cortex (ACC) and regions of the prefrontal cortex (PFC) (18, 21-24). Several models have been proposed for describing the anatomical and temporal interactions and ascribing the regions "roles" with respect to domains of pain processing. This is based on knowledge on the general function of the regions, where they receive and convey information from/to, observations from cases with brain lesions, and experimental pain research on humans and animals.

A large proportion of the secondary afferents transmitting nociceptive signals enter the CNS via the thalamus where integration and modulation occurs before signals are conveyed by post-thalamic neurons to the cortex (21). According to the classical model of lateral/medial pain system, ventrobasal and dorsolateral thalamic nuclei receive neurons ascending in the lateral spinothalamic tract, and transmit signals to cortical regions encoding the sensory-discriminative aspects of pain (somatosensory cortices (S1 and S2), insula, parietal operculum) (7, 25-27), while medial thalamic nuclei receive afferents from the spinothalamic, spinoreticular and spinomesencephalic tract and convey signals to regions encoding the affective dimension (S2, insula, parietal operculum, ACC/limbic system) (7, 21, 26).

The conscious experience of pain is constructed in the cortex. This can be conceptualized as a process aimed to interpret whether the incoming signal is a threat and hence to be experienced as pain, and that regions of the brain contribute with different aspects to this interpretation. In sum, a multidimensional experience is constructed: an integration of sensory-discriminative, affective-motivational, and cognitive-evaluative components.

The insular cortex is generally considered to be a seat of integration of sensory and cognitive information and interoception (the awareness/experience of bodily and emotional states) (28). In pain processing, the posterior and anterior insula have been ascribed somewhat different

contributions. The posterior insula has been linked to encoding the location and intensity of painful stimuli (the sensory-discriminative dimension), along with the S1 and inner operculum, based on observations of selective pain deficits or pain syndrome accompanying focal lesions in posterior insula and inner operculum (29), findings that stimulation of these regions can trigger pain (30), and that activation in posterior insula has been shown to be correlated with pain intensity ratings (31, 32). Meanwhile, one of these studies (32) also showed activation in S1, as well as S2, ACC and several other regions linked to other dimensions of pain, reflecting that nociception does not occur in isolation: it is integrated with the other dimensions (7).

The anterior insula, S2 and ACC are classically considered to be involved in encoding the affective dimension of pain, which is dependent on perception and context (20, 24). Correlation between activation in the insula and pain unpleasantness ratings has been shown (33), and studies on animals have found that the rostral anterior insula is connected with several regions linked to affective pain-related behavior and descending modulation (34) and correspondingly that injecting morphine into it reversed antinociceptive behavior and lowered firing in the dorsal horn (35). In mice with neuropathic pain, lesions in the ACC diminished pain-related anxio-depressive behaviors (36). In humans, functional magnetic resonance imaging (fMRI) studies have shown activation of the anterior insula and the ACC during pain and during empathy for others in pain, indicating these structures contribute to the affective experience of pain in others as well as in oneself (37, 38).

In addition to the affective-motivational, the ACC has also been linked to the cognitive-evaluative dimension of pain (24) which is in accordance with knowledge on the general function of the ACC: it has been linked to a variety of functions, including cognitive control when facing behavioral conflicts and comparison of values (39).

The general function of the prefrontal cortex in cognitive modulation and control also pertains to pain processing (21, 24). Activity in regions in the PFC is increased during placebo analgesia (40) and when using cognitive strategies for pain reduction (41). Findings from a study of controlled versus uncontrolled pain suggest that regions in PFC have roles in inhibiting or facilitating pain through an effect on the insula (42).

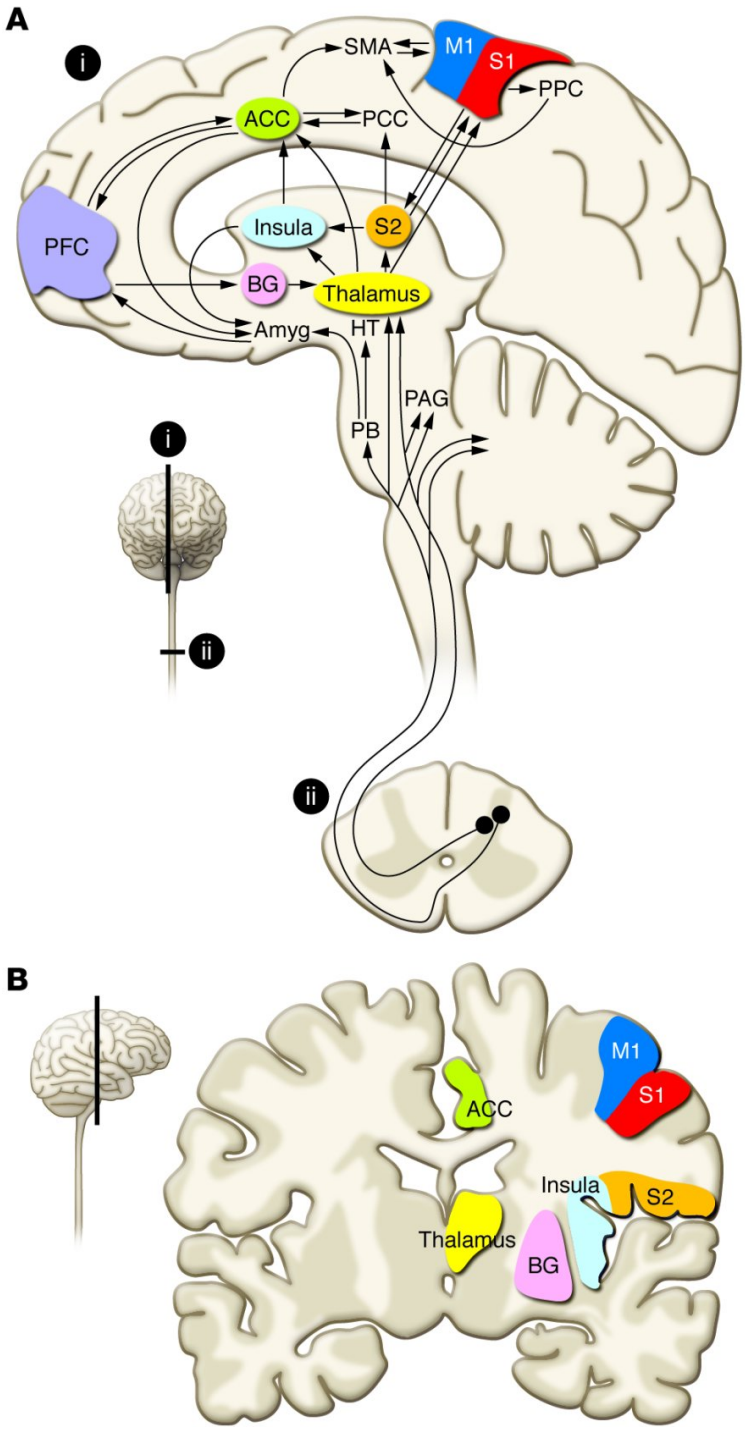
While these regions, due to their consistent activation in response to noxious stimuli, have been proposed to be the main components of a brain network for (acute) pain (22), broader

perspectives need to be considered: As these regions overlap with regions activated by other sensory stimuli that contrasts surroundings or expectations (43), there has been a debate regarding the existence of a specific pain processing system, which can serve as a neurobiological signature for pain (44), as opposed to a general salience system (43, 45, 46). The regions and networks identified as components of the pain processing system, as well as neurotransmitter and receptor systems, also overlap considerably with those involved in cognitive functions, including insular, anterior cingulate and prefrontal cortices (47). Moreover, several additional regions are found to be activated in response to pain, though less consistently, including the cerebellum, motor and supplementary motor cortices, amygdala, hippocampus and basal ganglia (20, 21). These observations have led to the view that the pain matrix cannot be unequivocally defined, but rather is a substrate subject to significant modulation, where the contribution from different brain regions depend on the many factors known to influence pain such as context (10, 48), cognitive and psychological factors (27, 49). This is supported by recent evidence of high interindividual variability in the predictive weight of some of the pain-related regions, including prefrontal and cerebellar regions (49). An example of factors that can influence the contribution of different regions is chronic pain, as neuroanatomic regions associated with pain unpleasantness is different in chronic pain cases than in healthy volunteers (24).

Furthermore, a core characteristic of this system is that the regions do not operate in isolation or on a simple linear timescale, but in a continuously ongoing interplay (27). There is increasing emphasis on network perspectives (50-52).

In addition to generating the conscious experience, brain processing influence pain by mechanisms of descending modulation. This includes direct connections to the spinal level, or via brainstem nuclei (21, 27, 53).

Figure 1: Brain regions involved in pain processing



From Schweinhardt, P. and M. C. Bushnell (2010). "Pain imaging in health and disease--how far have we come?" J Clin Invest 120(11*): 3788-3797*. Reproduced with permission from publisher.

1.3 Variation in the status of the brain

Given the neuronal basis in pain processing, it is feasible that variation in the status of the brain could contribute to differences in pain sensitivity and tolerance. The concept of brain health is increasingly used during the last decade, and while a definition has not been finalized, it is a multidimensional concept and not just the absence of disease (54). While the multidimensionality is reflected in a challenge in deciding on objective measurement of it, relevant characteristics include brain structure and cognition (54). A variety of factors influence brain structure and cognitive function in a positive or negative way, both within the normal spectrum and as risk factors for diseases. Regarding diseases, the most common conditions affecting brain structure and function are stroke and dementias (55).

With increasing availability, MRI has become a preferred method of imaging of brain structure as it safe and allows for distinguishing between gray and white matter and visualization of cortical and subcortical structures. Among challenges related to volumetric measurements of the brain is the relation between these volumes and overall body size. It is customary to adjust for a measure of head size, such as intracranial volume (ICV), as this is considered more valid for description of relationships between structure and function (56).

Increasing age is associated with an overall decrease in gray matter volume (GMV), while the amount and trajectory vary between brain regions and with age (57, 58). The overall loss of brain volume is estimated to range between 0.2% -0.5% per year, and for subcortical structures it has been estimated that age accounts for 5-45% of the variation (57). Increasing age is also related to decline in several cognitive domains (episodic and prospective memory, executive function, selective and divided attention, working memory and processing speed) (58).

The relationship between sex and GMV has been debated and largely depends on whether (and how) differences in body/head size are taken into account. While men have larger total brain volumes than women (59), this difference substantially decreases when controlling for brain size (60) and is dependent on method of correction for ICV (61). A recent study of a large sample from the UK Biobank found that males had higher raw volumes and surface areas while women had higher raw cortical thickness, and that these differences persisted when adjusting for total brain volume, however with attenuated effect sizes and fewer

significant regions (62). On tests of cognitive function, women score higher on some tasks and men on others (63).

Higher levels of education have been linked to larger cortical thickness (64) and GMV (65, 66), and are also associated with cognitive performance across the entire adult lifespan (67). Physical activity has been linked to higher cognitive test scores (68, 69).

While it is well established that age, sex, smoking, hypertension diabetes, obesity, high total cholesterol and low high-density lipoprotein (HDL) cholesterol are important risk factors for stroke (70), cardiovascular risk factors are associated with brain structure (71) and cognitive test results (68) even in the absence of stroke. Smoking has been linked to thinner cortical thickness (72), lower gray matter density (73), lower total brain volume (74), more rapid decrease in hippocampal and total brain volume (75), and with poorer performance on cognitive tests (68). Measures of obesity are associated with less gray matter (75-77) and lower cognitive performance (78). Hypertension has been linked to greater shrinkage of regional GMV (79) and steeper decline in corpus callosum volume (80), while higher diastolic blood pressure was related to smaller brain volume (74) and lower cognitive test scores (68). Diabetes or elevated glucose/HbA1c is shown to be associated with smaller brain volume (74), thinner overall (64) and regional cortical thickness (71), accelerated hippocampal atrophy (75), qualitatively assessed cortical atrophy (81) and with poorer cognitive performance (68). Higher level of HDL cholesterol (64) and lower levels of a score indicating "bad" cholesterol (71) have been linked to thinner cortex. No association between HDL cholesterol and cognitive performance was found in a population-based study (68) but total cholesterol has been linked to increased risk of Alzheimer's dementia (82).

1.4 Measurement of pain and variation in pain sensitivity

As pain is a subjective experience, objectively measuring it is inevitably an oxymoron. At the same time, operationalization is necessary to enable quantitative research.

With regards to clinical pain, chronic pain is defined as pain that lasts more than three months (83). Methods to assess clinical pain include rating scales, such as the numeric rating scale (NRS) or visual analogue scale (VAS), and questionnaires aimed to demarcate pain type, such as the DN4 for neuropathic pain, or to cover multiple aspects of the pain experience, such as the McGill questionnaire (84, 85). However, pain due to clinical conditions has inherent variation related to the severity of pathology causing it and is also influenced by the

consequences it has for the affected person and contextual factors (27). This entails that variation in clinical pain due to variation in the nervous system processing it, cannot be disentangled from variation related to these other factors.

Experimental pain assessments provide an opportunity to measure variation in response to a controlled nociceptive stimulus in a standardized setting (4). A variety of methods have been developed with the intention of measuring individual variation in pain sensitivity to such controlled nociceptive stimuli. To be precise, what these methods do is expose the subjects to a nociceptive stimulus and measure responses to it (86). Stimuli include cold, heat, mechanical, chemical, ischemic, and electrical stimuli. Responses that are measured include threshold (the intensity of stimulus required for the subject to experience pain), pain intensity ratings or tolerance (the maximum duration or intensity of the stimulus the participant can endure). A variety of "dynamic" paradigms are also used, including temporal summation, offset analgesia, and conditioned pain modulation, but for brevity these will not be discussed further here.

There are considerable individual differences in responses to noxious stimuli (4), but while some reported high correlation between pain thresholds for different stimuli within individuals and claimed that this supported a notion of individuals being generally stoic or complaining (87), others have found the opposite, namely poor correlation between stimuli (86, 88).

While pain is always multidimensional, it seems likely that threshold measurements might be more related to the nociceptive apparatus and sensory/discriminative dimension of pain, while suprathreshold measurements such as intensity ratings and tolerance assessments are likely to relate more strongly to also emotional and cognitive processing of the pain. Tolerance assessments have been suggested to reflect acute clinical pain better than other laboratory pain assessments, as they include emotional aspects in addition to nociception (89).

A commonly used pain tolerance assessment is the cold pressor test (CPT). This test is done by submerging a hand or leg in cold water for up to several minutes. It was initially developed for the study of blood pressure responses that could indicate risk of hypertension (90), but as it predictably induced pain it was adopted as a pain assessment (91) and has become an established method as such (92). It is considered a good proxy for clinical pain due to shared characteristics, sensitivity to psychological influence, and reliability (93, 94).

When the skin is put in contact with a cold and potentially noxious cold stimulus, this activates nociceptors (through TRPM8 and possibly/probably other receptors), and signals are transmitted in Aδ and C fibers to the dorsal horn. However, submerging the hand into cold water over an extended period also entails that tissues will gradually be cooled. It has been suggested that cold pain is mediated by nociceptors in cutaneous veins (95). CPT induces local vasoconstriction and activation of the sympathetic nervous system (96). It is known to activate descending modulatory mechanisms and is often used as conditioning stimulus in paradigms for study of DPM (97).

Functional MRI studies done with the CPT (foot immersed) have shown that it is associated with increased activation in regions including S1, S2, cingulate cortex, insula and PFC as well as in the midbrain and pons, suggesting that the PAG and the reticular formation were recruited (98), that it modulated responses to tonic heat pain in thalamus, insula and S2 (99), and shifted functional connectivity in several networks (100). These studies support the multidimensional aspects of CPT, with involvement of affective circuits likely reflecting psychological and cognitive components in this test, as well as activation of brainstem regions related to DPM.

Tolerance assessments can also be done with other pain stimuli, such as cuff pain algometry (CPA) which applies pressure around the full circumference of an arm or leg (101). CPA has been found to activate afferents in deep tissue with smaller contribution from cutaneous afferents (102), and is proposed to be a good model of musculoskeletal pain sensitivity (103). It also activates DPM (104).

While considerable efforts have been invested in examining factors that might explain differences in experimental pain sensitivity, how it relates to biological, life-style related, psychological, and cognitive factors, or to chronic pain and its comorbidities, much remains to be disentangled. With regard to age, one metanalysis concluded that pain threshold was higher in older subjects while tolerance thresholds were similar across age (105), while another systematic review and metanalysis found that findings were inconsistent and might differ across stimulus modalities (106). Sex differences in pain sensitivity are commonly reported, with women being more sensitive than men to the majority of pain stimuli (107). Meanwhile, it has been pointed out that there are nuances to be considered and that women may have greater habituation/adaptation to repeated/prolonged stimuli (108) and differences may be related to biological differences or to gender role (109). In a study designed for

another purpose, lower CPT tolerance time was observed in women as well as in those with lower education levels, smokers and statin users, and in people with emotional distress (110).

Blood pressure is linked to pain sensitivity, with higher systolic blood pressure (SBP) being related to lower pain sensitivity (111) including tolerance to CPT (112). A phenomenon described as blood-pressure related hypoalgesia is known, with possible mechanisms including a role of baroreceptors, overlapping brain areas (113, 114) and alterations in afferent sensory pathways, which may be present in hypertensive patients in particular (113). One study showed that within the normal range of SBP (<140/90) there was an inverse relationship with pain ratings, while there was no further effect in the hypertensive range (111).

Polyneuropathy is a common complication of diabetes and might affect pain sensitivity through reduced sensitivity or allodynia. A link between diabetes and sensitivity to pressure pain at the sternum has also been shown (115). Obese persons are found to be both more (116) or less (117, 118) sensitive to pain, possibly depending on pain stimulus (116) or examined body region (117). A recent study found no relationship between obesity and measures with ratings to heat and cold or tolerance to the CPT (119).

While nicotine has analgesic properties and its administration is shown to reduce sensitivity in experimental pain studies (120), pack-years of smoking were positively associated with ratings of experimental pain (121). People who are habitually more physically active have been found to be less sensitive to several pain modalities (122) and more tolerant to CPT in particular (123).

A relationship between mental health and pain sensitivity has been found, although with inconsistencies regarding direction of effect. An earlier review of six studies (n=11-66) found evidence that depressed subjects were less likely to perceive a stimulus as painful (124), while in a more recent study (735 patients and 456 healthy controls) depressed patients had lower thresholds and higher ratings, both indicating higher sensitivity (125).

A relationship between experimental pain sensitivity and chronic pain conditions has been shown. Irritable bowel syndrome was associated with shorter CPT tolerance time, higher intensity ratings to CPT, and lower heat-pain thresholds (126). Heat and pressure pain ratings correlated with clinical pain in fibromyalgia (127). In a systematic review, pressure pain thresholds were lower in people with osteoarthritis pain (128). CPT endurance time was

associated with analgesic use in cross-sectional analysis (129). These studies have mostly used cross-sectional data, and hence cannot infer causal effects. Chronic pain is associated with pain sensitization (130), implying that the association can be caused by chronic pain leading to increased sensitivity while it is also possible that increased pain sensitivity is a risk factor for development of chronic pain.

While it remains to be established how findings from experimental pain studies can be translated to clinical pain, it has been shown that CPT tolerance time was significantly longer in people who had undergone an unrecognized myocardial infarction compared to people who had had a symptomatic myocardial infarction (131). CPT tolerance time predicted postoperative pain in one study (132) and another found that CPT shortly after whiplash predicted nonrecovery one year later (133).

1.5 The relationship between variation in the status of the brain and pain

Chronic pain and the brain

Substantial evidence has shown that chronic pain patients have neuroanatomical differences compared to healthy controls – usually decreased gray matter, but regional increases have also been found (19, 134-136). An interpretation of this is that chronic pain causes alterations in brain structure, as findings often correlate with pain duration and overlap in different pain conditions (134). This is supported by longitudinal studies showing that persisting low back pain was associated with a decrease in gray matter density (137) and that such findings resolved in cases where pain improved after successful treatment of hip osteoarthritis (138-140) and low back pain (141). Meanwhile, increasing evidence suggests that maladaptive plasticity is likely to be an intrinsic part of the pathophysiology in the development of chronic pain (19, 142). Moreover, given the extensive processing in the brain and the variation in the brain across the population, it is possible that this variation could be a neurobiological underpinning of individual differences in pain sensitivity and possibly a risk factor for experiencing more or more severe acute pain or for developing chronic pain. It is difficult to assess this in chronic pain conditions, as the effect of brain status on the pain cannot be disentangled from an effect of pain on the brain, given that these probably participate in a maladaptive cycle and that it is likely that there is a confounding effect of inherent variation within and between clinical pain conditions. Experimental pain studies allow for assessment

of a relationship between pain sensitivity and the status of the brain, independent of these confounding effects related to chronic pain conditions.

Gray matter and experimental pain sensitivity

Given the widespread processing of pain in cortical and subcortical regions, it is likely that variation in gray matter in these regions could be reflected in variation in pain sensitivity. This relationship has been studied in several smaller healthy volunteer or case-control studies and two larger, population-based samples, with heterogenous findings with regards to the presence and direction of effect as well as in which brain regions it is found. This might be related to sample selection, sample size, power issues, and to methodological differences such as the use of different pain stimuli and assessments.

Most studies used threshold assessments (52, 135, 143-148), with heterogenous results in presence, direction, and regional location of effect. While most of these studies are small (n=23-92), two larger, population-based studies have been conducted. In the Rotterdam study, a significant correlation between GMV and heat pain threshold was found in 839 subjects with chronic musculoskeletal pain, indicating a relationship between pain sensitivity and GMV that was inverse in thalamus and hippocampus while positive in ACC, but the effect was only found in women (135). In a large, population-based healthy sample (n=501), no association was found between regional GMV and pressure pain thresholds (143).

Studies using suprathreshold stimuli (5 studies, n=28-116) have consistently found higher pain sensitivity to be associated with less gray matter in the insula (149-153) and with a variety of other regions including S2 (151), ACC (151), PCC (149, 150), hippocampus (151), precuneus (150), intraparietal sulcus and inferior parietal lobe (150). Pain tolerance assessment (CPT) is only reported by one study on 14 yoga practitioners and 14 controls (153), where a correlation between insular GMV and pain tolerance was found.

Cognitive function and pain sensitivity

Variation in cognitive function could be reflected in variation in pain processing, considering the overlapping brain networks and transmitter system and the cognitive dimension of pain itself. It has been shown that people with chronic pain have poorer performance on cognitive tests (47, 154, 155). This has been proposed to be caused by the overlap between processing system entailing that when someone is in pain, the resources in these regions are occupied by

the pain processing (156) and less is available for other functions (47). Alternatively or in addition, pain can induce maladaptive neuroplastic changes, or there could be a release of neurochemical substances that affect cognitive processing in an unfavorable way (154). While both explanations suggest pain as the cause of cognitive problems, a bidirectional relationship deserves consideration.

Most studies on the relationship between cognitive function and experimental pain sensitivity have been performed on samples of healthy volunteers and focused on the relationship between pain and specific cognitive functions. Several studies report associations between CPT tolerance time and response inhibition measured with tone detection (157) and Stroop color and word (158-160) tasks, while no association was found with other assessments of executive function (159, 160). One study using pressure pain threshold and CPA tolerance found no relationship between these measures and stop-signal or Stroop tasks (161).

While the design of these studies allows for studying the relationship between specific cognitive functions and pain tolerance, they cannot determine how variation in cognitive function across broad populations relates to pain processing. To my knowledge, only one previous study has been done on a population-based sample, using data from the sixth survey of the Tromsø study. In this study, an association between CPT tolerance time and 12-word immediate recall test and digit-symbol coding task was found, indicating that people with poorer performance on cognitive tests have lower pain tolerance (162).

Stroke and pain sensitivity

Brain health can be affected by brain diseases, of which stroke is the largest contributor to years lived with disability (55). It is well known that a stroke can cause post-stroke pain conditions including central post-stroke pain (CPSP) and post-stroke shoulder pain (PSSP). While CPSP was previously known as post-stroke thalamic pain, it is now known that this neuropathic pain condition can also result from vascular lesions in other parts of the somatosensory pathways (163). Lesions affecting motor pathways can cause arm paresis and/or spasticity, with PSSP as a possible complication.

Given the importance of the extensive brain network involved in pain processing, it is also likely that strokes can affect some of these regions or connections between them, which could affect pain processing beyond focal alterations corresponding to stroke lesion location, and

yield alterations in pain sensitivity that could be present irrespective of whether the patient has a chronic pain condition.

Experimental pain sensitivity in stroke survivors has previously been studied with the aim of disentangling the pathophysiology of specific post-stroke pain conditions, namely PSSP (164-166) or CPSP (167-169), or specific stroke lesion locations (170). Evidence of both focal and widespread alterations in pain sensitivity has been found in patients with PSSP (164-166) and CPSP (168, 169), and in control stroke patients with sensory deficits but no chronic pain (169). Few have studied pain sensitivity in stroke patients without pain, but intensity ratings in response to heat and pinprick stimuli were increased in patients with cerebellar strokes (170). In line with their aim, all these studies are case-control designs (n=20-60), and participants were included based on having a specified post-stroke pain condition or stroke lesion location, often from rehabilitation or pain clinics. This entails samples that are highly selected, and inference cannot directly be drawn to the general population of stroke survivors.

While most of these studies assessed pain with thresholds and/or intensity ratings, two studies used CPT as a conditioning stimulus in a CPM procedure and reported lower CPT tolerance in the unaffected side in PSSP patients (164, 166).

1.6 Knowledge gaps and rationale for the thesis

Previous research on the relationship between pain and the brain has identified brain regions and networks involved in pain. While chronic pain is associated with risk of confounding from variation in the chronic pain condition, experimental pain studies allow for assessment of the relationship between nociceptive stimuli in a controlled setting and measurements related to variation in the health status of the brain.

Studies on the relationship between gray matter and pain sensitivity are heterogenous and the two larger, population-based studies found either no association (143) or an association only in women with chronic pain, but not in men (135). This heterogeneity may be due to methodological differences and complexities in pain physiology, but possible contributors to heterogeneity between studies are also small sample sizes and sampling of selected populations.

Only one large population-based study has assessed the relationship between broad cognitive tests and pain (162). Corroboration of results and addition of other cognitive tests and pain stimuli is warranted.

There is evidence that cerebral stroke is associated with altered pain sensitivity in small samples selected by chronic pain condition (164-169) or stroke lesion location (170). Findings suggest that this is not restricted to body regions corresponding to stroke lesion location (164-166, 168, 169), and altered pain sensitivity can also be present in stroke survivors without chronic pain (170). This warrants investigation in a larger sample, in patients both with and without chronic pain.

Moreover, it has been shown that activation of some of the pain processing regions is different in subjects with high pain intensity ratings from those with low ratings (171). Functional brain connectivity in a pain-free resting state has been shown to predict pain thresholds (51). These findings suggest that differences in pain sensitivity between individuals have neurobiological underpinnings, but less is known on how this plays out with respect to whether or to what extent variation in the health status of the brain is related to the variation in pain sensitivity in people from the general population. Previous research done in healthy volunteers or case-control design cannot answer this question. This is an important knowledge gap, as knowledge on this relationship can enable identification of risk factors and populations at risk for pain.

2 Aims of the thesis

We aimed to study the relationship between pain tolerance and aspects of brain structure and function in the setting of a large, population-based epidemiological study. Specifically, we aimed to study:

1) if pain tolerance is related to variation in gray matter volume
2) if pain tolerance is related to cognitive function
3) whether pain tolerance is affected in people with previous cerebral stroke

3 Study population and methods

3.1 Study population

The Tromsø Study is a multi-purpose longitudinal cohort study which has been carried out with intervals of 6-7 years since 1974 (172, 173). The cohort consists of inhabitants of the municipality of Tromsø, which is the largest city in northern Norway. The population in 2015, when the 7th wave of the Tromsø Study (Tromsø 7) was started, was 73,000 (173). Initially, the Tromsø study started as The Tromsø Heart Study with the aim to study causes of cardiovascular disease, motivated by the high mortality of cardiovascular disease in Norway and particularly northern Norway. Progressively more examinations have been added, including experimental pain assessments (Tromsø 6 and Tromsø 7) and cognitive testing (Tromsø 5, 6 and 7). In Tromsø 7, a subsample was invited to MRI of the brain. Participants have been followed up for registration of endpoints/cardiovascular events including stroke.

Participants to the Tromsø study are selected as whole birth cohorts and/or by random sampling of specified age groups and invited by postal letter. The letter suggests a participation date, but participants can choose to drop in at any time for the duration of the study. Tromsø 6 was conducted in 2007 – 2008. To this wave, 19,762 inhabitants aged 30 years and older where invited, and 12,984 (65.7%) participated. In Tromsø 7, which was conducted in 2015-2016, all inhabitants aged 40 years or older (n=32,591) were invited to the first visit and 21,083 (64.7%) participated. Non-attenders were reminded twice. A subsample was premarked for invitation to a second visit if they attended the first (n=13,028 of whom 9925 selected by random sampling and 3103 drawn from participants of the second visit of Tromsø 6) (173). The second visit consisted of several examinations, including cognitive testing. From attendants to the second visit, a subsample was invited to the MRI study (174). Experimental pain assessments were performed as part of the first visit of both Tromsø 6 and Tromsø 7, and all participants were invited to do these tests.

For this thesis, participants were included from Tromsø 7 while paper 3 also included data from Tromsø 6 (Figure 2).

Paper I

In paper I we studied the association between GMV and CPT tolerance in participants from Tromsø 7 who had been examined with MRI of the brain and had sufficient image quality in addition to having completed CPT and available information on covariates. The sample included for this study was 1522 participants.

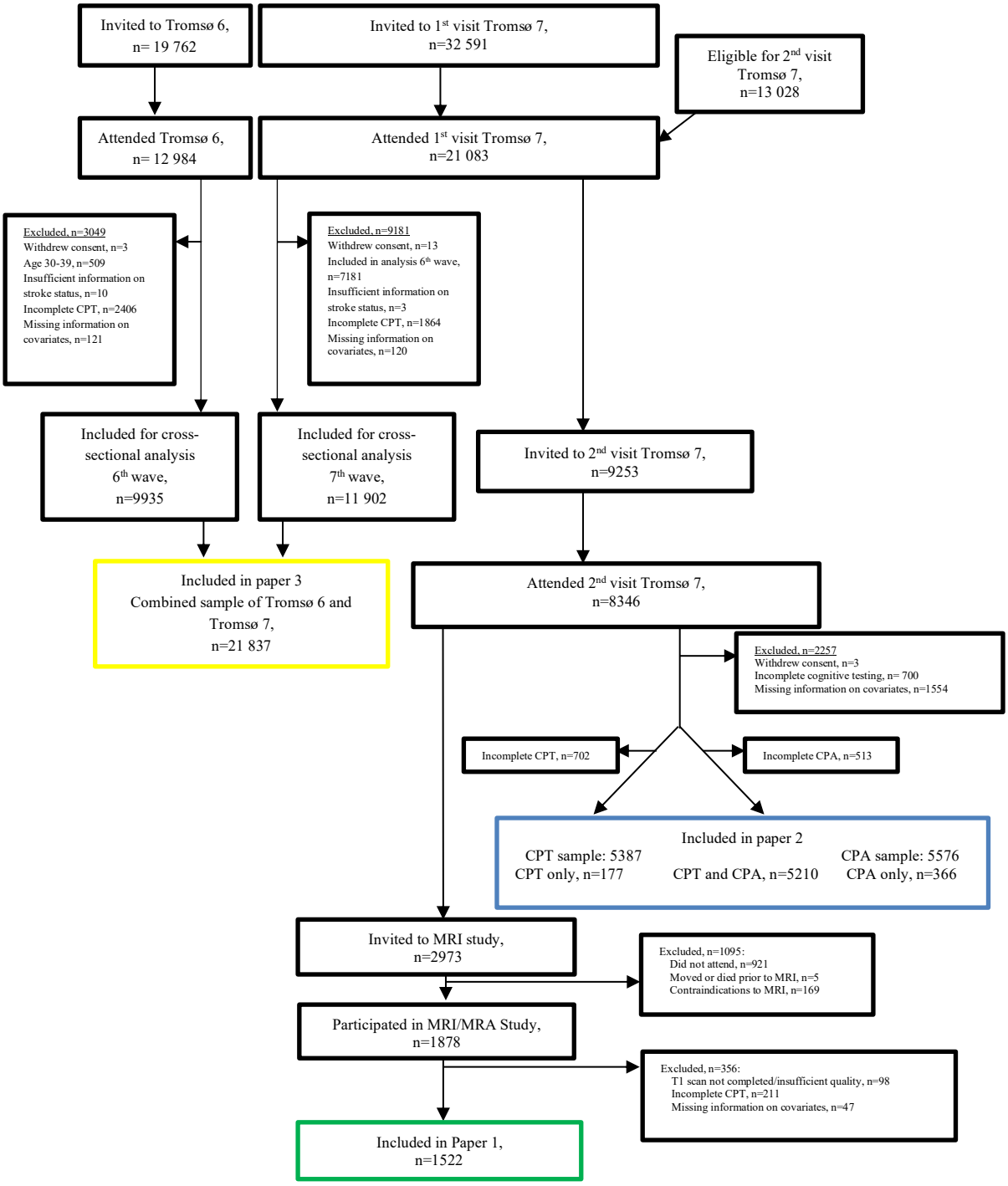
Paper II

In paper II we studied the relationship between cognitive test scores and pain tolerance assessed by CPT and CPA. We included 5753 Tromsø 7 participants who had completed cognitive testing and CPT and/or CPA tolerance test, and for whom information on covariates were available.

Paper III

In paper III we studied whether pain tolerance assessed with CPT was different in participants with or without previous stroke. We included participants from Tromsø 6 and Tromsø 7 who had completed CPT and for whom information on stroke status and covariates were available. Participants were excluded from the Tromsø 7 sample if they had been included in the Tromsø 6 sample to ensure independent samples. The sample from Tromsø 6 consisted of 9935 participants, the sample from Tromsø 7 of 11,902 participants, and the combined sample of 21,837 participants.

Figure 2: Flowchart of participants



3.2 Ethics

The Tromsø study, the MRI study and the present study were approved by the Regional Committee for Medical and Health Research Ethics (2014/940/REK Nord, 2014/1665/REK Nord and 2017/1951/REK Nord, respectively). All participants signed written informed consent.

3.3 Funding

This project was funded by a PhD grant from Northern Norway Regional Health Authority (grant number HNF1460-19).

3.4 Measurement of gray matter volume, cognitive tests and stroke status

3.4.1 Gray matter volume

Participants were scanned in a 3 Tesla (3T) Siemens Skyra MR scanner at the University Hospital of North Norway. The total scan time was approximately 22 minutes, and included T1-weighted, T2-weighted FLAIR (fluid-attenuated inversion recovery sequence), time-of-flight angiography, and susceptibility-weighted series. In this study, only T1 images were used (key parameters for this sequence: 3D magnetization prepared rapid acquisition gradient-echo (MPRAGE) sequence with flip angle 9°, time to repetition/echo time/time to inversion=2300/4.21/996 milliseconds, parallel acceleration factor 2). The images were acquired in the sagittal plane. Field of view was 256 mm, 256x256 image matrix, 176 slices, 1 mm slice thickness and 1 mm isotropic reconstructed resolution.

Estimation of intracranial volume (ICV) and cortical volumes (total and regional) was done using FreeSurfer (v.6.0, http://surfer.nmr.mgh.harvard.edu). ICV was computed from the scaling factor of the affine transformation of the T1-weighted image to the Talairach template in FreeSurfer (175). To estimate cortical GMV, skull stripping, segmentation of brain tissue, and placement of the gray/white and gray/cerebrospinal fluid borders was done (176, 177), while subcortical GMV was estimated by the method described by Fischl and coworkers (177). Participants with large lesions (cysts, tumors, etc.) were excluded, as FreeSurfer may be unreliable in these cases.

A difference from standard nomenclature must be noted for the subcortical segmentation: while the substantia nigra and red nuclei are generally assigned to the mesencephalon, FreeSurfer includes these in the ventral diencephalon along with the hypothalamus with mamillary bodies, the subthalamic, lateral geniculate, medial geniculate nuclei and surrounding white matter (178, 179).

3.4.2 Cognitive tests

Cognitive testing included a 12-word immediate recall test (180), digit-symbol-coding test (181), and the Mini-Mental Status Examination (MMS-E) (182, 183). In the 12-word immediate recall test, participants were presented 12 nouns written on a board and spelled out loud with 5 second intervals, before they were given 2 minutes to recall as many words as possible (score according to number of correctly recalled words, range 0-12).

In the digit-symbol-coding test, participants were given a key linking 9 symbols to 9 numbers and given 90 seconds to fill in as many symbols as possible in blank numbered squares. One point was given for each correct symbol, with a maximum of 96 points.

The MMS-E is a test often used for dementia screening and consists of 20 tasks assessing several cognitive domains. The maximum score is 30. A score of 28-30 points is considered normal, a score of 25-27 points as possible cognitive impairment, and a score of 24 or below as cognitive impairment (183).

3.4.3 Stroke status

Information on stroke status was obtained from two sources: The Tromsø Study Cardiovascular Disease Register contains information on incident ischemic, hemorrhagic, and unclassifiable strokes until 31.12.14 (until 31.12.17 for subarachnoid hemorrhages), while information on ischemic, hemorrhagic, and unclassifiable strokes from 01.01.15 was obtained from the Norwegian Stroke Register. The Tromsø Study Cardiovascular Disease Register was established for the purpose of collecting endpoints for the study of cardiovascular risk factors. Each participant´s first-ever event of stroke, as well as myocardial infarction, atrial fibrillation, and venous thromboembolism are registered (184). Data collection is by expert review of medical records: a search is done for relevant discharge and out-patient diagnoses in the hospital records of the University Hospital of Northern Norway, which is the only hospital within 300 km distance and hence likely to cover all strokes in participants in the Tromsø Study with few exceptions. All relevant discharge diagnoses are adjudicated by a trained physician, based on the medical record. Strokes are defined according to the WHO definition as rapidly developed clinical signs of focal or global disturbance of cerebral function, lasting more than 24 hours or until death, with no apparent cause other than vascular (185), and classified as ischemic or hemorrhagic stroke or subarachnoid hemorrhage according to findings on diagnostic imaging (as unclassifiable if no imaging was done in the acute phase). Strokes are defined as ischemic if hemorrhage is ruled out on diagnostic imaging. The Norwegian Stroke Register was established in 2012 and is a national medical quality register. Its purpose is to measure and contribute to improvement in quality of care for stroke patients, ensure equal and good-quality care in all Norwegian hospitals, and contribute to clinical and epidemiological research (186). Strokes are defined according to the WHO definition, and registration of patients with ischemic and hemorrhagic strokes is mandatory for all Norwegian hospitals. Registration is done by trained physicians and nurses.

For this study, participants with a diagnosis of ischemic, hemorrhagic, or unclassifiable stroke or subarachnoid hemorrhage prior to participation, were included as stroke cases. If diagnostic imaging revealed an ischemic lesion, transient ischemic attacks (TIA) were included as ischemic stroke cases.

3.5 Measurement of pain tolerance

3.5.1 Cold pressor test

The CPT was done with a setup consisting of a 13-liter vat filled with cold water (3°C) which was continuously exchanged with a circulating water cooler (FP40_HE, Julabo GmbH, Seelbach, Germany) to ensure constant temperature. The participants were asked to submerge their hand and wrist into the cold water and keep it there for as long as they were able or to a maximum time of 120 seconds in Tromsø 7 and 106 seconds in Tromsø 6. In Tromsø 7 the non-dominant hand was submerged while in Tromsø 6 it was the dominant hand. Prior to testing a brief screening interview was done to exclude participants who a) declined the test, b) were unable to comprehend instructions or c) had medical issues that were considered to interfere or put the participant at risk if exposed to cold, such as amputation or paresis of the hand, sensory disturbances, eczema, cold allergy or Raynaud syndrome, or if the participant had lost consciousness during the venipuncture performed before arriving at the test station.

If one hand was affected by a contraindication for testing, the other hand could be used.

3.5.2 Cuff pressure algometry

Cuff pain tolerance was assessed with a computerized cuff pressure algometer (NociTech, Aalborg, Denmark) and a blood pressure cuff. The cuff was fitted and inflated around the participants leg, one at a time. The pressure increased by one kilopascal (kPa) per second, until the participant pressed a button to stop the test or to a maximum of 100 kPa (100 seconds). Participants were excluded from testing if they declined, had difficulty understanding the instructions or reported a reason indicating they should not be tested such as sensory and motor dysfunction or problems with peripheral circulation.

3.6 Covariates

Information on covariates was collected from on-site measurements and questionnaires. Weight and height were measured with light clothing and no shoes. Body mass index (BMI) was calculated using the formula weight/height^2 (kg/m^2). Blood pressure was measured three times with the participant seated, and the mean of the last two measurements was used. HbA1c was analysed with high performance liquid chromatography and serum total cholesterol and high-density lipoprotein (HDL) cholesterol by standard enzymatic colorimetric methods. Questionnaires provided data on medical history, medication use, socioeconomic status, and lifestyle habits.

In paper I, hypertension was defined as self-reported current hypertension and/or use of antihypertensive medication and/or systolic blood pressure above 140 and/or diastolic blood pressure above 90. In paper II, systolic blood pressure was used to apply the same method as the previous study (162). In paper III, the definition was the same as for paper I except that self-report of previous hypertension was included as the strokes had occurred prior to participation. In paper I, diabetes was defined as self-reported current diabetes and/or use of anti-diabetic medication and/or HbA1c above 6.5%, while in paper III self-report of previous diabetes was also defined as diabetes. Hyperlipidemia was defined as use of lipid-lowering drugs or total cholesterol/HDL ratio above 5. If information on one of the criteria constituting the definition of hypertension, diabetes or hyperlipidemia was missing, the definition was based on available data. If all criteria were missing the covariate was set to missing.

Information on smoking was assessed as current, previous, or never daily smoking. Education was assessed by the question “What is the highest levels of education you have completed?” with the categories up to 10 years, 3 years of upper secondary, less than 4 years of college/university or 4 years or more of college/university. Exercise was assessed by the question “How often do you exercise (i.e., walking, skiing, swimming or training/sports?)” with the options never, less than once a week, once a week, 2-3 times a week, approximately every day. Mental health was assessed with the ten-item version of Hopkins Symptom Checklist (SCL-10), and depression was defined as SCL-10 average above 1.85 (187). Chronic pain was assessed by the question “Do you have persistent or recurrent pain that has lasted for 3 months or more?” (yes/no). Analgesic use was assessed by questionnaire on use of prescription/non-prescription analgesics the last four weeks, each with the options not used, less than weekly, weekly, or daily.

Participants with missing information on chosen covariates were excluded from all analyses.

3.7 Statistical methods

For descriptive purposes, participants were grouped according to whether or not they were able to tolerate CPT until the maximum time in paper I and II, while according to stroke status in paper III. Group differences were evaluated with t-test or Wilcoxon rank sum test for continuous variables, Pearson χ^2 for categorical variables. GMV and cognitive test scores were standardized by z-transformation to get manageable and comparable effect estimates. Kaplan-Meier plots were created to visualize CPT tolerance time by strata of the independent variables.

In the analyses, CPT tolerance time was used as the outcome while gray matter volume, cognitive test scores and stroke status were the independent variables in paper I, II and III respectively. Adjustment was made for putative confounders by adding them to the model.

As CPT tolerance time is a right-censored variable due to the maximum time, we used survival analysis with Cox proportional hazards models. Time with the hand in the water bath was used as the time variable, and hand withdrawal as the event. Interaction was tested for age and sex (paper I, II and III) and chronic pain (paper I and II) by adding interaction terms to the model (the respective variable multiplied with the respective independent variables). Statistical significance level was set to 0.05.

The assumption of proportional hazards (PH assumption) was assessed by inspection of observed versus expected survival plots and log-log survival curves and tested statistically using test of scaled Schoenfeld residuals.

In paper I, explorative analyses were performed to assess the relationship between pain tolerance and regional gray matter volume. This was done by fitting the fully adjusted Cox regression model vertex-vise for cortical volumes (188) and for each predefined region for subcortical structures. As this entailed a large number of statistical tests and consequently increased risk of type I error, correction for multiple testing was done by false discovery rate (FDR) correction.

Statistical analyses were performed using STATA version 16.1 for windows (StataCorp LLC, Texas, USA). In paper I, explorative analyses of the relationship between pain tolerance and

regional GMV was done using the “Survival toolbox” in FreeSurfer (188) and R with the ggseg package (https://github.com/ggseg/ggseg) for visualization.

4 Main results – summary of papers

4.1 Paper 1

In total 1522 participants were included for analysis in paper I. Of these, 612 (40.2%) kept their hand in the water until the maximum time and hence were considered pain tolerant, while 910 (59.8%) withdrew it at an earlier time and were therefore considered pain sensitive.

Among pain sensitive participants there were more women, education levels were lower, and a higher proportion had diabetes, while there were no differences in the other covariates.

GMV was associated with CPT tolerance time. For total GMV the hazard ratio (HR) was 0.81 (95 % confidence interval (CI) 0.71-0.93), in multivariate analysis. In subsamples with available information on chronic pain and depression, we added this as additional covariates, but this changed the effect estimates very little. We found no interaction effect of chronic pain.

Effect sizes were similar in the right and left hemisphere and in cortical and subcortical structures. Explorative vertex-wise analyses of cortical volumes showed that the effect was stronger and remained significant after FDR-correction for multiple testing, in clusters in locations including bilateral insula, bilateral postcentral, left anterior and right posterior cingulate, right superior frontal, precentral, medial and lateral orbitofrontal cortex, as well as bilateral ventral diencephalon and left nucleus accumbens.

4.2 Paper 2

For analyses on CPT tolerance time and cognitive test scores, 5387 participants were included, of whom 37% (1994) endured the test until the maximum time, while 63% (3393) withdrew it earlier. There was a significant association between CPT tolerance time and score on immediate recall test (HR 0.93, 95% CI 0.90 – 0,.97), coding test (HR 0.94, 95% CI 0.89 – 0.98) and MMS (HR 0.93, 95% CI 0.90 – 0.96) (HR and CI for multivariable analyses).

For analyses on cuff pain tolerance and cognitive test scores, 5576 participants were included. There was a significant association between cuff pain tolerance and immediate recall test (HR 0.94, 95% CI 0.91- 0.97) and coding test (HR 0.93, 95% CI 0.89 – 0.96) but not with MMS (HR 0.98, 95% CI 0.95 – 1.00) in multivariate analysis.

4.3 Paper 3

In total 21,837 participants were included for this study, 9935 from Tromsø 6 and 11,902 from Tromsø 7. Of these 311 had had a stroke prior to study participation, 181 of the Tromsø 6 participants and 130 of the Tromsø 7 participants. Overall, the proportion of participants who were pain tolerant was smaller in Tromsø 7 than in Tromsø 6, but in both study waves the proportion of pain tolerant subjects was smaller among those with a history of stroke than in those with no stroke: In Tromsø 6, 60% of those with stroke were pain tolerant while it was 68.3% of those without stroke. In Tromsø 7, 26.9% of those with stroke were pain tolerant while it was 36.4% of those with no stroke. Statistical analyses showed that having a history of stroke was associated with increased risk of hand withdrawal (HR 1.28, 95% CI 1.10 – 1.50) in multivariate analyses in combined sample Tromsø 6 and Tromsø 7). Subgroup analysis of participants with and without self-reported chronic pain showed similar results (HR 1.28, 95% CI 0.99 – 1.66 in participants with chronic pain and HR 1.29, 95% CI 1.04 – 1.59 in participants without chronic pain, in combined sample).

5 Discussion

5.1 Methodological considerations

5.1.1 Study design

By definition, epidemiology is «the study of the occurrence and distribution of health-related events, states, and processes in specified populations, including study of the determinants influencing such processes, and the application of this knowledge to control relevant health problems» (189). Knowledge on how the distribution of an outcome varies according to other factors of interest serves important purposes: identification of high-risk groups enables efforts aimed at risk reduction or treatment of diseases or symptoms, and knowledge on relationships can contribute to generation of hypotheses on causes and consequences (190).

The three papers included in this thesis have a population-based observational, cross-sectional design. Observational designs allow for studies on the relationship between an outcome of interest and presumed exposure(s) as they occur naturally in the life of the research subjects (191).

The population-based recruitment of participants through invitation of random samples and whole birth cohorts, along with the high response rates, entails that the sample is likely to be representative for the general population. This is an important addition to previous research on these topics, which has mainly, with a few exceptions, been done in healthy volunteers or case-control design. These samples might differ from the spectrum of the condition or characteristic as well as other health-related factors in the general population. The population-based sample in our study is therefore an important addition to previous research.

5.1.2 Validity

Application of knowledge from a study requires that findings can be inferred from the study to other settings, which has essential prerequisites: internal and external validity. Internal validity considers whether, or to what degree, a study is affected by bias or systematic error. Sources of bias that are important to consider include selection bias, which has to do with the procedure of selecting participants, and information bias, which arises if the measurement of variables is flawed. External validity builds on internal validity, and additionally requires that the study population is representative of the population to which one wants to infer the findings.

5.1.2.1 Selection bias

Selection bias occurs when the exposure and outcome of interest affect the inclusion of participants. This can arise from different mechanisms, some of which affect the internal and some the external validity.

In the Tromsø Study, participants are recruited through a postal invitation letter. To attend, they need to be willing and able to visit the study center, and it is likely that the health status of invited participants can affect their attendance. In general, healthy people are more likely to attend health surveys. This type of selection bias is termed the healthy volunteer effect or, correspondingly, non-respondent bias (192). The consequence is that the variation between attendants is smaller than within the population, which induces a bias toward "no difference". Although somewhat decreasing in the last two surveys, the participation rate in the Tromsø study is generally high (172). In Tromsø 6 the attendance rate was 66%, in Tromsø 7 it was 65%. In Tromsø 1-6, it has been found that compared to attendees, non-attendees tend to include higher proportions of men and single persons and be younger (172). Due to legal restrictions, it has not been possible to analyze morbidity and mortality in attendees compared to non-attendees, but consistent attendants to Tromsø 2-4 had lower mortality than those who attended only Tromsø 4 even though invited to all three study waves (172). A study of non-participants in the HUNT-study, which is another population-based Norwegian survey with similar design and recruitment methods, found a higher prevalence of cardiovascular diseases, diabetes, fibromyalgia, and psychiatric disorders in non-attendees (193). It is reasonable to assume that a similar non-respondent selection bias is present in the Tromsø study.

In the case of paper III, it is probable that individuals with severe strokes are less likely to attend the study. Additionally, participants who have had a stroke were less likely to have completed the experimental pain examination, even if they did attend the study. Among attendants, we found that the proportions of participants who had not completed CPT was larger in participants with a history of stroke. In Tromsø 6, 82% of those with no stroke had completed the test while only 62% among those who had had a stroke. In Tromsø 7, the proportions were 87% and 63.5% in no stroke/stroke participants, respectively. This suggests a selection bias where milder strokes, with less disability, are likely to be over-represented in the group of participants with a history of stroke. A similar selection bias can be assumed regarding individuals affected by brain disease with established, and particularly with extensive, changes in gray matter volume and cognitive function, both due to the likelihood of

attendance and that problems comprehending instructions was an exclusion criterion for experimental pain assessments.

In summary, there is likely to be a healthy volunteer effect/non-respondent bias in our study. As this usually entails smaller variation between participants than in the population, it is likely this would weaken our results rather than strengthen them.

5.1.2.2 Information bias

Information bias is a consequence of flaws in the definition or measurement of study variables (190). This can lead to misclassification: a variable is assigned to another category than it should have been (189). If this error/flaw is independent of exposure and outcome (non-differential), it usually entails a risk that the strength of an association is underestimated. If a flaw/error differs depending on the exposure, outcome, or both (differential misclassification), the strength of the association can be under- or overestimated.

Potential sources of information bias are related to the respective methods of measurement. Questionnaires and interviews on previous behaviors, exposures and diseases entail a risk of recall bias regarding previous exposures. It is considered that ill-advised behaviors such as smoking are generally more likely to be under-reported. Retrospective collection after a diagnosed outcome can entail that rumination has an impact on the recall of those who have been affected, and this can lead to either under – or over-reporting compared to those not affected. This can lead to differential misclassification. Interviews or tests performed by a technician entail a risk of observer bias: a systematic error in an observers measurement of the variable (189). The risk of recall or observer bias can increase if the participant or the observer is aware of the research question.

Moreover, measurements must be evaluated as to whether they accurately measure the concept they are intended to measure (validity) and whether they are consistent when repeated under identical circumstances (reliability) (194). Validity includes the extent to which the measurement covers all the content it was designed to measure (content validity), and whether the test score reflects the degree of the concept in question (construct validity). Reliability, whether the measure is consistent, requires that a measurement should be stable if repeated in the same participants under similar conditions (194).

Potential sources of bias in measurements of GMV, cognitive tests, and stroke status

GMV is an objective measurement, done by computer software. Information bias could occur if the technology was inaccurate and be differential only if this inaccuracy differed across the spectrum of GMV. Measurements of gray matter is challenging due to the folded nature of the cortex and to the variation in signal intensities and exact anatomical location for subcortical structures. In the FreeSurfer software, a technique has been developed for optimizing geometrical accuracy while preserving topological correctness (177). Comparisons with results from manual estimations from MRI data done by a trained anatomist and postmortem measurements, indicated similar estimates (176). Test-retest experiments with repeated assessments in the same scanner and in different scanners indicated robust findings (176). For subcortical structures the accuracy of the method is found to be comparable to manual tracing (178). The FreeSurfer method may be unreliable in cases with large lesions or other pathology, such as cysts and tumors, and these cases were excluded to avoid spurious measurements.

Cognitive tests were performed and scored according to standardized protocols in order to minimize bias.

Change of data source for information on stroke status might have introduced bias. Manual expert reviews of hospital records, as for The Tromsø Study Cardiovascular Disease Register, is considered the gold standard. Registration in the Norwegian Stroke Register is also done by trained doctors and nurses. A validation study (184) confirmed a high level of correctness (PPV 97.5%) and moderate sensitivity (79.8%) when compared to The Tromsø Study Cardiovascular Disease Register, using data from 2013 to 2014. The low coverage in 2013 (63%) was considered a likely cause of the moderate sensitivity, and this had improved to 88% in 2015 (195), and 84% in 2016 (196). As both registries are based on registration from hospitals, non-hospitalized strokes will not be registered. Hence, we might have missed some stroke cases among attendants, and these will have been included in the control group. This could have weakened the association, but the proportion misdiagnosed is likely to be small.

Potential sources of bias in pain tolerance measures

To minimize sources of bias, experimental pain assessments were performed using computer assisted standardized protocols, including step-by-step procedures, verbatim participant instructions, and automated registration of pain tolerance. Regular meetings were held with technicians to ensure uniform understanding and execution of the procedure. A test-retest

evaluation was done to assess the stability of CPT: A subsample of Tromsø 7 participants were invited to repeat the procedure, mean 277 days after the first. An intraclass correlation coefficient (ICC) of 0.837 for the two measurements indicates that CPT is a stable measure. This is in line with findings from another study, where a test-retest procedure was performed in sixty one healthy students and showed an ICC of 0.85 – 0.92 for CPT tolerance time, indicating excellent reliability (197). Likewise, test-retest of cuff pain tolerance on the lower leg is found to have excellent reliability with ICC of 0.87 (103).

The proportion of participants who were pain tolerant was lower in Tromsø 7 than in Tromsø 6. However, this did not affect the association between stroke and pain tolerance in paper III. In paper II, we found a similar association and effect size as in a previous study on Tromsø 6 data. This indicates that differences in CPT in Tromsø 6 and Tromsø 7 did not impact relationships with the outcomes we have studied.

Potential sources of bias in measurements of covariates

Information from questionnaires was used for information on several covariates. For some of these (diabetes, hypertension, hyperlipidemia), this information was combined with on-site measurements or blood samples, to minimize the effect of recall bias influencing these variables and ensure undiagnosed conditions would be classified according to standard criteria. Some variables were defined based on questionnaire information only, namely education, exercise, smoking, mental health, chronic pain, or use of analgesic medication. While it could be expected that participants with stroke or conditions affecting gray matter or cognitive performance could be more prone to not recalling correctly, it seems likely that this would be random rather than resulting in a systematic deviation of answers. It is possible that stroke affects the risk of recall bias on the question on smoking habits, resulting in some risk of differential misclassification in this variable in paper 3. With this exception, it is likely that misclassification in is non-differential. Non-differential misclassification of covariates may result in residual confounding (190).

5.1.3 Confounding

An observed association between two variables may be due to a causal relationship between the two, or due to another variable that is associated with both the presumed exposure and outcome and constitutes an alternative explanation of the relationship – a confounder. This is not bias: while not causal, a confounded association is real (190). An association can be strengthened, induced, weakened, or eliminated by confounding. When potential confounders

are identified, they can be dealt with by stratification, restriction or by including them in a regression model. Identification is done on the basis of knowledge of factors that are associated with exposure and outcome and the causal network in which exposure, outcome and associated variables are part (189), in order to decide whether an associated variable is likely to be a confounder, or a mediator or collider. A mediator is a variable that is on the causal pathway between exposure and outcome rather than an alternative explanation. Conditioning for a presumed mediator is not appropriate unless the aim is to study the effect of other pathways between exposure and outcome. A collider is a variable that is caused by exposure and outcome, and conditioning on it will tend to induce a non-causal association, i.e., collider bias (189).

We selected covariates known to be associated with pain tolerance as well as other measures of pain sensitivity, as it could be expected that this could impact pain tolerance. With regards to chronic pain, we considered that in paper III, this could have been a collider due to the fact that stroke can cause chronic pain conditions. Therefore, we chose not to adjust for it but rather to do stratified sensitivity analysis in this paper.

It is possible that the relationship between chronic pain and the variables we have studied is dependent on the type and severity of chronic pain. In our samples, about 34% report chronic pain. While this is close to the overall prevalence found in a metanalysis of 80 studies, which was 31% (2), it is likely to include pain conditions with low severity. It is possible that controlling for moderate to severe pain or widespread pain would have yielded somewhat different results.

While most research on the relationship between experimental pain assessment and blood pressure has focused on systolic blood pressure, we chose to also include those with a diagnosis of hypertension or had been prescribed antihypertensive medication in this variable, as we considered that elevated blood pressure over time was most relevant with regard to risk of stroke and variation in GMV. Moreover, as modulatory systems for pain and blood pressure overlap (113), it seems likely that a dysfunction in the cardiovascular system associated with hypertension might be related to altered pain tolerance. With regard to obesity, we could have considered using waist circumference rather than BMI, as one study found a correlation between GMV and both these measures, but the correlation with waist circumference was stronger in females (76) and another that, among components of metabolic syndrome, waist circumference had the strongest correlation with GMV (198).

While we did not adjust for physical activity in paper I, this is associated with pain tolerance (123) and accelerated brain age (199) and hence it could have been appropriate to include it in the model. However, we did additional adjustments for exercise post hoc, and this had minimal impact on results (HR 0.83, 95% CI 0.73 – 0.95). Moreover, we could have considered adjusting for a measure of sleep or sleep problems, as sleep deprivation is found to increase pain sensitivity (200) and insomnia is associated with shorter CPT tolerance time (201). However, we did additional adjustments for HSCL-10, which includes a question on insomnia, in paper I and II.

Adding covariates beyond age and sex generally had minimal impact on our results. It could be speculated whether these covariables have low impact on pain tolerance or on the brain, or that the brain is a major pathway through which these factors exert their influence on pain tolerance, in which case adding the covariates would have minimal impact as the variation related to them is already in the model.

5.1.4 Missing data

In all three papers, only complete cases where included. Missing data can be completely at random, random or not random, and the type of missingness has significance as to whether complete case analyses tend to be biased. As described above, participants with a history of stroke were less likely to perform CPT. As this systematic difference can be explained by observed data (the independent variable), this is missing of the type "missing at random" and complete case analysis is usually not biased (202).

5.1.5 External validity

The population of Tromsø mainly consists of white Caucasians. A systematic review and meta-analysis found lower tolerance to cold pain in African Americans, Hispanics, and Asians than in non-Hispanic whites (203). While it seems likely that the relationship between GMV, cognitive function and stroke and cold pain tolerance does not depend on the cold pain tolerance per se, some caution might be appropriate in inferring results to other racial or ethnic groups or racial/ethnic minorities.

5.1.6 Statistical considerations

5.1.6.1 Cox regression/survival analysis for analysis of CPT tolerance time

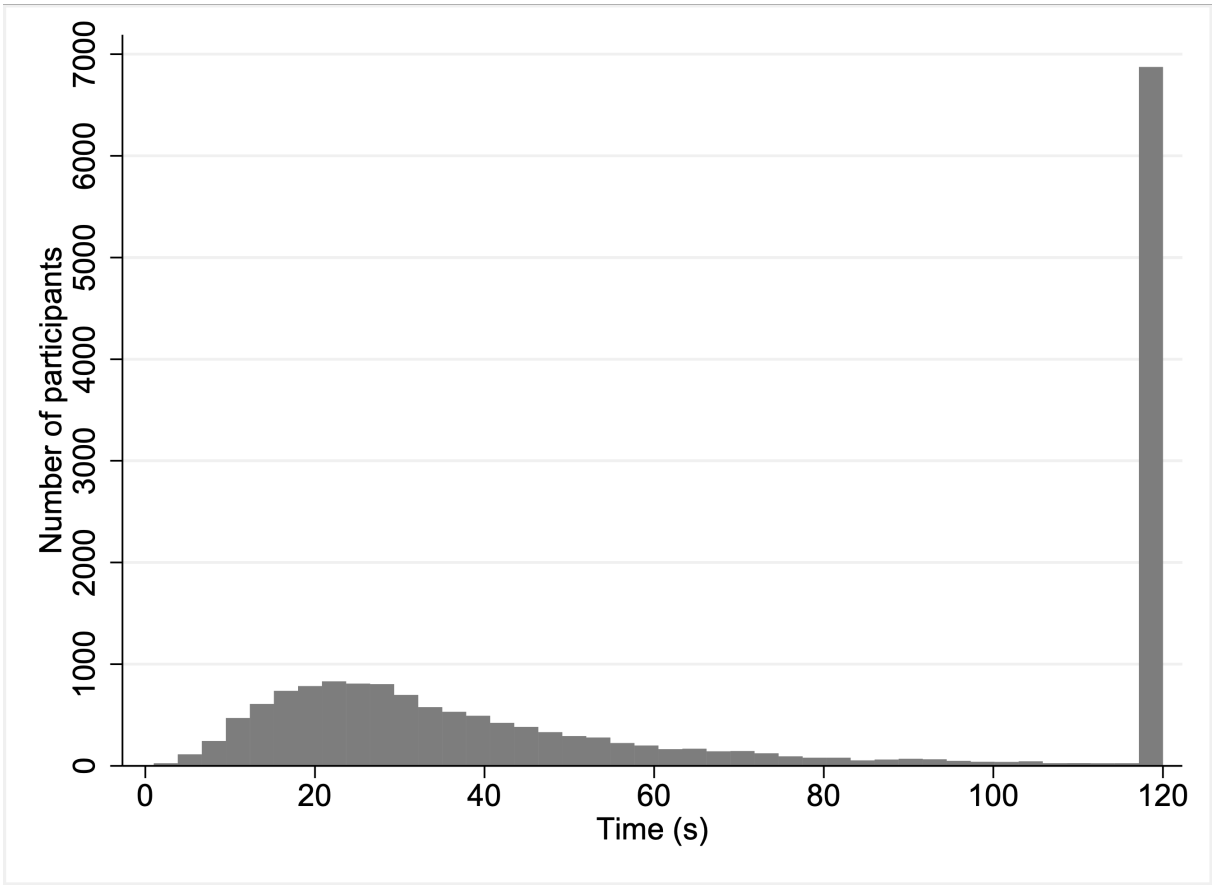
A research question in the form of a hypothesis on a presumed relationship, requires a statistical test to assess the probability of the hypothesis (or to be accurate, the corresponding

null hypothesis) being true or false. In the choice of the appropriate statistical model, the distribution of the data must be considered. Parametric tests, such as t-tests and analysis of variance, assume data are normally distributed, which allows for describing them by parameters, i.e. the mean and standard deviation, and use of parametric methods for estimation of parameters and hypothesis testing (204). Non-parametric tests generally rely on ranking the data and are not dependent on the distribution. They are suited for hypothesis testing, with power similar to that of parametric tests if samples are sufficiently large, however they do not give meaningful estimates without additional assumptions (204).

CPT tolerance time was the outcome in all three papers. Because this test had a maximum time limit, a large proportion of the participants withdrew their hand at this time, and the time they would have endured the test in absence of a maximum time is not known – that is; they are censored with regard to their tolerance time. Moreover, the distribution of CPT tolerance time was not normal (Figure 3), as previously described in data from the Tromsø study as well as in other data sets (205).

Survival analysis with the Cox proportional hazard model, is a semi-parametric model that provide a means for hypothesis testing, estimation of effect sizes and adjustment for covariates, without assumptions on the distribution of the data. The output is usually expressed as the hazard ratio (HR), which is the ratio of the hazards in individuals who differ in the predictor/covariate of interest with one unit (206). An important assumption is that the relationship between the variables and the hazard is constant over time, known as the proportional hazards (PH) assumption (206). This assumption can be evaluated using plots and statistical tests.

Figure 3: Histogram of CPT tolerance time in Tromsø 7 participants



Histogram of CPT tolerance time in all participants who completed the test in Tromsø 7, n=18,236.

5.1.6.2 Correction for multiple testing

To minimize the risk of false positive results, i.e., Type 1 error, the number of tests should be kept to a minimum. While a probability of Type 1 error (alpha) of 0.05 is generally accepted, as reflected by the convention of setting the threshold of significance at this level, the risk of at least one such error increases with the number of tests (the family-wise error rate increases) (207).

If one does many tests, steps can be taken towards decreasing this risk, by changing the cut-off level for statistical significance to be more stringent, i.e., a lower p-value. Simplistically, this could be done by choosing a lower p-value as limit for what is considered significant, as for example 0.01. However, the number of analyses should be considered. A known method of correction for multiple testing based on the number of tests is the Bonferroni correction, which adjusts the p-value by dividing the alpha by the number of independent

hypotheses/tests. However, this is often considered overly conservative as tests are rarely independent. Moreover, the drawback of procedures of correction for multiple testing is that they entail an increased risk of type 2 error, i.e., a loss of power to detect a true association (207, 208). The choice of method for correction must be calibrated to minimize the risk of both Type 1 and Type 2 error.

Our exploratory analyses in paper 1, where the Cox regression analysis was done in each vertex, entailed a very large number of analyses, and hence a large risk of type 1 error. Data from vertexes in the brain are correlated due to physical properties of the tissue (i.e., cortex volumes in the vicinity of a specific measurement will be similar) as well as properties and methods of the scanner and processing of images prior to analyses (such as smoothing). We therefore chose to use False Discovery Rate (FDR) correction, which is a method based on the expected proportion of false positives among those tests that uncorrected are declared as significant (208, 209). P values for the individual tests are calculated and then ranked in ascending order. Then, the number of expected false positives given the number of tests performed, are used to calculate the index k, which denotes the number of p values expected to be true positives. The p-value of the test in position k in the ranked order is the FDR corrected p-value.

5.2 Discussion of main results

5.2.1 Gray matter volume and pain tolerance

In paper I, we found that larger total and regional GMV was associated with longer CPT tolerance time. The effect was similar in both hemispheres and in both cortical and subcortical regions. Adding age and sex to the model resulted in increased effect size, while additional covariates had minimal impact on results. There was no interaction effect between GMV and age or sex. Additional adjustment for chronic pain or depression in subsamples with available information on these items did not change effect estimates, and there was no interaction effect of chronic pain. In post-hoc vertex-vise analyses, cortical clusters with larger effect estimates and significant association after FDR correction for multiple testing included bilateral insula, bilateral postcentral, left anterior and right posterior cingulate cortex, right superior frontal, precentral, medial and lateral orbitofrontal cortex. Among subcortical regions, significant associations after correction for multiple testing were seen in bilateral ventral diencephalon and left accumbens.

The presence of an association fits well with previous research. The importance of brain regions and networks in pain processing has been firmly established by functional studies. While the relationship between structural and functional characteristics of brain regions and their relationship with behavioral outcomes is not fully understood (210), it is likely that there is a relationship and emerging evidence has started to illuminate it in other fields of cognitive neuroscience (210-213), and recently also in the pain field: a link between structure and function has been shown by one study which found pain threshold correlated with both gray matter volume and local clustering coefficient in a cluster of voxels in the left posterior insular cortex to left parietal operculum including S2 (52).

Here we confirm a relationship between pain tolerance and total GMV, and in particular with GMV in cortical regions previously identified by functional imaging studies to be activated consistently across several pain assessments (22, 24), and with CPT in particular (98), including the postcentral gyrus (containing S1), insula, ACC and regions in the PFC). There is increasing recognition of the importance of the brain as a whole and that a core quality of the pain processing system is that it is distributed across many regions in the brain (18). There has been a shift from approaches mapping one-to-one relationships, and increased focus on brain networks in the pain field (50-52), as has been the case in other fields, such as cognitive

neuropsychology (210). Moreover, on a population level, differences in GMV are likely to be related to the distribution of risk factors and conditions affecting the brain, some of which likely affect it on a local and some on a global level. The finding of an association with total GMV is relevant with regards to these considerations.

CPT tolerance time is likely to be influenced by the sensory-discriminative, affective-motivational, and cognitive-evaluative dimensions of the pain experience and by descending modulation mechanisms. The finding of association across broad regions of the brain fits well with CPT tolerance time as a pain assessment. The postcentral gyri contain S1, which receives nociceptive signals conveyed through the spinothalamic tract and has been ascribed a role in the sensory-discriminative dimension of pain processing. An association between gray matter in these regions and pain sensitivity has previously been found by one other study, where a positive relationship between S1 and pain sensitivity was found for heat and cold pain thresholds (145), i.e., the opposite direction of effect. This difference might relate to the inherent differences between threshold and suprathreshold assessments.

The association with the insula and ACC fit well with the affective components likely involved in this assessment. An association between less gray matter in the insula and higher pain sensitivity has consistently been found by other studies using suprathreshold pain assessments (149-153). A positive relationship between gray matter in the ACC and pain sensitivity measured by heat pain threshold was found in women with chronic musculoskeletal pain from the Rotterdam study, i.e., opposite direction of effect than in our study. However, given the poor correlation between experimental pain assessment methods (86, 88) and indications of alterations in pain processing in chronic pain populations, the findings are not necessarily mutually exclusive. Possible explanations may be that the Rotterdam substudy included only participants with chronic pain, used a different pain stimulus and measured threshold.

Regions in the PFC relate to the cognitive-evaluative dimension of pain. We found an association between pain tolerance and two small clusters in OFC: while a cluster in the right medial OFC was negatively associated with tolerance time, like most of the rest of the cortex, a cluster in the right lateral orbitofrontal cortex had the opposite direction of effect. These inconsistencies and the small size of the clusters indicate that the findings in these regions should be interpreted with some caution. Meanwhile, one study has suggested that different

regions within PFC can have inhibitory or facilitatory effects on pain (42), supporting a possibility of opposite directions of effect in different regions.

In addition to these regions which are known to be most consistently activated, we found an association with the right posterior cingulate cortex, also often reported as activated by pain stimuli (21), and with the bilateral inferior parietal cortex. Associations between pain sensitivity assessed with intensity ratings and gray matter in these regions were also reported in another study using a suprathreshold pain assessment (150). These regions are part of the default mode network, where altered dynamics have been found in chronic pain as well during a sustained noxious stimulus (5-20 minutes) in healthy volunteers (214).

In addition to a role in constructing the multidimensional pain experience, regions related to affect and cognition can modulate pain through an effect on descending pathways which inhibit or facilitate spinal nociception (DPM) (27). Subcortical regions also exert descending modulatory effects. We found higher GMV in the ventral diencephalon (VDE) to be associated with longer CPT tolerance time, and while the resolution and contrast in our images do not allow for disentangling what parts of the VDE contribute to the effect, it does contain regions known to partake in DPM (i.e., the hypothalamus). Among subcortical regions, we also found an association between CPT tolerance time and left accumbens, which is part of aversion-reward systems (142) and has been ascribed a key role in action selection in ambiguous situations (215).

In summary, the main pattern emerging from our study is that pain tolerance is associated with GMV, with stronger effect sizes in regions previously shown to be associated with pain, and findings are to a large part in line with previous research in the area. With regards to differences in findings between studies on gray matter and pain sensitivity, there are several explanations that need to be considered. Many of the previous studies had small samples, often consisting of healthy volunteers, which entails smaller variability, and in combination with small or moderate effect sizes, this can lead to insufficient power and poor reliability. However, there is also likely to be heterogeneities reflecting the actual complexity of pain physiology. The poor correlation between experimental pain modalities is a logical explanation that studies using different modalities (i.e., heat, cold, and pressure), outcome measures (threshold, tolerance, intensity ratings) or paradigms, have somewhat different findings (86, 88). Moreover, in reflection of the multidimensional experience of pain, it is also influenced by a number of factors other than the nociceptive stimulus, such as attention,

psychological and physical condition (27). This entails pain and pain tolerance might be related to a number of factors of the individual and the test situation.

One of the factors that could influence the relationship between gray matter and pain tolerance could be chronic pain. Chronic pain is associated with alterations in gray matter volume (135, 136), with shorter CPT tolerance time (126) and with increased pain sensitivity measured by heat and pressure pain ratings (127) and pressure pain thresholds (128). This entails chronic pain could be a confounder. It is also feasible that brain regions involved in pain processing could differ between chronic pain patients and healthy controls, as suggested from findings from case-control studies of musculoskeletal pain (147) and migraine (146). In our study, adjustment for chronic pain as a covariate had minimal influence on the effect estimate. Nor did we find interaction effect between GMV and chronic pain. It is possible that an impact of chronic pain on the relationship between GMV and pain tolerance depends on the type and severity of chronic pain. Meanwhile, our findings suggest that there is a relationship that is not explained by chronic pain. Moreover, although our cross-sectional observational design does not allow for causal inference, it seems more likely that variation in GMV could cause variation in pain tolerance, than that pain tolerance, in absence of chronic pain, should alter brain structure. This entails variation in pain tolerance due to variation in GMV could be a risk factor for experiencing more severe pain or have more difficulty in coping with it. A possibility that pre-existing brain structural differences could be a risk factor or predisposition to chronic pain is supported by findings that smaller amygdala volume and white matter corticolimbic connectivity (216) higher functional connectivity within dmPFC-amygdala-accumbens circuit (137), and white matter structural properties (217) predicted transition to chronic pain.

The resolution and contrast in our imaging does not allow for differentiation of constituents of gray matter, and hence it is not known whether variation is caused by variation in the number or size of neurons or glia, or density of synapses or dendritic spines (13). While the available neuronal resources seem likely to influence pain processing, glia cells have also been linked to pain (218).

The dominant pattern emerging from this study is that larger GMV is associated with longer pain tolerance in a general population. This adds to previous research, as while there is considerable amount of variation in gray matter volume across the general population, studies on the relationship between gray matter and pain sensitivity have mainly studied healthy

volunteers (52, 144, 145, 148, 150, 152) or case-control studies in which cases were chronic pain patients (146, 147, 149) or yoga/meditation practitioners (151, 153). In the two larger, population-based samples, association was not found (143) or present only in women (135). Our findings suggest that people with lower GMV might be more sensitive to pain or less able to cope with it.

5.2.2 Cognitive test scores and pain tolerance

In paper II, we found that higher CPT and CPA tolerance time was associated with higher scores on immediate recall and coding test. A similar association was found between CPT and MMS while the relationship between CPA and MMS was weaker. Adding covariates to the model, including chronic pain and depression, had minimal impact on the relationships. There was no significant interaction effect with chronic pain.

For CPT, the association with immediate recall and coding tests is a replication of findings from the previous wave of the Tromsø Study (162), showing that this is a consistent association. The consistency of this association is in itself a valuable contribution, given the poor reproducibility currently discussed in many cognitive and social psychology studies (219). While the previous study (162) found age group differences in the association, this pattern could not be replicated in the present study, indicating that age-group differences are less consistent than the overall association, and might even reflect type I error in the previous study. The addition of MMS-E indicates that the association is also consistent across another cognitive test.

The similar association we found with CPA show that the relationship is also consistent across another pain modality. Given that the association between cognitive function and pain tolerance is likely to be more related to the cognitive dimension of pain processing, it fits well that change of nociceptive stimulus has little impact on the relationship. The only other study that have published results on relationship between CPA and cognitive testing found no association with score on the cognitive task (Stroop interference task) (161).

Adding chronic pain or depression to the model had minimal impact on the relationship and there was no interaction effect of chronic pain, indicating that our findings are not explained by an effect of chronic pain on pain sensitivity. We used a broad definition of chronic pain, and it cannot be excluded that the relationship may differ across pain conditions or depend on the severity or distribution of pain. However, it is also possible that reduced cognitive

function is a risk factor for pain rather than or in addition to a possible consequence of it. This fits well with findings that cognitive function at baseline predicted chronic pain after surgery (even in patients who had no pain before surgery) and development of chronic pain in a community-based cohort (220, 221).

While the cognitive tests in our study are broad and hence not appropriate for study of the relationship between pain tolerance and specific cognitive functions, tests of general cognitive deficit might be well suited for identifying populations at risk.

5.2.3 Stroke and pain tolerance

In paper III, we found that previous stroke was associated with decreased CPT tolerance time. The association was similar in participants with and without chronic pain. These findings fit well with previous research, where several studies have shown focal (164-166) and widespread (164-166, 168, 169) alterations in pain sensitivity after stroke, and add to them by showing the association is present in a large, population-based sample.

As the stroke registries do not contain information on stroke lesion location, we cannot assess whether or in how many cases this association is related to alterations in somatosensory function corresponding to stroke lesion deficits, which is a major limitation to this study. If participants reported sensory or motor dysfunction that could entail risk or interference with the test, they were tested on the other hand or excluded, and hence most, if not all, participants are likely to have been tested on a non-affected hand. Considering that we used a tolerance measure, it is also likely this is influenced by altered affective-motivational and cognitive processing rather than just focal somatosensory deficits.

Previous studies using threshold assessments have somewhat heterogenous findings regarding presence and direction of association, while reports on suprathreshold assessments with intensity ratings and CPT tolerance time indicate higher pain sensitivity in stroke patients. In PSSP patients, thresholds were found to be lower in both the contralesional (164-166) and ipsilesional (164, 165) side, but with somewhat different findings with regard to presence and direction of effect for different pain modalities (164). In CPSP patients, thresholds are found to be lower (167, 169) or higher (168) on the ipsilesional side. Intensity ratings were higher in CPSP patients (168, 169) as well as in pain-free stroke controls (168) in the affected (169) and unaffected (168, 169) sides. In 30 pain-free patients with cerebellar stroke, it was found that they had higher intensity ratings in response to heat and pinprick stimuli than healthy

controls while there was no significant difference in heat and pressure thresholds (170). CPT tolerance time is reported by two PSSP studies who used it as a conditioning stimulus in a CPM procedure, applied to the unaffected side. Both found that PSSP patients had lower CPT tolerance time compared to pain-free stroke controls (164, 166) and healthy, stroke-free controls (164). In the latter study, descriptive data suggest pain-free stroke controls had shorter pain tolerance than healthy controls, but statistical test of this difference is not reported (164).

One consequence of the widespread nature of pain processing in the brain is that if one region is affected by a lesion, large parts of the system are still intact, which entails a system that is highly resilient (18). With few exceptions, focal lesions generally do not eliminate the ability to experience pain. Considering the warning signal function of pain, this is beneficial. Meanwhile, a distributed system with a high degree of interconnectivity across multiple anatomical, physiological and temporal scales might be vulnerable to disruption. A lesion affecting the function of one region might have effects on other regions and the network as a whole, that might be difficult to predict (19). Emerging evidence is showing that a localized stroke lesion, in addition to causing focal deficits and disconnection syndromes related to the specific function of affected gray and white matter, might have widespread and global effects on the brain and its networks, including degeneration and atrophy (222). A longitudinal study showed that greater atrophy was associated with cognitive decline (223). Given the widespread nature of pain processing and its physiological overlap with cognitive function, it seems feasible that a similar pattern pertains to effects of stroke and subsequent atrophy might impact on pain processing over time. This fits with reports of increasing prevalence of pain as time passes after the stroke (224). As the Tromsø Study Cardiovascular Disease Register only registered first-ever strokes of each subtype, and the risk of recurrent stroke within 10 years has been found to be as high as 39.2% (225), we were not able to assess whether time since stroke was related to pain tolerance.

In PSSP and CPSP patients, it cannot be disentangled whether hypersensitivity is due to the pain or due to the stroke. One study report decreased pain thresholds in patients with sensory deficits but no pain (169). Another study which specifically assessed pain sensitivity in pain-free stroke patients, reported that cerebellar stroke patients had higher intensity ratings in response to heat and pinprick stimuli compared to healthy controls (170).

In our study, stratified analyses showed similar results for participants with and without chronic pain. In both groups, stroke survivors had shorter CPT tolerance time than participants without stroke. While it is possible that the relationship can differ across types, distribution and/or severity of chronic pain, this finding suggests that lower pain tolerance is not dependent of chronic pain. Although we do not have specific information on post-stroke pain conditions, the broad definition of chronic pain in our study ensures that patients with these conditions, regardless of severity, have been excluded from the group without chronic pain. This means that stroke survivors might be more sensitive to pain or have less ability to cope with it, even in the absence of chronic pain. If so, it is possible that increased pain sensitivity could be a risk factor or mediator of development of chronic pain after stroke. It is well described that patients who have had a stroke commonly report pain, both in the early (224, 226, 227) and later stages (224, 228). While this includes post-stroke pain conditions such as PSSP and CPSP, there are also several reports of pain considered not to be stroke-related as it is distributed in the unaffected side (229, 230) or for other reasons (224, 226, 230, 231). With respect to PSSP and CPSP, overlap in the features of these conditions have led to suggestions of shared pathophysiology (232). Increased pain sensitivity due to stroke could be an explanation for these observations.

5.2.4 The relationship between the status of the brain and pain tolerance

In papers I and II we found that higher GMV and cognitive function are associated with higher pain tolerance, while in paper I we found that cerebral stroke is associated with lower pain tolerance. Taken together, it can be said that our findings indicate that a healthy brain is associated with higher pain tolerance, and correspondingly: that risk factors or diseases affecting brain health could entail higher pain sensitivity or lower ability to tolerate it.

Our findings fit well with those of previous research and add to current body of evidence by showing these associations in a large, population-based sample, which increases power and is likely to show robust findings. Hence, it is a contribution to greater knowledge on the neurobiological underpinnings of differences in pain experienced in response to the same stimulus. Moreover, demonstrating these associations in a general population indicate that these factors might be relevant in identifying populations at risk for pain.

The use of tolerance measure enabled us to show an association with an assessment that is likely to involve all dimensions of the pain experience and to induce activation of modulatory mechanisms. What we measure when we measure pain tolerance is likely a function of the

individual pain sensitivity, i.e., the amount of pain the person feels in response to the stimulus, the motivation and the ability to tolerate it. It has been suggested that the multidimensional nature of tolerance assessments entail that such tests might be better reflections of clinical pain than other pain assessments (89).

As chronic pain is known to be associated with pain sensitivity as well as our main independent variables (GMV, cognitive function and stroke), this could have been expected to be a confounder (or in paper III, a collider) explaining or contributing to the associations. However, controlling for chronic pain by adjustment and testing for interaction (in papers I and II) or stratification (in paper III) had little impact on results. While it is possible that this might differ across type, distribution and severity of chronic pain, our results suggest a relationship between pain sensitivity and the brain that is not a result of chronic pain. This entails that the status of the brain – due to genetic, environmental and behavioral factors as well as diseases – could be a neurobiological underpinning for variation in pain sensitivity or ability to cope with it.

5.2.5 Implications

The findings from our studies are relevant with regards to identifying populations at risk of pain. There is an ongoing search for biomarkers of pain. While there are several practical and ethical considerations that need to be considered (233) especially with respect to the use of a "biomarker" to decide whether an individual is in pain, biomarkers used for the purpose of risk assessment and treatment stratification would be highly valuable. While functional imaging has been invaluable in gaining knowledge on pain processing in the brain, it is very resource-intensive and impractical for extensive clinical use. Structural MRI has broader applicability. In the clinic, stroke status and cognitive function is usually known or can easily be obtained.

It is likely that individuals with dementia and larger strokes are underrepresented in our study. However, it is possible that the associations we find are present, and may even be larger, also in these groups. This entails patients with dementia or stroke might be more sensitive to pain in clinical settings or have lower ability to cope with it. This is supported by previous research. There is evidence for altered, mostly increased, sensitivity to experimental pain in the elderly and in patients with neurodegenerative diseases (234). Moreover, self-reported pain is associated with subsequent development of dementia (235, 236), with stronger association seen closer to time of diagnosis (236) suggesting that pain could be a symptom in

the phase of preclinical brain disease. This has important clinical relevance, especially considering these patient groups have considerable comorbidity, might have difficulty expressing pain if they do experience it in the setting of injuries, diseases and procedures, and are vulnerable to side effects of medication. This calls for awareness in health professionals to provide timely and well considered management.

Increasing knowledge and understanding of pain physiology, and the underpinnings of its complexity and differences, is valuable in encounters with all pain patients. It enables awareness, understanding, validation, communication and patient education opportunities, and underscores the need for multidimensional treatment opportunities (18, 20).

5.3 Importance and relevance for public health

The corroboration of a relationship between pain tolerance and GMV, cognitive function and cerebral stroke in a general population is important as it is a contribution towards identifying populations at risk of pain, which is a significant burden to the individuals affected as well as costs for society (237). In addition to increased awareness when facing patient groups, knowledge on the underpinnings of differences in pain sensitivity might contribute to improvements in treatment and prevention.

6 Conclusions, implications, and future perspectives

We found an association between pain tolerance and GMV, cognitive test scores and having a history of cerebral stroke, in a general population. Larger total and regional GMV and higher scores on cognitive testing were associated with decreased risk of hand withdrawal, i.e., higher pain tolerance, while having a history of stroke was associated with increased risk of hand withdrawal, i.e., lower pain tolerance. These findings suggest brain health is important for pain tolerance, which is likely to be influenced by sensitivity to painful stimuli and the ability to tolerate them.

While it is possible that the role of chronic pain in the relationship between pain and the brain might depend on the type and severity of chronic pain, our findings suggest that there is a relationship between pain tolerance and brain health that does not depend on chronic pain.

Our findings have possible clinical implications. Variation in the status of the brain could be a contributing factor to individual differences in sensitivity to painful stimuli. Increased pain sensitivity might be a part of the explanation of the relationship between baseline brain characteristics and cognitive function and subsequent development of chronic pain shown by others.

Individuals with stroke and other brain diseases which are related to loss of GMV and cognitive function, such as Alzheimer's disease, could be more sensitive to or have more difficulty coping with pain when exposed to injuries, diseases, or medical procedures. Knowledge on this relationship can increase the ability of health professionals to recognize and respond to pain in these patients. Increased understanding may per se lead to improvement in the treatment of pain patients in general, because of increased awareness as well as improved validation and communication.

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Paper 1

Gray matter volume and pain tolerance in a general population: the Tromsø Study

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Gray matter volume and pain tolerance in a general population: The Tromsø Study

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ABSTRACT

As pain is processed by an extensive network of brain regions, the structural status of the brain may affect pain perception. We aimed to study the association between gray matter volume (GMV) and pain sensitivity in a general population. We used data from 1522 participants in the 7th wave of the Tromsø study, who had completed the cold pressor test (3℃, maximum time 120s), undergone MRI of the brain, and had complete information on covariates. Cox proportional hazards regression models were fitted with time to hand withdrawal from cold exposure as outcome. GMV was the independent variable and analyses were adjusted for intracranial volume, age, sex, education level and cardiovascular risk factors. Additional adjustment was made for chronic pain and depression in subsamples with available information on the respective item. FreeSurfer was used to estimate vertex-wise cortical and subcortical gray matter volumes from the T1-weighted MR image. Post-hoc analyses were performed on cortical and subcortical volume estimates. Standardized total GMV was associated with risk of hand withdrawal (Hazard Ratio (HR) 0.81, 95% confidence interval (CI) 0.71 to 0.93). The effect remained significant after additional adjustment for chronic pain (HR 0.84, 95% CI 0.72 – 0.97) or depression (HR 0.82, 95% CI 0.71 – 0.94). In post-hoc analyses, positive associations between standardized GMV and pain tolerance were seen in most brain regions, with larger effect sizes in regions previously shown to be associated with pain. In conclusion, our findings indicate that larger GMV is associated with longer pain tolerance in the general population.

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INTRODUCTION

As pain is processed by a network of brain regions(1-5), it would be expected that the structural and functional status of the brain is an important contributor to the considerable individual differences in pain experienced in response to the same disease or stimulus(6). In most clinical pain conditions, the effect of central nervous system processing on pain is conflated with the severity of the pathology causing it, and cannot be quantified. However, studies applying controlled experimental pain stimuli have revealed considerable differences in pain sensitivity in the general population(6), and that these have clinical implications: lower sensitivity has been linked to increased risk of unrecognized myocardial infarction(7), while higher sensitivity is associated with several chronic pain conditions(8-10), analgesic use(11), postoperative pain(12) and predicted non-recovery after acute whiplash(13).

Previous research on the relationship between gray matter and pain sensitivity has shown heterogeneous findings with regard to the presence of an effect, its direction, and in which brain regions it is found(14-26). These inconsistencies may be due to small sample sizes, but also to methodological differences in pain assessments and the complex nature of pain physiology. For example, many studies assessed pain by low-intensity threshold stimuli(14-21), which are by definition the lowest stimulus intensity that is perceived as painful. These are unlikely to activate the full range of pain processing and modulating mechanisms in the same ways as stimuli of higher intensity and longer duration.

Reports on association between total GMV and pain sensitivity are scarce, as previous studies have focused on brain regions, in line with earlier functional neuroimaging studies aimed at identifying regions participating in pain processing, conceptualized as the pain matrix(1, 2). However, evolving knowledge has made it clear that the pain matrix cannot be unequivocally defined(3, 4). Increased efforts have been directed at investigating how functional connectivity and network properties of the brain affect pain perception(16, 27, 28), accentuating the importance of the brain as a whole. It is increasingly clear that fundamental for the physiology of pain processing is its distributed properties(5). The brain shows morphological variations due to a number of factors, including lifestyle, ageing and brain diseases, some of which affect the brain on a regional and some on a global level.

A relationship between total and regional gray matter and pain sensitivity would be expected to have implications for the incidence, severity and persistence of pain, with potential clinical importance in the general population and notably in patients with degenerative brain diseases. Consequently, we aimed to study the relationship between total and regional gray matter volume (GMV) and pain sensitivity assessed with the cold pressor test (CPT), a test of pain tolerance, in a large population-based sample.

METHODS

Study design and participants

The Tromsø Study is a multi-purpose health study that has been carried out repeatedly in the municipality of Tromsø, Norway since 1974(29). The study design is of repeated cross-sectional health surveys to which whole birth cohorts and selected groups are invited. The seventh survey, conducted in 2015-2016, consisted of two visits. All inhabitants in the Tromsø municipality over the age of 40 (n=32 591) were invited to participate in the first visit, and 21 083 (64.7%) attended. A total of 9253 first-visit participants were invited to a second visit which included extended clinical examinations, and 8346 (90%) attended. From this subsample, we invited 2973 participants to take part in an MRI study of the brain which took place in 2016-2017. Of these, 921 declined, 169 were excluded because of contraindications for MRI, and five were excluded because they had moved or died before the MRI examination. Subjects were excluded if imaging revealed abnormalities that would interfere with volume estimations, i.e. (large) intracranial cysts, tumors or strokes. Of the 1878 subjects who underwent MRI scanning, we included 1522 subjects aged 40-84 years who had sufficient MRI image quality, had completed CPT, and where without missing information on covariates (Supplementary Figure 1). As the design of the Tromsø study is adapted to collect a comprehensive amount of health-related data, some of these are assessed at different visits. As for variables used in our study, CPT and information on all covariates was assessed at the first visit, while MRI was done on a separate visit at a later time.

Ethics

The Tromsø study, the MRI study and the present study were approved by the Regional Committee for Medical and Health Research Ethics (2014/940/REK Nord, 2014/1665/REK Nord and 2017/1951/REK Nord, respectively). All participants signed written informed consent before participating in the study.

Measurements and variables

Participants completed questionnaires which provided information on level of education, smoking habits, current or previous diabetes and hypertension, and use of medication for diabetes or hypertension (yes/no/previously). Chronic pain was assessed by the question “Do you have persistent or recurrent pain that has lasted for 3 months or more?” (yes/no). Weight and height were measured with light clothing and no shoes. Body mass index (BMI) was calculated using the formula weight/height^2 (kg/m^2). Blood pressure was measured three times with the participant seated, and the mean of the last two measurements was used. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol were analyzed by standard enzymatic colorimetric methods and HbA1c with high

performance liquid chromatography.

Mental health was assessed with SCL-10, and depression was defined as SCL-10 average above 1.85(30).

Smoking was defined as self-reported current daily smoking. Hypertension was defined as self-reported current hypertension and/or use of antihypertensive medication and/or systolic blood pressure above 140 and/or diastolic blood pressure above 90. Diabetes was defined as self-reported current diabetes and/or use of anti-diabetic medication and/or HbA1c above 6.5%. If information on one of the criteria constituting the definition of hypertension or diabetes was missing, the definition was based on available data. If all criteria were missing the covariate was set to missing.

Participants with missing information on education, hypertension, diabetes, cholesterol, HDL-cholesterol, BMI or smoking were excluded from all analyses.

Cold pressor test (CPT)

CPT tolerance time was tested by asking the participants to submerge their non-dominant hand and wrist in a thirteen-liter vat containing circulating 3°C water and keep it there as long as they were able, up to a maximum of 120 seconds. A constant water temperature was ensured by continuous exchange between the vat and a circulating cooler (FP40-HE, Julabo GmbH Germany). Participants were excluded from CPT testing if they declined to perform the test, had problems comprehending the instructions, reported medical issues that in their experience affected their response to cold (e.g. cold allergy, Raynaud`s syndrome, loss of sensitivity in both hands), or had open sores or eczema on both hands. For descriptive purposes, participants were categorized as pain tolerant if they could endure the full 120 seconds of CPT and as pain sensitive if they withdrew the hand from the water earlier than that.

The stability of CPT was assessed in a subsample of Tromsø 7 participants (n= 273, mean time between first and second CPT 277 days). The intraclass correlation coefficient of the two measurements was 0.837, indicating that pain tolerance assessed by CPT is relatively stable. Good test-retest stability of CPT has also been reported previously(31).

Magnetic resonance imaging

Participants were scanned at the University Hospital of North Norway with the same 3T Siemens Skyra MR scanner (Siemens Healthcare, Erlangen, Germany). The scan protocol included T1-weighted, T2-weighted FLAIR (fluid-attenuated inversion recovery sequence), time-of-flight

angiography and susceptibility weighted series with a total scan time of approximately 22 minutes. In this study, we used only the T1 images. Key parameters for the T1 3D magnetization prepared rapid acquisition gradient-echo sequence: flip angle 9°, time to repetition/echo time/time to inversion = 2300/4.21 /996 ms, parallel acceleration factor 2. The images were acquired sagittally, with a field of view = 256 mm, 256 x 256 image matrix, 176 slices, 1 mm slice thickness, and 1 mm isotropic reconstructed resolution.

We used FreeSurfer (v.6.0, http://surfer.nmr.mgh.harvard.edu) to estimate intracranial volume (ICV) and cortical volumes (total and in different brain regions) from the T1-weighted images. Briefly, ICV was derived from the affine transformation of the T1-weighted image to the Talairach template(32). Subcortical GMV was estimated with the segmentation method described by Fischl(33), while cortical GMV were estimated by skull stripping, segmentation of brain tissue, and placement of the gray/white and gray/cerebrospinal fluid borders(33, 34). Note that in the subcortical segmentation, the ventral diencephalon region segmented with FreeSurfer includes the hypothalamus with mammillary bodies, the subthalamic, lateral geniculate, medial geniculate and red nuclei, substantia nigra, and surrounding white matter(35, 36). This differs from standard anatomical nomenclature where the substantia nigra and red nuclei are located to the mesencephalon.

Statistical analyses

For descriptive purposes, participants were grouped as pain tolerant if they could endure the full 120 seconds of CTP, and as pain sensitive if not. Continuous variables are presented as means with standard deviations and categorical variables as numbers and percentages. Group differences were evaluated with t-tests for continuous variables and Chi square tests for categorical variables. Risk of hand withdrawal across quartiles of GMV was visualized by Kaplan-Meier curve.

In analysis of the association between GMV and pain tolerance, cold pressor pain tolerance time was used as a continuous variable. Because this variable is right-censored due to the maximum time limit, Cox proportional hazards models were fitted, with time in water bath as the time variable and hand withdrawal as event. GMV was standardized by z-transformation. Adjustment for ICV was done by adding it to the model as covariate of no interest. To assess the contribution of potential confounders to the relationship between GMV and pain sensitivity, we included covariates previously identified as possible causes of variance in both GMV and pain, namely age, sex, education level, and the cardiovascular risk factors hypertension, diabetes, cholesterol, HDL cholesterol, BMI and smoking. Potential confounders were added to the model in three steps: first age and sex, then education level, and then the cardiovascular risk factors. Analyses were performed on a sample consisting of participants with complete information on all the above-mentioned covariates. Additionally, chronic pain and depression were added to the full model in subsamples (n=1376 and 1460 respectively)

where information on the item was available. Interactions between GMV and age (dichotomized above/below mean), sex and chronic pain (subsample) were assessed by adding interaction terms to the model (the respective variable multiplied with standardized GMV). Interaction terms that did not reach statistical significance were excluded from the final models. Graphical check of the proportional hazards (PH) assumption confirmed overlapping observed versus expected survival plots and parallel log-log survival curves, while statistic test of scaled Schoenfeld residuals confirmed that the PH assumption was met for the relationship between standardized GMV and CPT tolerance time (Chi-square 1.17, p=0.280).

Explorative analyses were performed to examine the relationship between CPT and regional GMV. For subcortical GVM, we used the FreeSurfer segmentation volumes, i.e., accumbens area, amygdala, caudate, hippocampus, pallidum, putamen, thalamus, and ventral diencephalon. For cortical volumes, we used the vertex-wise cortical volume measurements from FreeSurfer that were smoothed with a 20 mm filter-width-half-maximum Gaussian kernel. The fully-adjusted Cox regression model was used to examine the association between the CPT time and the volume measurements. This was done in R for the subcortical measures, while a vertex-wise Cox regression model(37)was used on the cortical volume estimates. P-values were FDR-corrected across both hemispheres for both analyses.

Statistical analyses and visualizations were performed in STATA (version 16.1 for windows (StataCorp LLC, Texas, USA) and R (ver. 4.1.1.) together with the fsbrain (ver. 0.5.3) package.

Data availability

Data availability is restricted due to their sensitive nature. De-identified data can be obtained by application to the Tromsø study. Contact tromsous@uit.no for details.

RESULTS

Of the 1522 participants, 612 (40.2%) were pain tolerant and endured the full 120s, while 910 (59.8%) were pain sensitive and withdrew their hand from the cold water before the time limit (Figure 1). There were more women among the pain sensitive participants, education levels were lower and a higher proportion had diabetes (Table 1). There were no significant differences in age, cholesterol, HDL cholesterol, BMI, hypertension, current smoking, or in time interval from pain assessment to MRI. In subsamples with available information, there was no significant difference in presence of chronic pain or depression between the pain tolerant and pain sensitive groups.

Kaplan-Meier curve of CPT tolerance time according to quartiles of total GMV showed that participants in the lowest quartile of GMV had shorter CPT tolerance time while those in the highest quartile of GMV had longer CPT tolerance time (Figure 2). Results from the primary analysis are

presented in Table 2 (for effect sizes and statistical values for the covariates, see Supplementary Table 1). In the multivariable adjusted model, using CPT tolerance time and GMV as continuous variables, higher total GMV was associated with decreased risk of hand withdrawal (HR 0.81, 95% CI 0.71 – 0.93). As GMV was standardized by z-transformation in the analyses, this means that for one standard deviation increase in GMV, hazard of hand withdrawal decreased by 19%. Additional adjustment for the number of days between CPT and MRI assessments had little impact on the results (HR 0.82, 95% CI 0.71 – 0.94, p 0.004). There was no significant interaction between age and GMV. While more women were pain sensitive (Table 1, Supplementary Figure 2), there was no significant interaction effect of sex on the relationship between total GMV and CPT. Subgroup analyses of men and women showed similar results (Supplementary Table 2).

Chronic pain was reported by 467 (33.9%) of the 1376 participants with available information on this item (33.6% of the pain tolerant and 34.2% of the pain sensitive participants). Additional adjustment for chronic pain had negligible impact on effect estimates for the relationship between standardized total GMV and pain tolerance time (HR 0.84, 95% CI 0.72 – 0.97). We found no significant interaction between chronic pain and total GMV. In the subsample with available information on depression (n=1460), additional adjustment for this item produced similar results (HR 0.82, 95% CI 0.71 – 0.94). Likewise, the association remained significant and the effect estimate essentially unchanged (HR 0.84, 95% CI 0.73 – 0.97) when adjustment was made for chronic pain and depression in the subsample with available information on both items (n=1324).

Further exploration of the relationship between CPT tolerance time and GMV in brain regions showed that the association was similar in the right and left hemispheres and in cortical and subcortical regions (Table 3). Vertex-vise analyses showed association between GMV and pain tolerance which remained significant after FDR correction for multiple testing in bilateral insula, bilateral postcentral, bilateral inferior parietal, left fusiform, left rostral anterior cingulate(ACC), right posterior cingulate(PCC), right superior frontal, right precentral, right medial and lateral orbitofrontal (OFC), right pars opercularis and right superior temporal cortices (Table 4, Figure 3).

Vertex-vise analyses of cortical thickness showed similar findings for bilateral superior frontal, left postcentral, left posterior cingulate and right superior temporal cortex, while the effect was in opposite direction for left medial orbitofrontal cortex. Details are presented in Supplementary Table 3 and Supplementary Figure 3.

Results from analyses of subcortical structures are presented in Figure 4. Association that remained significant after FDR-correction for multiple testing was seen in bilateral ventral diencephalon and left accumbens.

There was no significant association between total white matter volume and CPT (Supplementary

Table 4).

DISCUSSION

Our main finding was that total GMV was associated with CPT tolerance time, indicating higher pain sensitivity with smaller GMV. The results remained essentially unchanged by adjustments for putative confounders, including chronic pain and depression. Results were similar for both hemispheres and for cortical and subcortical GMV. Vertex-wise analyses of volume measurements suggest HR below one across most of the cortex, while regions with larger effect estimates and significant association after FDR-correction for multiple testing include bilateral insula, bilateral postcentral, bilateral inferior parietal and right PCC as well as smaller clusters in left rostral ACC and right OFC. The effect size for total GMV was larger than for GMV in any individual clusters, in line with emerging evidence suggesting that the distributed nature is among the core characteristics of the pain processing system(5).

Other studies have assessed the relationship between GMV(14-18, 20, 23, 26), gray matter density(22) or cortical thickness(19, 21, 25) in brain regions and pain sensitivity using a variety of pain assessments. A large study (n= 501) found no association between regional GMV and pressure pain threshold in a population-based healthy sample(15). Results from a sub-study of 839 participants with chronic joint pain from the Rotterdam study showed a significant correlation between heat pain threshold and GMV in certain regions, but only in women, and the direction of effect varied between regions(14). A recent study of pain thresholds and GMV also found that the direction of the association differed across brain regions(16), while others have found smaller(17, 18, 21) or larger(19-21) regional gray matter to be associated with increased pain sensitivity. All of these studies measured pain sensitivity as pain thresholds, i.e. the lowest stimulus intensity that elicits a pain sensation. It is likely that this differs physiologically from supra-threshold stimuli and tolerance assessments. Studies of supra-threshold stimuli(22, 23, 25, 26) and pain tolerance(24) all indicate that less grey matter in a selection of regions is associated with increased sensitivity.

Functional neuroimaging studies have identified a set of regions consistently activated during acute pain, including somatosensory cortices (S1 and S2), insular cortex (IC), anterior cingulate cortex (ACC), prefrontal cortex (PFC) and thalamus(1, 2, 4, 5). Our results from the vertex-wise analyses were mostly consistent with this pattern. Largest effect size was seen in bilateral insula. Less gray matter in the insula has previously been linked to higher pain sensitivity in studies using supra-threshold pain stimuli(23, 25, 26) and pain tolerance assessments(24) but with lower pain sensitivity in a single study (n=39) assessing pain

thresholds(16). The insula is involved in both sensory and affective dimensions of pain and, along with the ACC, it is one of the regions most commonly activated by pain stimuli in functional imaging studies(2, 4). We also found a strong effect estimate in smaller clusters in left ACC, right OFC and bilateral postcentral cortex. The ACC is understood to be involved in affective and cognitive dimensions of pain, while OFC is part of the prefrontal cortex, which has been linked to cognitive dimensions of pain perception(4). The postcentral gyri contain the primary somatosensory cortices (S1), involved in the sensory dimension of pain(1, 2, 4).

Additionally, we found a significant effect in clusters in bilateral inferior parietal and right posterior cingulate cortex. Similar findings were reported by a previous study using suprathreshold pain assessment(22). These areas are part of the default mode network which has also been linked to pain(38).

As a tolerance test, CPT is likely to trigger the affective and motivational aspects of pain perception, hence a strong relationship with brain areas involved in affective processing, such as the insula, ACC and OFC, is expected. In addition to their role in the pain experience, these regions influence descending pain modulation (DPM) of pain transmission through connections to the brainstem and spinal cord. Among the structures with largest effect size in our study is the ventral diencephalon. The resolution in our imaging does not allow for identification of what part(s) of the ventral diencephalon contributes to the effect, but it does include the hypothalamus - also known to be involved in DPM(3). Large effect sizes for regions known to be important in DPM corresponds well with the characteristics of CPT: it is an intense stimulus of relatively long duration, which triggers endogenous pain inhibition, and is therefore often used in conditioned pain modulation research paradigms(39). Likewise, nucleus accumbens have been linked to integration of information and action selection(40), functions likely to be highly relevant in CPT.

Our findings indicate a relationship between GMV and pain tolerance across many regions in the brain, while regions linked to affective pain processing and pain inhibition stand out with larger effect sizes. What appears to be divergent findings in existing research on gray matter and pain sensitivity might be meaningful in light of the complex nature of pain physiology. In addition to the above-mentioned differences between thresholds, supra-threshold and tolerance assessments, the correlation between pain modalities (heat, cold and pressure) is poor(41, 42). Also, pain is heavily influenced by a number of internal and external factors(3). In light of this, it is conceivable that the most important brain regions and even the direction of effect might depend on stimulus modality, outcome measure (e.g. threshold, tolerance, rating), and the individual`s physical and psychological condition.

Chronic pain could constitute one of these individual factors. A number of clinical pain conditions have been associated with increased pain sensitivity in previous studies (8-10, 43), and it was therefore somewhat surprising that this was not found in this study. Most likely, this is related to the broad definition of chronic pain used in questionnaire surveys such as ours. While adjustment for chronic pain had negligible impact on effect estimates in the present study, this might depend on the type and severity of chronic pain. A similar reasoning pertains to the lack of association with symptoms of anxiety and depression.

Though our study is cross-sectional and does not support causal inference, there are good reasons to assume that variation in GMV affects pain sensitivity, rather than the other way around. While we see no plausible mechanism whereby higher pain sensitivity *per se*, in the absence of chronic pain, should affect brain structure, it is reasonable to assume that gray matter variation in brain areas known to be involved in pain perception and inhibition will affect pain sensitivity. The amount of neurons available for recruitment are likely to be relevant for function, and glia cells have also been linked to pain(44). The relationship between brain structure and function in the pain field is not clearly established, but some evidence is emerging(16) and in other fields of cognitive neuroscience there is more substantial evidence that such a relationship exists(45-47). Increased sensitivity to pain due to lower GMV could contribute to risk of developing chronic pain, as suggested by recent studies indicating that brain structural properties may predict transition from acute to chronic pain(48, 49). This is a potential contribution to an ongoing search of biomarkers(50) for use in patient stratification and personalized treatment strategies.

The dominating pattern emerging from our study is that less GMV is associated with increased pain sensitivity. This has important implications for patients with degenerative brain diseases, in whom loss of GMV is often accompanied by inability to adequately communicate pain and who may thus experience severe procedural, acute or chronic pain that goes undetected. Research on pain in this group is challenging and current evidence is inconsistent, but pain perception does seem to be altered in individuals with cognitive impairment, with more evidence suggesting higher pain sensitivity than lower(51). Our findings can contribute to illuminate possible neurobiological underpinnings of altered pain perception in this group.

The main limitation of this study is its cross-sectional design. Though it seems unlikely that higher pain sensitivity, in the absence of chronic pain, would lead to loss of GMV, it is possible that factors that contribute to higher pain sensitivity, such as chronic low-grade inflammation(52) could also affect GMV(53). Nor can other sources of unmeasured confounding be excluded. Another limitation is the time between CPT and assessments of covariates and MRI, though the test-retest reliability of the CPT was high and adjustment for elapsed time between the two examinations did not impact our findings. Furthermore, the MRI resolution and contrast did not permit identification of brain stem nuclei,

which play an important role in descending pain inhibition.

Major strengths include the large population-based sample, use of a pain stimulus which is known to involve multiple dimensions of the pain experience as well as elicit endogenous pain inhibition, and the consistency of findings between brain hemispheres, across regions and with the known atlas of pain-processing brain areas.

Conclusion

Lower GMV is associated with lower pain tolerance in the general population, independent of the presence of chronic pain. This implies that differences in total and regional GMV due to normal variation, ageing or, notably, degenerative brain diseases, could contribute to the incidence, severity and persistence of pain.

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None of the authors have declared any conflicts of interest.

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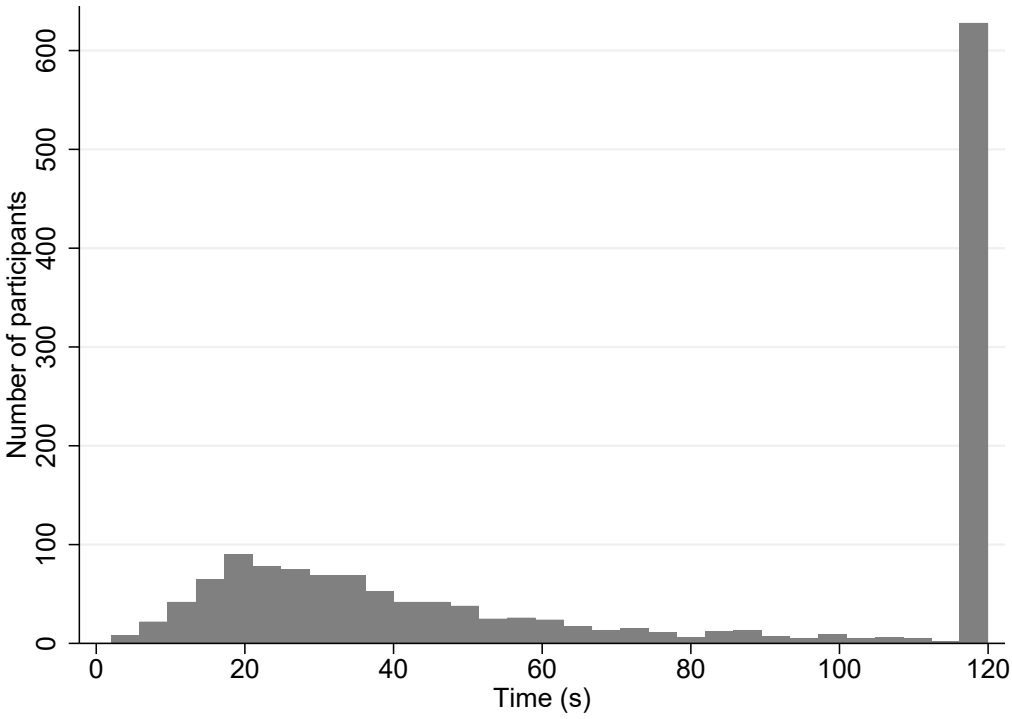
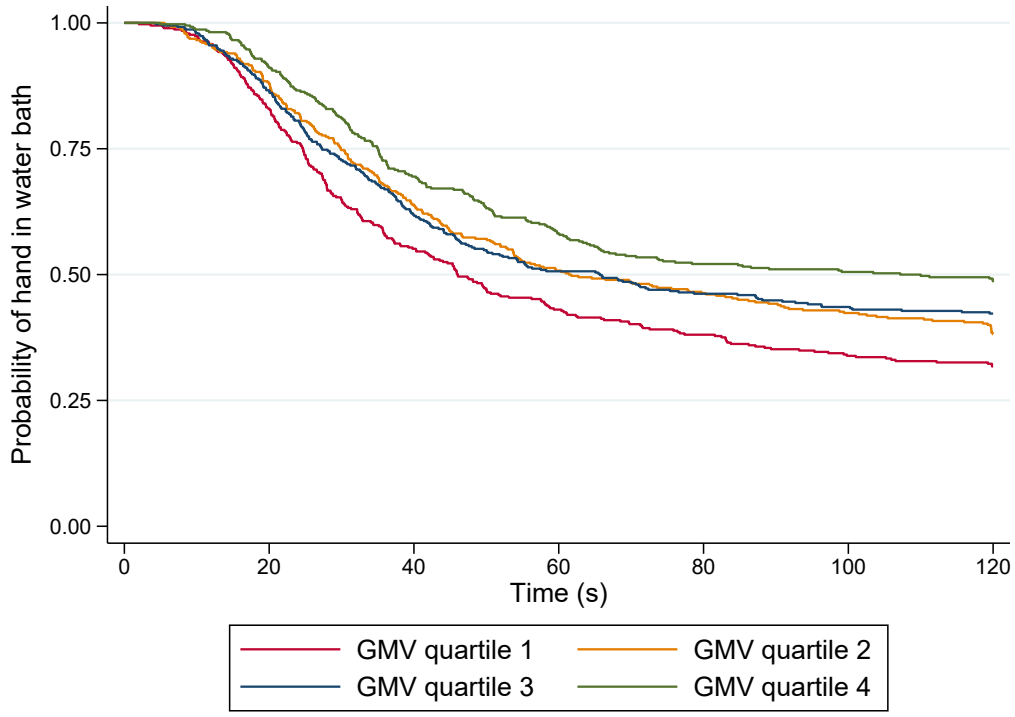
Figure 1: Histogram of CPT tolerance time



The maximum time of the test was 120 seconds.

*CPT: cold pressor test

Figure 2: Kaplan-Meier curve of CPT tolerance time stratified by quartiles of total GMV



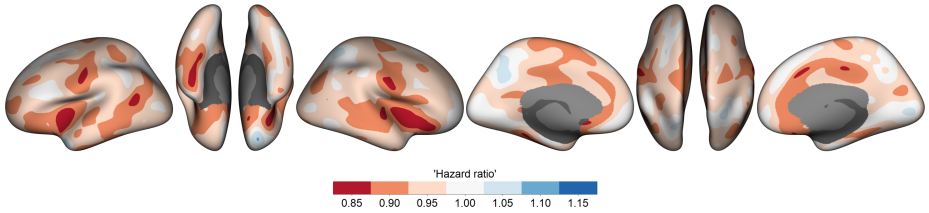
Participants grouped by quartiles of total GMV

*CPT: cold pressor test, GMV: gray matter volume

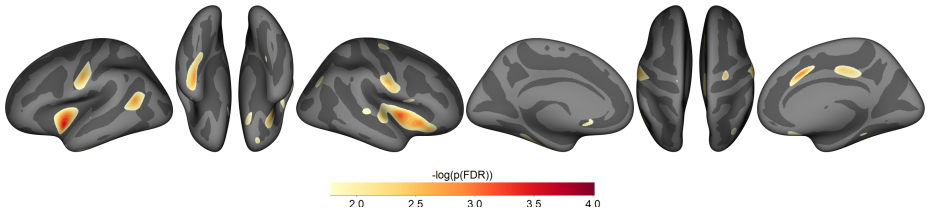
Figure 3: Visual presentation of association between CPT tolerance time and GMV in cortical regions

Figure 3:

3a



3b

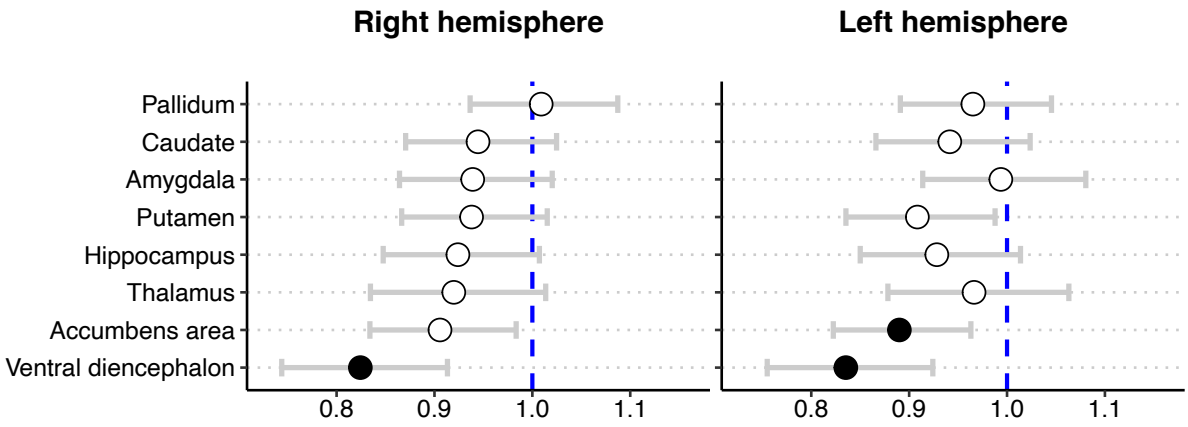


Vertex-wise Cox regression with gray matter volume, standardized by z-transformation, as independent variable and time with hand in cold-water bath as outcome, adjusted for age, sex, intracranial volume, education, hypertension, diabetes, cholesterol, HDL cholesterol, BMI and smoking.

3a: Hazard Ratios across cortical regions. Hazard ratio below one indicates lower hazard of hand withdrawal from cold water bath, i.e. higher pain tolerance, when GMV increases by one standard deviation.

3b: Clusters were the association remain significant after FDR correction for multiple testing. The scale is –log(p). The raw p-value for p(FDR) < 0.05 was p = 0.016 (-log(0.0016)= 1.78).

Figure 4: Forrest plot of hazard ratios in subcortical regions



Analyses are Cox regression with gray matter volume, standardized by z-transformation, as independent variable and time with hand in cold-water bath as outcome, adjusted for age, sex, intracranial volume, education, hypertension, diabetes, cholesterol, HDL cholesterol, BMI and smoking. Black/filled circles indicate significant association after FDR correction for multiple testing.

Table 1. Demographic and clinical characteristics of all participants, and according to pain tolerance*

	All participants n=1522	Pain tolerant n=612 (40.2%)	Pain sensitive n=910 (59.8%)	P value
Age in years, mean ± SD	63.0 ± 10.5	63.0 ± 10.5	63.0 ± 10,5	0.997
Female, n (%)	786 (51.6)	270 (44.1)	516 (56.7)	<0.001
Education, n (%)				0.016
Primary/secondary school, up to 10 years	425 (27.9)	151 (24.7)	274 (30.1)	
Upper secondary, 3 years	430 (28.3)	163 (26.6)	267 (29.3)	
College or university, 1-3 years	313 (20.6)	139 (22.7)	174 (19.12)	
College or university, 4 years or more	354 (23.3)	159 (26.0)	195 (21.4)	
Diabetes, n (%)	109 (7.2)	26 (4.3)	83 (9.1)	<0.001
Hypertension, n (%)	799 (52.5)	321 (52.5)	478 (52.5)	0.977
Total cholesterol in mmol/L , mean ± SD	5.5 ± 1.1	5.5 ± 1.1	5.5 ± 1.1	0.867
HDL cholesterol mmol/L, mean ± SD	1.6 ± 0.5	1.6 ± 0.5	1.6 ± 0.5	0.194
BMI in kg/cm^2 , mean ± SD	27.2 ± 4.1	27.2 ± 3.9	27.2 ± 4.3	0.761
Current smoking, n (%)	194 (12.8)	69 (11.3)	125 (13.7)	0.158
Total gray volume in ml, mean ± SD	617.4 ± 58.1	626.0 ± 57.9	611.6 ± 57.5	<0.001
Days from pain test to MRI, mean ± SD	368.5± 256.5	366.2± 254.9	370± 257.8	0.767
Chronic pain**	467 (30.7)	188 (30.7)	279 (30.7	0.842
Depression***	74 (4.9)	22 (3.6)	52 (5.7)	0.061

*Participants where categorized as pain tolerant if they endured the whole 120 seconds of the cold pressor test, and pain sensitive if they withdrew their hand at an earlier time. P-value is for difference between pain sensitive and pain tolerant group, assessed with t-test for continuous variables and with Pearson chi2 for categorical variables. **Chronic pain: 146 of the 1522 participants in the sample were missing for this variable, 53 of the pain tolerant and 93 of the pain sensitive. ***Depression: 62 of the 1522 participants in the sample were missing for this variable, 26 of the pain tolerant and 36 of the pain sensitive

HDL; high density lipoprotein, BMI; body mass index, MRI; magnetic resonance imaging

Table 2: Cox regression analyses of the association between standardized total GMV and pain tolerance time

	HR	95 % CI	p
Crude (adjusted for ICV as covariate of no interest)	0.87	0.79 – 0.96	0.004
Adjusted for ICV, age and sex	0.79	0.69 – 0.90	0.001
Adjusted for ICV, age, sex and education	0.80	0.70 – 0.91	0.001
Adjusted for ICV, age, sex, education and CVD risk factors*	0.81	0.71 – 0.93	0.003

Analyses are Cox regression with time with hand in cold-water bath as outcome and GMV, standardized by z-transformation, as independent variable. Hazard ratio below one indicates lower hazard of hand withdrawal from cold water bath, i.e. higher pain tolerance, when GMV increases by one standard deviation.

*the CVD risk factors included in the model were hypertension, diabetes, total cholesterol, high density lipoprotein cholesterol, BMI and smoking

GMV: gray matter volume, HR; hazard ratio, ICV; intracranial volume; CVD; cardiovascular disease. HDL; high density lipoprotein, BMI; body mass index, MRI; magnetic resonance imaging

Table 3: Cox regression analyses of the association between pain tolerance and GMV in right and left hemisphere and cortical and subcortical structures

	Right			**Left**			**Sum (right + left)**		
	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
Cortical	0.88	0.78 – 1.00	0.046	0.88	0.77 – 0.99	0.035	0.88	0.77 – 0.99	0.039
Subcortical	0.84	0.76 – 0.94	0.004	0.86	0.77 – 0.96	0.008	0.86	0.75 – 0.95	0.004
Sum	0.86	0.76 – 0.98	0.026	0.86	0.76 – 0.98	0.022	0.86	0.76 – 0.98	0.022

Analyses are Cox regression with gray matter volume, standardized by z-transformation, as independent variable and time with hand in cold-water bath as outcome, adjusted for age, sex, intracranial volume, education, hypertension, diabetes, BMI, smoking, cholesterol and HDL.

GMV; gray matter volume, HR; hazard ratio, CI: Confidence interval

Table 4: Significant clusters for the association between CPT and FreeSurfer cortical volume estimates

Region	Talairach coordinates			Cluster size (mm^2)	p-value	HR	95% CI
	x	y	z				
Left hemisphere							
Insula	-15.2	29.9	-33	648.56	<0.001	0.85	0.78 - 0.93
Fusiform	-13.5	-4.7	-57.3	568.46	0.002	0.88	0.81 - 0.95
Postcentral	-34.9	8.7	22.3	384.76	0.003	0.89	0.82 - 0.96
Inferior parietal	-28.3	-57.8	-2.4	335.87	0.002	0.89	0.82 - 0.96
Rostral anterior cingulate	36.7	42.6	-32.8	15.37	0.014	0.89	0.82 - 0.98
Right hemisphere							
Insula	15.3	23.9	-23.1	1432.05	<0.001	0.87	0.80 - 0.94
Postcentral	39.5	11.8	14.1	326.6	0.003	0.89	0.82 - 0.96
Posterior cingulate	-31.3	-2.2	30	274.96	0.006	0.90	0.83 - 0.97
Superior frontal	-31.7	51.4	21.8	218.97	0.001	0.88	0.82 - 0.95
Precentral	-2.2	6.8	56.4	82.5	0.006	0.91	0.85 - 0.97
Medial orbitofrontal	-24.7	56	-46	65.89	0.010	0.90	0.83 - 0.97
Inferior parietal	10.3	-74.4	20	63.38	0.011	0.91	0.84 - 0.98
Pars opercularis	25.3	35	-8.2	12.6	0.015	0.90	0.83 - 0.98
Lateral orbitofrontal	-12.6	86.1	-43.1	12.43	0.014	1.10	1.02 - 1.19
Superior temporal	38.6	-17.6	-16.7	3.73	0.016	0.91	0.83 - 0.98

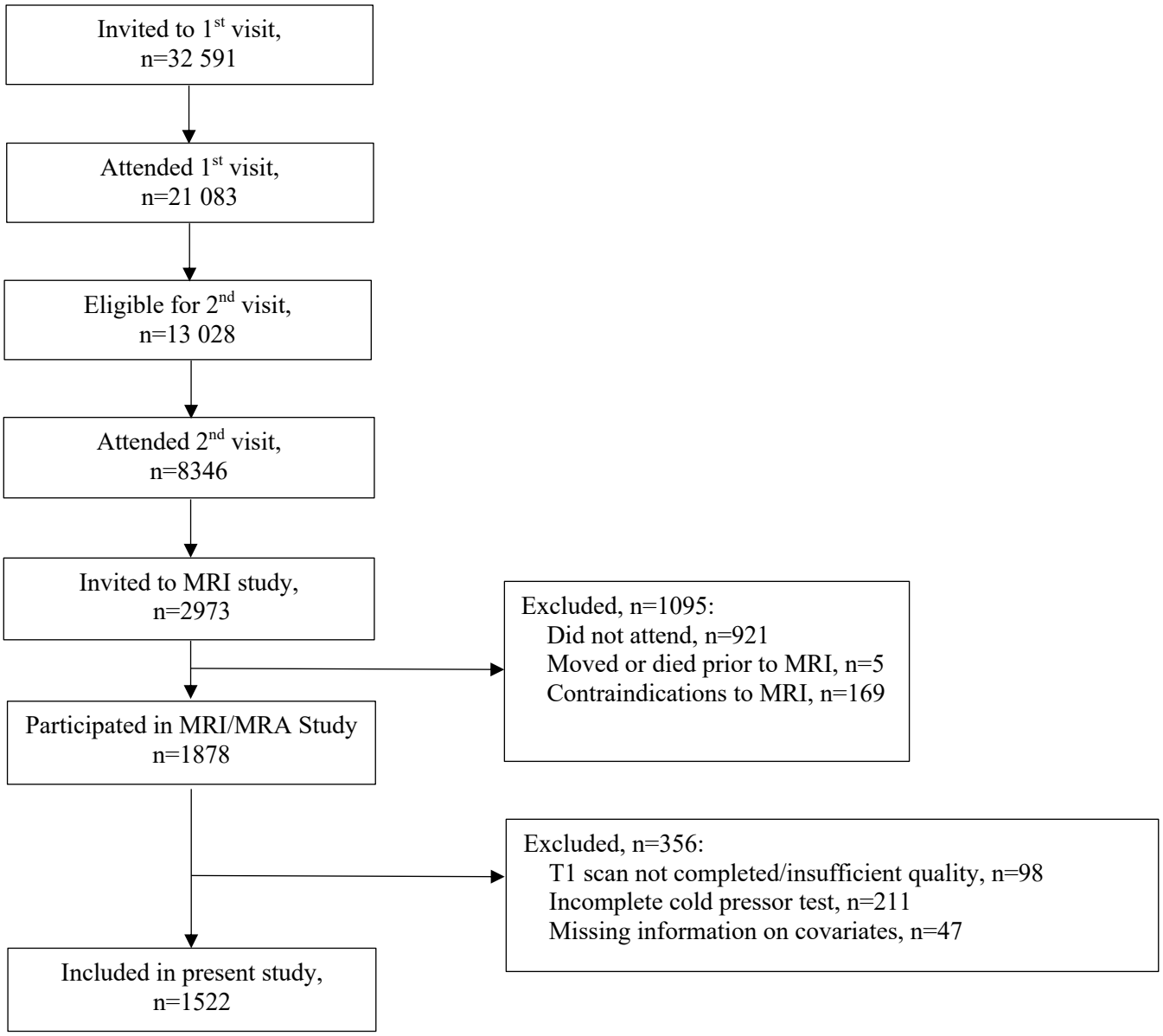
Clusters were the association remain significant after FDR correction for multiple testing. Analyses are Cox regression with time with hand in cold-water bath as outcome and GMV, standardized by z-transformation, as independent variable, adjusted for age, sex, intracranial volume, education, hypertension, diabetes, BMI, smoking, cholesterol and HDL.

Clusters were thresholded with a false discovery rate (FDR) corrected $p < 0.05$, across both hemispheres

The Talairach coordinates are the most significant vertex in the cluster for which the raw p-values are reported

SUPPLEMENTARY

Supplementary Figure 1. Flow chart of participants of the 7th wave of the Tromsø Study and the present study



All inhabitants in the municipality of Tromsø above the age of 40 were invited to part 1 of the 7th wave of the Tromsø study. Before invitation, a subsample of subjects was premarked to be invited to one or more examinations in part 2, if they participated in part 1. This subsample was selected by random (n=9925) plus an additional sample of participants in previous waves (n=3103). Those who were premarked for carotid ultrasound in part 2, and attended part 1 and part 2, were invited to the MRI substudy.

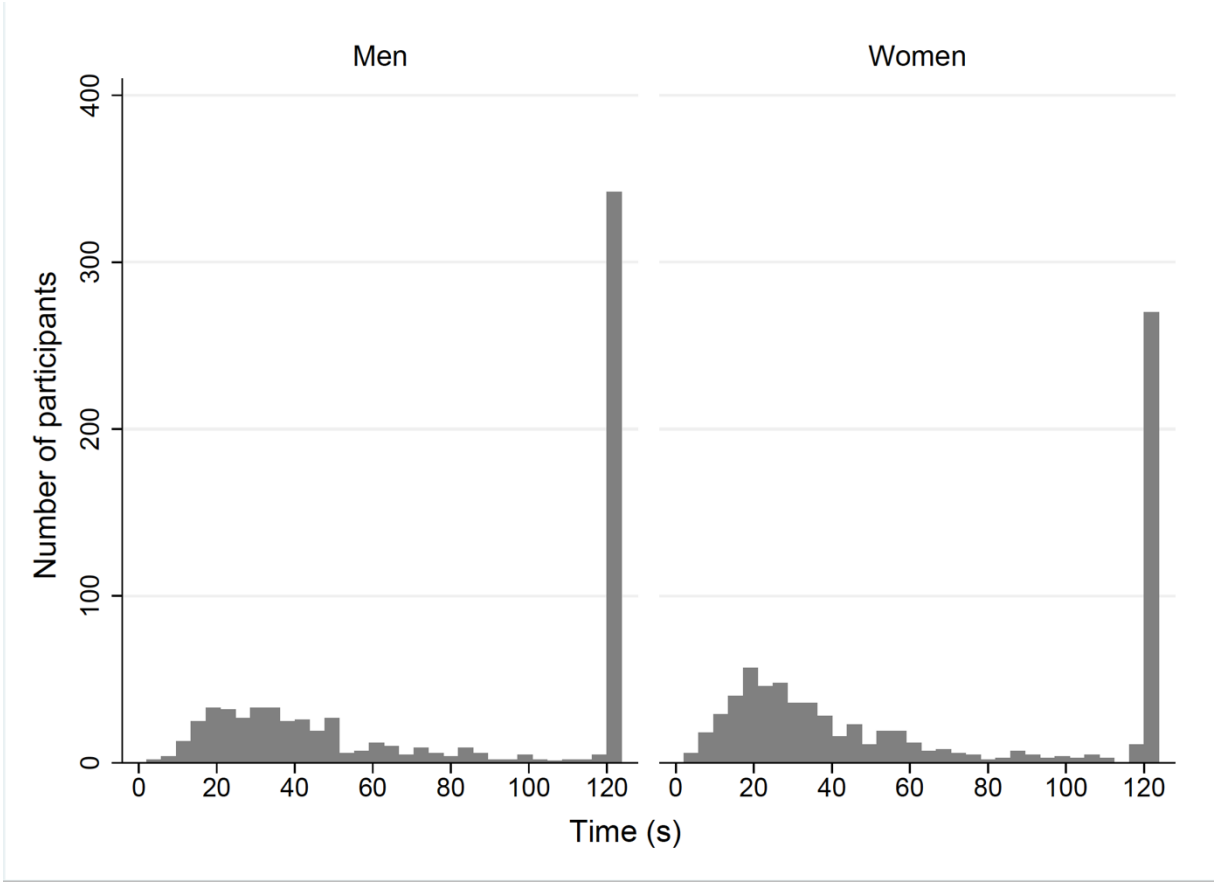
Supplementary Table 1: Cox regression analyses of the association between standardized total GMV and pain tolerance time

		HR	95 % CI	p
Crude (adjusted for ICV as covariate of no interest)				
	Standardized GMV	0.87	0.79 – 0.96	0.004
Adjusted for ICV, age and sex				
	Standardized GMV	0.79	0.69 – 0.90	0.001
	Age	0.99	0.98 – 1.00	0.030
	Female sex	1.33	1.13 – 1.58	0.001
Adjusted for ICV, age, sex and education				
	Standardized GMV	0.80	0.70 – 0.91	0.001
	Age	0.99	0.98 – 1.00	0.012
	Female sex	1.33	1.12 – 1.57	0.001
	Education			
	Upper secondary, 3 years	0.97	0.82 – 1.16	0.760
	College or university, 1-3 years	0.82	0.67 – 1.00	0.047
	College or university, 4 years or more	0.82	0.68 – 1.00	0.047
Adjusted for ICV, age, sex, education and CVD risk factors				
	Standardized GMV	0.81	0.71 – 0.93	0.003
	Age	0.99	0.98 – 1.00	0.087
	Female sex	1.45	1.22 – 1.74	<0.001
	Education			
	Upper secondary, 3 years	0.98	0.83 – 1.17	0.849
	College or university, 1-3 years	0.83	0.68 – 1.01	0.067
	College or university, 4 years or more	0.85	0.70 – 1.04	0.113
	Hypertension	0.91	0.78 – 1.06	0.235
	Diabetes	1.45	1.15 – 1.84	0.002
	Total cholesterol	1.02	0.96 – 1.09	0.504
	HDL cholesterol	0.77	0.66 – 0.90	0.001
	BMI	0.99	0.98 – 1.01	0.560
	Smoking	1.10	0.91 – 1.34	0.321

Analyses are Cox regression with time with hand in cold-water bath as outcome and GMV, standardized by z-transformation, as independent variable. Hazard ratio below one indicates lower hazard of hand withdrawal from cold water bath, i.e. higher pain tolerance, when GMV increases by one standard deviation.

GMV: gray matter volume, HR; hazard ratio, CI: Confidence interval, ICV; intracranial volume; CVD; cardiovascular disease. HDL; high density lipoprotein, BMI; body mass index, MRI; magnetic resonance imaging

Supplementary Figure 2: histogram of CPT tolerance time for men and women



The maximum time of the test was 120 seconds.

*CPT: cold pressor test

Supplementary Table 2: Subgroup analysis of the association between standardized total GMV and pain tolerance time in women and men

SUBGROUP ANALYSIS SEX	MEN (n=736)			WOMEN (n=786)		
	HR	95 % CI	p	HR	95 % CI	p
Crude (adjusted for ICV as covariate of no interest)	0.80	0.69 – 0.91	0.002	0.94	0.82 – 1.07	0.348
Adjusted for ICV and age	0.77	0.64 – 0.94	0.010	0.79	0.66 – 0.96	0.016
Adjusted for ICV, age and education	0.80	0.65 – 0.97	0.023	0.80	0.66 – 0.96	0.020
Adjusted for ICV, age, education and CVD risk factors*	0.85	0.70 – 1.04	0.122	0.80	0.66 – 0.97	0.024

Analyses are Cox regression with total gray matter volume, standardized by z-transformation, as independent variable and time with hand in cold-water bath as outcome,

*CVD risk factors; hypertension, diabetes, BMI, smoking, cholesterol and HDL.

HR; hazard ratio, CI: Confidence interval, CVD: cardiovascular disease.

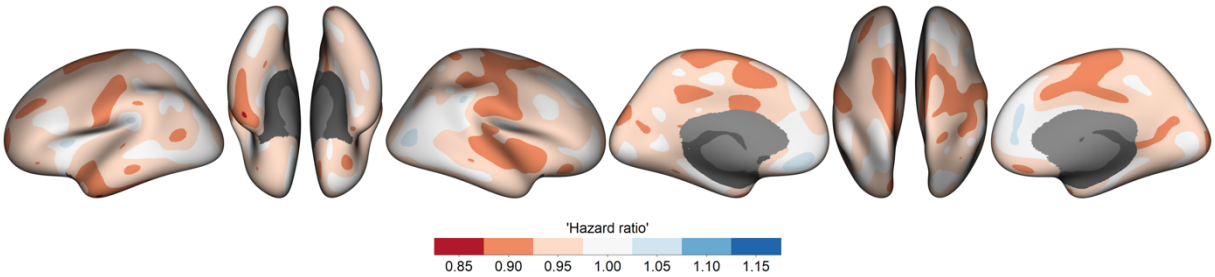
Supplementary Table 3: Significant clusters for the association between CPT and FreeSurfer cortical thickness estimates.

Region	Talairach coordinates			Cluster size (mm^2)	p-value	HR	95% CI
	x	y	z				
Left hemisphere							
Inferior temporal	-22.2	11.2	-60.5	639.38	0.001	0.90	0.84 – 0.96
Paracentral	25.7	-15.1	66	317.25	0.007	0.91	0.85 – 0.97
Superior frontal	14.5	31.1	56	297.92	0.010	0.91	0.85 - 0.98
Superior frontal	30.3	28.5	40.3	200.99	0.010	0.91	0.85 - 0.98
Postcentral	-32.2	7.8	26.3	193.47	0.006	0.90	0.84 - 0.97
Posteriorcingulate	30.4	-25.2	28	137.3	0.008	0.91	0.85 - 0.98
Medial orbitofrontal	32.1	73.7	-36.6	60.83	0.016	1.09	1.02 - 1.16
Rostral middle frontal	-3.6	80.2	11.1	15.8	0.023	0.92	0.86 - 0.99
Right hemisphere							
Precentral	29.3	12.9	27.2	381.72	0.005	0.90	0.84 - 0.97
Paracentral	-30.6	-1.9	58.5	219.99	0.006	0.91	0.85 - 0.97
Superior temporal	35.7	2.9	-26.1	161.03	0.010	0.91	0.84 - 0.98
Precentral	-0.1	7.6	54.9	125.3	0.015	0.92	0.85 - 0.98
Superior parietal	12.5	-28.7	40.4	100.01	0.016	0.92	0.86 - 0.98
Superior frontal	-31.3	48.1	27	92.55	0.012	0.91	0.85 - 0.98
Superior frontal	-30.3	16.2	40.8	75.33	0.018	0.92	0.86 - 0.99
Superior temporal	38.6	-16.6	-17.3	71.73	0.011	0.91	0.85 - 0.98

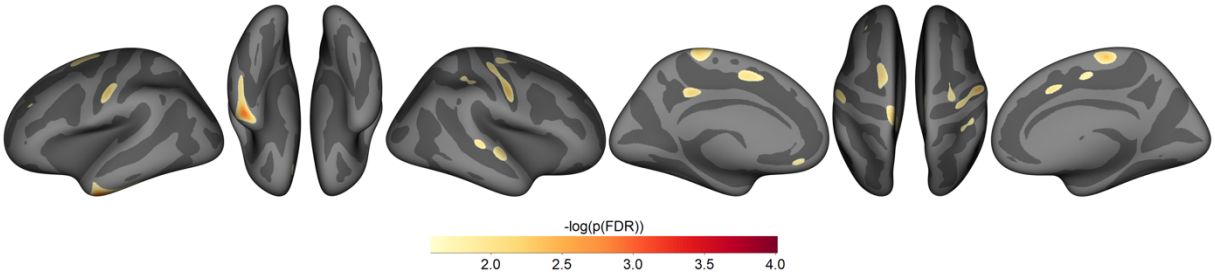
Clusters were thresholded with a false discovery rate (FDR) corrected $p < 0.05$, across both hemispheres. The Talairach coordinates are the most significant vertex in the cluster for which the raw p-values are reported.

Supplementary Figure 3: Visual representation of the association between CPT and cortical thickness

3a



3b



Vertex-wise Cox regression with time with hand in cold-water bath as outcome and cortical thickness, standardized by z-transformation, as independent variable adjusted for age, sex, intracranial volume, education, hypertension, diabetes, cholesterol, HDL cholesterol, BMI and smoking.

3a: Hazard Ratios across cortical regions. Hazard ratio below one indicates lower hazard of hand withdrawal from cold water bath, i.e. higher pain tolerance, when cortical thickness increases by one standard deviation.

3b: Clusters were the association remain significant after FDR correction for multiple testing across both hemispheres (corrected $p < 0.05$). The scale is log(p). The raw p-value for $p(FDR) < 0.05$ was $p = 0.0257$ ($-\log(0.0257) = 1.59$).

Supplementary Table 4: Cox regression analysis of association between CPT and total white matter volume

	HR	95 % CI	p
Crude (adjusted for ICV as covariate of no interest)	0.96	0.87 – 1.06	0.462
Adjusted for ICV, age and sex	0.98	0.88 – 1.10	0.799
Adjusted for ICV, age, sex and education	0.99	0.88 – 1.10	0.815
Adjusted for ICV, age, sex, education and CVD risk factors*	0.99	0.88 – 1.10	0.807

Analyses are Cox regression with total white matter volume, standardized by z-transformation, as independent variable and time with hand in cold-water bath as outcome, adjusted for age, sex, intracranial volume, education, hypertension, diabetes, BMI, smoking, cholesterol and HDL.

CPT: Cold pressor test; HR; hazard ratio, CI: Confidence interval

Paper 2

Associations between cognitive test scores and pain tolerance. The Tromsø Study.

Tonje Anita Melum, Ólöf A. Steingrímsdóttir, Henrik B Jacobsen, Bente Johnsen, Audun Stubhaug, Henrik Schirmer, Ellisiv B. Mathiesen, Christopher S. Nielsen

Submitted

Associations between cognitive test scores and pain tolerance. The Tromsø Study

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ABSTRACT

Objectives: Previous studies have suggested that experimental pain sensitivity is associated with cognitive function. We aimed to assess this relationship in a large population-based sample.

Methods: We included 5753 participants (aged 40-84 years) from the 7th wave of the population-based Tromsø Study who had been examined with cognitive tests and experimental pain assessments, and for whom information on covariates were available. Cox regression models were fitted using standardized scores on cognitive tests (12-word immediate recall test, digit symbol coding test and Mini Mental Status Examination) as the independent variable and cold pressor or cuff pressure pain tolerance as the dependent variables. Statistical adjustment was made for putative confounders, namely age, sex, education, smoking, exercise, systolic blood pressure, body mass index, symptoms indicating anxiety or depression, analgesic use, and chronic pain.

Results: In multivariate analysis, cold pressor tolerance time was significantly associated with test scores on the 12-word immediate recall test (HR 0.93, 95% CI 0.90 – 0.97), the digit symbol coding test (HR 0.94, 95% CI 0.89 – 0.98), and the Mini Mental Status Examination (HR 0.93, 95% CI 0.90 – 0.96). Tolerance to cuff pressure algometry was significantly associated with 12-word immediate recall (HR 0.94 – 0.97) and Digit Symbol Coding test scores (HR 0.93, 95% CI 0.89 – 0.96) while the association with Mini Mental State Examination test score was weaker (HR 0.98, 95% CI 0.95 – 1.00).

Conclusion: Lower pain tolerance was associated with poorer performance on cognitive tests.

Ethical committee number:
The present study: 2017/1951/REK Nord. The Tromsø study: 2014/940/REK Nord,

Keywords: pain, cognition, experimental pain, cold pressor test, cuff pressure algometry, immediate recall test, digit symbol coding test, Mini-Mental State Examination

INTRODUCTION

Pain and cognition are intertwined. They are both processed by a wide network of brain regions, with considerable overlap including in the insula, anterior cingulate and frontal cortices (1). Likewise, several neurotransmitters and receptor systems are involved in the processing of both pain and cognition (1). It is well documented that people with chronic pain perform worse on cognitive tests (1, 2, 3). Proposed explanations of this association include that pain occupies resources in brain regions important for cognitive processing (4), or induces unfavorable neuroplastic changes or release of neurochemical mediators (2) that have adverse consequences for cognitive processing. However, a bidirectional relationship must be considered. Given the shared neuroanatomical and neurochemical underpinnings (1) and the role of cognition in the evaluative component of pain, it is reasonable to hypothesize that variation in brain health and cognitive performance could affect pain perception and modulation. Experimental pain assessments provide a unique opportunity to examine responses to nociceptive stimuli, independent of the presence of chronic pain, providing a critical test of this hypothesis.

A relationship between cognitive test scores and experimental pain assessments has been found by several studies. In a previous study by our group, it was shown that longer pain tolerance, measured by the cold pressor test (CPT), is associated with higher performance on immediate recall and digit symbol coding task (5). Interaction effects and subgroup analyses indicated the effect was stronger in the oldest age groups for immediate recall, while in the youngest and oldest for coding test. Meanwhile, results from other studies (6, 7, 8, 9, 10) are heterogenous, suggesting the association might depend on type of cognitive or experimental pain assessment. While higher tolerance to the CPT was associated with better performance on measurements of cognitive inhibitory control, namely stop-signal (6) and Stroop (7, 8, 9) tasks, association with CPT tolerance was not found with other assessments of executive function (7, 8, 9). A study on pain sensitivity assessed by threshold and tolerance to cuff

pressure algometry (CPA) and thresholds to manual pressure pain found no significant correlation between these measures and stop-signal or Stroop tasks (10).

In the present study, we aimed to expand the tests used in our earlier work to include CPA tolerance as experimental pain method and MMS-E as an additional cognitive test. This allowed for assessment of whether the association is consistent across different pain stimuli (applied to different body parts) and another cognitive test, in addition to test whether our previous findings could be replicated in a new sample.

METHODS

Study design and participants

We included all 5753 participants of the seventh survey of the population-based Tromsø Study, who had completed cognitive testing and experimental pain assessment with CPT and/or CPA tolerance test, and for whom information on covariates were available (Fig 1). Details on design of the seventh wave of the Tromsø study have been published previously (11).

**********INSERT FIGURE 1 APPROXIMATELY HERE**********

Cognitive tests

The cognitive assessments included 3 tests: a) Immediate 12- word recall (12); 12 nouns were shown written on a board and read out loud with 5 second intervals, before the participant was asked to recall as many as possible within 2 minutes (score 0-12 according to number of words recalled), b) the digit symbol coding test also used in the Wechsler Adult Intelligence Scale (13); Nine numbers were paired with nine symbols, and participants were asked to fill in symbols in blank numbered squares using this key, c) Mini-Mental Status Examination (MMS-E), commonly used as a screening tool for dementia (14, 15).

Experimental pain assessment:

In the CPT, the participants were asked to keep their hand and wrist submerged in cold water (3°C) for as long as they were able or until the maximum time (120 seconds). Constant temperature was ensured by continuous exchange between the thirteen-liter cold water vat and a circulating cooler (FP40-HE, Julabo GmbH Germany). Time with hand in water bath was used as measure of CPT tolerance. CPA tolerance was assessed by inflating a blood pressure

cuff around one leg at a time, by 1kPa/s up to a maximum limit of 100 kPa. Inflation and pressure were controlled by a cuff pressure algometry device (NociTech, Aalborg, Denmark). The participant was instructed to press a button to stop the test if the pain became unbearable. Pain tolerance was recorded as kPa at button press (equal to endurance time as the pressure increased by 1 kPa per second) or at the maximum limit, whichever came first. For this study, result from CPA on the non-dominant leg was used as there were fewer missing on this variable than CPA on the dominant leg. Reasons for exclusion included participants decline, inability to comprehend instructions or medical issues that were considered to interfere or put the participant at risk if exposed to cold or pressure to the calf.

Covariates

Information on covariates were obtained from on-site measurements (systolic blood pressure and body mass index (BMI)) or questionnaire (education level, smoking (current, previous or never daily smoking), exercise frequency, symptoms of anxiety or depression measured with the 10-item version of Hopkins Symptom Checklist (HSCL-10) (16), frequency of analgesic use (prescription or non-prescription) and presence of chronic pain (yes or no)) (11).

Statistical analyses

For descriptive purposes, participants were categorized as pain tolerant or pain sensitive according to whether or not they endured the full 120 seconds of CPT. Group differences were evaluated with t-test or Wilcoxon rank-sum test for continuous variables and with Pearson chi2 for categorical variables. In the analyses, CPT and CPA tolerance was used as continuous, right-censored variables. Kaplan-Meier curves were created for visualization of CPT and CPA tolerance according to cognitive test scores (above or below mean for immediate recall and coding test, for MMS-E according to whether score indicated normal (score of 28-30 points), possible cognitive impairment (25-27) or cognitive impairment (≤24) (15). Cox proportional hazards models were fitted for analysis with pain tolerance time to CPT and CPA as time variables (censored at the 120 and 100 seconds maximum times) and test abortion as event. Cognitive test scores, standardized by z-transformation, were used as the independent variable. Putative confounders were added as covariates in three steps: first age, sex, and education (Model 1), then additional adjustment for smoking, exercise, BMI, blood pressure and depression (Model 2) and last additional adjustment for chronic pain and analgesic use (Model 3). Interaction terms were tested for age, sex, and chronic pain by adding the respective variable multiplied with cognitive test score. Sensitivity analysis was

performed by excluding those who had attended cognitive testing and CPT in the previous 6th survey of the Tromsø Study (5), to check whether the association was present in an independent sample.

Analyses were performed in STATA (version 16.1 for windows (StataCorp LLC, Texas, USA)).

RESULTS

A sample of 5387 participants were included for analysis on CPT tolerance time (Fig 1), of whom 1994 (37%) were categorized as pain tolerant and 3393 (63%) as pain sensitive. Median age was 63 years (range 40 – 84) and 51.9% were women. Pain tolerant participants were characterized by a lower proportion of women, fewer current smokers and lower mean BMI, while their education level and exercise frequency were higher, and mean systolic blood pressure was higher (Table 1). A higher proportion of the pain sensitive participants had a HSCL-10 score indicative of anxiety or depression. Pain sensitive participants reported more frequent use of analgesic medication, while the proportions who reported chronic pain were similar in the two groups. The pain tolerant participants had higher mean scores on immediate recall and coding test and for MMS-E the upper limit of the interquartile range was higher in this group indicating a distribution with more participants with higher scores. There was no significant difference in the proportions with possible or definite cognitive impairment according to levels of MMS-E scores.

**********INSERT TABLE 1 APPROXIMATELY HERE**********

Kaplan-Meier curves showing raw data of CPT tolerance time stratified by immediate recall and coding test scores indicated that participants with a score below the mean tended to withdraw their hand from the water at an earlier time (Fig 2). Kaplan-Meier curves of CPT tolerance time according to MMS score group, indicated participants in lower categories of cognitive function showed increased likelihood of hand withdrawal (Fig 2).

*********INSERT FIGURE 2 APPROXIMATELY HERE**********

Multivariable adjusted analysis on the relationship between cognitive test score and CPT tolerance time showed a significant association between pain tolerance time and cognitive test

scores for all three tests (Table 2). Adding covariates to the models had minimal impact on the relationships. For immediate recall, the hazard ratio (HR) was 0.93 (95% confidence interval (CI) 0.90 – 0.97) when adjusting for all covariates. The results for coding test and MMS-E were similar (HR 0.94, 95% CI 0.89 – 0.98 and HR 0.93, 95% CI 0.90 – 0.96, respectively). Sensitivity analysis showed that results were similar when participants who had attended cognitive testing and CPT in the previous 6th survey were excluded (results not shown), indicating presence of association across independent samples.

**********INSERT TABLE 2 APPROXIMATELY HERE**********

For analysis of CPA tolerance, 5576 participants where included (Fig 1), of whom 383 (6.9%) were CPA tolerant and endured the full time of the test, while the majority (n=5193, 93.1%) stopped the test at an earlier time. Kaplan Meier curves of CPA tolerance showed similar patterns as for CPT for immediate recall and coding test, while for MMS the pattern was less clear (Fig 3). Analysis of CPA tolerance showed similar findings as for CPT, with scores of immediate recall and coding test significantly associated with hazard of stopping the CPA test: when adjusting for all covariates HR was 0.94 (95% CI 0.91 – 0.97) for immediate recall and 0.93 (95% CI 0.89 - 0.96) for coding test. The association with MMS-E was weaker, with smaller effect size and borderline significance (HR 0.98, 95% CI 0.95 – 1.00 when adjusting for all covariates) (Table 2).

*********INSERT FIGURE 3 APPROXIMATELY HERE**********

In testing for interaction effects of age, we found a borderline significant interaction effect (p=0.050) on the relationship between immediate recall test and CPT and a significant interaction effect on the relationship between all three cognitive tests and CPA tolerance. Subgroup analysis indicated that the association between immediate recall test and CPT was stronger in the youngest age group (Table 3). For CPA tolerance, subgroup analysis suggested stronger effect in the younger participants for immediate recall and MMS-E, while stronger effect in the oldest age group for coding test (Table 3). We found significant interaction effect of sex on the association between MMS-E and CPT. Analyses on this relationship stratified by sex suggested somewhat stronger effect in men (Table 4). There was no significant interaction effect of chronic pain on the relationship between any of the cognitive test scores and CPT or CPA tolerance time.

**********INSERT TABLES 3 AND 4 APPROXIMATELY HERE**********

DISCUSSION

Our main finding was that pain tolerance for two different experimental pain modalities, applied to hand and leg, was associated with cognitive function across all three cognitive tests. The results are similar to observations from the 6th survey of Tromsø Study on the association between immediate recall test and coding test and CPT (5), indicating consistency across time and samples. While the addition of CPA and MMS provide new information on the association with these tests as well as consistency across pain and cognitive assessments, replication of previous findings is in itself an important contribution: while reproducibility is a cornerstone in science, it has been shown that less than half of original effects were reproduced in a large study on replication of results from cognitive and social psychology studies (17).

The pattern of age group differences in the previous study (5) were not supported in the present. In the previous study there was stronger association between immediate recall test and CPT in the two oldest age groups (5), while in the present the interaction effect was borderline significant and stronger association is suggested in the youngest. Age group differences in association between coding test and CPT where not replicated in the present study. We found interaction effects of age on the relationship between CPA tolerance and cognitive test scores, with heterogeneities regarding what age groups had stronger effects. Interaction effect of sex was only found for the relationship between MMS and CPT tolerance, with small difference between sexes. While it is possible that there is heterogeneity related to differences in cognitive tests and pain assessments and the neurobiological underpinnings of these differences, inconsistent findings regarding the presence of interaction effects and distribution of effect across strata suggest undue emphasis should not be placed on these effects, which may well reflect Type I error.

The main pattern emerging from our study is a consistent association between cognitive test scores and pain tolerance in a general population. These findings suggest that people with lower cognitive performance are less tolerant to pain. Inclusion in the present study required that participants responded to the invitation letter by taking themselves to the venue to attend at the Tromsø study, and participants who did not sufficiently understand the instructions

were excluded from experimental pain assessments. This entails individuals with cognitive impairments are underrepresented in our study, and correspondingly smaller variance within our sample, which could be expected to weaken our results. Meanwhile, it seems likely that the association we find in our sample is also present in these groups. This implies that particular care should be taken by health professionals in treatment of these groups, as pain might be experienced as more intense – or harder to deal with – by these persons.

As chronic pain is associated with impaired cognitive function (1, 2, 3) and with pain sensitivity (18, 19), it follows that chronic pain could be expected to be a confounder on the relationship between cognitive function and pain tolerance. However, adding chronic pain to the model had minimal impact on the effect estimates, and there was no interaction effect of chronic pain on the relationship. While we use a broad definition of chronic pain and it is possible that this depends on type and severity of chronic pain, a plausible interpretation of our findings is that cognitive function, and/or brain conditions affecting cognitive function, affect pain processing. Consistent with this, poorer cognitive performance has been shown to predict chronic pain in population-based and surgical cohorts (20, 21). The association between cognitive test scores and pain tolerance could illuminate a mechanistic underpinning for this relationship. Reduced cognitive function in chronic pain populations may be a cause or risk factor for, rather than or in addition to, a consequence of chronic pain.

Limitations and Strengths

The major strengths of our study are the large sample recruited from a general population, consistency with findings from the previous study, and consistency across pain modalities and cognitive tests. While there are some differences in CTP across the 6^{th} and 7^{th} wave of the Tromsø study, these do not affect the relationship with cognitive test scores. A limitation of our study is that the cognitive tests employed were broad and unsuited for identifying more specific cognitive deficits and hence we are unable to distinguish associations with specific cognitive functions from a more general deficit in cognitive ability.

Conclusion

Cognitive function assessed by immediate recall, coding test and MMS-E is associated with pain tolerance and these associations are independent of the presence of chronic pain. These findings replicate and extend previous findings, and as such appear to be robust. In summary,

there is a consistent association between cognitive test scores and pain tolerance, suggesting that people with poorer performance on cognitive tests are more sensitive to pain.

Ethical statement

Acknowledgements

Research funding

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Author contributions:

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests

Henrik Schirmer: Received a research grant to institution as part of a joint venture research collaboration on heart failure with Novartis, consultant fees from Norvartis and lecture fees from Amgen, Astra Zeneca, Boehringer, and Novartis in person

None of the other authors have declared any competing interests.

Informed consent

All participants signed written informed consent.

Ethical approval

The Tromsø study and the present study were approved the Regional Committee for Medical and Health Research Ethics (2014/940/REK Nord,and 2017/1951/REK Nord, respectively).

Figure 1: Flow chart of participants of the 7th wave of the Tromsø Study and the present study

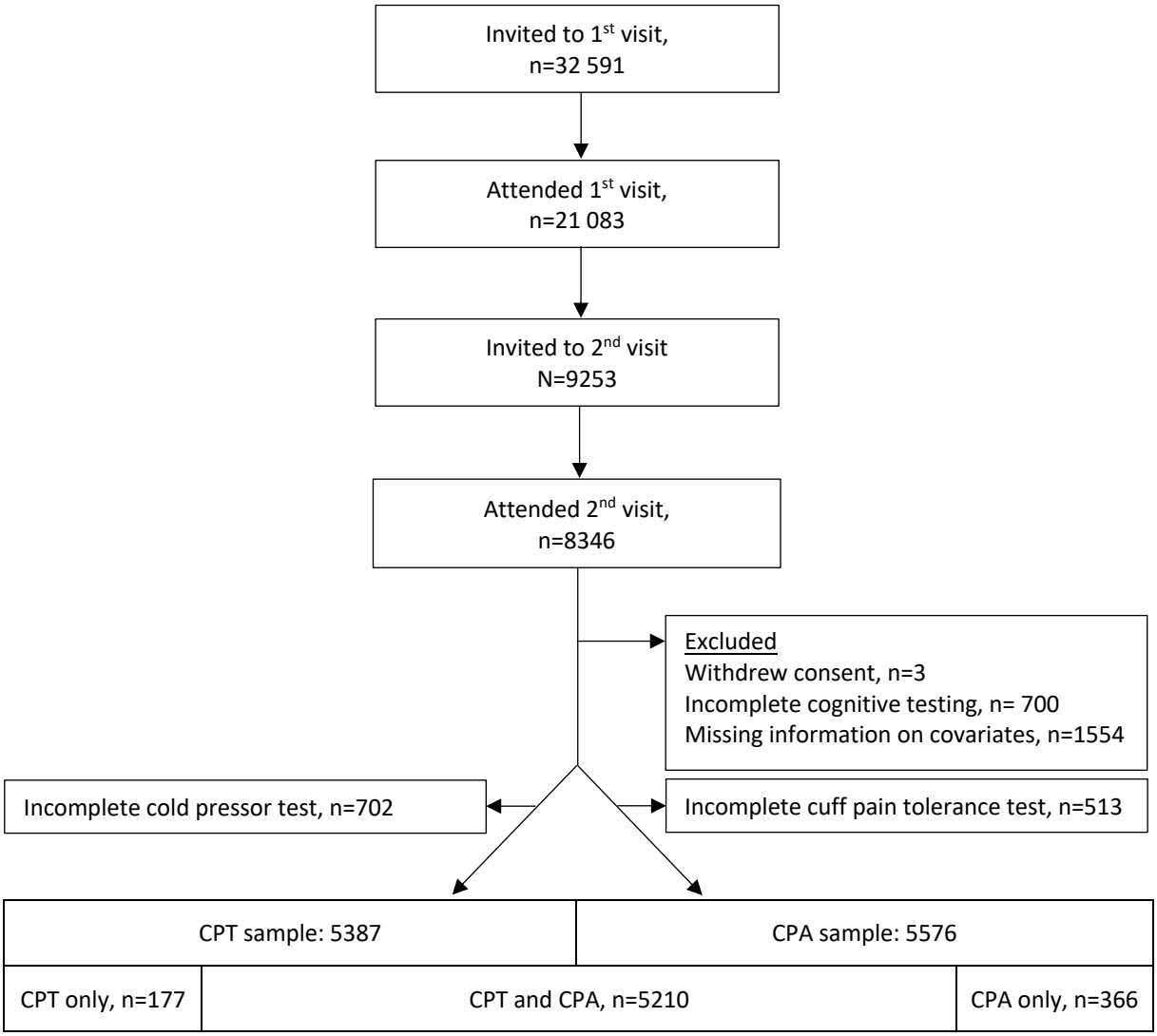
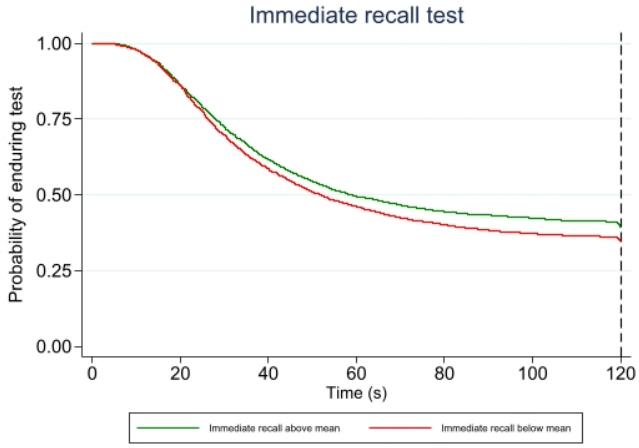


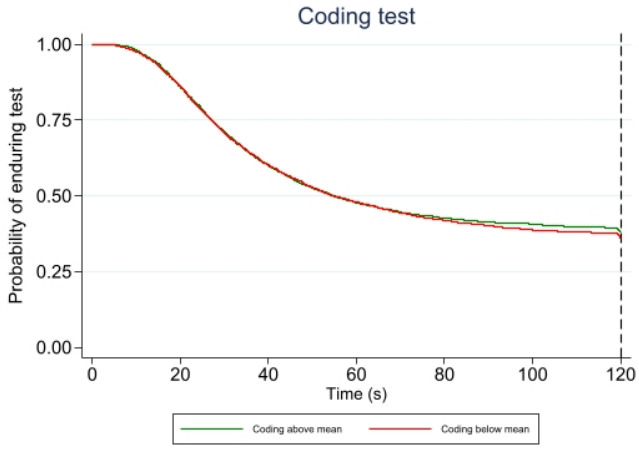
Figure 1: All inhabitants in the municipality of Tromsø aged 40 years or older were invited by postal letter and 64.7% participated. Among those invited, 13028 were premarked for invitation to a second visit, if they attended the first visit. At the first visit, participants completed questionnaires, and underwent blood sampling and clinical examinations, including the cold pressor test and cuff pressure algometry. At the second visit, extended examinations were performed, among them cognitive testing. Participants were included in the present study if they had completed cognitive testing (12-word immediate recall test, digit symbol coding test and Mini Mental State Examination), CPT and/or CPA, and had available information on covariates (age, sex, education, smoking, exercise, systolic blood pressure, body mass index, symptoms indicating anxiety or depression, analgesic use and chronic pain).
Abbreviations: CPT, cold pressor test. CPA, cuff pressure algometry.

Figure 2: Kaplan-Meier curves of CPT tolerance time by score on cognitive test

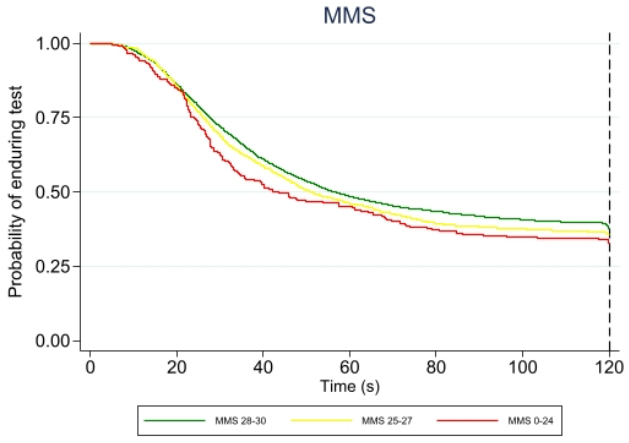
A)



B)



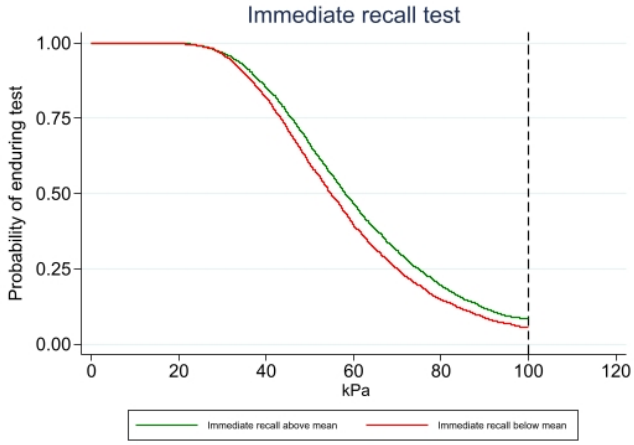
C)



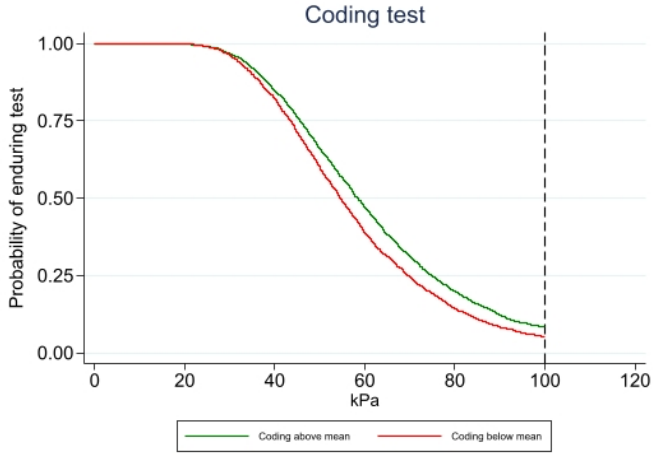
Probability of keeping the hand in the water bath in participants stratified by scores on cognitive test. The maximum time was 120s, indicated by the dotted reference line. A and B: For immediate recall and coding test, participants are grouped according to test score above or below mean. C: For MMS-E, participants are grouped according to whether the score is considered normal (score of 28-30 points), possible cognitive impairment (25-27) or cognitive impairment (≤24). CPT: Cold pressor test. MMS-E: Mini Mental State Examination

Figure 3: Kaplan-Meier curves of CPA tolerance by score on cognitive test

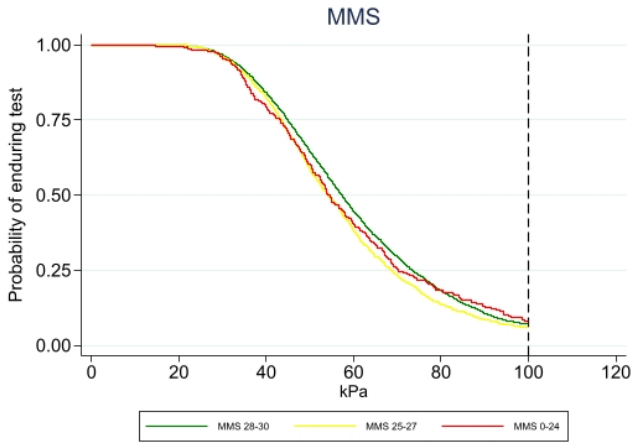
A)



B)



C)



Probability of enduring CPA in participants stratified by scores on cognitive test. The pressure increased by 1 kPa/s. The maximum pressure was 100 kPa, indicated by the dotted reference line.
A and B: CPA endurance by scores on immediate recall and coding test. Participants are grouped according to test score above or below mean. C: CPA endurance by MMS-E score. Participants are grouped according to whether the score is considered normal (score of 28-30 points), possible cognitive impairment (25-27) or cognitive impairment (≤24). CPA: Cuff pressure algometry. MMS-E: Mini Mental State Examination.

Table 1: Descriptive characteristics of all participants in CPT sample and according to CPT tolerance*

	All participants n=5387	Pain tolerant n= 1994 (37%)	Pain sensitive n=3393 (63%)	P value**
Age in years, median (interquartile range)	63 (54 – 69)	63 (53 – 69)	63 (55 – 69)	0.457
Women, n (%)	2793 (51.9)	904 (45.3)	1889 (55.7)	<0.001
Education, n (%)				0.003
Primary/secondary school, up to 10 years	1278 (23.7)	442 (22.2)	836 (24.6)	
Upper secondary, 3 years	1538 (28.6)	535 (26.8)	1003 (29.6)	
College or university, 1-3 years	1098 (20.4)	425 (21.3)	673 (19.8)	
College or university, 4 years or more	1473 (27.3)	592 (29.7)	881 (26.0)	
Exercise				<0.001
Never	176 (3.3)	46 (2.3)	130 (3.8)	
Less than once per week	591 (11.0)	197 (9.9)	394 (11.6)	
Once a week	748 (13.9)	246 (12.3)	502 (14.8)	
2-3 times a week	2316 (43.0)	887 (44.5)	1429 (72.4)	
Approximately every day	1556 (28.9)	618 (31.0)	938 (27.7)	
Smoking, n (%)				<0.001
Never	2169 (40.3)	910 (45.6)	1259 (37.1)	
Previous	2595 (48.2)	887 (44.5)	1708 (50.3)	
Current	623 (11.6)	197 (9.9)	426 (12.6)	
BMI in kg/cm2 , mean ± SD	27.3 ± 4.4	27.1 ± 4.2	27.4 ± 4.5	0.012
Systolic BP mean ± SD	131.8 ± 19.3	132.7 ± 18.9	131.3 ± 19-6	0.010
Anxiety or depression (HCSL-10 ≥ 1.85), n(%)	339 (6.3)	106 (5.3)	233 (6.9)	0.024
Chronic pain yes, n (%)	1854 (34.4)	668 (33.5)	1186 (35.0)	0.278
Analgesics last four weeks				<0.001
Not used	2907 (54.0)	1156 (58.0)	1751 (51.6)	
Less than weekly	1663 (30.9)	568 (28.5)	1095 (32.3)	
Weekly	597 (11.1)	199 (10.0)	398 (11.7)	
Daily	220 (4.08)	71 (3.6)	149 (4.4)	
Immediate recall test score, mean ± SD	7.5 ± 1.9	7.6 ±1.9	7.4 ± 1.9	<0.001
Coding test score, mean ± SD	44.8 ± 11.8	45.3 ± 11.7	44.5 ± 11.9	0.0145
MMS-E test score, median (interquartile range)	29 (27 – 29)	29 (27 – 30)	29 (27 – 29)	0.001
MMS-E deficit, n (%)				
Normal 28-30	3914 (72.7)	1477 (74.1)	2437 (71.8)	0.156
Possible impairment 25-27	1263 (23.5)	448 (22.5)	815 (24.0)	
Cognitive impairment 24 or lower	210 (3.9)	69 (3.5)	141 (4.2)	

*****Participants were categorized as pain tolerant if they endured the whole 120 seconds of the cold pressor test, and pain sensitive if they withdrew their hand at an earlier time.

**P-value is for difference between pain sensitive and pain tolerant group, assessed with t-test for continuous variables and with Pearson chi2 for categorical variables. As age and MMS was not normally distributed, Wilcoxon rank-sum test was used for difference between groups for these variables. Abbreviations: CPT, cold pressor test. BMI, body mass index. HSCL-10: Hopkins symptom check list (10-item version). MMS-E: Mini Mental State Examination.

Table 2: Cox regression analyses of the association between cognitive test scores and pain tolerance

	Immediate recall test score (standardized by z-transformation)			**Coding test score** (standardized by z-transformation)			**MMS-E test score** (standardized by z-transformation)		
Cold pressor test (n=5387)									
	HR	95 % CI	p	HR	95 % CI	p	HR	95%CI	p
Model 1:	0.92	0.89 - 0.96	<0.001	0.93	0.89 - 0.97	0.002	0.93	0.90 - 0.96	<0.001
Model 2:	0.93	0.90 - 0.97	<0.001	0.94	0.89 - 0.98	0.004	0.93	0.90 - 0.96	<0.001
Model 3:	0.93	0.90 - 0.97	<0.001	0.94	0.89 - 0.98	0.004	0.93	0.90 - 0.96	<0.001
Cuff pain tolerance test (n=5576)									
	HR	95 % CI	p	HR	95 % CI	p	HR	95 % CI	p
Model 1:	0.94	0.91 - 0.96	<0.001	0.92	0.89 - 0.95	<0.001	0.97	0.94 - 1.00	0.036
Model 2:	0.94	0.91 - 0.97	<0.001	0.93	0.89 - 0.96	<0.001	0.98	0.95 - 1.00	0.090
Model 3:	0.94	0.91 - 0.97	<0.001	0.93	0.89 - 0.96	<0.001	0.98	0.95 - 1.00	0.082

Analyses are Cox regression with cognitive test score, standardized by z-transformation, as independent variable and endurance time of CPT or CPA as outcome, adjusted for covariates as specified by model:
Model 1: adjusted for sex, age and education
Model 2: adjusted for age, sex, education, smoking, exercise, BMI, blood pressure and depression
Model 3: adjusted for age, sex, education, smoking, exercise, BMI, blood pressure, depression, chronic pain, and analgesic use. Abbreviations: BMI, body mass index. MMS-E: Mini Mental State Examination.
CPT; cold pressor test. CPA; Cuff pain algometry

Table 3: Subgroup analysis according to age groups

	Immediate recall test score (standardized by z-transformation)			**Coding test score** (standardized by z-transformation)			**MMS-E test score** (standardized by z-transformation)		
Cold pressor test (n=5387)									
	HR	95 % CI	p	HR	95 % CI	p	HR	95% CI	p
Age 40-59 (n=1959)	0.88	0.83 – 0.94	<0.001	0.96	0.89 – 1.03	0.289	0.88	0.83 – 0.94	<0.001
Age 60-69 (n=2233)	0.97	0.92 – 1.03	0.390	0.93	0.86 – 0.99	0.031	0.95	0.89 – 1.01	0.076
Age 70-84 (n=1195)	0.93	0.86 – 1.01	0.067	0.89	0.81 – 0.99	0.031	0.93	0.88 – 0.98	0.005
Cuff pain tolerance test (n=5576)									
	HR	95 % CI	p	HR	95 % CI	p	HR	95% CI	p
Age 40-59 (n= 2021)	0.91	0.87 – 0.96	<0.001	0.94	0.89 – 1.00	0.052	0.95	0.90 – 0.99	0.025
Age 60-69 (n=2334)	0.94	0.90 – 0.99	0.022	0.95	0.89 – 1.00	0.064	0.97	0.92 – 1.02	0.195
Age 70-84 (n=1221)	0.96	0.90 – 1.02	0.193	0.86	0.80 – 0.93	<0.001	0.99	0.95 – 1.03	0.634

Analyses are Cox regression with cognitive test score, standardized by z-transformation, as independent variable and endurance of CPT or CPA as outcome, adjusted for age, sex, education, smoking, exercise, BMI, blood pressure, depression, chronic pain and analgesic use.
CPT; cold pressor test. CPA; Cuff pain algometry.

Table 4: Subgroup analyses of the association between cognitive test scores and CPT tolerance time according to sex

	Immediate recall test score (standardized by z-transformation)			Coding test score (standardized by z-transformation)			MMS-E test score (standardized by z-transformation)		
Cold pressor test (n=5387)									
	HR	95 % CI	p	HR	95 % CI	p	HR	95%CI	p
Men (n=2954)	0.95	0.90 – 1.01	0.075	0.94	0.88 – 1.01	0.089	0.91	0.86 – 0.96	<0.001
Women (n= 2793)	0.92	0.88 – 0.97	0.001	0.94	0.88 – 0.99	0.029	0.95	0.91 – 0.99	0.016

Analyses are Cox regression with cognitive test score, standardized by z-transformation, as independent variable and time with hand in cold-water bath as outcome, adjusted for age, education, smoking, exercise, BMI, blood pressure, depression, chronic pain and analgesic use. Abbreviations: BMI, body mass index. MMS-E: Mini Mental State Examination.

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Paper 3

Pain tolerance after stroke: The Tromsø study

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ORIGINAL ARTICLE

Pain tolerance after stroke: The Tromsø study

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Abstract

Background: Stroke lesions might alter pain processing and modulation by affecting the widely distributed network of brain regions involved. We aimed to compare pain tolerance in stroke survivors and stroke-free persons in the general population, with and without chronic pain.

Methods: We included all participants of the sixth and seventh wave of the population-based Tromsø Study who had been tested with the cold pressor test (hand in cold water bath, 3°C, maximum time 106 s in the sixth wave and 120 s in the seventh) and who had information on previous stroke status and covariates. Data on stroke status were obtained from the Tromsø Study Cardiovascular Disease Register and the Norwegian Stroke Register. Cox regression models were fitted using stroke prior to study attendance as the independent variable, cold pressor endurance time as time variable and hand withdrawal from cold water as event. Statistical adjustments were made for age, sex, diabetes, hypertension, hyperlipidaemia, body mass index and smoking.

Results: In total 21,837 participants were included, 311 of them with previous stroke. Stroke was associated with decreased cold pain tolerance time, with 28% increased hazard of hand withdrawal (hazard ratio [HR] 1.28, 95% CI 1.10–1.50). The effect was similar in participants with (HR 1.28, 95% CI 0.99–1.66) and without chronic pain (HR 1.29, 95% CI 1.04–1.59).

Conclusions: Stroke survivors, with and without chronic pain, had lower cold pressor pain tolerance, with possible clinical implications for pain in this group.

Significance: We found lower pain tolerance in participants with previous stroke compared to stroke-free participants of a large, population-based study. The association was present both in those with and without chronic pain. The results may warrant increased awareness by health professionals towards pain experienced by stroke patients in response to injuries, diseases and procedures.

1 | INTRODUCTION

Pain is among the most common complications in the early phase after stroke (Bovim et al., 2018; Indredavik et al., 2008; Langhorne et al., 2000), and later many report presence (Klit et al., 2011) or development (Bovim et al., 2018) of chronic pain. Pain after stroke includes, but is not limited to, specified post-stroke pain syndromes. It is

also reported that stroke patients experience pain considered *not* to be stroke related (Bovim et al., 2018; Indredavik et al., 2008; Lundström et al., 2009; Naess et al., 2010), such as pain in the unaffected as well as the affected side (Jönsson et al., 2006; Naess et al., 2010). Several features of chronic post-stroke nociceptive or neuropathic pain syndromes are overlapping, and shared pathophysiology has been suggested (Zeilig et al., 2013). As pain is processed in a widespread network in the brain (Coghill, 2020; Mercer Lindsay et al., 2021), affection to any part of the network might alter pain processing, with possible implications for the occurrence and severity of acute, procedural and chronic pain in stroke patients.

Experimental pain studies allow for assessment of responses to controlled nociceptive stimuli, including pain thresholds, direct pain ratings or pain tolerance, reflecting various aspects of the individual's pain sensitivity. Such studies of stroke survivors are generally small and have often focused on the pathophysiology of specific post-stroke pain syndromes (i.e. post-stroke shoulder pain [PSSP] and central post-stroke pain [CPSP]) or the consequences of specific stroke lesion locations. Stroke participants are often recruited from rehabilitation centres (Roosink et al., 2011; Zeilig et al., 2013) or pain centres (Tuveson et al., 2009), or inclusion criteria require specified deficits (Casey et al., 2012; Krause et al., 2016; Roosink et al., 2012), post-stroke chronic pain condition (Roosink et al., 2011; Soo Hoo et al., 2013; Tuveson et al., 2009) or stroke lesion location (Ruscheweyh et al., 2014). Studies on pain sensitivity in stroke survivors without chronic pain are scarce, but increased sensitivity is reported in 30 pain-free cerebellar stroke patients (Ruscheweyh et al., 2014) and in pain-free stroke control subjects ($n < 30$) in two studies (Krause et al., 2016; Roosink et al., 2011).

We aimed to compare pain tolerance assessed by the cold pressor test (CPT) in subjects with and without prior stroke, and with and without chronic pain, in the setting of a large population-based study.

2 | METHODS

2.1 | Study design and participants

We conducted an epidemiological study using data from the Tromsø study, which is a population-based multipurpose health study that has been carried out with intervals of 6–7 years since 1974 (Jacobsen et al., 2012). Participants are recruited through an invitation letter sent to whole birth cohorts and age-stratified random samples living in the municipality of Tromsø in Northern Norway. We used data from the sixth and seventh survey, carried out in 2007–2008 and 2015–2016 respectively. In Tromsø 6, 19,762 inhabitants above 30 years of age were invited and 65.7% participated (Eggen et al., 2013), while in Tromsø 7, all 32,591 inhabitants above 40 years of age were invited and 64.7% participated (Hopstock et al., 2022) (Figure 1). Participants completed questionnaires, provided blood samples and completed a range of clinical examinations, among them tests of experimental pain tolerance. Included in this study were 9935 participants of Tromsø 6 aged 40 years and above, and 11,902 participants of Tromsø 7 who had not been included in the Tromsø 6 sample, who had completed the experimental pain examination. Thus, two independent samples from Tromsø 6 and Tromsø 7 were analysed, and subsequent combined analysis was performed by pooling the two samples. Participants for whom information on stroke status and covariates were unavailable were excluded (Figure 1).

2.2 | Ascertainment of stroke status

The Tromsø Study Cardiovascular Disease Register contains validated information on incident strokes of all subtypes in participants until 31 December 2014 (31 December 2017 for subarachnoid haemorrhage). Information on ischaemic and haemorrhagic strokes occurring after 01 January 2015, was obtained from the Norwegian Stroke Register (Varmdal et al., 2021). In both registries, strokes are defined in accordance with the WHO definition, as rapidly developed clinical signs of focal or global disturbance of cerebral function, lasting more than 24 h or until death, with no apparent cause other than vascular (WHO MONICA Project Principal Investigators, 1988). Strokes are classified as ischaemic if diagnostic imaging reveal an acute ischaemic lesion or rules out haemorrhage. Haemorrhagic strokes and subarachnoid haemorrhages are classified according to findings on diagnostic imaging. If no imaging has been performed in the acute phase, the stroke is defined as unclassifiable. The registries do not include information on stroke location or laterality. As the purpose of The Tromsø Study Cardiovascular Disease Register was to provide information on end points for study of cardiovascular risk factors, only the first of each stroke type were registered. Data collection were done by expert review of medical records and hence very resource-intensive. The Norwegian Stroke Register was established in 2012 and includes information on ischaemic, haemorrhagic and unclassifiable strokes. After a validation study confirming sufficient quality (Varmdal et al., 2021), it was decided that data from The Norwegian Stroke Register was to be used for information on stroke status in the Tromsø Study from 01 January 2015.

We included participants in the stroke group if they were registered with ischaemic, haemorrhagic or unclassifiable stroke or subarachnoid haemorrhage that had occurred prior to participation. Transient ischaemic attacks

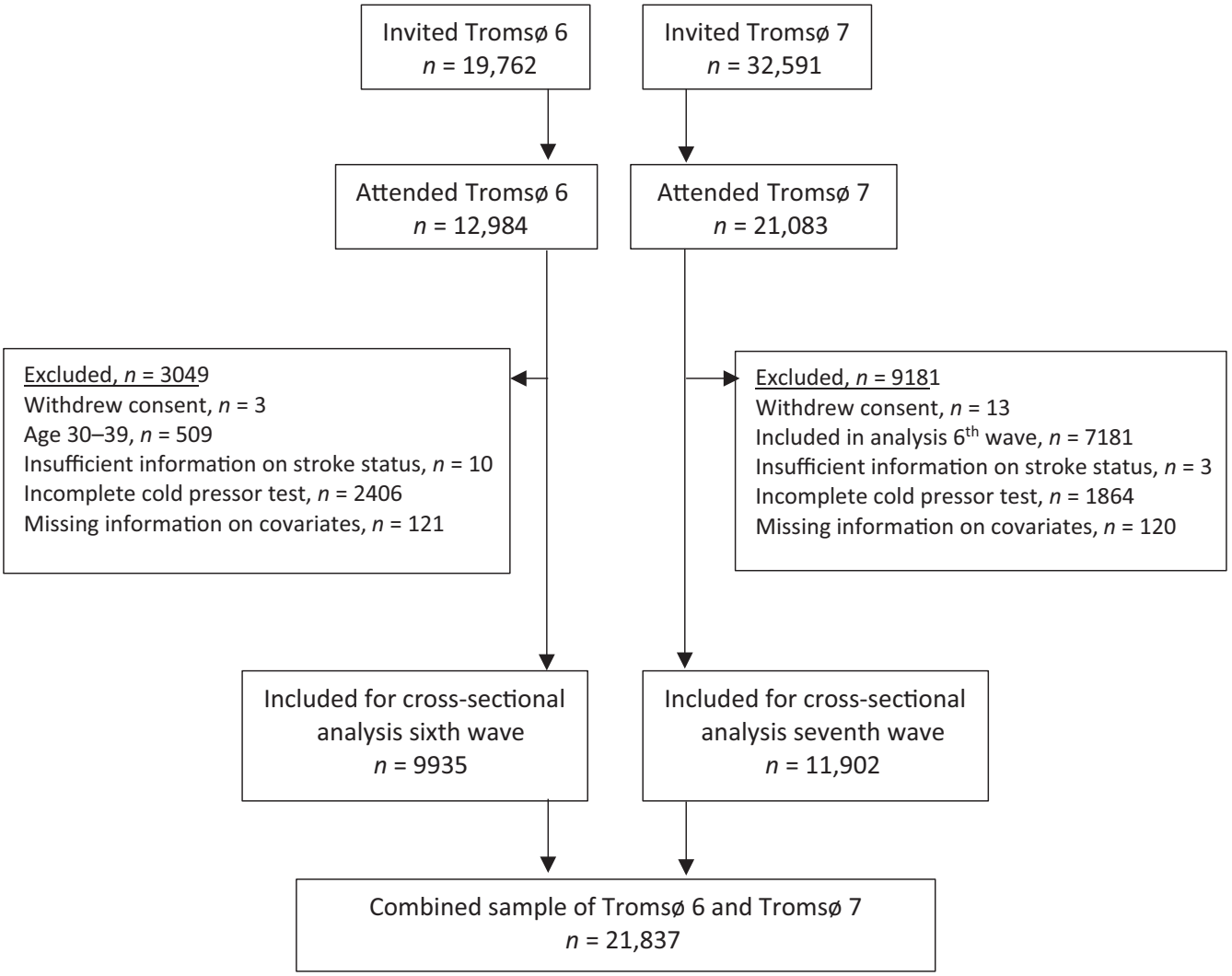

FIGURE 1 Flow chart of participants of Tromsø 6 and Tromsø 7 and this study. In Tromsø 6, 19,762 inhabitants above the age of 30 were invited, selected by random sampling, whole birth cohorts or due to participation in previous waves. Participants aged 30–39 were excluded from analyses, in order to get similar groups for comparison in the stroke versus no stroke group as well as in the Tromsø 6 and Tromsø 7 cross-sectional samples. There were no strokes in this age group. In Tromsø 7, all inhabitants above the age of 40 were invited.

were defined as ischaemic stroke when an acute ischaemic lesion was present on diagnostic imaging.

2.3 | Experimental pain examination

Pain tolerance was assessed with the CPT, where the participants were asked to hold their hand and wrist in circulating cold water, 3°C, for as long as they were able to or until a maximum time limit was reached. Controlled water temperature was ensured by using a cooling circulator (FP40-HE, Julabo GmbH Germany) with continuous exchange to the vat in which the participant held their hand. Exclusion criteria for CPT included (a) participants declining to perform the test; (b) inability to comprehend and follow instructions; (c) amputation or paresis of the hand; (d) open sores on the hand; (e) medical issues that, in the participants experience, cold exposure would put them at risk of negative side effects, such as cold allergy, Raynaud's syndrome; (f) sensory or motor dysfunction if this could interfere or put the participant at risk. In Tromsø 6, the maximum time limit was 106 s, the dominant hand was submerged. In Tromsø 7, the maximum time was 120 s, the non-dominant hand was submerged. For descriptive purposes, participants were classified as pain tolerant if they kept their hand in the cold water until the maximum time, and pain sensitive if they did not.

2.4 | Covariates

To adjust for putative confounders, risk factors for stroke that could also affect pain tolerance were selected as covariates, namely age, sex, diabetes, hypertension, hyperlipidaemia, body mass index (BMI) and smoking. Diabetes was defined as self-reported diabetes, use of

anti-diabetic medication or HbA1c ≥6.5%. Hypertension was defined as self-reported hypertension, use of antihypertensive medication or blood pressure above 140 systolic or above 90 diastolic. Hyperlipidaemia was defined as use of lipid-lowering drugs or total cholesterol/HDL ratio above 5. BMI was calculated using the formula weight/height^2 (kg/m^2). Smoking status was assessed by questionnaire as current, previous or never daily smoking. If any of the above-mentioned covariates were missing, the participant was excluded from all analyses. Chronic pain was assessed by questionnaire ('do you have persistent or constantly recurring pain that has lasted for 3 months or more?') and information on this item was used for subgroup analysis in subsamples with available information on this item. C-reactive protein (CRP) was added as an additional covariate in subsamples with available information on the item, to assess potential confounding from inflammation.

2.5 | Statistical methods

Descriptive statistics are reported as counts and percentages for categorical data, means and standard deviations for continuous variables. Group differences were evaluated with *t*-tests for continuous variables and chi-squared tests for categorical variables. While participants were classified according to pain tolerance for descriptive purposes, CPT tolerance time was used as a continuous right censored variable in the analyses, and hand withdrawal as the event. Kaplan–Meier plots were created for visualization of CPT tolerance time. The effect of stroke on CPT was modelled using Cox proportional hazard methods. Censoring time was the maximum time limit of the test—106 s in Tromsø 6 and 120 s in Tromsø 7. Putative confounders were added to the model as covariates. Interaction effects were tested for age and sex by adding interaction terms to the model (stroke status multiplied with age and sex respectively). Additional adjustment was done for CRP in subsamples with available information on this item. Graphical check of the proportional hazards (PH) assumption confirmed that observed versus expected survival plots were overlapping and that log–log survival curves were parallel. Statistic test of scaled Schoenfeld residuals confirmed that the PH assumption was met for the relationship between stroke and CPT tolerance time.

Separate analyses were performed for participants in Tromsø 6 and participants in Tromsø 7, as well as for the combined sample, with additional adjustment for study wave (Tromsø 6 or Tromsø 7). In the combined sample, subgroup analyses were performed for each stroke subtype separately (ischaemic stroke, haemorrhagic stroke or subarachnoid haemorrhage). Finally, chronic pain subgroup analysis was performed on subsamples consisting of participants with or without chronic pain for participants with available information on this item ($n = 9924$ in Tromsø 6 and $n = 11{,}086$ in Tromsø 7).

Statistical analyses were performed using STATA version 16.1 for windows (StataCorp LLC). Statistical significance level was set to 0.05.

3 | RESULTS

Descriptive characteristics of the samples are presented in Table 1. Among participants included from Tromsø 6, 181 had a history of stroke prior to attendance, while there were 130 participants with prior stroke in the Tromsø 7 sample. The majority of strokes were ischaemic (83%). Time between stroke and CPT was 35 days–44 years in Tromsø 6, and 30 days–44 years in Tromsø 7. Participants with a history of stroke were older and included fewer women, a higher proportion had diabetes, hypertension, hyperlipidaemia and were smokers and mean BMI was higher. The prevalence of chronic pain was similar in the two groups.

While the overall proportion who were pain tolerant was larger in Tromsø 6 than in Tromsø 7, the proportion of pain-tolerant subjects was lower in those with previous stroke than in those without stroke in both surveys (60.2% vs 68.3% in participants with and without previous stroke, respectively, in Tromsø 6, and 26.9% vs 36.4% in Tromsø 7). Kaplan–Meier plots of CPT tolerance time by stroke status and sex are presented in Figure 2. Plot of baseline hazard of hand withdrawal according to stroke status is presented in Figure S1. In Cox proportional hazard models, participants with a history of stroke had decreased pain tolerance compared to participants without stroke in all three samples (Table 2). In the Tromsø 6 sample, previous stroke was associated with a 33% increased risk of hand withdrawal (HR 1.33, 95% CI 1.05–1.68), and the relationship remained significant when adjusting for age and sex and when adding additional covariates in the multivariable model (HR 1.42, 95% CI 1.12–1.80). In the Tromsø 7 sample, the association was similar (HR 1.30, 95% CI 1.06–1.59) and remained significant when adjusting for age and sex, but not after adjustment for additional covariates (HR = 1.22, 95% CI 0.99–1.49). In the analysis of the combined sample, HR was 1.31 (95% CI 1.13–1.53) and remained significant in all models (HR 1.28, 95% CI 1.10–1.50 in multivariable analysis). Interaction terms for age or sex were not significant and were omitted from further analyses. Additional adjustment for CRP in subsamples with available information on this item had minimal impact on the results (HR 1.27, 95% CI 1.09–1.50 in combined sample, $n = 21{,}075$).

In the combined sample, the association between stroke and pain tolerance was similar in participants

TABLE 1 Demographic and clinical characteristics of cross-sectional samples in Tromsø 6 and Tromsø 7 and combined sample of Tromsø 6 and Tromsø 7, according to stroke status.

	Tromsø 6			Tromsø 7			Combined		
	Stroke $n=181$	No stroke $n=9754$	p	Stroke $n=130$	No stroke $n=11,772$	p	Stroke $n=311$	No stroke $n=21,526$	p
Age in years, mean ± SD	66.0 ± 10.1	57.1 ± 11.5	<0.001	63.3 ± 9.6	53.0 ± 9.6	<0.001	65.0 ± 9.9	54.9 ± 10.7	<0.001
Women, *n* (%)	63 (34.8)	5027 (51.5)	<0.001	45 (34.6)	6038 (51.3)	<0.001	108 (34.7)	11,065 (51.4)	<0.001
Diabetes, *n* (%)	27 (14.9)	633 (6.5)	<0.001	20 (15.4)	629 (5.3)	<0.001	47 (15.1)	1262 (5.9)	<0.001
Hypertension, *n* (%)	150 (82.9)	4774 (48.9)	<0.001	100 (76.9)	4312 (36.6)	<0.001	250 (80.4)	9086 (42.2)	<0.001
Hyperlipidaemia, *n* (%)	119 (65.8)	3075 (31.5)	<0.001	100 (76.9)	3248 (27.6)	<0.001	219 (70.4)	6323 (29.4)	<0.001
BMI, mean ± SD	27.5 ± 4.0	26.9 ± 4.2	0.069	28.3 ± 3.8	27.4 ± 4.6	0.017	27.9 ± 3.9	27.2 ± 4.4	0.007
Daily smoking, *n* (%)			0.422			0.059			0.049
Current	34 (18.8)	1996 (20.5)		25 (19.2)	1691 (14.4)		59 (19.0)	3687 (17.1)	
Previous	87 (48.1)	4212 (43.2)		61 (46.9)	4947 (42.0)		148 (47.6)	9159 (42.6)	
Pain tolerant	109 (60.2)	6661 (68.3)	0.021	35 (26.9)	4285 (36.4)	0.027	144 (46.3)	10,946 (50.9)	
Chronic pain[a]	58 (32)	3112 (31.9)	0.976	44 (33.9)	4088 (34.8)	0.375	102 (32.8)	7200 (33.5)	0.779
Stroke subtype[b]									
Ischaemic	147			110			257		
Intracerebral haemorrhage	16			7			23		
Subarachnoid haemorrhage	12			16			28		
Unclassified	7			0			7		

Note: Participants were classified as pain tolerant if they could endure the maximum time of the cold pressor test, which was 106 s in Tromsø 6 and 120 s in Tromsø 7. *p*-value is for difference between stroke and no stroke group.

Abbreviation: BMI, body mass index.

[a]Chronic pain is presented for participants with available information on this item. In Tromsø 6, 11 participants (no strokes) had missing information on this item, while in Tromsø 7, it was 816 (24 strokes).

[b]In Tromsø 6, one participant was registered with both an ischaemic and an unclassifiable stroke. In Tromsø 7, three participants were registered with both an ischaemic stroke and an intracerebral haemorrhage.

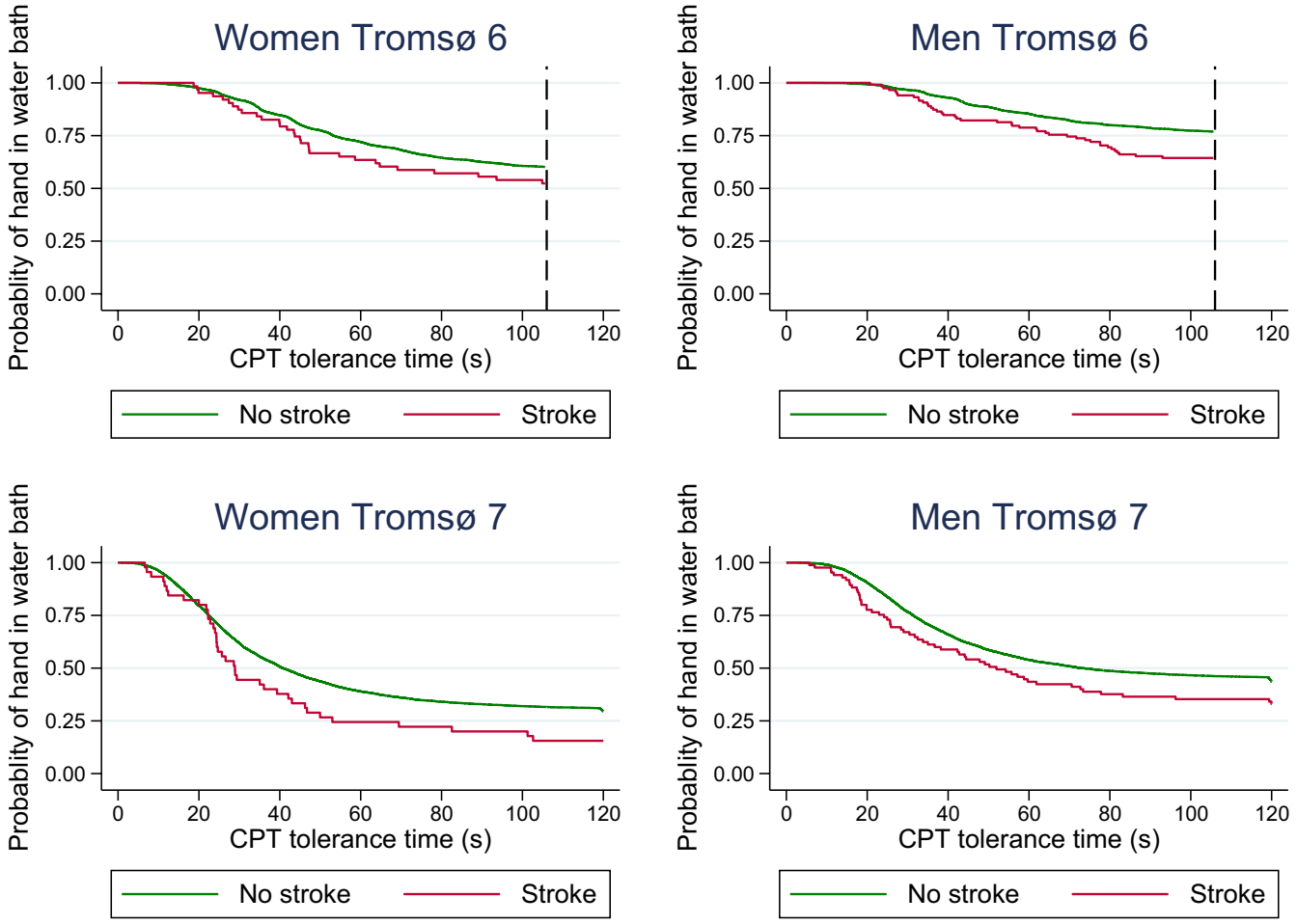

FIGURE 2 Kaplan–Meier plot of CPT tolerance time for women and men in Tromsø 6 and Tromsø 7. Probability of keeping the hand in the water bath for women and men, according to stroke status. In both study waves, participants with a history of stroke had increased rates of hand withdrawal from cold water bath. The maximum time was 106 s in Tromsø 6, indicated by reference line, while in Tromsø 7 it was 120 s. CPT, cold pressor test.

TABLE 2 Cox regression analyses of the association between stroke and pain tolerance time.

	Tromsø 6			Tromsø 7			Combined		
	HR	95% CI	*p*	HR	95% CI	*p*	HR	95% CI	*p*
Crude[a]	1.33	1.05–1.68	0.016	1.30	1.06–1.59	0.011	1.31	1.13–1.53	<0.001
Model 1[b]	1.50	1.18–1.90	0.001	1.31	1.07–1.61	0.009	1.38	1.18–1.61	<0.001
Model 2[c]	1.42	1.12–1.80	0.004	1.22	0.99–1.49	0.062	1.28	1.10–1.50	0.002

Note: Analyses are Cox proportional hazards model with time with hand in cold-water bath as outcome and stroke status as independent variable. Hazard ratios above 1 indicate higher hazard of hand withdrawal, that is, lower pain tolerance. Tromsø 6: max time 106 s, 181 strokes. Tromsø 7: max time 120 s, 130 strokes. Combined sample: 311 strokes.

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a]Crude: no adjustment in Tromsø 6 or Tromsø 7, in combined sample adjustment for study indicator (Tromsø 6 or Tromsø 7).

[b]Model 1: adjusted for age and sex. In combined sample additional adjustment for study indicator.

[c]Model 2: adjusted for age, sex, diabetes, hypertension, hyperlipidaemia, body mass index and smoking. In combined sample additional adjustment for study indicator.

with and without chronic pain (multivariable adjusted HR 1.28 [95% CI 0.99–1.66] in participants with chronic pain [$n = 7302$] and 1.29 [95% CI 1.04–1.59] in participants without chronic pain [$n = 13{,}708$] in combined sample). The association was somewhat stronger for participants with chronic pain in the Tromsø 6 sample, and weaker for participants with chronic pain in the Tromsø 7 sample, compared to participants without chronic pain (Table 3).

TABLE 3 Subgroup analysis of the association between stroke and pain tolerance time, in participants with or without self-reported chronic pain.

	Tromsø 6 ($n = 3170$)			Tromsø 7 ($n = 4132$)			Combined ($n = 7302$)		
With self-reported chronic pain	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Crude[a]	1.58	1.1–2.28	0.013	1.13	0.79–1.61	0.489	1.30	1.01–1.68	0.040
Model 1[b]	1.82	1.26–2.63	0.001	1.17	0.82–1.68	0.376	1.40	1.09–1.81	0.010
Model 2[c]	1.72	1.19–2.48	0.004	1.07	0.74–1.53	0.727	1.28	0.99–1.66	0.056
Without self-reported chronic pain	**Tromsø 6 ($n = 6754$)**			**Tromsø 7 ($n = 6954$)**			**Combined ($n = 13,708$)**		
	HR	**95% CI**	***p***	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Crude[a]	1.21	0.89–1.64	0.232	1.37	1.02–1.84	0.034	1.29	1.04–1.59	0.019
Model 1[b]	1.33	0.98–1.81	0.068	1.42	1.05–1.90	0.021	1.37	1.11–1.70	0.004
Model 2[c]	1.29	0.94–1.76	0.111	1.31	0.97–1.76	0.076	1.29	1.04–1.59	0.022

Note: Analyses are Cox proportional hazards model with time with hand in cold-water bath as outcome and stroke status as independent variable. Hazard ratios above 1 indicate higher hazard of hand withdrawal, that is, lower pain tolerance. Tromsø 6: max time 106 s, 181 strokes. Tromsø 7: max time 120 s, 130 strokes. Combined: 311 strokes.

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a]Crude: no adjustment in Tromsø 6 or Tromsø 7, in combined sample adjustment for study indicator (Tromsø 6 or Tromsø 7).

[b]Model 1: adjusted for age and sex. In combined sample additional adjustment for study indicator.

[c]Model 2: adjusted for age, sex, diabetes, hypertension, hyperlipidaemia, body mass index and smoking. In combined sample additional adjustment for study indicator.

TABLE 4 Subgroup analysis of association between stroke and pain tolerance in stroke subtypes, in combined sample of Tromsø 6 and Tromsø 7.

Stroke type	No. of strokes	HR	95% CI	*p*
Ischaemic	253	1.35	1.14–1.59	0.001
Haemorrhagic	20	0.65	0.29–1.45	0.294
Subarachnoid haemorrhage	28	1.50	0.97–2.35	0.071

Note: Analyses are Cox proportional hazards model with time with hand in cold-water bath as outcome and stroke status as independent variable, adjusted for study indicator (Tromsø 6 or Tromsø 7), age, sex, diabetes, hypertension, hyperlipidaemia, body mass index and smoking. Participants with other types of stroke were excluded from analysis (e.g. when doing analysis for ischaemic stroke, participants with haemorrhagic stroke or subarachnoid haemorrhage were excluded). Hazard ratios above 1 indicate higher hazard of hand withdrawal, that is, lower pain tolerance.

Abbreviations: CI, confidence interval; HR, hazard ratio.

In stroke type subgroup analysis in the combined sample (Table 4), the association was significant for ischaemic stroke (HR 1.35, 95% CI 1.14–1.59), but not for intracerebral (HR 0.65, 95% CI 0.29–1.45) or subarachnoid haemorrhage (HR 1.50, 95% CI 0.97–2.35). However, the number of participants with haemorrhagic stroke and subarachnoid haemorrhage was quite low, 20 and 28 participants, respectively, rendering this conclusion uncertain.

4 | DISCUSSION

Our main finding was that individuals with a history of stroke have lower pain tolerance compared to individuals without stroke, in a large, general population-based sample. The finding was similar across study samples, and in subgroups with and without chronic pain. The association was also found in subgroup analysis of participants with ischaemic stroke, which constituted 83% of all included strokes. While the overall CPT tolerance time was shorter in Tromsø 7 than in Tromsø 6 in all participants, this did not affect the results from analyses comparing participants with and without stroke. Inspection of Kaplan–Meier plots of probability of keeping the hand in the water bath also suggest that the difference did not influence the shape of the curves.

Pain is a multidimensional experience processed in an extensive network of brain regions (Mercer Lindsay et al., 2021) and pain tolerance is likely to be influenced by its sensory-discriminative, affective-motivational and cognitive-evaluative dimensions as well as modulatory mechanisms. Altered sensitivity to pain in stroke patients may be due to stroke lesions affecting the somatosensory pathway and distributed corresponding to lesion location. While key regions and corresponding contributions to other dimensions of pain processing have been identified (Apkarian et al., 2005; Mercer Lindsay et al., 2021), a core

quality of pain processing and modulation is its distributed nature and high degree of interconnectivity. This entails that it is highly resilient; the ability to experience pain is rarely extinguished despite focal or widespread injury in the brain (Coghill, 2020). However, the high degree of interconnectivity also implies that a disruption can have consequences across multiple anatomical and temporal scales (Kuner & Flor, 2016), leading to altered function and plasticity. In the light of this, it is reasonable that a stroke can affect pain in ways that do not necessarily correspond to stroke lesion location but rather reflect its impact on the pain processing network and the dynamic interplay in it.

Previous studies using experimental pain assessments in stroke patients have found altered pain sensitivity in body regions corresponding to stroke lesion location in patients with PSSP (Roosink et al., 2011, 2012; Soo Hoo et al., 2013; Zeilig et al., 2013) and CPSP (Krause et al., 2016). Evidence of higher pain sensitivity in the ipsilateral/unaffected side, suggesting widespread hypersensitivity, has been found in patients with PSSP (Roosink et al., 2011, 2012; Soo Hoo et al., 2013) and CPSP (Casey et al., 2012; Krause et al., 2016; Tuveson et al., 2009), as well as in stroke patients with sensory abnormalities, but not chronic pain (Krause et al., 2016) and pain-free cerebellar stroke patients (Ruscheweyh et al., 2014).

As previous evidence indicate that chronic pain is associated with increased pain sensitivity (Kosek & Ordeberg, 2000; Woolf, 2011), increased sensitivity (and correspondingly decreased CPT tolerance time) in stroke patients could conceivably be an effect of a post-stroke chronic pain condition such as CPSP or PSSP. However, we found similar association between stroke and pain tolerance in participants with and without chronic pain, suggesting that this was not the case. Increased pain sensitivity in stroke patients without chronic pain is previously reported by smaller studies (i.e. $n \leq 30$) (Krause et al., 2016; Ruscheweyh et al., 2014). This implies that increased pain sensitivity after stroke is not only a possible consequence of chronic post-stroke pain, but also a potential risk factor for it. This indicates that central sensitization could be a contributing factor in the pathophysiology of post-stroke pain syndromes, as previously suggested (Klit et al., 2009), and could also increase the risk and intensity of pain related to other diseases, injuries and medical procedures in stroke patients. Even though it is not yet clear how experimental pain assessments translate to clinical pain, relevance of CPT is supported by studies finding that lower CPT tolerance time is associated with increased risk of post-operative pain (Bisgaard et al., 2001) and with chronic pain (Stabell et al., 2013), while in one prospective study reduced CPT tolerance time at baseline was associated with non-recovery after whiplash (Kasch et al., 2005).

The possible clinical implications of lower pain tolerance have particular importance in stroke patients, considering the co-occurrence of aphasia and cognitive decline that render many of these patients with limited ability to communicate their pain (Edwards et al., 2020). If stroke patients are more sensitive to pain, or have more difficulty coping with it, this calls for awareness in health professionals.

4.1 | Limitations and strengths

The strengths of our study are the large representative samples which allows comparison with stroke-free participants of the general population, the inclusion of participants with and without chronic pain and consistency of findings across study samples.

The study also has several limitations, of which the lack of details on stroke location and deficits is the most substantial. This precluded analysis of whether reduced pain tolerance is more pronounced on the hand contralateral or ipsilateral to the stroke. However, hand paresis was an exclusion criterion for CPT and the participants were asked if they had sensory or motor dysfunction that could interfere with testing or put them at risk. If so, they were excluded or tested on the other hand. Due to this selection procedure, it is likely that stroke patients with severe deficits are underrepresented in our study, which would be expected to weaken our results. Whether recurrent stroke or time since stroke is a factor in the relationship could not be evaluated as we did not have sufficient information on the number of strokes in each participant. Our study is observational and consequently does not allow causal inference, nor can it be excluded that the association may be due to unmeasured confounding. Pain assessments are poorly correlated (Janal et al., 1994; Neziri et al., 2011) and inference cannot be made directly from CPT to other pain stimuli or assessments. A previous study found shorter CPT tolerance time in cases with mild to moderate Alzheimer, while supra-threshold pain ratings were lower in the same group (Jensen-Dahm et al., 2015). In our study, we cannot disentangle and explain the mechanistic underpinnings of lower CPT tolerance in stroke survivors.

5 | CONCLUSION

Participants with a history of stroke have lower pain tolerance. This association is present in stroke survivors both with and without chronic pain. This may have important clinical implications: these patients could be more sensitive to acute and procedural pain, and considering that many stroke patients have difficulty communicating

their symptoms, this calls for increased awareness among health professionals.

AUTHOR CONTRIBUTIONS

TA Melum executed the statistical analyses and drafted the article with supervision from CS Nielsen, EB Mathiesen and H Stigum. CS Nielsen, ÓA Steingrímsdóttir and A Stubhaug executed the collection of pain sensitivity data and EB Mathiesen was responsible for quality control of the stroke data from medical registries. AP Årnes assisted in the implementation of statistical models. All authors discussed the results and commented on the article. All authors have approved this article.

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CONFLICT OF INTEREST STATEMENT

None of the authors have declared any conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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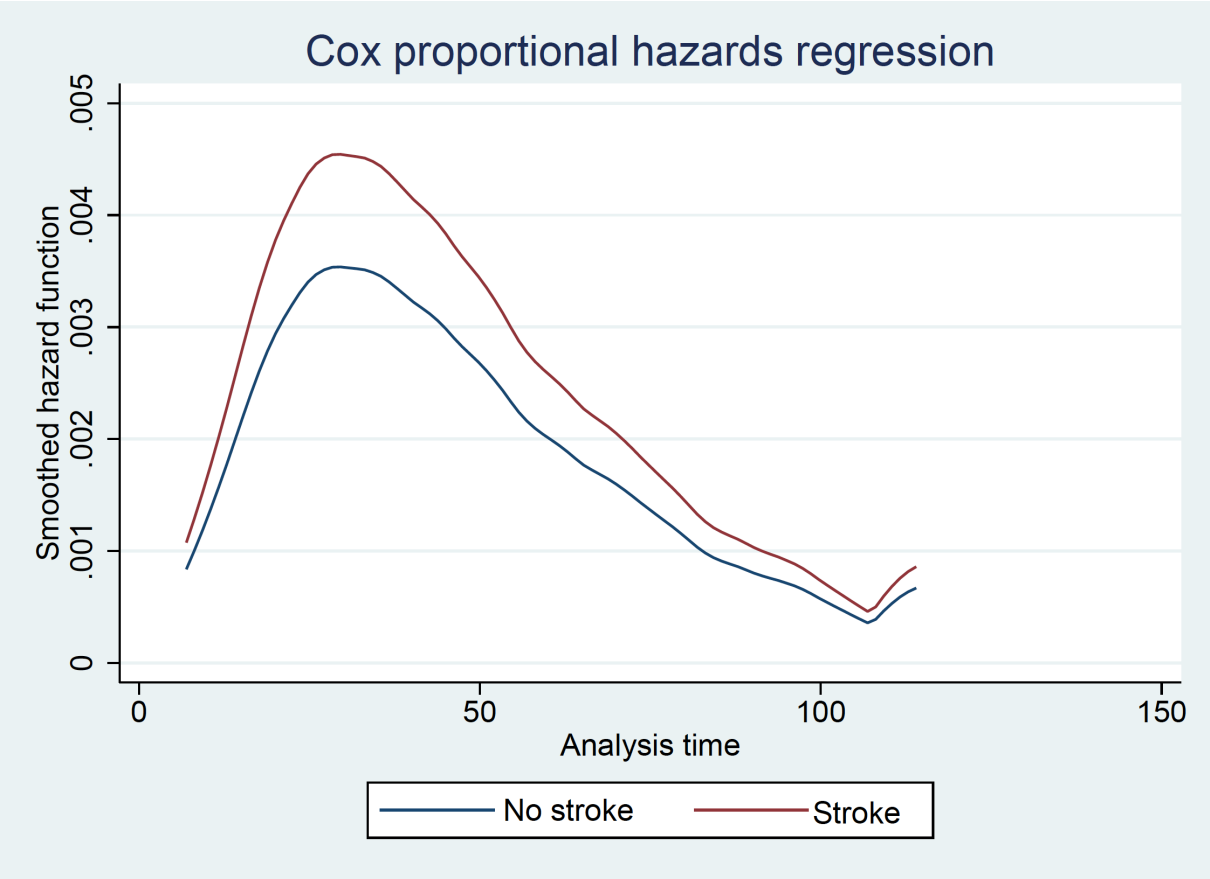
Supplementary Figure 1: Baseline hazard curve according to stroke status



Baseline Hazard curve according to stroke status, in the combined sample.
Cox regression model was fitted with time with hand in cold-water bath as outcome and stroke status as independent variable, adjusted for age, sex, diabetes, hypertension, hyperlipidemia, body mass index, smoking and study indicator (Tromsø 6 or Tromsø 7). Covariates were set to 0 (categorical variables) or minimum value in sample (continuous variables) for curve of baseline hazard.
The maximum time was 106 seconds in Tromsø 6, 120 seconds in Tromsø 7.

Appendix I

Links to papers I and III

Links to papers I and III

Paper I: Gray matter volume and pain tolerance in a general population: the Tromso study
https://journals.lww.com/pain/abstract/2023/08000/gray_matter_volume_and_pain_tolerance_in_a_general.12.aspx

Paper III: Pain tolerance after stroke: The Tromsø study
https://onlinelibrary.wiley.com/doi/10.1002/ejp.2124

Appendix II

Questionnaire 1 Tromsø 6

Tromsø-undersøkelsen

The form will be read electronically. Please use a blue or black pen
You can not use comas, use upper-case letters.

2007 - 2008 Confidential

HEALTH AND DISEASES

1 **How do you in general consider your own health to be?**

- ☐ Very good
- ☐ Good
- ☐ Neither good nor bad
- ☐ Bad
- ☐ Very bad

2 **How is your health compared to others in your age?**

- ☐ Much better
- ☐ A little better
- ☐ About the same
- ☐ A little worse
- ☐ Much worse

3 **Do you have, or have you had?**

	Yes	No	Age first time
Heart attack	☐	☐	
Angina pectoris	☐	☐	
Stroke/brain hemorrhage	☐	☐	
Atrial fibrillation	☐	☐	
High blood pressure	☐	☐	
Osteoporosis	☐	☐	
Asthma	☐	☐	
Chronic bronchitis/Emphysyma/COPD	☐	☐	
Diabetes mellitus	☐	☐	
Psychological problems *(for which you have sought help)*	☐	☐	
Low metabolism	☐	☐	
Kidney disease, *not including urinary tract infection (UTI)*	☐	☐	
Migraine	☐	☐	

4 **Do you have persistent or constantly recurring pain that has lasted for 3 months or more?**

☐ Yes ☐ No

5 **How often have you suffered from sleeplessness during the last 12 months?**

- ☐ Never, or just a few times
- ☐ 1-3 times a month
- ☐ Approximately once a week
- ☐ More that once a week

6 **Below you find a list of different situations. Have you experienced some of them in the last week (including today)?** (Tick once for each complaint)

	No complaint	Little complaint	Pretty much	Very much
Sudden fear without reason	☐	☐	☐	☐
You felt afraid or worried	☐	☐	☐	☐
Faintness or dizziness	☐	☐	☐	☐
You felt tense or upset	☐	☐	☐	☐
Easily blamed yourself	☐	☐	☐	☐
Sleeping problems	☐	☐	☐	☐
Depressed, sad	☐	☐	☐	☐
You felt useless, worthless	☐	☐	☐	☐
Feeling that life is a struggle	☐	☐	☐	☐
Feeling of hopelessness with regard to the future	☐	☐	☐	☐

USE OF HEALTH SERVICES

7 **Have you during the past year visited:**
If YES; how many times?

	Yes	No	No. of times
General practitioner (GP)	☐	☐	
Psychiatrist/psychologist	☐	☐	
Medical specialist outside hospital *(other than general practitioner/psychiatrist)*	☐	☐	
Physiotherapist	☐	☐	
Chiropractor	☐	☐	
Alternative medical practitioner *(homeopath, acupuncturist, foot zone therapist, herbal medical practitioner, laying on hands practitioner, healer, clairvoyant, etc.)*	☐	☐	
Dentist/dental service	☐	☐	

8 **Have you during the last 12 months been to a hospital?**

	Yes	No	No. of times
Admitted to a hospital	☐	☐	
Had consultation in a hospital without admission;			
At psychiatric out-patient clinic	☐	☐	
At another out-patient clinic	☐	☐	

9 **Have you undergone any surgery during the last 3 years?**

☐ Yes ☐ No

USE OF MEDICINE

10 **Do you take, or have you taken some of the following medications?** (Tick once for each line)

	Never used	Now	Earlier	Age first time
Drugs for high blood pressure	☐	☐	☐	
Lipid lowering drugs	☐	☐	☐	
Drugs for heart disease	☐	☐	☐	
Diuretics	☐	☐	☐	
Medications for osteoporosis	☐	☐	☐	
Insulin	☐	☐	☐	
Tablets for diabetes	☐	☐	☐	
Drugs for metabolism Thyroxine/levaxin	☐	☐	☐	

11 **How often have you during the last 4 weeks used the following medications?**(Tick once for each line)

	Not used the last 4 weeks	Less than every week	Every week, but not daily	Daily
Painkillers on prescription	☐	☐	☐	☐
Painkillers non-prescription	☐	☐	☐	☐
Sleeping pills	☐	☐	☐	☐
Tranquillizers	☐	☐	☐	☐
Antidepressants	☐	☐	☐	☐

12 **State the names of all medications -both those on prescription and non-prescription drugs- you have used regularly during the last 4 weeks.** Do not include vitamins, minerals, herbs, natural remedies, other nutritional supplements, etc.

If the space is not enough for all medications, use an additional paper of your own.

When attending the survey centre you will be asked whether you have used antibiotics or painkillers the last 24 hours. If you have, you will be asked to provide the name of the drug, strength, dose and time of use.

FAMILY AND FRIENDS

13 **Who do you live with?** (Tick for each question and give the number)

	Yes	No	Number
Spouse/cohabitant	☐	☐	
Other persons older than 18 years	☐	☐	
Persons younger than 18 years	☐	☐	

14 **Tick for relatives who have or have had**

	Parents	Children	Siblings
Myocardial infarction	☐	☐	☐
Myocardial infarction before 60 years	☐	☐	☐
Angina pectoris	☐	☐	☐
Stroke/brain haemorrhage	☐	☐	☐
Osteoporosis	☐	☐	☐
Stomach/duodenal ulcer	☐	☐	☐
Asthma	☐	☐	☐
Diabetes mellitus	☐	☐	☐
Dementia	☐	☐	☐
Psychological problems	☐	☐	☐
Drugs/substance abuse	☐	☐	☐

15 **Do you have enough friends who can give you help when you need it?**

☐ Yes ☐ No

16 **Do you have enough friends whom you can talk confidentially with?**

☐ Yes ☐ No

17 **How often do you normally take part in organised gatherings, e.g. sports clubs, political meetings, religious or other associations?**

☐ Never, or just a few times a year
☐ 1-2 times a month
☐ Approximately once a week
☐ More than once a week

WORK, SOCIAL SECURITY AND INCOME

18 **What is the highest level of education you have completed?** (Tick one)

☐ Primary, 1-2 years secondary school
☐ Vocational school
☐ High secondary school (A-level)
☐ College/university less than 4 years
☐ College/university 4 years or more

19 **What is your main occupation/activity?** (Tick one)

☐ Full time work ☐ Housekeeping
☐ Part time work ☐ Retired/benefit recipient
☐ Unemployed ☐ Student/military service

20 **Do you receive any of the following benefits?**

- ☐ Old-age, early retirement or survivor pension
- ☐ Sickness benefit (are in a sick leave)
- ☐ Rehabilitation benefit
- ☐ Full disability pension
- ☐ Partial disability pension
- ☐ Unemployment benefits
- ☐ Transition benefit for single parents
- ☐ Social welfare benefits

21 **What was the households total taxable income last year?** Include income from work, social benefits and similar

- ☐ Less than 125 000 NOK
- ☐ 125 000-200 000 NOK
- ☐ 201 000-300 000 NOK
- ☐ 301 000-400 000 NOK
- ☐ 401 000-550 000 NOK
- ☐ 551 000-700 000 NOK
- ☐ 701 000 -850 000 NOK
- ☐ More than 850 000 NOK

22 **Do you work outdoors at least 25% of the time, or in cold buildings (e.g. storehouse/industry buildings)?**

- ☐ Yes
- ☐ No

PHYSICAL ACTIVITY

23 **If you have paid or unpaid work, which statement describes your work best?**

- ☐ Mostly sedentary work *(e.g. office work, mounting)*
- ☐ Work that requires a lot of walking *(e.g. shop assistant, light industrial work, teaching)*
- ☐ Work that requires a lot of walking and lifting *(e.g. postman, nursing, construction)*
- ☐ Heavy manual labour

24 **Describe your exercise and physical exertion in leisure time. If you activity varies much, for example between summer and winter, then give an average. The question refers only to the last year.** (Tick the one that fits best)

- ☐ Reading, watching TV, or other sedentary activity.
- ☐ Walking, cycling, or other forms of exercise at least 4 hours a week *(here including walking or cycling to place of work, Sunday-walking, etc.)*
- ☐ Participation in recreational sports, heavy gardening, etc. *(note:duration of activity at least 4 hours a week)*
- ☐ Participation in hard training or sports competitions, regularly several times a week.

25 **How often do you exercise?** (With exercise we mean for example walking, skiing, swimming or training/sports)

- ☐ Never
- ☐ Less than once a week
- ☐ Once a week
- ☐ 2-3 times a week
- ☐ Approximately every day

26 **How hard do you exercise on average?**

- ☐ Easy- do not become short-winded or sweaty
- ☐ You become short-winded and sweaty
- ☐ Hard- you become exhausted

27 **For how long time do you exercise every time on average?**

- ☐ Less than 15 minutes
- ☐ 15-29 minutes
- ☐ 30-60 minutes
- ☐ More than 1 hour

ALCOHOL AND TOBACCO

28 **How often do you drink alcohol?**

- ☐ Never
- ☐ Monthly or more infrequently
- ☐ 2-4 times a month
- ☐ 2-3 times a week
- ☐ 4 or more times a week

29 **How many units of alcohol** (a beer, a glass of wine or a drink) **do you usually drink when you drink alcohol?**

- ☐ 1-2
- ☐ 3-4
- ☐ 5-6
- ☐ 7-9
- ☐ 10 or more

30 **How often do you drink 6 units of alcohol or more in one occasion?**

- ☐ Never
- ☐ Less frequently than monthly
- ☐ Monthly
- ☐ Weekly
- ☐ Daily or almost daily

31 **Do you smoke sometimes, but not daily?**

- ☐ Yes
- ☐ No

32 **Do you/did you smoke daily?**

- ☐ Yes, now
- ☐ Yes, previously
- ☐ Never

33 **If you previously smoked daily, how long is it since you stopped?**

Number of years ☐☐

34 **If you currently smoke, or have smoked before: How many cigarettes do you or did you usually smoke per day?**

Number of cigarettes ☐☐

35 **How old were you when you began smoking daily?**

Number of years ☐☐

36 **How many years in all have you smoked daily?**

Number of years ☐☐

37 **Do you use or have you used snuff or chewing tobacco?**

- ☐ No, never
- ☐ Yes, previously
- ☐ Yes, sometimes
- ☐ Yes, daily

DIET

38 **Do you usually eat breakfast every day?**

☐ Yes ☐ No

39 **How many units of fruits or vegetables do you eat on average per day?** (units means for example a fruit, a cup of juice, potatoes, vegetables)

Number of units ☐☐

40 **How many times per week do you eat hot dinner?**

Number ☐☐

41 **How often do you usually eat these products?**
(Tick once for each line)

	0-1 times/ mth	2-3 times/ mth	1-3 times/ week	4-6 times/ week	1-2 times/ day
Potatoes	☐	☐	☐	☐	☐
Pasta/rice	☐	☐	☐	☐	☐
Meat *(not processed)*	☐	☐	☐	☐	☐
Processed meat *(sausages/meatloaf/meatballs)*	☐	☐	☐	☐	☐
Fruits, vegetables, berries	☐	☐	☐	☐	☐
Lean fish	☐	☐	☐	☐	☐
Fat fish *(e.g. salmon, trout, mackerel, herring, halibut, redfish)*	☐	☐	☐	☐	☐

42 **How much do you normally drink the following?**
(Tick once for each line)

	Rarely/ never	1-6 glasses /week	1 glass /day	2-3 glasses /day	4 or more glasses /day
Milk, curdled milk, yoghurt	☐	☐	☐	☐	☐
Juice	☐	☐	☐	☐	☐
Soft drinks with sugar	☐	☐	☐	☐	☐

43 **How many cups of coffee and tea do you drink daily?** (Put 0 for the types you do not drink daily)

	Number of cups
Filtered coffee	☐☐
Boiled coffee (coarsely ground coffee for brewing)	☐☐
Other types of coffee	☐☐
Tea	☐☐

44 **How often do you usually eat cod liver and roe?** (i.e. "mølje")

☐ Rarely/never ☐ 1-3 times/year ☐ 4-6 times/year
☐ 7-12 times/year ☐ More than 12 times/year

45 **Do you use the following supplements?**

	Daily	Sometimes	No
Cod liver oil or fish oil capsules	☐	☐	☐
Omega 3 capsules *(fish oil, seal oil)*	☐	☐	☐
Vitamins and/or mineral supplements	☐	☐	☐

QUESTONS FOR WOMEN

46 **Are you currently pregnant?**

☐ Yes ☐ No ☐ Uncertain

47 **How many children have you given birth to?**

Number ☐☐

48 **If you have given birth, fill in for each child: birth year, birth weight and months of breastfeeding** (Fill in the best you can)

Child	Birth year	Birth weight in grams	Months of breastfeeding
1	☐☐☐☐	☐☐☐☐	☐☐
2	☐☐☐☐	☐☐☐☐	☐☐
3	☐☐☐☐	☐☐☐☐	☐☐
4	☐☐☐☐	☐☐☐☐	☐☐
5	☐☐☐☐	☐☐☐☐	☐☐
6	☐☐☐☐	☐☐☐☐	☐☐

49 **During pregnancy, have you had high blood pressure?**

☐ Yes ☐ No

50 **If yes, which pregnancy?**

☐ The first ☐ Second or later

51 **During pregnancy, have you had proteinuria?**

☐ Yes ☐ No

52 **If yes, which pregnancy?**

☐ The first ☐ Second or later

53 **Were any of your children delivered prematurely (a month or more before the due date) because of preeclampsia?**

☐ Yes ☐ No

54 **If yes, which child?**

1st child ☐ 2nd child ☐ 3rd child ☐ 4th child ☐ 5th child ☐ 6th child ☐

55 **How old were you when you started menstruating?**

Age ☐☐

56 **Do you currently use any prescribed drug influencing the menstruation?**

Oral contraceptives, hormonal IUD or similar ☐ Yes ☐ No

Hormone treatment for menopausal problems ☐ Yes ☐ No

When attending the survey centre you will get a questionnaire about menstruation and possible use of hormones. Write down on a paper the names of all the hormones you have used and bring the paper with you. You will also be asked whether your menstruation have ceased and possibly when and why.

Appendix III

Link to Questionnaire 2 Tromsø 6

Link to the second questionnaire in Tromsø 6:
https://uit.no/Content/531228/cache=20172908084211/Questionnaire_T6_2.pdf

Appendix IV

Questionnaire 1 Tromsø 7

The Tromsø Study
2015–2016

CONFIDENTIAL

The questionnaire will be optically read. Please, use blue or black inked pen only. Use block lettering. Refrain from the use of comma.

Date for filling in the questionnaire: ☐☐☐☐☐☐

HEALTH AND DISEASES

1.1 How do you in general consider your health to be?

Excellent	Good	Neither good nor bad	Bad	Very bad
☐	☐	☐	☐	☐

1.2 How is your health now compared to others of your age?

Excellent	Good	Neither good nor bad	Bad	Very bad
☐	☐	☐	☐	☐

1.3 Have you ever had, or do you have?
Tick once for each line.

	No	Yes, currently	Previously, not now	Age first time
High blood pressure	☐	☐	☐	☐☐
Heart attack	☐		☐	☐☐
Heart failure	☐	☐	☐	☐☐
Atrial fibrillation	☐	☐	☐	☐☐
Angina pectoris *(heart cramp)*	☐	☐	☐	☐☐
Cerebral stroke / brain haemorrhage	☐		☐	☐☐
Diabetes	☐	☐	☐	☐☐
Kidney disease, not including urinary tract infection *(UTI)*	☐	☐	☐	☐☐
Bronchitis / emphysema / COPD	☐	☐	☐	☐☐
Asthma	☐	☐	☐	☐☐
Cancer	☐	☐	☐	☐☐
Rheumatoid Arthritis	☐	☐	☐	☐☐
Arthrosis	☐	☐	☐	☐☐
Migraine	☐	☐	☐	☐☐
Psychological problems for which you have sought help	☐	☐	☐	☐☐

1.4 Do you have persistent or constantly recurring pain that has lasted for three months or more?

☐ No ☐ Yes

DENTAL HEALTH

2.1 How do you consider your own dental health to be?

	1	2	3	4	5	
Very bad	☐	☐	☐	☐	☐	Excellent

2.2 How satisfied or dissatisfied are you with your teeth or denture?

	1	2	3	4	5	
Very dissatisfied	☐	☐	☐	☐	☐	Very satisfied

USE OF HEALTH SERVICES

3.1 Have you during the past 12 months visited?

	Yes	No	Number of times
General practitioner *(GP)*	☐	☐	☐☐
Emergency room	☐	☐	☐☐
Psychiatrist / Psychologist	☐	☐	☐☐
Another medical specialist than a general practitioner *(GP)* or a psychologist or psychiatrist *(not at a hospital)*	☐	☐	☐☐
Dentist / dental services	☐	☐	☐☐
Pharmacy *(to buy / get advice about medicines / treatment)*	☐	☐	☐☐
Physiotherapist	☐	☐	☐☐
Chiropractor	☐	☐	☐☐
Acupuncturist	☐	☐	☐☐
CAM provider *(homeopath, reflexologist, spiritual healer etc.)*	☐	☐	☐☐
Traditional healer *(helper, "reader" etc.)*	☐	☐	☐☐
Have you during the past 12 months communicated with any of the services above by using the Internet?	☐	☐	☐☐

3.2 Have you over the past 12 months visited a hospital?

	Yes	No	Number of times
Hospital admission	☐	☐	☐☐
Visited an out-patient clinic:			
Psychiatric out-patient clinic	☐	☐	☐☐
Other out-patient clinics (not psychiatric department)	☐	☐	☐☐

USE OF MEDICIN

4.1 **Do you use or have you used?** *Tick once for each line.*

	Never	Now	Previously, not now	Age first time
Blood pressure lowering drugs	☐	☐	☐	
Cholesterol lowering drugs	☐	☐	☐	
Diuretics	☐	☐	☐	
Drugs for heart disease *(for example anticoagulants, antiarrhythmics, nitroglycerin)?*	☐	☐	☐	
Insulin	☐	☐	☐	
Tablets for diabetes	☐	☐	☐	
Drugs for hypothyroidism (Levaxin or thyroxine)?	☐	☐	☐	

4.2 **How often during the past four weeks have you used?** *Tick once for each line.*

	Not used in the past 4 weeks	Less than every week	Every week but not daily	Daily
Painkillers on prescription	☐	☐	☐	☐
Painkiller non-prescription	☐	☐	☐	☐
Acid suppressive medication	☐	☐	☐	☐
Sleeping pills	☐	☐	☐	☐
Tranquillizers	☐	☐	☐	☐
Antidepressants	☐	☐	☐	☐

4.3 **State the name of all medicines, both those on prescription and non-prescription drugs, you have used regularly during the last 4 weeks.** *Do not include nonprescription vitamin-, mineral- and food supplements, herbs, naturopathic remedies etc.*

If there is not enough space for all medicines, continue on a separate sheet.

DIET

5.1 **Do you usually eat breakfast every day?**

☐ No ☐ Yes

5.2 **How many units of fruit or vegetables do you eat on average per day?** *One unit is by example one apple, one salad bowl.*

Number of units

5.3 **How often do you eat these food items?** *Tick once for each line.*

	0–1 times per month	2–3 times per month	1–3 times per week	4–6 times per week	Once a day or more
Red meat (*All products from beef, mutton, pork)?*	☐	☐	☐	☐	☐
Fruits, vegetables, and berries?	☐	☐	☐	☐	☐
Lean fish *(Cod, Saithe)?*	☐	☐	☐	☐	☐
Fat fish *(salmon, trout, redfish, mackerel, herring, halibut)?*	☐	☐	☐	☐	☐

5.4 **How many glasses / containers of the following do you normally drink / eat?** *Tick once for each line.*

	Rarely / never	1–6 glasses per week	1 glass per day	2–3 glass per day	4 or more per day
Milk / Yogurt with probiotics (Biola, Cultura, Activia, Actimel, BioQ etc.)	☐	☐	☐	☐	☐
Fruit juice	☐	☐	☐	☐	☐
Soft drinks with sugar	☐	☐	☐	☐	☐
Soft drinks with artificial sweeteners	☐	☐	☐	☐	☐

5.5 **How many cups of coffee or tea do you usually drink daily?** *Put 0 for the types you <u>do not</u> drink daily.*

	Number of cups
Filtered coffee	
Boiled coffee / french plunger coffee *(coarsely ground coffee for brewing)*	
Instant coffee	
Cups of espresso-based coffee *(from coffee-machines, capsules etc.)*	
Black tea *(e.g. Earl Grey, Black currant)*	
Green tea / white tea / oolong tea	
Herbal tea *(e.g. rose hip tea, chamomile tea, Rooibos tea)*	

HEALTH ANXIETY

	Not at all	A little bit	Moderately	Quite a bit	A great deal
6.1 **Do you think there is something seriously wrong with your body?**	☐	☐	☐	☐	☐
6.2 **Do you worry a lot about your health?**	☐	☐	☐	☐	☐
6.3 **Is it hard for you to believe the doctor when he / she tells you there is nothing to worry about?**	☐	☐	☐	☐	☐
6.4 **Do you often worry about the possibility that you have a serious illness?**	☐	☐	☐	☐	☐
6.5 **If a disease is brought to your attention (e.g., on TV, radio, the internet, the newspapers, or by someone you know), do you worry about getting it yourself?**	☐	☐	☐	☐	☐
6.6 **Do you find that you are bothered by many different symptoms?**	☐	☐	☐	☐	☐
6.7 **Do you have recurring thoughts about having a disease that is difficult to be rid ofom?**	☐	☐	☐	☐	☐

PHYSICAL ACTIVITY

7.1 **If you are in paid or unpaid work, which statement describes your work best?** *Tick the most apprioate box.*

- ☐ Mostly sedentary work? *(e.g. office work, mounting))*
- ☐ Work that requires a lot of walking *(e.g. shop assistant, light industrial work, teaching)*
- ☐ Work that requires a lot of walking and lifting *(e.g. nursing, construction)*
- ☐ Heavy manual labour

7.2 **Describe your exercise and physical exertion in leisure time over the last year.** *If your activity varies throughout the year, give an average. Tick the most appropriate box.*

- ☐ Reading, watching TV / screen or other sedentary activity?
- ☐ Walking, cycling, or other forms of exercise at least 4 hours a week? (*including walking or cycling to place of work, Sunday-walking etc.)*
- ☐ Participation in recreational sports, heavy gardening, snow shoveling etc. at least 4 hours a week.
- ☐ Participation in hard training or sports competitions, regularly several times a week?

7.3 **During the last week, how much time did you spend sitting on a typical week or weekend day?** *E.g., at a desk, while visiting friends, while watching TV / screen.*

☐ Hours sitting on a weekday (both work and leisure hours*)*

☐ Hours on a weekend day

ALCOHOL

8.1 **How often do you drink alcohol??**

- ☐ Never
- ☐ Monthly or less frequently
- ☐ 2–4 times a month
- ☐ 2–3 times a week
- ☐ 4 or more times a week

8.2 **How many units of alcohol (1 beer, glass of wine or drink) do you usually drink when you drink alcohol?**

1–2	3–4	5–6	7–9	10 or more
☐	☐	☐	☐	☐

8.3 **How often do you have six or more units of alcohol in one occasion??**

- ☐ Never
- ☐ Less frequent than monthly
- ☐ Monthly
- ☐ Weekly
- ☐ Daily or almost daily

TOBACCO and SNUFF

9.1 **Do you / did you smoke daily?**

☐ Never ☐ Yes, now ☐ Yes, previously

9.2 **Have you used or do you use snuff or chewing tobacco daily?**

☐ Never ☐ Yes, now ☐ Yes, previously

QUESTIONS ABOUT CANCER

10.1 **Have you ever had**

	No	Yes	**If yes:** Age first time	**If yes:** Age last time
A mammogram	☐	☐		
Your PSA (Prostate Specific Antigen) level measured)	☐	☐		
A colon examination (colonoscopy, stool sample test)	☐	☐		

10.2 **Has anyone in your close <u>biological</u> family ever had**

	Children	Mother	Father	Maternal grandmother	Maternal grandfather	Paternal grandmother	Paternal grandfather	Aunt	Uncle	Sibling
Breast cancer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Prostate cancer	☐		☐		☐		☐		☐	☐
Colon cancer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

EDUCATION AND INCOME

11.1 **What is the highest levels of education you have completed?**
Tick one box only.

- ☐ Primary / partly secondary education. *(Up to 10 years of schooling)*
- ☐ Upper secondary education: *(a minimum of 3 years)*
- ☐ Tertiary education, short: College / university less than 4 years
- ☐ Tertiary education, long: College / university 4 years or more

11.2 **What was the household's total taxable income last year?**
Include income from work, social benefits and similar.

- ☐ Less than 150 000 kr
- ☐ 150 000–250 000 kr
- ☐ 251 000–350 000 kr
- ☐ 351 000–450 000 kr
- ☐ 451 000–550 000 kr
- ☐ 551 000–750 000 kr
- ☐ 751 000 –1 000 000 kr
- ☐ More than 1 000 000 kr

FAMILY AND FRIENDS

12.1 **Who do you live with?**

	Yes	No	Number
Spouse / partner	☐	☐	
Other persons over 18 years	☐	☐	
Persons under 18 years	☐	☐	

12.2 **Do you have enough friends who can give you help and support when you need it?**

☐ Yes ☐ No

12.3 **Do you have enough friends that you can talk confidentially with?**

☐ Yes ☐ No

12.4 **How often do you take part in organised gatherings, e.g., sports clubs, political meetings, religious or other associations?**

Never, or just a few times a year	1–2 times a month	Approximately once a week	More than once a week
☐	☐	☐	☐

WOMAN ONLY

13.1 **How old were you when you first started menstruating?**

Age

13.2 **Are you pregnant at the moment?**

☐ No ☐ Yes ☐ Uncertain

13.3 **How many children have you given birth to?**

Number

13.4 **If you have given birth, how many months did you breast-feed? Fill in for each child the birth year, birth weight and the number of months breast feeding.** *Fill in the best you can*

	Birth year	Birth weight in grams	Months of breastfeeding
Child 1			
Child 2			
Child 3			
Child 4			
Child 5			
Child 6			

MEN ONLY

14.1 **Have you ever had an inflammation of your prostate / urine bladder?**

☐ No ☐ Yes

14.2 **Have you ever had a vasectomy?**

☐ No ☐ Yes **If yes:** Which year was it

Thank you for your contribution.

Appendix V

Link to Questionnaire 2 Tromsø 7

Link to the second questionnaire in Tromsø 7:
https://uit.no/Content/709325/cache=20202011171303/FINAL Q2 translation20190307.pdf