Genetic factors associated with orthodontic pain in children and adolescents: a pilot study

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Acknowledgments

I write these acknowledgements with a heavy heart and a tear in the eye, as I park my large backside down within the Hogwarts Express for the final time. It seems surreal that my three-year journey at Hogwarts has finally come to an end.

Let’s kick off with the formalities. Thank you to everyone who has made this research project possible. A huge thank you to our inspiring teachers for so generously sharing their knowledge and passion for orthodontics with us. Thank you also to the administrative staff and dental assistants for always looking after us. A final thanks must go to my classmates, past and present, for being the best classmates that one could ask for. Life would have been so dull without the Harry Potters, librarians, Tiggers, walruses, snowies, and yellow snowies.

Whilst walking the corridors of Hogwarts late at night, hiding beneath Harry’s invisibility cloak, I overheard some students murmuring about the fact that all excellent acknowledgments sections should contain a quote. It would be rude not to heed good advice. Some wise man or woman drinking butterbeer at Hogsmeade once said something along the lines of, “Every action has an equal and opposite reaction”. This statement resonates with me because of its relevance not only to orthodontics and physics, but also to life in general.

That’s about all I have to say. I wish I had more time to give the acknowledgements section the justice that it deserved, but unfortunately, a completed version of this thesis was due right smack bang in the middle of our exam period. Perfect timing, as with everything in Hogwarts!

Dobby Potter signing out of Hogwarts, and back into the world of the Muggles.
Danke schön, xoxo
Abstract

Introduction: Pain is often reported as being the worst aspect of orthodontic treatment. Nearly all patients experience pain and discomfort at their teeth at some point during orthodontic treatment. Little information exists on the severity of pain in the latter stages of orthodontic treatment. In addition, no studies have investigated the role of genetic factors on pain caused by fixed appliances.

Objectives: To investigate whether demographic, clinical or genetic factors are associated with the severity of pain experienced following adjustment of fixed orthodontic appliances.

Methods: Eighty-two participants undergoing fixed orthodontic treatment were recruited. Baseline DNA was collected via blood or saliva samples. Immediately after bond-up or an adjustment of the fixed appliances, the participants used a smartphone app to record regular pain scores at their teeth over the following three days.

Results: Pain peaked approximately 19 hours after the orthodontic adjustment, then gradually returned toward baseline levels by day three. Pain on chewing was significantly greater than the resting pain at the teeth at all time points concerned. There was a significant difference in the total amount of pain at the teeth over the three days when comparing bond-ups to no arch wire changes (with or without bends placed). Gender, age, and time in treatment were not associated with the severity of pain experienced after an orthodontic adjustment. The rs931233 SNP of the HTR2A and the rs4646310 SNP of the COMT genes were significantly associated with pain severity. Haplotypes of the COMT gene also showed promising, although non-significant associations with pain severity.

Conclusions: Pain on chewing is significantly more painful compared to resting pain at the teeth after adjustment of fixed appliances. SNPs of the HTR2A and COMT gene were associated with the severity of pain following adjustment of fixed appliances. Therefore, it seems that genetic factors have a modifying effect on orthodontic pain (as is the case with many other pain conditions such as TMD, fibromyalgia, and experimental pain). Larger samples are required to investigate these associations further.
Overview

This research project focuses primarily on the clinical and genetic factors associated with orthodontic pain, and is divided into five main chapters that are organised as follows:

Chapter 1 – General introduction and review of the literature
A general overview of orthodontic pain and the genetics associated with various pain conditions are presented in the first chapter. This introductory chapter includes a review of the mechanism of orthodontic pain, the effect that pain has on patients, and the variables that are known to affect it. In addition, the genetics section focuses on the specific genetic factors associated with temporomandibular disorder, as well as the structure and physiology of the COMT gene and enzyme, one of the most commonly studied pain genes.

Chapter 2 – Core methods and materials
The methodological details of the present work are presented in the second chapter. The chapter covers aspects of study design, patient recruitment, app development and experimental procedure.

Chapter 3 – Ecological momentary assessment of pain profiles in adolescents undergoing orthodontic treatment
The orthodontic pain profile and limitations associated with previous pain research in orthodontics are briefly described. The methods section of this chapter includes a description of the specific methods used to measure and analyse pain at the teeth in the first three days following an orthodontic adjustment. Findings from this analysis are presented and discussed.

Chapter 4 – Genetic factors associated with orthodontic pain in adolescents
The role of genetic factors on TMD and general pain conditions are described briefly. The methods section of this chapter includes a description of the specific methods used in DNA collection and analysis. Finally, the findings of the genetic and pain analysis are presented and discussed.
Chapter 5 – Conclusions and future directions
The fifth and final chapter of this work highlights the study’s conclusions and the exciting avenues that could be investigated in the future.

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<th>Full Form</th>
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<tr>
<td>APS</td>
<td>Average Pain Sensitivity haplotype</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
</tr>
<tr>
<td>CAMK4</td>
<td>Calcium/Calmodulin-dependent protein kinase 4</td>
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<tr>
<td>CGRP</td>
<td>Calcitonin Gene-Related Peptide</td>
</tr>
<tr>
<td>CHRM2</td>
<td>Muscarinic cholinergic receptor 2</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>COMT</td>
<td>Catechol-O-Methyltransferase</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EMA</td>
<td>Ecological Momentary Assessment</td>
</tr>
<tr>
<td>GRK5</td>
<td>G protein-coupled receptor kinase 5</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-Pituitary-Adrenal system</td>
</tr>
<tr>
<td>HPS</td>
<td>High Pain Sensitivity haplotype</td>
</tr>
<tr>
<td>HTR2A</td>
<td>Serotonin 2A receptor</td>
</tr>
<tr>
<td>IFRD1</td>
<td>Interferon-related developmental regulator 1</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin-1β</td>
</tr>
<tr>
<td>IL-β2</td>
<td>Interleukin-β2</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>LPS</td>
<td>Low Pain Sensitivity haplotype</td>
</tr>
<tr>
<td>MAF</td>
<td>Minor Allele Frequency</td>
</tr>
<tr>
<td>MB-COMT</td>
<td>Membrane-bound catechol-O-methyltransferase</td>
</tr>
<tr>
<td>Met</td>
<td>Methionine</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
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<tr>
<td>NFP</td>
<td>Neurofilament protein</td>
</tr>
<tr>
<td>NiTi</td>
<td>Nickel-titanium</td>
</tr>
<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td>NR3C1</td>
<td>Glucocorticoid receptor</td>
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<tr>
<td>OHRQoL</td>
<td>Oral Health-Related Quality of Life</td>
</tr>
<tr>
<td>OPPERA</td>
<td>Orofacial Pain Prospective Evaluation and Risk Assessment</td>
</tr>
<tr>
<td>PDL</td>
<td>Periodontal ligament</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>S-COMT</td>
<td>Soluble catechol-O-methyltransferase</td>
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<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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<tr>
<td>SP</td>
<td>Substance P</td>
</tr>
<tr>
<td>TMD</td>
<td>Temporomandibular disorder</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour Necrosis Factor-α</td>
</tr>
<tr>
<td>Val</td>
<td>Valine</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual Analogue Scale</td>
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<tr>
<td>VIP</td>
<td>Vasoactive Intestinal Polypeptide</td>
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I. Literature review

1.1 Introduction
Orthodontic treatment can bring many positive benefits for patients, including improvements in dental appearance, facial appearance, and psychological well-being (Proffit et al., 2014). However, there are also several adverse effects of orthodontic treatment which patients must endure. Many patients report pain as being the worst aspect of orthodontic treatment (Oliver and Knapman, 1985). Pain is surprisingly common, with more than 90% of patients experiencing pain and discomfort at some point during their orthodontic treatment (Scheurer et al., 1996; Erdinc and Dinçer, 2004). It has been reported that the pain experienced following placement of an initial arch wire is usually more severe, and lasts for longer than the pain following premolar extractions (Jones and Chan, 1992). It is therefore not surprising that early pain experiences can trigger a small proportion of patients (8%) to discontinue their orthodontic treatment prematurely (Patel, 1989). Orthodontic fixed appliances have also been shown to reduce patients' oral health-related quality of life (OHRQoL) for a period of about one month following their insertion. Reasons for the reduction in OHRQoL included physical pain, psychological discomfort, and physical disability (Chen et al., 2010).

1.2 Definitions of pain and discomfort
The International Association for the Study of Pain defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Mersky and Bodguk, 1994). Pain functions as a warning signal, to alert the organism to tissue damage, thereby enabling the organism to avoid harm (Bergius et al., 2000). Discomfort is defined as “slight pain” (Oxford Dictionary, 2015). According to their definitions, pain and discomfort are simply two points on the same continuum. It stands to reason that discomfort is a mild form of pain, and that these two terms may be used interchangeably.
1.3 Mechanism of orthodontic pain

The act of applying forces to a tooth surface often results in pain which can be categorised as either an immediate or a delayed response. The immediate pain response is due to the compression of the periodontal ligament (PDL), while the delayed response is attributed to hyperalgesia of the PDL (Burstone, 1962). The application of orthodontic forces on teeth can cause pressure, ischaemia, inflammation and oedema in the PDL space, which ultimately results in pain (Furstman and Bernick, 1972). The mechanical forces on the teeth cause an acute inflammatory reaction within the PDL, leading to vasodilation and the release of inflammatory mediators. These inflammatory mediators are responsible for the hyperalgesia, causing the PDL to become more sensitive to pain (Yamasaki et al., 1984; Shanfeld et al., 1986; Nicolay et al., 1990; Saito et al., 1991; Vandesvka-Radunovic, 1999).

Thus, the PDL becomes more sensitive to algogens (pain-causing chemicals) like substance P, histamine, prostaglandin E, serotonin and bradykinin (Ferreira et al., 1978; Polat et al., 2005). These algogens then stimulate the afferent A-δ and C nerve fibres of the trigeminal nerve. Both the trigeminal nerve and the trigeminal nucleus caudalis are involved in processing orofacial sensory information (Krishnan, 2007). Action potentials from the first-order afferents enter the dorsal roots of the spinal cord and synapse with second-order neurons. The second-order neurons then ascend in the anterolateral tract and synapse with the third-order neurons in the thalamic nuclei. There are two nociception pathways: fast physiologic pain is conducted by the neospinothalamic system, while slow pathologic pain is conducted via the paleospinothalamic system (Skjelbred and Lökken, 1997). The third-order neurons in the thalamus then run to the cerebral cortex, where the pain is interpreted, and an appropriate response is prepared.

Neurons also play a part in the pain process. When afferent neurons are stimulated by an inflammatory reaction, there is an antidromic release of neuropeptides, which results in further inflammation. This process is known as “neurogenic inflammation” (Vandesvka-Radunovic, 1999). Neuropeptides such as neurofilament protein (NFP), calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), and substance P (SP) have all been implicated in this process. SP, released from sensory peripheral nerve endings, causes monocytes to secrete cytokines like interleukin-1β (IL-1β), IL-6 and tumour necrosis factor-α (TNF-α), which elicit acute or chronic inflammation, as
well as bone resorption (Nicolay et al., 1990; Norevall et al., 1995). In support of this theory, markedly increased levels of IL-1 and TNF have been found in the PDL and alveolar bone cells of cat canines which had been moved orthodontically (Davidovitch et al., 1988; Davidovitch, 1991). These biochemical mediators are also released in the gingival crevicular fluid of humans following the placement of elastic separators. Interestingly, the intensity of pain one hour after placement of separators was correlated with the levels of prostaglandin E2, while the intensity of pain one day following placement of separators was associated with IL- β2 levels (Giannopoulou et al., 2006).

1.4 Impact of pain and discomfort on jaw function
There have been several studies which have investigated the onset of pain following the application of fixed appliances. It is generally accepted that pain begins a few hours after an orthodontic force is applied, peaks around 24 hours, lasts for between three to five days, and returns to baseline levels after about seven days (Ngan et al., 1989; Jones and Chan, 1992).

After 24 hours, 94% of patients reported pain from their orthodontic appliances (transpalatal arch, partial appliances, upper and/or lower fixed appliances). For those reporting pain, a mean pain score of 42 out of 100 (range of 0-100, no standard deviation given) on the visual analogue scale (VAS) has been reported (Scheurer et al., 1996). Therefore, orthodontic pain caused by fixed appliances can be regarded as significant, and of moderate intensity for the average patient. During orthodontic treatment, patients describe feeling tension, pressure, soreness of teeth, and pain. The most severe pain is induced by incising and chewing food, with VAS scores approaching 50-55 as early as four hours following the placement of an initial arch wire (Ngan et al., 1989). Because of the pain evoked during incising and chewing, many patients struggle to bite and chew food of a firm or hard consistency. As a result, these patients are forced to change the consistency of their diet for a few days (Krishnan, 2007).

In addition, studies have demonstrated that orthodontic pain arising from the periodontal nociceptors can effect changes in motor neuron output, which results in a decrease in the activity of jaw-closing muscles such as the masseter and temporalis. It is hypothesised that these changes are mediated in the brain stem, and act as a protective mechanism to prevent further damage to the injured part of the masticatory system (Goldreich et al., 1994;
Michelotti et al., 1999).

It is believed that patients adapt to the continuous pain and discomfort of orthodontic treatment, because the sensations soon cease or disappear from their focus of attention (Gosney, 1985; Jones and Chan, 1992). Indeed, the pain experienced from fixed appliances has been shown to cause disturbances in a patient’s sleep pattern immediately after placement, with significant improvements over the following days (Scheurer et al., 1996). One explanation for this observation is that patients adapt to their new appliances within the first seven days, with most patients reporting significant decreases in the amount of perceived pressure, tension, sensitivity of teeth, and pain, after as little as three to five days following the insertion of appliances. Assuming that forces stay relatively constant within the first seven days of appliance insertion, the adaptation to pain and discomfort may be due to changes in perception of adverse stimulation by the patients (Sergl et al., 1998).

1.5 Variables that affect orthodontic pain

1.5.1 Sociodemographic variables

There is a huge variation in pain response among individuals undergoing orthodontic treatment. Some patients experience high levels of pain, while others experience only mild discomfort (Ngan et al., 1989; Bergius et al., 2008). The sensory reaction to pain is complex, partly because pain is modulated by the higher centres of the nervous system (Polat, 2007).

The vast majority of studies on orthodontic pain do not show any sex-related differences in pain perception (Ngan et al., 1989; Jones and Chan, 1992; Fernandes et al., 1998; Sergl et al., 1998; Erdinc and Dinçer, 2004; Scott et al., 2008; Abdelrahman et al., 2015; Rahman et al., 2015). However, there are some studies that report greater levels of orthodontic pain among females (Scheurer et al., 1996; Bergius et al., 2002). In terms of general, experimentally induced pain, women are more likely to report pain of greater severity and longer duration than men. It is thought that males may under-report the level of pain they have experienced due to social and cultural pressures which leads them to minimise any outward display of distress or discomfort (Riley III et al., 1998).

It is very difficult to determine whether age has any influence on the perceived severity of pain during orthodontic treatment, since adolescents and adults may be treated with different protocols. Some studies have found that younger patients experience less pain
than older patients (Jones, 1984; Jones and Richmond, 1985; Jones and Chan, 1992; Fernandes et al., 1998). However, numerous studies have also demonstrated that there is no relationship between age and the severity of pain experienced during orthodontic treatment (Ngan et al., 1989; Scott et al., 2008; Abdelrahman et al., 2015; Rahman et al., 2015). One study has even reported that adolescents (patients between 14 and 17 years of age) reported the highest intensities of pain during orthodontic treatment when compared to pre-adolescents and adults (Brown and Moerenhout, 1991). Overall, there seems to be no consensus regarding the effects of age on pain perception in the orthodontic literature.

1.5.2 Psychological variables

The psychological well-being of patients has a significant impact on the severity of perceived pain (Brown and Moerenhout, 1991; Jones and Chan, 1992). Stress can play a part in the perception of pain. Momentary stress responses were designed as responses to aid in survival. However, prolonged stress, particularly of the mental variety, can have adverse effects on an individual by increasing sympathetic nervous activity in the absence of a physical threat. There is some evidence of a correlation between stress and the frequency/severity of perceived pain (Korszun, 2002; Okeson, 2005). Anxiety has also been shown to play a part in pain perception by lowering the pain threshold so that normal painless impulses are perceived as painful (Litt, 1996). Restorative procedures are reported to evoke greater levels of pain among highly anxious patients (Klages et al., 2006). Interestingly, simple dental procedures, such as the placement of orthodontic separators, have also been shown to be associated with greater pain levels among dentally anxious patients (Beck et al., 2014).

It has been suggested that individuals with greater concerns about the severity of their malocclusion perceive lower intensities of pain and discomfort in the seven days following appliance insertion (Sergl et al., 1998). In addition, patients who demonstrated low motivation for orthodontic treatment experienced higher levels of orthodontic pain after placement of elastic separators (Bergius et al., 2008). However, conflicting evidence exists regarding the relationship between the perceived severity of a malocclusion and the reported levels of pain during orthodontic treatment (Bergius et al., 2002).
Catastrophising has been shown to be associated with high-pain responders in a study utilising elastic separators to induce orthodontic pain. In particular, individuals with higher “magnification” scores (one part of the Pain Catastrophising Scale) experienced higher levels of pain. Magnification refers to “a person’s likelihood to exaggerate the threat value or seriousness of pain sensations” (Beck et al., 2014). The results from this study suggest that cortical pain processing may play an important role in pain perception, demonstrating that certain psychological characteristics can influence pain severity. Another study has shown that expectations of lower pain resulted in reduced levels of perceived pain, while expectations of higher pain resulted in amplified pain levels (Koyama et al., 2005).

1.5.3 Clinical variables
Several orthodontic studies have failed to demonstrate any significant association between the type of arch wire used at bond-up and the amount of pain experienced. No significant differences in perceived pain have been found between a superelastic and conventional NiTi arch wire (Fernandes et al., 1998), a NiTi and multistranded steel arch wire (Jones and Chan, 1992), a 0.014” and 0.016” NiTi arch wire (Erdinc and Dincer, 2004), and 0.014” superelastic, thermoelastic and conventional NiTi wires (Abdelrahman et al., 2015). In addition, no relationship between the severity of reported pain and the amount of anterior or overall crowding has been found (Jones and Richmond, 1985).

1.6 Measuring orthodontic pain and discomfort
Pain is a subjective phenomenon, which is best assessed using a self-report approach. The VAS is the most commonly used method to measure orthodontic pain (Ngan et al., 1989; Jones and Chan, 1992; Scheurer et al., 1996; Bergius et al., 2000). It is a non-verbal measure, and consists of a line which is usually 10 cm long. Each end of the scale represents the limits of the pain experience. For example, the left end might be labelled “no pain”, while the right end is labelled “severe pain” (Duncan et al., 1989). Respondents are asked to place a vertical mark on the line corresponding to the level of pain they are experiencing. The distance of the mark from the left end of the scale is measured, and represents the severity of pain. The VAS is designed to offer the respondent a rating scale that has minimal constraints. The main advantage of this is that it provides respondents with the freedom to choose the exact intensity of pain that they are experiencing (Linacre, 1998). The VAS is easy for most patients to grasp, and children aged five and above are able to use it (McGrath, 1990). Some of the strengths of the VAS include its ability to discriminate
between small increments of pain intensity (Seymour, 1982; Langley and Sheppeard, 1983), its sensitivity to measuring successive responses to treatment (Melzack and Torgerson, 1971), as well as its reproducibility (Scott and Huskisson, 1979) and reliability (Revill et al., 1976).

1.6.1 Limitations associated with measuring orthodontic pain
The vast majority of studies which have investigated orthodontic pain have utilised paper-based surveys to record participants' pain at various time intervals following orthodontic arch wire adjustments (Jones and Chan, 1992; Scheurer et al., 1996; Erdinc and Dincer, 2004; Fleming et al., 2009; Benson et al., 2012; Woodhouse et al., 2015). This approach has inherent limitations because pain surveys are often filled in retrospectively, and the memory of pain may be inaccurate, as memory recall is not always reliable (Bradburn et al., 1987). Furthermore, during paper-based surveys, participants are able to look back at their previous pain scores, which could influence their current and future pain ratings. Some studies have attempted to eliminate participants being able to look at their past pain scores by calling patients at the required times to ask about pain scores (Bergius et al., 2008), as well as asking patients to mail back their pain scores after the completion of each survey (Scheurer et al., 1996; Bergius et al., 2002).

1.7 Genetics associated with pain
The perception of pain is an incredibly complex process which is influenced by environmental and genetic factors (Mogil, 1999). In recent times, there has been increasing interest in the role of genetic factors that influence pain perception. To date, no studies have investigated whether or not there are associations between certain genetic polymorphisms and the intensity of pain experienced by patients undergoing orthodontic treatment. However, there is ample evidence that single nucleotide polymorphisms (SNPs) of certain genes can influence the severity of pain caused by fibromyalgia and experimental pain, and can even contribute to temporomandibular disorders (TMD).

1.7.1 Genetics and experimental pain
Given the lack of studies investigating the genetic basis of orthodontic pain, there is a need to consider other similar models in order to identify selected candidate genes. Several haplotypes, which refer to a set of single nucleotide polymorphisms (SNPs) on one
chromosome that tend to occur together, have been identified in previous studies focusing on experimental pain and TMD. Catechol-O-methyltransferase (COMT) is one of the most widely studied genes involved in human pain. COMT catalyzes the degradation of catecholamines such as dopamine, adrenaline and noradrenaline, and as such, is involved in pain processing (Belfer and Segall, 2011). The three most common COMT haplotypes are termed high pain sensitivity (HPS), average pain sensitivity (APS) and low pain sensitivity (LPS). The four SNPs of the COMT gene that contribute to these COMT haplotypes include rs6269, rs4633, rs4818 and rs4680. These COMT haplotypes were named according to their association with sensitivity to experimental pain in humans. The experimental pain consisted of pressure pain thresholds, thermal pain thresholds and tolerances, as well as ischaemic pain thresholds and tolerances. Some 11% of the variation in perception of experimental pain in a sample of 202 healthy females could be attributed to combinations of these three common haplotypes (Diatchenko et al., 2005).

1.7.2 Genetics and TMD
Numerous studies have investigated the association between genetics and TMD. The role of several COMT haplotypes on the incidence of TMD was recently investigated in a three-year prospective study involving 202 women. Females who had only HPS and/or APS haplotypes had a 2.3-fold greater risk of developing TMD when compared to females who had one or two LPS haplotypes (Diatchenko et al., 2005).

Interestingly, in an extension of this study, approximately a quarter of the patients with pain-sensitive haplotypes of the COMT gene with a history of orthodontic treatment developed TMD during this three-year period. However, none of the 20 patients with pain-sensitive haplotypes who did not have a history of orthodontic treatment developed TMD during these three years. Thus, orthodontic treatment may increase the risk of developing TMD in patients with pain-sensitive haplotypes of the COMT gene – this represents a gene-environment interaction. In patients with pain-resistant haplotypes, orthodontic treatment was not associated with an increase in risk of developing TMD (Slade et al., 2008).

The Orofacial Pain Prospective Evaluation and Risk Assessment (OPPERA) project carried out a large case-control study in America, which included 348 patients with chronic TMD and 1612 controls. Some 3295 SNPs from 358 genes implicated in human pain perception
were tested. Nine SNPs from six different genes were associated with TMD: rs2963155, rs9324918 and rs33389 SNPs of the glucocorticoid receptor (NR3C1) gene, the rs9316233 SNP of the serotonin 2A receptor (HTR2A) gene, the rs7800170 SNP of the muscarinic cholinergic receptor 2 (CHRM2) gene, the rs3756612 and rs10491334 SNPs of the calcium/calmodulin-dependent protein kinase 4 (CAMK4) gene, the rs728273 SNP of the interferon-related developmental regulator 1 (IFRD1) gene, and the rs12415832 SNP of the G protein-coupled receptor kinase 5 (GRK5) gene (Smith et al., 2011). This study also tested the association between COMT haplotypes and TMD. Participants with HPS haplotypes had an increased risk of TMD (OR = 1.28, P = 0.05). Interestingly, there was no significant difference between the APS and LPS haplotypes in this case-control study.

The NR3C1 gene, located on chromosome five, codes for the glucocorticoid receptor, which is a binding site for cortisol. The glucocorticoid receptor is a significant component of the hypothalamic-pituitary-adrenal (HPA) system, the primary endocrine stress axis in humans. This system has been previously been implicated in the development of TMD (Korszun et al., 2002). The OPPERA project found that the rs2963155 SNP, located in the long intron of the NR3C1 gene, was associated with TMD (OR = 0.62, P = 6.15 x 10^-5). The minor allele is G, and the minor allele frequency (MAF) is about 20-24% in both Caucasians and African-Americans. The minor G allele had a protective effect against TMD (Smith et al., 2011).

The HTR2A gene, located on chromosome 13, codes for a serotonin receptor. The serotonergic system is known to influence both nociceptive and affective pathways. The OPPERA project found that the rs9316233 SNP, an intronic polymorphism, was also associated with TMD (OR = 0.64, P = 3.44 x 10^-4). The minor allele is G, and the MAF is about 30%. Interestingly, the minor G allele was protective against TMD in females, but not in males. For white females, the OR was 0.39, P = 2.3 x 10^-4 (Smith et al., 2011). An earlier study found that rs6313, a synonymous polymorphism located on exon one of the HTR2A gene, was associated with TMD (Mutlu et al., 2002).

A smaller case-control study conducted in Italy recruited 50 patients affected by TMD and 132 controls. The entire COMT gene was sequenced for all of these participants. Some 40 COMT variants were found, 18 of which were novel discoveries. The SNPs rs1655656,
rs165722 and rs4646310 were found significantly more frequently in TMD patients when compared to the controls. All of these SNPs are located in the promoter region of the COMT gene. The SNPs rs1655656 and rs4646310 were novel discoveries - patients with the CC genotype of rs1655656 were 5.3 times more likely to suffer from TMD when compared to the patients with the GG genotype, while patients with the AG genotype of rs4646310 were 2.6 times more likely to suffer from TMD when compared to patients with the GG genotype (Michelotti et al., 2014). It has been proposed that the SNPs located in the promoter region of the COMT gene could alter DNA transcription, RNA splicing, mRNA stability, or mRNA transport and translation, ultimately leading to an increased risk of TMD (Nackley et al., 2006). Intriguingly, this study did not report on the effect of the three most common COMT haplotypes, as mentioned previously, on the risk of TMD.

1.8 Catechol-O-methyltransferase (COMT)

Catechol-O-methyltransferase (COMT) is one of the most widely studied genes involved in human pain (Belfer and Segall, 2011). COMT is an enzyme that transfers a methyl group from S-adenosyl methionine to a catechol substrate. This results in the production of S-adenosyl-L-homocysteine, as well as an O-methylated catechol (Axelrod and Tomchick, 1958; Guldberg and Marsden, 1975). COMT catalyzes the degradation of catecholamines such as dopamine, adrenaline and noradrenaline. These neurotransmitters are involved in a vast array of physiological processes such as cognition, cardiovascular function, stress response, and pain processing (Belfer and Segall, 2011). COMT is involved in the regulation of extracellular catecholamine concentrations, especially in the prefrontal brain region and peripheral tissues. The concentration of catecholamines can affect the pain tract at multiple levels (Tammimaki and Manniesto, 2012).

The COMT gene codes for two COMT protein isoforms – soluble COMT (S-COMT) and membrane-bound COMT (MB-COMT) (Salminen et al., 1990; Lundstrom, 1991). The COMT gene is located on the long arm of chromosome 22, at the gene map locus of 22q11.2. It spans about 27kb, and contains two promoters. These two promoters direct the synthesis of two distinct transcripts:

1) The most distal 5’ promoter (P2) regulates the synthesis of a 1.5kb transcript, which encodes both MB-COMT (271 amino acids) and S-COMT (221 amino acids) (Tenhunen et al., 1993; Tenhunen et al., 1994).
2) The second promoter (P1) is located between two separate ATG (translation initiation) codons in exon 3. P1 regulates the synthesis of a 1.3kb transcript, which encodes only S-COMT (Tenhunen and Ulmanen, 1993; Tenhunen et al., 1994).

Translation of MB-COMT and S-COMT are initiated from two separate ATG translation initiation codons in exon 3 (Berrocci et al., 1991; Lundstrom et al., 1991). The sequence downstream of the S-COMT start codon is identical in both P1 and P2. The COMT gene contains a single translation stop codon, located in exon 6. A diagram of the structure of the COMT gene is displayed below in Figure 1.1.

Numerous splice variants of the 1.5kb COMT transcript have been found in the human brain (Tunbridge et al., 2007). Currently, the functional significance of these splice variants is unknown. However, the detection of their presence adds further complexity to COMT biology.

Figure 1.1 Source: Andersen and Skorpen, 2009. Structure of the human COMT gene, but not to scale. The line represents introns, while the boxes represent exons. The approximate sizes of introns and exons are indicated. Filled boxes correspond to protein coding regions. White boxes are untranslated regions. Translation start codons for MB-COMT (MB-ATG) and S-COMT (S-ATG) are indicated, as are the two promoters, P1 and P2. The TGA stop codon is located in exon 6 (Tenhunen et al., 1994; Weinshilboum, 2006).

In the brain, the ratio of MB-COMT to S-COMT enzyme is about 70:30. However, in peripheral tissues, the S-COMT form of the enzyme is predominant (Tenhunen et al., 1994; Chen et al., 2004). There are high levels of the 1.5kb COMT transcript (which encodes both the MB-COMT and S-COMT enzymes) and very low levels of S-COMT transcripts within the brain, demonstrating the importance of the 1.5kb transcripts for translation of both the COMT isoforms. The MB-COMT enzyme has a much higher affinity
(approximately ten-fold) for dopamine and adrenaline when compared with the S-COMT enzyme (Lotta et al., 1995). Therefore, MB-COMT is better suited to metabolise the concentrations of catecholamines found in the brain (Roth, 1992). COMT has been detected in a number of cell types located within the brain: these include non-neuronal, glial and neuronal cells (Kaplan et al., 1979; Karhunen et al., 1994; Kastner et al., 1994). The prefrontal cortex and striatum neurons seem to be the main cell populations in the brain that express COMT (Matsumoto et al., 2003). In vitro studies utilising cultured cells with overexpression of COMT have demonstrated that MB-COMT is associated with intracellular membranes, for example, the rough endoplasmic reticulum. S-COMT is located in the nucleus and cytoplasm (Ulmanen et al., 1997). However, it is not known whether this in vitro situation is representative of what actually occurs in vivo.

1.8.1 COMT activity and pain in animal studies

Numerous studies have investigated the effects of altering COMT activity on pain, using genetically modified mice. These studies have used COMT knockout mice, as well as transgenic mice that overexpress the Val158 (high COMT activity) variant of the human COMT gene in the prefrontal cortex. The homozygous COMT knockout mice (with no COMT activity) had increased pain sensitivity, while the transgenic mice with overexpression of the Val158 variant had reduced sensitivity to painful stimuli (Papaleo et al., 2008). In addition, when COMT inhibitors were administered to mice and rats, there was an increase in experimental pain sensitivity (Diatchenko et al., 2005; Nackley et al., 2007; Kambur et al., 2010).

1.8.2 Regulation of catecholamine concentrations in the brain (derived from animal studies)

There are regions within the brain that contain a high dopamine transporter density, for example, the striatum. In these high dopamine transporter regions, the primary method of terminating the dopamine signal is by uptake of dopamine into the dopamine nerve terminals via dopamine transporters. Therefore, COMT plays a minor role in terminating dopamine signals in these regions (Cass et al., 1993; Giros et al., 1996; Moron et al., 2002; Eisenhofer et al., 2004). However, there are areas in the brain with a low dopamine transporter density, for example, in the prefrontal cortex (Sesack et al., 1998). In these regions, the dopamine signal is terminated by dopamine uptake into postsynaptic neurons or non-neuronal cells (Wilson et al., 1988; Trendelenburg, 1990; Manniesto et al., 1992). After uptake into these cells, the dopamine is metabolised by COMT (Matsumoto et al.,
Thus, areas which rely on COMT for termination of dopamine signals, such as the prefrontal cortex, are affected by variations in COMT activity (Cass et al., 1993; Giros et al., 1996; Moron et al., 2002; Eisenhofer et al., 2004; Yavich et al., 2007; Kaenmaki et al., 2010). It therefore stands to reason that COMT is likely to play a key role in the modulation of dopamine neurotransmission in the prefrontal cortex.

1.8.3 COMT genetic variation

The COMT gene locus contains numerous single nucleotide polymorphisms with minor allele frequency greater than 1%. Most of these SNPs are located in noncoding regions, or if they are located in coding regions, do not alter the amino acid sequence of the COMT protein. It must be noted that SNPs in noncoding regions, or silent SNPs, can have significant effects on processes such as DNA transcription, RNA splicing, mRNA stability, as well as mRNA transport and translation (Andersen and Skorpen, 2009).

Genetic variation in the COMT gene has been associated with several different pain conditions. These include differences in pain perception caused by experimental pain stimuli (Diatchenko et al., 2005) and variable susceptibility to common pain conditions such as fibromyalgia (Gursoy et al., 2003; Vargas-Alarcon et al., 2007; Tammimaki and Manniesto, 2012), migraine (Emin Erdal et al., 2001) and TMD (Diatchenko et al., 2005) to name a few. A more comprehensive table of COMT SNPs and their associated pain conditions is listed in Table 1.1.

The most studied SNP in the COMT gene is rs4680. This polymorphism results in a substitution from valine (Val) to a methionine (Met) at codon 158 for MB-COMT, and codon 108 for S-COMT. The consequence of this amino acid substitution is an enzyme with a lower thermostability, ultimately resulting in a dramatic three to four-fold decrease in the activity of the COMT enzyme (Lotta et al., 1995). The Val158Met polymorphism therefore governs the level of the COMT activity (Lotta et al., 1995; Chen et al., 2004), and has been associated with sustained muscular pain (Zubieta et al., 2003), experimental pain (Diatchenko et al., 2006), headache (Hagen et al., 2006), migraine (Emin Erdal et al., 2001), and fibromyalgia (Gursoy et al., 2003; Vargas-Alarcon et al., 2007).

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<table>
<thead>
<tr>
<th>SNP</th>
<th>Study</th>
<th>Number of participants</th>
<th>Type of pain</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4680</td>
<td>Zubieta et al. 2003</td>
<td>29</td>
<td>Sustained muscular pain</td>
<td>Met allele is associated with pain ratings as well as reduced activation of µ-opioid system</td>
</tr>
<tr>
<td></td>
<td>Diatchenko et al., 2006</td>
<td>202</td>
<td>Experimental pain</td>
<td>rs4680 associated with temporal summation of heat pain left</td>
</tr>
<tr>
<td></td>
<td>Hagen et al., 2006</td>
<td>2451</td>
<td>Headache</td>
<td>Val/Val genotype had lower prevalence of non-migrainous headache</td>
</tr>
<tr>
<td></td>
<td>Emin Erdal et al., 2001</td>
<td>126*</td>
<td>Migraine</td>
<td>Met allele (homozygous and heterozygous) associated with migraine</td>
</tr>
<tr>
<td></td>
<td>Vargas-Alarcon et al., 2007</td>
<td>248*</td>
<td>Fibromyalgia</td>
<td>rs4680 associated with fibromyalgia in Spaniards, but not Mexicans</td>
</tr>
<tr>
<td></td>
<td>Gursoy et al., 2003</td>
<td>122*</td>
<td>Fibromyalgia</td>
<td>Val/Met and Val/Val genotypes associated with fibromyalgia</td>
</tr>
<tr>
<td></td>
<td>rs6269</td>
<td>Diatchenko et al., 2005</td>
<td>202 Experimental pain (thermal, pressure, ischaemic pain)</td>
<td>rs6269 associated with experimental pain</td>
</tr>
<tr>
<td></td>
<td>Kim et al., 2006</td>
<td>735</td>
<td>Heat and cold pain</td>
<td>rs6269 weakly associated with cold pain sensitivity</td>
</tr>
<tr>
<td></td>
<td>Vargas-Alarcon et al., 2007</td>
<td>248*</td>
<td>Fibromyalgia</td>
<td>rs6269 associated with fibromyalgia in Spaniards, but not Mexicans</td>
</tr>
<tr>
<td></td>
<td>rs4818</td>
<td>Diatchenko et al., 2005</td>
<td>202 Experimental pain (thermal, pressure, ischaemic pain)</td>
<td>rs4818 associated with experimental pain</td>
</tr>
<tr>
<td></td>
<td>George et al., 2008</td>
<td>58</td>
<td>Shoulder pain</td>
<td>High pain catastrophising and COMT APS/HPS diplotype associated with increased pre and post-op persistent pain</td>
</tr>
<tr>
<td></td>
<td>rs740603</td>
<td>Kim et al., 2006</td>
<td>Third molar extraction</td>
<td>rs740603 associated with third molar extraction pain</td>
</tr>
<tr>
<td></td>
<td>rs4646312</td>
<td>Kim et al., 2006</td>
<td>Heat and cold pain</td>
<td>rs4646312 weakly associated with cold pain sensitivity</td>
</tr>
</tbody>
</table>

* case control study
A recent study has investigated the complex pattern of variation and linkage disequilibrium (nonrandom allelic association) across the COMT gene in 45 human populations using 28 SNPs. Common haplotypes (sets of associated SNP alleles) from diverse evolutionary lineages potentially harbour undetected variants with functional consequences (Mukherjee et al., 2010). Linkage disequilibrium analysis has revealed that in the case of most human populations, COMT contains three haploblocks. In most instances, three to four common haplotypes cover the entire genetic diversity across each haploblock (Gabriel et al., 2002).

As described previously, Diatchenko and colleagues have described three common haplotypes which account for 96% of all haplotypes observed (Diatchenko et al., 2005). These haplotypes consist of four SNPs – rs6269, rs4633, rs4818 and rs4680. The three haplotypes were named low pain sensitivity (LPS), average pain sensitivity (APS) and high pain sensitivity (HPS), with up to a 20-fold difference in COMT activity between the LPS and HPS haplotypes. The APS haplotype containing the Met158 allele correlated with intermediate activity of the COMT enzyme. The Val158 allele correlated with either high or low COMT enzyme activity, depending on the three other SNP alleles that contribute to the haplotype. This occurs via alteration of the mRNA secondary structure, which either permits or restricts the translation of COMT (Nackley et al., 2006).

The secondary structure is the two-dimensional pairing of nucleotides within an RNA sequence, which results in characteristic folds, stems and loops, which ultimately determine the rate of mRNA translation into protein. Despite both the HPS and LPS haplotypes containing the Val158 allele (high COMT activity), the HPS haplotype (low COMT activity) has restricted translation of COMT, which is believed to be because of its long, rigid stem loops that inhibit protein synthesis. The resultant COMT has also shown to metabolise 11.4 times less catecholamine compared with that of the LPS haplotype in a cell culture assay (Nackley and Diatchenko, 2010). Therefore, individuals who are homozygous for the LPS haplotype will metabolise catecholamines more efficiently, and as a result, will have reduced catecholamine-mediated neurotransmission (Voelker et al., 2009). This is illustrated in Figure 1.2. Catecholamines are essential components of both the ascending and descending pain tracts, and any changes in catecholamine levels may increase or decrease nociception.

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Figure 1.2 Source: Belfer and Segall, 2011. A warrior-worrier model of the relationship between COMT metabolism and COMT “pain sensitivity” alleles in different species: COMT enzyme is depicted as “pacman” and catecholamines dopamine, adrenaline and noradrenaline as small black dots. High COMT metabolism associated with human or mouse alleles or certain rat strains, likely results in less catecholamine signaling in the adrenergic receptor.

Because no previous orthodontic research has investigated the role of genetics on pain associated with fixed appliances, we used this literature review to select six candidate SNPs and haplotypes from three different genes. More information about these chosen candidate genes is listed in Table 1.2.
Table 1.2 The SNPs and haplotypes of the three candidate genes investigated in this research

<table>
<thead>
<tr>
<th>Candidate gene</th>
<th>SNP</th>
<th>Minor allele</th>
<th>Minor allele frequency</th>
<th>Type of pain</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMT</td>
<td>rs6269</td>
<td>G</td>
<td>0.3568</td>
<td>Experimental pain</td>
<td>Diatchenko et al., 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Heat and cold pain</td>
<td>Kim et al., 2006</td>
</tr>
<tr>
<td></td>
<td>rs4680</td>
<td>A</td>
<td>0.3692</td>
<td>Sustained muscular</td>
<td>Zubieta et al., 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Experimental pain</td>
<td>Diatchenko et al., 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Headache</td>
<td>Hagen et al., 2006</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Migraine</td>
<td>Emin Erdal et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fibromyalgia</td>
<td>Vargas-Alarcon et al., 2007; Gursoy et al., 2003</td>
</tr>
<tr>
<td></td>
<td>rs4646310</td>
<td>A</td>
<td>0.1056</td>
<td>TMD</td>
<td>Michelotti et al., 2014</td>
</tr>
<tr>
<td>HPS/APS/LPS (haplotype)</td>
<td></td>
<td></td>
<td></td>
<td>Experimental pain</td>
<td>Diatchenko et al., 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TMD</td>
<td>Diatchenko et al., 2005</td>
</tr>
<tr>
<td>NR3C1</td>
<td>rs2963155</td>
<td>G</td>
<td>0.2228</td>
<td>TMD</td>
<td>Smith et al., 2011</td>
</tr>
<tr>
<td>HTR2A</td>
<td>rs9316233</td>
<td>G</td>
<td>0.2977</td>
<td>TMD</td>
<td>Smith et al., 2011</td>
</tr>
</tbody>
</table>
I.9 Aims and objectives
To investigate the relationship between demographic, clinical, genetic factors, and the severity of pain experienced by patients during orthodontic fixed appliance treatment.

The specific objectives of the study were:
1. To develop an Android smartphone application to measure pain in real-time at the teeth after an orthodontic adjustment.
2. To describe the pain profile of participants after the fixed appliances are adjusted, and investigate whether gender, age, time in treatment, or details of the orthodontic adjustment are predictors of the pain experienced.
3. To investigate whether certain genotypes/haplotypes of the COMT, HTR2A and NR3C1 genes (which are associated with TMD) are associated with the pain experienced during orthodontic treatment with fixed appliances.

I.10 Hypotheses
1) The severity of pain during orthodontic fixed appliance treatment is influenced by gender, age, time in treatment with fixed appliances, and the details of the orthodontic adjustment visit.
2) The severity of pain during orthodontic fixed appliance treatment is associated with certain genotypes/haplotypes of the COMT, HTR2A and NR3C1 genes.
2. Core Methods

2.1 Research approach
A prospective longitudinal study design was employed using the STROBE guidelines (von Elm et al., 2007). Some of the patients recruited in this research had already been enrolled in an ongoing genetics study within the Sir John Walsh Research Institute, University of Otago.

2.2 Study sample
A convenience sample of 82 patients (mean age = 15.2 years; 56.1% female) undergoing treatment at the orthodontic clinic, Faculty of Dentistry (University of Otago), participated in this study. Patients were invited to participate in the study by approaching them in the clinic, as well as contacting them by telephone.

2.2.1 Inclusion criteria
Participants were included if they were:
   1) Currently undergoing, or about to commence, orthodontic treatment with fixed appliances in at least one arch
   2) Younger than 18 years of age
   3) Willing to participate and provide informed consent

2.2.2 Exclusion criteria
Participants were excluded if they had:
   1) Craniofacial syndromes such as cleft lip and/or palate
   2) Undergoing orthognathic surgery
   3) A diagnosed depressive disorder
   4) Any chronic pain syndrome
   5) Used any neurologically-acting medication or medication that could potentially affect pain sensitivity (such as antidepressants)
   6) Active caries or periodontal disease
Participants were provided with an explanation of the research goals and procedures, and the investigator was available to all participants during the project, should any stress, harm, or related concerns have arisen.

2.2.3 Sample size

With a minor allele frequency of the COMT gene estimated at 0.35, and type I error set at 5%, it was estimated that a cohort of more than 300 participants would allow 80% power to detect a relative risk of 1.5 or greater. However, since this project was a pilot study, our goal was to recruit as many participants as we could in the available timeframe. It is expected that further patient recruitment will continue over the next few years until the target sample size is reached.

2.3 Development of smartphone app

To date, all orthodontic pain studies have used paper-based visual analogue scales (VAS). Therefore, it is impossible to determine whether participants had completed the VAS scores at the correct time, or if the VAS scores were completed retrospectively. This leads to potential concerns regarding the validity and reliability of the data. Additionally, paper-based versions of the VAS allow participants to look back at their past pain scores, which could potentially influence the next set of pain scores that they are required to complete. In order to overcome these limitations, a smartphone app was developed to measure pain.

WSH, MF, JA and MA were involved in designing and developing the app. MA was employed to code and produce the app. The app was designed to be simple and easy to use for the young patients who would be participating in this research project. In order to maximise the likelihood of participants completing all of the pain questionnaires, the app was designed to send audio alerts to the participants at the times the pain questionnaires needed to be filled in. The app only allowed participants to complete the pain questionnaires up to three hours before, and up to three hours after the scheduled times. Participants were not able to enter pain scores outside this window. If the participants did not enter their pain scores within this window, a missing score was recorded for that session. The app allowed us to determine whether or not participants filled in the pain questionnaires at each time point, and also recorded exactly what time the participants filled in the pain questionnaires. This method guaranteed the accuracy of the collected data, because we were able to verify that the pain questionnaires were filled in at the correct
time. By using an app, participants were also unable to refer back to their previous pain scores, which could have potentially influenced their current pain scores (leading to bias).

### 2.3.1 Technical details of app development

The Android pain app was developed using the Eclipse integrated development environment software (Luna version 4.4.2\(^1\)) with the Android Eclipse Plugin, to target devices running Android versions 3.0 to 6.0.

The app would present the participant with an Android system notification (including a vibration and audio cue, and a text display) at the scheduled questionnaire session time. Clicking the notification would launch the app, or else the app could be launched from its icon on the smartphone’s application list.

Users would iterate through each page of questions, entering their answers via radio buttons for mutually exclusive answers, check buttons for mutually inclusive answers, sliders to answer on a continuum (VAS scores), and text and numerical entry boxes using the default Android onscreen keyboard and number pad.

Questionnaire answers were stored on a local database (SQLite\(^2\)) at the completion of each session. Once all the sessions were complete, the data was retrieved from the database, compiled into a single comma-separated-values file, and emailed automatically from within the pain app, using the JavaMail API\(^3\) to a Google Mail account under control of the questionnaire administrator, from where it could be downloaded and analysed. If the app detected no internet connection when attempting to send the email, the user would be alerted and prompted to connect to the internet from within the app. This would continue until internet connection was made, upon which time the app would send the email.

### 2.3.2 Testing phase

WSH was involved in testing the app on 10 smartphones before the participants started using the app. WSH tested for any bugs, or malfunctioning of the app, as well as the

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1. [https://eclipse.org/ide/](https://eclipse.org/ide/)
2. [https://www.sqlite.org/](https://www.sqlite.org/)
accuracy of the exported data when compared to the input data. Under ideal testing conditions, the app was deemed to be ready for use in the study.

2.3.3 Some of the app-related problems encountered during the study
The app was more problematic while collecting data in the actual study. Data was lost if the app was shut down unexpectedly, if the phone was turned off, or if the phone ran out of battery. As a result of the lost data, approximately 12 patients were required to repeat the pain questionnaires on the pain app.

In addition, some participants missed a number of pain questionnaires, despite the app sending out alerts at every time point that pain scores were required. In order to obtain fairly complete data, participants were asked to completely repeat the three-day pain survey at a subsequent adjustment appointment of their fixed appliances, if three or more (out of the seven) pain entries were missing. Unfortunately, the exact number of the participants who had to repeat the pain questionnaires was not recorded. However, it was less than 15% of the sample.

2.4 Experimental procedure
This study consisted of two phases. Phase one involved collection of demographic data and DNA from participants, while phase two involved the use of a mobile phone app to assess the pain in the three days after the participants’ fixed appliances were adjusted.

2.4.1 Phase one
Participants completed a self-report questionnaire which provided information regarding sociodemographic details (such as age, gender and ethnicity). Participants also completed a psychological questionnaire which included the Pain Catastrophising Scale for Children, the Corah Dental Anxiety Scale, and the State-Trait Anxiety Inventory for Children (see Appendix 7.1). However, the information collected from these psychological questionnaires was not utilised in this thesis.

Study participants were asked to provide a blood sample in the first instance. If participants declined to give blood, they could provide a saliva sample instead. A blood sample was preferred over a saliva sample, as blood contains a greater quantity and quality of DNA when compared to that of saliva. A registered nurse collected blood samples on-site using
standard venepuncture procedures. The samples included a 10 mL EDTA tube that was used for DNA preparation, and a 5 mL gold top SST tube for serum. The SST vacutainers were centrifuged at 3,500 rpm on-site, and then taken to the Merriman Laboratory (University of Otago) for storage. When venepuncture was refused, 10 mL of saliva was collected instead, using specific saliva kits (DNA genotek™ Oragene-500 kits).

The DNA was extracted using a chloroform extraction with an ethanol precipitation method on whole blood (buccal cells for the saliva samples). Five SNPs from the candidate genes COMT (rs4680, rs6269, rs4646310), HTR2A (rs9316233) and NR3C1 (rs2963155) were genotyped for all of the participants. Genotyping was conducted using TaqMan SNP genotyping assays (purchased from Thermo Fisher; 5 Caribbean Dv, Scoresby, VIC 3179, Australia) in conjunction with KAPA Probe Fast Master mix on a Lightcycler 480 real-time PCA machine. Genotyping was replicated in 10% of the population as a quality control. Haplotypes were phased using PLINK1.9.

2.4.2 Phase two
Immediately after participants had their fixed appliances adjusted, they were issued with an Android smartphone (Vodafone Smart Prime 6 with a 5” colour display). They then logged onto a specially designed pain application and completed the baseline pain questionnaires under the supervision of the primary investigator (WSH). Participants were able to ask WSH for help if they did not understand any of the questions asked by the pain application. Participants were also given an information sheet with details about the app and pain questionnaires (see Appendix 7.2).

VAS’s were used to quantify the amount of pain experienced (Figure 2.1). For this study, an adapted version of the traditional paper-based VAS was used, in the form of a digital VAS. Instead of using a pen to place a mark on the line, participants used their finger to drag a small dot to represent their pain level. On the standardised smartphones that we issued, the VAS line was 9.35 cm long, and the dot was 1.5 mm in diameter.
The application asked participants to rate pain at (1) baseline; (2) 8pm on the first evening; (3) 8am on the second morning; (4) 8pm on the second evening; (5) 8am on the third morning; (6) 8pm on the third evening; and (7) 8am on the fourth morning. Overall, there were a total of seven pain questionnaires over approximately 72 hours after the participants’ fixed appliances were adjusted. An audio alert rang out at each of the seven time points to alert participants to complete the questionnaire. Participants were able to enter pain scores up to three hours before, and three hours after each scheduled time. Participants were not able to enter pain scores outside these times.

At each pain questionnaire, participants were asked about consumption of pain relief, resting pain at their teeth, pain at their teeth immediately after chewing a piece of gum twenty times (Wrigley Extra, peppermint or spearmint flavour), orofacial pain, and headaches (refer to Appendix 7.3 for the exact flow of the app). Participants were asked not to chew gum unless the app asked them to, in the three days that they were undertaking the pain questionnaires. In addition, participants were not given specific instructions on whether to chew the gum with their front teeth or back teeth.

It is important to note that the type of orthodontic activation (i.e. details of what was performed at the adjustment visit) and time in treatment was not standardised for participants. For example, it was not possible to have all of our patients record their pain immediately after bond-up with fixed appliances. However, details regarding the amount of time in fixed appliances, and the exact procedures performed at each adjustment were recorded.
Figure 2.2 A patient using the Android pain application
2.5 Data storage

2.5.1 Storage of questionnaires
Hard-copy baseline questionnaires filled out by all participants were kept in secure storage within the Faculty of Dentistry, University of Otago. These questionnaires will be retained for up to 10 years at the above location. Only the investigators involved in this study were able to access these questionnaires.

2.5.2 Storage of DNA samples
All DNA samples obtained during the study were securely stored in such a way that only the investigators of the study were able to gain access to them. These DNA samples will be retained for up to 10 years in the Merriman Laboratory at the Biochemistry Department at the University of Otago. No other external source, commercial or non-commercial, will have access to any of this information without the permission of the study participants/parents.

2.5.3 Storage of pain application data
The data from the pain application was automatically emailed to a secure Gmail account. Only the investigators were able to access this account.

2.6 Ethical approval
This study was approved by the University of Otago Human Ethics Committee in February 2016 (reference H15/124). Written and informed consent forms were collected from all study participants. In addition, parental consent was obtained for study participants under the age of 17 years. Appendix 7.4 contains a copy of the ethics approval. Appendix 7.5 contains a copy of the information sheets for parents and participants, and the participant/parental consent forms.

2.7 Maori consultation
Consultation with the Ngai Tahu Research Consultation Committee was completed in December 2015 (Appendix 7.6).
2.8 Funding
This study was supported by grants from the Sir John Walsh Research Institute (Fuller Scholarship), the New Zealand Association of Orthodontists, and the New Zealand Dental Research Foundation.
3. Ecological momentary assessment of orthodontic pain profiles in children and adolescents undergoing orthodontic treatment

3.1 Introduction

Many patients report pain as being the worst aspect of orthodontic treatment (Oliver and Knapman, 1985). Pain is incredibly common, with more than 90% of patients experiencing pain and discomfort at some point during their orthodontic treatment (Scheurer et al., 1996; Erdinc and Dinçer, 2004). Surprisingly, it has been reported that the pain following placement of an initial arch wire is usually more severe, and lasts for longer than the pain following orthodontic premolar extractions (Jones and Chan, 1992).

A multitude of studies have investigated the onset of pain following the application of fixed appliances. It is generally accepted that pain begins a few hours after an orthodontic force is applied, peaks around 24 hours, lasts for between three to five days, and returns to baseline levels after about seven days (Ngan et al., 1989; Jones and Chan, 1992). A mean value of approximately 50 mm on a 100 mm visual analogue scale was reported 24 hours after bond-up with full upper and lower fixed appliances (Woodhouse et al., 2015). Therefore, pain at the beginning of orthodontic treatment with fixed appliances can be regarded as significant and of moderate intensity for the average patient. However, it must be noted that pain levels experienced during orthodontic treatment can vary drastically between different individuals (Krishnan, 2007).

Most studies investigating orthodontic pain have used paper-based surveys to record participants’ pain at various time intervals following orthodontic adjustments (Jones and Chan, 1992; Scheurer et al., 1996; Erdinc and Dinçer, 2004; Fleming et al., 2009; Benson et al., 2012; Woodhouse et al., 2015). This approach has inherent limitations because pain surveys are often filled in retrospectively, and the memory of pain may be inaccurate, as memory recall is not always reliable (Bradburn et al., 1987). Furthermore, during paper-based surveys, participants are able to look back at their previous pain scores which could influence their current and future pain ratings. Some studies have attempted to resolve this problem by calling patients at the required times to ask about their current pain scores.

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1 Chapter has been submitted to Seminars in Orthodontics for publication in a special issue.
(Bergius et al., 2008), as well as asking patients to mail back their pain scores at each time point (Scheurer et al., 1996; Bergius et al., 2002).

Ecological Momentary Assessment (EMA) allows participants to report repeatedly on various experiences “in real-time, in real-world settings, over time and across contexts”. EMA enables researchers to minimise subjects’ recall bias and maximise ecological validity since the data is collected in the subjects’ natural environment. EMA studies are often used to assess particular events in subjects’ lives, or to assess subjects at periodic intervals. Various technological devices such as written diaries, telephones, electronic diaries and physiological sensors can be used in EMA (Shiffman et al., 2008).

The aim of this study was to design a smartphone app that could regularly measure pain levels of participants following the adjustment of their fixed appliances. This app would provide ratings of current pain, information about the time that participants filled in their surveys, as well as information on when participants missed their pain surveys. We have used this app to investigate pain profiles in a sample of patients undergoing fixed orthodontic treatment. Specifically, we aim to determine the association between pain and several demographic and treatment factors such as gender, age, time in treatment, and type of orthodontic adjustment.
3.2 Methods

The study utilised a longitudinal prospective design, which was developed according to the STROBE guidelines (von Elm et al., 2007). The study was set between June 2016 and April 2017 and was based in the orthodontic clinic at the Faculty of Dentistry, University of Otago, New Zealand.

A convenience sample of 82 orthodontic patients who fulfilled the inclusion and exclusion criteria were recruited (see Chapter 2 for more details about the recruitment process). Eligible participants filled in a questionnaire that asked about basic demographic information. Following this questionnaire, participants were shown how to use an Android smartphone application that was developed as part of this study to record the severity of pain at the teeth following an adjustment of the fixed appliances.

Immediately following an orthodontic adjustment appointment, regardless of the stage of treatment that they were in, the participants were issued with an Android smartphone (Vodafone Smart Prime 6, with a 5” colour display). Participants then filled out the first pain survey on the pain app, under the supervision of the primary investigator (WSH). Participants were required to fill out pain surveys at (1) baseline (immediately after an orthodontic adjustment); (2) 8pm on day one; (3) 8am on day two; (4) 8pm on day two; (5) 8am on day three; (6) 8pm on day three; and (7) 8am on day four. Each pain survey asked about analgesic consumption, resting pain at the teeth, and pain at the teeth immediately after chewing a piece of chewing gum twenty times (Wrigley Extra, peppermint or spearmint flavour, Wrigley, Chicago, Illinois, USA). A digital sliding VAS was used to record the severity of pain experienced - the left side of the VAS was labelled “no pain at all”, while the right side was labelled “worst pain imaginable”. The digital VAS was 9.35 cm long, which differs slightly from the 10 cm long VAS diagrams used in traditional paper-based forms. Participants dragged a small circular slider (measuring 1.5 mm in diameter) to rate the severity of pain felt at their teeth. They were asked not to chew gum unless the app asked them to.
3.2.1 Outcomes
The primary outcome measurements were pain at the teeth at rest and after chewing gum, in the 72 hours after an orthodontic adjustment. Pain scores were assessed timewise and across the whole three-day observation period as area under the curve (AUC) of perceived level of pain. Secondary outcomes included reported use of oral analgesics, headache, and facial pain.

3.2.2 Statistical analysis
Statistical analyses were carried out using SPSS Software (version 23, IBM, NY, USA). Preliminary analyses entailed normality tests and tests for equality of variances – assumption of normal distribution was tested using a one sample Kolmogorov-Smirnov test.

A repeated measurement Friedman analysis of variance was used to test the effects of time (seven time points over 72 hours) on the two VAS variables (“current pain at teeth” and “pain at teeth after chewing”). The square root of the area under the curve (AUC, normally distributed) was calculated for VAS scores of current pain at teeth and pain at teeth after chewing, and entered as dependent variables in a general linear model, with age, gender, details of orthodontic adjustment, and time in treatment as covariates. Type I error was set at 0.05.
3.3 Results

3.3.1 Sample
The mean age of participants was 15.2 years (SD = 1.6 years) and ranged between 10.8 and 18.2 years. There were a greater number of female participants compared to male participants (F = 56.1%). Seventy-one (86.6%) participants identified themselves as being NZ European only, while seventy-six (92.7%) participants identified as being NZ European and Maori/Pacific Islanders. Two participants (2.4%) identified as being Maori or Pacific Island only, while four participants (4.8%) were of “other” ethnicity (Niuean, Chinese, Bangladeshi and Colombian). The mean time in orthodontic treatment was 12 months (SD = 8.4 months), with a range of 0 to 32 months. The vast majority of participants (95.1%) had fixed appliances in two arches, while 4.9% had fixed appliances in one arch only.

3.3.2 Details of orthodontic adjustments
The distribution of the study sample by the type of orthodontic adjustments are presented in Table 3.1.

Table 3.1 Type of orthodontic adjustment across the study sample.

<table>
<thead>
<tr>
<th>Type of orthodontic adjustment</th>
<th>No. of patients</th>
<th>% of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>No arch wire changes +/- minor bends in arch wire</td>
<td>5</td>
<td>6.1</td>
</tr>
<tr>
<td>Power chain replacement +/- minor bends in arch wire</td>
<td>27</td>
<td>32.9</td>
</tr>
<tr>
<td>One arch wire changed</td>
<td>27</td>
<td>32.9</td>
</tr>
<tr>
<td>Two arch wires changed</td>
<td>14</td>
<td>17.1</td>
</tr>
<tr>
<td>New bond up in at least one arch</td>
<td>9</td>
<td>11</td>
</tr>
</tbody>
</table>

The majority of participants had one arch wire change, or power chain replaced with or without minor bends in the arch wires. A small number of participants had no arch wire changes with or without bends in the arch wires.

3.3.3 Missing data
Overall, 6.4% of pain surveys were not completed by the participants. Missing data was handled by substituting the missing pain score with that from the previous pain survey, based on the principle of last observation carried forward.
3.3.4 Timeliness of pain scores
Some 93.6% of the pain surveys were filled in by the participants. On average, participants filled in the pain surveys 21 minutes later than expected (ranging from 167 minutes early to 177 minutes late, SD = 56 minutes).

3.3.5 Pain relief
Only 37.8% of the participants reported using analgesics (paracetamol and/or ibuprofen) at some point in the three days following adjustment of their fixed appliances.

3.3.6 Headache
Nearly one-quarter of the participants reported headaches (left or right temple areas) at least once during the three-day study period.

3.3.7 Pain and time
Pain levels (by VAS) varied significantly over the 72-hour assessment period for both current pain at teeth (F=19.4; P<0.001), and pain at the teeth after gum chewing (F=22.9; p<0.001; Figure 3.1).

Participants reported mild levels of current pain at the teeth immediately after the orthodontic baseline appointment, with a mean VAS score of 10.6 % (standard error = 1.6 %). Compared to baseline, the pain scores rose steadily in the first day, and peaked on the morning of the second day (approximately 19 hours after the fixed appliances were adjusted) where the mean VAS score increased to 26.5 % (standard error = 2.6%; p=0.004). The pain scores then reduced steadily between 19 and 72 hours after the orthodontic adjustment. On the evening of day three and the morning of day four, the mean VAS scores did not differ significantly from the baseline scores (p>0.05; Figure 3.1).

Mild levels of pain at the teeth after gum chewing were also reported immediately after the orthodontic baseline appointment, with a mean VAS score of 12.7% (standard error = 2.0%). VAS pain on chewing increased rapidly, with a mean VAS score of 33.7% by 8pm on the first evening (p<0.001). The pain levels after chewing peaked on the morning following the orthodontic adjustment (approximately 19 hours after), with a VAS score of 36.8% (standard error = 3.4%; p<0.001). By 8pm on day two, the gum chewing VAS scores had only decreased slightly, with a mean VAS score of 31.9 (p=0.003). Pain on chewing
decreased steadily after this. At 8am on day four, the pain on chewing did not differ from the baseline pain on chewing score ($p>0.05$; Figure 3.1). Chewing gum triggered greater amounts of pain compared to the resting pain at the teeth, across all the time points.
Figure 3.1 Time profile of current pain at teeth and pain at teeth after chewing gum over the three-day study period (error bars represent the 95% confidence interval).
Cumulative pain levels (i.e. total pain) experienced at the teeth over the three days of testing were assessed using the area under the curve (AUC) of VAS scores for both current pain at the teeth and pain at the teeth after gum chewing. The AUC of current pain and pain on chewing was not significantly influenced by age ($F \leq 1.9; p \geq 0.172$), gender ($F \leq 0.6; p \geq 0.452$), or number of months in treatment ($F \leq 1.3; p \geq 0.254$). Conversely, the AUC of pain experienced at the teeth was significantly influenced by details of what was performed at the orthodontic adjustment visits. This applied to both the AUC for current pain ($F=2.9; p=0.028$) and pain after gum chewing ($F=6.0; p<0.001$) (Figures 3.2 and 3.3).

There was greater total current (resting) pain at the teeth when comparing “new bond-ups in at least one arch” to “no arch wire changes +/- bends placed in the arch wire” ($p = 0.009$). There were no other statistically significant differences in the total amount of pain experienced at the teeth when comparing the other details of adjustment appointments. Similarly, there was a statistically significant difference in the total amount of pain experienced at the teeth after gum chewing when “new bond ups in at least one arch” were compared to “no arch wire changes +/- bends placed in the arch wire” ($P < 0.001$). There were no other statistically significant differences in the total amount of pain experienced at the teeth after chewing gum, when comparing the other details of adjustment appointments.
**Figure 3.2** Total amount of current (resting) pain experienced at the teeth when compared to what was performed at the adjustment visit (error bars represent the 95% confidence interval)
Figure 3.3 Total amount of pain experienced at the teeth after chewing gum when compared to what was performed at the adjustment visit (error bars represent the 95% confidence interval).
3.4 Discussion

This longitudinal prospective study utilised an EMA approach using a smartphone application to investigate orthodontic pain profile following an orthodontic adjustment. In general, pain steadily increased following the adjustment, peaked at 8am on day two, and then gradually returned toward baseline levels by day four. This pain profile is similar to those reported in previous studies (Ngan et al., 1989; Jones and Chan, 1992).

Our findings indicate that using an EMA approach via smartphones is an effective method of assessing pain because it allows measurements to be completed at each intended time point. Indeed, pain surveys were filled on average 21 minutes after the scheduled time. In addition, participants were unable to look at their past pain scores, thereby reducing the risk of bias while rating their current pain scores.

One weakness of this study was the relatively small sample size (82 participants). When analysed by details of the actual adjustments, the smallest category only included five participants. Another weakness was the fact that the use of pain relief could have affected the VAS pain scores of the participants. Unfortunately, we did not have time to conduct a statistical analysis that could have adjusted for the effects of pain relief on the pain scores. Participates who used pain relief were not excluded from the statistical analysis. Therefore, we have simply described the proportion of patients in our sample who took analgesics during the study period. In addition, the study participants were treated by a number of different clinicians. We failed to control for “operator bias” in our statistical analysis. A further shortcoming of this study is that we used a VAS that was only 93.5 mm long. It is possible that VAS’s shorter than 100 mm may not be as accurate at reporting pain levels (Revill et al., 1976). In the future, this issue may be addressed by using a smartphone or tablet with a larger screen, so that a 100 mm long VAS can be used. An important strength of this study was the use of a smartphone application to collect data and minimise the number of pain surveys that were missed. Indeed, 93.6% of the pain surveys were completed, with only 6.4% of pain surveys not filled in.

Pain on chewing gum was significantly higher than resting pain at the teeth. The peak pain when chewing gum was 36.8% at 8am on day two, while the peak resting pain at the teeth was 26.5% at 8am on day two. The gum that was issued was soft in texture, so it would not be unreasonable to expect that chewing food of harder consistencies would cause
significantly more pain. Two studies have demonstrated that chewing gum may reduce the amount of pain after bond-up with fixed appliances and may also reduce the consumption of ibuprofen following bond-up (Benson et al., 2012; Ireland et al., 2016). Because we wanted to use the gum to elicit chewing discomfort (and not for its potential pain relief properties), we asked the participants to avoid chewing it except for when the app asked them to (two times per day, twenty chews per occasion).

This study found that gender does not affect the magnitude of orthodontic pain. This finding is consistent with the vast majority of studies on orthodontic pain which have not demonstrated any sex-related differences (Ngan et al., 1989; Jones and Chan, 1992; Fernandes et al., 1998; Sergl et al., 1998; Erdinc and Dinçer, 2004; Scott et al., 2008; Abdelrahman et al., 2015; Rahman et al., 2015). By comparison, only a few studies have found females to experience greater levels of orthodontic pain (Scheurer et al., 1996; Bergius et al., 2002).

Despite there being no consensus regarding the effects of age on pain perception in the orthodontic literature, this study found that age has no relationship with the severity of pain experienced during orthodontic treatment, which agrees with a number of studies (Ngan et al., 1989; Scott et al., 2008; Abdelrahman et al., 2015; Rahman et al., 2015). Some authors believe it is hard to assess the relationship between age and pain perception because adolescents and adults may be treated with different protocols. One reason this study may not have detected a difference is because the age range was limited (10.8 to 18.2 years), and the treatment protocols and philosophies were quite similar between all of the participants.

This study also investigated the effect of time in treatment on pain severity, and found no association. As far as we are aware, no previous study has tested the effect of time in treatment and the severity of orthodontic pain. Nonetheless, there was a statistically significant association between details of the orthodontic adjustment and the severity of pain experienced. Patients who had just been bonded up experienced significantly more total resting pain at the teeth, and significantly more total chewing pain at the teeth in the three days after bond-up, when compared to patients who had no arch wire changes, with or without minor bends placed in the arch wires. It is possible that patients who had just been bonded up could have been stressed by the use of cheek retractors and the long
appointment time of the bond-up. In addition, oral lesions and ulcers may have caused significant pain in the first few days after bond-up. However, this should not have had any bearing on our results as the app clearly asks about pain at the teeth, and not about pain at the cheeks or soft tissues. Furthermore, given that we didn’t collect pain data from participants throughout their entire course of orthodontic treatment, it may be incorrect to state that bond-ups caused more pain than other orthodontic adjustments. This could easily be addressed in further research by collecting pain data from participants over the entire course of their orthodontic treatment, which has never been done before. This is the first study that has investigated the association between orthodontic pain, time in treatment and details of the adjustment. Accordingly, our findings cannot be compared to those from previous studies.

In conclusion, EMA is a promising approach for investigating the effects of multiple factors on orthodontic pain in real-world settings. Peak pain at the teeth occurred approximately 19 hours after the orthodontic adjustment, and decreased steadily toward baseline levels after three days. Gender, age and treatment duration were not associated with the severity of pain at the teeth. However, patients undergoing bond-ups experienced significantly more pain at their teeth when compared to patients having no arch wire changes, with or without minor bends in the arch wire, regardless of the time spent in treatment.
4. Genetic factors associated with orthodontic pain in children and adolescents

4.1 Introduction

The perception of pain is an incredibly complex process which is influenced by many environmental and genetic factors (Mogil, 1999). In recent times, there has been increasing interest in the role of genetic factors which influence pain perception. To date, no studies have investigated whether there are associations between certain genetic polymorphisms and the intensity of pain experienced by patients undergoing orthodontic treatment. However, there is ample evidence that several single nucleotide polymorphisms (SNPs) influence the severity of pain caused by fibromyalgia and experimental pain, and can even contribute to temporomandibular disorders (TMD).

One of the most commonly studied genes in pain research is the Catechol-O-methyltransferase (COMT) gene. The COMT gene is located on the long arm of chromosome 22, and encodes the COMT enzyme, which is responsible for catalysing the degradation of catecholamines such as dopamine, adrenaline and noradrenaline. These neurotransmitters are involved in a vast array of physiological processes such as cognition, cardiovascular function, stress response, and pain processing (Belfer and Segall, 2011). COMT is especially involved in the regulation of extracellular catecholamine concentrations in the prefrontal region of the brain. Catecholamines are essential components of both the ascending and descending pain tracts, and any changes in catecholamine levels may increase or decrease nociception. Interestingly, variations in the level of COMT activity result in compensatory effects within the central nervous system. Subjects with low COMT activity experience reduced analgesia by endogenous opioids via µ-opioid pathways (Zubieta et al., 2003).

The COMT gene locus contains numerous single nucleotide polymorphisms with minor allele frequency greater than 1%\(^1\). Most of these SNPs are located in noncoding regions, or if they are located in coding regions, do not alter the amino acid sequence of the COMT protein. It must be noted that SNPs in noncoding regions, or silent SNPs, can have

significant effects on processes such as DNA transcription, RNA splicing, mRNA stability, as well as mRNA transport and translation (Andersen and Skorpen, 2009). Genetic variation in the COMT gene has been associated with a number of pain conditions. These include differences in pain perception caused by experimental pain stimuli (Zubieta et al., 2003; Diatchenko et al., 2005), and variable susceptibility to common pain conditions such as fibromyalgia (Gursoy et al., 2003; Vargas-Alarcon et al., 2007), migraines (Emin Erdal et al., 2001) and temporomandibular disorders (Diatchenko et al., 2005). It is also worth noting that numerous splice variants of the 1.5kb COMT transcript have been found in the human brain (Tunbridge et al., 2007). At this point in time, the functional significance of these splice variants is unknown, but the detection of their presence adds further complexity to COMT biology.

Numerous studies have investigated the association between genetics and experimental pain and TMD. These probably represent the closest models that we have when beginning to investigate the role of genetics on orthodontic pain. Several haplotypes, which refers to a set of SNPs on one chromosome inherited together, have been linked to experimental pain. The three most common COMT haplotypes are termed high pain sensitivity (HPS), average pain sensitivity (APS), and low pain sensitivity (LPS). The four SNPs of the COMT gene that contribute to these COMT haplotypes include rs6269, rs4633, rs4818 and rs4680. These COMT haplotypes were categorized according to their association with sensitivity to experimental pain in humans. Some 11% of the variation in perception of experimental pain could be attributed to combinations of these three common haplotypes (Diatchenko et al., 2005). Further research has focused on the role of these COMT haplotypes on the incidence of TMD in a three-year prospective study containing 202 women. Females who had only HPS and/or APS haplotypes had a 2.3-fold greater risk of developing TMD when compared to females who had one or two LPS haplotypes (Diatchenko et al., 2005).

Genetic markers such as SNPs, or combinations of SNPs, are important because they implicate enzymes and shed light on the mechanisms behind the aetiology of pain. In addition, they also enable us to study gene-environment interactions, whereby an environmental influence on disease is only expressed in people with a specific genotype (Khoury, 1998). For example, an environmental factor such as depression could be a risk factor for TMD among patients with a certain combination of alleles for a SNP. However,
depression may not be a risk factor for TMD among patients with differing combinations of alleles for this SNP. It is possible that orthodontic treatment with fixed appliances may act as an environmental risk factor in the development of TMD. In patients with pain-sensitive haplotypes of the COMT gene, eight out of 36 patients with a history of orthodontic treatment developed TMD during a three-year period. However, none of the 20 patients with pain-sensitive haplotypes who didn’t have a history of orthodontic treatment developed TMD during these three years. This was statistically significant (p = 0.04). Thus, orthodontic treatment may increase the risk of developing TMD in patients with pain-sensitive haplotypes of the COMT gene – this represents a gene-environment interaction. In patients with pain-resistant haplotypes, orthodontic treatment was not associated with an increase in risk of developing TMD (Slade et al., 2008).

The OPPERA project carried out a large case-control study in America. Some 348 patients were affected by chronic TMD, while 1612 control patients were included. 3295 SNPs from 358 genes implicated in human pain perception were tested for all of these patients. Nine SNPs from six different genes were associated with TMD. These included the rs2963155, rs9324918 and rs33389 SNPs of the NR3C1 gene, the rs9316233 SNP of the HTR2A gene, the rs7800170 SNP of the CHRM2 gene, the rs3756612 and rs10491334 SNPs of the CAMK4 gene, the rs728273 SNP of the IFRD1 gene, and the rs12415832 SNP of the GRK5 gene (Smith et al., 2011). This study also tested the association between COMT haplotypes and TMD. Participants with HPS haplotypes had an increased risk of TMD (OR = 1.28, p = 0.05). Interestingly, there was no significant difference between the APS and LPS haplotypes in their case-control study.

A smaller case-control study conducted in Italy recruited 50 patients affected by TMD and 132 controls. The entire COMT gene was sequenced for all participants. 40 COMT variants were found, 18 of which were novel discoveries. The SNPs rs1655656, rs165722 and rs4646310 were found significantly more frequently in TMD patients when compared to the controls. All of these SNPs are located in the promoter region of the COMT gene. The SNPs rs1655656 and rs4646310 were novel - patients with the CC genotype of rs1655656 were 5.3 times more likely to suffer from TMD when compared to the patients with the GG genotype, while patients with the AG genotype of rs4646310 were 2.6 times more likely to suffer from TMD when compared to patients with the GG genotype (Michelotti et al., 2014). It is proposed that the SNPs located in the promoter region of
the COMT gene could alter DNA transcription, RNA splicing, mRNA stability, or mRNA transport and translation (Nackley et al., 2006), ultimately leading to an increased risk of TMD. Interestingly, this study did not test the effect of the three most common COMT haplotypes, as mentioned previously, on the risk of TMD.

Given that patients with pain-sensitive haplotypes of the COMT gene perceive higher intensities of pain during experimental pain studies, as well as having an increased risk of developing TMD, it is plausible that these same patients could also experience more pain during orthodontic treatment with fixed appliances. Therefore, our aims were to determine whether certain SNPs/haplotypes of the COMT, HTR2A and NR3C1 genes are associated with the severity of orthodontic pain experienced by patients undergoing orthodontic fixed appliance treatment.
4.2 Methods

The study utilised a longitudinal prospective design, which was developed according to the STROBE guidelines (von Elm et al., 2007). The study was set between June 2016 and April 2017 and was based in the orthodontic clinic at the Faculty of Dentistry, University of Otago, New Zealand.

A convenience sample of 82 orthodontic patients who fulfilled the inclusion and exclusion criteria were recruited (see Chapter 2 for more details about the recruitment process).

4.2.1 DNA collection

Participants were asked to provide a DNA sample in the form of blood or saliva. Blood was encouraged, as it contains a greater quality and quantity of DNA when compared to saliva. A registered nurse collected blood samples on-site using standard venepuncture procedures. The samples included a 10 mL EDTA tube that was used for DNA preparation, and a 5 mL gold top SST tube for serum. The SST vacutainers were centrifuged at 3,500 rpm on-site, and then taken to the Merriman Laboratory (University of Otago) for storage. When venepuncture was refused, 10 mL of saliva was collected instead, using specific saliva kits (DNA genotek™ Oragene-500 kits).

4.2.2 DNA extraction

The DNA was extracted using a chloroform extraction with an ethanol precipitation method on whole blood (buccal cells for saliva).

4.2.3 Candidate gene and SNP selection

Selected SNPs from the COMT (rs6269, rs4680, rs4646310), NR3C1 (rs2963155) and HTR2A (rs9316233) genes were selected after a review of the literature. Please see Table 1.3 in Chapter 1, which highlights some of the pain conditions that these SNPs have been associated with.

4.2.4 Genotyping

Genotyping of the above SNPs was conducted using TaqMan SNP genotyping assays (from Thermo Fisher; 5 Caribbean Dv, Scoresby, VIC 3179, Australia) in conjunction with KAPA Probe Fast Master mix on a Lightcycler 480 real-time PCA machine. Genotyping was replicated in 10% of the population as a quality control. Haplotypes were phased using
4.2.5 Assessing pain outcomes
Immediately following an orthodontic adjustment appointment, regardless of the stage of treatment that they were in, the participants were issued with an Android smartphone (Vodafone Smart Prime 6, with a 5” colour display). They then filled out regular pain surveys in the first three days after their fixed appliances were adjusted (please refer back to Chapters 2 and 3 for more details).

4.2.6 Statistical analysis
Data were analysed using conventional descriptive methods. One-Way ANOVA was used to assess differences in pain severity across genotypes and haplotypes. Analyses were carried out using the Statistical Package for the Social Sciences (SPSS v22.0, SPSS Inc, Chicago IL, USA), and Stata (version 13.1; Stata Corp LP, College Station, TX, USA). Haplotypes were phased using PLINK1.9.
4.3 Results
The various SNP genotypes and mean pain values experienced at the teeth are presented in Table 4.1. Participants with the AA genotype of the rs4646310 SNP of the COMT gene experienced almost triple the total resting (current) pain at the teeth compared to the AG and GG genotypes (p = 0.022). In addition, participants with the CG genotype of the rs9316233 SNP of the HTR2A gene experienced almost double the total chewing pain at the teeth when compared to those with the CC and GG genotypes (p = 0.018). The remainder of the SNPs did not show statistically significant differences in pain at rest (current pain) or on chewing (p > 0.05; Table 4.1).
Table 4.1 SNPs of COMT, serotonin 2A receptor and glucocorticoid receptor genes by mean reported pain at rest and on chewing (max and overall)

<table>
<thead>
<tr>
<th>Candidate SNPs/Genotypes</th>
<th>Frequency (Percentage)</th>
<th>Resting pain at T3 (Peak)</th>
<th>Chewing pain at T3 (Peak)</th>
<th>Resting pain T0 – T7 (Overall)</th>
<th>Chewing pain T0 – T7 (Overall)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rs6269 (COMT)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>32 (40)</td>
<td>28.3 ± 23.1</td>
<td>41.8 ± 27.8</td>
<td>111.9 ± 86.4</td>
<td>192.1 ± 120.0</td>
</tr>
<tr>
<td>AG</td>
<td>41 (51.25)</td>
<td>24.6 ± 25.6</td>
<td>33.1 ± 32.4</td>
<td>109.6 ± 115.1</td>
<td>176.0 ± 156.7</td>
</tr>
<tr>
<td>GG</td>
<td>7 (8.75)</td>
<td>21.3 ± 20.6</td>
<td>25.1 ± 23.1</td>
<td>69.3 ± 51.2</td>
<td>103.7 ± 76.7</td>
</tr>
<tr>
<td><strong>rs4680 (COMT)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>23 (29.87)</td>
<td>28.2 ± 24.3</td>
<td>37.9 ± 28.4</td>
<td>100.9 ± 68.3</td>
<td>169.5 ± 102.7</td>
</tr>
<tr>
<td>AG</td>
<td>41 (53.25)</td>
<td>23.8 ± 24.3</td>
<td>34.8 ± 31.7</td>
<td>109.7 ± 114.7</td>
<td>184.8 ± 159.0</td>
</tr>
<tr>
<td>GG</td>
<td>13 (16.88)</td>
<td>30.3 ± 23.9</td>
<td>36.9 ± 27.2</td>
<td>110.5 ± 93.2</td>
<td>169.8 ± 124.6</td>
</tr>
<tr>
<td><strong>rs4646310 (COMT)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>1 (1.28)</td>
<td>63.0 ± NA</td>
<td>64.0 ± NA</td>
<td>366.0 ± NA</td>
<td>349.0 ± NA</td>
</tr>
<tr>
<td>AG</td>
<td>30 (38.46)</td>
<td>20.6 ± 19.8</td>
<td>32.1 ± 30.5</td>
<td>93.6 ± 82.7</td>
<td>156.4 ± 136.1</td>
</tr>
<tr>
<td>GG</td>
<td>47 (60.26)</td>
<td>29.4 ± 25.6</td>
<td>39.0 ± 29.7</td>
<td>114.1 ± 104.0</td>
<td>190.8 ± 138.5</td>
</tr>
<tr>
<td><strong>rs2963155 (NR3C1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>43 (54.43)</td>
<td>23.3 ± 24.1</td>
<td>32.4 ± 30.5</td>
<td>96.9 ± 94.7</td>
<td>167.0 ± 143.8</td>
</tr>
<tr>
<td>AG</td>
<td>32 (40.51)</td>
<td>28.1 ± 24.1</td>
<td>40.4 ± 30.5</td>
<td>114.1 ± 102.7</td>
<td>187.7 ± 135.9</td>
</tr>
<tr>
<td>GG</td>
<td>4 (5.06)</td>
<td>28.8 ± 26.3</td>
<td>32.3 ± 25.1</td>
<td>149.8 ± 152.0</td>
<td>162.3 ± 132.7</td>
</tr>
<tr>
<td><strong>rs9316233 (HTR2A)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>47 (59.49)</td>
<td>23.4 ± 23.7</td>
<td>29.6 ± 28.3</td>
<td>86.3 ± 78.0</td>
<td>140.0 ± 108.4</td>
</tr>
<tr>
<td>CG</td>
<td>30 (37.97)</td>
<td>29.1 ± 24.7</td>
<td>44.0 ± 30.2</td>
<td>134.3 ± 120.5</td>
<td>229.4 ± 162.6</td>
</tr>
<tr>
<td>GG</td>
<td>2 (2.53)</td>
<td>23.5 ± 30.4</td>
<td>37.5 ± 46.0</td>
<td>93.5 ± 101.1</td>
<td>138.5 ± 146.4</td>
</tr>
</tbody>
</table>

*P = 0.022, b P = 0.018
The mean chewing pain at T3 (peak pain during the three-day observation period) is plotted against the COMT haplotypes in Figure 4.1. The greatest chewing pain was observed in participants with the APS/HPS and HPS/LPS haplotypes, while the lowest pain was observed in those with the LPS/LPS haplotype. However, there was no significant difference between pain at T3 and haplotypes of the COMT gene ($p = 0.184$; Figure 4.1).
Figure 4.1 Mean reported pain score after chewing by different COMT haplotypes at T3 (peak pain). Error bars represent standard error.

P = 0.184
Figure 4.2 is very similar to Figure 4.1, but shows the mean total chewing pain over three days against the COMT haplotypes. Once again, the greatest chewing pain was observed in participants with the APS/HPS and HPS/LPS haplotypes, while LPS/LPS experienced the least amount of total chewing pain. However, this was not statistically significant ($p = 0.173$; Figure 4.2).
Figure 4.2 Mean total reported pain score after chewing by different COMT haplotypes (T0-T7; overall). Error bars represent standard error.

P = 0.173
Figure 4.3 from the work by Diatchenko and colleagues (2005) demonstrates that the APS/HPS haplotype was associated with the greatest experimental pain sensitivity, while LPS/LPS was associated with the lowest experimental pain sensitivity. When comparing Figures 4.1, 4.2 and 4.3, it can be seen that there is a similar gradient between the COMT haplotypes and the severity of pain experienced. The APS/HPS haplotypes experienced the greatest amount of orthodontic and experimental pain, the LPS/LPS haplotypes experienced the least amount of orthodontic and experimental pain, while the other three haplotypes were somewhere in the middle.
Figure 4.3 Experimental pain responsiveness categorised by different COMT haplotypes (Diatchenko et al., 2005)

$P = 0.0004$
4.4 Discussion

This is the first study to investigate the role of genetic influences on the intensity of pain following the adjustment of orthodontic fixed appliances. Since previous studies have not investigated the role of genetics on orthodontic pain, genes implicated in TMD were used to test for this association.

The main weakness of this study was the small sample size (82 participants). In genetic research, large sample sizes are required, as genes often only show minor effects. However, this weakness will be addressed in the future as we increase our study sample. The aim is to recruit 300 participants, which will greatly enhance the statistical power to detect common genetic variants with minor effects. Another weakness is the subjective nature of the VAS used to assess pain severity. This is not just a shortcoming of the VAS, but a weakness of all available instruments since the perception of pain is largely subjective. In addition, approximately 10 subjects had to be excluded from this study because we could not analyse the DNA from their saliva samples. The only way to ensure high-quality DNA is through blood sampling, which not all study participants were happy to provide. The strengths of this study include the use of a specialized smartphone app, which ensured that the pain data collected was reliable. The latter is important since reported pain scores can be inaccurate if patients fill them in retrospectively. In addition, participants were unable to look at their past pain scores, thus reducing any bias or error when it came to recording their current pain scores.

There was a statistically significant difference in the total amount of chewing pain at the teeth for the rs9316233 SNP of the HTR2A serotonin 2A receptor gene. Participants with the CG genotype experienced significantly more total chewing pain at the teeth in the first three days after the adjustment of their fixed appliances when compared to participants with the CC and GG genotypes. This finding would also be considered clinically significant, as the patients with the CG genotype experienced approximately 64% more pain on chewing than the CC and GG genotypes in the three days after adjustment of their fixed appliances. The rs9316233 SNP is an intronic polymorphism of the HTR2A gene. In the OPPERA study, the minor G allele was protective against TMD (OR = 0.64). Interestingly, the effect of this SNP applied only to females, and was not observed in males. For white females alone, the protective effect of the minor G allele was even more pronounced (OR = 0.39, P = 2.3 x 10^-4). In addition, there seemed to be a race-specific effect, with no effect
seen in African Americans (OR = 0.9, P = 0.77). For non-Hispanic whites, the OR was 0.56, P = 0.0053 (Smith et al., 2011). However, this race-specific effect would have had minimal influence on the present study, as the vast majority of participants identified as being New Zealand Caucasian. Interestingly, the G allele was not protective against pain in this study, with the CG genotype associated with the most pain. The genetic analysis was completed for both males and females together, so it is unclear whether a gender-based effect exists in our sample.

The rs6313 SNP of the HTR2A gene, a synonymous polymorphism in the first exon, has also been associated with TMD in another study (Mutlu et al., 2004). The rs6313 SNP lies 40kb upstream from the rs9316233 SNP, and the two SNPs are not in strong linkage disequilibrium (D' = 0.29, r² = 0.02). A more recent prospective cohort study identified two SNPs (rs12584920 and rs17289394) of the HTR2A gene associated with chronic widespread musculoskeletal pain (Nicholl et al., 2011). This gene has been implicated in influencing both nociceptive and affective pathways (Slade et al., 2016), which suggests that it is biologically plausible for the rs9316233 SNP to affect the total amount of chewing pain at the teeth in our study.

There was also a statistically significant difference in the total amount of resting (current) pain at the teeth for the rs4646310 SNP of the COMT gene. The AA genotype (366 total pain units) experienced significantly more total pain at the teeth when compared to the AG (93.6 total pain units) and GG (114.1 total pain units) genotypes. However, there was only one participant who had the AA genotype. In addition, this participant underwent a bond-up, which we have shown produces the highest levels of pain compared to any other orthodontic adjustment. Therefore, larger sample sizes would be required to investigate this association further.

This study also investigated the role of COMT haplotypes on pain caused by fixed appliances. These were not significantly associated with pain severity in this study. However, there was a distinct pattern evident – the APS/HPS haplotypes experienced the greatest total amount of chewing pain, while the LPS/LPS haplotypes experienced the least pain. This is consistent with the findings of Diatchenko and colleagues (2005), who found that females with the APS/HPS haplotypes were 2.3 times more likely to develop TMD when compared to females who had one or two LPS haplotypes. We did not analyze our data
by gender, as males and females showed a similar amount of pain at the teeth after their fixed appliances were adjusted. Even though the differences in perceived pain between the two haplotypes were not statistically significant, they are arguably clinically significant. Indeed, participants with the HPS/APS haplotypes experienced 2.4 times more pain at their teeth than the LPS/LPS haplotypes.

It has been shown that the different haplotypes of the COMT gene do not lead to differences in the amount of COMT mRNA. This suggests that the differences in enzymatic activity are a result of differences in protein translation – the LPS haplotype results in COMT activity which is 4.8 times higher than that of the APS haplotype, while the LPS haplotype results in COMT activity 11.4 times higher than the HPS haplotype. One theory for this effect is that interactions between the rs4633, rs4818 and rs4680 SNPs result in changes of the mRNA secondary structure, which ultimately affects the efficacy of protein translation (Diatchenko et al., 2005). However, it is unclear as to how differences in COMT activity affect pain perception. Reduction in COMT activity associated with a met allele at codon 158 has been shown to decrease levels of endogenous opioids in certain regions of the CNS, leading to increased pain (Zubieta et al., 2003). Additionally, reduced COMT activity could result in increased levels of catecholamines such as adrenaline, leading to persistent pain states through stimulation of B2-adrenergic receptors in both the CNS and PNS (Khasar et al., 2003).

Findings from this preliminary study are promising, and include important associations between orthodontic pain and the rs9316233 SNP of the HTR2A gene and the rs4646310 SNP of the COMT gene. In addition, there was some evidence of a pattern for orthodontic pain across haplotypes of the COMT gene, even though this did not reach statistical significance. In the future, it will be interesting to investigate these associations further using a larger sample size. In summary, certain SNPs and haplotypes of the HTR2A and COMT genes appear to play some role in the severity of orthodontic pain following the adjustment of orthodontic fixed appliances.
5. Future directions and conclusions

This research utilised an EMA approach using a smartphone app to measure pain at the teeth in the three days after an orthodontic adjustment. We believe that this is currently the most accurate and reliable method of measuring orthodontic pain. Further research could assess pain after every adjustment of participants’ fixed appliances, which would certainly provide greater insight into the pain profiles of orthodontic patients as treatment progresses. In addition, this method has the potential to be very useful in measuring other forms of pain, such as TMD and headaches. Measuring pain in real-time should be much more accurate and reliable when compared to retrospective pain reports.

This study found exciting associations between the rs9316233 SNP of HTR2A gene, the rs4646310 of the COMT gene, and the severity of pain after an orthodontic adjustment. In addition, there were promising associations between haplotypes of the COMT gene and the severity of orthodontic pain. Our aim is to significantly increase the sample size over the next two years. In the future, with reducing costs of genetic testing, it may be possible to perform genome-wide testing on a larger sample which would help uncover novel associations between common genetic polymorphisms and orthodontic pain.

If there is a genetic influence on the severity of pain following the adjustment of orthodontic appliances, routine saliva samples of all our patients could identify those who are likely to experience higher levels of pain and discomfort. This paves the way towards personalised medicine. We may be able to tailor our treatment to minimise pain for these susceptible individuals. For example, clinicians may consider increasing the time between successive adjustment appointments, preparing patients and their parents psychologically, using materials with different force properties, and providing individualised pain relief protocols. This may enhance compliance and improve quality of life during treatment.
6. References


7. Appendices

7.1 Participant questionnaire

Genetic and psychological factors associated with orthodontic pain

Date: ______/____/____
ID: ______________
Date of Birth ______/____/____
Gender (please circle) Male/Female

For clinician to fill in:

- Treatment start date (mm/yyyy) __________
- Previous archwire (Mx) __________
- Previous archwire (Md) __________
- New archwire (Mx) __________
- New archwire (Md) __________
- Time and date of archwire change __________
- FULFA Yes/No
- Fixed appliances in one arch Mx/Md

The clinician will go through this section with you:

- Have access to an Android smartphone Yes/No
- Craniofacial syndromes such as cleft lip and palate Yes/No
- Anxiety disorders or depressive illness Yes/No
- Chronic pain syndromes Yes/No
- Use of neurologically-acting medication Yes/No
- Active decay or gum disease Yes/No
- Are you less than 18 years of age? Yes/No
- Previous history of orthodontic treatment (before current braces) Yes/No
  - If yes, did you have a plate, full upper and lower braces, braces on the top teeth, braces on the bottom teeth?
Pain Catastrophising Scale (Child version)

Thoughts and feelings during pain

We are interested in what you think and how strong the feelings are when you are in pain. Below are 13 sentences of different thoughts and feelings you can have when you are in pain. Try to show us as clearly as possible what you think and feel, by putting a circle around the word under each sentence that best reflects how strongly you have each thought.

1. When I am in pain, I worry all the time about whether the pain will end

   Not at all  Mildly  Moderately  Severely  Extremely

2. When I am in pain, I feel I can’t go on like this much longer

   Not at all  Mildly  Moderately  Severely  Extremely

3. When I am in pain, it’s terrible and I think it’s never going to get better

   Not at all  Mildly  Moderately  Severely  Extremely

4. When I am in pain, it’s awful and I feel that it takes over me

   Not at all  Mildly  Moderately  Severely  Extremely

5. When I am in pain, I can’t stand it anymore

   Not at all  Mildly  Moderately  Severely  Extremely

6. When I am in pain, I become afraid that the pain will get worse

   Not at all  Mildly  Moderately  Severely  Extremely
7. When I am in pain, I keep thinking of other painful events

Not at all  Mildly  Moderately  Severely  Extremely

8. When I am in pain, I want the pain to go away

Not at all  Mildly  Moderately  Severely  Extremely

9. When I am in pain, I can’t keep it out of my mind

Not at all  Mildly  Moderately  Severely  Extremely

10. When I am in pain, I keep thinking about how much it hurts

Not at all  Mildly  Moderately  Severely  Extremely

11. When I am in pain, I keep thinking about how much I want the pain to stop

Not at all  Mildly  Moderately  Severely  Extremely

12. When I am in pain, there is nothing I can do to stop the pain

Not at all  Mildly  Moderately  Severely  Extremely

13. When I am in pain, I wonder whether something serious may happen

Not at all  Mildly  Moderately  Severely  Extremely
Corah Dental Anxiety Scale

This next section asks more about how you feel when you go to the dentist. For each question, please tick the box of the answer which comes closest to how you feel.

If you had to go to the dentist tomorrow, how would you feel about it?
- I would look forward to it as a reasonably enjoyable experience
- I wouldn’t care one way or the other
- I would be a little uneasy about it
- I would be afraid that it would be unpleasant and painful
- I would be very frightened of what the dentist might do

When you are waiting in the dentist’s surgery for your turn in the chair, how do you feel?
- Relaxed
- A little uneasy
- Tense
- Anxious
- So anxious that I sometimes break out in a sweat or almost feel physically sick

When you are waiting in the dentist’s chair while they get their drill ready to begin working on your teeth, how do you feel?
- Relaxed
- A little uneasy
- Tense
- Anxious
- So anxious that I sometimes break out in a sweat or almost feel physically sick

You are waiting in the dentist’s chair to have your teeth cleaned. While you are waiting and the dentist is getting out the instruments which they will use to scrape your teeth around the gums, how do you feel?
- Relaxed
- A little uneasy
- Tense
- Anxious
- So anxious that I sometimes break out in a sweat or almost feel physically sick
HOW-I-FEEL QUESTIONNAIRE
Developed by C.D. Spielberger, C.D. Edwards, J. Montuori, and R. Lushene
STAIC Form C-1

Name: _____________________________ Age: _______ Date: _______

DIRECTIONS: A number of statements which boys and girls use to describe themselves are given below. Read each statement carefully and decide how you feel right now. Then put an X in the box in front of the word or phrase which best describes how you feel. There are no right or wrong answers. Don’t spend too much time on any one statement. Remember, find the word or phrase which best describes how you feel right now, at this very moment.

1. I feel ___________________________ □ very calm □ calm □ not calm
2. I feel ___________________________ □ very upset □ upset □ not upset
3. I feel ___________________________ □ very pleasant □ pleasant □ not pleasant
4. I feel ___________________________ □ very nervous □ nervous □ not nervous
5. I feel ___________________________ □ very jittery □ jittery □ not jittery
6. I feel ___________________________ □ very rested □ rested □ not rested
7. I feel ___________________________ □ very scared □ scared □ not scared
8. I feel ___________________________ □ very relaxed □ relaxed □ not relaxed
9. I feel ___________________________ □ very worried □ worried □ not worried
10. I feel ___________________________ □ very satisfied □ satisfied □ not satisfied
11. I feel ___________________________ □ very frightened □ frightened □ not frightened
12. I feel ___________________________ □ very happy □ happy □ not happy
13. I feel ___________________________ □ very sure □ sure □ not sure
14. I feel ___________________________ □ very good □ good □ not good
15. I feel ___________________________ □ very troubled □ troubled □ not troubled
16. I feel ___________________________ □ very bothered □ bothered □ not bothered
17. I feel ___________________________ □ very nice □ nice □ not nice
18. I feel ___________________________ □ very terrified □ terrified □ not terrified
19. I feel ___________________________ □ very mixed-up □ mixed-up □ not mixed-up
20. I feel ___________________________ □ very cheerful □ cheerful □ not cheerful
HOW-I-FEEL QUESTIONNAIRE

STAIC  Form C-2

Name: ____________________________ Age: _________ Date: __________

DIRECTIONS: A number of statements which boys and girls use to describe themselves are given below. Read each statement carefully and decide if it is hardly-ever, or sometimes, or often true for you. Then for each statement, put an X in the box in front of the word that seems to describe you best. There are no right or wrong answers. Don’t spend too much time on any one statement. Remember, choose the word which seems to describe how you usually feel.

1. I worry about making mistakes ......................  □ hardly-ever  □ sometimes  □ often
2. I feel like crying ............................................ □ hardly-ever  □ sometimes  □ often
3. I feel unhappy .............................................. □ hardly-ever  □ sometimes  □ often
4. I have trouble making up my mind .................. □ hardly-ever  □ sometimes  □ often
5. It is difficult for me to face my problems ......... □ hardly-ever  □ sometimes  □ often
6. I worry too much .......................................... □ hardly-ever  □ sometimes  □ often
7. I get upset at home ....................................... □ hardly-ever  □ sometimes  □ often
8. I am shy ..................................................... □ hardly-ever  □ sometimes  □ often
9. I feel troubled .............................................. □ hardly-ever  □ sometimes  □ often
10. Unimportant thoughts run through my mind and bother me ........................................... □ hardly-ever  □ sometimes  □ often
11. I worry about school ..................................... □ hardly-ever  □ sometimes  □ often
12. I have trouble deciding what to do ............... □ hardly-ever  □ sometimes  □ often
13. I notice my heart beats fast ............................. □ hardly-ever  □ sometimes  □ often
14. I am secretly afraid ....................................... □ hardly-ever  □ sometimes  □ often
15. I worry about my parents .............................. □ hardly-ever  □ sometimes  □ often
16. My hands get sweaty .................................... □ hardly-ever  □ sometimes  □ often
17. I worry about things that may happen .......... □ hardly-ever  □ sometimes  □ often
18. It is hard for me to fall asleep at night .......... □ hardly-ever  □ sometimes  □ often
19. I get a funny feeling in my stomach .............. □ hardly-ever  □ sometimes  □ often
20. I worry about what others think of me .......... □ hardly-ever  □ sometimes  □ often

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**Overall, how anxious are you about dental treatment?** Please circle most appropriate answer.

<table>
<thead>
<tr>
<th>Not at all</th>
<th>Mildly anxious</th>
<th>Moderately anxious</th>
<th>Very anxious</th>
<th>Extremely Anxious</th>
</tr>
</thead>
</table>
7.2 Information sheet given to participants following orthodontic adjustment

**Information regarding “My Braces Experience” application**

Thank you for agreeing to participate in our study!

Please start the “My Braces Experience” app and fill in the first questionnaire before you leave the clinic. If you have any questions or problems, do not hesitate to ask Will for help. My patient ID number is ________________

*If you start using the application after 5pm, the application will let you fill in the second questionnaire straight away. Please refrain from filling this in until 8pm or later. After this, you will be required to fill in a questionnaire at the following times:*

- On day 1 at 8pm
- On day 2 at 8am
- On day 2 at 8pm
- On day 3 at 8am
- On day 3 at 8pm
- On day 4 at 8am

*The application will allow you to fill in each questionnaire up to three hours before the scheduled time, and up to three hours after the scheduled time.*

If you turn your phone off, or if it runs out of battery, the app will not be able to send you alerts. Please remember that you will need to fill in the questionnaires around 8am and 8pm each day, for three days.

Please also note that once you click “proceed” for each question, you cannot go back. Therefore, you will need to ensure that the answer you have entered is correct before you hit the “proceed” button for each question.

Could you please try to avoid chewing gum at all times except for when the application asks you to chew gum. Please spit the gum out after you have chewed it for 20 strokes. After you have completed all of the questionnaires from the “My Braces Experience” app, you are welcome to chew as much or as little gum as you like!

After you have completed all of the questionnaires, please ensure that your smartphone is connected to Wi-Fi so that the data from the questionnaires can be sent back to us. If you are out at the time, please open the app when you are back on Wi-Fi so that the data can be sent to us.

If you have any questions or run into any problems, please do not hesitate to email Will at williamsewhoy@gmail.com
Diagram of pain sites

Jaw

Temple

In the ear

In front of the ear
7.3 Flow of “My Braces Experience” app

Figure 1. Participants are sent to WSH as soon as they have finished getting their braces adjusted by their orthodontic postgraduate student.

The participant’s user ID, first name, last name and date of birth are entered.

Figure 2. Scroll to the bottom of this screen, then enter the participant’s sex (male or female).
Figure 3.
Some information about this research project is provided for participants. NB, we changed the inclusion criteria to include any adjustment of the braces (as including only patient’s having arch wire changes would have reduced the number of participants that we could collect data from during the limited duration of this project).

Figure 4.
This screen is no longer relevant, as participant’s do not need to have had an arch wire change to be eligible to start this app. The text should really read, “Has your orthodontist confirmed that you have just had your braces adjusted?”. Participants can then select “no” or “yes”. The app will not let participants proceed unless “yes” is selected.
Figure 5.
More information for the participants. They are now ready to start the app!

Figure 6.
The first question asks about consumption of pain relief. “No” or “Yes” can be selected.
Figure 7. If “Yes” is selected, participants can select what pain relief they have taken in the last 4 hours.

Figure 8. Using a visual analogue scale, participants are asked to describe the current pain level at their teeth. The pointer can be moved anywhere along the horizontal line from, “No pain at all” to “Worst pain imaginable”.

Question 1
Have you taken any pain relief in the last 4 hours?
Yes

Please select which pain relief you have taken

☐ Paracetemol
e.g. PARADOL, PARACARE, PARAPÆDI, PAMOL

☐ Ibuprofen
e.g. IBUDESIC, I-PROFEN, NUROFEN, ADVIL, ACT-3, MEDIX

☐ Diclofenac
e.g. VOLTAREN

☐ Aspirin
e.g. ASPEC, CARTIA, CARDIPRIN

☐ Other

NEXT QUESTION

Question 2
On the line below, move the slider to describe the current pain level at your teeth

No pain at all

Worst pain imaginable

NEXT QUESTION
Figure 9.
This is an example of the participant moving the slider to “Worst pain imaginable”.

Figure 10.
This question asks participants about pain in the jaw, temple, in the ear, or in front of the ear. Participants can select “No” or “Yes” in response to this question.
**Figure 11.** If “Yes” was selected, participants can select what site and what side they have had pain on.

**Figure 12.** As an example, this participant has experienced pain in the jaw region on the left side.
Figure 13.
This patient experienced the “worst pain imaginable” at his/her left jaw.

Figure 14.
This question asks participants about headaches that include the temple areas of the head. Participants can answer “No” or “Yes” in response to this question.
Figure 15.
If “Yes” was selected, participants can select what side they experienced headache pain on.

Figure 16.
As an example, this participant experienced headache pain in the right temple region.
Figure 17.
In terms of headache pain, this patient experienced the “worst pain imaginable” at his/her right temple.

Figure 18.
Participants are then asked to chew one piece of Extra gum for 20 strokes.
Figure 19.
This participant experienced the “worst pain imaginable” when chewing the gum.

Figure 20.
This screen alerts participants that their next survey is tonight at 8pm.
Figure 21. At 8pm on the first day, the application will play an audio alert to inform the participant that the second survey is ready to be filled in.

Figure 22. Again, participants are asked about pain relief consumption. However, from the second survey onwards, the survey asks about pain relief consumption in the previous 12 hours (the first survey asks about pain relief consumption in the previous four hours).
Figure 23.
Again, participants are asked to describe the current pain level at their teeth.

Figure 24.
This question is not included in the first survey. It asks participants about the maximum pain experienced at their teeth since the first questionnaire.
Once again, participants are asked about pain in the jaw, temple, in the ear, or in front of the ear, on either side. Participants can select “No” or “Yes” in response to this question.

As an example, this participant has experienced pain in front of the ear on the right side.
Figure 27.
This patient is experiencing the “worst pain imaginable” in front of his/her ear on the right side.

Figure 28.
This question is not included in the first survey. It asks participants about the maximum pain at the relevant pain site since the first questionnaire.
Figure 29.
Once again, participants are asked about headaches that include the temple areas of the head. Participants can answer “No” or “Yes” in response to this question.

Figure 30.
As an example, this participant experienced headache pain in the left temple region.
Figure 31.
In terms of headache pain, this patient is experiencing the “worst pain imaginable” at his/her left temple.

Figure 32.
This question is not included in the first survey. It asks participants about the maximum headache pain since the first questionnaire.
Figure 33.
Once again, participants are asked to chew one piece of Extra gum for 20 strokes.

Figure 34.
This participant experienced the “worst pain imaginable” when chewing the gum.
Figure 35.
This screen alerts participants that their next survey is tomorrow at 8am. At the subsequent surveys, figures 21-35 are repeated. All in all, participants fill in 7 surveys over approximately 72 hours.
7.4 Ethics approval

H15/124

15 February 2016

Professor M Farella
Department of Oral Sciences
Faculty of Dentistry

Dear Professor Farella,

I am again writing to you concerning your proposal entitled “Genetic and psychological factors associated with orthodontic pain”, Ethics Committee reference number H15/124.

Thank you for your e-mail of 10th February 2016, with attached revised documentation, addressing the issues raised by the Committee.

On the basis of this response, I am pleased to confirm that the proposal now has full ethical approval to proceed.

The standard conditions of approval for all human research projects reviewed and approved by the Committee are the following:

Conduct the research project strictly in accordance with the research proposal submitted and granted ethics approval, including any amendments required to be made to the proposal by the Human Research Ethics Committee.

Inform the Human Research Ethics Committee immediately of anything which may warrant review of ethics approval of the research project, including: serious or unexpected adverse effects on participants; unforeseen events that might affect continued ethical acceptability of the project; and a written report about these matters must be submitted to the Academic Committees Office by no later than the next working day after recognition of an adverse occurrence/event. Please note that in cases of adverse events an incident report should also be made to the Health and Safety Office:

http://www.otago.ac.nz/healthandsafety/index.html

Advise the Committee in writing as soon as practicable if the research project is discontinued.

Make no change to the project as approved in its entirety by the Committee, including any wording in any document approved as part of the project, without prior written approval of the Committee for any change. If you are applying for an amendment to your approved research, please email your request to the Academic Committees Office:
Genetic and Psychological Factors Associated with Orthodontic Pain

Information sheet for participants

Introduction
Thank you for showing an interest in this project. Please read this information sheet carefully. Take time to consider and, if you wish, talk with relatives or friends, before deciding whether or not to participate. If you decide to participate, we thank you. If you decide not to take part, there will be no disadvantage to you, and we thank you for considering our request.

What is the aim of this research project?
We are inviting you to take part in this study, which has been designed to identify whether certain genes and psychological factors are associated with the amount of pain caused by braces. Pain is considered the worst aspect of orthodontic treatment, with more than 90% of patients reporting pain or discomfort at some point during their orthodontic treatment.

This research project will help to improve our understanding of orthodontic pain, and may enable orthodontists to identify patients who are likely to suffer from high levels of pain and discomfort before orthodontic treatment begins. This could enable personalised orthodontic treatment, which may reduce the pain experience of orthodontic patients, and therefore improve their quality of life during the orthodontic treatment process.

Who is funding this project?
This study is being funded by the Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, as well as by the New Zealand Association of Orthodontists.

Who are we seeking to participate in the project?
We are looking for patients who are currently undergoing orthodontic treatment with braces, and patients who are about to commence treatment with braces. You will be invited to participate in this project if you meet the study’s selection requirements.

Unfortunately, not everyone will be suitable for this study. Patients with certain conditions such as cleft lip and/or palate, a diagnosed depressive illness, any chronic pain syndrome, active tooth decay or gum disease, or patients using neurologically-acting medication or medication that could affect pain sensitivity, will not be appropriate for our study, as they may affect or distract us from what we are trying to find out.
If you participate, what will you be asked to do?
Should you agree to take part in this project, you will be asked to provide a sample of your blood for genetic testing, which will be collected in the Orthodontics Department by our research assistant, who is a registered nurse. If you are not willing to provide a blood sample, we will ask you to provide a saliva sample. Blood samples are generally encouraged due to the higher quality of DNA that can be extracted from them. Good quality DNA will greatly help us find these genes involved in orthodontic pain. DNA (via a blood or saliva sample) will only be collected once. Please note that if you have already provided a blood or saliva sample as part of an existing research project within the orthodontics department, you will not be required to provide us with a further blood or saliva sample.

In addition to providing us with some personal information, such as age and ethnicity, you will be asked to fill in some questionnaires, that aim to assess psychological characteristics which may be related to orthodontic pain. The total time to collect the DNA sample and fill in the questionnaires should take between 30-45 minutes.

Finally, you will be asked to fill in pain scores on seven separate occasions in the three days following an adjustment of your braces. At each of these seven occasions, we ask that you chew a sugar-free chewing gum (which we will provide to you) for 20 strokes, before filling in the amount of pain experienced. We will help you to install a specialised application on your smartphone, which will alert you to fill in pain scores at the appropriate times.

Once you have filled in all of your pain scores over the three-day period, we will send you a movie voucher as a thank you for participating in our project.

Once again, participation is entirely voluntary. If you decide not to take part in this project, there will be no disadvantage to yourself of any kind.

Is there any risk of discomfort or harm from participation?
Having a blood sample taken may hurt a little, and some people may get a small bruise at the site where the blood is withdrawn. Although very rare, this site may become infected. However, most people have no problems from this routine procedure. If you have had any bad experiences with giving blood samples, please let the nurse know beforehand, so she can accommodate for your special circumstances.

What specimens, data or information will be collected, and how will they be used?
We will collect personal information such as gender, ethnicity and age. In addition, we will collect clinical information (such as the stage of orthodontic treatment that you are at, what new arch wire has been used in your mouth), information from the questionnaires, as well as the pain scores in the three days following an adjustment of your braces. This data will mainly help us during the analysis stage, when we are trying to make sense of the results. If further information is required, we may need to access your dental/orthodontic records. All of this information will stay strictly private.

DNA will be extracted from blood or saliva samples as described previously. By-products from this procedure are usually disposed of using medical waste contractors. Please indicate on the consent form if you would prefer that a suitable Karakia be used for disposing of this genetic material. The samples, which may be used to study any related genes in the future, will be stored and tested in Associate Professor Merriman’s laboratory at the University of Otago. Rest assured that all of the information that we store will be de-identified, and will not be able to be traced back to you.
The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand). Every attempt will be made to preserve your anonymity. You will also be offered the opportunity to review the main findings of the study through the project’s website.

What about anonymity and confidentiality?
The data collected will be securely stored in such a way that only those mentioned below will be able to gain access to it. Data and DNA samples obtained as a result of the research will be retained for at least 10 years in secure storage. Any personal information held on the participants (such as contact details) will be destroyed at the completion of the research. However, the data derived from the research will most likely be kept for much longer, in a de-identified form.

Only the research team will be able to access the above data and DNA samples. No other external source, commercial or non-commercial, will have access to any personal data or information.

If you agree to participate, can you withdraw later?
Yes, you can. You may withdraw from participation in this project at any time, and without any disadvantage to yourself of any kind.

What if I have any questions?
If you have any questions about our project, either now, or in the future, please feel free to contact either:

Mr William Sew Hoy/Mrs Cindy Mullens
Department of Oral Sciences
Faculty of Dentistry
Tel: +64 3 479 7071
Email: sewwil843@student.otago.ac.nz
  cindy.mullens@otago.ac.nz

Dr Joseph Antoun
Department of Oral Sciences
Faculty of Dentistry
Tel: +64 3 479 7071
Email: joseph.antoun@otago.ac.nz

This study has been approved by the University of Otago Human Ethics Committee (Health). If you have any questions about the ethical conduct of the research, you may contact the Committee through the Human Ethics Committee Administrator (phone +64 3 479 8256, or email gary.witte@otago.ac.nz). Any issues you raise will be treated in confidence, and investigated. You will be informed of the outcome.
Genetic and Psychological Factors Associated with Orthodontic Pain

Information sheet for parents/guardians

Introduction
Thank you to you and your child for showing an interest in this project. Please read this information sheet carefully. Take time to consider and, if you wish, talk with relatives or friends, before deciding whether or not your child should participate. If you and your child decide to participate, we thank you both. If you decide not to take part, there will be no disadvantage to you or your child, and we thank you for considering our request.

What is the aim of this research project?
We are inviting your child to take part in this study, which has been designed to identify whether certain genes and psychological factors are associated with the amount of pain caused by braces. Pain is considered the worst aspect of orthodontic treatment, with more than 90% of patients reporting pain or discomfort at some point during their orthodontic treatment.

This research project will help to improve our understanding of orthodontic pain, and may enable orthodontists to identify patients who are likely to suffer from high levels of pain and discomfort before orthodontic treatment begins. This could enable personalised orthodontic treatment, which may reduce the pain experience of orthodontic patients, and therefore improve their quality of life during the orthodontic treatment process.

Who is funding this project?
This study is being funded by the Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, as well as by the New Zealand Association of Orthodontists.

Who are we seeking to participate in the project?
We are looking for patients who are currently undergoing orthodontic treatment with braces, and patients who are about to commence treatment with braces. Your child will be invited to participate in this project if he/she meets the study’s selection requirements.

Unfortunately, not everyone will be suitable for this study. Patients with certain conditions such as cleft lip and/or palate, a diagnosed depressive illness, any chronic pain syndrome, active tooth decay or gum disease, or patients using neurologically-acting medication or medication that could affect pain sensitivity, will not be appropriate for our study, as they may affect or distract us from what we are trying to find out.
If your child participates, what will he/she be asked to do?

Should you and your child agree to take part in this project, he/she will be asked to provide a sample of his/her blood for genetic testing, which will be collected in the Orthodontics Department by our research assistant, who is a registered nurse. If he/she is not willing to provide a blood sample, we will ask him/her to provide a saliva sample. Blood samples are generally encouraged due to the higher quality of DNA that can be extracted from them. Good quality DNA will greatly help us find these genes involved in orthodontic pain. DNA (via a blood or saliva sample) will only be collected once. Please note that if your child has already provided a blood or saliva sample as part of an existing research project within the orthodontics department, he/she will not be required to provide us with an additional blood or saliva sample.

In addition to providing us with some personal information, such as age and ethnicity, your child will be asked to fill in some questionnaires, that aim to assess psychological characteristics which may be related to orthodontic pain. The total time to collect the DNA sample and fill in the questionnaires should take between 30-45 minutes.

Finally, your child will be asked to fill in pain scores on seven separate occasions in the three days following an adjustment of his/her braces. At each of these seven occasions, we ask that your child chews a sugar-free chewing gum (which we will provide to you) for 20 strokes, before filling in the amount of pain experienced. We will help your child to install a specialised application on his/her smartphone, which will alert him/her to fill in pain scores at the appropriate times.

Once your child has filled in all of his/her pain scores over the three-day period, we will send him/her a movie voucher as a thank you for participating in our project.

Once again, participation is entirely voluntary. If you and your child decide not to take part in this project, there will be no disadvantage to you or your child in any way.

Is there any risk of discomfort or harm from participation?

Having a blood sample taken may hurt a little, and some people may get a small bruise at the site where the blood is withdrawn. Although very rare, this site may become infected. However, most people have no problems from this routine procedure. If your child has had any bad experiences with giving blood samples, please let the nurse know beforehand, so she can accommodate for your child’s special circumstances.

What specimens, data or information will be collected, and how will they be used?

We will collect personal information such as gender, ethnicity and age. In addition, we will collect clinical information (such as the stage of orthodontic treatment that your child is at, what new arch wire has been used in his/her mouth), information from the questionnaires, as well as the pain scores in the three days following an adjustment of your child’s braces. This data will mainly help us during the analysis stage, when we are trying to make sense of the results. If further information is required, we may need to access your child’s dental/orthodontic records. All of this information will stay strictly private.

DNA will be extracted from blood or saliva samples as described previously. By-products from this procedure are usually disposed of using medical waste contractors. Please indicate on the consent form if you would prefer that a suitable Karakia be used for disposing of your child’s genetic material. The samples, which may be used to study any related genes in the future, will be stored and tested in Associate Professor Merriman’s laboratory at the University of Otago. Rest assured that all of the information that we store will be de-identified, and will not be able to be traced back to you.
The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand). Every attempt will be made to preserve your child’s anonymity. You and your child will also be offered the opportunity to review the main findings of the study through the project’s website.

What about anonymity and confidentiality?
The data collected will be securely stored in such a way that only those mentioned below will be able to gain access to it. Data and DNA samples obtained as a result of the research will be retained for at least 10 years in secure storage. Any personal information held on the participants (such as contact details) will be destroyed at the completion of the research. However, the data derived from the research will most likely be kept for much longer, in a de-identified form.

Only the research team will be able to access the above data and DNA samples. No other external source, commercial or non-commercial, will have access to any personal data or information.

If you and your child agree to participate, can you withdraw later?
Yes, you can. Your child may withdraw from participation in this project at any time, and without any disadvantage of any kind.

What if I have any questions?
If you have any questions about our project, either now, or in the future, please feel free to contact either:

Mr William Sew Hoy/Mrs Cindy Mullens
Department of Oral Sciences
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Cindy.mullens@otago.ac.nz

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Genetic and Psychological Factors Associated with Orthodontic Pain

Information sheet for child participants

Thank you for agreeing to consider helping us out. This sheet will explain to you what we are trying to do, and help you to decide whether or not to participate. In either case, we thank you for considering our request. Please remember, there is nothing wrong with not participating if that’s what you prefer.

What are we trying to do?
Braces can be painful sometimes, especially in the first few days after an orthodontist has adjusted them. We are trying to find out whether certain genes, or the way we think, can influence the amount of pain caused by braces. This could eventually help orthodontists to reduce the amount of pain that their patients have to endure.

Who are we looking for?
We are looking for volunteers who currently have braces, and volunteers who are about to receive braces.

What will you be asked to do?
We need three things from you – something to extract the DNA from, information about the way you think, and information about the pain you have experienced after you have had your braces adjusted.

Your DNA, which contains the genes we want to study, is found either in blood or saliva. We would like to take a very small sample of your blood to extract this DNA. This will involve you visiting a nurse who will do this for you. We prefer the DNA that we get from your blood as it helps us a lot more, but we can also collect some saliva instead, if you really don’t want to give blood. Saliva samples involve spitting some of your saliva into a small tube. We will only need to collect your DNA once (either through blood or saliva). If you have already given us blood or saliva as part of an existing research project within the orthodontics department, we will not require any further DNA from you.
The second part involves you answering some questions about the way you think. The total time to collect the DNA and fill in the questionnaires should take about 30-45 minutes.

Finally, we will ask you to record the amount of pain you are feeling in the three days after adjustment of your braces. You can enter the amount of pain you are feeling on your smartphone. Each time you are asked to record the amount of pain you are feeling, you will also be asked to chew on some chewing gum, and record the amount of pain caused by the chewing.

**What will we do with your information?**
We will use your DNA sample and other information you have given us to learn more about orthodontic pain caused by braces. Your DNA sample will be stored and tested in Associate Professor Merriman’s laboratory at the University of Otago. We will keep this information for at least 10 years. Please note that we may use the information collected from this research project for future related research projects. However, all information that you have provided to us will be de-identified.

We will write up the results from this study for our university work. The results may also be written up in journals and talked about at conferences, but your name will not be on anything written up about this study.

**Who will see my answers and other bits of information?**
Only the research team and the people we work with will look at the information you have kindly given to us.

**Can I change my mind and pull out from the project?**
Yes, you can. You may pull out from participation in the project at any time, and without any disadvantage to yourself of any kind.

**What if I have any questions?**
If you have any questions about what we are doing, either now or in the future, please let us know:

William Sew Hoy/Cindy Mullens  
Phone: +64 3 479 7071  
Email: sewwii43@student.otago.ac.nz  
cindy.mullens@otago.ac.nz

Joseph Antoun  
Phone: +64 3 479 7071  
Email: joseph.antoun@otago.ac.nz
Genetic and Psychological Factors Associated with Orthodontic Pain

CONSENT FORM for participants > 16 years of age

1. I have read the Information Sheet concerning this study and understand the aims of this research project.

2. I have had sufficient time to talk with other people of my choice about participating in the study.

3. I confirm that I meet the criteria for participation, which are explained in the Information Sheet.

4. All of my questions about the project have been answered to my satisfaction, and I understand that I am free to request further information at any stage.

5. I know that my participation in the project is entirely voluntary, and that I am free to withdraw from the project at any time without disadvantage.

6. I know that as a participant, I will be providing the study researchers with information such as my medical history, the questionnaires that I will be completing, the DNA from my blood/saliva sample, and the pain scores after adjustment of my braces, as listed in the Information Sheet.

7. I know that the questionnaires will explore psychological factors which may be associated with orthodontic pain, and that if the line of questioning develops in such a way that I feel hesitant or uncomfortable, I may decline to answer any particular question(s), and/or may withdraw from the project without disadvantage of any kind.

8. I understand the nature and size of the risks of discomfort or harm which are explained in the Information Sheet.

9. I know that when the project is completed, all personal identifying information will be removed from the paper records and electronic files which represent the data from
the project, and that these will be placed in secure storage and kept for at least ten years.

10. I understand that the results of the project may be published and be available in the University of Otago Library, but any personal identifying information will remain confidential between myself and the researchers during the study, and will not appear in any spoken or written report of the study.

11. I know there is no remuneration offered for this study, and that no commercial use will be made of the data.

12. I understand that the DNA samples will be tested and stored in Associate Professor Tony Merriman’s laboratory for at least ten years. I also understand that the information I have provided as part of this study may be used for future related studies, but this data will be de-identified and will not be able to be traced back to me.

13. At the end of the study, I consent to any remaining samples being disposed of using:

- [ ] Standard disposal methods
- [ ] Disposed with appropriate karakia

14. I am happy being contacted again in the future

- [ ] No, I do not wish to be contacted again
- [ ] Yes, but I understand that I do not have to participate in further studies

15. In the unlikely event of exposure to blood products by staff, I consent to allow for testing of blood borne diseases to be undertaken

I agree to take part in this project.

…………………………………………………………………………………………………..
(Name of participant)

……………………………………………………………………………………………………
(Signature of participant) .......................................................... (Date)

This study has been approved by the University of Otago Human Ethics Committee (Health). If you have any questions about the ethical conduct of the research, you may contact the Committee through the Human Ethics Committee Administrator (phone +64 3 479 8256, or email gary.witte@otago.ac.nz). Any issues you raise will be treated in confidence, and investigated. You will be informed of the outcome.
Genetic and Psychological Factors Associated with Orthodontic Pain

CONSENT FORM for parents/guardians of participants <16 years of age

1. I have read the Information Sheet concerning this study and understand the aims of this research project.

2. I have had sufficient time to talk with other people about my child’s choice to participate in the study.

3. I confirm that my child meets the criteria for participation, which are explained in the Information Sheet.

4. All of my questions about the project have been answered to my satisfaction, and I understand that I am free to request further information at any stage.

5. I know that my child’s participation in the project is entirely voluntary, and that he/she is free to withdraw from the project at any time without disadvantage.

6. I know that as a participant, my child will be providing the study researchers with information such as his/her medical history, the questionnaires that he/she will be completing, the DNA from his/her blood/saliva sample, and the pain scores after adjustment of his/her braces, as listed in the Information Sheet.

7. I know that the questionnaires will explore psychological factors which may be associated with orthodontic pain, and that if the line of questioning develops in such a way that my child feels hesitant or uncomfortable, he/she may decline to answer any particular question(s), and/or may withdraw from the project without disadvantage of any kind.

8. I understand the nature and size of the risks of discomfort or harm for my child, which are explained in the Information Sheet.

9. I know that when the project is completed, all personal identifying information will be removed from the paper records and electronic files which represent the data from
the project, and that these will be placed in secure storage and kept for at least ten years.

10. I understand that the results of the project may be published and be available in the University of Otago Library, but any personal identifying information will remain confidential between my child and the researchers during the study, and will not appear in any spoken or written report of the study.

11. I know there is no remuneration offered for this study, and that no commercial use will be made of the data.

12. I understand that the DNA samples will be tested and stored in Associate Professor Tony Merriman’s laboratory for at least ten years. I also understand that the information my child has provided as part of this study may be used for future related studies, but this data will be de-identified and will not be able to be traced back to my child.

13. At the end of the study, I consent to any remaining samples being disposed of using:

☐ Standard disposal methods
☐ Disposed with appropriate karakia

14. I am happy being contacted again in the future:

☐ No, I do not wish to be contacted again
☐ Yes, but I understand that I do not have to participate in further studies

15. In the unlikely event of exposure to blood products by staff, I consent to allow for testing of blood borne diseases to be undertaken.

I agree for my child to take part in this project.

..........................................................................................................................................................  ........................................
(Signature of parent/guardian) (Date)

..........................................................................................................................................................
(Name of child)

This study has been approved by the University of Otago Human Ethics Committee (Health). If you have any questions about the ethical conduct of the research, you may contact the Committee through the Human Ethics Committee Administrator (phone +64 3 479 8256, or email gary.witte@otago.ac.nz). Any issues you raise will be treated in confidence, and investigated. You will be informed of the outcome.
Genetic and Psychological Factors Associated with Orthodontic Pain

CONSENT FORM for child participants

I have been told about this study and understand what it is about. All my questions have been answered in a way that makes sense.

I know that:

1. Participation in this study is voluntary, which means that I do not have to take part if I don’t want to, and nothing will happen to me. I can also stop taking part at any time and don’t have to give a reason.

2. Any time I want to stop, that's OK.

3. If I don’t want to answer some of the questions, that’s fine.

4. If I have any worries or if I have any other questions, then I can talk about these with the research team.

5. The paper and computer file with my answers will only be seen by the research team and the people they work with. They will keep whatever I say private.

6. The research team will write up the results from this study for their university work. The results may also be written up in journals and talked about at conferences. My name will not be on anything written up about this study.

7. The research team may use the information that I have given for similar research projects in the future. I understand that this information will be de-identified and will not be able to be traced back to me.

I agree to take part in the study.

.................................................................  ........................................
(Signed)                                           (Date)
7.6 Maori consultation

Tuesday, 15 December 2015.

Professor Mauro Farella,
Faculty of Dentistry - Department of Oral Science,
DUNEDIN.

Tēnā Koe Professor Mauro Farella,

Factors associated with orthodontic pain

The Ngāi Tahu Research Consultation Committee (the committee) met on Tuesday, 15 December 2015 to discuss your research proposition.

By way of introduction, this response from The Committee is provided as part of the Memorandum of Understanding between Te Rūnanga o Ngāi Tahu and the University. In the statement of principles of the memorandum it states "Ngāi Tahu acknowledges that the consultation process outline in this policy provides no power of veto by Ngāi Tahu to research undertaken at the University of Otago". As such, this response is not "approval" or "mandate" for the research, rather it is a mandated response from a Ngāi Tahu appointed committee. This process is part of a number of requirements for researchers to undertake and does not cover other issues relating to ethics, including methodology they are separate requirements with other committees, for example the Human Ethics Committee, etc.

Within the context of the Policy for Research Consultation with Māori, the Committee bases consultation on that defined by Justice McGechan:

"Consultation does not mean negotiation or agreement. It means: setting out a proposal not fully decided upon; adequately informing a party about relevant information upon which the proposal is based; listening to what the others have to say with an open mind (in that there is room to be persuaded against the proposal); undertaking that task in a genuine and not cosmetic manner. Reaching a decision that may or may not alter the original proposal."

The Committee considers the research to be of importance to Māori health.

As this study involves human participants, the Committee strongly encourage that ethnicity data be collected as part of the research project as a right to express their self-identity. That is the questions on self-identified ethnicity and descent, these questions are contained in the latest census.

The Committee notes the researchers have identified that, "Maori blood samples will be disposed with appropriate karakia, if requested", and asks what has been implemented to enable this and how are the researchers identifying those participants who wish karakia.

The Committee suggests researchers consider the Southern District Health Board’s Tikaka Best Practice document, in particular patient engagement. The document also covers the collection, storage and disposal of blood and tissue samples. This document is available on the Southern District Health Board website.

The Ngāi Tahu Research Consultation Committee has membership from:
Te Rūnanga o Ōtākou Incorporated
Kāti Huirapa Rūnanga ki Puaretariki
Te Rūnanga o Moeraki