

**IDENTIFICATION OF POTENTIAL GENETIC MARKERS OF FACIAL ASYMMETRY
AND TMD IN ORTHOGNATHIC SURGERY PATIENTS**

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ABSTRACT

Temporomandibular disorders (TMD) are comorbid conditions. Most are related to anxiety-induced muscular pain, but some are associated with facial asymmetry resulting from condylar resorption (CR) or condylar hyperplasia (CH). The etiology of the most common forms of CH and CR are still unknown. CR can be caused by rheumatoid arthritis (RA) or more commonly osteoarthritis (OA) of the TMJ, and inflammatory mediators have been previously implicated. Previous studies have identified pain/inflammatory genes related to chronic TMD while others have demonstrated potential genetic markers for RA. Similarly, genome-wide association (GWA) studies have identified genes associated with height, some of which may participate in craniofacial growth, CH, and the development of asymmetry. Masseter muscle is frequently involved in TMD of muscular origin, and left/right fiber-type differences have been previously found in subjects with facial asymmetry. A human transcriptome microarray was used to evaluate whether genes involved with height, pain, or inflammation were differentially expressed in masseter muscle from facially asymmetric patients with and without TMD.

This study evaluated orthognathic surgery patients with varying skeletal malocclusions, including subjects with and without facial asymmetry and TMD (n= 93). Masseter muscle samples were collected from ten orthognathic surgery patients treated to correct skeletal malocclusions. Two of whom were classified with facial asymmetry with or without TMD, with one of the two showing positive evidence of CR. Samples were

disrupted in QIAzol Lysis Reagent, RNA was isolated using a Qiagen miRNeasy Mini Kit according to the manufacturer's instructions, and quality of the total RNA was tested by Agilent Bioanalyzer and Nanodrop spectrophotometry. Samples were used for quantitative Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and protocols for microarray analysis were conducted as described in the Ambion WT Expression Manual and the Affymetrix GeneChip Expression Analysis Technical Manual.

Principal Components Analysis (PCA) was completed to detect fold-changes for each transcript to determine differences in global gene expression between the two asymmetric and eight remaining subjects. To find differentially expressed transcripts step-up t-tests were performed to correct for false discovery rate (FDR) comparing the two asymmetric samples to the eight symmetric samples. Differences were considered significant if step-up p-values were <0.05 and fold differences were $>\pm 2$ between groups. This study evaluated 847 height-related genes and 551 genes associated in pain/inflammatory processes. Genes of interest were determined a priori from GWA studies and the Algenomics Pain Research Panel v.2.0 partially derived from the Orofacial Pain: Prospective Evaluation and Risk Assessment (OPPERA) study.

Two hundred and eight transcripts of 847 height associated genes and 132 of 551 pain/inflammatory genes were significant for expression ($P < 0.05$) and displayed $>\pm 2.0$ fold differences in facial asymmetry and/or TMD specimens. Among genes specifically reported to be associated with pain/inflammation, NPY5R (+2.11 fold), GABRA6 (+2.14 fold), CACNA2D1 (-12.51 fold) and EREG (+2.12 fold) showed significantly different ($P < 0.001$) expression levels in the two asymmetric versus the remaining eight symmetric

patients. CACNA2D1 expression was significantly increased in symmetric male subjects versus symmetric females ($P < 0.05$) as well as in asymmetric females versus asymmetric males ($P < 0.05$). CACNA2D1 expression was also significantly increased in symmetric male subjects versus symmetric females ($P < 0.05$) and was differentially expressed at lower levels, however not significantly, in asymmetric males ($p = 0.51$).

Based on the results collected, the following conclusions were drawn. These methods provide a novel approach to study TMD and/or facial asymmetry in human subjects. To our knowledge, this is the first study to demonstrate that significant expression variation in human height genes may contribute to facial asymmetry with or without TMD, possibly through decreased expression of CACNA2D1. These data suggest TMD patients with facial asymmetry associated with condylar resorption may show significant differential expression of certain inflammatory marker genes such as EREG and CACNA2D1. These data support that gender may play a key role in the development of TMD, possibly through increased CACNA2D1 expression providing protective effects in TMD-free males but deleterious effect in females with TMD. These results support previous findings of pain/inflammatory genes associated with TMD derived from muscular pain. Further studies are needed to understand the genetic contributions to TMD, which may play an important role in future clinical intervention.

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CHAPTER 1

INTRODUCTION

In the age of genomics, much research has been devoted to find genetic markers of medical conditions in hope of developing therapeutic regimens on the molecular level to treat polygenetic disease phenotypes. Unfortunately, the literature falls short on documenting the genetic and epigenetic influences on the craniofacial complex. Facial asymmetry, TMD, CH, and CR all may be influenced by complex inheritance with multifactorial etiology between genetic makeup and environmental factors.

Future genetic testing that could assess the risk of, or intervene in the development of, facial asymmetry of orthodontic patients may have multiple benefits. Clinicians would be able to eliminate ambiguity in diagnosis and treatment as well as improve functional and esthetic orthodontic outcomes. It may also provide crucial diagnostic information of potential comorbidities to medical colleagues. An example of such is would be predilection toward a deviated septum increasing risk of chronic sinusitis or sleep apnea.

TMD is the most common orofacial pain disorder (Dworkin et al., 1992). It is believed that the condition's economic impact to society is approximately 17 million lost days of work for every 100 million workers (Dworkin & LeResche, 1993). It occurs mainly between 20 and 40 years of age (Williamson & Simmons, 1979), and approximately 50% of individuals have a least one sign or symptom of this heterogeneous condition (Okeson, 2013). Prevalence of severe TMD is thought to range between 20-30%, but surprisingly only about 5% of these patients require treatment (Henriksson et al., 2002). This discrepancy is thought to be due to the wide variance of

perceived pain (Epker & Gatchel, 2000). Women have been found to show higher frequency of TMD than males (Bush et al., 1993). Age and gender are considered two consistent demographic risk factors related to TMD, while race remains controversial (Edwards et al., 2001). Plesh et al. reported that Caucasian women showed higher rates of TMD than African Americans after adjusting for socioeconomic status (2002).

The etiology of TMD is multi-factorial. Most TMD is muscular in origin (Schiffman et al., 1990), and recent studies have found an association between TMD and inflammatory/pain-related genes (Maixner et al., 2011). Other studies have found associations between TMD and facial asymmetry (Fushima et al., 1999). TMD with facial asymmetry is less common and may be associated with condylar resorption (Okeson, 2013) or condylar hyperplasia (Wolford et al., 2014). Condylar resorption can be caused by Rheumatoid Arthritis (Okeson, 2013) while the etiology of the most common form of condylar hyperplasia is still unknown (Wolford et al., 2014). Previous studies have identified potential pain/inflammatory-related genetic markers for RA (Eleftherohorinou et al., 2011). Genome-wide association studies have identified genes associated with height (Allen et al., 2010), some of which may participate in craniofacial growth, condylar hyperplasia, and development of facial asymmetry (Kwon et al., 2007). Masseter muscle is frequently involved in muscular-associated TMD together with left/right fiber-type differences in subjects with facial asymmetry (Raoul et al, 2011).

Advances in biomedical science holds the promise for the possible ability to determine an individual's likelihood of acquiring facial asymmetry with and without TMD, the potential level of severity, and proper therapeutic intervention based on genetic

analysis. Such promise may be achieved through a saliva sample taken from a patient on initial exam. Patients treated in such a proactive fashion would not only decrease the economic burden of medical treatment of TMD, but vastly improve the quality of care by providing precise, successful clinical intervention. Unfortunately TMD are comorbid conditions, sometimes involving dentofacial deformities. Often they are plagued by underdiagnosis, misdiagnosis, and mistreatment. There is lack of scientific predictability in the non-surgical management of TMD to successful outcomes, and this scenario has not only has created insufficient informed consent, but has also led to patient decisions that significantly affect their quality-of-life. As medical schools' training on the oral cavity and the TMJ is often sparse, orthopedists, general physicians, and rheumatologists are often hesitant to treat TMD. If orthodontists are truly experts in dento-facial orthopedics, the orthodontic community should 'quarterback' the proper diagnosis and treatment of TMD with justification through evidence-based research.

In this case control study, a human transcriptome microarray was used to evaluate whether genes involved with height, pain, or inflammation were differentially expressed in masseter muscle from facially asymmetric patients with and without TMD.

CHAPTER 2

REVIEW OF THE LITERATURE

2.1 TMD of Muscular Origin

The most common form of TMD is muscular in origin, and its main symptom is masticatory muscle pain (Schiffman et al., 1990). Pain may also be perceived in other head and neck muscles, through headaches, at the TMJ, and in the dentition. A second symptom associated with muscular TMD is muscle dysfunction which elicits pain upon any further contraction outside a limited range of motion. The patient often will present with limited mouth opening because surpassing that range would induce myalgia. Sometimes acute malocclusion may occur, resulting from muscle dysfunction, as patients show a deviation of their normal occlusion upon closure. This dental sign is derived from a muscle contraction leading to temporary shortening of muscle resting length (Okeson, 2013).

All muscular disorder TMDs are precipitated by local or systemic factors. Examples of local factors include tooth or facial trauma and extended masticatory muscle activity. Main systemic factors are believed to be stress and genetics. A less common systemic factor is deep pain input. Any chronic, deep pain input may trigger the muscle splinting (Carlson et al., 1993). Also called muscular protective co-contraction, muscle splinting is the biological safety mechanism which responds to a systemic or local event (Bell, 1990). During muscle splinting, mouth opening and closing results in increased muscle activity of elevator and depressor muscles outside the normal range. An example of a local acute factor that induces co-contraction is the placement of a high filling. The

masticatory muscles compensate for the interference and fatigue themselves. If the co-contraction is short, muscle function is returned to normal. However, extended co-contraction leads to the development of a masticatory muscle disorder. Little information is known about other examples of systemic factors which include bacterial and viral infections.

Emotional stress is a predominant systemic etiology behind muscular disorder TMD. It was previously thought that poor occlusion was the primary cause of masticatory muscle disorders. It is now believed that the majority of parafunctional activity which accentuates TMD signs and symptoms is due to anxiety as opposed to occlusal condition (Okeson, 2013). Stressors are defined as anything that causes stress, and they incorporate both good and bad events. Interestingly, the body handles both good and bad stress the same way (Selye, 1974). The hypothalamus is the emotional center of the brain. It processes stress using the hypothalamus-pituitary-adrenal (HPA) axis. Stress stimulates the sympathetic nervous system (SNS) to activate the HPA axis. The HPA axis excites somatic gamma efferents, leading to increased activation peripheral skeletal muscles. This directly leads to increased muscle tension over time and is the most common complication of internal stress release. Internal stress is the release of stress internally and is generally believed to be something that humans should strive to avoid. Certain forms of external stress release are inappropriate in society, such as shouting and object breaking. Physical exercise is an acceptable form of external stress release. Stress is a normal part of life that can be a healthy motivating factor. Long-term internal stress release can be damaging to the body. Studies have shown that it

upregulates the sympathetic nervous system (SNS), and prolonged sensitization of the SNS is thought to increase muscle tone (Grassi & Passatore, 1988).

2.2 Genomics and TMD

In addition to studying hormonal differences and psychosocial factors that influence pain transmission in TMD, the age of genomics has led some to look for genetic markers of chronic pain. Interestingly, it has been reported that the main clinical factor found on initial examination of a TMD patient is previous history of a separate painful condition (Andersson, 2004). Previous studies have evaluated genes associated with pain transmission, pain perception, and inflammation, however due to their often cross sectional design, science has failed to uncover if these factors are an etiology or consequence of TMD (Diatchenko et al., 2005). To answer this significant problem, the Orofacial Pain Prospective Evaluation and Risk Assessment (OPPERA) consortium was begun in 2006.

OPPERA proposes two phenotypes in the development of TMD. Pain amplification and psychological stress may act synergistic to one another in determining the onset and persistence of TMD (Maixner et al, 2011). Pain amplification is characterized by increased sensitization of the autonomic and central nervous systems, decreased inhibitory input during pain perception, and an increased inflammatory state. The result is an increased response to nociceptive input. Previous case-control studies has found pain amplification to be related to TMD risk (Maixner et al., 1995). High

levels of stress or depression have been linked to TMD (Carlson et al., 1993). Somatic awareness, defined as the ability to perceive increased signs and symptoms that cannot be explained clinically, has also been implicated in developing TMD by greater than twice the normal rate (Rammelsberg et al., 2003). Unfortunately, studies have not determined with confidence whether the psychological characteristics seen in certain TMD patients were preexisting or not. One prospective study found that TMD-free females were significantly more likely to develop TMD over a three year period if they had anxiety and depression at baseline (Slade et al., 2007).

The first part of the OPPERA project was a baseline case-control study which recruited individuals with and without chronic TMD from 2006 to 2008. Its goals were to determine associations between TMD and six possible risk factors including sociodemographics, pain amplification, cardiovascular function, psychosocial profile, and a series of over 300 genes believed to be involved in the perception of pain (Maixner et al., 2011). This initial study led to the publication of eight subsequent papers that detail the background, methods, and findings of the case-control study as well as the future direction of the OPPERA project. OPPERA's main study is a prospective cohort also begun in 2006 of 3,200 initially TMD-free subjects over a seven year period. This study will differ from the baseline case-control OPPERA as it will be evaluating for etiological determinants of initial-onset TMD. Goals of the prospective study are to determine if psychosocial profile, pain amplification, elevated cardiovascular parameters, and specific pain-related genes of interest are risk factors for initial-onset TMD (Maixner et al., 2011). Results of the prospective study are expected to be published in 2014.

The baseline case-control OPPERA study found that Caucasians and females had the greatest risk of developing TMD (Slade et al., 2011). Certain autonomic nervous system (ANS) dysfunction was also noted. These included decreased cardioparasympathetic and increased cardiosympathetic activity in TMD patients (Maixner et al., 2011). The strongest, significant associations with TMD case status were found with pain amplification of sensitivity to pressure-point pain and increased somatic awareness physiological profile (Greenspan et al., 2011, Fillingim et al., 2011).

The OPPERA study which sought to find genetic risk factors for chronic TMD used 166 chronic TMD subjects from the baseline case-control OPPERA study and 1,442 TMD-free controls recruited from the prospective OPPERA cohort. To increase the power of the study, 183 chronic TMD cases and 170 controls were added from a University of North Carolina (UNC) case-control study. While the genotyping and phenotyping procedures were mainly consistent with the procedures of OPPERA, recruitment of subjects differed. While OPPERA used Caucasian, African Americans, and other races, UNC used only non-Hispanic white females for both their cases and controls. Also, UNC TMD subjects were selected from the UNC Orofacial Pain Clinic while OPPERA's TMD subjects were selected from the population. Blood genotyping was carried out and samples were genotyped using the Algynomics Pain Research Panel v.2.0 (complete list provided through <http://www.algynomics.com/pain-research-panel.html>) assessing 3,295 single nucleotide polymorphisms (SNPs).

Significant associations were found in the following pain/inflammatory genes: calcium channel, voltage-dependent, alpha 2/delta subunit 1 (CACNA2D1) which

mediates the influx of calcium ions into the cell upon membrane polarization; epiregulin (EREG) which is a ligand of epidermal growth factor receptor (EGFR) family; Catechol-*O*-methyltransferase (COMT) involved in neurotransmission of dopamine, norepinephrine, and epinephrine; 5-hydroxytryptamine (serotonin) receptor 2A (HTR2A) involved in neurotransmission of serotonin; Nuclear receptor subfamily 3, group C, member 1 (NR3C1) involved in the glucocorticoid response; Calcium/calmodulin-dependent protein kinase type IV (CAMK4) implicated in transcriptional regulation in lymphocytes and neurons; G protein-coupled Cholinergic receptor, muscarinic 2 (CHRM2) involved in the binding of acetylcholine; Interferon-related developmental regulator 1 (IFRD1) involved in regulating gene activity in the proliferative and/or differentiation pathways induced by NGF; G protein-coupled receptor kinase 5 (GRK5) functioning to regulate motility of leukocytes; adrenoceptor alpha 2C and 1D (ADRA2C, ADRA1D) regulating neurotransmitter release from sympathetic nerves and from adrenergic neurons in the central nervous system; opioid receptor, delta 1(OPRD1) functions in analgesia, motor integration, cognitive function, and mood driven behavior; interleukin 10 (IL-10) which is a cytokine involved in inflammation; and glutamate receptor, ionotropic, N-methyl D-aspartate 2A (GRIN2A) involved in long-term potentiation which is an activity-dependent increase in the efficiency of synaptic transmission thought to underlie certain kinds of memory and learning (Smith et al., 2011).

The authors reiterate that casual relationships between chronic TMD and clinical, psychosocial, and autonomic findings cannot be inferred due to the case-control nature of these combined studies. These findings will be further evaluated in the prospective

cohort OPPERA study. While previous literature has implicated many gene associations with TMD, what has yet to be determined are causative genetic factors of TMD with and without facial asymmetry.

2.3 Muscular Disorder TMD Diagnosis and Treatment

To date, four types of masticatory muscle TMD disorders have been identified. These include acute non-inflammatory local muscle disorders, acute central nervous system (CNS)-mediated local muscle disorders, chronic CNS-mediated local muscle disorders, and chronic systemic muscle disorders.

2.3.1 Acute Non-Inflammatory Local Muscle Disorders

Non-inflammatory myalgia is local muscle soreness that occurs from overuse of a muscle, short-withstanding stress that has not significantly stimulated the autonomic nervous system (ANS), other regional change in the orofacial environment such as trauma, or even deep pain (Okeson, 2013). It is the most common acute muscle disorder, and this condition left untreated may cause deep pain which can progress to cyclical chronic pain after the original regional insult has healed. Generally there is no pain at rest, and it is believed that this somatic pain on motion is caused by a regional inflammatory-repair response. Treatment includes limiting mandibular movements to non-painful motions, educating the patient of the impact of stress, instructing the patient to limit non-functional tooth contact, the use of occlusal splints, and prescribing anti-inflammatory medications if deep pain input is suspected.

2.3.2 Acute Central Nervous System (CNS)-Mediated Local Muscle Disorders

Acute muscle disorders involving the CNS include tonic contraction myalgia and trigger-point myalgia. These disorders are primarily thought to be brought on by stimulation of the autonomic nervous system (ANS) due to stress, however they can also occur from deep pain input. When anxiety is the etiological agent, the CNS stimulates peripheral afferent nerves, causing a release of nociceptive mediators into peripheral tissues to cause pain (Mense, 1993). If acute muscle disorders are left untreated with continuity of pain, these disorders can become chronic. Chronic muscle disorders often involve broader areas of muscular pain with secondary clinical depression and involve sensitization of the CNS. (Marbach & Lund, 1981). Untreated chronic masticatory muscle disorders may lead to functional, internal derangements of the TMJ which may require surgery for treatment.

Tonic contraction myalgias are rare events and are categorized by myospasms (Okeson, 2013). These can be induced by muscle fatigue or electrolyte imbalances and often present with pain at rest. Myospasms are easily differentiated from other muscle disorders as they involve a firm, fully-contracted muscle which causes muscle shortening and sometimes acute malocclusion. In most cases, they only last for a few minutes and are rare events. If the myospasm occurs frequently, the muscular disorder is classified as a dystonia which requires a different treatment strategy. The most effective treatment includes allowing the muscle to stretch through massage or even through injection of local anesthetic into the myospasm region.

Trigger point myalgia is categorized by myofascial pain (Okeson, 2013).

Although its etiology is not fully understood, it is believed that certain muscle bands in trigger zones become hypersensitized upon palpation, causing referred pain through central excitatory effects. Trigger points can either be active or inactive. Most lie in areas of cervical neck and shoulder muscles, but sometimes lie on the face. An active trigger point is one that immediately refers pain to a different region when touched. A dormant trigger point will not elicit a painful response when provoked. Trigger point myalgia may be derived from overuse of a muscle, psychological stress, environmental factors, infections, or even deep-pain input (Travell et al., 1999). It is important to note that the patient's main symptom will be the area of referred pain and not the trigger point itself. Treatment involves localizing the trigger point, stretching the trigger point region via massage, ultrasound treatment, or injection of local anesthesia.

2.3.3 Chronic CNS-Mediated Local Muscle Disorders

Chronic muscle disorders consist of centrally mediated myalgia and fibromyalgia. Centrally mediated myalgia is a chronic local muscle disorder that involves a history of constant, long-standing pain primarily through CNS effects. This constant neurogenic pain is present both at rest and during activity and is usually of long-standing history. Muscles are sore upon palpation and may atrophy as a consequence of disuse. It is mainly caused by exacerbation of an untreated acute local muscle disorder which leads to the antidromic effect. Here, peripheral muscle ANS afferents become CNS afferents to the muscle. Instead of pain transmission from the periphery to the CNS, transmission of

pain signals occurs from the CNS to the muscle by release of mediators such as bradykinin and substance P into the peripheral tissue. Treatment should not be carried out on the peripheral tissue that is receiving referred pain signals but instead should be focused on the central pain mechanism. Options for treatment include increasing rest of muscles, restricting the mandible to non-painful movements, avoiding exercise and injections into local tissues, and teaching patients to disclude teeth during the day along with possible occlusal splint therapy during sleep. Medications such as anti-inflammatories, tricyclic antidepressants, and anticonvulsants have also been documented to improve sleep and help desensitize the CNS. Centrally mediated myalgia treatment often progresses slowly. Once the patient is out of pain, physical therapy stretching of the masticatory muscles can be started (Okeson, 2013).

2.3.4 Chronic Systemic Muscle Disorders

One chronic systemic myalgia that dentists should be aware of is fibromyalgia. Although its etiology is controversial, fibromyalgia is a systemic musculoskeletal pain disorder which involves CNS hypersensitization to peripheral muscle input. Patients experience non-trigger point pain in all four quadrants of the body and often live sedentary lifestyles as muscle function increases pain. It should not be confused with TMD, as approximately 42% of its patients show TMD symptoms (Korszun et al., 1998). Treatment should be referred to a medical colleague.

2.4 TMD Associated with Condylar Resorption

Condylar resorption has several etiologies, some of which include trauma, infection, osteoarthritis (OA), and rheumatoid arthritis (RA). Proper diagnosis of these joint disorders through a detailed medical history and clinical exam are critical for effective treatment. No matter the etiology, all condylar resorptive diseases result in activation of the molecular bone resorption pathway. This is characterized by the release of cytokines that promote osteoblast recruitment of osteoclasts, resulting in enzymatic degradation of hydroxyapatite.

2.4.1 Osteoarthritis of the TMJ

Although its etiology remains unknown, OA of the TMJ is thought to be caused by an increased loading of the TMJ and is the most common arthritides of the TMJ (Okeson, 2013). Physiological bone loading of the TMJ helps to deliver nutrients to and from local tissues while pathological loading of the subarticular condylar bone is believed to create free radicals. These free radicals then undergo oxidation reactions which destroy articular tissues (Gunson et al., 2011), leading to the recruitment of cytokines. Numerous cytokines and matrix metalloproteinases (MMPs) have been implicated in condylar resorption including: Tumor Necrosis Factor – alpha (TNF – alpha) which functions as a pro-inflammatory cytokine involved with a variety of diseases; Interleukin 6 (IL-6) which is a cytokine that regulates cell growth and differentiation and plays an important role in the immune response; Receptor activator of nuclear factor kappa-B ligand (RANKL) which is a member of the TNF family involved with bone remodeling; matrix metalloproteinase 1 (MMP-1) involved in the breakdown of extracellular matrix (Muroi et al., 2007). OA is a non-inflammatory disease and occurs in two forms. The

less common form, primary OA, presents alone with an unknown etiology. Secondary OA is more common and is often secondary to progressive anterior disc displacement without reduction (DeBont et al., 1986). Anterior displacement of the condyle is thought to occur from hyperplasia of the synovial tissues causing a breakdown of the supporting disc ligaments (Wolford, 2001). This leads to an acceleration of abnormal loading of the condyle as it articulates directly with the glenoid fossa. Clinical symptoms of OA commonly include constant joint pain that increases with mandibular movement and secondary cyclic pain is often a comorbidity. Signs of the disease include limited mandibular opening and crepitus. Diagnosis is confirmed by radiographic evaluation usually limited to subcortical bone loss of the condyle caused by the pathologic loading force (Quinn & Stover, 1998). It is important to note that radiographic changes are only seen after approximately six months of active OA when significant demineralization has occurred.

In severe OA, bone resorption is extended to the articular surfaces of the condyle and glenoid fossa resulting in osteophyte formation and increased joint space. The results of severe bilateral resorption are heavy posterior occlusal contact, bilateral anterior open bite, possible mandibular asymmetry due to differential resorption, and backward rotation of the mandible. Unilateral OA is called idiopathic condylar resorption (ICR). ICR is characterized by moderate to severe facial asymmetry with ipsilateral midline deviation, ipsilateral heavy posterior contact, contralateral open bite, and often TMJ pain.

Interestingly, it is more common in young females and estrogen modulation has been implicated (Gunson et al., 2009). Recently, Gunson et al. proposed that ICR patients may be positive for various inflammatory factors locally in their TMJ synovial fluid as

opposed to through blood analysis. They suggest that these patients might benefit from anti-inflammatory pharmacological treatment similar to that of autoimmune disease patients (2012).

Most OA cases are self-limiting (de Leeuw et al., 1995), and 80% occur predictably in three stages (Okeson, 2013). The first stage is categorized by clicking and crepitus with or without pain. During the second stage pain emerges with limited mandibular range of motion. The third stage occurs in two parts. The first shows a decrease of pain with continued joint sounds. The final stage shows a return to normal mandibular range of movement with no pain. OA becomes osteoarthritis as the remodeling becomes stable and the joint begins to adapt, often with deposition of sclerotic bone of the articular surface of the condyle. From initial appearance of symptoms to termination, the usual time period is approximately one year.

Osteoarthritis is a stable condition that does not require treatment. Understanding the stages of OA are critical for the clinician to help discourage aggressive therapy.

Conservative treatment for active OA consists of splint therapy, stress release protocols, painless muscle stretching, and anti-inflammatory medications. In some cases where these therapies do not allow for cessation of symptoms, intracapsular corticosteroid injections are indicated. If this supportive therapy fails, surgical intervention may be necessary. In severe OA cases, especially in ICR, the patient reaches the osteoarthritis period with significant facial asymmetry, and openbite malocclusion. In these cases, orthognathic surgery is required to restore the patient to normal esthetics and function.

2.4 TMD Associated with Rheumatoid Arthritis

Prevalence of RA is estimated at approximately 0.5-1% of the general population (Silman & Hochberg, 2001). RA is an autoimmune polyarthritis, an arthritis which involves over five joints in the body, which causes hyperplasia of the synovial membrane. The synovial fibroblasts secrete enzymes that destroy cartilage and in severe cases, synovial osteoclast proliferation causes subsequent bone loss. Currently, diagnosis involves a grading system where points are given for certain criteria. Examples include positive joint involvement of a given joint and positive serology factors such as rheumatoid factor, C-reactive protein, erythrocyte sedimentation rate, and anti-citrullinated protein antibodies. Within three months of diagnosis, patients are usually treated with non-biologic disease-modifying anti rheumatic drugs (DMARDs). These drugs work to reduce positive serology factors and decrease damage to bone and cartilage. While the etiology of RA remains unknown, there is evidence that the disease is strongly dictated by genetics. Genes that have been implicated include Major Histocompatibility Complex, class II, DR beta 1 and DR4 (HLA-DRB1 & HLA-DR4) of the human leukocyte antigen (HLA) class II genes and Protein Tyrosine Phosphatase, non-receptor type 22 (PTPN22) which affects B and T cell receptors (Scott et al., 2010). Others include EREG and CACNA2D1 (Eleftherohorinou et al., 2011, Murakami et al., 2013). Some have found an association between the onset RA and emotional stress (Boscarino et al., 2010).

A common dentofacial consequence of RA is condylar resorption. In juvenile rheumatoid arthritis (JRA) patients, resorption of the condyles has been estimated at 62 percent (Pedersen et al., 2001). Approximately 50% of RA patients have TMJ symptoms

(Tabeling & Dolwick, 1984). RA condylar resorption is commonly bilateral and accompanied by multiple joints of the body demonstrating painful symptoms. Clinical signs are similar to bilateral OA condylar resorption which includes increased posterior contact, facial asymmetry, and development of a bilateral openbite (Sato et al., 1997). Diagnosis of RA condylar resorption is confirmed with RA diagnosis and radiographic findings. While pharmacological treatment should be differed to a rheumatologist, the dental profession can provide supportive treatment. This includes a stabilization appliance and possible arthrocentesis if symptoms do not improve. RA condylar resorption does not follow the fairly predictable course of OA condylar resorption. In OA, if the resulting malocclusion and esthetic change are severe enough, the patient can be treated surgically during osteoarthrosis with predictable outcomes. RA is characterized by periods of activity and remission. Therefore, if orthognathic surgery is completed after an active period, the results are unpredictable.

2.5 Condylar Hyperplasia

Condylar hyperplasia (CH) was first recorded in the literature by Humphey and Adams in 1856 (Adams, 1873). Signs and symptoms of bilateral CH may include TMD, facial asymmetry due to differential condylar growth, a developing prognathic mandible with compensatory maxillary downward growth, anterior and posterior crossbite, and open bite. Similar to unilateral CR, unilateral CH shows exacerbated functional and esthetics deformities. Unilateral CH may show TMD, ipsilateral open bite, contralateral lingual and anterior crossbite, ipsilateral compensatory downward maxillary growth, and

contralateral midline shift. TMD symptoms of CH include both masticatory muscle disorders and internal derangements. Previously the term CH has been used in the literature to describe many different growth aberrations of the mandibular condyle. Wolford has classified four types of CH (2013), with type 1 as the most prevalent and type 4 as the least.

CH type 1 is the most common form of CH. This class occurs during the pubertal growth as an accelerated and accentuated growth deviation. It is self-limiting and often ends in the patients second decade of life. CH type 1A depicts bilateral accelerated mandibular growth where patients will often develop Class III malocclusions due to the predominately horizontal growth vector of the condyle. CH type 1B is the unilateral version of CH type 1A. Radiographic imaging reveals slight elongation and rounding of the head and neck of the condyle. Disc displacements are also common, especially on the contralateral side, and caused by functional overload of the pathology. Hand-wrist radiographs do not provide diagnostic insight, as CH is a pathologic process and does not follow normal physiology. Upon histological analysis, the condyle resembles a normally growing condyle with the exception of hyperplasia of the proliferative zone. The proliferative zone of the condyle is responsible for the remodeling of the condylar head and neck. Its role is to form cartilage which is transformed into bone. It is believed that when the proliferative zone has ceased activity that progression of CH type 1 has ended (Wolford et al., 2009).

Treatment of CH type 1 is depended on active versus completed condylar growth. Previously, isotopic bone scans have used to detect activity of CH to aid as a diagnostic

tool. Some argue that Single Photon Emission Computed Tomography (SPECT) and bone scintigraphy are not diagnostic of “hot” condyles due to high false-positive rates (Wolford, 2013). Reasons include difficulty differentiating on bone scans normal condylar activity from abnormal condylar growth. It is also believed that displaced discs show increased activity. Wolford states that the most accurate way to diagnosis CH type 1 is through serial cephalometry, serial dental models, and serial clinical evaluations. Treatment of arrested CH type 1 includes orthognathic surgery to restore functional and esthetic concerns. There are two options for treating active CH type 1. Surgery may be deferred until condylar growth is completed. This may sometimes compromise treatment outcome as greater surgical movements will be required to correct the facial and dental deformities. The preferred option is to perform a high condylectomy with combined surgery and articular disc repositioning if necessary. High condylectomy has been demonstrated as a stable treatment to arrest condylar growth. If the patient only requires mandibular surgery, care must be taken with this option to perform surgery at an age where the vast majority of mandibular growth is completed (approximately age 15 in girls and 18 in boys) to prevent the maxilla from continued growth and development of a potential malocclusion (Wolford, 2013). Similarly, age requirements are recommended for CH type 1B to prevent the contralateral condyle from continuing normal growth and causing a malocclusion.

CH type 2 through 4 has unilateral, tumorous origins and may or may not be self-limiting. The origin of CH type 2 is an osteochondroma, and it is not self-limiting. CH type 2B involves an exophytic growth off the head of the condyle while type 2A does not. There is a predilection toward females, and the majority of cases occur in the second

decade of life after mandibular growth has been completed. Signs and symptoms are similar to that of CH type 1B, however there is a greater vertical growth vector of the ipsilateral mandible. Therefore, there is an increased risk of ipsilateral open bite. Additionally, there is a deposit of soft tissue and lengthening of the muscles of mastication ipsilaterally. Radiographic evaluation of CH type 2 depicts an elongated head and condylar neck as well as an extremely rounded head. Histological analysis is usually necessary to confirm the diagnosis. Osteochondroma is the most common tumor of the condyle. It typically shows hyperplasia and hypertrophy of cartilage, endochondral ossification, and islands of chondrocytes in irregularly thick subchondral trabecular bone (Gray et al., 1994).

Two treatment options exist for CH type 2, both of which include a low condylectomy which predictably removes the tumor and halts condylar growth. Wolford suggests additional articular disc repositioning if necessary and orthognathic surgery to correct the facial deformity. Historically, low condylectomy alone has been the most popular treatment option, however it can compromise functional and esthetic outcome. The condylar neck is recontoured after the low condylectomy to function as the new condyle. Both treatment options may fail to achieve optimal facial harmony without additional procedures to address the excessive soft tissue. If CH type 2 is discovered in a pubertal child, treatment should be deferred following similar guidelines to type 1B. It is important to note that CH type 2B may have greater risk of surgical morbidity.

Etiology of CH type 1 remains unknown. Genetic and hormonal factors, involving estrogen and growth hormone, have been implicated (Wolford, 2009). This

theory is due to the high incidence of female patients and onset during pubertal growth. Some believe that approximately one-third of CH cases are genetically determined with the other two-thirds occur spontaneously (Gottlieb, 1951). It is thought that a great deal of relapse in Class III orthognathic mandibular setback procedures in patients who were thought to have completed growth is actually due to CH type 1A (Wolford, 2013). Similarly, patients who relapse after surgical correction of CH type 1B may have CH type 2 and need a subsequent low condylectomy.

CH type 3 and 4 involve rare tumors, some of which may be malignant. Both types are treated based on the diagnosis of the tumor. Any patient that experiences paresthesia, paralysis, numbness, or extreme pain in the condylar area should be immediately evaluated for a potential malignant tumor.

While CH involves abnormal craniofacial growth, facial asymmetry may also develop without subsequent TMD as a consequence of development. Over the years, many have published on theories of craniofacial growth and bone remodeling in the skull.

2.6 Theories of Craniofacial Growth and Remodeling

The practice of orthodontics, similar to other fields in medicine, improves its knowledge of craniofacial growth and development through empirical evidence in order to translate into clinical applications to improve the quality of care. In the 1700s, John Hunter, the father of comparative anatomy, became arguably the first scientist to research craniofacial growth. He concluded the mandible grew by apposition and resorption at the

circumference of bones through vital staining in pigs (Hunter, 1771). His observations of bone remodeling were later supported Humphry in 1866 who, using implanted wires in pigs, found posterior ramus deposition and anterior ramus resorption of periosteal bone (Meikle, 2002). Similarly, Brash's Remodeling Theory was grounded in Hunter's earlier work. It described that craniofacial bones mature solely by appositional growth at its surfaces (Brash, 1934).

In the 1940s two anatomists, Weinmann and Sicher developed the Sutural Theory of craniofacial growth. Here, craniofacial growth was based on intrinsic potential of the connective tissue and cartilage of the craniofacial complex. They believed that the sutures, synchondroses, and the mandibular condylar cartilage were all primary growth centers and essentially grew in an equivalent fashion to the epiphysis of long bones (Weinmann & Sicher, 1948). Similarly during this time, Brodie concluded that facial form and growth could not be altered in his Genetic Theory (Brodie, 1946). He believed that the orthodontist only had control of the final position of the teeth.

By the 1950s, the notion that sutures were centers of intrinsic growth potential was being questioned, and Scott put forth a revised theory of craniofacial growth. His Nasal Septum Theory proposed that sutural growth was compensatory and not a primary determinant of growth. Secondly, he concluded that nasal septum cartilage grew anterior-inferiorly, displacing the maxillary complex down and forward. The mandibular condyle, according to Scott, behaved similar to synchondroses and nasal septum cartilage. During the 1960s and 1970s, concurrent with the birth of molecular genetics through identification of the double-helix (Carlson, 2005), a new, highly controversial

paradigm gave rise. The long-standing genomic paradigm of craniofacial growth, based on the belief that humans were born with innate growth potential that could not be orthodontically modified, was now challenged by the functional paradigm.

The dentist-anatomist Melvin Moss gave birth to the functional matrix of craniofacial growth. This theory promotes that underlying bone growth can be modified by a clinician as a result of the functional matrix hypothesis (FMH). The FMH concludes that craniofacial growth is the result of functional matrices and skeletal units. Functional matrices include capsular matrices periosteal matrices. Capsular matrices are both craniofacial spaces and organs such as the brain. Examples of periosteal matrices include muscles and bones. Capsular matrices allow for the translation of skeletal units. Periosteal matrices undergo apposition and resorption. Two types of skeletal units exist, and they include microskeletal and macroskeletal units. Changes in periosteal matrices influence microskeletal units such as the maxillary tuberosity, while changes in capsular matrices influence macroskeletal units such as the neurocranium.

By the 1970s, Enlow had described the positional movements of bone in response to localized apposition and deposition. Bone remodeling is responsible for the maintenance of bone shape and size during human growth. Cortical drift occurs when a bone moves toward the appositional surface and away from the resorbing surface. Displacement is the movement of hard tissue, and its associated soft tissue, through space due to growth. Relocation is the result bone that moves to a different space while never changing its anatomic position. Lastly, the V-principle implies that V-shape structures

grow toward the wide end, with bone deposition on the inner side of the ‘V’ and bone resorption along the outside.

Today in the genomic era, there is an increased desire to find genetic markers of both physiological and pathological processes. Two physiological processes that have recently come under genetic evaluation are craniofacial malocclusion and human height.

2.7 Heritability of Malocclusion and Human Height

Certain skeletal malocclusions have been associated with genetic and epigenetic variations, some of which are associated with distinct variations of skeletal masticatory muscle fiber phenotype (Rowlerson et al., 2005). Similarly, the etiology of facial asymmetry may involve bone and/or muscle growth and function (Kwon et al., 2007).

Single nucleotide polymorphisms (SNPs) of the gene myosin IH (MYO1H), an actin-based motor molecule with ATPase activity, has been found to be associated with Class III mandibular prognathism (Tassopoulou-Fishell et al., 2012). The phenotype has an autosomal dominant pattern of inheritance with incomplete penetrance (El-Gheriani et al., 2003). Similarly K (lysine) acetyltransferase 6B (KAT6B), a histone acetyltransferase which may be involved in brain development, has been found to be associated with mandibular prognathism (Huh et al, 2013). In one recent study, MYO1H did not show high expression levels in mature adult masseter muscle, while MYO1C and KAT6B continued to show statistically significant associations with mandibular prognathism subjects (Desh et al., 2013). This suggests that MYO1H may

show increased expression during mandibular growth and then return to normal expression levels. However, KAT6B and MYO1C may continue to show significantly different expression throughout life.

Although the influence of masseter muscle genotype on malocclusion has yet to be determined, masseter fiber type phenotype has been shown to be associated with skeletal malocclusion which may elucidate masseter genotype relationships through future studies. The greatest association is the increased amount of type II fast-contracting fibers in masseter muscle samples from deep bite patients (Sciote et al., 2012). Here, type II fiber percentage showed an inverse relationship with facial vertical dimension. Patients with facial asymmetry have also demonstrated statistically significant increased type II fibers on the side of the deviation (Raoul et al., 2011). The shorter mandibular side may have a greater amount of Type II fibers due to a demand for greater contraction force. However, it is not yet determined if masticatory muscle phenotype is dictated by the underlying skeletal deformity or if muscle phenotype will correct to normal fiber composition with corrective orthognathic surgery.

Human height is estimated as 81% heritable (Perola et al., 2007) and 180 loci have been cited as potential sites for genes involved with adult height (Allen et al., 2010). There is some evidence that quantitative trait loci (QTLs) for height are located on chromosome X, 7, and 20. Some of these loci contain genes that have previously been shown to influence height. Fibroblast growth factor receptor 3 (FGFR3) binds acidic and basic fibroblast growth hormone and is involved in skeletal dysplasia. Signal transducer and activator of transcription 5B (STAT5B), which acts as a transcription activator in

response to cytokines, is associated with human growth defects. Unfortunately, genes involved with the majority of the 108 loci have not been linked to height. It is believed that human height is mostly determined by genes involved with growth plate chondrogenesis and growth plate senescence.

Approximately 900 genes associated with human height have previously been reported, many of which are contained within GWA studies (Lui et al., 2012). Lui et al. used microarray studies of mice and rat growth plate, a knockout mice phenotype database, and a human disease database to determine GWA genes required for normal growth plate function.

The role of human height-associated genes on the development of skeletal malocclusion remains unclear and is a new area of research. Unlike its etiology, the history of diagnosis and treatment of skeletal malocclusion is rich in the literature.

2.8 Skeletal Malocclusion Prevalence and History of Treatment

The term dentofacial describes how the dental arches relate to facial contours (MacNalty, 1961). Dentofacial deformities are skeletal malocclusions in which there is an improper vertical or horizontal relationship of the maxilla and mandible. Skeletal malocclusions have been recorded dating back to the Roman Empire and affect approximately 20% of the population (Booth et al., 1999). Sagittal facial deformities (SFDs), identified in a profile view, are classified by maxillary and mandibular jaw length. A Class II consists of either maxillary excess, mandibular deficiency, or a

combination of both. A Class III SFD is comprised of maxillary deficiency, mandibular excess, or a combination of both. Although dental compensations of skeletal abnormalities likely underestimate the severity of skeletal abnormalities, the greatest prevalence of skeletal malocclusion in the United States is mandibular deficiency. Approximately, 2.7 % of the U.S. population has a skeletal malocclusion significant enough to require orthognathic surgery, with mandibular deficiency attributing to 2% (White et al., 2003).

Dysgnathia, a general term which describes an abnormality of the teeth as well as at least one offending jaw, was mainly treated with dental correction prior to the 1950s. The first records of orthognathic surgery, a surgical attempt to correct the underlying skeletal deformity through surgical re-positioning of the jaws, was presented by Simon P. Hullihen in 1849 in which he described a mandibular osteotomy that was performed on a burn victim. In 1897, surgery was performed by Vilray Papin Blair for orthodontic indication in a patient with mandibular prognathism. This marked the first collaborative effort between an orthodontist, Edward Angle, and a maxillofacial surgeon in the treatment of skeletal malocclusion. Maxillary-repositioning surgery was first described by Martin Wassmund's (1927) Le Fort I osteotomy. However, it was not till the 1960s that Obwegeser helped to propel the Le Fort I to become a favorable surgery due to its prior high surgical morbidity. He was also responsible for describing the sagittal split ramus osteotomy (SSRO) – the first intraoral-approach, mandibular-repositioning surgery (Trauner & Obwegeser, 1957) which is regarded today as the gold-standard of mandibular surgery (Bill et al., 2006). Currently, comprehensive fixed orthodontic

therapy combined with orthognathic surgery is the mainstay for correcting severe skeletal malocclusion.

Less severe SFDs allow for possible non-surgical orthodontic treatment achieved through dental compensation. Greater amounts of dental compensation may be achieved through growth modification in a growing child than through camouflage in a patient with minimal growth potential. Proffit et al. describes an envelope of discrepancy in which hard tissue changes can be produced through orthodontics alone, orthodontic growth modification, or orthognathic surgery (2013). Facemask therapy as growth modification to correct Class III SFD has shown mixed results. Some studies have shown it as an effective (Kim et al., 1999), whereas others have discussed the possibility of different types of Class III SFDs as the factor leading to unsuccessful results (Delaire, 1997). Westwood et al. argues that facemask therapy should be aggressively overcorrected due to the likelihood that post-treatment growth will continue in the Class III direction (2003). Similar mixed findings have been recorded in the treatment of Class II SFDs. Studies have demonstrated that although most headgear and fixed functional appliances improve jaw discrepancies, there is a range of response to early Class II SFD treatment (Tulloch et al., 1997). Variation in growth has been noted in the growth of untreated Class II SFDs, therefore a means to determine which patients will have favorable outcomes is needed. When growth modification is no longer achievable due to a lack of active growth, dental camouflage is an acceptable option to correct the dental malocclusion if the SFD is less severe or if the patient rejects surgical treatment.

Patterns of Class II camouflage include non-extraction treatment with Class II elastics, retraction of upper anterior teeth into a premolar extraction space, or distal movement of upper molars. Class III camouflage may include non-extraction treatment with Class III elastics or retraction of lower anterior teeth into a lower premolar or anterior extraction space. Although relapse in Class II camouflage has shown to be minimal (Colin et al., 2003), Class III camouflage orthodontic treatment relapse tends to be more significant (Battagel 1993, 1994).

CHAPTER 3

AIMS OF THE INVESTIGATION

This study has three specific aims. First, a microarray analysis will be done to compare global gene expression in masseter muscle between subjects with facial symmetry and asymmetry with or without TMD. Second, expression data for sets of genes associated with height and sets of genes associated with pain and inflammation will be collected, sorted and summarized according to their significance and fold difference between subjects with facial symmetry and asymmetry with or without TMD. Third, expression of significant genes of interest previously associated with TMD of muscular origin will be confirmed by quantitative RT-PCR of masseter samples from the microarray as well as from other orthognathic surgery subjects with facial symmetry or asymmetry with or without TMD.

CHAPTER 4

MATERIALS & METHODS

4.1 The Sample

Masseter muscle came from 93 patients (males =30, females = 63) undergoing orthognathic surgery at the University of Lille Hospital, Lille France. Consent for subject participation in this study was obtained in accordance with the Research Ethics Committee of the University of Lille and the Institutional Review Board of Temple University, and all subjects had a non-contributory medical history. Diagnosis of skeletal classification, based on the surgical repositioning needed for each patient, was done by the French surgical team before orthognathic surgery. Orthodontic diagnoses were made before surgery using the Delaire analysis (Delaire, et al., 1981) as well as the type of surgical repositioning needed to correct jaw deformation and malocclusion. A summary of malocclusion types are shown in Table 1 and 2. Vertical malocclusions included openbite and deepbite malocclusions, sagittal malocclusion included skeletal Class II and III, and transverse malocclusion included both posterior facial asymmetry and mandibular asymmetry. Patients were checked for facial skeletal symmetry using a posterior-anterior cephalogram and/or submental vertex radiographs. All patients received the Jaw Pain and Function (JPF) questionnaire, translated into French, with a self-rating scale for evidence of TMD. The JPF Questionnaire was created to diagnose TMD (Clark et al., 1989) and has been found to be 98% sensitive and 100% specific if a cut off score of 6 is used for responses (Gerstner et al., 1994, Undt et al., 2006). TMD signs and symptoms were recorded, which included positive findings of muscular pain

upon palpation, crepitus, bruxism, and onychophagia. Based upon the several diagnoses and evaluations, patients were sorted by gender, malocclusion type, facial symmetry, and TMD status (Table 4, 5).

All subjects had at minimum a mandibular bilateral sagittal split osteotomy using Epker's technique. Depending on the correction needed, it is sometimes necessary to cut the pterygo-masseteric sling to reposition bones. A Tessier's distractor was used to completely separate the two bony pieces by more than one inch. Before closing the surgical approach, lacerated muscle fibers are removed as clinical waste to avoid being interpositioned between the bony pieces or being introduced in the suction drain. During this procedure, approximately 0.5cm³ of masseter muscle tissue was excised on left and right sides from a consistent site in the middle of the deep layer 1.5 cm from the lowest point of the mandible's angle as described previously by Rowlerson et al. (2005). Muscle samples were mounted for sectioning, quickly frozen and stored at -80°C. Muscle was shipped on dry ice in lots 60 specimens to Dr. Sciote's laboratory at the Kornberg School of Dentistry at Temple University. Upon arrival, muscle was stored at -80°C until sectioning and isolation of total RNA for microarray analysis and RT-PCR.

Eleven of the 93 patient masseter samples were selected for microarray analysis based on operator analysis of subjects with the greatest mandibular growth rotation as describe in Skieller et al (1984). The eleven samples (mean subject age 28) included three Class II deep bite subjects, four Class II open bite, two Class III open bite, one Class III deep bite, and one Class II malocclusion with normal vertical dimension (Table 3). Two of the eleven patients (Subject 4 &10) were classified with posterior facial

asymmetry of the pterygoid plates, vomer bone, and septal cartilage from evaluation of submental vertex and view radiographs and P-A cephalograms (Figure 1 & 3). Both patients had masseter muscle samples taken from the short side (i.e. the same side that the chin deviated) and none reported a history of facial trauma. Patient 4 also had evidence of condylar resorption as determined by evaluation of panoramic radiography with resulting mandibular asymmetry to the left (Figure 2). The radiographic reveals evidence of flattening of both condylar heads, greater on the left side. The sequela of this finding was greater, progressive mandibular retrusion with clockwise rotation of the mandible on the left due to decreased ramus height, which contributed to the diagnosis of mandibular midline left of facial midline. This patient was diagnosed with TMD according to their JPF questionnaire score of 14 and exhibited clinical signs of TMD, including crepitus (Figure 4). Patient 4 was classified as a skeletal Class II anterior open bite with a mandibular asymmetry (<3 mm) with significant posterior facial asymmetry. Patient 10 was diagnosed as a skeletal Class III anterior open bite with mandibular asymmetry (<3 mm) and significant posterior facial asymmetry (Figure 3). No TMD signs/symptoms were exhibited by Patient 10 upon clinical exam and JPF = 0. Subject 3, a 41 year old Class II open bite woman, was confirmed to have obstructive sleep apnea.

Table 1. Distribution of Subjects by Sagittal Skeletal Malocclusion Classification.		
Malocclusion	Number of Subjects	Percent of Total Subjects (n = 93)
Class II	67	72%
Class III	26	28%

Table 2. Distribution of Subjects by Vertical Skeletal Malocclusion Classification.		
Malocclusion	Number of Subjects	Percent of Total Subjects (n = 93)
Open Bite	36	39%
Normal Bite	31	33%
Deep Bite	26	28%

Table 3. Orthognathic Surgery Subjects Included in the Microarray Analysis of Masseter Muscle Gene Expression *							
Subject	Gender	Age	Skeletal Classification		Asymmetry	JPF Score	TMD Signs
			Sagittal	Vertical			
1	F	15	Class II	Open	Midline R	5	Yes
2	F	17	Class II	Open	Minor	2	No
3*	F	41	Class II	Open	Minor	2	No
4	F	41	Class II	Open	Posterior facial/Midline L	14	Yes
5	F	24	Class II	Deep	Midline L	0	No
6	F	47	Class II	Deep	No	9	Yes
7	M	17	Class II	Normal	No	5	Yes
8	F	34	Class II	Deep	No	4	No
9	F	16	Class III	Open	No	2	No
10	F	53	Class III	Open	Posterior facial	0	No
11	F	18	Class III	Deep	Midline R	0	No

***Subject 3 was diagnosed with obstructive sleep apnea and excluded from the present study.**

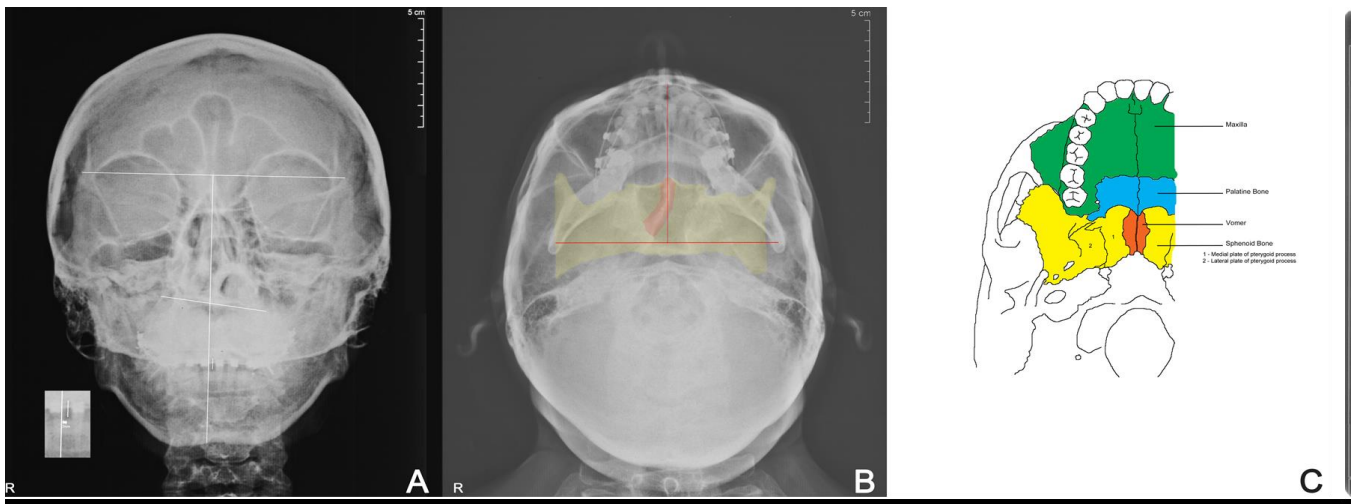


Figure 1. Radiographs of Subject 4 demonstrating posterior facial asymmetry.



Figure 2. Panoramic Radiograph of Subject 4 demonstrating greater condylar resorption on the left than the right, causing greater progressive mandibular retrusion and clockwise mandibular rotation on the left, leading to midline deviation to the left.

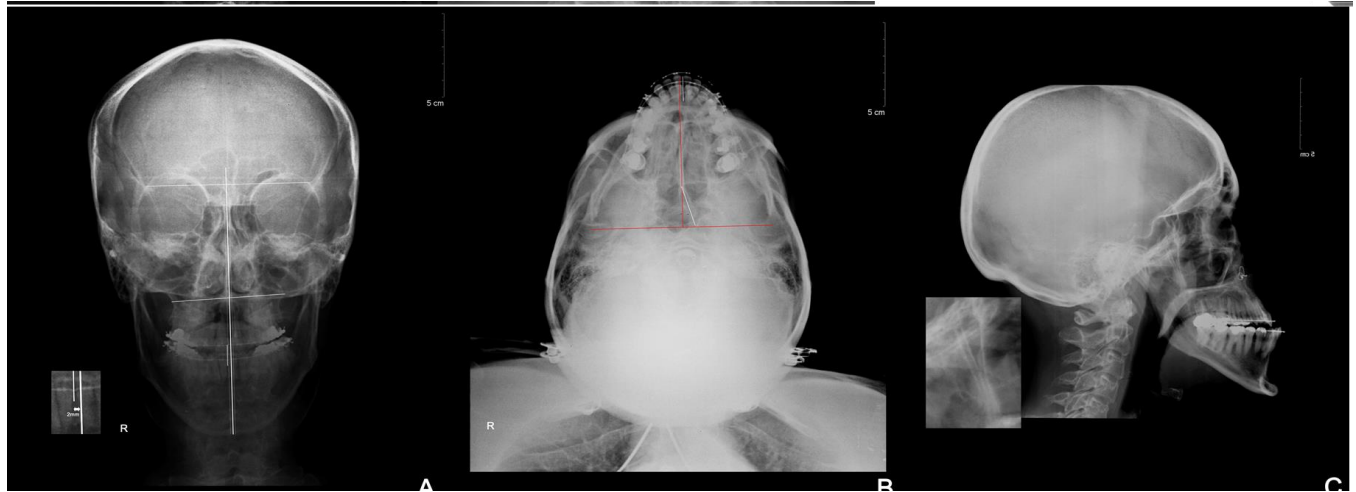


Figure 3. Radiographs of Subject 10 demonstrating posterior facial asymmetry and a Class III malocclusion.

Jaw Pain and Function Questionnaire – English Version

Jaw symptom and oral habit questionnaire First name : I Last name : L Identification : 038 Date : 17/06/2013 Instructions: Please check the appropriate answer to the following questions Examiner : R. Nicot						
A	Jaw pain questions	Doesn't hurt at all	Hurts a little	Hurts a lot	Almost unbearable	Unbearable pain without relief
1	Does it hurt when you open wide or yawn ?	X				
2	Does it hurt when you chew or use the jaws ?			X		
3	Does it hurt when you are not chewing or using the jaws ?		X			
4	Is your pain worse on waking ?	X				
5	Do you have pain in front of the ears or earaches ?			X		
6	Do you have jaw muscle (cheek) pain?			X		
7	Do you have pain in the temples ?		X			
8	Do you have pain or soreness in the teeth ?	X				
B	Jaw function questions	No	Maybe a little	Quite a lot	Almost all the time	All the time without stopping
9	Do your jaw joints make noise so that it bothers you or others?			X		
10	Do you find it difficult to open your mouth wide ?			X		
11	Does your jaw ever lock closed so you cannot open it?	X				
12	Does your jaw ever lock open so you cannot close it?	X				
13	Do you have a problem with your bite being uncomfortable?			X		

Figure 4. JPF questionnaire of Subject 4 with a positive diagnosis of TMD (total score = 14).

4.2 RNA Isolation for Microarray Analysis

Single masseter samples from either the left or right side of each of the selected subjects were transported on dry ice to the University of Pennsylvania Molecular Profiling Facility and University of Pennsylvania Center for Musculoskeletal Disorders where total RNA was isolated using Qiagen miRNeasy® procedures (Qiagen Inc., Valencia, CA) according to the manufacturer's specifications. Tissue was disrupted in QIAzol Lysis® Reagent (700 µl) with a generator probe. Chloroform was added to the homogenates at room temperature to promote dissociation of nucleoprotein complexes. Homogenate mixes were centrifuged and the upper aqueous phase containing RNA was transferred to a clean collection tube. RNA was precipitated by the addition of 100% ethanol to the aqueous phase tubes and collected by centrifugation onto RNeasy Mini Spin® columns. The total RNA, retained on the spin columns after a series of washes, was digested with a freshly prepared deoxyribonuclease I (DNase) stock solution (80ul) at room temperature for 15 min. Spin columns were washed several times with Qiagen-specified reagents and the purified RNA was eluted with ribonuclease (RNase)-free water by centrifugation. Control tests for the quality of isolated total RNA samples were done by Agilent Bioanalyzer® (Agilent, Santa Clara, CA) and Nanodrop (Thermo Fisher, Waltham, MA) Nanodrop spectrophotometry.

4.3 Microarray Preparation and Analysis

All protocols for sample preparation and microchip hybridization were conducted as described in the Ambion WT Expression Manual and the Affymetrix GeneChip Expression Analysis Technical Manual. Two hundred and fifty nano grams of total RNA was converted to first-strand cDNA using reverse transcriptase primed by poly(T) and random oligomers that incorporated the T7 promoter sequence. Second-strand cDNA synthesis was followed by in vitro transcription with T7 RNA polymerase for linear amplification of each transcript, and the resulting cRNA was converted to cDNA, fragmented, assessed by Bioanalyzer®, and biotinylated by terminal transferase end labeling. Five and a half micrograms of labeled cDNA were added to Affymetrix hybridization cocktails, heated at 99°C for five minutes and hybridized for sixteen hours at 45°C to Human Transcriptome 2.0® (HTA-2.0) GeneChips (Affymetrix Inc., Santa Clara CA). The microarrays were then washed at low stringency with 0.9M sodium chloride, 60mM sodium phosphate, 6mM ethylenediaminetetraacetic acid (EDTA), pH 7.4 (6X SSPE) and high stringency with 100mM 2-(N-morpholino) ethanesulfonic acid (MES), 0.1M sodium chloride buffer (NaCl) and stained with streptavidin-phycoerythrin. Fluorescence was amplified by adding biotinylated anti-streptavidin and an additional aliquot of streptavidin-phycoerythrin stain. A GeneChip 3000 7G® (Affymetrix Inc., Santa Clara CA) scanner was used to collect fluorescence signal. Fluorescence data from the 70,534 transcripts of the array were normalized and converted to Log₂-transformed values by Affymetrix Command Console with Partek Genomics Suite, v6.6 software.

Genes that were of particular biological relevance to this study were culled from the microarray data and organized into subsets. Selection criteria for these subsets were based upon previously cited evidence of genes that contribute to human height, growth, pain and inflammation. Growth and height genes of interest in this study were determined a priori using microarray studies of mice and rat growth plate, a knockout mice phenotype database, and a human disease database to determine GWA genes required for normal growth plate function (Lui et al., 2012). Selection of pain and inflammatory genes was based primarily on genes found in the Algynomics Pain Research Panel v.2.0 (complete list provided through <http://www.algynomics.com/pain-research-panel.html>). Eight hundred and forty seven height-associated genes and 551 genes associated in pain/inflammatory processes were evaluated.

Analysis was then narrowed to evaluate the genes, and associated proteins, that the baseline OPPERA genetic study found strong significant associations with chronic TMD regardless of this study's criteria for significance and fold expression difference (Table 7). Lastly, previously reported pain/inflammatory genes that were found to show significant expression levels and fold expression differences in this study were sorted for (Table 8).

4.4 Principle Components Analysis

A Principle Components Analysis (PCA) was done on the microarray data by Dr. John Tobias at the University of Pennsylvania array core facility in search of common

patterns of global gene expression in masseter muscle of the test eleven subjects. Based upon the clustering of similar patients by PCA, Subject 3 with obstructive sleep apnea was not included in further analyses of this study. Thereafter, data from the two subjects with posterior facial and eight remaining symmetry subjects were re-analyzed for expression differences.

4.5 Additional RNA Isolations and RT-PCR

Total RNA was also isolated in the laboratory of Dr. Sciote from the muscles that were on the opposite side of the samples used for the microarray analysis and from additional biopsies provided by the surgical team in Lille, France. Muscle was disrupted in TRIzol® reagent (Invitrogen, Carlsbad, CA), digested with DNase I, re-isolated with RNAqueous® (Ambion, Austin, TX) and quantified by absorbance at A260 as described by Horton et al. (2008).

Masseter muscle RNA from both left and right sides of the microarray subjects and from twelve other subjects were used for RT-PCR to compare expression of a selected gene of interest with the results of the microarray. EREG, which has been previously associated with RA, TMD, and pain (Eleftherohorinou et al., 2011, Smith et al., 2011, PAIN, 2012) was first selected. However, EREG did not show significant expression levels to quantify by this study's means. Thereafter, CACNA2D1 was selected as a specific gene of interest due previous literature which implicates its

involvement in TMD (Smith et al., 2011) and inflammatory conditions such as RA which can cause condylar resorption (Eleftherohorinou et al., 2011).

Quantification was done by TaqMan® (Applied Biosystems, Foster City, CA) RT-PCR methodology on the muscle RNA samples in triplicate for all assays. Reverse transcription and amplification steps were performed sequentially in the same assay using RNA-to-CT 1-Step® reagent in an Applied Biosystems Step One Plus® instrument. Quantification was done by the $\Delta\Delta CT$ method of Livak and Schmittgen (2001). The $\Delta\Delta CT$ method measures relative expression quantities based on the concentration threshold (C_T) of target gene amplification compared to a ‘housekeeping gene’ reference gene that is constitutively expressed in all tissues. For the masseter muscle samples, the target gene was CACNA2D1 that was normalized to the reference gene HPRT1. To use this method, an initial assay using standard plots was required to establish whether of CACNA2D1 and HPRT1 amplifications are greater than 90% efficiency and have approximately parallel slopes. A pure commercial preparation of human skeletal muscle RNA (Ambion, Austin, TX) was used as a standard calibrator for all quantification assays. Standards for comparison of initial amplification plots were 1, 10 and 100ng of the skeletal muscle RNA. Figure 5 shows the standard amplification plots for HPRT1 (A) and CACNA2D1 (B). The horizontal lines are the threshold cycles where fluorescence is above background and amplification is in log phase.

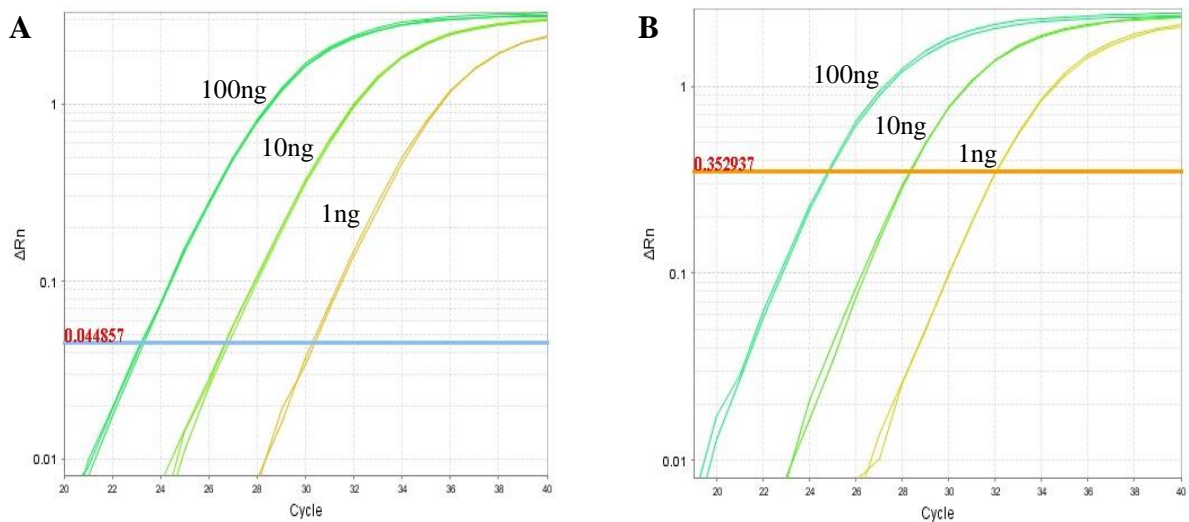


Figure 5. Standard Amplification Plots of *HPRT1* and *CACNA2D1*. Standards were 100, 10 and 1 ng of skeletal muscle RNA. **A.** Plot of the reference gene *HPRT1*. **B.** Plot of the target gene *CACNA2D1*. Horizontal lines through the plots show the amplification threshold cycle of *HPRT1* and *CACNA2D1*.

Amplification efficiency for both genes was greater than 90% and the slopes of the standard curves were within log phase of amplification and approximately 7% of one another. Based upon these results, the criteria for quantification by the $\Delta\Delta CT$ method were met and further assays were done using a 15ng skeletal muscle RNA calibrator.

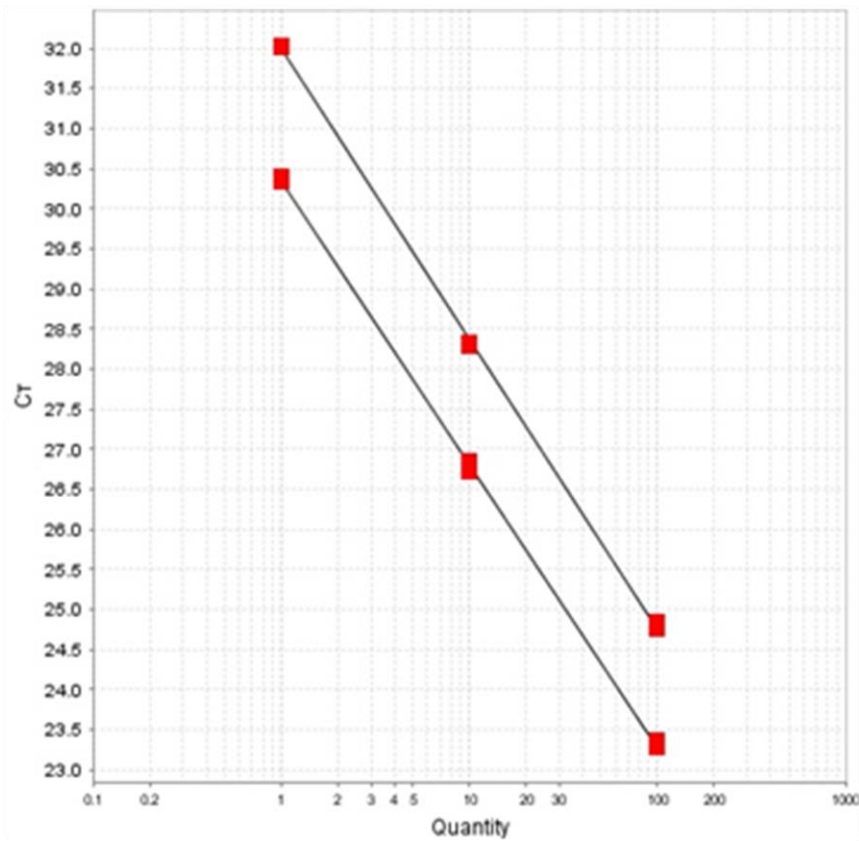


Figure 6. Amplification Standard Curves of for *HPRT1* and *CACNA2D1*. Slope of the curve for *HPRT1* was -3.53 with % amplification efficiency = 92.16. Slope of the curve for *CACNA2D1* was -3.35 with % amplification efficiency = 92.20.

4.6 Statistical Analyses

Descriptive statistics including mean and standard deviation were calculated to analyze the data set. Microarray analyses included a statistical test for significance as part of the Partek Genomics Suite. Values were considered significant if step-up p -values were <0.05 and fold differences were $>\pm 2$ between groups. Resulting p -values were corrected for false discovery rate (FDR) by using step-up p -values through the method of Benjamini and Hochberg as implemented in Partek Genomics Suite. This statistical approach is necessary to correct FDR since relatively small sample sizes are used to compare thousands of differences in gene expression levels.

To find differentially expressed height-related and pain/inflammatory-related transcripts a Student's two sample t -test was performed comparing the two asymmetric samples (Table 6) to the eight symmetric samples.

Student's two sample t -tests were also used to determine significance for values derived from RT-PCR. RT-PCR of CACNA2D1 expression by gender in TMD versus non-TMD subjects was carried out (Figure 8). Second, RT-PCR of CACNA2D1 by gender in symmetric versus asymmetric subjects was completed (Figure 9). Lastly, RT-PCR calculated lateral differences, between left and right samples, of CACNA2D1 expression in subjects while not sorting for gender (Table 9).

CHAPTER 5

RESULTS

5.1 Comparison of Symmetry, Gender, and TMD

When sorting for symmetry, of the 93 patients involved in the study 35% were diagnosed as asymmetric (Table 4). Thirty five percent of all TMD subjects were asymmetric, 30% of all Class II patients were asymmetric, and 50% of all Class III patients were asymmetric.

When sorting for gender, 87% of subjects diagnosed with TMD were female (Table 5). Eighty nine percent of Class II TMD subjects, 86% of Class III TMD subjects, 91% of openbite TMD subjects, 88% of normal vertical bite TMD subjects, and 86% of deepbite TMD subjects were female.

Table 4. Comparison of Symmetry and TMD in Subjects by Skeletal Malocclusion Classification.				
	Total	TMD	Class II	Class III
Symmetric	60	17	47	13
Asymmetric	33	9	20	13
Total Subjects	93	26	67	26

Table 5. Comparison of Gender and TMD in Subjects by Skeletal Malocclusion Classification.							
	Gender	Total	Class II	Class III	Open Bite	Normal Bite	Deep Bite
		TMD	TMD	TMD	TMD	TMD	TMD
Female	63	23	17	6	10	7	6
Male	30	3	2	1	1	1	1
Total Subjects	93	26	19	7	11	8	7

5.2 PCA

Figure 7 shows inter-sample differences by visualizing high dimensional variation in three dimensions. Each axis is a linear combination of all 70,534 transcripts, each with its own coefficient chosen in such a way to best show sample variations. RNA expression data of the two asymmetric patients, Subject 4 and 10, clustered separate from subjects with other malocclusion classifications by PCA. Although Subject 3 also sorted separately, the patient had a positive diagnosis of sleep apnea and was excluded from the study.

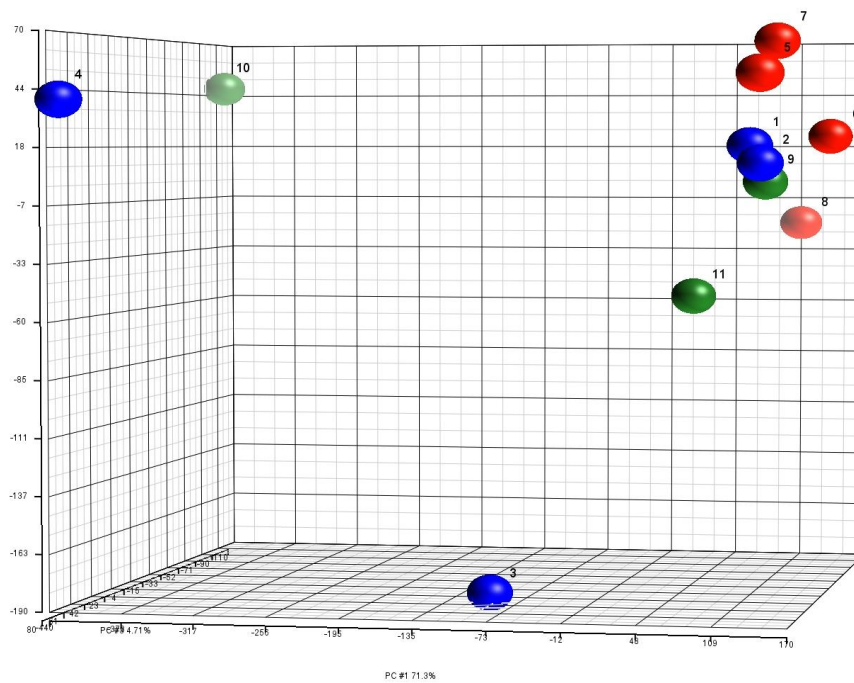


Figure 7. PCA of RNA expression levels between symmetric and asymmetric malocclusion subjects (Number signifies subject number).

5.3 Expression of Height and Pain/Inflammatory Genes in Asymmetric Subjects

Microarray transcripts were evaluated for differential expression and considered significant if they showed greater than ± 2 fold difference in gene expression and a step-up p-value < 0.05 . Applying this cutoff value between groups, approximately 22,000 gene transcripts were found to be significantly different, representing about 1/3 of the entire array. This reflects statistical cutoffs for significantly differentially expressed transcripts and is not related to results found from the PCA comparisons. Two hundred and eight transcripts of 847 height-associated genes (Appendix B) and 132 of 551 pain/inflammatory genes (Appendix A) were significant for expression ($P < 0.05$) and displayed $> \pm 2.0$ fold differences in facial asymmetry patients with or without TMD (Table 6).

Criteria for candidate genes was then expanded to include genes, and associated proteins, with strong significant association with chronic TMD reported from OPPERA that did not meet our significant expression criteria or greater than ± 2 fold difference in gene expression in asymmetric patients with or without TMD (Table 7). Here, glutamate receptor, ionotropic, N-methyl D-aspartate 2B (GRIN2B), the predominant excitatory neurotransmitter receptor in the mammalian brain, and glutamate receptor interacting protein 1 (GRIP1), an associated glutamate receptor protein that mediates neurotransmission, approximately met this study's criteria of significant expression ($P < 0.05$) and display of $> \pm 2.0$ fold differences in facial asymmetry patients with or without TMD. GRIN2B showed a +1.94 fold increase and GRIP1 showed a +2.32 fold increase in expression.

CACNA2D1 and EREG were the only genes from the OPPERA study that were previously found to be significantly associated with chronic TMD that met the criteria of showing significant differential gene expression and a greater than ± 2 fold difference in gene expression in this study (Table 8). CACNA2D1 (-12.51 fold) and EREG (+2.12 fold) showed significantly different ($P < 0.001$) expression levels in the two asymmetric versus the remaining eight symmetric patients.

Among other genes previously reported to be associated with pain/inflammation GABA A receptor subunit alpha 6 (GABRA6), involved in mediating inhibitory neurotransmission in the CNS, has been previously associated with decreased expression in rats with TMJ inflammation (Kramer & Bellinger, 2013). This finding is not consistent with the results of this study which reported a +2.14 fold increase in expression in asymmetric subjects with and without TMD. Decreased expression of neuropeptide Y receptor Y5 (NPY5R), a G-protein receptor involved in pain transmission, has been previously associated with increased psychological stress and depression (Mickey et al., 2010). However, this study found a +2.11 of increase of NPY5R expression in asymmetric subjects with and without TMD.

Table 6. Comparison of Significant Expression ($P < 0.05$) and $> \pm 2.0$ fold differences in Height and Pain/Inflammatory Genes of Interest in Asymmetric Patients versus Symmetric Patients.	
Number of Transcripts Analyzed	Number of Significantly Different Transcripts
847	208
551	132

Table 7. Baseline OPPERA Genes and Associated Proteins with Significant Associations (P <0.05) in Patients with Chronic TMD that did not Meet this Study's Significant Expression Criteria (P <0.05) or Greater than ± 2 Fold Expression Difference in Asymmetric Patients with or without TMD.				
Gene	Transcript ID	Gene Ontology	Asym vs Fold Diff	Sym P Value
ADRA1D	TC20000584.hg.1	mediates the influx of extracellular calcium	-1.04437	4.47E-01
ADRA2C	TC04000052.hg.1	mediates the influx of extracellular calcium	1.17094	1.46E-03
CAMK4	TC05000521.hg.1	transcriptional regulation of inflammation and in neurons	1.37779	1.27E-06
COMT	TC22000050.hg.1	degradation of neurotransmitters in postsynaptic neurons	1.03883	5.37E-01
GRIN2A	TC16000854.hg.1	increase in the efficiency of synaptic transmission	1.35694	8.85E-06
GRIN2B	TC12001265.hg.1	increase in the efficiency of synaptic transmission	1.93513	1.20E-06
GRIN2C	TC17001863.hg.1	increase in the efficiency of synaptic transmission	1.357	9.86E-05
GRIN2D	TC19000702.hg.1	increase in the efficiency of synaptic transmission	1.39886	9.07E-07
GRIN3A	TC09001423.hg.1	increase in the efficiency of synaptic transmission	1.39796	4.26E-04
GRIN3B	TC19000025.hg.1	increase in the efficiency of synaptic transmission	1.30484	0.000194
GRINA	TC08000835.hg.1	increase in the efficiency of synaptic transmission	-1.55113	0.001244
GRIP1	TC12001689.hg.1	mediator of the trafficking at specific subcellular location in neurons	2.32713	1.32E-07
GRIP2	TC03001197.hg.1	mediator of the trafficking at specific subcellular location in neurons	1.06542	0.24809
GRK1	TC13000427.hg.1	Retina-specific kinase	1.49171	1.08E-04
GRK4	TC04000041.hg.1	Retina-specific kinase	1.22416	4.28E-07
GRK5	TC10000865.hg.1	Retina-specific kinase	-1.27224	3.45E-03
IFRD1	TC07003291.hg.1	associated with sensory/motor neuropathy with ataxia	-1.60064	5.90E-02
IFRD2	TC03003410.hg.1	associated with sensory/motor neuropathy with ataxia	1.20875	1.55E-04
NR3C1	TC05001887.hg.1	glucocorticoid receptor involved in inflammatory responses	-1.68689	7.61E-05
NR3C2	TC04001627.hg.1	glucocorticoid receptor involved in inflammatory responses	-1.44531	2.72E-04
OPRD1	TC01000392.hg.1	Inhibits neurotransmitter release/ involved in analgesia	1.22732	1.91E-03
OPRK1	TC08001212.hg.1	Inhibits neurotransmitter release/ involved in analgesia	1.40553	2.92E-05
OPRL1	TC20000534.hg.1	Inhibits neurotransmitter release/ involved in analgesia	1.42965	8.95E-06
Abbreviations: Asym, asymmetric (Subject 4 and 10);				
Sym, symmetric (8 subjects with skeletal malocclusion without asymmetry);				
Fold Diff, fold difference between average expression in Asym vs Sym.				
Significant for step-up P-value with fold differences >±2 between groups.				

Table 8. Significant RNA Expression (P < 0.05) and ± 2.0 Fold Difference of Previously Reported Pain/Inflammatory Genes in Masseter Muscle Samples of Asymmetric and TMD Subjects from Microarray Analysis. *				
Gene	Transcript ID	Gene Ontology	Asym vs Fold Diff	Sym P Value
NPY5R	TC04000818.hg.1	positive regulation of acute inflammatory response	2.11	<0.001
GABRA6	TC05000902.hg.1	major inhibitory neurotransmitter in the vertebrate brain	2.14	<0.002
CACNA2D1	TC07001563.hg.1	mediate the influx of calcium ions into the cell	-12.51	<0.003
EREG	TC04000414.hg.1	positive regulation of cytokine production	2.12	<0.004
Abbreviations: Asym, asymmetric (Subject 4 and 10);				
Sym, symmetric (8 subjects with skeletal malocclusion without asymmetry);				
Fold Diff, fold difference between average expression in Asym vs Sym.				
Significant for step-up P-value with fold differences >±2 between groups.				

5.3.1 RT-PCR of CACNA2D1 by Gender in TMD Versus non-TMD Subjects

CACNA2D1 expression was increased, and almost significantly different ($p = 0.05$), in females with TMD versus females without TMD (Figure 8). Significantly increased CACNA2D1 expression ($p < 0.05$) was seen in male subjects without TMD versus female subjects without TMD (Figure 8).

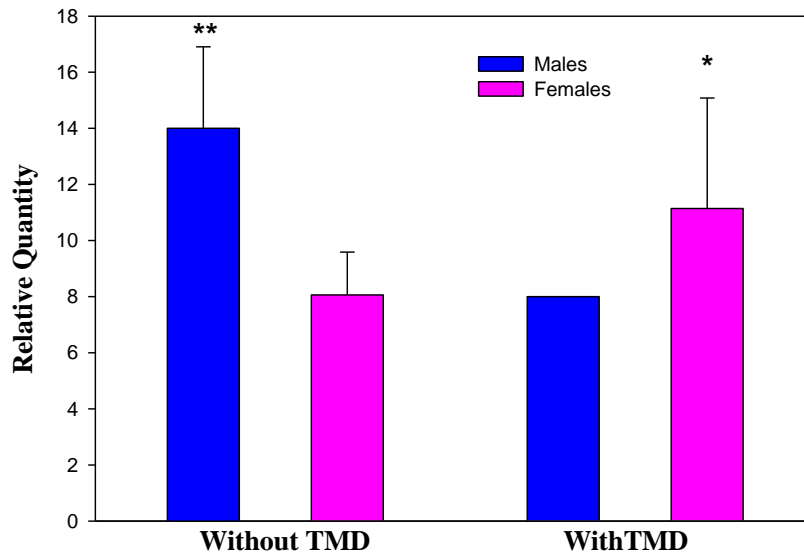


Figure 8. Comparison of CACNA2D1 Expression in Male and Female Subjects with and without TMD Associated Pain. ** $p = 0.0008$ between male & female subjects without TMD. * $p = 0.05$ between female subjects with & without TMD. Subjects without TMD, males $n = 4$, females $n = 8$; Subjects with TMD, males $n = 1$, females $n = 9$.

5.3.2 RT-PCR of CACNA2D1 by Gender in Symmetric versus Non-Symmetric Subjects

Significantly increased CACNA2D1 expression ($p < 0.05$) was found in male symmetric subjects versus female symmetric subjects (Figure 9). Decreased CACNA2D1 expression was seen in asymmetric male subjects versus symmetric male subjects, however it was not significant ($p = 0.51$) (Figure 9).

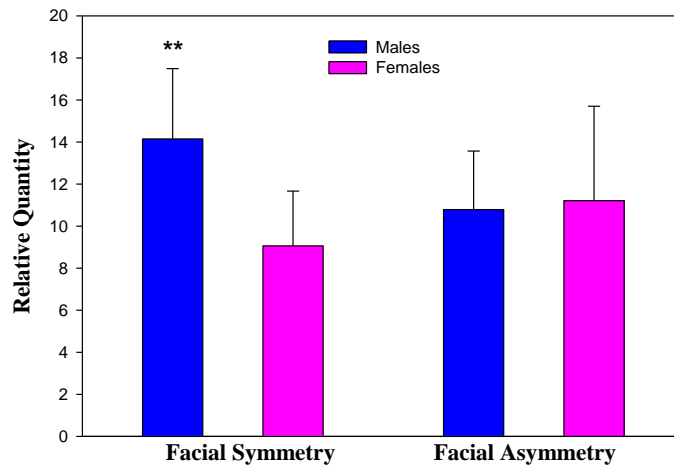


Figure 9. Comparison of CACNA2D1 Expression in Male and Female Subjects with Facial Symmetry or Asymmetry. $**p = 0.0127$ between male & female subjects with facial symmetry. Subjects: Facial symmetry males $n = 3$, females $n = 12$; Facial asymmetry males $n = 2$, females $n = 5$.

5.3.3 Lateral Differences of CACNA2D1 Expression by RT-PCR between Subjects

Average expression differences of CACNA2D1 between TMD and non-TMD subjects as well as between symmetric and asymmetric subjects were not significantly different (Table 9). However, in microarray subjects, the two asymmetric subjects showed significantly different CACNA2D1 expression than symmetric subjects.

Table 9. Comparison of CACNA2D1 Expression by Symmetry and TMD Status *	
Average Expression Differences in Right and Left Side Masseter Muscle Samples between Asymmetric (n=6) versus Symmetric (n=11) Subjects	P = 0.95
Average Expression Differences in Right and Left Side Masseter Muscle Samples between Microarray Asymmetric (n=2) versus Symmetric (n=6) Subjects	P = 0.02 *
Average Expression Differences in Right and Left Side Masseter Muscle Samples between TMD (n=10) versus non-TMD (n=12) Subjects	P= 0.62

* Significant ($p < 0.05$) using Student T-test Analysis

CHAPTER 6

DISCUSSION

This study aimed to quantify height gene expression and pain/inflammatory gene expression in masseter muscle to determine differences between asymmetric and symmetric subjects with/without TMD. Secondly, previously reported associations between pain/inflammatory genes and muscular TMD were confirmed through human transcriptome microarray analysis. These methods provide a novel approach to study TMD and/or facial asymmetry in human subjects.

The etiology of TMD is multi-factorial, and the majority of TMD involves masticatory muscle disorders (Schiffman et al., 1990). Masseter muscle is often involved in muscular TMD. Fiber-type differences in left and right masseter samples have in subjects with facial asymmetry have been reported (Raoul et al, 2011).

Previous studies have associated TMD with inflammatory/pain-related genes (Maixner et al., 2011). Two lesser common types of TMD include CR and CH, both of which can present with facial asymmetry.

The cause of the most common form of CH unknown (Wolford et al., 2014). Genome-wide association studies have identified potential genetic markers associated with height (Allen et al., 2010). Some of these genes may participate in craniofacial growth, condylar hyperplasia, and development of facial asymmetry (Kwon et al., 2007).

RA has been found to cause some cases of CR. Previous studies have identified potential pain/inflammatory-related genetic markers for RA (Eleftherohorinou et al., 2011). Although the etiology of OA of the TMJ is not well understood, some believe that inflammatory factors may be present in the TMJ synovial fluid but undetectable in the systemic bloodstream (Gunson et al., 2012). Therefore, it is possible that CR caused by OA as well as RA may be associated with inflammatory factors. This warrants further investigation for possible therapeutic intervention in the future.

6.1 Genetic Variation in Asymmetric Subjects

It was found that the asymmetric subjects (Subject 4 and 10) clustered separately than the symmetric subjects, regardless of sagittal malocclusion, vertical malocclusion, or TMD status (Figure 7). This suggests that although each subject presents with a unique malocclusion, the presence or absence of facial asymmetry provides the greatest genetic difference. Future studies with larger power will be necessary to verify this finding.

6.2 Expression of Height-Related Genes in Asymmetric Subjects

Two hundred and eight transcripts of 847 height-associated genes were significant for expression ($P < 0.05$) and displayed $> \pm 2.0$ fold differences in facial asymmetry patients with or without TMD (Table 6). These data are the first to suggest that human height-related genes may play an important role in the development of facial asymmetry. Further studies will be necessary to verify these findings. Genetic testing of height-

related genes delineating facial asymmetry of orthodontic patients in the future may be able to eliminate ambiguity in the diagnosis and treatment of skeletal upper midline deviations and cants. It may also help to discover genes associated with a deviated nasal septum which is a risk factor for sinusitis and sleep apnea.

Although no subject in this study was diagnosed with CH, future studies would benefit from including subjects with facial asymmetry with and without CH. This is especially important because the cause of the most common form of CH is currently unknown (Wolford, 2013). In this way, gene expression could be compared between symmetric controls, asymmetric patients without CH, and asymmetric patients with CH. A study of this design may also elucidate marker genes in CH patients with symptomatic versus asymptomatic TMD.

6.3 Expression of Pain/Inflammatory Genes in Asymmetric Subjects

One hundred and thirty two of 551 pain/inflammatory genes were significant for expression ($P < 0.05$) and displayed $> \pm 2.0$ fold differences in facial asymmetry patients with or without TMD (Table 6). These data suggest TMD patients with facial asymmetry associated with condylar resorption may show significantly different expression of certain inflammatory marker genes such as EREG and CACNA2D1 in masseter muscle regardless of a non-contributory medical history. There are limitations to making conclusions of expression of pain/inflammatory genes in asymmetric patients in the current study. First, future microarray data should separate the expression levels of TMD-free and TMD-positive subjects if one is to make conclusions about TMD from

microarray data. Second, the percent of subjects with TMD (n = 2) selected for the microarray analysis (n = 11) should be proportional to the total number of TMD subjects (n=26) in the entire study (n=93) for more reflective results. Future studies will be necessary to verify findings with greater sample size. It would also be beneficial to uncover genes that delineate asymptomatic from symptomatic CR. Furthermore, genetic comparison of expression levels between asymmetric patients with muscular TMD versus asymmetric CR patients would be of clinical significance.

6.4 Comparison of Symmetry, Gender, and TMD

Sixty five percent of all TMD subjects had symmetric skeletal malocclusions (Table 4). These data are consistent with the literature which states that the majority of TMD cases are masticatory muscle disorders (Schiffman et al., 1990), which do not involve facial asymmetry.

Eighty seven percent of subjects diagnosed with TMD were female (Table 5). While estrogen has been implicated in TMD, its role remains controversial. Recently it has been found that increased estrogen supplementation decreased inflammation and TMD symptoms (Torres-Chávez et al., 2012), however Wu et al. found that an increase in estradiol increases TMD symptoms in rats (2010). Future studies are needed to uncover the relationship between estrogen and TMD.

While at initial glance it seems that CACNA2D1 expression in males may have a protective effect against TMD symptoms while increasing risk of TMD symptoms in

females (Figure 8), there are several factors that first must be ruled out. Microarray analysis found an approximately 12-fold decrease in CACNA2D1 expression in asymmetric subjects with and without TMD versus symmetric subjects (Table 8, Appendix A) which seems inconsistent with RT-PCR findings that showed increased CACNA2D1 expression in female TMD subjects (Figure 8). It is possible that because masseter samples were only taken from the short side, decreased CACNA2D1 expression was found in microarray subjects as opposed to possible net increase in expression if samples were taken from the longer side or averaged. Secondly, it is possible that the facial asymmetry of both microarray subjects acted as a confounding variable on CACNA2D1 expression in TMD patients. This may have led to an underestimation of the fold difference exhibited by the two asymmetric subjects with and without TMD. Third, the microarray subjects (n=10) included both male and female subjects and the two asymmetric subjects were both female. Lastly, only three of a total of 26 TMD were male, which decreases validity of any assumption made between genders due to low sample size. Therefore, it is unwise to make any conclusion on gender differences without controlling for left and right side masseter samples, facial asymmetry, gender, and small sample size.

Without controlling for gender, CACNA2D1 expression in symmetric versus asymmetric subjects as well as in TMD versus non-TMD subjects were not found to be significantly different (Table 9). When sorting for gender, CACNA2D1 expression in facially symmetric patients may be significantly elevated in males compared to females and may also show decreased expression in those that are asymmetric despite that this study did not find the decrease significant (Figure 9). These results support the 12-fold

decrease in expression in asymmetric subjects that was found through microarray analysis (Table 6, Appendix A). However, these findings need to be verified with larger sample size and proportional numbers of male and female subjects.

6.5 Expression of Previously Reported Pain/Inflammatory Genes

Of baseline OPPERA genes and associated proteins with a strong significant association with chronic TMD that did not meet our significant expression criteria or greater than ± 2 fold expression difference in asymmetric patients with or without TMD, GRIP1 and GRIN2B were the closest to meeting this study's criteria (Table 7). Glutamate is the body's most abundant neurotransmitter, and further analysis is necessary to determine if its receptors and associated proteins play a greater role in determining a patient's risk for chronic TMD.

Of other genes previously reported to be associated with pain/inflammation, NPY5R (+2.11 fold), GABRA6 (+2.14 fold), and EREG (+2.12 fold) showed significantly different ($P < 0.001$) expression levels in the two asymmetric versus the remaining eight symmetric patients (Table 8). NPY has been implicated in inflammation as well as in emotion depressive disorders (Ji et al., 1994, Mickey et al., 2011). High concentrations of NPY in TMJ synovial fluid have been found to potentiate TMJ pain and inflammation in RA patients (Appelgren et al., 1995), and this is consistent with the increased level of expression found in this study. The increased expression of GABRA6 is contrary to previous findings of decreased GABRA6 expression in rats with TMD

(Kramer & Bellinger, 2013). This may be due to lack of sorting for left and right side masseter sample differences, facial asymmetry, gender, and small sample size. Lastly, EREG has been implicated as having a significant association with chronic TMD and pain (Smith et al., 2011, PAIN, 2012). Also, EREG has previously been found to show increased expression levels when associated with RA and general inflammation (Murakami et al., 2013). This finding is supported by our study which found increased level of expression in asymmetric subjects with and without TMD. Future studies will be necessary to elucidate the relationships between chronic TMD and NPY, GABRA6, and EREG.

6.6 Future Direction

The OPPERA study's proposed two phenotypes, psychosocial makeup and pain amplification, which influence the onset of and the progression of chronic TMD each have associated potential genetic markers (Figure 10).

In this study, of the 132 significant pain/inflammatory genes that displayed $>\pm 2.0$ fold expression differences in asymmetric subjects with and without TMD (Appendix A), clusters of genes of similar function were noted. Approximately 13 are associated with nervous system development, approximately 12 are associated with synaptic transmission, and approximately five are associated with toll-like receptors. Toll-like receptors are found on innate immune system cells such as macrophages and dendritic cells. These receptors detect foreign organisms that break through mucosal barriers causing their immune cell to activate cytokines or other immune cells. Interestingly, 5 genes are associated with systemic lupus erythematosus, suggesting a possible auto-immune link to chronic TMD phenotypes. These three groups of similarly functioning genes require future analysis for a greater understanding of the genetic influences on the progression of chronic TMD.

Of the 208 significant height-related genes that displayed $>\pm 2.0$ fold expression differences in asymmetric subjects with and without TMD (Appendix B), groupings of genes of similar function were also noted. Approximately 20 are associated with mitosis and/or apoptosis, approximately 20 are associated with the immune response, and approximately 20 are associated with G-protein coupled olfactory receptors. Future

studies will determine if these genes of similar function play a greater role in determining facial asymmetry with and without TMD.

While the OPPERA study will bring new light on the genetics of TMD, its design is not without its shortcomings. Firstly, OPPERA fails to account for the possible epigenetic influence of TMD. The authors should not rule out the possibility that a stressful environment may play a role in changing expression levels of pain sensitization and psychosocial behavior genes to contribute to or potentiate TMD.

Secondly, OPPERA downplays the significance of ‘genetic variants’ associated with TMD. Although somatic awareness and pressure-point pain had stronger associations with TMD, these conditions may be caused by inheritance of ‘genetic variants’ or even epigenetics as a result of increased stress. In other words, it should not be assumed that the changes in the psychological phenotype and pain sensitivity phenotype are the sole result of changes in the neuroendocrine system.

Lastly, OPPERA does not give thought to the idea that genetic changes in pain amplification genes may cause the psychological phenotype of somatic awareness by influencing psychosocial gene expression. This would support a more stepwise and causative paradigm.

Future OPPERA studies will not only have to be prospective, but control for previous psychological medical history and stress in the subjects’ environment. Similarly, the notion that only 33% of CH cases are caused by genetics (Gottlieb, 1951),

requires further investigation in the age of genomics. CH may have a large genetic or epigenetic influence that has yet to be uncovered.

One goal of this research is to determine an individual's likelihood of acquiring TMD with and without facial asymmetry through saliva sample genetic analysis, its potential severity level, and possible future genetic therapeutic treatment. Such protocols would greatly improve the quality of care of TMD. Currently, the conditions are often underdiagnosed, misdiagnosed, and mistreated. Treatment is often reactive, if treatment is carried out at all. Non-surgical management of TMD with and without facial asymmetry to successful outcomes is sometimes possible but not predictable. Improper diagnosis of TMD and informed consent has led to litigation against the orthodontist despite literature which shows that orthodontic treatment does not cause TMD (Mohlin et al., 2007). Proper diagnosis and treatment of TMD with and without facial asymmetry by the orthodontist can only be accomplished by following evidence-based indications and continuing to fully investigate its genetic component.

A second, equally important goal of this research is to determine a patient's risk of developing posterior facial asymmetry to discover future genetic therapeutic interventions based on genetic diagnosis through saliva samples. Detection of a deviated nasal septum will be especially important in light of the developing role of the orthodontist with diagnosis and treatment of childhood and adult sleep apnea. Lastly, genetic detection and of a deviated nasal septum will contribute to improved treatment of sinusitis and facial cants.

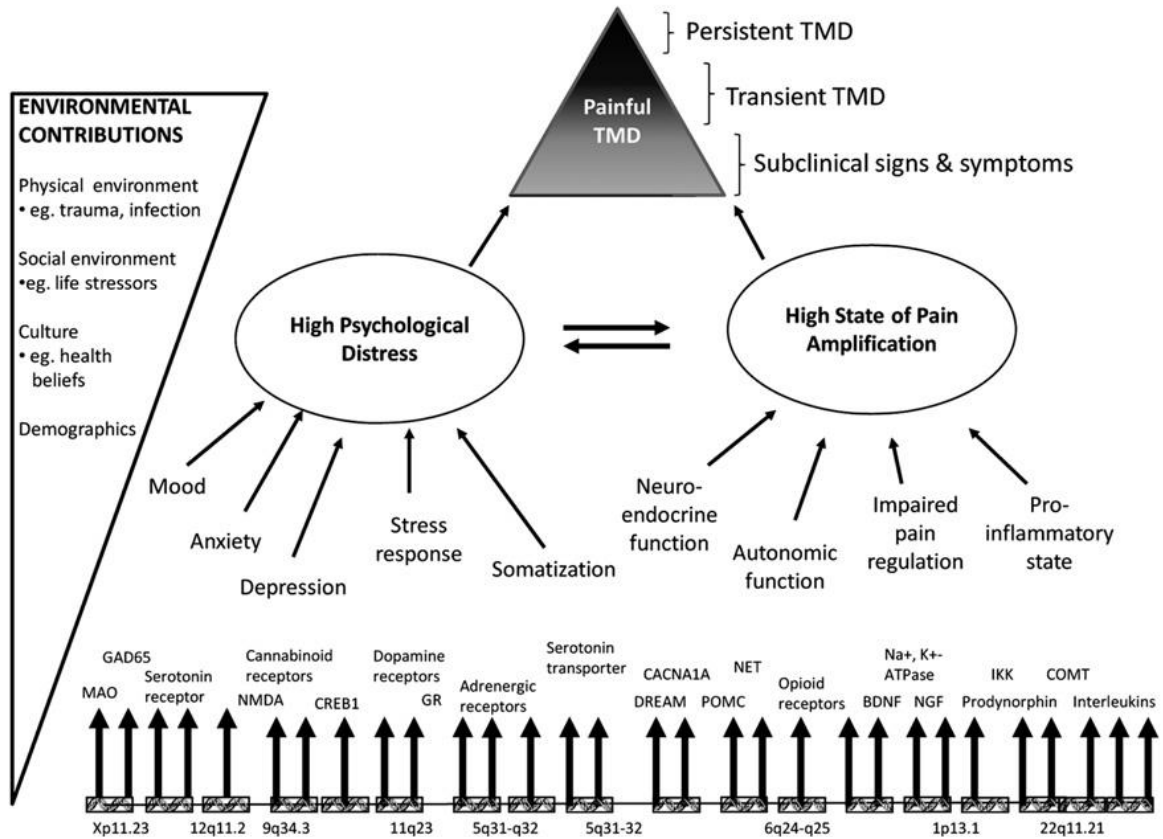


Figure 10. Depiction from Maixner et al. (2011) of the OPPERA study’s proposed two phenotypes of the onset and progression of chronic TMD (psychological distress and pain amplification), associated contributing risk factors of the phenotypes, and potential genetic regulators.

CHAPTER 7

CONCLUSIONS

- These methods provide a novel approach to study TMD and/or facial asymmetry in human subjects.
- To our knowledge, this is the first study to demonstrate that significant expression variation in human height genes may contribute to facial asymmetry with or without TMD, possibly through decreased expression of CACNA2D1.
- TMD patients with facial asymmetry associated with condylar resorption may show significantly different expression of certain inflammatory marker genes such as EREG and CACNA2D1.
- These results support previous findings of pain/inflammatory genes associated with TMD derived from muscular pain.
- These results support that gender may play a key role in the development of TMD, possibly through increased CACNA2D1 expression providing protective effects in TMD-free males but deleterious effect in females with TMD.
- Further studies are needed to understand the genetic contributions to TMD, which may play an important role in future clinical intervention.

BIBIOGRAPHY

1. Adams, R. (1873). The disease in the temporomandibular articulation, or joint of the lower jaw. In, *A Treatise on Rheumatic Gout or Chronic Rheumatic Arthritis of All the Joints* (2nd ed). London, UK: Churchill.
2. Allen, H. L., Estrada, K., Lettre, G., Berndt, S. I., Weedon, M. N., Rivadeneira, F., ... & Hayward, C. (2010). Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature*, 467(7317), 832-838.
3. Appelgren, A., Appelgren, B., Kopp, S., Lundberg, T., & Theodoreson, E. (1995). Neuropeptides in the arthritic TMJ and symptoms and signs from the stomatognathic system with special consideration to rheumatoid arthritis. *Journal of Orofacial Pain*, 9(3).
4. Andersson, H. I. (2004). The course of non-malignant chronic pain: a 12-year follow-up of a cohort from the general population. *European Journal of Pain*, 8(1), 47-53.
5. Battagel, J. M., & Orton, H. S. (1993). Class III malocclusion: the post-retention findings following a non-extraction treatment approach. *The European Journal of Orthodontics*, 15(1), 45-55.
6. Battagel, J. M. (1994). Predictors of relapse in orthodontically-treated Class III malocclusions. *Journal of Orthodontics*, 21(1), 1-13.
7. Bell, W. E. (1990). *Temporomandibular Disorders* (3rd ed.). Chicago, IL: Year Book.
8. Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, 289-300.
9. Bill, J., Proff, P., Bayerlein, T., Blens, T., Gedrange, T., & Reuther, J. (2006). Orthognathic surgery in cleft patients. *Journal of Cranio-Maxillofacial Surgery*, 34, 77-81.
10. Booth, P. W., Schendel, S. A., & Hausamen, J. E. (Eds.). (1999). *Maxillofacial surgery* (Vol. 1). Churchill Livingstone.
11. Boscarino, J. A., Forsberg, C. W., & Goldberg, J. (2010). A twin study of the association between PTSD symptoms and rheumatoid arthritis. *Psychosomatic Medicine*, 72(5), 481-486.
12. Brash J.C (1934). Some problems in the growth and developmental mechanics of bone. *Edinburgh Medical Journal*, 41(5), 305-387.

13. Brodie, A. G. (1946). Facial Patterns* A Theme on Variation. *The Angle Orthodontist*, 16(3), 75-87.
14. Bush, F. M., Harkins, S. W., Harrington, W. G., & Price, D. D. (1993). Analysis of gender effects on pain perception and symptom presentation in temporomandibular pain. *Pain*, 53(1), 73-80.
15. Carlson, D. S. (2005, December). Theories of craniofacial growth in the postgenomic era. In *Seminars in Orthodontics* (Vol. 11, No. 4, pp. 172-183). WB Saunders.
16. Carlson, C. R., Okeson, J. P., Falace, D. A., Nitz, A. J., Curran, S. L., & Anderson, D. (1993). Comparison of psychologic and physiologic functioning between patients with masticatory muscle pain and matched controls. *Journal of Orofacial Pain*, 7(1).
17. Carlson, C. R., Okeson, J. P., Falace, D. A., Nitz, A. J., & Lindroth, J. E. (1993). Reduction of pain and EMG activity in the masseter region by trapezius trigger point injection. *Pain*, 55(3), 397-400.
18. Clark, G. T., Seligman, D. A., Solberg, W. K., & Pullinger, A. C. (1989). Guidelines for the examination and diagnosis of temporomandibular disorders. *Journal of Craniomandibular Disorders*, 3(1).
19. de Bont, L. G., Boering, G., Liem, R. S., Eulerink, F., & Westesson, P. L. (1986). Osteoarthritis and internal derangement of the temporomandibular joint: A light microscopic study. *Journal of Oral and Maxillofacial Surgery*, 44(8), 634-643.
20. Delaire, J., Schendel, S. A., & Tulasne, J. F. (1981). An architectural and structural craniofacial analysis: a new lateral cephalometric analysis. *Oral Surgery, Oral Medicine, Oral Pathology*, 52(3), 226-238.
21. Delaire, J. (1997). Maxillary development revisited: relevance to the orthopaedic treatment of Class III malocclusions. *The European Journal of Orthodontics*, 19(3), 289-311.
22. De Leeuw, R., Boering, G., Stegenga, B., & De Bont, L. G. (1995). Symptoms of temporomandibular joint osteoarthrosis and internal derangement 30 years after non-surgical treatment. *Cranio: The Journal of Craniomandibular Practice*, 13(2), 81-88.
23. Desh, H., Gray, S. L., Horton, M. J., Raoul, G., Rowlerson, A. M., Ferri, J., ... & Sciote, J. J. (2014). Molecular motor MYO1C, acetyltransferase KAT6B and osteogenetic transcription factor RUNX2 expression in human masseter muscle contributes to development of malocclusion. *Archives of Oral Biology*, 59(6), 601-607.

24. Diatchenko, L., Slade, G. D., Nackley, A. G., Bhalang, K., Sigurdsson, A., Belfer, I., ... & Maixner, W. (2005). Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Human Molecular Genetics*, *14*(1), 135-143.
25. Dworkin, S. F., & LeResche, L. (1991). Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. *Journal of Craniomandibular Disorders: Facial & Oral Pain*, *6*(4), 301-355.
26. Dworkin, S. F., & LeResche, L. (1993). Temporomandibular disorder pain: Epidemiologic data. *APS bulletin*, *12*.
27. Edwards, C. L., Fillingim, R. B., & Keefe, F. (2001). Race, ethnicity and pain. *Pain*, *94*(2), 133-137.
28. Epker, J., & Gatchel, R. J. (2000). Prediction of treatment-seeking behavior in acute TMD patients: practical application in clinical settings. *Journal of Orofacial Pain*, *14*(4).
29. Eleftherohorinou, H., Hoggart, C. J., Wright, V. J., Levin, M., & Coin, L. J. (2011). Pathway-driven gene stability selection of two rheumatoid arthritis GWAS identifies and validates new susceptibility genes in receptor mediated signalling pathways. *Human Molecular Genetics*, *20*(17), 3494-3506.
30. El-Gheriani, A. A., Maher, B. S., El-Gheriani, A. S., Sciote, J. J., Abu-Shahba, F. A., Al-Azemi, R., & Marazita, M. L. (2003). Segregation analysis of mandibular prognathism in Libya. *Journal of Dental Research*, *82*(7), 523-527.
31. Enlow, D. H., & Hans, M. G. (1996). *Essentials of facial growth* (pp. 259-260). Philadelphia: Saunders.
32. Fillingim, R. B., Ohrbach, R., Greenspan, J. D., Knott, C., Dubner, R., Bair, E., ... & Maixner, W. (2011). Potential psychosocial risk factors for chronic TMD: descriptive data and empirically identified domains from the OPPERA case-control study. *The Journal of Pain*, *12*(11), T46-T60.
33. Fushima, K., Inui, M., & Sato, S. (1999). Dental asymmetry in temporomandibular disorders. *Journal of Oral Rehabilitation*, *26*(9), 752-756.
34. Gerstner, G. E., Clark, G. T., & Goulet, J. P. (1994). Validity of a brief questionnaire in screening asymptomatic subjects from subjects with tension-type headaches or temporomandibular disorders. *Community Dentistry and Oral Epidemiology*, *22*(4), 235-242.
35. Gottlieb, O. (1951). Hyperplasia of the mandibular condyle. *Journal of Oral Surgery*, *9*(2), 118.

36. Grassi, C., & Passatore, M. (1988). Action of the sympathetic system on skeletal muscle. *The Italian Journal of Neurological Sciences*, 9(1), 23-28.
37. Gray, R. J., Horner, K., Testa, H. J., Lloyd, J. J., & Sloan, P. (1994). Condylar hyperplasia: correlation of histological and scintigraphic features. *Dentomaxillofacial Radiology*, 23(2), 103-107.
38. Greenspan, J. D., Slade, G. D., Bair, E., Dubner, R., Fillingim, R. B., Ohrbach, R., ... & Maixner, W. (2011). Pain sensitivity risk factors for chronic TMD: descriptive data and empirically identified domains from the OPPERA case control study. *The Journal of Pain*, 12(11), T61-T74.
39. Gunson, M. J., Arnett, G. W., Formby, B., Falzone, C., Mathur, R., & Alexander, C. (2009). Oral contraceptive pill use and abnormal menstrual cycles in women with severe condylar resorption: A case for low serum 17 β -estradiol as a major factor in progressive condylar resorption. *American Journal of Orthodontics and Dentofacial Orthopedics*, 136(6), 772-779.
40. Gunson, M. J., Arnett, G. W., & Milam, S. B. (2012). Pathophysiology and pharmacologic control of osseous mandibular condylar resorption. *Journal of Oral and Maxillofacial Surgery*, 70(8), 1918-1934.
41. Hans, M. G., Enlow, D. H., & Noachtar, R. (1995). Age-related differences in mandibular ramus growth: a histologic study. *The Angle Orthodontist*, 65(5), 335-340.
42. Henrikson, T., Nilner, M., & Kurol, J. (2000). Signs of temporomandibular disorders in girls receiving orthodontic treatment. A prospective and longitudinal comparison with untreated Class II malocclusions and normal occlusion subjects. *The European Journal of Orthodontics*, 22(3), 271-281.
43. Horton, M. J., Rosen, C., Close, J. M., & Sciote, J. J. (2008). Quantification of myosin heavy chain RNA in human laryngeal muscles: differential expression in the vertical and horizontal posterior cricoarytenoid and thyroarytenoid. *The Laryngoscope*, 118(3), 472-477.
44. Huh, A., Horton, M. J., Cuenco, K. T., Raoul, G., Rowlerson, A. M., Ferri, J., & Sciote, J. J. (2013). Epigenetic influence of *KAT6B* and *HDAC4* in the development of skeletal malocclusion. *American Journal of Orthodontics and Dentofacial Orthopedics*, 144(4), 568-576.
45. Hunter, J., & Combe, W. (1778). *The Natural History of the Human Teeth...* J. Johnson.

46. Ji, R. R., Zhang, X., Wiesenfeld-Hallin, Z., & Hokfelt, T. (1994). Expression of neuropeptide Y and neuropeptide Y (Y1) receptor mRNA in rat spinal cord and dorsal root ganglia following peripheral tissue inflammation. *The Journal of Neuroscience*, *14*(11), 6423-6434.
47. Korszun, A., Papadopoulos, E., Demitrack, M., Engleberg, C., & Crofford, L. (1998). The relationship between temporomandibular disorders and stress-associated syndromes. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, *86*(4), 416-420.
48. Kramer, P. R., & Bellinger, L. L. (2013). Reduced GABA_A receptor $\alpha 6$ expression in the trigeminal ganglion enhanced myofascial nociceptive response. *Neuroscience*, *245*, 1-11.
49. Kwon, T. G., Lee, K. H., Park, H. S., Ryoo, H. M., Kim, H. J., & Lee, S. H. (2007). Relationship between the masticatory muscles and mandibular skeleton in mandibular prognathism with and without asymmetry. *Journal of Oral and Maxillofacial Surgery*, *65*(8), 1538-1543.
50. Lui, J. C., Nilsson, O., Chan, Y., Palmer, C. D., Andrade, A. C., Hirschhorn, J. N., & Baron, J. (2012). Synthesizing genome-wide association studies and expression microarray reveals novel genes that act in the human growth plate to modulate height. *Human Molecular Genetics*, *21*(23), 5193-5201.
51. MacNalty, S. A. S. (Ed.). (1961). *The British medical dictionary*. Caxton
52. Maixner, W., Diatchenko, L., Dubner, R., Fillingim, R. B., Greenspan, J. D., Knott, C., ... & Slade, G. D. (2011). Orofacial pain prospective evaluation and risk assessment study—the OPPERA study. *The Journal of Pain: Official journal of the American Pain Society*, *12*(11 Suppl), T4.
53. Maixner, W., Fillingim, R., Booker, D., & Sigurdsson, A. (1995). Sensitivity of patients with painful temporomandibular disorders to experimentally evoked pain. *Pain*, *63*(3), 341-351.
54. Maixner, W., Greenspan, J. D., Dubner, R., Bair, E., Mulkey, F., Miller, V., ... & Fillingim, R. B. (2011). Potential autonomic risk factors for chronic TMD: descriptive data and empirically identified domains from the OPPERA case-control study. *The Journal of Pain*, *12*(11), T75-T91.
55. Marbach, J. J., & Lund, P. (1981). Depression, anhedonia and anxiety in temporomandibular joint and other facial pain syndromes. *Pain*, *11*(1), 73-84.
56. Meikle, M. C. (2002). *Craniofacial development, growth and evolution*. Bateson.
57. Mense, S. (1993). Nociception from skeletal muscle in relation to clinical muscle pain. *Pain*, *54*(3), 241-289.

58. Mickey, B. J., Zhou, Z., Heitzeg, M. M., Heinz, E., Hodgkinson, C. A., Hsu, D. T., ... & Zubieta, J. K. (2011). Emotion processing, major depression, and functional genetic variation of neuropeptide Y. *Archives of General Psychiatry*, 68(2), 158-166.
59. Mihalik, C. A., Proffit, W. R., & Phillips, C. (2003). Long-term follow-up of Class II adults treated with orthodontic camouflage: a comparison with orthognathic surgery outcomes. *American Journal of Orthodontics and Dentofacial Orthopedics*, 123(3), 266-278.
60. Mohlin, B., Axelsson, S., Paulin, G., Pietilä, T., Bondemark, L., Brattström, V., ... & Holm, A. K. (2007). TMD in relation to malocclusion and orthodontic treatment: A systematic review. *The Angle Orthodontist*, 77(3), 542-548.
61. Murakami, M., Harada, M., Kamimura, D., Ogura, H., Okuyama, Y., Kumai, N., ... & Hirano, T. (2013). Disease-association analysis of an inflammation-related feedback loop. *Cell Reports*, 3(3), 946-959.
62. Muroi, Y., Kakudo, K., & Nakata, K. (2007). Effects of compressive loading on human synovium-derived cells. *Journal of Dental Research*, 86(8), 786-791.
63. Okeson, J. P. (2013). *Management of temporomandibular disorders and occlusion*. (7th ed). Elsevier Health Sciences.
64. PAIN, S. O. N. (2012). Canadian Pain Society Abstracts, 2012. *Pain*, 17(3), 180.
65. Pedersen, T. K., Jensen, J. J., Melsen, B. I. R. T. E., & Herlin, T. (2001). Resorption of the temporomandibular condylar bone according to subtypes of juvenile chronic arthritis. *The Journal of Rheumatology*, 28(9), 2109-2115.
66. Percipalle, P., Fomproix, N., Cavellan, E., Voit, R., Reimer, G., Krüger, T., ... & Östlund Farrants, A. K. (2006). The chromatin remodelling complex WSTF-SNF2h interacts with nuclear myosin 1 and has a role in RNA polymerase I transcription. *EMBO Reports*, 7(5), 525-530.
67. Perola, M., Sammalisto, S., Hiekkalinna, T., Martin, N. G., Visscher, P. M., Montgomery, G. W., ... & Peltonen, L. (2007). Combined genome scans for body stature in 6,602 European twins: evidence for common Caucasian loci. *PLoS Genetics*, 3(6), e97.
68. Plesh, O., Crawford, P. B., & Gansky, S. A. (2002). Chronic pain in a biracial population of young women. *Pain*, 99(3), 515-523.
69. Proffit, W. R., Fields Jr, H. W., & Sarver, D. M. (2013). *Contemporary orthodontics* (4th ed). St. Louis, MO: Elsevier Health Sciences.
70. Puri, J., Bellinger, L. L., & Kramer, P. R. (2011). Estrogen in cycling rats alters gene expression in the temporomandibular joint, trigeminal ganglia and trigeminal

- subnucleus caudalis/upper cervical cord junction. *Journal of Cellular Physiology*, 226(12), 3169-3180.
71. Quinn, J. H., & Stover, J. D. (1998). Arthroscopic management of temporomandibular joint disc perforations and associated advanced chondromalacia by discoplasty and abrasion arthroplasty: a supplemental report. *Journal of Oral and Maxillofacial Surgery*, 56(11), 1237-1239.
 72. Rammelsberg, P., LeResche, L., Dworkin, S., & Mancl, L. (2003). Longitudinal outcome of temporomandibular disorders: a 5-year epidemiologic study of muscle disorders defined by research diagnostic criteria for temporomandibular disorders. *Journal of Orofacial Pain*, 17(1).
 73. Raoul, G., Rowlerson, A., Sciote, J., Codaccioni, E., Stevens, L., Maurage, C. A., ... & Ferri, J. (2011). Masseter myosin heavy chain composition varies with mandibular asymmetry. *Journal of Craniofacial Surgery*, 22(3), 1093-1098.
 74. Rowlerson, A., Raoul, G., Daniel, Y., Close, J., Maurage, C. A., Ferri, J., & Sciote, J. J. (2005). Fiber-type differences in masseter muscle associated with different facial morphologies. *American Journal of Orthodontics and Dentofacial Orthopedics*, 127(1), 37-46.
 75. Sato, S., Goto, S., Kawamura, H., & Motegi, K. (1997). The natural course of nonreducing disc displacement of the TMJ: relationship of clinical findings at initial visit to outcome after 12 months without treatment. *Journal of Orofacial Pain*, 11(4).
 76. Schiffman, E. L., Haley, D. P., & Shapiro, B. L. (1990). The prevalence and treatment needs of subjects with temporomandibular disorders. *The Journal of the American Dental Association*, 120(3), 295-303.
 77. Sciote, J. J., Horton, M. J., Rowlerson, A. M., Ferri, J., Close, J. M., & Raoul, G. (2012). Human masseter muscle fiber type properties, skeletal malocclusions, and muscle growth factor expression. *Journal of Oral and Maxillofacial Surgery*, 70(2), 440-448.
 78. Scott, D.L., Wolfe, F., & Huizinga, T.W. (2010). Rheumatoid arthritis. *Lancet*, 376(9746):1094-108.
 79. Selye, H. (1974). *Stress without distress*. Philadelphia, PA: Lippincott.
 80. Silman, A. J., & Hochberg, M. C. (2001). *Epidemiology of the rheumatic diseases* (2nd ed). Oxford University Press.
 81. Slade, G. D., Bair, E., By, K., Mulkey, F., Baraian, C., Rothwell, R., ... & Ohrbach, R. (2011). Study methods, recruitment, sociodemographic findings, and demographic representativeness in the OPPERA study. *The Journal of Pain*, 12(11), T12-T26.

82. Slade, G. D., Diatchenko, L., Bhalang, K., Sigurdsson, A., Fillingim, R. B., Belfer, I., ... & Maixner, W. (2007). Influence of psychological factors on risk of temporomandibular disorders. *Journal of Dental Research*, 86(11), 1120-1125.
83. Skieller, V., Björk, A., & Linde-Hansen, T. (1984). Prediction of mandibular growth rotation evaluated from a longitudinal implant sample. *American Journal of Orthodontics*, 86(5), 359-370.
84. Smith, S. B., Maixner, D. W., Greenspan, J. D., Dubner, R., Fillingim, R. B., Ohrbach, R., ... & Diatchenko, L. (2011). Potential genetic risk factors for chronic TMD: genetic associations from the OPPERA case control study. *The Journal of Pain*, 12(11), T92-T101.
85. Tabeling, H. J., & Dolwick, M. F. (1984). Rheumatoid arthritis: diagnosis and treatment. *Florida Dental Journal*, 56(1), 16-18.
86. Tassopoulou-Fishell, M., Deeley, K., Harvey, E. M., Sciote, J., & Vieira, A. R. (2012). Genetic variation in Myosin 1H contributes to mandibular prognathism. *American Journal of Orthodontics and Dentofacial Orthopedics*, 141(1), 51-59.
87. Torres-Chávez, K. E., Sanfins, J. M., Clemente-Napimoga, J. T., Pelegrini-Da-Silva, A., Parada, C. A., Fischer, L., & Tambeli, C. H. (2012). Effect of gonadal steroid hormones on formalin-induced temporomandibular joint inflammation. *European Journal of Pain*, 16(2), 204-216.
88. Trauner, R., & Obwegeser, H. (1957). The surgical correction of mandibular prognathism and retrognathia with consideration of genioplasty: Part I. Surgical procedures to correct mandibular prognathism and reshaping of the chin. *Oral Surgery, Oral Medicine, Oral Pathology*, 10(7), 677-689.
89. Travell, J. G., & Simons, D. G. (1999). *Myofascial pain and dysfunction: the trigger point manual* (Vol. 2). Lippincott Williams & Wilkins.
90. Tulloch, J. F., Proffit, W. R., & Phillips, C. (1997). Influences on the outcome of early treatment for Class II malocclusion. *American Journal of Orthodontics and Dentofacial Orthopedics*, 111(5), 533-542.
91. Undt, G., Murakami, K. I., Clark, G. T., Ploder, O., Dem, A., Lang, T., & Wiesinger, G. F. (2006). Cross-cultural adaptation of the JPF-Questionnaire for German-speaking patients with functional temporomandibular joint disorders. *Journal of Cranio-Maxillofacial Surgery*, 34(4), 226-233.
92. Weinman, J. P., & Sicher, H. (1948). Bone and bones. Fundamentals of bone biology. *The American Journal of the Medical Sciences*, 215(1), 113.
93. White, R. P., & Sarver, D. M. (2003). *Contemporary treatment of dentofacial deformity* (Vol. 751). St Louis, Mo: Mosby.

94. Wolford, L. M. (2001). Idiopathic condylar resorption of the temporomandibular joint in teenage girls (cheerleaders' syndrome). *Proceedings (Baylor University Medical Center)*, 14(3), 246.
95. Wolford, L. M., Morales-Ryan, C. A., García-Morales, P., & Perez, D. (2009). Surgical management of mandibular condylar hyperplasia type 1. *Proceedings (Baylor University Medical Center)*, 22(4), 321.
96. Wolford, L. M., Movahed, R., & Perez, D. E. (2014). A Classification System for Conditions Causing Condylar Hyperplasia. *Journal of Oral and Maxillofacial Surgery*, 72(3), 567-95.
97. Wu, Y. W., Bi, Y. P., Kou, X. X., Xu, W., Ma, L. Q., Wang, K. W., ... & Ma, X. C. (2010). 17- β -estradiol enhanced allodynia of inflammatory temporomandibular joint through up-regulation of hippocampal TRPV1 in ovariectomized rats. *The Journal of Neuroscience*, 30(26), 8710-8719.

APPENDICES

APPENDIX A

SIGNIFICANT DIFFERENCES (P <0.05) IN PAIN/INFLAMMATORY RNA GENE EXPRESSION IN MASSETER MUSCLE FROM SUBJECTS WITH SKELETAL MALOCCLUSION AND FACIAL ASYMMETRY.				
Gene	Transcript ID	Gene Ontology	Asym	vs Sym
			Fold Diff	P Value
HSPA8	TC11002390.hg.1	synaptic transmission	-28.404	6.50E-08
BTG2	TC01001685.hg.1	response to mechanical stimulus	24.4172	4.50E-06
PRKAA2	TC01000685.hg.1	protein phosphorylation	23.1139	1.23E-06
HSP90AB1	TC06000615.hg.1	axon guidance	19.2708	6.69E-06
EGR1	TC05000701.hg.1	cytokine-mediated signaling pathway	16.4677	0.000628688
ZFAND5	TC09001209.hg.1	platelet-derived growth factor receptor signaling pathway	-14.208	3.55E-06
CACNA2D1	TC07001563.hg.1	calcium ion transport	12.5127	4.10E-07
COQ10A	TC12000509.hg.1	mitochondrial inner membrane	11.9548	1.01E-06
NFKBIA	TC14001036.hg.1	toll-like receptor signaling pathway	9.91595	5.82E-07
NPY6R	TC05000691.hg.1	neuropeptide Y receptor activity	9.79721	9.76E-06
MAPK1	TC22000547.hg.1	toll-like receptor signaling pathway	9.74363	1.10E-07
ACSL1	TC04001809.hg.1	long-chain fatty acid metabolic process	8.93311	8.12E-06
CAT	TC11000327.hg.1	response to reactive oxygen species	7.53333	6.72E-07
FKBP5	TC06004150.hg.1	protein folding	6.93992	0.00256572
FOS	TC14000471.hg.1	toll-like receptor signaling pathway	6.21266	0.00753702
SPARC	TC05001955.hg.1	platelet activation	5.91797	1.77E-07
GABARAPL1	TC12000159.hg.1	GABA receptor binding	5.78613	0.000155956
TMSB10	TC02000500.hg.1	actin cytoskeleton organization	-5.5694	2.66E-05
WNK1	TC12000012.hg.1	ion transport	5.51789	6.20E-05
SOD2	TC06004141.hg.1	vasodilation by acetylcholine	-5.2533	9.05E-06
HNRNPU	TC01006391.hg.1	nuclear mRNA splicing	5.03368	3.99E-05

PPP3CA	TC04001425.hg.1	response to stress	5.00693	9.70E-06
ADRB2	TC05000814.hg.1	vasodilation by norepinephrine-epinephrine	-4.6172	0.000642058
ATF1	TC12000397.hg.1	toll-like receptor signaling pathway	4.50431	9.14E-06
MAPK14	TC06000523.hg.1	toll-like receptor signaling pathway	4.46377	3.32E-06
HSPA9	TC05001832.hg.1	protein folding	4.42076	5.10E-06
TUBB	TC06000347.hg.1	neuron differentiation	4.26379	7.40E-07
ARL5B	TC10000140.hg.1	small GTPase mediated signal transduction	4.14984	0.00134871
GPX4	TC19000030.hg.1	response to oxidative stress	-4.0607	7.99E-05
WNK1	TC12000010.hg.1	neuron development	3.89214	1.23E-05
CSNK1A1	TC05001924.hg.1	Wnt receptor signaling pathway	3.88347	1.58E-06
MTDH	TC08000594.hg.1	positive regulation of I-kappaB kinase/NF-kappaB cascade	3.67207	0.00026617
MAOB	TC0X000978.hg.1	negative regulation of serotonin secretion	3.63272	1.01E-05
PRKACA	TC19001231.hg.1	protein phosphorylation	3.33769	2.03E-06
DDX24	TC14001462.hg.1	RNA metabolic process	3.25062	0.00479207
ANXA2	TC15001505.hg.1	fibrinolysis	-3.2363	3.83E-05
CTSD	TC11003472.hg.1	cell death	3.21581	1.38E-05
VPS4A	TC16000567.hg.1	protein transport	3.13672	0.000811281
ERBB4	TC02002735.hg.1	nervous system development	3.06741	4.38E-05
ATP1A1	TC01001018.hg.1	regulation of sodium ion transport	3.05178	3.83E-05
CALCRL	TC02002605.hg.1	calcium ion transport	3.01113	0.000135872
IGF1	TC12001890.hg.1	anti-apoptosis	3.00121	0.00136137
APP	TC21000340.hg.1	protein phosphorylation	-2.9748	6.68E-05
PIP5K1A	TC01001204.hg.1	cell migration	2.97475	2.86E-06
ATP6V1B2	TC08000151.hg.1	transmembrane transport	2.96525	9.50E-06
PPP3CC	TC08000172.hg.1	activation of pro-apoptotic gene products	2.95391	2.03E-05
IFNGR1	TC06002152.hg.1	interferon-gamma-mediated signaling pathway	2.90727	1.14E-05
CALM2	TC02005060.hg.1	epidermal growth factor receptor signaling pathway	2.83394	7.19E-06
SLC12A2	TC05000612.hg.1	potassium ion transport/ sodium ion transport	2.83334	0.000605859

VPS52	TC6_mann_hap4000173.hg.1	protein transport	2.81684	2.65E-06
MSN	TC0X000353.hg.1	leukocyte migration	2.74924	3.13E-05
JUN	TC01002708.hg.1	microglial cell activation	2.73228	0.00203818
PRKG1	TC10000347.hg.1	forebrain development	2.70255	7.05E-06
TUBB	TC6_apd_hap1000029.hg.1	neuron differentiation	-2.6001	2.63E-06
TUBB	TC6_dbb_hap3000038.hg.1	neuron differentiation	-2.6001	2.63E-06
TUBB	TC6_mann_hap4000040.hg.1	neuron differentiation	-2.6001	2.63E-06
TUBB	TC6_mcf_hap5000032.hg.1	neuron differentiation	-2.6001	2.63E-06
TUBB	TC6_qbl_hap6000038.hg.1	neuron differentiation	-2.6001	2.63E-06
TUBB	TC6_ssto_hap7000038.hg.1	neuron differentiation	-2.6001	2.63E-06
TUBB	TC6_cox_hap2000045.hg.1	neuron differentiation	2.57334	3.76E-06
ARRB1	TC11002092.hg.1	Notch signaling pathway	2.55135	0.00018025
RAP1A	TC01000979.hg.1	platelet activation	2.49815	2.77E-05
EGFR	TC07000328.hg.1	activation of MAPKK activity	2.48298	2.67E-05
STAU1	TC20000923.hg.1	intracellular mRNA localization	-2.4055	0.000368089
HNRNPD	TC04001332.hg.1	RNA processing	2.39845	0.000483471
VEGFA	TC06000608.hg.1	positive regulation of protein phosphorylation	2.35792	0.000257369
EPHX1	TC01001856.hg.1	cellular response to glucocorticoid stimulus	2.23205	5.90E-05
P2RY1	TC03000835.hg.1	cell surface receptor signaling pathway	2.16535	8.02E-05
SC5DL	TC11001106.hg.1	small molecule metabolic process	2.14276	7.75E-05
TNXB	TC6_mann_hap4000149.hg.1	signal transduction	2.09414	0.00573483
MAOA	TC0X000207.hg.1	synaptic transmission	2.07615	0.0864106
IKBKAP	TC09001455.hg.1	immune response/ I-kappaB phosphorylation	2.05909	5.15E-05
TNFAIP3	TC06001027.hg.1	B-1 B cell homeostasis/response to molecule of bacterial origin	-2.046	0.0420318
MME	TC03000846.hg.1	sensory perception of pain	2.03852	0.000175863
STAU2	TC08002586.hg.1	mRNA transport	-2.0327	0.00131523
VPS52	TC6_mcf_hap5000180.hg.1	protein transport	2.02786	2.10E-05
VPS52	TC6_ssto_hap7000174.hg.1	protein transport	2.02786	2.10E-05
VPS52	TC6_qbl_hap6000193.hg.1	protein transport	2.02046	2.42E-05
MPDZ	TC09000913.hg.1	synaptic plasticity	2.01397	0.0157814

ANXA1	TC09000335.hg.1	negative regulation of acute inflammatory response	2.00393	0.00628716
P2RY2	TC11000772.hg.1	positive regulation of neuron projection development	-2.0026	0.000116096
VPS52	TC6_cox_hap2000205.hg.1	protein transport	1.99394	2.68E-05
VPS52	TC6_dbb_hap3000191.hg.1	protein transport	1.99394	2.68E-05
ATP2B1	TC12001798.hg.1	ion transmembrane transport	1.98966	0.000191141
EDNRA	TC04000729.hg.1	positive regulation of protein phosphorylation	1.97479	0.0181122
RAB5A	TC03000111.hg.1	nervous system development	1.97241	5.39E-05
VPS52	TC06001581.hg.1	protein transport	1.96119	2.97E-05
RAG1	TC11000347.hg.1	B cell differentiation/T cell differentiation	1.95202	6.63E-07
PROK2	TC03001550.hg.1	inflammatory response	1.95758	2.33E-05
CCK	TC03001318.hg.1	behavioral fear response/axonogenesis	1.96692	3.97E-06
GRIA4	TC11000968.hg.1	synaptic transmissio	1.9788	3.85E-07
PRL	TC06001320.hg.1	cell surface receptor signaling pathway	1.9955	3.83E-06
TRIM40	TC06000333.hg.1	E3 ubiquitin-protein ligase	1.99663	4.67E-06
TPH2	TC12000629.hg.1	serotonin biosynthetic process/response to stress	1.99758	4.01E-06
IL22RA2	TC06002151.hg.1	regulation of tyrosine phosphorylation	1.99885	3.57E-07
TRIM40	TC6_cox_hap2000032.hg.1	E3 ubiquitin-protein ligase	2.00201	3.43E-06
TRIM40	TC6_qbl_hap6000025.hg.1	E3 ubiquitin-protein ligase	2.00201	3.43E-06
TRIM40	TC6_dbb_hap3000025.hg.1	E3 ubiquitin-protein ligase	2.01424	2.07E-06
MC4R	TC18000550.hg.1	response to insulin stimulus	2.02556	0.000559985
PTGER3	TC01002774.hg.1	cell death	2.02881	2.30E-05
HRH1	TC03000064.hg.1	inflammatory response/synaptic transmission	2.0336	4.15E-06
P2RY13	TC03001906.hg.1	hematopoiesis and the immune system	2.0344	0.0168278
SLC17A6	TC11000262.hg.1	sodium ion transport /neurotransmitter uptake	2.07715	3.37E-07
PDYN	TC20000558.hg.1	neuropeptide signaling pathway/synaptic transmission	2.10483	2.94E-06
NPY5R	TC04000818.hg.1	positive regulation of acute inflammatory response	2.10573	2.03E-05
EREG	TC04000414.hg.1	positive regulation of cytokine production	2.12244	8.71E-07
GABRA6	TC05000902.hg.1	synaptic transmission	2.14136	7.98E-07
C6orf15	TC6_cox_hap2000149.hg.1	hyaluronic acid binding	2.1487	9.09E-05
LIPM	TC10000630.hg.1	lipid catabolic process	2.15933	3.80E-07
DPCR1	TC6_qbl_hap6000043.hg.1	associated with systemic lupus erythematosus	2.1668	6.05E-06
SCN2A	TC02000985.hg.1	sodium ion transport/myelination	2.17245	1.59E-06
TAAR5	TC06002115.hg.1	synaptic transmission	2.1769	6.89E-05
CHRM5	TC15000231.hg.1	dopamine transport	2.17728	2.27E-06
DPCR1	TC06000352.hg.1	associated with systemic lupus erythematosus	2.19461	4.25E-06
GRIA2	TC04000799.hg.1	synaptic transmission	2.19823	5.12E-07

GABRG2	TC05000904.hg.1	synaptic transmission, GABAergic	2.22665	3.99E-07
RGS4	TC01001427.hg.1	inactivation of MAPK activity	2.2456	4.01E-06
DPCR1	TC6_cox_hap2000050.hg.1	associated with systemic lupus erythematosus	2.25148	5.83E-06
PTGIR	TC19001650.hg.1	cell-cell signaling	2.25368	1.20E-05
DPCR1	TC6_mcf_hap5000037.hg.1	associated with systemic lupus erythematosus	2.25548	3.54E-06
DPCR1	TC6_dbb_hap3000043.hg.1	associated with systemic lupus erythematosus	2.30111	4.02E-06
AGTR2	TC0X000570.hg.1	cell surface receptor signaling pathway	2.3595	2.49E-07
C6orf15	TC6_qbl_hap6000139.hg.1	hyaluronic acid binding	2.39331	5.09E-05
IAPP	TC12000230.hg.1	apoptotic process	2.43736	6.11E-05
F2RL2	TC05001502.hg.1	response to wounding	2.46935	1.49E-06
CYP2C9	TC10000675.hg.1	steroid metabolic process	2.57275	8.86E-07
TAAR6	TC06000994.hg.1	G-protein coupled receptor signaling pathway	2.58968	2.14E-05
GABRG3	TC15000126.hg.1	synaptic transmission	2.60197	8.69E-05
UGT2B7	TC04000371.hg.1	cellular glucuronidation	3.52804	6.27E-07
TAAR8	TC06000993.hg.1	G-protein coupled receptor signaling pathway	3.53377	1.55E-05
TAAR9	TC06000992.hg.1	G-protein coupled receptor signaling pathway	3.85475	7.64E-05
TAAR1	TC06002118.hg.1	G-protein coupled receptor signaling pathway	4.91418	3.77E-06

Abbreviations: Asym, asymmetric (Subject 4 and 10); Sym, symmetric (8 subjects with skeletal malocclusion without asymmetry);

Fold Diff, fold difference between average expression in Asym vs Sym.

Significant for step-up P-value with fold differences $>\pm 2$ between groups.

APPENDIX B

**SIGNIFICANT DIFFERENCES (P <0.05) IN HEIGHT-RELATED RNA GENE EXPRESSION
IN MASSETER MUSCLE FROM SUBJECTS WITH SKELETAL MALOCCLUSION AND FACIAL ASYMMETRY.**

Gene	Transcript ID	Gene Ontology	Asym vs	Sym
			Fold Diff	P Value
TBX15	TC01003042.hg.1	embryonic cranial skeleton morphogenesis	21.7709	6.65E-08
DYM	TC18000501.hg.1	bone development	10.8843	9.45E-08
MEF2C	TC05001579.hg.1	osteoblast differentiation	6.53951	3.79E-06
KCNJ2	TC17000813.hg.1	synaptic transmission	5.28547	3.48E-05
PPAP2A	TC05001363.hg.1	germ cell migration	4.67171	1.65E-05
ZBTB20	TC03001670.hg.1	regulation of transcription	4.42276	1.82E-06
NF1	TC17000362.hg.1	osteoblast differentiation	2.71001	4.48E-06
IGF1R	TC15000949.hg.1	positive regulation of cell proliferation	2.50759	1.41E-05
ZFP36L1	TC14001253.hg.1	regulation of transcription	2.49478	4.31E-05
ZNF462	TC09000544.hg.1	positive regulation of transcription	2.28125	0.00723781
TGFB2	TC01001805.hg.1	cell morphogenesis	2.24114	0.00131003
DDX42	TC17000771.hg.1	regulation of anti-apoptosis	2.14568	0.000419246
EFEMP1	TC02001867.hg.1	negative regulation of chondrocyte differentiation	2.13209	0.00393153
PPIF	TC10000566.hg.1	protein folding	1.98223	1.70E-05
PRKG2	TC04001330.hg.1	protein phosphorylation	2.2365	5.15E-07
CENPW	TC06000966.hg.1	mitotic cell cycle	2.28036	7.24E-07
COL11A1	TC01002932.hg.1	cartilage condensation/ chondrocyte development	2.56935	3.15E-07
TEAD1	TC11000203.hg.1	regulation of transcription	12.1657	4.45E-07
DYM	TC18000501.hg.1	bone development	10.8843	9.45E-08
ZBTB20	TC03001670.hg.1	regulation of transcription	4.42276	1.82E-06
NFIC	TC19000069.hg.1	DNA replication	4.03863	3.98E-06
TNPO1	TC05000340.hg.1	protein import into nucleus	3.95058	1.71E-06
PIP4K2B	TC17001433.hg.1	cell surface receptor signaling pathway	-	1.19E-05

			3.67011	
			-	
SUCLG2	TC03001533.hg.1	tricarboxylic acid cycle	3.26113	8.51E-06
			-	
IGF1R	TC15000949.hg.1	positive regulation of cell proliferation	2.50759	1.41E-05
			-	
TULP4	TC06001132.hg.1	regulation of transcription	2.33318	6.04E-05
			-	
TGFB2	TC01001805.hg.1	cell morphogenesis	2.24114	0.00131003
			-	
SEPT2	TC02001477.hg.1	mitosis	2.23749	1.88E-05
			-	
LTBP1	TC02000219.hg.1	sequestering of TGFbeta in extracellular matrix	2.14919	0.000486433
			-	
EFEMP1	TC02001867.hg.1	epidermal growth factor receptor signaling pathway	2.13209	0.00393153
			-	
MYO9A	TC15001620.hg.1	small GTPase mediated signal transduction	2.10254	0.000785253
			-	
INSR	TC19001111.hg.1	regulation of transcription	2.05499	0.0067507
			-	
SBNO1	TC12002087.hg.1	protein-coding	1.97449	0.000708249
KCNJ16	TC17000812.hg.1	synaptic transmission	1.95074	1.45E-05
DTL	TC01001767.hg.1	response to DNA damage stimulus	2.15074	7.00E-07
COL11A1	TC01002932.hg.1	cartilage condensation	2.56935	3.15E-07
GHSR	TC03002005.hg.1	growth hormone secretion	2.73044	1.51E-05
			-	
CASQ1	TC01001363.hg.1	skeletal muscle tissue development	29.3112	3.98E-07
			-	
CS	TC12003213.hg.1	carbohydrate metabolic process	20.1905	3.80E-08
			-	
BVES	TC06001976.hg.1	muscle organ development	19.9989	5.64E-07
			-	
HIST1H1E	TC06000168.hg.1	nucleosome assembly	14.1342	0.000874154
			-	
CSE1L	TC20000384.hg.1	cell proliferation	12.6044	6.56E-07
			-	
PPP2R3A	TC03000732.hg.1	protein dephosphorylation	12.3889	3.71E-07
			-	
COQ10A	TC12000509.hg.1	function of coenzyme Q	11.9548	1.01E-06
			-	
CD164	TC06002000.hg.1	muscle organ development	10.2437	1.03E-06
			-	
UBQLN1	TC09001280.hg.1	apoptotic process	9.30829	7.96E-07
			-	
PREPL	TC02001798.hg.1	proteolysis	7.77091	2.80E-07
			-	
KIAA0368	TC09001482.hg.1	ER-associated protein catabolic process	7.29893	8.88E-06
			-	
DDX6	TC11002353.hg.1	RNA metabolic process	7.00319	2.34E-07
			-	
HLA-DRA	TC06000399.hg.1	immune response	6.97513	2.27E-06
			-	
FKBP5	TC06004150.hg.1	protein folding	6.93992	0.00256572

HLA-DRA	TC6_dbb_hap3000083.hg.1	immune response	6.80288	1.93E-06
HLA-DRA	TC6_cox_hap2000090.hg.1	immune response	6.27495	1.60E-06
IGFBP7	TC04001226.hg.1	regulation of cell growth	6.08102	4.02E-06
HLA-DRA	TC6_mcf_hap5000075.hg.1	immune response	5.69508	2.60E-06
HLA-DRA	TC6_qbl_hap6000081.hg.1	immune response	5.69508	2.60E-06
HLA-DRA	TC6_ssto_hap7000076.hg.1	immune response	5.69508	2.60E-06
HLA-DRA	TC6_mann_hap4000074.hg.1	immune response	5.61789	2.75E-06
MAP2K6	TC17000807.hg.1	activation of MAPK activity	5.36902	6.13E-06
PSMB3	TC17000466.hg.1	mitotic cell cycle	5.30353	3.69E-06
MRPL42	TC12000725.hg.1	translation	5.16824	0.000117519
COPA	TC01003400.hg.1	intracellular protein transport	5.10702	2.28E-06
PIK3C2A	TC11003476.hg.1	epidermal growth factor receptor signaling pathway	4.84471	3.34E-06
RAD23B	TC09000545.hg.1	response to DNA damage stimulus	4.77301	4.38E-06
PJA2	TC05001664.hg.1	regulation of protein kinase A signaling cascades	4.72755	1.16E-06
MIR7-1	TC09001284.hg.1	nuclear mRNA splicing	4.61865	1.37E-07
MAPK14	TC06000523.hg.1	apoptotic process	4.46377	3.32E-06
N4BP2L2	TC13001723.hg.1	associated with neutropenia	4.40464	1.19E-06
HLA-DRB1	TC6_ssto_hap7000164.hg.1	immune response	4.37257	0.000250441
RHOT1	TC17000373.hg.1	apoptotic process	4.36527	6.74E-07
FILIP1	TC06001882.hg.1	controls neocortical cell migration	4.22493	2.07E-05
ERGIC2	TC12001348.hg.1	vesicle-mediated transport	4.21123	0.000109458
TMEM181	TC06001133.hg.1	G protein-coupled receptor on cell surface	4.09981	2.34E-05
IARS	TC09001331.hg.1	tRNA aminoacylation	4.01725	1.35E-06
HINT3	TC06000963.hg.1	nucleotide hydrolase and transferase	3.96955	9.94E-06
MIRLET7F1	TC09000457.hg.1	non-coding RNA that involved in post-transcriptional regulation	-3.9121	0.00117178
HLA-DRB1	TC6_apd_hap1000101.hg.1	immune response	3.85966	0.000159393
TRA2B	TC03002080.hg.1	RNA splicing, via transesterification reactions	3.76443	1.08E-05

HIST1H3E	TC06000174.hg.1	regulation of gene silencing	3.76188	0.00133807
C1orf21	TC01001591.hg.1	associated with prostatic hypertrophy	-3.7338	1.14E-05
CDC5L	TC06000617.hg.1	nuclear mRNA splicing	3.72952	0.00668212
PANK3	TC05002041.hg.1	coenzyme A biosynthetic process	3.47816	0.000132504
DCAF16	TC04001069.hg.1	protein ubiquitination	3.46245	4.48E-07
VPS13C	TC15001510.hg.1	protein localization	3.36674	1.47E-05
TRA2A	TC07001197.hg.1	nuclear mRNA splicing	3.36267	4.51E-06
TAF2	TC08001559.hg.1	G2/M transition of mitotic cell cycle	3.35188	9.19E-06
TNS1	TC02002765.hg.1	fibroblast migration	3.31357	4.88E-07
POLR2B	TC04000336.hg.1	nuclear mRNA splicing	3.29296	1.80E-06
LAMC1	TC01001583.hg.1	cell adhesion	3.28327	9.22E-06
CG030	TC13000552.hg.1	affiliated with the lncRNA class	3.24605	0.00129279
SNORD21	TC01000854.hg.1	nuclear-transcribed mRNA catabolic process	3.23198	4.09E-06
MLXIP	TC12000969.hg.1	nucleocytoplasmic transport	3.22477	5.57E-06
HLA-B	TC6_qbl_hap6000147.hg.1	immune response	3.22392	3.80E-06
RNF138P1	TC05001364.hg.1	pseudogene affiliated with the lncRNA class	3.18073	0.000482158
SDHB	TC01002286.hg.1	aerobic respiration	3.11663	1.14E-05
HLA-DRB1	TC6_ssto_hap7000163.hg.1	immune response	-3.0582	0.000357093
ANKRD17	TC04001279.hg.1	hepatic differentiation during embryogenesis	3.02463	7.40E-05
SNTA1	TC20000770.hg.1	neuromuscular junction development	-3.0158	3.05E-06
FNDC3B	TC03000926.hg.1	positive regulation of fat cell differentiation	2.93026	9.76E-06
CCDC47	TC17001785.hg.1	post-embryonic development	-2.9258	0.000261727
FBXL17	TC05001660.hg.1	Substrate-recognition component of the SCF-type E3 ubiquitin ligase complex	2.82952	7.03E-06
GTF2H5	TC06001130.hg.1	regulation of transcription	2.76923	4.22E-05
FBLN2	TC03000077.hg.1	organ development (skeletal and neuronal structures)	2.71086	3.24E-06
HLA-B	TC06004060.hg.1	immune response	2.66219	1.51E-05
PGP	TC16000780.hg.1	carbohydrate metabolic process	2.63051	1.84E-05
PKN2	TC01000831.hg.1	cell cycle	2.60906	1.55E-05
RTF1	TC15000308.hg.1	stem cell maintenance	-	0.0212003

			2.56615	
			-	
EPDR1	TC07003323.hg.1	cell-matrix adhesion	2.56259	9.60E-07
			-	
HLA-B	TC6_ssto_hap7000191.hg.1	immune response	2.55282	1.26E-06
			-	
DCAF7	TC17000768.hg.1	multicellular organismal development	2.49426	2.03E-05
			-	
ARFGEF2	TC20000382.hg.1	intracellular signal transduction	2.48405	0.00013559
			-	
VGLL2	TC06000928.hg.1	skeletal muscle tissue development	2.48261	0.0264613
			-	
HDLBP	TC02002948.hg.1	cholesterol metabolic process	2.48146	0.00903578
			-	
HLA-B	TC6_mcf_hap5000195.hg.1	immune response	2.45825	6.90E-06
			-	
HLA-B	TC6_cox_hap2000237.hg.1	immune response	2.45539	2.25E-05
			-	
MAPKAPK3	TC03000316.hg.1	toll-like receptor signaling pathway	2.44325	1.57E-06
			-	
AGTPBP1	TC09001287.hg.1	mitochondrion organization	2.42461	2.34E-05
STAU1	TC20000923.hg.1	intracellular mRNA localization	-2.4055	0.000368089
			-	
STAG1	TC03001812.hg.1	mitotic cell cycle	2.40546	8.05E-05
			-	
HLA-B	TC6_mann_hap4000188.hg.1	immune response	2.39196	1.70E-06
			-	
PEA15	TC01001364.hg.1	regulation of apoptotic process	2.38739	4.83E-06
SENP6	TC06000745.hg.1	proteolysis	-2.3859	8.46E-06
			-	
KDM2A	TC11000680.hg.1	regulation of transcription	2.38489	1.45E-05
			-	
OPTN	TC10000097.hg.1	cell death	2.36495	0.00694018
NCSTN	TC01001367.hg.1	Notch signaling pathway	-2.346	2.03E-05
			-	
FBXO7	TC22000245.hg.1	cell death	2.32075	0.000475418
			-	
PARVA	TC11000201.hg.1	actin cytoskeleton organization	2.31833	2.33E-05
			-	
SOCS5	TC02000286.hg.1	epidermal growth factor receptor signaling pathway	2.24241	8.64E-06
			-	
NDUFAF1	TC15001249.hg.1	mitochondrial electron transport	2.24198	0.0103522
			-	
FBXW11	TC05002058.hg.1	protein dephosphorylation	2.21788	3.80E-05
MSL2	TC03001811.hg.1	histone H4-K16 acetylation	-2.2065	5.20E-05
			-	
CLIC4	TC01000314.hg.1	cell differentiation	2.19831	0.000145091
			-	
RNPC3	TC01000917.hg.1	mRNA processing	2.19216	0.000183481
			-	
MUSK	TC09000553.hg.1	regulation of synaptic growth at neuromuscular junction	2.10842	0.00878214
HLA-DRB1	TC6_qbl_hap6000182.hg.1	immune response	-2.0701	0.00587168

TMBIM4	TC12003283.hg.1	apoptotic process	2.04788	9.44E-05
FCHO2	TC05000343.hg.1	membrane invagination	2.02729	4.09E-06
CRIP1	TC02000285.hg.1	cytoplasmic microtubule organization	2.01092	7.48E-05
EPRS	TC01003833.hg.1	tRNA aminoacylation	2.00435	0.0132669
SRRM1	TC01000313.hg.1	RNA splicing	2.00267	0.0186343
SUZ12	TC17000369.hg.1	positive regulation of cell proliferation	2.00253	1.73E-05
ATP2B1	TC12001798.hg.1	ATP biosynthetic process	1.98966	0.000191141
ANKRD52	TC12001599.hg.1	recognition of phosphoprotein substrates	1.97005	0.000103946
KIAA0317	TC14001310.hg.1	protein ubiquitination	1.96807	7.04E-05
H1FX	TC03001767.hg.1	nucleosome assembly	1.96445	7.49E-05
PARP6	TC15002777.hg.1	catalyzes the post-translational modification of proteins	1.96211	3.94E-06
HIST1H4E	TC06000172.hg.1	telomere maintenance	1.95455	0.00184868
PROK2	TC03001550.hg.1	anti-apoptosis	1.95758	2.33E-05
GAP43	TC03000595.hg.1	nervous system development	2.0016	4.58E-07
GYPE	TC04001605.hg.1	sialoglycoprotein and a type I membrane protein	2.01385	4.08E-06
FAM169B	TC15001978.hg.1	associated with arthritis	2.01982	2.26E-06
MC4R	TC18000550.hg.1	positive regulation of cAMP biosynthetic process	2.02556	0.000559985
HRH1	TC03000064.hg.1	G-protein coupled receptor signaling pathway	2.0336	4.15E-06
KCNQ1DN	TC11000067.hg.1	imprinted gene	2.06601	7.75E-06
TCAM1P	TC17000773.hg.1	germ cell-Sertoli cell interactions	2.07745	2.90E-06
LOC100271832	TC02000218.hg.1	RNA gene, and is affiliated with the lncRNA class	2.07779	0.000155964
MGC27382	TC01000791.hg.1	RNA gene, and is affiliated with the lncRNA class	2.07977	3.31E-06
OMG	TC17001340.hg.1	nerve growth factor receptor signaling pathway	2.10284	0.000264153
LOC388948	TC02004963.hg.1	protein-coding gene, and is affiliated with the lncRNA class	2.10366	1.56E-05
MIR3143	TC06000226.hg.1	involved in post-transcriptional regulation of gene expression	2.10476	2.81E-05
LOC728724	TC08001635.hg.1	RNA gene, and is affiliated with the lncRNA class	2.1082	7.26E-06
LOC401242	TC6_mcf_hap5000097.hg.1	RNA gene, and is affiliated with the lncRNA class	2.11467	2.98E-05
LOC401242	TC6_ssto_hap7000096.hg.1	RNA gene, and is affiliated with the lncRNA class	2.11467	2.98E-05
DNM1P46	TC15001989.hg.1	pseudogene, and is affiliated with the lncRNA class	2.12774	6.48E-06
TMEM202	TC15000658.hg.1	transmembrane protein 202) is a protein-coding gene	2.13494	9.69E-06
FOLH1	TC11001758.hg.1	proteolysis	2.14351	2.31E-07
MIR193A	TC17000365.hg.1	post-transcriptional regulation of gene expression	2.15767	2.24E-05
KCNE2	TC21000136.hg.1	muscle contraction	2.16056	2.62E-05
GPC5	TC13000323.hg.1	Metabolism of carbohydrates and Heparan sulfate/heparin	2.1739	2.50E-06

ZSCAN12P1	TC06000274.hg.1	pseudogene, and is affiliated with the lncRNA class.	2.19571	2.16E-05
LOC340113	TC05000145.hg.1	RNA gene, and is affiliated with the lncRNA class	2.20861	1.17E-06
MIR1244-1	TC05000561.hg.1	post-transcriptional regulation of gene expression	2.22096	7.61E-06
MIR1244-1	TC12001190.hg.1	post-transcriptional regulation of gene expression	2.22096	7.61E-06
LIN28B	TC06000840.hg.1	suppressor of microRNA (miRNA) biogenesis	2.22229	1.19E-06
OPN5	TC06000637.hg.1	regulation of transcription	2.23719	2.74E-06
MIR216A	TC02001871.hg.1	post-transcriptional regulation of gene expression	2.25513	7.71E-05
ENPP2	TC08001557.hg.1	regulation of cell migration	2.2828	0.000291477
GYPB	TC04002926.hg.1	sialoglycoprotein of the human erythrocyte membrane	2.28978	1.33E-06
LOC440040	TC11000421.hg.1	RNA gene, and is affiliated with the lncRNA class	2.29481	1.34E-05
ZNF847P	TC01003906.hg.1	pseudogene	2.31397	8.61E-07
OR4C45	TC11001754.hg.1	G-protein coupled receptor activity and olfactory receptor activity	2.32837	1.82E-07
HIST1H4I	TC06000224.hg.1	transcription regulation, DNA repair, DNA replication and chromosomal stability	2.45061	8.73E-06
MIR3679	TC02000875.hg.1	post-transcriptional regulation of gene expression	2.83662	4.31E-05
FAM19A1	TC03000412.hg.1	brain-specific chemokines/neurokines acting as regulators of immune and nervous cells	2.84835	2.12E-06
OR4X1	TC11000415.hg.1	G-protein coupled receptor activity and olfactory receptor activity	2.85649	1.27E-05
OR5V1	TC06004147.hg.1	G-protein coupled receptor activity and olfactory receptor activity	3.26566	5.02E-05
OR2B6	TC06000271.hg.1	G-protein coupled receptor activity and olfactory receptor activity	3.33265	4.61E-05
MIR3122	TC01001768.hg.1	post-transcriptional regulation of gene expression	3.34252	5.17E-07
OR4S1	TC11000416.hg.1	G-protein coupled receptor activity and olfactory receptor activity	3.61331	3.64E-05
OR4C46	TC11000427.hg.1	G-protein coupled receptor activity and olfactory receptor activity	3.68545	1.50E-07
OR4C13	TC11000422.hg.1	G-protein coupled receptor activity and olfactory receptor activity	3.71248	0.000149152
OR5V1	TC6_ssto_hap7000214.hg.1	G-protein coupled receptor activity and olfactory receptor activity	3.83127	8.78E-06
OR5V1	TC6_cox_hap2000247.hg.1	G-protein coupled receptor activity and olfactory receptor activity	3.84348	2.18E-06
OR5V1	TC6_mann_hap4000211.hg.1	G-protein coupled receptor activity and olfactory receptor activity	3.84348	2.18E-06
OR2K2	TC09001481.hg.1	G-protein coupled receptor activity and olfactory receptor activity	4.04371	8.65E-07
OR4C12	TC11001760.hg.1	G-protein coupled receptor activity and olfactory receptor activity	4.37757	1.17E-06
OR4B1	TC11000413.hg.1	G-protein coupled receptor activity and olfactory receptor activity	4.37921	5.54E-07
OR12D3	TC06001483.hg.1	G-protein coupled receptor activity and olfactory receptor activity	4.84073	8.82E-07
OR12D3	TC6_ssto_hap7000102.hg.1	G-protein coupled receptor activity and olfactory receptor activity	4.89941	2.28E-06
OR4X2	TC11000414.hg.1	G-protein coupled receptor activity and olfactory receptor activity	4.90115	1.07E-05

OR12D3	TC6_cox_hap2000120.hg.1	G-protein coupled receptor activity and olfactory receptor activity	5.14165	2.16E-06
OR12D3	TC6_mcf_hap5000102.hg.1	G-protein coupled receptor activity and olfactory receptor activity	5.24391	2.28E-06
OR12D3	TC6_mann_hap4000098.hg.1	G-protein coupled receptor activity and olfactory receptor activity	5.36786	1.23E-06
OR12D3	TC6_dbb_hap3000107.hg.1	G-protein coupled receptor activity and olfactory receptor activity	5.37862	1.32E-06
DNAJA1P5	TC01000913.hg.1	pseudogene, and is affiliated with the lncRNA class	6.27799	5.87E-07
OR14J1	TC06000314.hg.1	G-protein coupled receptor activity and olfactory receptor activity	7.57988	2.73E-07
OR4A5	TC11001763.hg.1	G-protein coupled receptor activity and olfactory receptor activity	9.18742	1.52E-06
OR4A47	TC11000418.hg.1	G-protein coupled receptor activity and olfactory receptor activity	17.3128	1.00E-06

Abbreviations: Asym, asymmetric (Subject 4 and 10); Sym, symmetric (8 subjects with skeletal malocclusion without asymmetry);

Fold Diff, fold difference between average expression in Asym vs Sym.

Significant for step-up P-value with fold differences $\geq \pm 2$ between groups.