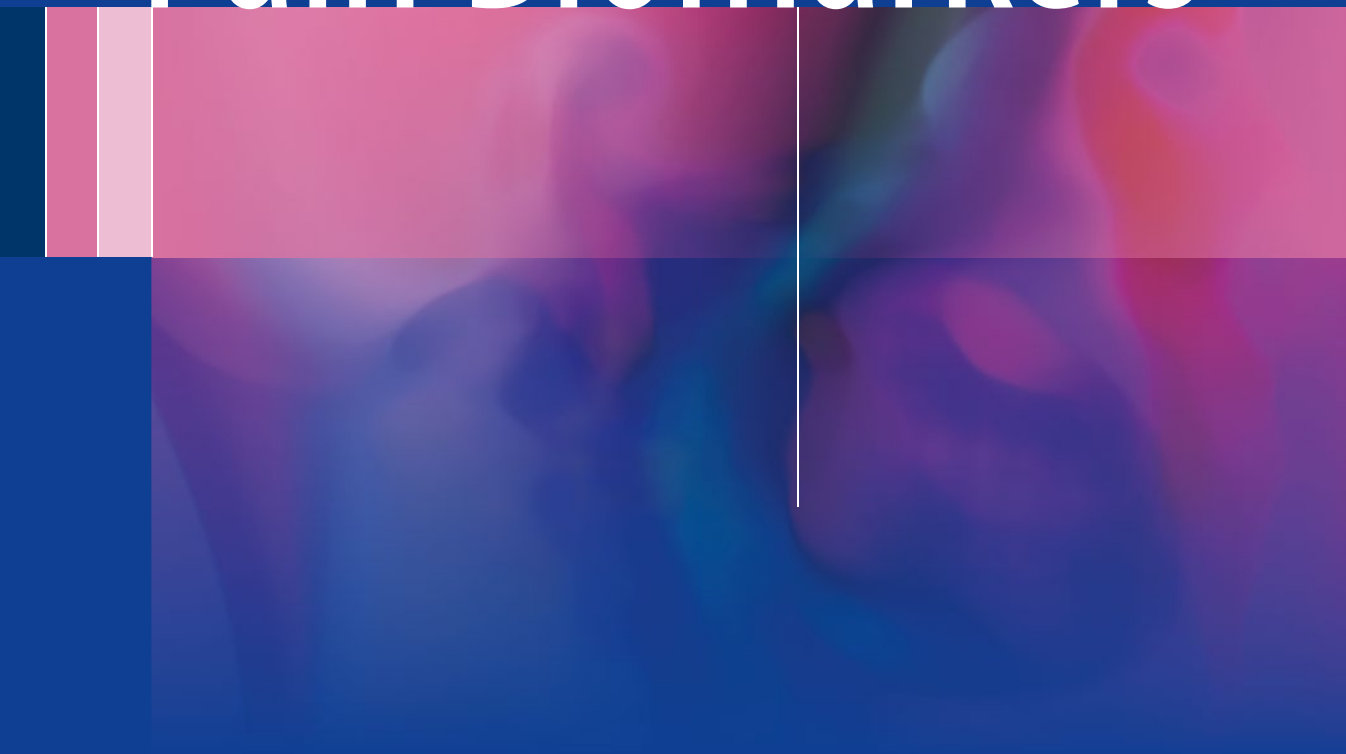
Jean-Paul Goulet
Ana Miriam Velly
Editors

Orofacial Pain Biomarkers



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Preface

The thread that led to the edition of this volume was a Neuroscience Group Symposium held in 2012 during the 90th General Session and Exhibition of the International Association for Dental Research in Iguaçu Falls, Brazil, entitled "Orofacial pain biomarkers: Implication on pain prevention, management and research." Things have continued to evolve, and within a limited space, our intent with this book is to cover some of the many different aspects of pain biomarkers in the context of orofacial pain hoping it can contribute to improve our understanding and management strategies through better and more targeted research.

It is not an overstatement to say that, over an entire life span, everyone will experience orofacial pain at least on few if not several occasions. We may not recall but for most of us the first occurrence is at an early age with the eruption of our deciduous teeth. At least we must all remember the pain associated with the loss of one of those teeth, and while growing up, we learned the important role that pain plays in the presence of a threat or tissue damage that impairs our well-being. Clearly, being able to give a meaning to an orofacial pain serves to dissipate the anguish that accompanies such a personal and unpleasant sensory experience. No wonder that early iteration of the classic paradigm of pain disappearing with things returning to what they were after a normal healing timeline becomes expected. However, a much more complex pain experience will emerge in a scenario where the sufferer's cognitive construct offers no reasonable explanation for an orofacial pain condition that is refractory or has a recurrent timeline. Unfortunately, this is not uncommon considering that chronic orofacial pain conditions that are mostly inexplicable and mainly characterized by nonspecific physical findings have an estimated prevalence in the range of 10% in the general population. Hence, this brings into play all the emotional and psychosocial factors that contribute to the multidimensional aspect of the patient's condition.

As a whole, our understanding of chronic orofacial pain conditions is still very limited though the progress made during recent decades has contributed to better diagnosis, classification, and identification of risk and prognostic factors. However, most patients with chronic orofacial pain are facing uncertainty due to the lack of clear organic causes that could explain the symptoms, and though we may be better at labeling a patient's condition so it can legitimate the symptoms, still psychological disturbances and a lower quality of life are commonalities among those afflicted. Adding to this burden, a return to normal functioning and previous levels of health is unlikely for many.

Despite considerable advancements, we still need to improve treatment strategies for common chronic orofacial pain conditions so patients can have more predictable therapeutic benefits. Early diagnosis and better understanding of the underlying pain mechanisms become imperative. A problem in orofacial pain studies aimed to diagnose, classify, and identify the risk factors, as well as establish better treatments, is that pain is fundamentally a multifaceted subjective phenomenon. This is well depicted in the definition given by the International Association for the Study of Pain (IASP) (1986), "pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage."

Therefore, identification of biomarkers as measures for ensuring the presence of chronic orofacial pain conditions could contribute to a more objective, valid, and reliable multiaxial diagnosis. Biomarkers could also help to the identification of putative risk factors, elucidate mechanisms associated with chronic orofacial pain, and aid in the identification of the most appropriate pain management approaches.

The subject of this book is about recent advances in orofacial pain studies and biomarkers. The content is divided into four thematically distinct parts that include 12 chapters. In the first part, "Clinical and Epidemiological Aspects of Orofacial Pain," Goulet and Woda (Chap. 1) explain what makes pain in the orofacial region so unique, address classification issues and clinical phenotypes, and describe the features of the most common chronic orofacial pain conditions. In their chapter, Velly and Fricton (Chap. 2) review the prevalence of painful and non-painful comorbidities among individuals with orofacial pain and discuss the implication of comorbidities in the identification of biomarkers for chronic orofacial pain, which is largely unknown.

In the second part, "Mechanisms of Chronic Orofacial Pain," Barry J. Sessle (Chap. 3) reviews relevant orofacial pain mechanisms and trigeminal nociceptive pathways before focusing on peripheral and central processes involved in chronic orofacial pain. Bourgeais and colleagues (Chap. 4) outline the anatomo-functional relationship between cortical regions; address central regulation mechanisms, hypothalamic excitability disturbances, and dysfunctions of medullary trigeminovascular regions; and analyze the impact of such dysfunctions as putative biomarkers of central sensitization phenomena on the origin of sustained trigeminal pain.

Part III, "Biomarkers in Orofacial Pain," comprises five chapters. Satu Jääskeläinen (Chap. 5) addresses the neurophysiologic markers of neuropathic orofacial pain and demonstrates how neurophysiologic and psychophysical examination provides sensitive and specific information about trigeminal neuropathy in patients presenting orofacial pain symptoms. After a brief description of the sampling methods, Malin Ernberg (Chap. 6) presents the main categories of muscle biomarkers and describes potential biomarkers for masticatory muscle pain. Per Alstergren (Chap. 7) addresses immunological biomarkers for inflammatory types of temporomandibular joint pain focusing on candidate for early diagnosis, prognosis, and monitoring of disease activity. Seltzer and Diehl (Chap. 8) review genes that are candidate biomarkers for the major persistent orofacial pain disorders and potential key elements for the development of "precision medicine". After an

overview of the different biofluids, Katsiougiannis and colleagues (Chap. 9) focus on saliva, serum, and synovial fluid as reservoirs of biochemical information and discuss their respective utilization and value in the identification of disease states associated with chronic orofacial pain.

In Part IV, "Study Designs and Statistical Analysis for the Identification of Biomarkers, and Future Direction," Velly and colleagues (Chap. 10) provide a generic-case definition and classification of biomarkers, and propose guidelines for the assessement of biomarkers before adressing factors that may influence the discovery and validation of pain biomarkers. In chapter 11 Russell Steele addresses the complexity of data analysis in pain biomarkers research and the impact of relying on surrogate measures relevant to the pain experience. Finally in Chapter 12, the editors present their closing remarks and future direction.

It is our hope that this book will benefit not only to researchers in the field of orofacial pain and biomarkers but also clinicians, educators, and students who are part of the whole community that are instrumental in the development of new knowledge. This work has been possible only because all the authors were willing to invest time and energy on top of their usual duties and we want to express our sincere gratitude to each of them. Finally, we want to thank the publisher and more specifically Elektra McDermott and her staff who supported us all along this endeavor.

Québec, Canada

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Part I

Clinical and Epidemiological Aspects of Orofacial Pain

1 Orofacial Pain: Classification and Road Map to Clinical Phenotypes

Jean-Paul Goulet and Alain Woda

Abstract

The orofacial region consists of heterogeneous tissues that make diagnosing and treating pain conditions a challenging task. Vital to these processes are well-structured classification systems that cover the breadth of chronic orofacial pain conditions and provide diagnostic criteria to enhance our ability to properly identify and categorize clinical events in an agreed pattern. A revision of the classification systems for orofacial pain disorders developed respectively by the International Association for the Study of Pain, the International Headache Society, the American Academy of Orofacial Pain, and the American Academy of Craniofacial Pain reveals a number of deficiencies and inconsistencies ranging from terminology to the structure itself and the set of diagnostic criteria. To improve communication and enable effective collaborative work, we are at the crossroads for the development of a new multiaxial classification system using ontological principles to build a realistic and comprehensive representation of orofacial pain disorders. With research focusing on pain biomarkers, optimizing the systematization of data collection may contribute to identifying clinical phenotypes of chronic orofacial pain conditions that have the most impact on patient life.

1.1 Introduction

Pain in the orofacial region is a puzzling health concern, particularly when the pain is persistent and devoid of any explanation in terms of tissue damage and pathobiological cause. What makes it even more challenging for clinicians is the number and diversity of conditions for which orofacial pain is a prominent symptom that makes it difficult to distinguish many of these disorders clinically. Population-based cross-sectional surveys have shown that a 1-month prevalence rate of self-reported orofacial pain ranges from 19% to 26% [1, 2]. In most instances sudden pain in the orofacial region has a clear somatic cause with overt clinical manifestations generally associated to recognizable pathophysiological processes. The diagnosis may not always be

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straightforward; however, established treatment modalities through dental procedures and pharmacological methods normally lead to complete resolution of the pain with no major or prolonged inconveniences.

On the other hand, between 5% and 14% of adults suffer from recurrent or persistent orofacial pain with an onset going back months or even years before consulting [3, 4]. The incidence of a new onset of chronic orofacial pain is estimated at 4.6% over a 2-year time span [3]. Middle-aged adults are more likely to be afflicted, and the proportion of women outnumbers men by a ratio of 2:1. Chronic orofacial pain conditions are not uncommon, and yet effective treatments are often limited by inaccurate diagnosis and poorly understood mechanisms of the pain signaling system when overt pathobiological processes remain elusive. In such a scenario, treatment response is more generic than specific and is greatly influenced by personal and environmental factors if not by any chance.

The study of biomarkers is one of many strategies to reveal what can be singular about a disorder or disease and to uncover mechanisms that can contribute to better patient care. What we currently call biomarkers are characteristics that can be objectively measured and evaluated as indicators of normal biological or pathogenic processes or pharmacological responses to a therapeutic intervention [5] (see Chap. 10). Common chronic orofacial pain conditions with equivocal treatment response and a clear impact on the patient's quality of life are thus likely candidates for biomarker research, bearing in mind the multidimensional aspect of chronic pain which broadens the scope beyond the sole biological continuum to include the clinical pathway and, more specifically, psychological and behavioral domains. Current investigative paths and methods should focus on combinations of biomarkers for a given condition rather than looking for an answer through a single one. Hence, the scope of measures for orofacial pain biomarkers goes from clinic to genetic and includes physiological parameters, psychological or behavioral characteristics, imaging modalities, molecular and protein gradients, and genomics [6].

This chapter first presents what makes pain occurring in the orofacial region so intricate by evoking the various source and categories of persistent pain conditions while addressing issues regarding the concept of chronicity. This is followed by an appraisal of the current classification systems for orofacial pain disorders before focusing finally on the most common clinical phenotypes of persistent orofacial pain which represent target conditions for biomarker research.

1.2 The Orofacial Region as a Unique Body Part

Before going any further, it is important to recall that the anatomical confinement of pain in the orofacial area corresponds to the region below the orbitomeatal line, above the neck and anterior to the ears, including pain within the oral cavity [1]. Of significance is that this topographical definition refers to the location of the pain whatever the source. For example, brain tumors and neck problems represent potential source of orofacial pain that lies outside the anatomical boundaries defined above. Such heterotopic pains are commonly qualified "referred" or "projected" orofacial pain conditions and must at some point be considered in the differential diagnosis. While referred pain to the orofacial region can occur, the opposite also happens when the pain spreads outside the orofacial area and becomes more "diffuse."

What also makes the orofacial region unique are the highly specialized structures that lay in close proximity and receive complex sensory and autonomic supply from the cranial and upper cervical nerves to support a variety of specialized functions (taste, feeding, swallowing, speech, smell, breathing, hearing, vision) that contribute to the general well-being of patients. Having good knowledges of the various face and neck tissues and structures, such as the salivary glands, muscles, nerves, major blood vessels, lymph nodes, bones, teeth, mucosa, and sinuses, helps to address the arduous task of uncovering the source of orofacial pain. Moreover, one must be aware that activation of the parasympathetic nervous

system (which results in swelling, flushing, lacrimation, and rhinorrhea) is a prominent feature of a number of orofacial pain conditions [7]. The fact that these manifestations may be confined to the oral mucosa and not the skin increases the difference with similar conditions affecting other parts of the body. For example, parasympathetically induced redness is less visible in the mouth than on a limb.

From a neurophysiological and psychophysical perspective, studies show both similarities and differences in the dynamic and specific responsiveness of the trigeminal system to tissue injury and inflammation, as compared to the spinal system [8]. The many distinct orofacial tissue constituents that are candidate causes of acute pain states can lead to hyperalgesic priming of the trigeminal pain system through cellular signaling pathways at the primary nociceptor level, with subsequent changes in brain stem activity. Preclinical studies have clearly demonstrated that plastic changes at one or more levels of the pain signaling pathways are likely to contribute to the transition from acute to chronic pain [9–11].

1.3 Type of Orofacial Pain

Orofacial pain is a sensory experience within a specific anatomical region, and as in any other part of the body, it is perceived as a symptom that is mostly tied to the belief that something is wrong. However, pain is not simply what goes on in the body part that is hurting but depends also on brain activity. The International Association for the Study of Pain (IASP) thus avoids linking pain to a noxious stimulus by defining it as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" [12]. What best depicts pain as primarily a "brain activity" is the classic example of a patient who undergoes minor surgery under hypnosis without receiving any local anesthesia. Although peripheral inputs still reach the brain, hypnosis enables the patient to exert some type of control on brain activity so they feel no pain [13]. While inhibition of pain sensation can take place with no blockage of nociceptive peripheral inputs, the opposite is also true. Creating a mind state of tissue damage under hypnosis is enough to induce pain sensations in the absence of nociceptive peripheral inputs [13–15].

When orofacial pain is induced through the activation of peripheral nociceptors, it signals the brain of the presence of stimuli that may result in tissue damage, and as such the pain is self-protective and possesses a biologic role. This scenario depicts a stimulus-dependent pain, namely, "nociceptive pain," which is usually short lasting. Common examples are sharp tooth pain elicited when biting inadvertently on something hard or when drinking cold water. We learn by experience to avoid orofacial pain linked to noxious mechanical and thermal stimuli. Another type of protective pain driven by the activity of the immune system follows tissue injuries or infections, or is induced by autoimmune disorders. This gives rise to "inflammatory pain" which is better qualified as being adaptive [16, 17]. This pain has also a biologic role by promoting healing and a functional role by signaling inappropriate action to prevent further damage or discomfort during the recovery process. Inflammatory pain can be acute, such as dental pain induced by pulpitis or apical infection, or chronic, as seen with temporomandibular joint arthralgia associated with degenerative joint disease or rheumatoid arthritis. As a general rule, "inflammatory pain" has a readily identifiable etiology and pathophysiological processes.

There are instances when pain has no protective role and therefore no real purpose. The pain is then disserviceable and represents a disease of its own. This pain is labeled as being maladaptive and may follow an injury to the nervous system or result from a dysfunction caused by an impaired regulation of one or more components of the neurobiological apparatus [18, 19]. Maladaptive pain in the trigeminal region can be broadly categorized as being either neuropathic, dysfunctional, or neurovascular in nature. *Neuropathic* pain occurs when the pain is the consequence of injuries or damage to the nervous system. This may occur following dentoalveolar nerve encroachment during the placement of a

dental implant or following orthognathic surgery. *Dysfunctional* pain is the result of a malfunction of the pain signaling, modulating, or analyzing system in the absence of nerve injury. It is not a stimulus-dependent pain and is often in no way related to peripheral inflammation [20]. Conditions displaying features of dysfunctional orofacial pain include burning mouth syndrome and persistent idiopathic facial pain (see Chap. 5). *Neurovascular* pain is unique to the head and face region. Unlike the other pain types, it pertains to abnormal brain stem sensory processing following aberrant cortical activity or poorly understood mechanisms that activate the posterior hypothalamus [21]. Neurovascular orofacial pain is likely the consequence of episodic neurologic conditions that share clinical features with migraine and trigeminal autonomic cephalalgia. As opposed to the classical migraine and cluster headache, the epicenter of neurovascular pain in the face regards the distribution of the maxillary or mandibular branch of the trigeminal nerve. Furthermore, the classical autonomic signs and symptoms that usually accompany these conditions are not as prominent [22].

Better understanding and reliable means to identify pain mechanisms are key approaches to improve treatment strategies. Animal and human studies have shown that chronic pain can be induced or maintained by disturbed functions of the nervous system [23]. Upregulation of neuronal proteins through the selective alteration of gene transcription can alter the excitability of second-order brain stem neurons. Chronic pain can also stem from a disturbance of the conditioned pain modulation system, or an alteration of brain processes through the hypothalamic-pituitary-adrenal axis. Still, a degree of uncertainty remains as to whether these pain mechanisms represent a true intrinsic malfunctioning of the pain pathways or are the result of a subclinical biological process that escapes detection with existing test methods. Assessing chronic pain mechanisms with minimal accuracy in the clinical setting is a difficult task, and little is known regarding the reliability and validity of clinical features potentially associated with malfunction at the peripheral and central nervous system levels. Parallel efforts to improve patient management will be successful through a better understanding of the etiology from a biological, psychological, and behavioral perspective that considers the impact of comorbid conditions, as well as the role of personal beliefs and emotional status as moderators or mediators of the pain experience.

1.4 Chronicity and Orofacial Pain: An Evolving Concept

Categorizing pain conditions as acute or chronic is routine in clinical settings. Although this is a widely accepted notion, new insights raise concerns regarding the practice of resorting on temporal cutoffs to distinguish acute from chronic. Not surprisingly, the definition of chronicity varies among clinicians, researchers, patients, and healthcare administrators. In most instances the different viewpoints concern the time elapsed since the onset of the pain and less often the frequency and severity of pain that persists and the number of pain days over a predefined period.

Generally speaking, most endorse the IASP definition that "chronic" denotes the "persistence of pain beyond the normal time of healing" [12]. For clinical practicality, the IASP states that for nonmalignant pain, a duration exceeding 3 months is a reasonable time period, but for research purposes, a duration of 6 months is deemed a more convenient point at which a pain state can be declared as being chronic. The notion of healing generally ties pain to tissue injury and the activation of pain pathways. It is well established, however, that a malfunction of pain signaling systems also occurs in the absence of tissue injury [20]. On the other hand, the International Headache Society (HIS) makes a distinction between pain that is episodic and that which is chronic by adding a notion of frequency. In headache terminology, the qualifier "chronic" applies to primary headache disorders, when attacks occur repeatedly for more than 3 months on more days than not. The only exception pertains to trigeminal autonomic cephalalgias, where "chronic" is used only when the disorder has been unremitting for more than 1 year. It is clear that how we currently define chronicity by a range of calendar-based periods is largely

reminiscent of heuristic views and there is no universally accepted operational definition to satisfy everyone.

Aside from the intrinsic nature of what we traditionally call chronic pain, it has been suggested that the lack of significant improvement to render a pain condition more endurable has prognostic relevancy and may indeed require consideration [24]. A definition of chronic based solely on duration (number of pain days) is far from being optimal, because it is difficult to apply to intermittent, recurring pains and provides no clues as to the clinical significance of long-lasting pain. A reappraisal of what chronic pain is should better capture the multidimensional experience that goes beyond the severity and duration of the pain and also includes the behavioral and psychological aspects that influence the course and the patient's well-being.

New knowledge on the plasticity of the nervous system has shifted our focus toward the transition between acute and chronic pain and the possibility that certain pain conditions may be chronic from the onset. For example, it has been shown that acute inflammatory insults and environmental stressors produce a long-lasting hypersensitivity of peripheral nociceptors to pro-inflammatory cytokines and that this hypersensitivity is later responsible for a dramatically enhanced hyperalgesic response to subclinical traumatic events [11]. The clinical course of burning mouth syndrome that is described later may represent one among other similar conditions that does not show a transition from acute to chronic. Broadening the case definition of chronic pain beyond pain duration and frequency may thus provide new avenues for clinical phenotyping of orofacial pain conditions. It can be noted, however, that the occurrence of a pain condition that is dysfunctional from the onset has little chance of being immediately diagnosed because no somatic cause is uncovered by the patient or the doctor.

1.5 Terminology and Orofacial Pain Entities

We learn about the breadth of conditions responsible for pain in the trigeminal region through taxonomy and classification systems that are developed by mandated committees and groups of experts. Although everyone aims to be as close to the truth as it can be, the reality that is depicted in a classification system depends on the purposes and mission of a group or organization. When comparing classification systems, one can see that similar terms are not utilized following the same reasoning, similar entities are given different names or case definitions, and not uncommonly distinctive entities may share the same name. Various views are also expressed by the way classes that regroup the different entities belonging to a classification system are labeled and structured.

A common pitfall seen in clinical and research setting is the misuse of terms such as "temporomandibular disorder" (TMD) and "neuropathic pain" which gives the false impression of dealing with a specific diagnosis. In fact these terms designate a number of clinical entities that must be distinguished from one another for appropriate prognosis appraisal and treatment decisions. Such misuse is counterproductive as it perpetuates ambiguity in the message one wants to convey regarding the true diagnosis. Not all the organizations committed to the relief of temporomandibular pain and dysfunction agree on a common list of clinical entities, and once again it emphasizes that how one sees the reality is greatly influenced by its own background and beliefs.

As illustrated in Table 1.1, the list of masticatory muscle-related pain entities from different sources has undergone a significant number of changes over the years [25, 27–35]. Only myofascial pain and muscle spasm remain common to current classification systems. This supports the different conceptual views experts have regarding the etiology, pathogenesis, and mechanisms of masticatory muscle pain disorders. With a greater concerted effort, however, we may get closer to a more consensual taxonomy. For example, "protective co-contraction" and "muscle splinting" are probably different names given to the same phenomenon observed in a number of pain-related muscle conditions [36]. While this phenomenon may indeed exist physiologically, its recognition as being a distinct clinical entity is questionable.

Table 1.1 Past and current terminology for masticatory muscle disorders

W. E. Bell				
(*1982*)		(*1986*)		
Protective co-contraction		Protective muscle splinting		
Myospasm		Muscle spasm		
Myositis		Muscle inflammation		
American Academy of Orofacial Pain				
(*1990*) Myofascial pain Myositis Sprain Reflex splinting	(*1993*) Myofascial pain Myositis Myospasm Protective muscle spasm	(*1996*) Local myalgia Myofascial pain Myospasm Myositis	(*2008*) Local myalgia Myofascial pain Centrally mediated myalgia Myospasm Myositis	(*2014*) See DC/TMD
Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD)				
(RDC/TMD 1992) Myofascial pain Myofascial pain with limited opening		*(DC/TMD 2014)* Myalgia Local myalgia Myofascial pain Myofascial pain with referral Tendonitis Spasm Myositis		
American Academy of Craniofacial Pain [25]				
(*Extracapsular TMDs*) Myalgia Muscle splinting Muscle spasm Myositis Trismus Myofascial pain syndrome Temporal tendonitis Ernest syndrome		(*Adapted from* Pertes and Gross [26]) Acute Myositis Reflex muscle splinting Muscle spasm Chronic Myofascial pain Muscle contracture Hypertrophy Myalgia secondary to systemic disease		
Okeson (2014, [27]***)***				
Protective co-contraction		Myofascial pain		
Local muscle soreness		Myospasm		
Centrally mediated myalgia		Systemic chronic myalgia		

Bell [28]; de Leeuw [29]; de Leeuw and Klasser [30]; Dworkin and LeResche [31]; McNeill [32, 33]; Okeson [27, 34]; American Academy of Craniofacial Pain [25]; Pertes and Gross [26]; Schiffman et al. [35]

On the other hand, consensus and standardization go beyond the sole denomination of specific clinical entities and extend to the case definition, key clinical features, and diagnostic criteria. "Myofascial pain" is a clinical entity listed in most classification systems, yet the diagnostic criteria vary substantially across organizations; some clearly refer to the presence of an active muscle trigger point, whereas for others, it remains a non-issue [25, 27, 35]. Comparing research data is therefore compromised, which unfortunately hinders progress in our understanding of the condition. The fact that the American Academy of Orofacial Pain (AAOP) and the International RDC/TMD Consortium Network are now endorsing the recently published expanded taxonomy for TMD and the new validated diagnostic criteria for the most common disorders is a move in the right direction, and hopefully other organizations will follow suit [30, 35, 37].

Another important aspect is the impact that new knowledge and understanding have on current terminology. At the 2011 World Workshop on Oral Medicine, a committee of international experts was

given the task of conducting a systematic review of the pathophysiology of chronic myalgia of the masticatory system [38]. Although the committee recognized that the existing terminology "myofascial pain" was widely accepted, they expressed concern regarding the accuracy of the denomination, which implies that the pain arises from muscle and fascia. The committee suggested a name change for the descriptive term "Persistent Orofacial Muscle Pain" (POMP), terminology reminiscent of ontological principles. Whether POMP is a "particular," therefore singular, entity or designates rather a wider "type" of orofacial muscle pain is not really clear and remains a legitimate question [39]. Obviously, any change in terminology must be exercised with great caution, as it risks adding confusion for researchers and even more so for nonspecialized practitioners. It should at least not be undertaken without revisiting the other entities belonging to the same taxonomic cluster, thereby enabling everyone to understand what it really means and how it relates to the other entities. In short, unless research brings forth decisive new data, knowledge, or treatment, avoiding a change of denomination or taxonomy may represent the best option.

1.6 Current State of Operational Classification Systems for Orofacial Pain Disorders

A carefully reasoned classification system depicts the reality and the many faces of a domain and how it is organized. It shows the array and diversity of diagnoses, assists in treatment decision making, and provides insight regarding prognosis. It is a communication tool as well as an invaluable source of information for research. On the other hand, a classification system is also dynamic and is exposed to changes as progress is made through the accumulation of new data. Development and updating, however, have more to do with coherence and pragmatism than with the absolute truth. The inherent guiding principles include biological plausibility, exhaustiveness, mutual exclusivity of items, reliability, and simplicity of use for anyone within or gravitating around a specialized field [39, 40].

Before the emergence of multivariate analysis and ontology principles, taxonomic entities were empirically classified by groups of experts using preconceived theoretical approaches based on distinguishing features that best fitted the purpose of the classification system. For practicality in the clinical setting, signs and symptoms, body region, and structures are thus frequently used. The reality of orofacial pain disorders is unique but not everyone sees it the same way; conceptual differences are therefore responsible for divergent views of how entities are defined, labeled, and organized. With data that best reflects the reality, cluster analysis is regarded as the most appropriate first step to establish an evidence-based classification system [41]. Clinical entities making up each cluster are likely to show more homogeneity, and a better hierarchical order of the taxonomy is thus expected. Except for the taxonomy proposed by Woda et al. [42] for chronic orofacial pain and by Pimenta e Sylva Machado et al. [43] for TMD patients, all of the classification systems elaborated by professional organizations are empirical and expert-driven [42, 43]. Moreover, entities are defined according to theoretical concepts, and the diagnostic criteria are subsequently validated [31, 35, 44].

The strengths and weaknesses of existing methods for taxonomic research and classification purposes are described in detail elsewhere [41]. Obviously, all have limitations, yet it is possible to use them in a complementary way. Briefly, *cluster* analysis (multivariate analysis) enables us to identify which entities actually exist in a breadth of orofacial pain characterized by a broad continuum containing a combination of signs and symptoms with largely overlapping clinical depictions. Although this analysis should be used first, this requires a large representative sample of patients. Even when this condition is satisfied, entities with low prevalence rates cannot be extracted easily and or not at all if very rare. The method of classifying subjects according to *diagnostic criteria* should follow the cluster analysis, when an entity has already been singled out. Thus far, the major issue in all studies is that groups of subjects with a presumed condition are already preselected prior to their characterization by diagnostic criteria.

When testing the sensitivity and specificity of a set of diagnostic criteria for a given entity, subjects are chosen based on preexisting inclusion criteria; clearly, this approach is suggestive of circular reasoning. As seen with cluster analysis, a number of cases will be left unclassified by diagnostic criteria, particularly clinical entities that have a broad spectrum of clinical presentations. The advantage of using diagnostic criteria is undeniable, as it allows for the standardized inclusion of subjects in clinical studies. Thus, an ideal classification should rely on the association of the two methods described above. Existing entities should be initially identified through cluster analyses, followed by a definition of their diagnostic criteria. That said, however, these two methods would still leave many entities with a low prevalence rate without a label or description. There is therefore an unavoidable need for a more subjective approach in which expert opinion (another name for authority-based consensus) plays the primary role. *Expert opinion* makes it possible to propose a more exhaustive classification, yet it cannot rely on science only. In addition, authority-based consensus is not automatically less subjective when carried out by a committee than when proposed by an individual. Finally, recognizing ontology as the basis for a coherent description of a clinically and scientifically established reality has given rise to new rules and recommendations regarding the classification and denomination of orofacial pain disorders. A detailed description of the ontology concept lies beyond the scope of this chapter and is covered in a recent publication [39].

Owing to their respective missions, two international organizations include orofacial pain disorders in their classification systems, namely, the International Association for the Study of Pain (IASP), with its multiaxial classification and coding system (http://www.iasp-pain.org/files/Content/ContentFolders/Publications2/FreeBooks/Classification-of-Chronic-Pain.pdf), and the International Headache Society (IHS), with its classification of headache disorders (ICHD-3) [12, 45]. The IASP categorizes pain syndromes into generalized or localized conditions. Disorders giving rise to orofacial pain appear under the heading "Relatively localized syndromes of the head and neck" and are classified into five subcategories, each listing orofacial pain conditions specific to an organ system or body structure regardless of pathobiological processes (Table 1.2).

Each pain condition can be coded according to five axes referring respectively to anatomical region, system involved, temporal characteristic, intensity, and etiology. The IHS classification system (ICHD-3) pertains to primary and secondary headaches (Part I and Part II) as well as painful cranial neuropathies and other facial pains not causing secondary headaches (Part III) (Table 1.3).

Despite the progress in recent decades in the clinical diagnosis of chronic orofacial pain, we have seen very few changes to the IASP and IHS classification systems. While more similarities than differences would generally be expected, both taxonomies differ in several regards. First, each classification system includes a number of conditions that are exclusive to them; second, the number of conditions listed under similar subcategories differs; and finally, similar clinical entities have different denominations. Except for osteoarthritis and rheumatoid arthritis, the IASP continues to enclose all temporomandibular pain disorders under the generic diagnosis "Temporomandibular Pain and Dysfunction Syndrome," thereby failing to acknowledge other distinctive entities. For neuropathic pain of the head and neck, the IASP has a list of 16 conditions, whereas 21 are included in the IHS/ICHD-3 and only six denominations are common to both

Table 1.2 Classification structure of orofacial pain syndromes by the International Association for the Study of Pain (IASP) (Merskey and Bugduk [12])

Relatively localized syndromes of the head and neck	Number of entities
1. Neuralgia of the head and neck	16
2. Craniofacial pain of musculoskeletal origin	7
3. Lesions of the ear, nose, and oral cavity	13
4. Primary headache syndromes, vascular disorders, and cerebrospinal fluid syndromes	16
5. Pain of psychological origin in the head, face, and neck	3

Table 1.3 Classification structure of orofacial pain entities by the International Headache Society (ICHD-3) (International Headache Society Classification Committee [45])

CATEGORY 11: Headache or facial pain attributed to disorder of the cranium, neck, eyes, ears, nose sinuses, teeth, mouth, or other facial or cervical structure
11.1–11.9: Headache and facial pain attributed to disorder from the sources above or to other disorder
CATEGORY 13: Painful cranial neuropathies and other facial pains
13.1 Trigeminal neuralgia
Classical trigeminal neuralgia
Classical trigeminal neuralgia, purely paroxysmal
Classical trigeminal neuralgia with concomitant persistent facial pain
Painful trigeminal neuropathy
Painful trigeminal neuropathy attributed to acute Herpes zoster
Post-herpetic trigeminal neuropathy
Painful posttraumatic trigeminal neuropathy (PPTTN)
Painful trigeminal neuropathy attributed to multiple sclerosis (MS) plaque
Painful trigeminal neuropathy attributed to a space-occupying lesion
Painful trigeminal neuropathy attributed to other disorder
13.2 Glossopharyngeal neuralgia
13.3 Nervus intermedius (facial nerve) neuralgia
Classical nervus intermedius neuralgia
Nervus intermedius neuropathy attributed to Herpes zoster
13.4 Occipital neuralgia
13.5 Optic neuritis
13.6 Headache attributed to ischaemic ocular motor nerve palsy
13.7 Tolosa-Hunt syndrome
13.8 Paratrigeminal oculosympathetic (Raeder's) syndrome
13.9 Recurrent painful ophthalmoplegic neuropathy
13.10 Burning mouth syndrome (BMS)
13.11 Persistent idiopathic facial pain (PIFP)
13.12 Central neuropathic pain
Central neuropathic pain attributed to multiple sclerosis (MS)
Central post-stroke pain (CPSP)

classification systems. These discrepancies led the IASP to edit a crosswalk to the classification of the IHS for conditions that have a different name or are classified under a different subcategory. For example, "Glossodynia and sore mouth" listed by the IASP under "Lesions of the ear, nose, and oral cavity" refers to "Burning mouth syndrome" found in the ICHD-3 under "Painful cranial neuropathy and other facial pain." Additional joint efforts between organizations could easily resolve many of these existing differences, for everyone's benefit.

The IASP and IHS taxonomies are falling short of fulfilling the needs of clinicians and researchers in the field of orofacial pain [46]. The diagnostic criteria they propose are mostly derived from empirical data, and although they may indeed have face and content validity, their validation awaits prospective field studies. The usefulness of these classification systems in the diagnosis of chronic orofacial pain has been recently studied. When the ICHD classification system was tested in a neurological tertiary care center, up to 29% of patients with facial pain could not be classified [47]. Used in a dental clinic for orofacial pain, the ICHD classification system yielded a definitive diagnosis for only 56% of patients; not surprisingly, the major limitation regarded the diagnosis of pain-related temporomandibular disorders [48]. Obviously, a referral to the expanded taxonomy for temporomandibular disorders codeveloped by the International RDC/TMD Consortium Network (http://www.rdc-tmdinternational.org) and the American Academy of Orofacial Pain could easily fill this gap [35, 37]. The expanded TMD taxonomy stems from a multisite project that addressed the validity of the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) published by Dworkin and LeResche in 1992 [31, 44].

Briefly, the RDC/TMD is an empirically derived dual-axis classification system for the most common TMDs that is based on the biopsychosocial model of pain. Although primarily intended for research purposes, the RDC/TMD gained acceptance among clinician who started using them in clinical setting. Axis I allows to yield a physical diagnosis for the most common pain and non-pain-related TMDs by applying diagnostic criteria derived from the history and clinical examination. Axis II on the

other hand enables to assess and grade TMD pain-related disability. The multisite Validation Project that assessed the criterion validity of the original Axis I RDC/TMD led to what is now known as the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) that were recently implemented for use in clinical and research setting [35]. The newly validated Axis I diagnostic criteria for the most common temporomandibular disorders are now part of the expanded TMD taxonomy that also includes less common disorders.

The American Academy of Orofacial Pain (AAOP) and the American Academy of Craniofacial Pain (AACP) are professional organizations that provide a scheme for classifying orofacial pain through their official publications [25, 30]. The AAOP assorts orofacial pain conditions to six major categories and 15 subcategories referring primarily to pain source (Table 1.4) [30].

For temporomandibular disorders, the AAOP follows the expanded TMD taxonomy and refers to the ICHD-3 classification system for headache disorders and painful cranial neuropathies, thus avoiding the pitfall of inconsistencies. The AACP regroups craniofacial pain conditions under six headings (Table 1.4). The AACP classification of TMDs is adapted from Pertes and Gross [26]; however, a different list of muscle disorders appears under "Extracapsular TMDs" (Table 1.1) [26]. All of the TMDs included in the expanded TMD taxonomy do not appear in the AACP classification, and a number of conditions bear a different name. Moreover, for common TMDs, none of the diagnostic criteria have been field-tested, and those listed for myalgia represent potential causes rather than key elements specifically linked to the nature of the condition. For headache disorders and neuralgias of the head and neck, the AACP refers to the IHS classification system and the IASP taxonomy, respectively.

Finally, the classification system developed by Okeson integrates both a physical and a psychological axis [27]. The structure for the physical axis uses a dichotomous approach that defines at the outset orofacial pain as being either "somatic" or "neuropathic," with the former being further categorized into "superficial or deep pain" and the latter into "episodic or continuous pain." The

Table 1.4 Classification structure of the American Academy of Orofacial Pain (AAOP) and the American Academy of Craniofacial Pain (AACP) (de Leeuw and Klasser [5]; American Academy of Craniofacial Pain [19])

AAOP	AACP
1. Intraoral pain disorders Odontogenic pain Non-odontogenic pain Oral mucosal pain	**1. Extracapsular temporomandibular disorders** Muscles disorders Ligament/tendon disorders
2. Temporomandibular disorders (expanded taxonomy) Temporomandibular joint disorders Masticatory muscle disorders	**2. Headache pain** Primary headache (ICHD list) Secondary headache (ICHD list)
3. Extracranial causes of orofacial pain and headaches Pain stemming from tissues or organs in the head and neck Pain stemming from systemic disease	**3. Neuralgia of the head and face** (IASP list)
4. Neuropathic pain (ICHD list) Episodic neuropathic pain Continuous neuropathic pain Dysesthesia	4. *Temporomandibular disorders* Adapted from Pertes and Gross [26]
5. Primary headache disorders (ICHD list) Migraine Tension-type headache Cluster headache and other trigeminal autonomic cephalalgias	**5. Additional structures that can cause craniofacial pain** Eye pain Ear pain
6. Vascular and nonvascular intracranial cause of orofacial pain (ICHD list) Headache associated with vascular intracranial disorders Headache associated with nonvascular intracranial disorders	**6. Non-odontogenic intraoral pain disorders** Cutaneous and mucogingival pain BMS Glossodynia

next levels regroup orofacial pain conditions under one of several categories based on tissue or system involved.

1.7 Common Chronic Orofacial Pain Entities and Clinical Phenotypes for Biomarker Research

The starting point for the study of pain biomarkers is selecting an entity from every potential disorder. Empirical data from cross-sectional studies show that only a limited number of entities account for the most common chronic orofacial pain conditions and these can be regrouped under either temporomandibular disorders or neuropathic pain disorders [1, 4, 49–51]. TMD myalgia and TMD arthralgia are conditions that frequently coexist and present overlapping manifestations. On the other hand, atypical odontalgia (persistent dentoalveolar pain disorder), burning mouth syndrome, and persistent idiopathic facial pain pertain to the category of neuropathic pain disorders. What these five conditions have in common are the increased odds in females relative to males, the poor correlation between physical findings and the level of pain reported, no known etiology, a poorly understood pathophysiology, and the significant psychosocial and psychological impact on patient life. Many will lump these conditions under the label "Idiopathic chronic orofacial pain syndromes"; however, enough distinguishing features exist between them when the location, character, temporal pattern, and modifying factors of the pain are compared [22, 42]. A brief description of these conditions is of particular interest to highlight potential clinical phenotypes that may help the planning and selection processes of patients for research purposes.

1.7.1 TMD Myalgia

Temporomandibular myalgia represents the most common diagnosis among all of the chronic pain-related disorders affecting the orofacial region. As defined by the DC/TMD, myalgia is a clinical entity characterized by pain of masticatory muscle origin affected by jaw movement, function, or parafunction, and replication of this pain occurs with provocation testing of the masticatory muscles. When this pain is not better accounted for by another pain diagnosis, the recently validated diagnostic criteria show false-positive and false-negative rates of 1% and 10% respectively [35]. Other denominations found in the literature for "myalgia" as per the DC/TMD include local muscle soreness, chronic myalgia, masticatory myofascial pain, and persistent orofacial muscle pain [22, 27, 38, 42]. Patients with masticatory muscle myalgia complain mostly of a unilateral dull aching pain in the cheek area, body, and angle of the mandible which may extend to the ear and forehead. Headache is a frequent associated complaint with the involvement of the temporalis muscle. Duration, severity, and fluctuation of the pain during the day tend to vary, as some patients report their worse pain upon awakening in the morning, while others claim the pain builds up as the day goes on [22, 38, 42].

The systematic palpation of the masseter and temporalis muscles with at least 1 kg of pressure for 5 s makes it possible to identify three subtypes of myalgia patients [35]. Whether the pain evoked by palpation remains localized under the finger pad, spreads inside the muscle, or is referred outside the confinement of the muscle boundaries, the diagnostic subtypes are respectively local myalgia, myofascial pain, and myofascial pain with referral (Table 1.5). Only myalgia, as an umbrella disorder, and myofascial pain with referral have validated diagnostic criteria with a respective sensitivity of 0.90 and 0.86 and specificity of 0.99 and 0.98. The likelihood of different pain mechanisms and response to treatment are among the reasons to support the study of the three myalgia subtypes. Despite the fact that it remains a highly controversial subject, more attention should be paid to muscle trigger points, as defined for myofascial pain syndrome occurring in other body parts [52, 53]. The "active"

Table 1.5 DC/TMD for myalgia and its subtypes and arthralgia (Schiffman et al. [35])

Diagnostic criteria for Myalgia		
History positive for both: 1. Pain in the jaw, temple, in the ear, or in front of ear; 2. Pain modified with jaw movement, function or parafunction	*Clinical examination positive for both:* 1. Confirmation of pain locations(s) in the temporalis or masseter muscle(s); 2. Report of familiar pain in the temporalis or masseter muscle(s) with at least one of the following provocation tests: (a) Palpation of the temporalis or masseter muscle; (b) Maximum unassisted or assisted opening movements(s)	
The pain is not better accounted for by another pain diagnosis		
Subtypes of myalgia as differentiated by provocation testing with palpation		
Local myalgia 1. History criteria as for "Myalgia"; 2. Clinical examination criteria as for "Myalgia"; AND (a) Report of pain localized to the site of palpation	*Myofascial pain* 1. History criteria as for "Myalgia"; 2. Clinical examination criteria as for "Myalgia"; AND (a) Report of pain spreading beyond the site of palpation but within the boundary of the muscle	*Myofascial pain with referral* 1. History criteria as for "Myalgia"; 2. Clinical examination criteria as for "Myalgia"; AND (a) Report of pain at a site beyond the boundary of the muscle being palpated
The pain is not better accounted for by another pain diagnosis		
Diagnostic criteria for arthralgia		
History positive for both: 1. Pain in the jaw, temple, in the ear, or in front of ear; 2. Pain modified with jaw movement, function or parafunction	*Clinical examination positive for both:* 1. Confirmation of pain locations(s) in the area of the TMJ(s); 2. Report of familiar pain in the TMJ with at least one of the following provocation tests: (a) Palpation of the lateral pole or around the lateral pole; (b) Maximum unassisted or assisted opening, right or left lateral, or protrusive movements(s)	
The pain is not better accounted for by another pain diagnosis		

muscle trigger point phenomena appear to be associated with local changes in the biochemical milieu, which may contribute to complex neurobiological mechanisms in the peripheral and central nervous systems [54–56]. These active muscle trigger points have been documented in the masseter and temporalis muscle of patients with chronic masticatory muscle myalgia and are thought to develop from latent trigger points in response to altered muscle demand because of overload, overuse, or prolonged contraction [57, 58]. Current views regarding other possible etiologies include such extrinsic factors as trauma, anxiety, and adverse environmental conditions [59].

Aside from active trigger points that reproduce the patient's orofacial pain, potential candidates for clinical phenotype of myalgia subtypes are concomitant arthralgia and neck pain, level of physical and psychological disabilities, widespread pain and comorbid conditions, extraterritorial allodynia, and hyperalgesia determined by quantitative sensory testing [60–63].

1.7.2 TMJ Arthralgia

Temporomandibular joint arthralgia corresponds to joint origin pain felt in front of or inside the ear that is affected by jaw movement, function, or parafunction, and replication of this pain occurs with provocation testing of the TMJ. To that end, familiar joint pain should be evoked during full and assisted mandibular opening or by palpation of the lateral pole of the TMJ with at least 0.5 kg of pressure for 5 s while the mandible is in a comfortable position or palpation around the lateral pole with a pressure of 1 kg for 5 s when the mandible is in a forward position. The recently validated DC/TMD for TMJ arthralgia has a sensitivity of 0.89 and a specificity of 0.98 when the pain is not better accounted for by another orofacial pain diagnosis (Table 1.5). Contrary to TMJ arthritis, a diagnosis of arthralgia means no clinical signs of edema, erythema, and/or increased temperature associated with joint inflammation or infection. TMJ arthralgia can exist by itself with no other joint disorder;

however, the presence of a concomitant disk displacement or joint disease may suggest a different etiology, clinical course, and prognosis. Moreover, TMJ arthralgia is commonly seen in conjunction with chronic TMD myalgia [64, 65]. Hence, for research purposes, different clinical phenotypes of TMJ arthralgia may exist, with such possible discriminating factors as (1) pain at rest and/or during jaw function, (2) concomitant diagnosis of another TMD, (3) history of jaw injury or trauma, and (4) degree of jaw disability and psychosocial impact. These distinctive features may prove to be useful in uncovering more specific pain biomarkers.

1.7.3 Atypical Odontalgia (AO) or Persistent Dentoalveolar Pain Disorder (PDAP)

The IASP defines "atypical odontalgia" under the heading "Odontalgia: Toothache 4," as tooth pain not associated with lesions. According to the IHS, AO represents a more localized intraoral subform of "persistent idiopathic facial pain" (PIFP) or a subform of "painful posttraumatic trigeminal neuropathy" (PPTTN), two nosologic entities listed under the heading of "Painful Cranial Neuropathies and Other Facial Pains." Other terms used to define AO have included phantom tooth pain and idiopathic tooth pain. More recently and using an ontologically based taxonomic approach, persistent dentoalveolar pain disorder (PDAP) was suggested for tooth pain of non-odontogenic origin [66]. To differentiate PDAP caused by nondental factors such as facial trauma, dental procedures, trigeminal neuralgia, migraine toothache, or post-herpes zoster infection from PDAP arising in the absence of thereof PDAP is subdivided into primary and secondary with the former referring to unexplained or idiopathic cases (primary PDAP).

Typically, patients with AO have persistent pain involving a single tooth or site where a tooth has been extracted, and for which clinical and radiographic investigations reveal no hard and soft tissue pathologies [67, 68]. At the outset, the presumed offending tooth often has a filling, and despite negative clinical and radiographic findings for apical periodontitis, a series of treatments usually follow because the pain does not go away. Hence, in many instances, the painful tooth shows a technically successful root canal treatment subsequent to several other equally successful ones. Moreover, before a diagnosis of AO is finally made, some patients may elect to undergo apical surgery or an extraction because they are convinced that it is a tooth-related pain due to an occult odontogenic inflammation [69, 70].

AO may thus have one of three clinical presentations and that is pain to (1) a tooth with a vital pulp whether it has a filling or not, (2) a devitalized tooth with a technically successful root canal treatment, or (3) an edentulous site where the presumed offending tooth was located. All too frequently, AO has been a wastebasket diagnosis for any unexplainable toothache by a local factor, with no consideration given to various forms of heterotopic tooth pain. Conditions besides the one already mentioned above that must be excluded by appropriate investigations include referred cardiac pain, cluster headache, and hemicrania continua [71, 72]. Therefore, an effective diagnosis of AO or more appropriately "primary PDAP" signifies that all local and remote causes have been ruled out and that the tooth pain cannot be explained by another diagnosis. While there are no universally accepted and validated diagnostic criteria for this subtype of non-odontogenic toothache, the one proposed by Nixdorf et al. [66] for PDAP subtypes represents the best available alternative (Table 1.6). Distinctive phenotypes of primary PDAP (AO) may however exist based on response to local anesthetic, somatosensory profile determined by intraoral quantitative sensory testing, psychosocial disability, and the presence of psychiatric comorbidity [74, 75].

1.7.4 Burning Mouth Syndrome

According to the International Headache Society and the International Association for the Study of Pain, burning mouth syndrome (BMS) is a dis-

Table 1.6 Diagnostic criteria for persistent dentoalveolar pain (PDAP), burning mouth syndrome (BMS), and persistent idiopathic face pain (PIFP)

Diagnostic criteria for persistent dentoalveolar pain (Atypical odontalgia) (Nixdorf et al. [66])	
A. Persistent pain (including dysesthesia) present at least 8 h per day, 15 days or more per month for 3 or more months B. Localized in the dentoalveolar region(s), and C. Not caused by another disease or disorder	
Primary Not in close temporal relationship with a causal event	*Secondary* In close temporal relationship with a causal event
Diagnostic criteria for Burning Mouth Syndrome International Headache Society Classification Committee [73]	
A. Oral pain fulfilling criteria B and C B. Recurring daily for >2 h per day for >3 months C. Pain has both of the following characteristics: 1. Burning quality 2. Felt superficially in the oral mucosa D. Oral mucosa is of normal appearance and clinical examination including sensory testing is normal E. Not better accounted for by another ICHD-3 diagnosis	
Diagnostic criteria for Persistent Idiopathic Face Pain International Headache Society Classification Committee [73]	
A. Facial and/or oral pain fulfilling criteria B and C B. Recurring daily for >2 h/day for >3 months C. Pain has both of the following characteristics: 1. Poorly localized, and not following the distribution of a peripheral nerve 2. Dull, aching or nagging quality D. Clinical neurological examination is normal E. A dental cause has been excluded by appropriate investigation F. Not better accounted for by another ICHD-3 diagnosis	

tinctive nosologic entity characterized by a daily recurring intraoral burning or dysesthetic sensation. Occurring on an intact intraoral mucosa, BMS is unexplained by local factors, systemic disorders, laboratory abnormalities, or psychiatric disorders [76]. BMS is therefore a diagnosis made after excluding all potential causes, and not all agree that this condition really fits the definition of a true syndrome. This syndrome appears under the heading of "Painful cranial neuropathies" in the ICHD-3 and is listed in the IASP classification system under "Group IV: Lesions of the ear, nose, and oral cavity" as "Glossodynia and sore mouth." The various denominations given to this condition over the years include stomatodynia, stomatopyrosis, glossopyrosis, and primary BMS. Only the ICHD-3 provides a set of operationalized diagnostic criteria, although their reliability and validity have yet to be tested in field studies (Table 1.6).

The burning pain in BMS is usually bilateral and begins on the anterior third of the dorsal surface of the tongue. It may also involve other intraoral sites, most likely the labial mucosa and the anterior palate. BMS must be distinguished from the symptoms of burning mouth caused by local and systemic disorders often referred to as "secondary burning mouth syndrome" [76, 77]. In such cases, mucosal changes are usually detected, with such possible causes as denture-related mechanical irritation, candida infection, allergic mucosal reactions, autoimmune mucosal or salivary gland disease, posttraumatic neuropathy, drug-induced hyposalivation, anemia, vitamin B12 or folic acid deficiency, diabetes, gastroesophageal reflux disorder, and Parkinson disease [76, 78].

Of particular interest, BMS predominantly affects perimenopausal women, with a spontaneous onset unrelated to any precipitating events, and it usually starts from few months before to several years after the beginning of the menopause [76, 79]. In addition, a majority of BMS patients complain of dry mouth and altered taste sensation despite normal salivation and somatosensory tests [80, 81]. Different temporal patterns of burning pain have been described [82]. With the exception of stress, patients with BMS usually report no aggravating factors. On the other hand, many will report that the burning sensation disappears when they eat, chew gum, or suck candies (personal unpublished data). Moreover, disrupted sleep is rarely a problem, and when questioned, BMS patients cannot tell if the burning is present when they wake up during the night. BMS patients will frequently exhibit considerable distress because of their fear of oral cancer. Finally, there is no significant evidence that anxiety, depression, and somatization problems cause BMS; however, different psychosocial profiles may exist and thus may influence the clinical course and treatment response.

1.7.5 Persistent Idiopathic Facial Pain

Formally called "atypical facial pain," the IHS defines persistent idiopathic facial pain (PIFP) as a distinct entity under the heading "Painful Cranial Neuropathies and Other Facial Pains," which is not to be confused with the same denomination used by many to qualify the entire group of unexplained chronic orofacial pain conditions. The IASP has deleted atypical facial pain from its current taxonomy, affirming that it was too often used to designate a variety of conditions and thus could be better diagnosed under either temporomandibular pain syndrome, atypical odontalgia, or pain of psychological origin. The IASP decision for not coming forth with another denomination and case definition for "atypical facial pain" is questionable considering the occurrence of chronic orofacial pain in the general population that does not fit the description of any existing clinical entity [68, 83, 84].

Patients with PIFP usually present a dull, poorly localized facial and/or oral pain that can have sharp exacerbations and be aggravated by stress. The pain may be superficial but is most often deep, is uni- or bilateral, and does not follow the distribution of a peripheral nerve, as does trigeminal neuralgia or painful posttraumatic trigeminal neuropathy. No significant events are associated with the outset, but in many instances, patients may report a history of minor injury or trauma to the face, despite no observed deficit during a clinical neurological examination. Most importantly, the diagnostic criteria listed in the ICHD-3 indicates that the pain must have been present daily for more than 2 h over at least 3 months, that all dental causes have been excluded, and that the pain cannot be accounted for by another ICHD-3 diagnosis (Table 1.6). Also not unusual, patients may report pain following palpation of the masticatory muscles or the temporomandibular joint, but when asked about the evoked pain, they will say that it does not reproduce their pain.

Beyond being primarily a diagnosis of exclusion based on the ICHD-3 criteria, it is likely that the central sensitization of the nervous system and the psychosocial impact of PIFP lead to a specific clinical phenotype. Thus certain sensory abnormality profiles may indeed be uncovered by quantitative sensory testing [85]. It is also possible that PIFP patients with different levels of psychosocial disability and the presence of psychiatric comorbidity represent distinctive phenotypes [84].

Conclusion

The clinical reality of orofacial pain is complex due to the broad continuum of sign and symptom combinations arising from heterogeneous tissues with different anatomical, electrophysiological, and pharmacological properties. Improving the patient's care flow and clinical pathway goes through the recognition of the disorder identity, an understanding of the pain mechanisms, and the delivery of proper treatment. Classification systems can help by giving insight into the breadth of orofacial pain conditions that exist but even more when providing validated diagnostic criteria for better standardization of the decision process. More advancement will come through the development of a multidimensional framework enabling to classify patient with persistent pain by integrating within the biological continuum subsets of clinical phenotypes that take into account the psychosocial and psychological aspects of the pain experience. A better systematization and representation of data linked to clinical phenotypes can help reduce the blurring effect of less than optimal data collection that results in poor signal-to-noise ratios when conducting research aiming to identify orofacial pain biomarkers.

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2 Comorbidities in Individuals with Orofacial Pain and Their Impact on Biomarkers

Orofacial Pain Comorbidities and Their Impact on Biomarkers

Ana Miriam Velly and James Fricton

Abstract

This chapter covers the epidemiology of orofacial pain, the comorbidities implicated in chronic orofacial pain, as well as their relationship with specific biomarkers. More specifically, it reviews the prevalence of orofacial pain and of painful and non-painful comorbidities among individuals with orofacial pain. It also examines the implication of comorbidities in the onset and persistence of chronic orofacial pain. This chapter further discusses the role of comorbidities in the identification of biomarkers for chronic orofacial pain, which is largely unknown, and the clinical and research impacts of these findings.

2.1 Introduction

Orofacial pain is a common condition and one for which the diagnosis and management are not simple tasks. It has been noted that 30% of individuals with orofacial pain [temporomandibular disorders (TMD)] still had pain 5 years later [1]. Moreover, the presence of other pain conditions increases the risk of chronic orofacial pain [2–6] and its persistence [1, 7–10], as well as complicates the diagnosis and treatment effectiveness [11].

This chapter first reviews the epidemiology of orofacial pain and comorbidities among individuals with orofacial pain. It also reviews the implication of comorbidities on the onset and persistence of orofacial pain. This is followed by an evaluation of the implication of comorbidities in the identification of biomarkers for orofacial pain conditions. Comorbidity is defined as the "concurrent existence and occurrence of two or more medically diagnosed diseases in the same individual" [12].

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2.2 Epidemiology of Orofacial Pain

As described in Chap. 1, the 1-month prevalence of orofacial pain is estimated to range from 19 to 26% among adults [6, 13, 14]. Among orofacial pain groups, toothache (12–14%) [15, 16] and TMD-related pain (5–12%) [15, 17–21] are more common than oral sores (8%) [15, 22], burning mouth (1%) [15, 22], trigeminal neuralgia (0.3%) [23], and persistent idiopathic facial pain (0.03%) [23]. The incidence of orofacial pain is 4.6% [3]. The incidence of TMD-related pain ranges from approximately 3.8–6.5% [20, 24]. Women (4.5%) are at greater risk of developing TMD-related pain than men (1.3%) [25–27].

Fifty to sixty-six percent of people with TMD will seek treatment. However, it is shocking to note that, regardless of treatment received, 30% of individuals with TMD still reported to have pain 5 years later [1] and 16% are worse [28]. One factor that contributes to the persistence and lack of improvement is the presence of comorbidities [1, 7, 8, 11].

2.3 Comorbidities Related to Orofacial Pain

Comorbidities are common among individuals with chronic orofacial pain [1, 7, 10, 29–47], with higher prevalence among women [34, 48] and among those with low socioeconomic status [33].

A cross-sectional study showed that females with painful TMD were 40% (odds ratio [OR] = 1.4, 95% confidence intervals [CI] 1.3–1.6) more likely to present two to three comorbid pain conditions than males [34]. African-Americans (OR = 1.4, 95% CI: 1.3–1.8) and Hispanics (odds ratio [OR] = 1.6, 95% CI: 1.2–1.6) were also more likely than non-Hispanic whites to report comorbid pain conditions [33]. The following section describes a number of painful and psychological comorbidities that not only co-occur with orofacial pain but that also increase its onset or persistence risks (See Figs. 2.1, 2.2).

2.3.1 Headaches

Headaches are common in the general population [22, 49] and among individuals with orofacial pain [13, 50–52]. More specifically, the frequency of headaches in TMD subjects ranges from 9 to 97% in adults [34, 40–42, 47, 53–55] and between 30 and 94% for adolescents [2, 56, 57]. Studies demonstrated that individuals with painful TMD are more likely (OR = 1.5–8.8) to have headaches than individuals without TMD (Fig. 2.1) [31, 39, 41, 47, 55, 57]. Females with TMD have a greater likelihood of having headaches than males [34].

Epidemiological studies clearly demonstrated that headaches not only co-occur among patients with orofacial pain but also increased its risk. Studies also showed that the chance of developing TMD-related pain was higher among adolescents (OR = 2.7; 95% CI: 1.6–4.4) [2] and adults (OR = 2.1, 95% CI: 1.2–3.7) [6] with headaches. This risk appears to be higher among those with headaches in the previous year (OR = 8.8; 95% CI: 3.8–20.1) [55] or with a headache once a week or more (OR = 3.7; 95% CI: 1.6–8.4) [6]. Headaches were also implicated in the aggravation of TMD signs [54] and in the emotional functioning of subjects [58].

2.3.2 Widespread Pain and Fibromyalgia Syndrome

Widespread pain also frequently co-occurs with chronic orofacial pain (39%) [59], especially TMD-related pain (16–54%) [7, 9, 10]. The prevalence of fibromyalgia syndrome (FM) among TMD individuals ranges between 7 and 18% [7, 32, 53, 60], and most individuals with FM exhibit painful TMD [61], showing again that fibromyalgia frequently co-occurs with TMD.

Cohort studies demonstrated that widespread pain also increased the risk of orofacial pain among adults (risk ratio [RR] = 4.0, 95% CI: 2.2–7.4) [3] and adolescents (OR = 3.2, 95% CI: 1.7–6.1) [2]. Widespread pain also increases the risk of persistent orofacial pain (RR = 2.0, 95%

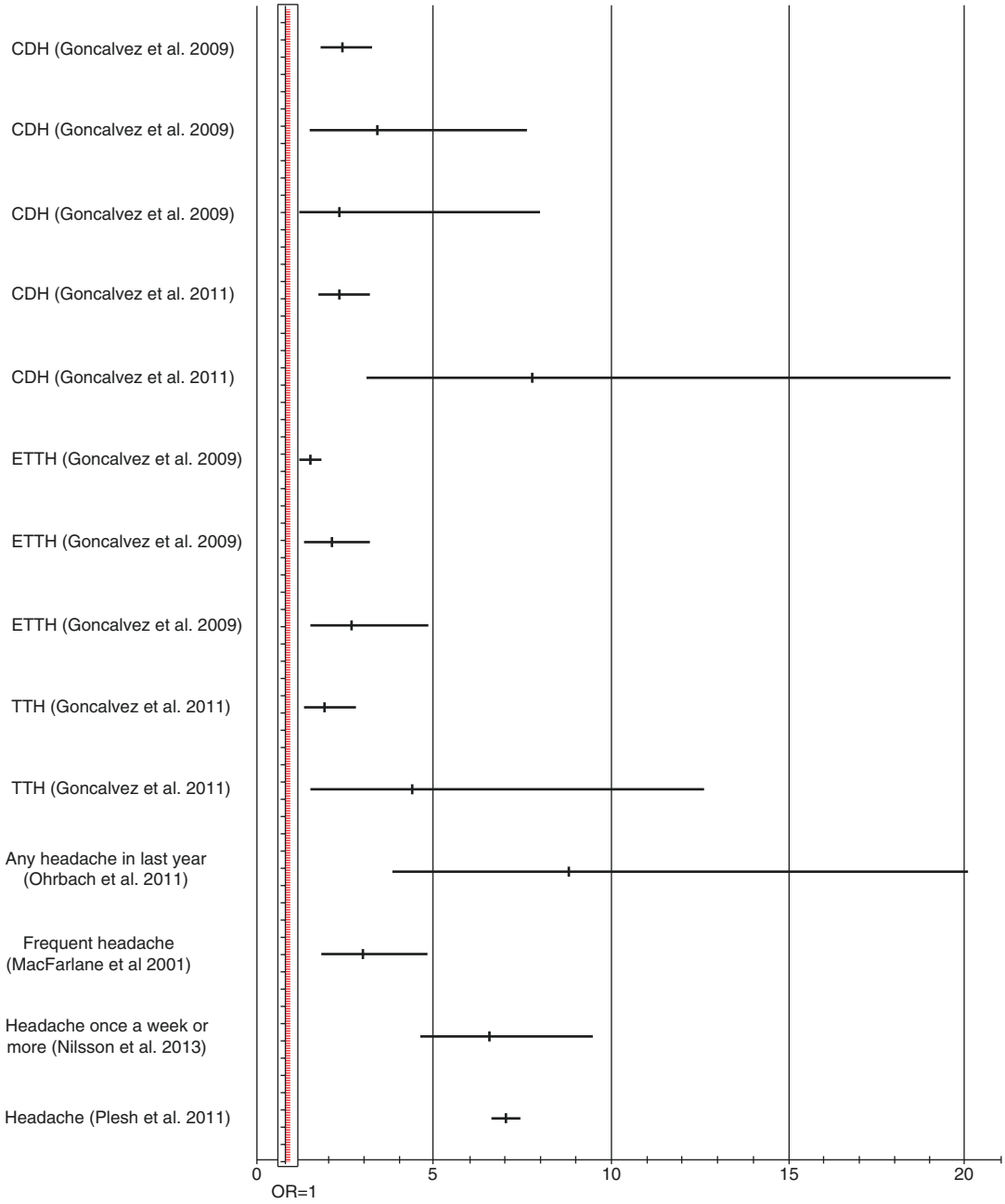

Fig. 2.1 Odds ratio and 95% confidence intervals illustrating the magnitude of the association between headache and orofacial pain. Abbreviations: *CDH* chronic daily headache, *ETTH* episodic tension-type headache, *TTH* tension-type headache, *OR* odds ratio. The horizontal line represents the 95% confidence intervals. The line crossing the confidence interval represents the OR

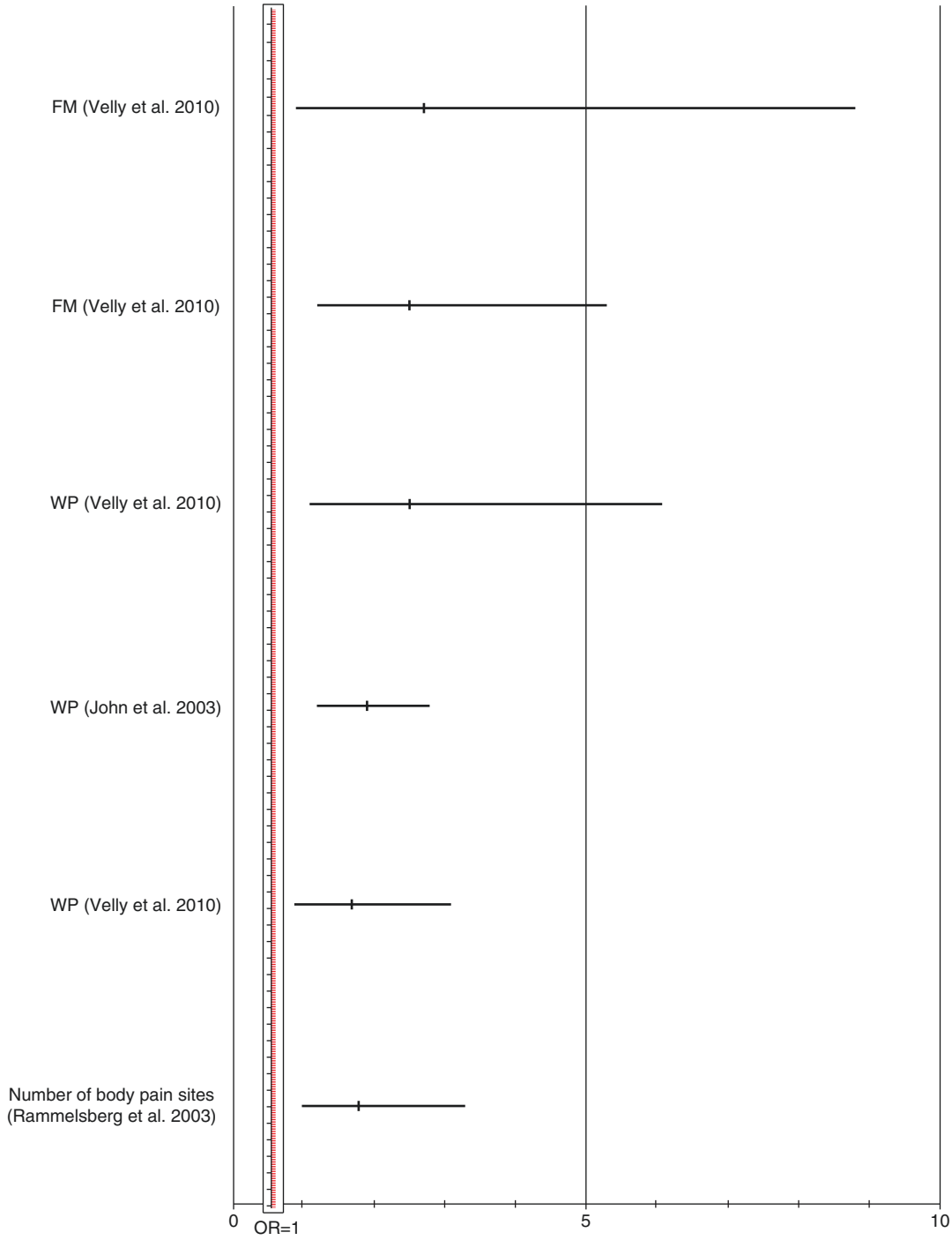

Fig. 2.2 Odds ratio and 95% confidence intervals showing the relationship between comorbid pain conditions and the persistence of orofacial pain. Abbreviations: *FM* fibromyalgia, *WP* widespread pain, *OR* odds ratio. The horizontal line represents the 95% confidence intervals. The line crossing the confidence interval represents the OR

CI: 1.4–2.8) [9], including painful TMD (Fig. 2.2) [1, 7].

2.3.3 Neck and Back Pain

Neck and back pains are also common symptoms reported by adults with orofacial pain (16–93%) [6, 34, 52, 53, 55, 62]. Previous epidemiological studies demonstrated that individuals with TMD-related pain were more likely (OR = 2.6–5) to have low back pain than individuals without (Fig. 2.3) [34, 55, 57]. The odds of joint pain (OR = 4.0, 95% CI: 3.7–4.3) are greater among adults with TMD-related pain than among those without [34]. Individuals with TMD-related pain are also more likely to experience neck pain (OR = 4.0–7.9) [34, 57]. Back (OR = 1.2; 95% CI: 1.2–1.3) and neck pains (OR = 1.5; 95% CI: 1.4–1.8) are also more likely to occur among females with TMD-related pain than males [34].

Moreover, a prospective-cohort study found an increased risk of painful TMD among adolescents with back pain compared to those without (OR = 3.9, 95% CI: 2.2–6.8) [2]. The risk increase among adolescents appears to be a little higher than for adults since the OR found among adults was close to 3 [(OR = 2.7; 95% CI: 1.1–7.0) [6] and 2.9 (95% CI: 2.0–4.3)] [55].

2.3.4 Visceral Comorbid Pain Conditions

Individuals with orofacial pain such as TMD and burning mouth syndrome [52] frequently report abdominal pain. For example, chronic pelvic pain (8%), irritable bowel syndrome (IBS; 9–16%), interstitial cystitis (17%), and stomach pain (34%) are noted among individuals with TMD [2, 53, 55]. The neural and sensory conditions include earache or ringing in the ear, hearing loss, fainting, or dizzy spells; respiratory conditions include sinusitis, allergies or hives, asthma, or breathing difficulties [55].

Furthermore, adolescents with painful stomach pain are 50% (OR = 1.5; 95% CI: 1.0–2.1) [25] to 90% (OR = 1.9, 95% CI: 1.2–3.1) [2] more likely to have painful TMD than those without stomach pain. Another study found an increased risk of painful TMD among adults with IBS compared to those without (OR = 2.7, 95% CI: 1.4–5.1) [55].

2.3.5 Psychological Factors

There is evidence that psychological factors are associated with chronic pain [63–65] and, in particular, with chronic painful TMD [66, 67]. Higher levels of stress [6, 63, 68, 69], anxiety [68, 70], depression [6, 63, 70–73], somatic awareness [2, 13, 63, 70–72, 74], and pain catastrophizing [63, 75, 76] have been noted among individuals with chronic orofacial pain.

Furthermore, affective disturbance (RR = 1.6, 95% CI: 1,1–2.2) [9], irritability (RR = 1.5, 95% CI: 1.1–2.1) [9], anxiety (RR = 2.8, 95% CI 1.3–6.2) [3], depression (OR = 2.2, 95% CI: 1.2–4.0), and perceived stress (OR = 2.2, 95% CI: 1.2–4.0) increase the risk of orofacial pain [6]. A higher risk is also related to painful TMD when individuals are exposed to depression (incidence density ratio [IDR] = 3.2, 95% CI: 1.5–6.7) [73] (hazard ratio [HR]= 1.31, 95% CI: 1.19–1.42)] [77], perceived stress [(IDR = 2.6, 95% CI: 1.2–5.5) [73] HR = 1.31, 95% CI: 1.16, 1.48)] [77], mood (IDR = 3.7, 95% CI: 1.7–8.1) [73], somatization [(OR = 1.8, 95% CI = 1.1–2.8) [2], HR = 1.38, 95% CI: 1.27–1.49)] [77], and life dissatisfaction (OR = 4.1, 95% C = 1.9–9.0) [2]. Psychological comorbidities also contribute to the persistence of TMD-related pain, regardless of the presence of painful comorbidities [7, 8].

2.4 Implications of Comorbidities on Biomarkers Identification

A number of candidate biomarkers cited in Chaps. 6, 7, 8, and 9 are associated with orofacial pain. It is important to note that these biomarkers are also associated with orofacial pain comorbidities. For example, NGF is elevated in many clinical pain conditions, such as headaches

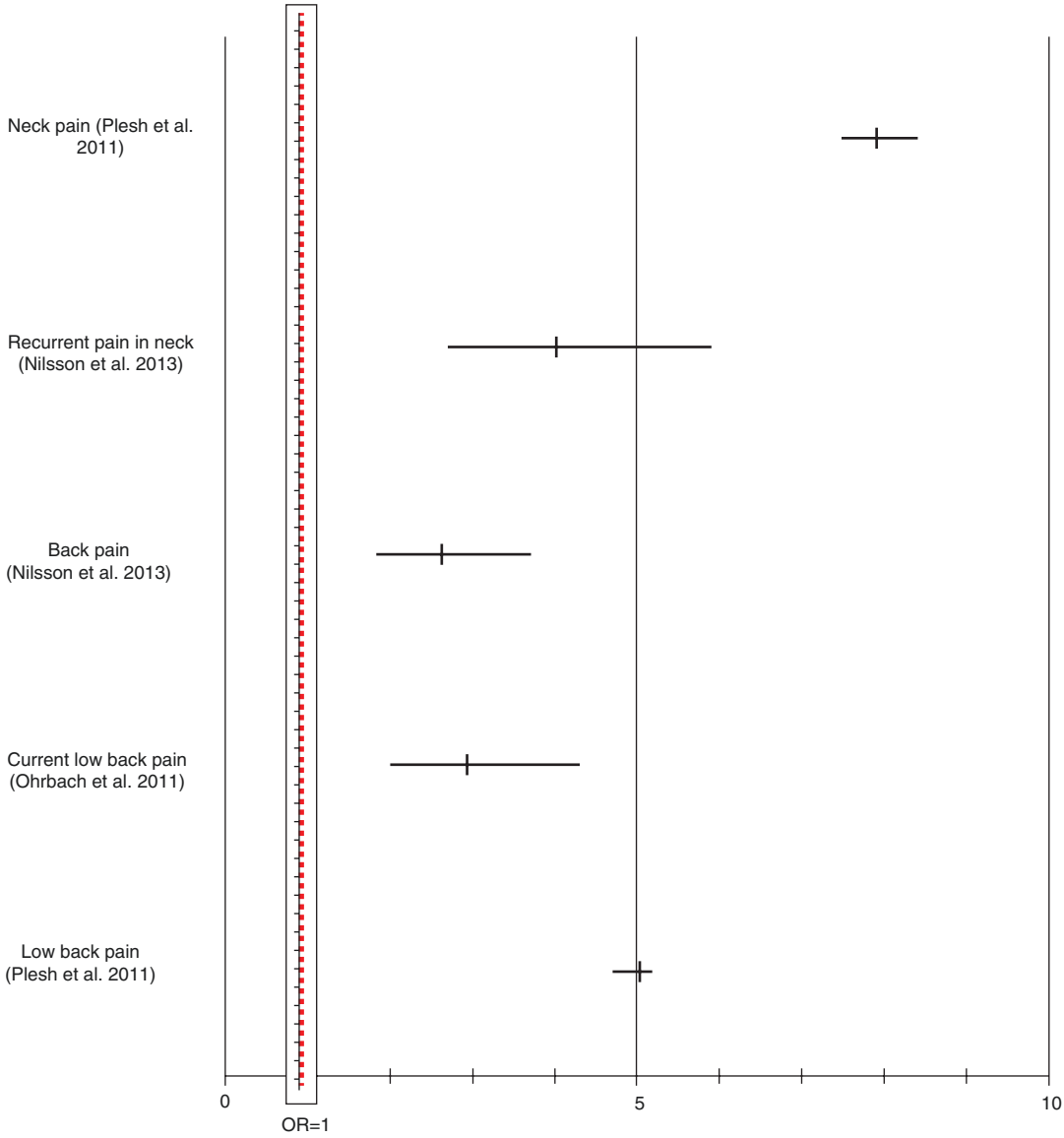

Fig. 2.3 Odds ratio and 95% confidence intervals illustrating the magnitude of the association between back pain or neck pain and orofacial pain. The horizontal line represents the 95% confidence intervals. The line crossing the confidence interval represents the OR

[78, 79], rheumatoid arthritis [80], fibromyalgia [81], visceral pain [82–84], and psychological comorbities [85, 86]. Elevated glutamate levels were also noted among patients with fibromyalgia [87], back pain [88], and headaches [89]. High levels of pro-inflammatory cytokines (e.g., IL-8, TNF, IL-6) are correlated with fibromyalgia [90–92], back pain [93], neck pain [94, 95], headaches [96, 97], visceral pain [84, 98–102], and psychological comorbidities [103]. Individuals with headaches [104] and neck pain [94] also presented higher levels of serotonin. Substance P levels were found to be positively related to neck pain [94], headaches [78, 105], visceral pain [106], and rheumatoid arthritis [105]. Elevated levels of CGRP were noted

among individuals with headaches [78, 107–109]. High levels of prostaglandins were identified among headaches [110], fibromyalgia [111], visceral pain [110], and depression cases [112–115].

Therefore, comorbidities probably can confound and/or modify the relationship between a candidate biomarker and orofacial pain. Hence, the studies assessing the putative biomarkers need to recruit a specific population and/or perform suitable statistical analysis to assess the impact of comorbidities on the relationship between a biomarker and orofacial pain (see Chaps. 10 and 11).

The identification of biomarkers for orofacial pain or comorbidities are very relevant since, based on cohort studies, comorbidities contribute to the onset [2–6] and the persistence of chronic orofacial pain over and beyond the expected healing time (Table 2.1) [1, 8–10].

2.5 Clinical Implications of Comorbidities in Orofacial Pain

In the treatment of orofacial pain, health-care providers need to identify the comorbidities and address them within a well-designed, multimodal, interdisciplinary integrative treatment plan that may involve dentists, physicians, physical therapists, and psychologists [116] since

Table 2.1 Examples of candidate biomarker of orofacial pain associated with orofacial pain comorbidities

Candidate biomarker	Comorbidity	Biofluid
Nerve growth factor [78, 79]	Migraine	Plasma
Nerve growth factor [84]	Interstitial cystitis	Serum
Nerve growth factor [82, 83]	Interstitial cystitis	Urine
Nerve growth factor [80]	Rheumatoid arthritis and psoriatic arthritis	Synovial fluid
Nerve growth factor [85]	Psychological stress	Saliva
Nerve growth factor [86]	Depression	Serum
Glutamate [89]	Headache	Plasma
IL-8 [92]	Fibromyalgia	Serum
IL-5 and IL-4 [96]	Migraine	Plasma
IL-6 and IL-10 [97]	Migraine	Serum
IL-17 [99]	Chronic pelvic pain syndrome	Urine
IL-1β, IL-6, TNF-α, IL-8 [84]	Interstitial cystitis	Serum
IL-6, IL-8, IL-1β (Pike et al. 2015) [100]	Irritable bowel syndrome	Serum
IL-10, IL-12, TGF-β [101]	Irritable bowel syndrome	Plasma
IL-6, IL-10, IL-13, IL-17, TNF-α, TGF-β [102]	Ulcerative colitis	Serum
IL-6 [103]	Depression	Serum
Serotonin higher [104]	Headache attack	Serum
Serotonin [94]	Neck pain	Microdialysis
Substance P [94]	Neck pain	Microdialysis
Substance P [106]	Interstitial cystitis	Urine
Substance P [105]	Migraine	Plasma
Substance P [78]	Migraine	Plasma and saliva
Substance P [105]	Rheumatoid arthritis	Synovial fluid and serum
Calcitonin gene-related peptide [78, 107, 108]	Migraine	Plasma
Calcitonin gene-related peptide [78, 109]	Migraine, cluster headache	Saliva
Prostaglandin E2 (Clarke et al. 2010) [114]	Irritable bowel syndrome	Serum
8-iso-prostaglandin F2α [110]	Headache	Urine
Prostaglandin E2 [111]	Fibromyalgia	Serum
Prostaglandin D2, prostaglandin E2 [115]	Major depression	Saliva

comorbidities not only contribute to the onset [2, 4–6, 59] but also have an effect on the persistence of pain [1, 7, 9, 10].

All interventions need to be tailored to the patient's unique characteristics and focus on treating the primary condition while addressing relevant risk factors and enhancing protective factors [116]. The ultimate goal of this strategy is to prevent the transition from acute to chronic orofacial pain as well as to promote tertiary prevention.

Conclusions

The findings presented in this chapter establish that a number of painful and non-painful comorbidities are common among patients with orofacial pain and, more specifically, painful TMD. Furthermore, epidemiological studies show that these comorbidities not only co-occur with orofacial pain but also contribute to the onset and persistence of orofacial pain. In addition, studies demonstrated that a series of candidate biomarkers for orofacial pain were also associated with the comorbidities of orofacial pain. It is therefore vital that researchers identify comorbid conditions and assess how these comorbidities affect the relationship between a candidate biomarker and orofacial pain.

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Part II

Mechanisms of Chronic Orofacial Pain

Neurobiological Mechanisms of Chronic Orofacial Pain

3

Barry J. Sessle

Abstract

This chapter reviews the several mechanisms in orofacial tissues and trigeminal nociceptive pathways in the brain that may account for chronic orofacial pain. Peripheral sensitization and central sensitization are particularly emphasized since they have characteristics that can explain the spontaneous nature, hyperalgesia, allodynia, and spread and referral of pain resulting from injury or inflammation of orofacial tissues and nerves. The chapter also notes several neural and non-neural modulatory factors influencing these mechanisms and their clinical implications.

3.1 Introduction

Pain is a multidimensional experience encompassing sensory-discriminative, cognitive, affective, and motivational dimensions, the expression of which can vary from one individual to another [1, 2]. The face, mouth, and jaws represent some of the most common areas of pain in the body, and epidemiological studies have documented the high prevalence of several acute or chronic orofacial pain conditions [3–5]. These chronic pain conditions in particular can present diagnostic and management challenges to the clinician. This is because of (i) the complex, even bizarre, nature of some of these pains; (ii) the multidimensional experience of pain itself that reflects a host of biopsychosocial influences; (iii) the special biological, emotional, and psychological meaning that the face and mouth have to humans; and (iv) the limited knowledge of the etiology, pathogenesis, and mechanisms underlying the initiation and progression of these pain conditions. This chapter reviews recent advances in our understanding of the mechanisms underlying orofacial pain, and chronic orofacial pain in particular, in order to assist clinicians in their management of the various chronic orofacial pain conditions. It first outlines relevant orofacial pathways and mechanisms and then focusses on processes involved in chronic orofacial pain.

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3.2 Overview of Orofacial Nociceptive Pathways and Mechanisms

3.2.1 Peripheral Processes

The fifth cranial nerve, the trigeminal nerve, provides the major sensory innervation of the face, mouth, and jaws. These tissues are densely innervated by trigeminal primary afferent (i.e., sensory) nerve fibers, each of which terminates peripherally as nerve endings termed receptors that "sense" stimuli applied to the face, mouth, and jaws. As a result of these stimuli, action potentials may be generated by these receptors in their associated afferent fibers, which conduct the action potentials into the brainstem. The orofacial receptors can be broadly categorized into two types: specialized or corpuscular receptors, of which several anatomically distinct types exist, and free nerve endings.

Many of the receptors associated primarily with large-diameter, fast-conducting myelinated primary afferent fibers (A-β afferents, some A-δ afferents) function as low-threshold mechanoreceptors since they respond to innocuous mechanical stimuli applied to the localized orofacial area supplied by the afferent (i.e., the receptive field of the afferent). There are mechanoreceptors in the facial skin, oral mucosa, periodontal tissues, periosteum, jaw muscles, and temporomandibular joint (TMJ), and their mechanoreceptive primary afferents provide sensory inputs into the central nervous system (CNS) that reflect detailed information of the quality, location, intensity, duration, and rate of movement of an orofacial mechanical stimulus [6, 7]. Mechanoreceptors located in the periodontal tissues, TMJ, and jaw muscles also account for our ability to detect and discriminate the size of small objects placed between the teeth, their hardness and texture, and bite force. Receptors in the TMJ and jaw muscles also underlie our conscious perception of jaw position (mandibular kinesthesia). The orofacial region also has thermoreceptors that are specifically activated by a small thermal change in either a cooling (cold receptors) or warming (warm receptors) direction. They are associated with some of the small-diameter, slow-conducting primary afferent fibers that are either myelinated (e.g., A-δ afferents) or unmyelinated (C-fiber afferents), and these thermoreceptive primary afferents provide the CNS with accurate information on the location, magnitude, and rate of the temperature change. There are of course also chemoreceptors in the oral cavity and nose that through other cranial nerves provide the CNS with information related to taste and smell.

Many of the free nerve endings in the orofacial tissues function as receptors (nociceptors) that sense the occurrence of a noxious stimulus [6–8]. They are the endings of primary afferents that are small diameter and slowly conducting (Aδ and C fibers). Several chemical mediators and cellular changes occur following the noxious stimulus, resulting in the activation of the nociceptive endings and their associated nociceptive afferents, which conduct the nociceptive signals into the CNS and thus may lead to the experience of pain. A prolonged increase in their excitability (so-called nociceptor or peripheral sensitization) may also occur, to such an extent that they become more responsive to subsequent noxious stimuli or even start responding to stimuli that normally are innocuous; they may also develop spontaneous (background) activity. In addition, some mechanically or thermally insensitive endings ("silent nociceptors") may be activated or sensitized by noxious chemical stimuli and then become responsive to noxious stimuli.

It has become apparent over the past two decades that the mechanisms involved in the activation or peripheral sensitization of orofacial nociceptive endings are very complex [7, 9, 10]. Ion channels or membrane receptors occur on the nociceptive afferent endings and include serotonergic, cholinergic, opioid, purinergic, bradykinin, histamine, prostaglandin, anandamide, excitatory amino acid and acid-sensitive receptors, adrenoreceptors, and vanilloid receptors [11–13]. Some of these ion channels and membrane receptors are activated, or their afferent endings are sensitized relatively directly by several types of noxious mechanical, chemical, and thermal stimuli (e.g., some vanilloid receptors [TRPVI] respond to protons (H^+), heat, and alge-

sic chemicals such as capsaicin), whereas others are acted upon by intermediary chemical mediators that are released in the peripheral tissues as a result of the noxious stimulus causing injury to the tissues. In addition, noxious stimuli producing tissue damage may cause the release of neurochemicals that are synthesized in the trigeminal ganglion cell bodies of the primary afferents themselves and released from their afferent endings; these include substance P, calcitonin gene-related peptide (CGRP), somatostatin, glutamate, and nerve growth factors. Some of these neurochemicals act on platelets, macrophages, mast cells, and other cells of the immune system to cause them to release inflammatory mediators such as serotonin (5-HT), histamine, bradykinins, and cytokines. The resulting redness, edema, and local temperature increases reflect what has been termed neurogenic inflammation since the inflammation may be initiated from these chemical mediators released from the nerve fibers themselves. Many of the chemical mediators also spread through the tissues and act on the ion channels and membrane receptors of adjacent nociceptive afferent endings and contribute to their peripheral sensitization. Glutamate, for example, is synthesized in the primary afferent cell bodies in the trigeminal ganglion and is released from not only the central endings of the primary afferents in the CNS (i.e., brainstem) but also from their endings in the orofacial tissues. Some afferent endings in peripheral tissues have glutamatergic receptors (N-methyl-D-aspartate [NMDA] and non-NMDA receptors) by which glutamate may excite or sensitize the nociceptive afferents. Some other chemical mediators (e.g., opioids and δ-amino butyric acid [GABA]) in contrast may decrease afferent excitability by acting on GABA and opiate receptors on the afferent endings. Interestingly, there is a sex difference in the peripheral action of glutamate and the opiate-related drug morphine; e.g., jaw or TMJ muscle nociceptive afferents show a greater sensitivity in females than in males to the application of glutamate, but females are less sensitive than males to the peripheral application of morphine [7, 8]. There is also increasing evidence that orofacial tissue inflammation or injury, especially of nerve fibers, can also cause changes in the properties of trigeminal ganglion cell bodies that may contribute to an abnormal sensory input into the brainstem (see Sect. 3.3).

There are several clinically relevant aspects of these peripheral processes. Peripheral sensitization of the nociceptive afferents is an important process contributing to the increased sensitivity that is usually a feature of a peripheral injury or inflammation site, e.g., as in the pain of a sunburn, arthritis, myositis, and pulpitis. The increased sensitivity may be reflected as an exaggerated perceptual response to a noxious stimulus ("hyperalgesia) or as a pain response to a stimulus (e.g., tactile) that is normally innocuous ("allodynia") or as an ongoing spontaneous pain; the sensitization of adjacent afferent endings beyond the initial injury site is a peripheral process contributing to the spread of pain in these tissues. Furthermore, the identification of substances released in painful tissues (e.g., glutamate, 5-HT, etc.) suggests that they may prove useful as biomarkers for certain types of pain states [13, 14], and other chapters in this book discuss this further (Chaps. 6, 7, 8, and 9). Also, the physiologically based sex differences noted above in the sensitivity of jaw muscle and TMJ nociceptive afferents to glutamate and opiate-related substances (i.e., morphine) may also contribute, along with the sex differences documented in environmental and psychosocial influences and the sex differences in CNS nociceptive mechanisms [4], to the sex differences in many orofacial pain conditions involving these tissues.

Another clinically significant point is that some drugs that are commonly used to relieve orofacial pain may exert their analgesic action by interfering with some of these peripheral mechanisms. Indeed, many common nonsteroidal anti-inflammatory drugs (NSAIDs) as well as several recently developed analgesics (e.g., cyclooxygenase-2 [COX-2] inhibitors) have their principal analgesic action by their influence on processes that enhance the excitability of nociceptive afferent endings. Furthermore, local anesthetics are effective for nerve blocks in eliminating pain resulting from peripheral tissue injury because they interfere with the ionic channels and currents

involved in the initiation and conduction of action potentials along the nociceptive afferents into the CNS [12, 13].

3.2.2 Central Pathways and Processes

The primary afferent nerve fibers in the trigeminal nerve project via the trigeminal ganglion and the trigeminal sensory nerve root into the trigeminal brainstem sensory nuclear complex, which can be subdivided into a main sensory nucleus and a spinal tract nucleus; the latter is subdivided further into the subnuclei oralis, interpolaris, and caudalis. The neural signals evoked by a light mechanical stimulus (e.g., tactile) of an orofacial tissue are transferred (via synaptic transmission) from the brainstem endings of the mechanoreceptive primary afferents to low-threshold mechanosensitive (LTM) neurons at all levels of the trigeminal brainstem sensory nuclear complex [2, 6, 10]. These second-order neurons conduct the signals onward to local brainstem regions, including those responsible for activating or suppressing muscles, and thereby serve as interneurons involved in reflexes or more complex sensorimotor behaviors. Another major projection from the LTM neurons in the trigeminal spinal tract nucleus and especially the main sensory nucleus is to LTM neurons in the ventroposterior thalamus (termed the ventrobasal thalamus in subprimates), principally on the contralateral side of the brain [2, 15]. Many of these thalamic LTM neurons project to parts of the overlying cerebral cortex, including the so-called somatosensory cortex involved in the perception of an orofacial touch stimulus. It has an extensive and disproportionate representation of the face and mouth relative to other body regions, reflecting the importance of sensory information from orofacial tissues compared to most other body regions. It is also noteworthy that the complex ultrastructure and regulatory processes that exist at each of the brainstem, thalamus, and cortical relay sites underlie the considerable modification of the synaptic transmission of the tactile-related signals that can occur at each of these CNS levels. Such modulatory mechanisms may explain how distraction or focusing one's attention on a particular task at hand can depress our awareness, for example, of the extensive mechanosensory inputs into the CNS from the mechanoreceptors that are being activated by our clothing.

In the case of orofacial thermosensation, the main brainstem relay site of the signals carried in the orofacial thermoreceptive primary afferent fibers is the trigeminal subnucleus caudalis. Some caudalis neurons appear to be exclusively activated by thermal stimulation of localized parts of the face and mouth and relay this thermal-related information to the contralateral thalamus and then to the somatosensory cerebral cortex. Subnucleus caudalis also is the major brainstem relay site of orofacial pain-related information, as noted below.

The vast majority of the nociceptive primary afferent fibers supplying the face and mouth project via the trigeminal ganglion to the trigeminal brainstem sensory nuclear complex, especially to the subnucleus caudalis where they release the chemical mediators that are synthesized in the primary afferent trigeminal ganglion cell bodies (see above). These include glutamate and the neuropeptide substance P which activate neurons in the trigeminal brainstem complex by acting, respectively, on glutamatergic receptors (N-methyl-D-aspartate [NMDA] and non-NMDA receptor subtypes) and neurokin receptors on the neurons. Many caudalis neurons receive the signals from these orofacial nociceptive primary afferents and thus can be excited by noxious stimulation of the face and mouth, TMJ, masticatory muscles, or meninges [6, 10]. These caudalis nociceptive neurons have been categorized as either wide dynamic range (WDR) neurons or nociceptive-specific (NS) neurons, and analogous neurons exist in the spinal dorsal horn of the spinal nociceptive pathways. The WDR neurons are activated by non-noxious (e.g., tactile) stimuli as well as by noxious stimuli applied to an orofacial receptive field and receive large-diameter (A-β) and small-diameter (A-δ and C fiber) afferent inputs. In contrast, NS neurons normally respond only to noxious stimuli (e.g., pinch, heat) and receive small-diameter afferent

inputs from A-δ and/or C fibers. Both types of neurons relay nociceptive information to other brainstem regions and also to the contralateral thalamus from where it is then relayed from analogous WDR or NS neurons to the overlying cerebral cortex or other thalamic regions [2, 6, 10, 15] where the information is processed and expressed as one or more of the many dimensions of the pain experience (see Sect. 3.1).

Although some differences between the two structures do exist, there is a close structural and functional homology between subnucleus caudalis and the spinal dorsal horn, and so subnucleus caudalis has become known also as the medullary dorsal horn [6, 16]. Nonetheless, subnucleus caudalis is not the only or essential brainstem element in orofacial nociceptive transmission since there is evidence that some of the more rostral subdivisions of the trigeminal brainstem complex, especially subnuclei interpolaris and oralis, may also play an important role [2, 17]. For example, afferent fibers from the tooth pulp, generally assumed to represent a nociceptive input, synapse with neurons present not only in subnucleus caudalis but also in the more rostral components of the complex, and the transitional zone between subnuclei caudalis and interpolaris has recently been shown to be important in muscle, autonomic, and endocrine responses to noxious orofacial stimuli and in intrinsic CNS modulatory influences on orofacial nociceptive transmission.

3.2.2.1 Modulatory Processes and Influences

At each relay in the trigeminal somatosensory pathways, the transmission process may vary depending on such diverse factors as maturational stage, age and sex, and behavioral state of the individual, plus genetic, nutritional, and immunological influences [2, 10, 18]. The intricate organization of the trigeminal brainstem complex, especially subnucleus caudalis, as well as the numerous afferent inputs to the trigeminal brainstem complex from peripheral tissues and from several CNS regions, provides the neural circuitry for the several interactions between these many inputs that influence somatosensory transmission. Some of these processes are involved in modifying touch, as noted above, but modulation of nociceptive transmission in the trigeminal system can also occur. For example, the responses of caudalis nociceptive neurons to small-fiber nociceptive afferent inputs can be markedly suppressed by large-fiber afferent inputs to caudalis that are activated by tactile stimulation of orofacial tissues (so-called sensory interaction); in some situations, even small-fiber nociceptive afferent inputs from other parts of the body may also suppress their activity. Their activity can also be suppressed by intrinsic inputs to caudalis from the spinal cord, brainstem, and higher CNS centers, such as the reticular formation, the periaqueductal gray, rostroventral medial medulla, and sensorimotor cortex. These modulatory influences result from endogenous neurochemicals, such as opioids, 5-HT, norepinephrine, and GABA, being released from these inputs and acting on the caudalis nociceptive neurons. Modulatory influences on trigeminal nociceptive transmission may also occur at thalamic and cortical levels [2, 10, 18].

There are several clinically relevant points about these modulatory influences. The influences on the nociceptive neurons of state of alertness, sleep, distraction and attention, and cognitive behavioral therapy are examples of behavioral factors whereby descending influences emanating from CNS regions involved in these behavioral functions and operating at the trigeminal brainstem complex and at higher brain levels may affect orofacial pain [2, 10, 19]. Placebo analgesia, which contributes to the effect of most pain-relieving procedures, also involves some of these systems [10, 20, 21]. Descending influences also have been implicated as intrinsic mechanisms contributing to the analgesic effects of several other procedures used to control pain. Morphine, for example, suppresses the activity of the nociceptive neurons by mimicking the action of the endogenous opioid chemical enkephalin which is a peptide that is pharmacologically similar to the opiate drugs such as morphine and which acts on opiate receptors existing on the nociceptive neurons and on neurons in some of the intrinsic modulatory pathways. Other pain-relieving drugs act

on other receptor processes to suppress the neurons, for example, amitriptyline on 5-HT receptor processes and pregabalin on voltage-gated calcium channels [22, 23]. The analgesic effects of some physical procedures (such as acupuncture or transcutaneous electrical nerve stimulation) appear also to involve some of these endogenous neurochemical processes and intrinsic pain-modulatory circuits [2, 10].

On the other hand, some modulatory CNS pathways have the opposite effect, i.e., facilitation of the nociceptive neurons, and contribute to the enhancement of pain, as might occur, for example, in the development and persistence of a chronic pain state or in the enhanced pain levels associated with fear, anxiety, and catastrophizing (Chap. 2). Facilitatory interactions also occur between various convergent afferent inputs to trigeminal nociceptive neurons in the CNS and contribute to the so-called referral of pain that may sometimes occur following tissue injury or inflammation (see Sect. 3.3.2 below). An especially noteworthy facilitatory effect may be initiated by injury or inflammation of peripheral tissues and can result in a prolonged increase in excitability of nociceptive neurons in the CNS. This so-called central sensitization is thought to be an important process contributing to the hyperalgesia, allodynia, and pain referral that characterize pain resulting from an orofacial injury or inflammation [2, 10, 24]. Furthermore, the development and maintenance of a central sensitization state appears to underlie most chronic pain conditions. Central sensitization reflects a neuroplasticity of the nociceptive pathways in the CNS and emphasizes that the nociceptive system is not hard wired but is dynamic and plastic, such that its excitability can change from one moment to another depending on the signals that its constituent WDR and NS neurons receive from peripheral tissues and on the CNS state of the individual. Central sensitization is manifested as an increase in excitability (e.g., spontaneous activity, increased receptive field size and responses to noxious stimuli, decreased activation threshold) of WDR and NS neurons.

In the trigeminal nociceptive system, central sensitization has been most studied in subnucleus caudalis and especially involves the release from the caudalis endings of trigeminal nociceptive afferents of excitatory amino acids (e.g., glutamate) that act via NMDA receptor mechanisms to induce a cascade of intracellular events in caudalis nociceptive neurons [2, 10, 24, 25]. A number of other brain chemicals such as those operating through neurokinin, opioid, GABA, and 5-HT receptor mechanisms contribute to or modulate these central neuroplastic changes induced by peripheral injury or inflammation. Other factors that influence these changes include genetic and environmental factors as well as non-neural (e.g., glial) cells, as noted below.

3.3 Chronic Orofacial Pain Mechanisms

With this background in processes underlying orofacial nociceptive transmission and its modulation, we can now focus on the peripheral and CNS mechanisms contributing to chronic orofacial pain states.

As Chap. 2 notes, chronic orofacial pain may accompany several types of painful and non-painful comorbidities. Chronic orofacial pain also can arise following injury or inflammation of orofacial tissues, including that associated with dental treatments (e.g., following endodontic treatment, dental implant placement, orthognathic surgery, tooth extraction), emphasizing the importance of trying to provide appropriate and timely management of acute pain so as to reduce the likelihood that it will transition into a chronic pain state (see Chap. 2). But for several chronic orofacial pain conditions (e.g., temporomandibular disorders [TMD], burning mouth syndrome, trigeminal neuralgia, so-called atypical odontalgia or persistent idiopathic facial pain), the etiology and pathogenesis are still unclear. The following sections on peripheral processes and central processes outline what is known of the peripheral and CNS processes that are associated with chronic inflammatory or neuropathic pain states and how they may explain chronic orofacial pain conditions. It is noteworthy that while many of these conditions may involve processes similar

to those in the spinal nociceptive system, there are some differences between trigeminal and spinal systems [6, 16]. For example, recovery from injury or inflammation may be faster in the trigeminal system, autonomic responses differ (e.g., no sprouting of sympathetic terminals on trigeminal ganglion cells following peripheral nerve injury), and the specific patterns of up- and downregulation of some ion channels and neurochemicals in primary afferents appear to be different between the two systems following chronic inflammation or injury. Thus, it cannot be assumed that processes involved in chronic inflammatory or neuropathic pain states in the spinal system can be automatically applied to the trigeminal system.

3.3.1 Peripheral Processes

It was noted earlier in the peripheral processes section that peripheral sensitization is reflected in enhanced spontaneous firing, an increase in responsiveness to noxious stimuli, and a decrease in activation threshold of nociceptive primary afferents, features that may contribute to the spontaneous pain, hyperalgesia, and allodynia that characterize many pain states, such as the increased sensitivity of the temporomandibular tissues in TMD, and the thermal sensitivity and spontaneous pain of an inflamed tooth [7, 9, 10, 13]. In addition, the spread of pain that occurs following tissue injury or inflammation may be explained by the chemical mediators released as part of the peripheral sensitization process that may spread through the tissues to act upon adjacent nociceptive afferent endings. Peripheral sensitization is normally reversible and gradually dissipates as the injured or inflamed tissue heals. But persistence of a peripheral inflammatory state and the continual sensitizing effect of chemical mediators on nociceptive afferent endings (e.g., as in an arthritic joint) can lead to accompanying CNS changes (see below) and thereby to a chronic pain state. Likewise, nerve injury may affect the nociceptive endings by producing prolonged changes in the expression and activity of voltage-gated calcium, sodium, or potassium ion channels on the endings and contribute, for example, to spontaneous or ectopic discharges that are conducted along the afferents into the brainstem; such changes have been implicated in the development of many types of neuropathic pain including those manifested in the orofacial region [9, 24, 26]. On the other hand, if the nerve injury transects afferent nerve fibers, there may be loss of sensation in the peripheral area supplied by the transected afferents, but the neuropathology may still produce a neuropathic pain state because of the central consequences of the nerve injury (see below). Nociceptive afferents may also become sensitive to sympathetic modulation following nerve injury, and this is thought to contribute to some pain conditions, e.g., some types of complex regional pain syndrome [9, 27].

It is important to note that changes are not limited to the peripheral endings of the primary afferents. Injury or inflammation of orofacial tissues, including primary afferent nerve fibers, can also be associated with persistent physiological and neurochemical changes in the neuronal cell bodies of the primary afferents in the trigeminal ganglion and involve modulatory influences on the ganglion neuronal cell bodies from non-neural (satellite glial cells) that are closely associated with the cell bodies [24, 28]. The injury or inflammation can send signals via the involved afferent nerve fibers to the trigeminal ganglion and produce alterations in gene expression, intracellular signaling (e.g., ERK, p38MAPK, phosphatases), and excitability of the ganglion neurons. The satellite glial cells may also show intracellular changes and themselves can be acted upon by chemical mediators (e.g., substance P, CGRP, ATP) released from the affected neurons. The existence of gap junctions between these cells, and between them and the neurons, provides an additional process by which the satellite glial cells and neurons may communicate and contribute to the spread of excitation in the trigeminal ganglion. These forms of communication between them may explain recent findings that injury to sensory nerves or inflammation of one trigeminal division (e.g., V3) can lead to excitability changes in trigeminal ganglion neurons subserving another division (e.g., V2) [24, 25]. It is however not yet

clear if the neurons involved in these changes are nociceptive and/or non-nociceptive neurons. Nonetheless, these cellular events in the trigeminal ganglion likely are important processes involved in the generation of increased or abnormal trigeminal afferent inputs to the brainstem that can influence neuronal and glial cell functions in the central trigeminal nociceptive processes underlying orofacial chronic pain mechanisms.

The clinical implications of these events in peripheral orofacial tissues and trigeminal ganglion are several. As noted above, the alterations in the properties of trigeminal nociceptive afferents as part of the peripheral sensitization process may contribute to spontaneous pain, hyperalgesia, allodynia, and pain spread in chronic pain states. In addition, it was noted earlier (Sect. 3.3.1) that the several chemical mediators and cellular processes involved in the activation or sensitization of the nociceptive afferents represent potential or realized targets of peripherally acting analgesic (e.g., COX-2 inhibitors, local anesthetics). Nonetheless, the multiplicity of processes, often acting in parallel, implies that targeting only one or a few of them is unlikely to have a significant analgesic impact [12]. The recent findings of spread of excitation to other trigeminal division(s) within the ganglion following inflammation or injury within another trigeminal division also have clinical relevance since such a process could conceivably be important in the extraterritorial sensory changes reported in some clinical cases of chronic pain [29–34]; as noted below, central processes may also contribute to such extraterritorial spread. Also of clinical relevance are recent findings in animal models mimicking the compression of the trigeminal ganglion or trigeminal sensory root that has been reported to occur in many trigeminal neuralgia patients and to be of etiological significance. Such compression produces nociceptive behavior in the animals and trigeminal brainstem cellular changes apparently reflecting the consequences of the abnormal afferent inputs to the brainstem produced by the compression [35]. These findings further emphasize the importance of trigeminal ganglion changes and the generation of abnormal afferent inputs in the production of an altered CNS state that, as the following section indicates, underpins the development and maintenance of a chronic orofacial pain condition.

3.3.2 Central Processes

As noted above, a number of alterations can occur in the CNS in association with tissue injury or inflammation and contribute to the development and maintenance of a chronic orofacial pain condition. Central sensitization appears to be the dominant central neural change associated with these pain states.

Central sensitization reflected in a hyperexcitability of brainstem nociceptive neurons in trigeminal subnucleus caudalis has been well documented in several chronic as well as acute inflammatory or neuropathic pain models [10, 24, 25]. Central sensitization also occurs in other components of the trigeminal brainstem complex (e.g., subnucleus oralis and the interpolaris/caudalis transitional zone) as well as at higher levels of the trigeminal nociceptive system (e.g., thalamus) although it appears to depend on the functional integrity of subnucleus caudalis for its expression since it can be abolished in these CNS sites by experimentally blocking the synaptic function of subnucleus caudalis [36]. It is also noteworthy that excitability changes following trigeminal nerve injury are not limited to the trigeminal somatosensory system but may also occur in CNS regions involved in the psychosocial functioning of the individual or in motor functions such as motor cortex pathways projecting to trigeminal motoneurons [37–39] and thus contribute to comorbid psychosocial and motor disruptions that are frequently associated with chronic pain states.

Like peripheral sensitization (see above), central sensitization appears to be normal physiological reaction to sustained noxious stimulation, and in most situations it is reversible and the pain state resolves. However, if central sensitization becomes maintained, chronic or persistent pain may develop [2, 10, 24, 25]. Unfortunately, the factors that predispose to the prolongation of

these reactions to tissue injury or inflammation are not yet well understood, but there is emerging evidence that they include genetic as well as environmental, immunological, and psychophysiological factors [10, 40]. For example, different rodent strains may express different levels of trigeminal central sensitization and nociceptive orofacial behavior, and environmental influences related to stress may also modify the behavior [41, 42]. Recent findings also point to changes in the inhibitory or facilitatory intrinsic modulatory processes that were noted earlier to influence trigeminal nociceptive processing in the CNS. An increase in descending facilitatory influences or a decrease in inhibitory influences can enhance trigeminal neuronal excitability. Another related mechanism is that in some circumstances, the normal inhibitory action of the neurotransmitter GABA is switched in chronic pain models to an action that facilitates neuronal excitability leading to a centrally sensitized state [43].

Recent findings also point to another factor important in the development and maintenance of a centrally sensitized state. Like the involvement of non-neural cells in peripheral tissues and the trigeminal ganglion in chronic inflammatory and neuropathic pain states (see above), central sensitization in subnucleus caudalis nociceptive neurons also involves non-neural cells. Indeed it is dependent on the functional integrity of glial cells in the brainstem. There are two types of CNS glial cells that are particularly involved, namely, astrocytes and microglia. Glial cells are even more numerous than neurons in most CNS areas, and they normally serve to nurture neurons, maintaining the chemical environment around them and protecting and assisting in their repair and regeneration following injury, infection, or inflammation. In the brainstem and spinal cord, they are in close proximity to neurons and the afferent inputs to the neurons and so are uniquely placed to interact with them. Indeed, following injury or inflammation of orofacial tissues, those in subnucleus caudalis and adjacent regions become "activated" and release inflammatory cytokines and other substances that can influence the excitability of the nociceptive neurons. Recent electrophysiological, immunocytochemical, and behavioral studies in animal models of chronic orofacial inflammatory or neuropathic pain have documented a role for both astrocytes and microglia in trigeminal central sensitization. For example, interfering with glial cell function in the medulla can prevent the development of trigeminal central sensitization in caudalis nociceptive neurons and the associated nociceptive behavior of the animal and can also reverse the sustained central sensitization and nociceptive behavior that are a feature of chronic orofacial pain models [10, 24, 25, 28]. The normal or baseline nociceptive processing, in caudalis neurons, for example, is not affected by blockade of glial cells; only the hyperexcitable state of the sensitized nociceptive neurons is affected.

These recent findings are of clinical importance from several perspectives. The documentation of the critical role in trigeminal central sensitization of glial cells offers the possibility of new therapeutic targets for pain control, which pharmacologically in the past has been dominated by drugs targeting neuronal mechanisms. In addition, central sensitization reflects a neuroplasticity of the trigeminal nociceptive system, and more and more evidence is emerging from brain imaging and other approaches in humans that such neuroplasticity in certain CNS regions is associated with a chronic pain state such as TMD, trigeminal neuralgia, or other neuropathic pain conditions [44] and may prove useful as a pain biomarker.

The recent findings in chronic pain states of changes in excitability occurring in orofacial motor pathways (e.g., motor cortex) and the changes that may occur in CNS regions involved in psychosocial functions are also clinically relevant. Such alterations may contribute to the motor limitations and psychosocial problems that are often seen in chronic orofacial pain conditions.

Also of clinical relevance are the features of central sensitization of nociceptive neurons in trigeminal nociceptive pathways in chronic orofacial pain models, namely, spontaneous activity, hyperexcitable responses to noxious stimuli, and decreased activation threshold, which also reflect

features that, along with peripheral sensitization (see above), can explain the spontaneous pain, hyperalgesia, and allodynia that characterize several orofacial chronic pain conditions and may through quantitative sensory testing (QST) reflect biomarkers for some of these conditions (see Chap. 5). An example is the pain of a chronic arthritic condition, which may involve central sensitization of nociceptive neurons as well as peripheral sensitization of the afferents in the inflamed region. Another example is TMD, since these central and peripheral processes can explain the ongoing pain, increased pain sensitivity (i.e., hyperalgesia), and the lowered threshold for evoking pain (i.e., allodynia). The diffuse character of TMD pain can also be explained by involvement of adjacent afferents as part of the peripheral sensitization process, but also by central sensitization since neuronal receptive field expansion is a major feature of trigeminal central sensitization in chronic as well as acute orofacial pain models. As a consequence of the expansion of its receptive field, the centrally sensitized nociceptive neuron starts sending signals to higher brain centers from more widespread parts of the orofacial region and thereby contributes to the perception of a diffuse pain. On a related point, central sensitization also appears to be important in the referral of pain, which is a common feature of TMD and some other types of chronic orofacial pain states (e.g., headaches). Trigeminal nociceptive afferent inputs relayed to many caudalis nociceptive neurons appear to derive exclusively from cutaneous (and oral mucosal) tissues and endow these neurons with coding properties important for the detection and discrimination of superficial orofacial pain, which is usually well localized. In contrast, nociceptive information from deeper tissues (e.g., tooth pulp, TMJ, muscle, meninges) is predominantly processed by other subsets of caudalis nociceptive neurons (both NS and WDR) receiving extensive convergent afferent inputs from these tissues as well as cutaneous afferent inputs [2, 25]. These convergence patterns are also a feature of analogous nociceptive neurons in the thalamus and cortex and reflect processes contributing to deep pain and also to the poor localization, extraterritorial spread, and referral of pain from these deep tissues. Nonetheless, the pain referral mechanisms may depend not only on the convergent afferent input patterns to the nociceptive neurons but also on the neuroplastic changes expressed as central sensitization generated in the neurons by these inputs as a result of injury or inflammation. There is evidence suggesting that some of the widespread afferent inputs to the nociceptive neurons are normally "weak" and held in check by inhibitory processes but become "unmasked" in pathophysiological situations and are more effective in exciting the nociceptive neurons that have become hyperexcitable through the central sensitization process and a decrease of the inhibitory processes.

Also clinically relevant is evidence that central sensitization depends on nociceptive afferent inputs for its initiation and perhaps also for its maintenance. This underpins the now standard incorporation into dental restorative and surgical procedures of approaches such as local anesthesia and pre- and postoperative analgesic drugs that reduce nociceptive afferent inputs into the CNS and thus reduce the risk for the development of central sensitization and a persistent pain state. It also emphasizes again the point made above of the importance of timely and appropriate treatment of an acute pain state to reduce the possibility that it could lead to persistent sensitization processes and a chronic pain condition. Moreover, caudalis central sensitization and the accompanying nociceptive behavior that occur in animal models of orofacial inflammatory or neuropathic pain can be prevented from developing, or attenuated once developed, by analgesic drugs (e.g., morphine, pregabalin) used clinically in chronic orofacial pain patients [2, 13, 45, 46]. These findings emphasize the crucial role that central sensitization plays in the development and maintenance of chronic orofacial pain states.

Summary

Several mechanisms accounting for chronic orofacial pain have been identified in orofacial tissues and trigeminal nociceptive pathways in the CNS. These include peripheral sensitization and

central sensitization that have characteristics that can explain the spontaneous nature, hyperalgesia, allodynia, and spread and referral of pain resulting from injury or inflammation of orofacial tissues and nerves. A number of neural and non-neural modulatory factors influencing these mechanisms have been documented. Further elucidation of these mechanisms holds out promise for the development of new or improved diagnostic and management approaches for orofacial pain states.

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Oral and Craniofacial Pain: Contribution of Endogenous, Central Modulation Mechanisms

4

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Abstract

In this chapter, we will describe the main endogenous, central modulation and related maladaptive mechanisms involved in processing oral and craniofacial pain. In particular, we will explain how the functional anatomy and pathophysiology of brainstem, hypothalamic, and corticofugal networks may alter the excitability of the trigeminal system. We will describe our recent findings showing a direct anatomo-functional relationship between cortical, hypothalamic excitability disturbances and dysfunctions of medullary trigeminovascular regions. We will analyze the impact of such dysfunctions as putative biomarkers of central sensitization phenomena at the origin of sustained trigeminal pain.

4.1 Introduction

The trigeminal system is involved in processing nociceptive information from oral, facial, and cranial territories. The inputs from tissue-damaging events transduced by trigeminal nociceptors are similar to those throughout the rest of the body. The specific features of trigeminal pain experience probably result from activities generated by endogenous central modulation mechanisms involved in the processing of pain sensations and reactions.

The sensory pathways that convey craniofacial nociceptive inputs to higher levels of the brain originate in trigeminal ganglion nociceptors and their associated nuclei within the trigeminal brainstem sensory complex (Sp5) and upper cervical spinal cord (see Chap. 3). These structures are simultaneously collecting basic somesthetic activities from many sources that are not only relevant for pain but that could also have a role in the continual transmission of crucial information to maintain the integrity of oral and craniofacial regions. This information is constantly being selected and modulated in the context of an appropriate response by endogenous modulation networks

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originating from several central nervous system (CNS) structures. These regulation circuits can act at many levels to specifically discriminate the incoming messages. Some of the particular features of trigeminal nociception could thus result not only from the unique anatomo-functional organization of trigeminal brainstem nuclei but also from the interaction between bottom-up and top-down central mechanisms located upstream.

This chapter outlines the more relevant features of central regulation mechanisms of trigeminal nociception, on the basis of animal studies that provide valuable models in our understanding of human oral and craniofacial pain disorders.

4.2 Endogenous Modulation Mechanisms

4.2.1 Segmental Modulation: A Medullary Locus for Central Sensitization and Analgesia

A number of clinical and preclinical findings support the involvement of Sp5 neurons in oral and craniofacial nociceptive processing. In the last century, neurosurgical procedures showed that transection of the trigeminal descending tract at the level of the rostral pole of the trigeminal nucleus caudalis (Sp5C) produced thermoanalgesia of the face, without affecting significantly tactile sensations. However, painful sensations from the oral cavity were partially preserved following tractotomy, suggesting that craniofacial noxious inputs are conveyed also by neurons located more rostrally. As detailed in Chap. 3, animal studies have confirmed that the orofacial tissues have multiple representations in the Sp5C, in the transition zone between Sp5C/interpolaris (Sp5I) and oralis (Sp5O). The Sp5C projects also to the ipsilateral junction of Sp5C/Sp5I, Sp5O, and principalis (Pr5) nuclei over their whole caudal–rostral extent [1–3]. Such intratrigeminal connections are somatotopically organized, as observed both in animals [4]; however, the functional significance of these topographically organized, intratrigeminal connections is not fully elucidated. Ipsilateral inputs from Sp5C neurons to rostral trigeminal nuclei could contribute to the amplification of nociceptive outputs to supramedullary structures via the interpolar, oral, and principal subdivisions, since these regions convey orofacial inputs to brainstem and thalamic areas [5, 6].

As in spinal nociceptive processing, glutamatergic transmission is very important in the Sp5C since the local application of glutamate activates nociceptive neurons [7]. In addition, systemic or local application of NMDA antagonists in the Sp5C inhibits c-fos expression following corneal stimulation [8]. There is also strong evidence that rostral trigeminal nuclei, especially Sp5O, convey both extra- and intraoral nociceptive inputs, which are dependent on glutamatergic inputs from Sp5C (see also Chap. 3) [1, 9]. The excitatory receptive fields of wide dynamic range (WDR) Sp5C trigeminal neurons that convey noxious messages to upper CNS structures show a gradient of responsiveness, with the center responding to both innocuous and noxious stimuli and the remaining area responding only to strong stimuli. This phenomenon can be interpreted as if an innocuous stimulus would excite only few neurons, whereas a noxious one applied on the same somatic area would excite all the neurons showing an overlapping of their receptive fields. Based on this view, one could argue that the multiplication of innocuous stimuli applied over a large area, including a sufficient number of "centers" of receptive fields, would induce pain. However, since WDR neurons also have an adjacent inhibitory receptive field distinct from the excitatory one, innocuous mechanical stimuli applied to this inhibitory field are able to inhibit WDR activity. These observations led to the formulation of the gate-control theory of pain, which proposed that segmental inhibitions are elicited by activity in large-diameter, Aβ cutaneous afferent fibers and can be activated naturally by innocuous mechanical stimuli [10, 11]. The great majority of nociceptive primary afferents terminate in superficial layers (laminae I and II), but some Aδ-fibers also terminate in lamina V of the Sp5C (Fig. 4.1). Recent studies have shown the existence of a different distribution of two subsets of fine primary afferents: (1) Aδ and C peptidergic fibers contacting lamina I neurons at the origin of ascending projections and local

interneurons in outer lamina II and (2) non-peptidergic nociceptive primary afferents that terminate in the inner part of lamina II. In contrast, large myelinated Aβ fibers that convey innocuous inputs contact lamina V projection neurons and local protein kinase C gamma, (PKCγ), interneurons in inner lamina II [12]. Recent studies showed that following intense noxious stimulation or nerve injury, fine primary afferents release glutamate and several other peptides and neuromodulators onto lamina I neurons. Normally silent NMDA receptors become activated leading to a cascade of calcium-dependent and second messenger signaling that increases the excitability of lamina I neurons and thus facilitates the transmission of noxious messages to the brain. Under such circumstances, lamina I nociceptive neurons could be activated also by Aβ non-nociceptive primary afferents that usually drive inhibitory interneurons. Following injury, Aβ-fibers could activate PKCγ-expressing interneurons in inner lamina II which become disinhibited and in turn activate lamina I neurons (Fig. 4.1) [12–14].

The activation of Aδ- and C-fibers not only elicits pain but also both segmental and heterosegmental inhibitory mechanisms that must have a functional basis beyond the dorsal horn scope of the original gate-control hypothesis. Accordingly, several studies have shown the importance of small fiber activation in the production of analgesia by somatic electrical stimulation [15, 16]. In fact, percutaneous electrical stimuli can elicit both segmental and extrasegmental postsynaptic inhibitory processes that affect trigeminal WDR neurons, which are triggered exclusively by Aδ or both Aδ- and C-fibers [17]. Although transcutaneous electrical nerve stimulation (TENS) can be effective when applied at frequencies and intensities that activate mainly Aβ-fibers, the resulting pain relief is localized and often limited to the stimulated segment [18]. It has also been shown that stronger analgesic effects can be obtained with TENS by using a stimulation intensity that produces an unpleasant, but not quite painful, sensation [18, 19]. In summary, a substantial amount of data has implicated the activation of fine-diameter fibers in analgesic procedures based on segmental, percutaneous electrical stimulation. This conclusion is supported by studies

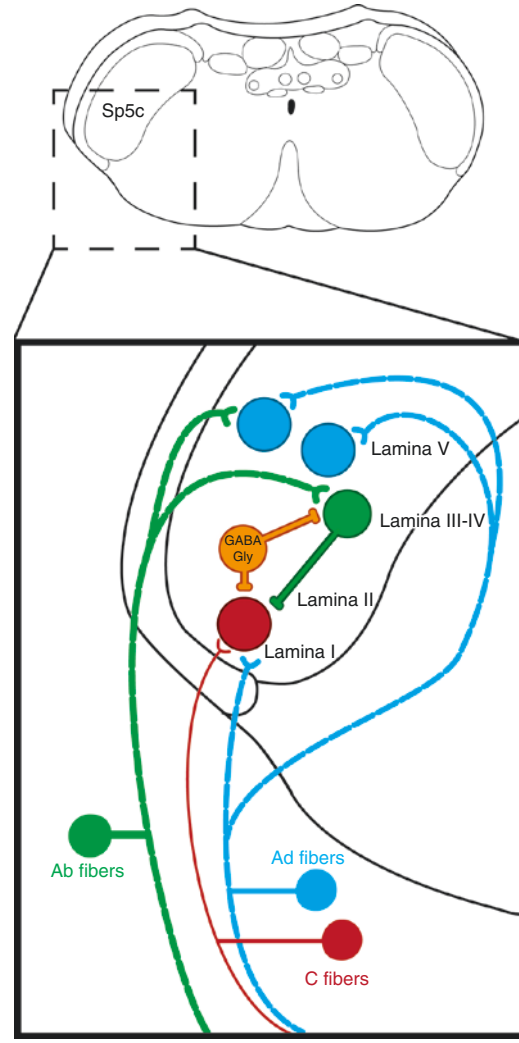

Fig. 4.1 Simplified representation of segmental modulatory influences onto cervicomedullary trigeminal neurons (Sp5C). After entering the trigeminal tract, most nociceptive (A and C) afferents pass caudally while giving off collaterals that terminate in the subdivisions of the spinal trigeminal nucleus and upper cervical cord. Second-order nociceptive neurons located in laminae I and V are activated by Aδ and C primary afferents. These fibers terminate mostly in lamina I, while a proportion of Aδ and Aβ non-nociceptive fibers contact also deep, lamina V neurons. Under normal circumstances, lamina V nociceptive neurons have a center (excitatory) surrounded by inhibitory receptive fields driven by Aβ fibers, via deep inhibitory interneurons. Following intense noxious stimulation or nerve injury, this inhibition could be lost, and lamina I nociceptive-specific neurons which are unresponsive to innocuous stimuli could be in turn activated also by Aβ non-nociceptive primary afferents. This activation could be partly mediated via PKCγ-expressing interneurons in inner lamina II, which become disinhibited. Abbreviations: *GABA* gamma-aminobutyric acid, *Gly* glycine, *PKCγ* protein kinase C gamma

showing that the intensity of stimulation is a critical parameter for obtaining greater analgesia using segmental TENS [20, 21].

4.3 Descending Modulation from the Brainstem

4.3.1 Diffuse Noxious Inhibitory Controls (DNIC)

In contrast to segmental controls, heterosegmental controls are elicited mainly by noxious stimuli. These inhibitions are mediated by descending brainstem-mediated mechanisms, such as diffuse noxious inhibitory controls (DNIC). Since the pioneering work of Le Bars and colleagues [22] which demonstrated that DNIC could induce widespread inhibitory controls on rat dorsal horn and medullary trigeminal neurons [23, 24], a number of studies showed that these controls have common anatomical and functional features in animals and humans. The supraspinal structures responsible for DNIC include the rat subnucleus reticularis dorsalis (SRD) in the caudal–dorsal medulla, which contains a homogeneous population of neurons whose properties mirror the functional characteristics of DNIC, viz., they are activated exclusively by noxious stimuli applied to any region of the body and precisely encode the intensity of these stimuli [25, 26]. Moreover, lesions of the caudal medulla reduce DNIC in both animals [27] and humans [28]. These caudal medullary networks have been proposed to facilitate the extraction of nociceptive information by increasing the signal-to-noise ratio between a pool of deep dorsal horn neurons that are activated from a painful focus and the remaining population of such neurons, which are simultaneously inhibited. Accordingly, the spatial summation of nociceptive peripheral inputs results in an initial increase in the number of activated neurons, which beyond a critical level of surface covered by the stimulus, is followed by a decrease in the responses of these WDR neurons [29]. In humans, similar antagonistic processes elicited by interactions of spatial summation and DNIC were recently reported [30]. In addition, DNIC also inhibits lamina I neurons, suggesting that a broader modulatory role is exerted by DNIC, probably via additional networks located in the rostral brainstem, such as the periaqueductal gray (PAG) matter and the rostral ventromedial medulla (RVM, see below). Thus, noxious inputs can modulate spinal outflow via these brainstem structures, in a bidirectional fashion [31].

4.3.2 DNIC and Counter-stimulation-Induced Analgesia

Further studies also suggested that DNIC mediates the "pain-inhibits-pain" or "counter-stimulation" phenomenon, whereby there is a mutual inhibition between the pathways that generate sensations elicited concomitantly by two separate painful foci. DNIC reduce both spinal [32], trigeminal reflexes [33] and the perception of experimental, acute pain following heterotopic noxious stimulation in man [34]. In addition to spino-bulbospinal loops involved in the DNIC circuitry, human brain imaging studies combined with psychophysics and electrophysiology have shown an important contribution of cortical regions belonging to the so-called pain matrix in the regulation of DNIC networks located downstream, during the analgesia produced by counter-stimulation [35, 36].

Chronic pain patient studies suggest that several mechanisms other than DNIC could also be implicated in counter-stimulation phenomena. For example, the effects of counter-stimulation are altered in neuropathic pain patients, thus showing that DNIC mechanisms differ in health and disease [37]. Light pressure applied to an allodynic area induced inhibitions of both the spinal RIII reflex and the concomitant painful sensation, whereas brushing on the same allodynic area, eliciting a similar level of pain, induced a reduction of the painful sensation but not a modification of the RIII reflex. One can conclude that in this latter situation, dynamic mechano-allodynia elicited a counter-stimulation effect

involving supraspinal rather than spinal circuitry. Furthermore, DNIC effects against temporally and spatially summated pain are reduced in fibromyalgia patients [38]. Also, significant reductions in the strength of DNIC are detected in some chronic, trigeminal painful conditions such as temporomandibular disorder and atypical trigeminal neuralgia [39, 40]. These observations suggest that the reduced ability to inhibit pain in patients with chronic pain is probably mediated by a dysfunction of endogenous pain inhibitory systems. Moreover, some studies suggest that such disturbances could also contribute to head pain processing as illustrated by a reduction of DNIC in chronic tension-type headache patients [41, 42] and a loss of DNIC acting on trigeminovascular Sp5C neurons in an animal model of medication-overuse headache [43].

4.3.3 The Rostral Ventromedial Medulla (RVM)

Early systematic studies of what was originally termed "stimulation-produced analgesia" in animals showed that localized microstimulation of the ventral periaqueductal gray (PAG) matter and rostral ventromedial medulla (RVM) effectively elicited strong behavioral antinociceptive effects as shown by the inhibition of jaw-opening reflexes elicited by tooth pulp stimulation [44]. Since the PAG projects minimally to the spinal and trigeminal dorsal horns but densely to the RVM, RVM neurons constitute a direct link for the descending modulation observed in these early studies. The RVM sends dense descending projections to superficial dorsal horn neurons, and these neurons, in turn, modulate the activity of deep dorsal horn cells at the origin of spinal ascending nociceptive pathways [45]. In contrast to the caudal SRD-medullary systems that preferentially modulate deep dorsal horn neurons, RVM cells modulate not only deep dorsal horn but also lamina I neurons, a key relay for nociceptive inputs to CNS areas that process signals relevant to homeostasis [46], suggesting a broader modulatory role by the RVM. In this respect, it was proposed that under appropriate environmental circumstances, RVM neurons integrate activities from the somatomotor and autonomic systems in response to different bodily needs. They could contribute not only to the modulation of pain but also to arousal reactions and homeostatic regulations such as changes in vasomotor, temperature, and sexual function in a manner appropriate to the behavioral status of the body [47, 48].

Electrophysiological studies in anesthetized rats have suggested a role of RVM neurons in a bidirectional, descending control of nociception. There are three classes of RVM neuron: off cells, which pause just prior to withdrawal reflexes; on cells, which show a burst of activity prior to such reflexes; and neutral cells which have no reflex-related activity. On and off cells project directly to dorsal horn laminae I, II, and V. Off cells are activated by local infusions of mu opioid agonists or $GABA_A$ antagonists, and their activity is correlated with inhibition of nociceptive transmission. In contrast, on cells, whose activity correlates with enhanced nociceptive transmission, are inhibited by local or systemic opioids [49].

Electrophysiological studies in unanesthetized rats are also illuminating in this regard because they demonstrate powerful state-dependent changes in RVM neurons. For example, RVM off cells are only intermittently active during waking but become continuously active when animals transition to slow-wave sleep [50] or when they are given barbiturate anesthesia [51] or morphine [52]. On cells show a reciprocal pattern, becoming much less active during slow-wave sleep. Interestingly, compared to the anesthetized and sleeping state, in awake rats, both on and off cells are more responsive to a variety of innocuous stimuli. Accordingly, unanesthetized rats display robust pro-nociceptive effects while being manipulated or submitted to stressful, threatening situations such as inescapable noxious stimuli, the presence of a predator, or contextual cues associated with intense or prolonged noxious stimuli [53]. In many of these situations, the behavioral pro-nociceptive effect probably involves the PAG-RVM network (see also Chap. 3).

4.3.4 The Cortex as a Widespread Source of Top-Down Modulation

Powerful endogenous control of nociception probably originates from the cortex since most nociceptive relays within the CNS are under corticofugal modulation, including downstream networks involved in segmental and heterosegmental modulation of medullary trigeminal neurons (see above, also Figs. 4.2 and 4.3). In contrast to bulbo-trigeminal descending controls, corticofugal modulation often occurs in the absence of a painful stimulus. For example, the insular cortex contributes to the processing of paradoxical pain elicited by the concurrent application of innocuous cold and warm stimuli [54], whereas frontal and primary somatosensory cortical areas may selectively alter the unpleasantness of pain perception following manipulation of attention, expectation, empathy, or the analgesia produced by placebo or hypnotic suggestions (for reviews, see 55–57). However, the mechanisms underlying these modulations remain poorly understood.

4.3.4.1 Corticofugal Modulation of Trigeminal, Medullary Dorsal Horn Activities

Early electrophysiological studies showed that stimulation of the primary somatosensory cortex inhibited the evoked responses of a proportion of medullary nociceptive neurons in the Sp5C [58]. Although the mediating pathways have not been identified, corticofugal controls are likely involved in the modulation, by behaviorally significant stimuli, of neurons in the trigeminal nucleus caudalis of trained monkeys. This type of modulation, termed "task related," may produce a greater neuronal response than that produced by equivalent stimuli in the absence of the relevant behavioral state [59]. In this regard, thermally responsive cells in the Sp5C exhibit an additional task-related response to visual or motor cues involved in the behavioral task, but not to similar stimuli presented outside the task [60]. The fact that many of these task-related responses exhibit a preferential association with either the visual stimulus or the motor response (hand movement) indicates that the mechanism of the behavioral modulation is mediated by distinct networks involved in either sensory or motor preparation. Interestingly, neither the detection of the visual stimulus nor the movement of the hand was related to the functions normally ascribed to trigeminal nucleus caudalis. This indicates that relevant information regarding the environment is disseminated to parts of the nervous system that may be involved directly or indirectly in the animal's ongoing behavior. Thus, neuronal responsiveness may bear no relationship to the features of stimuli that a sensory nucleus is capable of processing and may be dependent entirely on the behavioral context within which a sensory signal is received. As similar task-related responses have been demonstrated in several cortical areas, the task-related changes in trigeminal neuronal activity could represent a corticofugal reiteration of the paradigm instructions.

4.3.4.2 Corticofugal Modulation, Maladaptive Changes, and Impaired Orofacial Functions

Based on a number of preclinical and clinical studies, Avivi-Arber and colleagues [61] proposed that face primary motor (M1) and primary somatosensory (S1) cortices undergo plastic changes that can be induced by either peripheral or central inputs. In everyday life, such influences are produced by sensory stimulation and are involved in training and the learning of new motor skills. However, under pathological circumstances, maladaptive changes are produced not only by peripheral injury but also following progressive changes both in the chemistry and morphology of the human brain [62, 63].

Cortical plasticity must be highly dependent on reciprocal interactions with thalamic relays, since there are nearly ten times as many fibers projecting back from the cortex to the thalamus as there are in the forward direction from the thalamus to the cortex [64]. The function of this massive feedback network from S1 on the organization of whisker-barrel receptive fields in the ventroposteromedial thalamic nucleus (VPM)

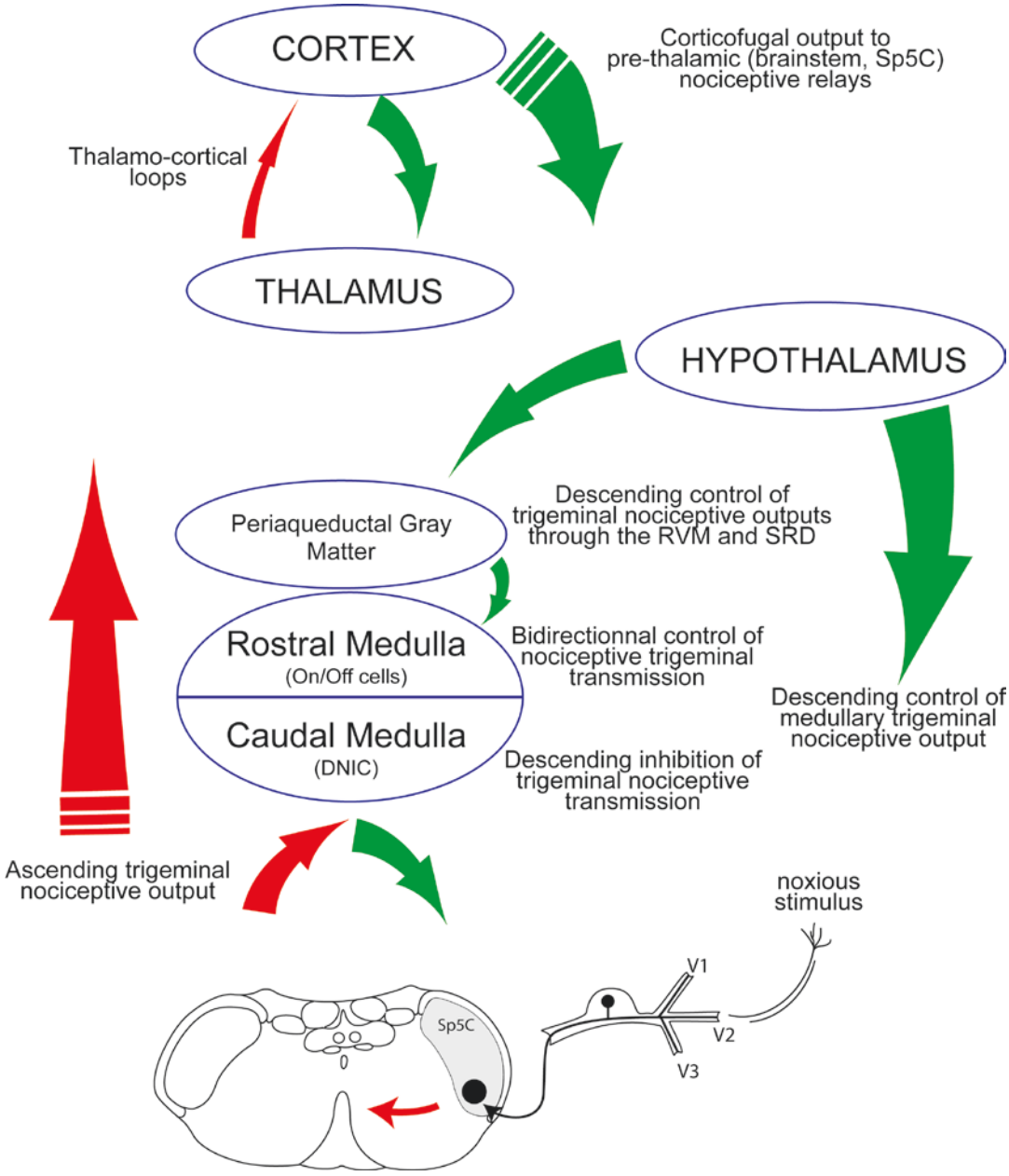

Fig. 4.2 Schematic representation of the main CNS descending networks of trigeminal pain modulation. A noxious stimulus carried by the trigeminal nerves activates segmental, bulbospinal, hypothalamic, and corticofugal modulatory mechanisms by which nociceptive signals may attenuate or increase their own magnitudes. The most important, widespread source of top-down modulation arises from the cortex since both thalamic and prethalamic nociceptive relays are under corticofugal modulation (see text). *Sp5C* spinal trigeminal nucleus, caudalis part, *V1* ophthalmic, *V2* maxillary, *V3* mandibular divisions of the trigeminal nerve

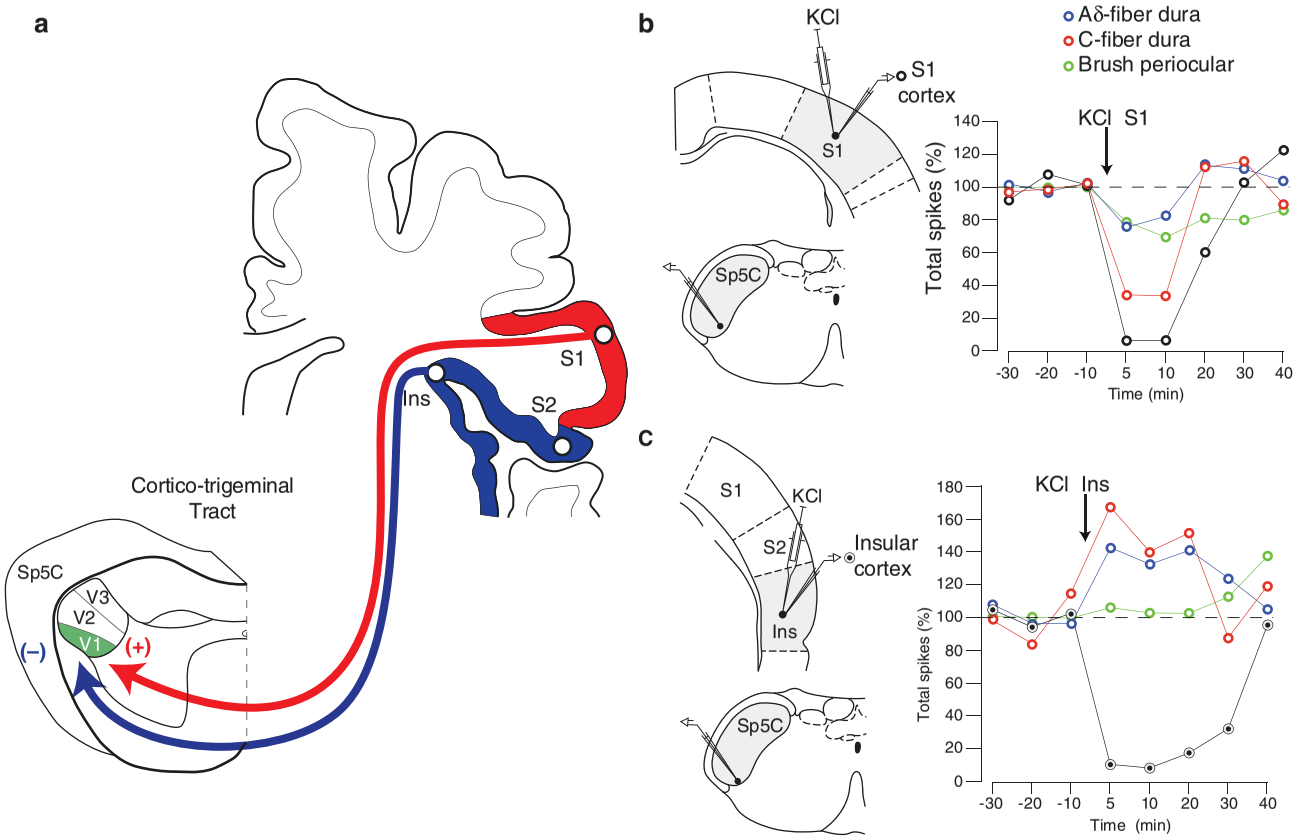

Fig. 4.3 Direct, corticofugal modulation of cervicomedullary trigeminovascular neurons (Sp5C), which convey nociceptive signals from meningeal nociceptors to CNS regions implicated in headache pain processing. (**a**) Corticofugal projections to Sp5C originate from the contralateral primary somatosensory (S1) and insular (Ins) cortices. S1 and Ins cortices innervate both deep and superficial layers of the Sp5C, respectively. (**b**) Cortical spreading depression (CSD) initiated in S1 cortex induces inhibition of cutaneous and meningeal-evoked responses of Sp5C neurons. (**c**) CSD initiated in Ins cortex induces a selective activation of meningeal-evoked responses of Sp5C neurons

and on the organization of limb tactile receptive fields in the ventroposterolateral nucleus (VPL) has been clearly established. The rat ventrobasal thalamus contains only excitatory neurons that project mainly to layer IV of the S1 cortex. Descending projections in the thalamocortical loop originate primarily in layer VI of the S1 cortex. Distal dendrites are densely innervated by these projections, which activate both ionotropic and metabotropic glutamate receptors. As in the visual and auditory systems, cortical feedback from S1 serves to amplify the effects of sensory stimulation to the classical center-surround receptive fields and helps to sharpen and adjust the profile of thalamic receptive fields (the "egocentric selection") [65]. Attempts to address this question by Krupa and colleagues [66] have shown that inactivation of the S1 cortex resulted not only in rapid changes in the receptive field properties of VPM cells driven by facial whisker pads but also in a significant reduction of their ability to reorganize their receptive fields following reversible deafferentation of trigeminal primary afferents.

Anatomo-functional studies also indicate that the capability to discriminate noxious inputs by S1 cannot be explained only by the projections or the response properties of ventrobasal thalamocortical afferents. Simultaneous thalamic and cortical recordings and pharmacological manipulation of corticothalamic feedback have shown that stimulus-driven, modality-specific influences from the S1 cortex are required to discriminate between innocuous and noxious cutaneous inputs. Corticothalamic feedback consists of either an enhancement of innocuous- or a reduction of noxious-evoked cutaneous responses. S1 produces such a selective, top-down modulation of thalamic ventrobasal responses to somatosensory inputs by engaging specific, GABAergic-mediated, corticothalamic modulation [67].

S1 neuronal activity can be related to a specific movement and may be suppressed during that movement by M1 modulatory influences [61]. It is tempting to speculate that such mechanisms could be involved in pain relief following electrical or repetitive transcranial magnetic stimulation (rTMS) of the motor cortex. Selective analgesia can be elicited following rTMS stimulation of either the motor or the dorsolateral prefrontal cortex. Such effects are not topographically distributed and probably occur at supraspinal levels, since rTMS does not affect spinal nociceptive processing as assessed with the RIII reflex [68]. The relevance of cortical plasticity in the changes of orofacial somatosensory perception is further underlined by the maladaptive changes that may occur following deafferentation. As shown by Ramachandran [69], light touch on an amputee's face referred sensations from the face to a precise area on the phantom hand. He suggested that these changes could be due to modifications in cortical topography and thus when the region of the somatosensory cortex formerly receiving inputs from the hand becomes silent, synapses from neighboring regions which had previously been subliminal become active – a process that can be reinforced later by sprouting of neurites. This idea is supported by the fact that a facial map of the phantom hand may be present immediately after surgery [70] and by psychophysical studies showing that, in healthy subjects, complete local anesthesia of the thumb did not affect the perception of the adjacent finger or digits on the contralateral side, whereas the perceived size of the unanesthetized lips increased by approximately 50% [71].

4.3.4.3 Corticotrigeminal Modulation and Migraine

Several lines of evidence from animal and human studies indicate that cortical spreading depression (CSD) is the pathophysiological substrate of migraine aura [72, 73] and migraine may thus be a result of maladaptive plasticity of corticofugal modulation [74]. CSD, which in animals can be induced by focal stimulation of the cerebral cortex, is a slowly propagating wave of neuronal depolarization and glial activation, whose mechanisms of initiation and propagation remain unclear. There are essentially no biomarkers of migraine progression, and although numerous findings indicate a substantial influence of CSD on peripheral, meningeal nociceptors [75], this issue is still subject of strong controversy [76].

Moreover, the existence of a direct relationship between cortical excitability changes and modifications of central, trigeminovascular neuronal

activities was also established. Our findings showed that restricted, lateralized regions within the rat S1 and insular (Ins) cortices send descending projections confined to the Sp5C area innervated by the ophthalmic branch of the trigeminal nerve (Sp5C). CSD-elicited corticofugal influences from Ins and S1 evoked, respectively, an enhancement and an inhibition of activities of Sp5C neurons induced by the activation of meningeal nociceptors. It is possible that such corticofugal influences could contribute to the development of migraine pain both in terms of topographic localization and pain tuning during an attack. We observed also that CSD triggered in the primary visual cortex selectively affects interoceptive (meningeal) over exteroceptive (cutaneous) nociceptive inputs onto Sp5C neurons [77]. More recently, by assessing cortical excitability and hemodynamic changes induced by somatosensory stimulation of the corresponding peripheral receptive fields, Theriot et al. [78] demonstrated that CSD induces a reduction of both electrophysiological and hemodynamic maps in the somatosensory cortex. Electrophysiological responses to somatosensory inputs were enhanced at the receptive field center but suppressed in surround regions. Because such sharpening can be seen on chronic time scales as a marker of sensory plasticity, these observations suggest that such profound alterations of sensory processing after CSD could contribute to chronic migraine-related sensitization. These findings shed new light on the role of corticofugal mechanisms as a direct link for topographically organized, differential, "top-down" processing mechanisms that modulate specifically trigeminovascular activities at the origin of headache pain (Fig. 4.3).

4.3.4.4 Hypothalamic Regulation in Headaches: Main or Supporting Actor in the Pathogenesis?

Although significant advances have been made over the past decade in understanding primary headaches, such as migraine and trigeminal autonomic cephalalgias (TACs), the discovery of effective treatments for patients has been hampered by the fact that their pathogenesis remains largely unknown. The migraine attacks are believed to involve activation of the trigeminovascular pathway (the peripheral trigeminal nerve innervations of the meningeal vessels and its central projections that form the trigeminothalamic tract), particularly its central components. Imaging studies showed that during the headache phase, there is consistent brainstem, pons, thalamic, and cortical activation. Recent studies have identified hypothalamic activation during the premonitory phase that can occur hours before the onset of the actual migraine headache [79]. Premonitory symptoms preceding a migraine attack, such as sleep disturbances, excessive yawning, changes in mood, alertness, appetite, and thirst, are all functions regulated by the hypothalamus. Additionally, the episodic nature of migraine attacks, the circadian rhythmicity, endocrine fluctuations, common triggers of migraine such as stress, and female hormonal fluctuations further implicate a hypothalamic involvement in the initiation of migraine. Importantly, these findings prompted the successful use of hypothalamic stimulation to treat cluster headache [80]. PET studies detected an activation of the ipsilateral posterior inferior hypothalamic gray matter during CH attacks, and voxel-based morphometric MRI showed alteration of the same area [79]. Cranial autonomic manifestations, including reddening of the eye, tearing, rhinorrhea, and eyelid edema, that accompany CH and also to a lesser extent occur in migraine could be produced by changes in the activity of hypothalamic structures that integrate signals which drive autonomic responses. Although migraine and TACs are different types of headache disorders, they both appear to have hypothalamic involvement in their pathogenic mechanisms.

4.3.4.5 The Paraventricular Hypothalamic Nucleus as a Major Source of Top-Down, Trigeminovascular Modulation

We recently identified the paraventricular hypothalamic nucleus (PVN), a region implicated both in neurohormonal and autonomic integration of stress responses (hypothalamic–pituitary–adrenal, HPA axis), as a likely hub that coordinates and integrates pain/anxiety comorbidity mechanisms involved in several primary headaches (Fig. 4.4) [81]. PVN descending projections are

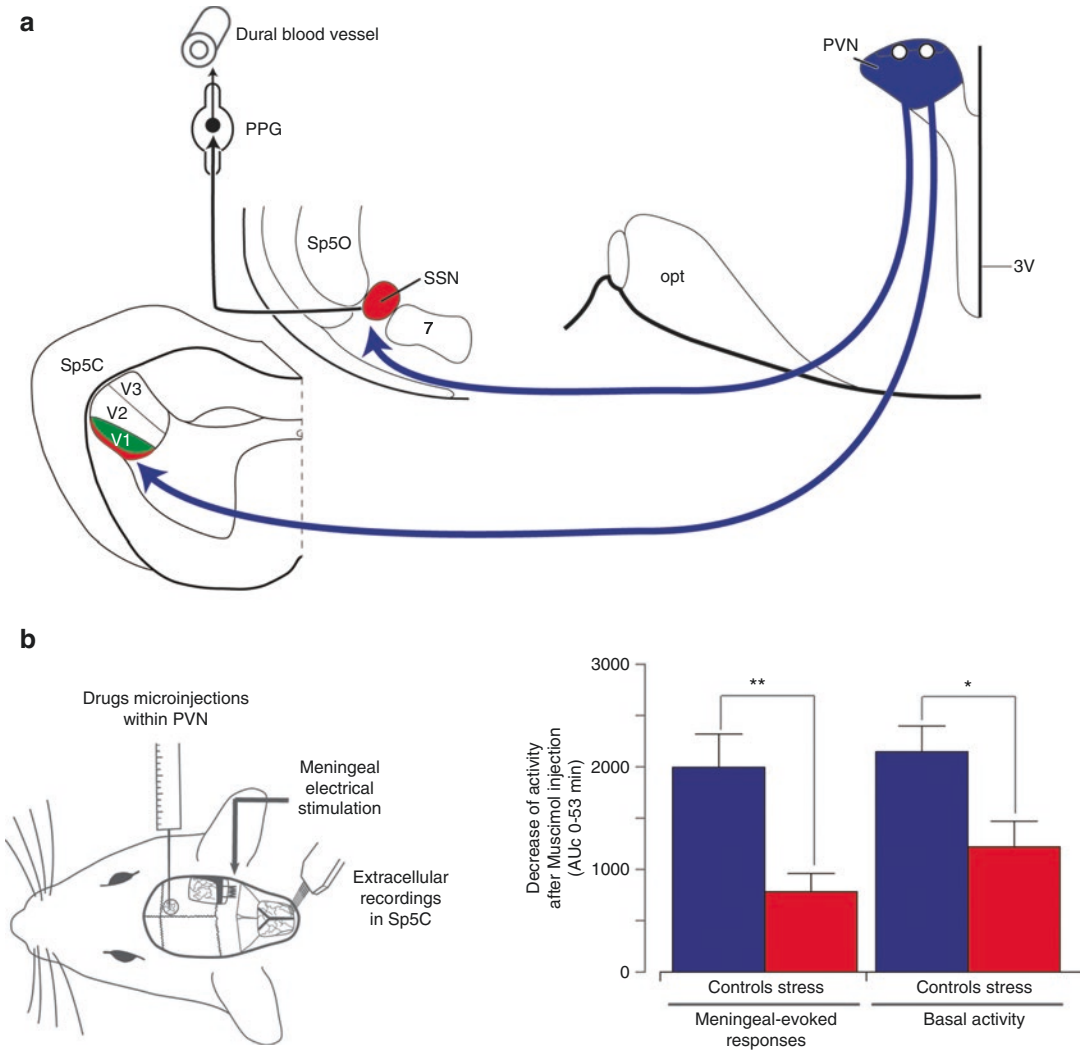

Fig. 4.4 Direct, hypothalamic modulation of cervicomedullary trigeminovascular neurons (Sp5C), which convey nociceptive signals from meningeal nociceptors to CNS regions implicated in headache pain processing. (**a**) Hypothalamic projections to Sp5C originate from the ipsilateral paraventricular nucleus (PVN), a key link of the hypothalamic–pituitary–adrenal axis. PVN innervates both the superficial layers of the Sp5C and the superior salivatory nucleus (SSN) which regulates cranial parasympathetic outflow via the pterygopalatine ganglion (PPG). (**b**) Acute stress (*red bars*) reduces the depressive effects of PVN microinjections of the $GABA_A$ agonist muscimol (*blue bars*), on both basal and meningeal-evoked responses of neurons simultaneously recorded in the Sp5C. Abbreviations: *3V* third ventricle; *7* facial nerve nucleus; *opt* optical tract; *Sp5O* spinal trigeminal nucleus, oralis part; *V1* ophthalmic; *V2* maxillary; *V3* mandibular divisions of the trigeminal nerve

confined to laminae I and II of the Sp5C and the superior salivatory nucleus (SSN). PVN cells can elicit, via SSN influences onto postganglionic parasympathetic neurons in the sphenopalatine ganglion, vasodilation and local release of inflammatory molecules that activate meningeal nociceptors. In this respect, a recent study has shown that SSN stimulation activates both Sp5C neurons and elicited cranial autonomic reactions, which were inhibited by drugs currently effective in trigeminal autonomic TACs treatments [82]. Interestingly, the same clusters of parvocellular PVN-Sp5C projecting cells are also densely labeled with corticotropin-releasing hormone (CRH) [83] and project to sympathetic and parasympathetic preganglionic neurons in the

brainstem and spinal cord implicated in the autonomic aspect of the stress responses [84].

Our recent findings showed that depression of PVN cells by the $GABA_A$ agonist muscimol inhibited both basal and nociceptive, meningeal-evoked activities of Sp5C neurons. A parallel processing of both HPA and trigeminovascular activities at the PVN level is further supported by our data indicating that $GABA_A$-mediated inhibition of the excitatory output of PVN cells onto Sp5C neurons is significantly reduced in a model of acute restrained stress [81]. As previously shown [85] acute stress reduces the properties of $GABA_A$ inhibitory synapses impinging on parvocellular PVN neurons by downregulating the transmembrane anion transporter KCC2, which maintains low intracellular Cl^- concentration, a prerequisite for the generation of Cl^- hyperpolarizing $GABA_A$-mediated responses. Such a loss of inhibition mediated by changes in the expression of KCC2 could thus constitute one of the mechanisms by which headaches may be generated primarily within the hypothalamus.

As a whole, these findings raise the possibility of top-down modulation conveyed by PVN cells, acting either on basal Sp5C activities or once meningeal nociceptors are stimulated, and mediated by sensory and autonomic PVN/trigeminal-SSN outflow mechanisms that are modified by stress. The question that remains is whether such hypothalamic links could be simply interpreted as putative "attack generators," since they are also likely to influence, or be influenced by, sensory information of the trigeminovascular pathway. Identification of migraine and TAC central maladaptive mechanisms will aid our understanding on the induction of attacks, will suggest new specific prophylactic therapies, and may prove useful tools for diagnosis of different headache types.

Summary

Taken together, these studies support the concept that CNS mechanisms which process trigeminal pain do not consist only of a bottom-up process whereby a painful focus modifies the inputs to the next higher level. Indeed, a number of CNS regions mediate subtle forms of plasticity by adjusting neural maps downstream and consequently altering all the modulatory mechanisms as a result of sensory experiences. Disturbances in normal sensory processing within these sensorimotor loops could lead to maladaptive changes, impaired oral and craniofacial functions, and consequent modifications in pain perception. Such ideas will help to bring together "bottom-up" and "top-down" mechanisms of trigeminal nociception and should be taken into account in future developments of therapeutic strategies aimed at improving life quality of patients suffering from impaired oral and craniofacial functions related to chronic pain.

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Part III

Biomarkers in Orofacial Pain

Neurophysiologic Markers of Neuropathic Orofacial Pain

5

Satu K. Jääskeläinen

Abstract

Comprehensive neurophysiologic and psychophysical examination provides unique, sensitive, and specific information about an underlying neuropathy in patients presenting with orofacial pain symptoms. These tests consist of special electroneuromyography techniques, brainstem reflex examinations, sensory and motor evoked potential recordings, as well as quantitative sensory testing of different sensory modalities (tactile, thermal, vibratory). The neurophysiologic diagnostic biomarkers for large and small nerve fiber systems can confirm definite diagnosis of neuropathic pain, also within the trigeminal distribution with up to 95–100% accuracy. They thus provide valuable differential diagnostic markers of neuropathic vs. musculoskeletal pain within the orofacial area. When used in appropriate combinations, these neurophysiologic markers allow accurate topographic-level diagnosis along the neuraxis from peripheral nerves to the cortex and help in guiding further imaging studies to the most likely region of underlying pathology. These tests have already elucidated neural mechanisms of various orofacial pain conditions including trigeminal neuropathic pain, trigeminal neuralgia, persistent idiopathic orofacial pain, primary burning mouth syndrome, and atypical odontalgia (or persistent dentoalveolar pain). In the future, neurophysiologic markers will hopefully open a way for individually tailored, mechanism-based treatment approaches. In addition, recent research indicates that neurophysiologic and psychophysical markers can provide invaluable prognostic information as regards, e.g., recovery and individual risk for persistent pain after nerve injury.

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5.1 Introduction to Neurophysiologic Diagnostics in Pain Patients

The diagnosis of neuropathic pain requires objective verification of a lesion or disease of the somatosensory system [1]. Neurological deficits, the hallmark of neuropathy and neuropathic pain, result from reduced signaling within the neuraxis after injury. However, negative sensory signs are difficult to confirm in clinical examination, especially in case of subtle or old injuries, or when positive symptoms and signs complicate the examination [2, 3]. False normal findings in clinical examination may occur in up to 94% of intraoperatively verified iatrogenic nerve injuries at late recovery [3, 4]. Similarly, clinical symptoms and signs alone give only modest to weak evidence for peripheral neuropathy [5]. In addition, pure small fiber damage may be difficult to verify in clinical examination with rough suprathreshold stimuli. As regards correct diagnosis and classification of pain on individual patient level, the importance of clinical neurophysiology and quantitative sensory testing (QST) is based on their ability to detect signs of subtle, even subclinical neuropathy from the peripheral receptors and nerves to the cerebral cortex [3, 6, 7]. Proper use of neurophysiologic techniques can also increase the yield of structural imaging via focusing to the "neurophysiologic region of interest" [8]. For definite diagnosis of neuropathic orofacial pain, adequate utilization of various combinations of these diagnostic techniques is often mandatory. As the trigeminal nerve provides sensory innervation for most of the orofacial region, this chapter will focus on neurophysiologic evaluation of the trigeminal system in neuropathic orofacial pain.

Clinical neurophysiology offers several sensitive and specific diagnostic markers for peripheral or central nervous system lesions and diseases, enabling detailed testing of both the large and small fiber sensory systems [1, 3, 6, 7, 9–11]. It provides objective and quantitative data that do not depend on patient's subjective report or cooperation. Neurophysiologic techniques are especially capable to reveal loss of function, and the cause for negative signs can be accurately localized along the neuraxis [7, 11]. Within orofacial region, in addition to electroneuromyography (ENMG) and evoked potential (EP) techniques, recording of brainstem reflexes provides invaluable information of the function of the trigeminal and facial nerves, their central connections, and top-down control of the brainstem circuits [3, 11]. Unlike neurophysiologic recordings, QST is a psychophysical measurement that requires good cooperation and is influenced by several other factors than capacity for sensory discrimination. Alterations in vigilance or motor reaction time as well as the inner subjective criterion or even malingering may have an effect on the results of QST [3, 12–15], and findings may thus need further confirmation with objective neurophysiologic or neuropathological investigations. QST methods and reference values have been published for several trigeminal distributions and for all sensory modalities including tactile, vibratory, and thermal sensations [3, 11, 14, 16, 17]. The tests can be performed either at chairside or, with better quality control and appropriate reference values, at the departments of clinical neurophysiology in order to detect and profile both negative (loss of function) and positive sensory signs (gain of function). In addition to neurophysiologic examination and QST, skin and mucosal biopsies can be done at optimally involved sites showing negative signs in the preceding neurophysiologic tests to confirm peripheral small fiber damage by measuring epithelial nerve fiber density (ENFD) [18]. Comprehensive reference values for epithelial small fibers and dermal myelinated fibers at all trigeminal skin distributions [19] should facilitate application of nerve fiber density measurements in the study of orofacial pain.

Although small fiber system damage of the peripheral small Aδ and C fibers or their central pathways is considered a prerequisite for the occurrence of neuropathic pain [1, 2], conventional neurophysiologic tests for large fibers constitute the first step in the differential diagnostic workout of possible neuropathic pain [1, 3, 7, 10, 11]. As all peripheral nerves include both large and small fibers, both fiber types are injured to various extents in most neuropathic processes. Thus, recordings investigating the large fibers can be used to detect, quantify, and locate potential underlying nerve lesion in orofacial pain

patients. They also enable assessment of prognosis and recovery. Specific neurophysiologic recordings or psychophysical tests for small fiber function are the next step in the diagnostic process [3, 7]. However, most of the small fiber tests do not allow topographic-level diagnosis; a lesion anywhere within the pathway from the skin to the somatosensory cortex will give rise to an abnormal result in thermal QST and laser (LEP) or contact heat evoked potential (CHEP) testing. Skin or mucosal biopsy for ENFD is needed to confirm or exclude peripheral small fiber neuropathy in case of abnormal QST or EP results [18]. Advanced immunohistochemical analyses of the biopsies will allow detection of functional changes in subepithelial fibers related to neuropathic pain such as increased expression of TRPV1 ion channels and purinergic receptors in burning mouth syndrome (BMS) [20, 21].

Besides their established value as diagnostic markers for neuropathic pain, neurophysiologic and QST methods have elucidated etiology and pathophysiology of many chronic orofacial pain conditions. These include trigeminal neuropathic pain after peripheral injury [22] or brainstem lesions [23], classical trigeminal neuralgia (CTN) and atypical or symptomatic trigeminal neuralgia (STN) [24, 25], postherpetic neuralgia [26], BMS [6, 27, 28], persistent idiopathic facial pain [PIFP; former atypical facial pain (AFP)] [29–31], and atypical odontalgia (AO) [9, 32]. Furthermore, neurophysiologic methods (somatosensory evoked potentials (SEP) or somatosensory evoked fields (SEF) recorded with electroencephalography or magnetoencephalography and navigated transcranial magnetic stimulation (TMS)) allow the study of cortical reorganization [31, 33] and alterations of intracortical excitability [34] associated with deafferentation pain.

5.2 Neurophysiologic Markers of the Orofacial Large Fiber System

ENMG of the trigeminal system requires special techniques for neurography as the main trunks of the nerve are located within deep, bony tissues. Motor action potentials can be elicited from muscles of mastication with needle stimulation of the masseter nerve [35, 36]. Sensory recording techniques have been described for the mandibular [37] and maxillary [38] distributions, with high diagnostic accuracy for iatrogenic inferior alveolar lesions and posttraumatic neuropathic orofacial pain [11, 22, 39]. Needle EMG can easily be performed at almost all cranial nerve motor distributions, including the laryngeal muscles.

Large fiber-mediated brainstem reflexes are useful diagnostic markers of neuropathic orofacial pain. Blink reflex (BR) can be elicited from eye closing muscles bilaterally after unilateral tactile or electrical stimulation of the trigeminal distributions (supraorbital, infraorbital, mental, and lingual nerves). The standard electrically elicited BR consists of two components, early ipsilateral R1 and later bilateral R2, all mediated via trigeminal tactile Aβ afferents (with higher intensities, also Aδ fibers may participate), brainstem nuclei, and motor fibers of the facial nerve. Corneal reflex (CR) and its recording resemble BR, but its afferent arc consists solely of nociceptive Aδ fibers that can be activated, e.g., with air puffs given to the cornea. Despite its probable usefulness, CR recording has only rarely been applied in the study of chronic orofacial pain. Masseter inhibitory reflex (MIR), inhibition of ongoing masticatory muscle activity after tactile stimulation of the infraorbital or mental nerve distributions, consists of two components, early silent period or exteroceptive suppression (SP1/ES1) and late SP2/ES2 recorded from masseter muscles bilaterally. The MIR arc involves trigeminal afferent and motor efferent fibers and their circuit within the pons. Jaw jerk reflex (JJR) is a tendon reflex elicited with a chin tap and recorded bilaterally from masseter muscles. It is mediated by trigeminal muscle spindle afferents and motor neurons, via trigeminal mesencephalic and pontine nuclei. It is often abnormal in extra-axial lesions compressing the mandibular nerve and causing demyelination. In case of abnormal JJR, needle EMG of the masseter muscles aids in localizing the lesion either to the rostral brainstem or the trigeminal motor efferents [3, 10, 11, 40, 41].

Revised NeuPSIG guideline on neuropathic pain assessment [1] recommends the trigeminal reflexes for orofacial pain diagnosis with level A evidence. Their diagnostic values in trigeminal

neuropathy and pain are shown in Table 5.1. The brainstem reflexes are accurate in revealing etiological lesions in STN and useful in localizing it within the neuraxis [42]. They aid in differential diagnostics, as in classical TN, the Aβ-fiber-mediated BR and MIR responses are normal [1, 11, 40]. Due to larger between-subject variability in R2 compared to R1 latencies, the R2 components of the BR are less sensitive although specific to trigeminal system lesions. Their sensitivity is best at acute stages, but they mostly normalize by 6 months if there are no obstacles to nerve regeneration [3, 11]. In orofacial neuropathic conditions, the various distinct patterns of abnormal brainstem reflex components allow very precise diagnosis and localization of lesions within the trigeminal system [11, 22, 28, 37, 40, 43]. Combined recording of JJR, BR, and MIR further increases topographic localizing accuracy [11, 23, 40, 43–45]. Using brain MRI as the reference, studies have shown excellent diagnostic precision for a combination of brainstem reflex recordings (JJR, BR, MIR) in detecting trigeminal lesions [sensitivity 100% and specificity 81% [44] and traumatic brainstem injuries [45]]. Similarly, combined use of trigeminal neurography, BR tests, and thermal QST leads to very high diagnostic accuracy (> 95–100%) for peripheral trigeminal lesions with or without pain [3, 4, 6, 11, 22, 39]. On the group level, abnormalities in BR recordings such as high reflex thresholds and prolonged response latencies have been found in patients with BMS, PIFP [6, 27, 29], and AO [32] giving support to the neuropathic nature of these pain states. On individual patient level, abnormal BR responses compared to reference values can be found in distinct subgroups of BMS and PIFP patients [6, 29]. The patterns of reflex abnormalities indicate that subclinical lesions within the trigeminal system either at the peripheral or the brainstem level can give rise to clinically typical BMS or PIFP symptoms, as similar although more severe findings occur in clinically obvious trigeminal neuropathic pain [3, 6, 22, 29, 36].

Table 5.1. Clinical and research indications of clinical neurophysiologic and psychophysical tests for orofacial neuropathic conditions and their diagnostic accuracy as appropriate or available

Test	Sensitivity[b]	Specificity[b]	Application in the orofacial region	Abnormal findings
Electromyography (EMG)	80–90%	> 90%	Needle EMG of muscles innervated by cranial nerves V, VII, IX, X, XI, XII – cranial neuropathy and neuropathic pain	Loss of function: signs of denervation in case of loss of motoneurons; signs of collateral and axonal reinnervation; localization, activity and age of the lesion
Neurography (nerve conduction velocity and response amplitude)	80–90%	60–100%	Infraorbital, inferior alveolar (mental), lingual, and major auricular sensory nerves, and masseteric motor nerve; neuropathic pain related to their peripheral lesions	Loss of function: small or absent responses, slow conduction velocity, conduction blocks, type of injury (axonal or demyelinating), extent of axonal injury, level of injury
Transcranial magnetic stimulation: motor evoked potential			Trigeminal, facial, and accessory spinal nerves (pyramidal tract and α motoneurons) – lesions within the pyramidal tract and peripheral nerves	Loss of function: small or absent responses, slow central or peripheral motor conduction
Transcranial magnetic stimulation: mapping of the motor cortex			Mapping of the representation of hand and facial or masticatory muscles within the M1 cortex – study of cortical reorganization	Quantification of maladaptive cortical plasticity: shrinkage of representation area in pain, recovery after successful treatment

Table 5.1. (continued)

Test	Sensitivity[b]	Specificity[b]	Application in the orofacial region	Abnormal findings
Transcranial magnetic stimulation: paired-pulse techniques			Study of intracortical inhibition and facilitation – changes in cortical excitability in neuropathic pain	Gain of function: decrease in cortical inhibition in neuropathic pain; differential diagnostics of neuropathic and musculoskeletal pain
Blink reflex with electrical stimulation	R1 59–100% R2 27–59%	R1 93–100% R2 60–100%	Trigeminal sensory divisions (Aβ afferents in SON, ION, MN, LN), pontine and medullary brainstem, facial nerve – peripheral and central nervous system lesions and neuropathic pain	Loss of function: delayed, small, or absent responses; accurate topographic-level diagnostics in orofacial pain and peripheral or brainstem lesions within the trigeminofacial system
Habituation of the blink reflex, paired-pulse techniques			Dopaminergic nigrostriatal and serotonergic striatoreticular inhibitory control of the brainstem – increased excitability/ decreased inhibition in neuropathic orofacial pain	Gain of function: deficient habituation of the R2 component due to decreased top-down inhibition; defects in the nigrostriatal dopamine system function in BMS and PIFP, verified with PET[a]
Blink reflex with laser or nociceptive electrical stimulation			Trigeminal small fiber system: nociceptive Aδ afferents and their CNS pathways – neuropathic pain	Loss of function: small or absent or delayed responses in trigeminal system lesions and neuropathic orofacial pain
Corneal reflex			Corneal Aδ afferents, brainstem, facial nerve	Loss of function: absent or delayed responses
Jaw jerk (masseter reflex)			Muscle spindle Ia afferents from masticatory muscles, mandibular nerve, mesencephalic and pontine nuclei of the trigeminal nerve	Loss of function: absent or delayed responses
Masseter inhibitory reflex	SP1 59–100%	SP1 93–100%	Trigeminal sensory afferents (ION, MN), pontine trigeminal motor nucleus, and α motoneurons	Loss of function: absent or delayed responses
Trigeminal somatosensory evoked potential (TSEP)			Needle stimulation of the SON, ION, and MN – early waves (up to 10 ms) useful in demonstrating trigeminal Aβ-fiber dysfunction and brainstem pathology	Loss of function: small or absent or delayed responses in trigeminal system lesions and neuropathic orofacial pain, subtle abnormalities in classical trigeminal neuralgia, level diagnosis
Laser evoked potential (LEP)	64%	83%	Trigeminal Aδ (heat pain) and C (warm) small fibers; peripheral and central pathways; cortical sources within anterior cingulum and insular cortex	Loss of function: small or absent or delayed responses in trigeminal system lesions and neuropathic orofacial pain, abnormalities even in classical trigeminal neuralgia, no level diagnosis Gain of function: abnormal habituation in migraine

(continued)

Table 5.1. (continued)

Test	Sensitivity[b]	Specificity[b]	Application in the orofacial region	Abnormal findings
Contact heat evoked potential (CHEP)	Same as LEP	Same as LEP	Same as LEP, but also Aδ (cool) fibers can be studied	Loss of function: small or absent or delayed responses in trigeminal system lesions and neuropathic orofacial pain, no level diagnosis
Quantitative sensory testing (QST): warm detection threshold	40–60%	80–100%	Small fiber system for innocuous warming (C fibers), from the periphery to the cerebral cortex	Both loss (hypoesthesia, anesthesia) and gain of function (hyperesthesia, allodynia), no level diagnosis
QST: cool detection threshold	40–65%	80–100%	Small fiber system for innocuous cooling (Aδ fibers), from the periphery to the cerebral cortex	Both loss (hypoesthesia, anesthesia) and gain of function (hyperesthesia, allodynia), no level
QST: heat pain detection threshold	10–40%	90–100%	Small fiber system for noxious heat (Aδ fibers), from the periphery to the cerebral cortex	Both loss (hypoalgesia, analgesia) and gain of function (hyperalgesia, allodynia), no level
QST: cold pain detection threshold			Small fiber system for noxious cold (Aδ fibers), from the periphery to the cerebral cortex	Both loss (hypoalgesia, analgesia) and gain of function (hyperalgesia, allodynia), no level
QST: tactile detection threshold	30–60%	50–90%	Large fiber system (Aβ afferents), from the periphery to the cerebral cortex	Both loss (hypoesthesia, anesthesia) and gain of function (hyperesthesia, allodynia), no level

SON supraorbital nerve, *ION* infraorbital nerve, *MN* mental nerve, *LN* lingual nerve, *dx* diagnosis, *R1* early blink reflex component, *R2* late BR components, *SP1* early component of masseter inhibitory reflex, *BMS* primary burning mouth syndrome, *PIFP* persistent idiopathic orofacial pain

[a]Neurotransmitter positron emission tomography (PET)

[b]Diagnostic values gathered from: [1, 3, 4, 7, 11, 39, 42, 44, 45, 57] all figures not exclusively for orofacial region

While brainstem reflexes are convincingly useful markers for neuropathic orofacial pain, they do not provide reliable positive diagnostic markers for temporomandibular pain [41, 46]. Regarding musculoskeletal or nociceptive orofacial pain, neurophysiologic methods are currently useful only in their differential diagnosis from neuropathic pain.

Somatosensory EPs to electrical stimuli may be applied to study trigeminal Aβ afferent fibers (TSEP), but this requires special needle stimulation technique at low intensities for reliable recording of the early potentials arising from the brainstem, thalamocortical radiation, and the S1 cortex and occurring within the first 10 ms of stimulation [10, 47]. Most studies reporting on TSEP findings in orofacial patients with neuropathy or pain have used imprecise surface stimulation and unreliable analysis of the variable middle or long latency TSEP components or muscle and reflex activity contaminating the recording (for detailed discussion, see [10, 47, 48]). Nevertheless, appropriately performed TSEP recordings are able to reveal subtle abnormalities in large fiber function, such as focal demyelination in CTN patients [48]. Transcranial magnetic stimulation (TMS) is used to elicit motor evoked potentials (MEP) for the investigation of central and peripheral motor pathways, also within the cranial nerve distributions. While abnormal MEP may aid in localizing neuropathic lesions of the trigeminal and facial systems [35], the results are normal in TMD [46]. So far, the TMS method has only rarely been applied to study orofacial pain. Yet, new navigated TMS devices hold promising potential for the study of

central mechanisms of neuropathic pain, and repetitive TMS can even be used to treat intractable trigeminal pain.

5.3 Neurophysiologic Markers of the Small Fiber System

Neurophysiologic markers of small fiber dysfunction include brainstem reflex recordings (BR and MIR) and EP recordings with painful electrical, laser, or contact heat stimuli [7, 26, 48–51]. The possibility to directly measure the small fiber system, the lesions of which are considered responsible for most of the sensory phenomena related to neuropathic pain, has raised considerable interest during the last decade. Neuropathic orofacial pain offers an optimal target for the application of these techniques as contact heat and laser stimuli to the trigeminal distributions elicit the largest and most stable CHEP and LEP responses at the central EEG derivations. After stimulation of the epithelial small Aδ-fiber endings, the main nociceptive N2-P2 complex arises from the anterior cingulum, with preceding small bilateral early components of insular origin [7, 26, 52].

With specific settings, laser stimulators allow separate analysis of the function of the Aδ- and C-fiber tracts [26, 48, 53]. C-fiber stimulation seems to be technically more demanding with contact heat stimulator [26] that may, however, allow recording of cold evoked potentials. The price of the new laser stimulators (neodymium- or thulium-YAG/YAP) able to give short stimuli with a rise time of 1000 °C/s that is optimal for Aδ LEP recordings is rather high. In addition, laser stimulators bear a risk for burn injuries as well as for retinal damage. These facts currently limit the availability of neurophysiologic techniques utilizing laser stimulation although they have been suggested to be the most useful diagnostic markers of neuropathic pain [1, 7]. Contact heat stimulators have the advantage that the skin temperature is always controlled, and burn injuries do not occur. In CHEP recording, the stimulus risetime is slower (70 °C/s), and the responses therefore are more dispersed and latencies longer. A recent study [51] using both laser and nociceptive electrical stimulation to evoke pain-related evoked potentials (PREP) showed that electrical stimulation might offer a safer and better tolerated means than LEP to study small fiber system. However, unlike laser and contact heat stimuli, electrical stimuli always simultaneously activate the large Aβ afferents in addition to small Aδ pain fibers, and thus, PREP is not a pure measure of small fiber function.

Table 5.1 summarizes the indications and diagnostic values of LEP and CHEP recordings in verifying loss of function within the Aδ- and C-fiber-mediated small fiber systems in neuropathic pain and trigeminal system damage [26, 49]. Specifically, Aδ-fiber-mediated LEP and CHEP recordings are helpful in detecting sensory neuropathy and, consequently, recommended for the diagnosis of neuropathic pain with level A evidence [1, 7]. The use of specific nociceptive stimuli increases the diagnostic sensitivity for an underlying neuropathy, as LEP recordings have demonstrated abnormal small fiber function in up to 100% of STN patients and even in half of the CTN patients irrespective of the findings in clinical examination [24]. In addition, PREP recordings have shown that patients with CTN (only paroxysmal pain) have delayed nociceptive EPs and BR and reduced amplitudes, whereas the PREP responses are larger and have shorter latencies in patients with atypical TN [25]. These large pain-related EPs probably reflect deficient habituation of single consecutive EP responses in atypical TN, similar to the phenomena found in LEP studies on headache [54] and BR studies on BMS and PIFP [3, 6, 27, 29, 31]. Furthermore, in trigeminal neuropathy with persistent burning pain, multimodal EP recordings have demonstrated specific sparing of C-fiber responses in the absence of Aβ and Aδ responses [10], indicating that the remaining C fibers are responsible for mediating the ongoing burning pain sensation. In line, abnormalities in Aδ- and C-fiber LEPs are associated with ongoing burning pain in postherpetic neuralgia, whereas abnormal Aβ-fiber-mediated BR responses occur in those PHN patients who have paroxysmal pain symptoms [26]. Clinical neurophysiologic techniques

are evidently able to elucidate distinct pathophysiological mechanisms of chronic trigeminal and orofacial pain and their association with subjective symptoms. However, there are still scarce data on LEP findings in different orofacial pain conditions and even less on CHEP or PREP techniques.

On group-level comparisons, BR responses to nociceptive-specific electrical stimulation [50] of the supraorbital, infraorbital, or mental nerves have been found to be smaller and have longer latencies both in CTN [25] and AO patients [32], which may indicate neuropathic etiology at least in part of the AO patients. It remains to be investigated whether nociceptive-specific BR or MIR can be utilized for individual patient diagnostics in orofacial pain conditions. In AO, this would probably require very focal stimulation of the distal alveolar nerve branches that may be damaged after dental procedures.

5.4 Psychophysical Markers in Orofacial Pain

QST, with appropriately gathered and applied reference values, improves diagnostic accuracy for trigeminal neuropathy compared to standardized clinical sensory examination (Table 5.2) [3, 4, 22, 27, 29, 39]. In the majority of neuropathic pain patients, loss of function can be confirmed with QST. Thermal hypoesthesia first indicated small fiber system hypofunction in BMS [27]. This finding was later verified with ENFD to be due to focal small fiber neuropathy of the tongue mucosa [18]. Similar thermal hypoesthesia has been reported to occur in PIFP and neuropathic facial pain due to trigeminal injuries [22, 29]. However, QST is only moderately sensitive compared to objective and more accurate neurophysiologic methods [3, 4, 39] or ENFD measurements [55]. Best diagnostic accuracy for neuropathy and neuropathic pain is reached when neurophysiologic recordings are combined with thermal QST or ENFD measurements, all complementing each other [3, 4, 7, 27, 29, 39, 56, 57].

The sensitivity of orofacial QST in neuropathy diagnosis varies according to sensory modality tested, type of change (loss or gain of function), and time of testing. As shown in Tables 5.1 and 5.2, elevation of tactile and innocuous thermal detection thresholds (hypoesthesia) offers better diagnostic yield for trigeminal neuropathic pain than heat pain detection thresholds (HPT) or cold pain detection thresholds (CPT) that, due to large between-subject variation (in the order of 10–30 °C), have wide reference limits and, thus, are insensitive diagnostic tools.

Table 5.2. Comparison of diagnostic values (%) of qualitative clinical sensory tests, quantitative sensory testing, and neurophysiologic recordings in the diagnosis of inferior alveolar nerve (IAN) neuropathy: results of intraoperative neurophysiological monitoring (at 2 weeks) combined with subjective report of sensory alteration (at 1 year) were used as the "gold standard" of nerve injury

Test	Sensitivity at 2 weeks/1 year	Specificity at 2 weeks/1 year
Brush stroke direction	40/0	89/100
Sharp/blunt discrimination	40/0	89/100
Warm/cold discrimination	44/7	100/100
Grating orientation discrimination	59/27	73/88
Tactile detection threshold	58/33	56/88
Cool detection threshold	64/40	100/88
Warm detection threshold	50/47	100/92
Heat pain detection threshold	43/13	100/96
Blink reflex of the mental nerve	59/27	60/100
Neurography of the IAN	88/82	55/100

Source: Modified from [3, 4, 39]. Normality of all QST and clinical neurophysiologic (CN) test results was determined according to own reference values gathered in the same CN laboratory (with a quality-control system accredited since 2003 according to the ISO/IEC 17025:2005 standard) with exactly same equipment, device settings, and instructions in healthy subjects

At individual patient level, thermal pain detection most often remains normal, especially in subtle or old injuries (>6 months), whereas tactile detection thresholds (TDT), cool detection thresholds (CDT), and warm detection thresholds (WDT) may help in confirming the diagnosis even at late stages of recovery [3, 4, 22]. Similarly, hypofunction in thermal QST (loss of function in innocuous modalities) most often occurs in patients with neuropathic pain as recently reported in a large multicenter study [58].

Appropriate reference values are extremely important in diagnostic use of QST. Due to spatial summation effect, thermode size has significant influence on pain and warm detection thresholds that are higher with smaller thermode [3, 11, 59]. Furthermore, thermode size should always be appropriate to the nerve distributions under study in order not to stimulate the neighboring intact territories [3, 11, 14, 60]. Density of small fiber endings varies between body sites, being highest in the fingertips and the lips and lowest on the trunk and proximal parts of the extremities – this variation is reflected in QST detection thresholds that are lowest on the palmar skin of the hands and near midline of the face [16, 61]. Consequently, reference values gathered, e.g., laterally at the cheek with large thermode cannot be applied to the small mental nerve distribution near midline [11, 16]. Inappropriate thermode size or reference site may blur diagnostic accuracy of QST; abnormalities in QST profiles were reported in 41% of healthy subjects with a recently launched protocol [58] in which reference values gathered at distal legs or hands may be applied to investigate proximal symptoms.

A major limitation of QST method is the lack of topographic-level diagnostic efficacy. A lesion anywhere along the neuraxis from the skin to the cortex may cause abnormalities in QST, and specific diagnosis requires additional investigations [3, 7, 15, 56, 57]. Similar to the spread of pain symptoms, thermal hypoesthesia is liable to extend beyond the original neuroanatomical borders both extrasegmentally and across the midline especially in neuropathic pain [22, 62], which makes the use of homologous contralateral site an unreliable reference in QST. The same applies to positive sensory signs in QST showing a pronounced tendency to contralateral and extrasegmental spread both in musculoskeletal [63] and neuropathic pain. The use of peripheral anesthetic blocks may aid in differentiating peripheral from central nervous system pathology [64], but neurophysiologic recordings, ENFD, or radiological imaging is crucial for exact localization of the cause of pain [3, 6, 7, 11].

Hypoesthesia to innocuous thermal stimuli is rather specific for neuropathic pain [2, 3, 58, 65], whereas tactile hypoesthesia may occur in chronic musculoskeletal and inflammatory pain [1]. QST offers a unique possibility to measure and quantify positive sensory phenomena (hyperesthesia, hyperalgesia, allodynia) in humans. None of these are specific to neuropathic conditions, though occurring also in musculoskeletal and inflammatory pain [2, 3]. Thus, gain of function does not differentiate neuropathic and nociceptive pain, although cold allodynia is mostly found in neuropathic pain [1, 2, 15]. However, thermal allodynia seems to be very rarely present in trigeminal neuropathic pain [22, 27, 29, 60, 65].

QST findings can provide clues to the type and severity of trigeminal nerve injury and, hence, prognostic information about recovery and risk of neuropathic pain. Hypoesthesia in thermal QST indicates small fiber injury and moderate to severe axonal nerve lesion [4, 11, 65], whereas tactile hypoesthesia occurs both in demyelinating and axonal nerve damage [4, 36]. At the acute stage, loss of function in thermal QST reflects axonal injury and small fiber deafferentation, which predicts later development of chronic neuropathic pain [36, 65]. Initially severe loss of thermal sensibility (i.e., severe axonal damage) predicts poor overall recovery from peripheral nerve injury [36, 65]. Furthermore, in line with observations on inferior alveolar nerve injuries with neurography [22] and experimental evidence from spinal cord injuries [66], QST findings have demonstrated that less severe, partial nerve injury may be more frequently associated with the occurrence of neuropathic pain than severe or total loss of function [65]. Less extensive axonal injury may also cause gain, instead of loss, of function in distinct subgroups of AO [60], BMS [27], and PIFP [29] patients, since very

minor nerve injuries seem to induce mainly positive signs [22]. In addition, originally low HPT, i.e., high thermal pain sensitivity, is considered an independent risk factor for development of persistent postsurgical [67] and TMD pain [65].

Pain measures in QST vary widely between subjects due to differences in subjective criterion [13] but are rather stable within a subject between repeated tests [59]. The within-subject consistency forms the basis for the use of QST and gain of function profiles in the follow-up of treatment effects and disease progression or recovery [1, 15]. This potential of QST still mainly waits for future application to orofacial pain.

5.5 Markers of Altered Excitability Within the Neuraxis

Brainstem reflex recordings have provided markers to study increased excitability within the trigeminal system in neuropathic orofacial pain. Normally, the BR responses habituate, i.e., the area under R2 response decreases with steady repetition of stimuli at ≥1 Hz frequency. Habituation of BR is under nigrostriatal dopaminergic inhibitory control [6]. Deficient habituation of the BR has been found in patients with trigeminal neuropathic pain[29], BMS [27, 28], and PIFP [29, 31], suggesting deficient inhibitory control of the brainstem. Subsequent neurotransmitter PET studies have shown defects in nigrostriatal dopamine system reminiscent of early Parkinson's disease in both BMS and PIFP patients [6, 68]. This increased trigeminal excitability extrasegmental to the distribution of peripheral neuropathy may represent a marker of deficient top-down inhibition that in turn might be a risk trait for the development of neuropathic pain. Similarly, reduced habituation of pain-related LEPs has been interpreted to indicate increased excitability of the somatosensory cortex in migraine [54]. Excitability of the trigeminal system may also be evaluated with paired-pulse stimulation and brainstem reflex recordings, as has been done for the study of TMD patients with normal results [46].

Conclusions

Neurophysiologic and psychophysical tests provide useful and accurate diagnostic, mechanism-related, and prognostic functional markers for neuropathic pain, but their full potential has not yet been explored in orofacial pain states. The diagnostic sensitivity of the tests is best at the acute stage after nerve damage, but they are superior to clinical examination also in chronic conditions and at late stages of recovery. Reports on deficient habituation of EPs to multimodal stimuli in migraine patients [54] raise the quest for studies on habituation of EPs, utilizing single-trial analyses and multimodal salient stimuli, also in different orofacial pain entities. Neurophysiologic demonstration of deficient central inhibition or increased excitability could serve as a marker for increased risk of chronic neuropathic pain after injury. It might also provide a marker for patient selection to pain treatment with new noninvasive neuromodulation techniques such as repetitive TMS, known to release endogenous dopamine. For future work, there are additional exciting neurophysiologic markers that can evaluate phenomena occurring in neuropathic pain such as cortical reorganization measured with source analysis of SEF or SEP responses [31, 33], as well as specific tests for cortical excitability and intracortical inhibition, assessed in detail with paired-pulse TMS techniques [34]. With a few exceptions [31], these have not been explored in chronic orofacial pain yet. TMS can also be used to assess training-induced plasticity within the primary motor cortex, as has been shown, e.g., for tongue musculature in healthy subjects [14]. With the recently introduced neuronavigated TMS devices, cortical mapping of the motor representation areas with an accuracy of a few millimeters [69] offers a precision tool for the study of injury or disease as well as plasticity- and treatment-related changes in cortical motor maps. These novel neurophysiologic markers of neuropathic pain-related brain level alterations still mostly wait for application to the study of chronic orofacial pain. In

the future, proper combinations of neurophysiologic, psychophysical, and neuropathological tests and biomarkers for assessment of neuropathic changes at the peripheral, brainstem, and cortical levels together with detailed profiling of functional alterations along the neuraxis will provide an accurate outline of structural and functional markers related to different orofacial pain conditions both on group and individual patient levels for precise tailored treatment approaches.

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Masticatory Muscle Pain Biomarkers

6

Malin Ernberg

Abstract

This chapter focuses on the potential role of biomarkers for masticatory muscle pain, i.e., myalgia. To date, no biomarkers have been identified that can be used clinically for diagnosis or treatment of myalgia of jaw muscles. There is evidence from microdialysis studies that intramuscular levels of glutamate and serotonin are elevated in patients with chronic myalgia, including myalgia of jaw muscles. High muscle levels of glutamate and serotonin correlate to pain intensity and mechanical allodynia, and both glutamate and serotonin have been shown to induce pain and mechanical hyperalgesia when injected into jaw muscles. This pain, consequently, can be blocked with specific receptor antagonists, indicating that glutamate and serotonin may be promising biomarker candidates. However, muscle levels of glutamate and serotonin do not correlate to plasma levels, which is a disadvantage since measuring intramuscular biomarker levels with currently available techniques is too complicated to be clinically useful. Nerve growth factor (NGF) has also been shown to cause long-lasting hyperalgesia, albeit with no pain, when injected into jaw muscles, but muscle biopsies did not show any differences in NGF levels between patients with jaw myalgia and pain-free controls. Additionally, muscle levels of prostaglandins, bradykinin, or substance P, commonly characterized pain mediators, do not seem to be elevated in myalgic jaw muscles. Because pain mediation and peripheral sensitization are complex events that involve many substances, future research should focus on investigating intramuscular profiles of multiple biomarkers. This, in turn, is possible with newly developed methods, such as proteomics and metabolomics.

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6.1 Introduction

Chronic masticatory muscle pain, i.e., myalgia, is a disorder affecting approximately 10% of the population and of which two thirds are women [1]. It is therefore a commonly treated disorder among

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clinicians, and although extensive research has focused on the pathogenesis of chronic muscle pain in the last decades, the nociceptive mechanisms that underlie the pain are still largely unknown [2]. This, in turn, requires that clinicians rely on patient reports, questionnaires, and semi-objective findings for diagnostics. For example, in the recently published Diagnostic Criteria for Temporomandibular Disorders (DC/TMD), there are three subclasses of myalgia; local myalgia, myofascial pain, and myofascial pain with referral [1]. The same main criteria are used for all diagnoses, and they differ only regarding the presence of pain spread with palpation and if the pain spreads within or beyond the boundary of the muscle. However, the pathogenesis underlying these diagnoses may not be the same and may also differ between patients with the same diagnosis. Pain is always a subjective experience, and semi-objective clinical methods like muscle palpation or assessment of pressure pain thresholds (PPT) have limited sensitivity and correlate only weakly with ongoing pain ratings [3]. There is therefore a need for more objective and sensitive tools, which has led to an increasing research interest in biomarkers for pain. Biomarkers are specific biochemicals in the body with a well-defined molecular feature that may be used in disease diagnosis and disease progress and to evaluate treatment effects [4] (see Chap. 10).

Biomarkers in muscle pain can be divided into three main categories; algesic biomarkers, tissue metabolites, and inflammatory mediators [5]. Algesic biomarkers are substances that can directly activate muscle nociceptors, such as glutamate and protons, whereas tissue metabolites are important substances in the cell metabolism, such as lactate and pyruvate. Inflammatory biomarkers are substances released in the tissue during inflammation, such as prostaglandins and cytokines (see further below).

6.2 Methods for Sampling and Analyzing Muscle Biomarkers

To be clinically useful, potential biomarkers have to be easily and reliably measured and also need to correlate to pain variables (e.g., pain intensity). Thus, the ideal biomarker could be measured in body fluids that are easily sampled at a low cost – such as blood, urine, or saliva (see Chaps. 9 and 10).

Conversely, since the events leading to pain may occur within the muscle itself, any change in intramuscular biomarker levels may not significantly alter systemic levels. Therefore, techniques that could measure biomarker levels in the muscle, such as muscle biopsies, may be useful. The disadvantages of biopsies, however, are both the tissue trauma induced and the difficulty of accessing certain muscle groups.

Microbiopsy techniques, which are used, for example, in cancer diagnosis, may reduce tissue trauma and thus be more useful but have not been adopted in muscle pain research until recently and need to be validated (Fig. 6.1).

Another technique of potential use is microdialysis. This technique allows for continuous monitoring of biomarkers in tissues in vivo, which is the major advantage over biopsies. By inserting a hollow microdialysis catheter with a dialysis membrane at its tip into the tissue and very slowly perfuse it with a saline buffer, mole-

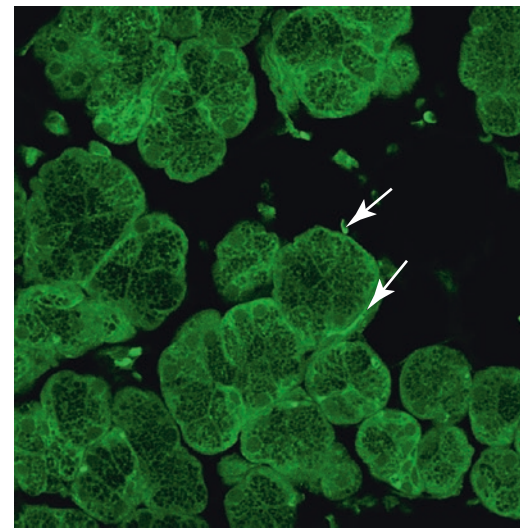

Fig. 6.1 A representative confocal image showing the expression of 5-HT_3 receptors on sensory nerve fibers surrounding myocytes in the human masseter muscle. The biopsy was taken from a pain-free volunteer with microbiopsy technique. The fluorescent intense *green color* marked by *arrows* represents 5-HT_3 receptor expression (Courtesy of Dr. Nikolaos Christidis)

cules may diffuse passively across the dialysis membrane and be sampled for later analysis. Using different techniques, the true tissue concentration of the biomarkers may be calculated. The major disadvantages of microdialysis are that the technique is time-consuming, complicated, and expensive and that it requires sensitive analyses due to the limited volumes of fluid that can be collected.

A problem with all the sampling methods outlined is potential diurnal variations in biomarker levels, which either must be ruled out or controlled for by sampling at the same time of the day (see Chap. 10). Tissue fluid levels of biomarkers may also be influenced by, e.g., age, gender, and body mass, which must also be considered. Furthermore, in most commercial kits, only single biomarkers can be analyzed, which makes analyses expensive and time-consuming if multiple biomarkers are to be assessed. More recent analyses that can combine several biomarkers have been developed. Bio-Plex, for example, uses color-coated polystyrene beads that allow analyses of up to 100 biomarkers in one sample using as little as 25 μL sample. The rapid development of proteomics and metabolomics with multi-panel analyses using protein or DNA microarrays, additionally, is also a promising tool for biomarker analyses.

6.3 Muscle Pain Pathophysiology

Myalgia is characterized by spontaneous pain that is aggravated by function and muscle soreness (hyperalgesia); pain referral is also frequently noted. It was previously believed that myalgia was caused by muscle inflammation, but in most myalgic pain conditions, there are no gross signs of inflammation or tissue trauma [6]. However, inflammation may still be present on a molecular level (neurogenic inflammation). There seems to be agreement about both peripheral and central mechanisms participating in muscle pain development and that the longer pain persists, the role of the central mechanisms becomes more pronounced. However, many researchers believe that even in chronic myalgic conditions, peripheral input is needed to drive pain. There is good evidence from animal studies that muscle trauma and ischemia may cause neurogenic inflammation, which is a normal response that promotes healing [2]. During this process, peripheral sensory afferent nerves are activated which leads to antidromic release of neuropeptides, such as substance P (SP), calcitonin gene-related peptide (CGRP), neurokinin A (NKA), and vasoactive intestinal peptide (VIP). These substances promote the release of other chemicals, e.g., prostaglandins, nerve growth factor (NGF), bradykinin, and serotonin (5-HT) that activate and sensitize the neuron by binding to specific receptors on the peripheral nerve terminal (Table 6.1) [2, 7]. Another important molecule is the vanilloid receptor (TRPV1) that seems to have a key role in initiating the neurochemical cascade associated with neurogenic inflammation [2, 7]. There is also evidence of a role for

Table 6.1 Potential biomarkers for muscle pain and their peripheral receptors

Endogenous ligand	Peripheral receptor
Glutamate	NMDA, AMPA, mGlu
Serotonin	5-HT1, 5-TH2, 5-HT3, 5-HT7
Nerve growth factor	TrkA, p75
Protons	TRPV1, ASIC
Bradykinin	BK1, BK2
Eicosanoids	
PGE2	EP1, EP2, EP3, EP4
LTB4	LTB41, LTB42
Neuropeptides	
SP	NK1
CGRP	CGRP1
NPY	Y1, Y2, Y4
Cytokines	
IL-1beta	IL1RI, IL1RII
TNF	TNFRI, TNFRII
IL-6	IL-6R
IL-8	IL-8R alpha, IL-8R beta

PGE2 prostaglandin E2, *LTB4* leukotriene B4, *SP* substance P, *CGRP* calcitonin gene-related peptide, *NPY* neuropeptide Y, *IL* interleukin, *TNF* tumor necrosis factor, *NMDA* *N*-methyl-*D*-aspartate, *AMPA* α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, *mGlu* metabotropic glutamate receptor, *5-HT* 5 hydroxytryptamine, *TrKA* tyrosine kinase A, *p75* p75 neurotrophin receptor, *EP* E-prostanoid, *TRPV1* transient receptor potential vanilloid 1, *ASIC* acid-sensing ion channel, *BK* bradykinin receptor, *NK* neurokinin 1

peripheral glutamate receptors in this peripheral sensitization. If peripheral sensitization continues for a prolonged period, central sensitization develops during which sensitization of second order *N*-methyl-*D*-aspartate (NMDA) glutamate receptors seems to play an important role [2, 7].

Besides these pain biomarkers, of which many have been of interest as candidates for muscle biomarkers, there are also inflammatory mediators, such as cytokines, that may also serve as biomarkers in jaw myalgia. Most cytokines are released from circulating immune cells, e.g., neutrophils, monocytes, and mast cells during inflammation, but some may also participate in neurogenic inflammation. Other potential biomarkers include metabolites, such as lactate, pyruvate, glucose, and glycerol. To date, no single biomarkers have been identified for jaw myalgia, but there are a few promising candidates. The following section of this chapter will deal with substances that have been a target for research as potential biomarkers for jaw myalgia.

6.4 Potential Biomarkers in Jaw Myalgia

6.4.1 Glutamate

Glutamate is an excitatory amino acid present in afferent sensory nerves where it acts as the main neurotransmitter for conveying sensory information to the central nervous system (CNS). It is present both in the trigeminal ganglion and in the central and peripheral nerve terminals and released in response to intense noxious stimulation or inflammation [8]. Immunohistochemistry has shown that glutamate receptors (GluRs) are present on peripheral nerve terminals, which makes it likely that glutamate would interact with these receptors. Three types of ionotropic GluR subtypes have been identified, all of which are present on peripheral nerve terminals: NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate (Table 6.1). There are also metabotropic GluR present on peripheral nerve terminals. Animal studies have shown that tissue glutamate levels are increased and that ionotropic GluRs are upregulated during inflammation. By activation of peripheral GluRs (AMPA, NMDA), glutamate excites peripheral sensory afferents and sensitizes them to thermal and mechanical stimuli [8].

Additionally, in human tissue, levels of glutamate are elevated in inflammatory conditions, such as rheumatoid arthritis [8], and glutamate injection evokes pain, mechanical sensitization, and increased blood flow [8]. In addition, ingestion of glutamate increased muscle glutamate levels more in patients with jaw myalgia than controls. However, thus far no specific GluR antagonist for clinical use has been developed and local administration of the noncompetitive NMDA antagonist ketamine shows contradictory results.

6.4.2 Serotonin

Serotonin, or 5-hydroxytryptamine (5-HT), is a small molecule of the monoamine family that exerts a range of biological effects in the human body and modulates physiological processes in both the central and peripheral nervous systems. 5-HT is synthesized both peripherally and in the CNS from the essential amino acid tryptophan, which is derived from the diet. Peripherally, the major sources of 5-HT are the enterochromaffin cells of the small intestines and enteric neurons, but 5-HT is also present in platelets and mast cells, and a small fraction of 5-HT is also unbound in the blood. In the CNS, 5-HT is found in serotonergic neurons, including those in the nucleus raphe magnus [9].

In response to tissue trauma and inflammation, 5-HT is released and, by activating receptors on peripheral sensory nerves, may activate and sensitize these peripheral neurons. Of special importance is the 5-HT_3 receptor, which seems to be important for mediating pain from the periphery [9]. Recent data shows that 5-HT_3 receptors are present on sensory nerves in the human masseter muscle (Fig. 6.1), and in patients with jaw myalgia more nerve fibers in the masseter muscle express 5-HT_3 receptors compared to controls. This suggests a potential role of 5-HT in myalgia.

Indeed, tender point injection with the 5-HT_3 receptor antagonist granisetron into the masseter muscle alleviate jaw myalgia. 5-HT also sensitizes peripheral mechanoreceptive afferent fibers to other chemicals, e.g., glutamate, SP, and CGRP, by enhancing the efficiency of tetrodotoxin-resistant sodium channels and lowering the threshold of TRPV1 receptors, which results in primary hyperalgesia [9].

6.4.3 Nerve Growth Factor

Nerve growth factor (NGF) belongs to the family of neurotrophins. NGF binds both to the low-affinity transmembrane receptor p75 and also to the high-affinity tyrosine kinase receptor A (TrkA), both of which are expressed on sensory neurons. It has been shown that the expression of these receptors in the trigeminal system is higher than in the spinal system [10]. Activation of TrkA receptors by NGF results in a cascade of intracellular and extracellular events leading to peripheral sensitization.

Animal research has shown an increase of endogenous levels of NGF after tissue injury and that inflammation and intramuscular administration of NGF in rodents is associated with mechanical and thermal hyperalgesia and nociceptive processing within the CNS [10].

Furthermore, levels of NGF are elevated in many clinical pain conditions, such as rheumatoid arthritis, cancer pain, and degenerative disc disease. TrkA receptors are co-localized with TRPV1 receptors, and NGF increases TRPV1 and NMDA expression and induces the release of other pain mediators, for example, prostaglandins [10]. In addition, via a positive feedback loop, NGF also induces the release of NGF itself that may sensitize adjacent nociceptors [10].

6.4.4 Bradykinin

Bradykinin is a peptide involved in the inflammatory response. Bradykinin is synthesized in the kinin-kallikrein system from the kininogen precursor by the enzyme kallikrein and metabolized by three kinases, among them angiotensin-converting enzyme (ACE). Bradykinin is a potent vasodilator and bronchoconstrictor that increases vascular permeability and facilitates pain transmission. Additionally, bradykinin further promotes the release of glutamate from astrocytes. During ischemic contractions, bradykinin is released in muscle tissue and numerous animal studies have shown that it has a role in muscle nociception and sensitization [11].

6.4.5 TRPV1

TRPV1 is a nonselective cation channel that is activated by a variety of exogenous and endogenous stimuli, for example, noxious heat (43–52 °C), chemical compounds, and protons [12]. Polymodal C-fibers normally express TRPV1 receptors, and during inflammation TRPV1 receptors are upregulated. Interestingly, TRPV1 receptors are not present on Aδ fibers under normal conditions but are expressed during inflammation. The pungent ingredient of chili pepper, capsaicin, but also mechanical stimuli activates TRPV1 receptors on sensory afferents that contain various pro-inflammatory neuropeptides. This causes the antidromic release of neuropeptides from the nerve endings that induce vasodilation and sensitize the neuron to other nociceptive chemicals, e.g., prostaglandins, NGF, bradykinin, and 5-HT.

6.4.6 Neuropeptides

Since the cloning of the first neuropeptide receptors in the late 1980s, neuropeptides have been a target for extensive research, especially in the pharmaceutical industry in search for new drug targets [13]. As the name implies, neuropeptides are small peptides that are released from neuronal cells and are commonly used by neurons to communicate with each other. Many neuropeptides are co-released with other neurotransmitters, such as glutamate, acetylcholine, and norepinephrine. Aside from their functions as neurotransmitters, neuropeptides have a variety of other functions,

for example, as neurohormones and growth factors. Some neuropeptides are released peripherally and have a key role in neurogenic inflammation, such as SP, CGRP, NKA, and VIP, whereas others are released in central brain regions, such as galanin, cholecystokinin, and neurotensin. Many neuropeptides affect vessel tone. For example, SP, CGRP, and VIP act as vasodilators, and neuropeptide Y (NPY), which is co-released with norepinephrine, is a strong vasoconstrictor. Several animal studies have shown that SP and CGRP relay nociceptive information and that sensitized peripheral neurons release SP and CGRP in response to innocuous stimulation. It is also believed that SP and CGRP act synergistically in inflammation and nociception [13].

6.4.7 Eicosanoids

During inflammation, eicosanoids play an essential role. Eicosanoids are substances produced in various cell types, e.g., endothelial cells, fibroblasts, and leukocytes, by the breakdown of arachidonic acid in the cell membrane in response to tissue trauma, leading to the formation of prostaglandins, thromboxanes, and leukotrienes. The formation of prostaglandins and thromboxanes from arachidonic acid depends on cyclooxygenase enzymes that catalyze the conversion. However, while prostaglandins may sensitize nociceptors, induce vasodilation, and inhibit trombocyte aggregation, thromboxanes have the opposite effect in that they facilitate trombocyte aggregation and induce vasoconstriction.

Leukotrienes, conversely, depend on the enzyme lipoxygenase for their production. Leukotrienes are strong vasoconstrictors, increase blood vessel permeability, induce chemotaxis, but also sensitize nociceptors [14].

The prostaglandin PGE_2 has been of particular interest in inflammatory pain studies and is an important biomarker in delayed onset muscle soreness [15]. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, reduce inflammatory pain at a peripheral level by inhibiting cyclooxygenases and, hence, the synthesis of prostaglandins. Thus, one may speculate that peripheral release of prostaglandins and leukotrienes could be part of the pathogenesis of jaw myalgia. However, the dose of PGE_2 needed for excitation of muscle nociceptors is so high that it is unlikely that it exerts a nociceptive effect during pathologic conditions. Therefore it is more likely that PGE_2 is involved in sensitization of nociceptors [16].

6.4.8 Cytokines

Cytokines are small peptides that are produced by most nucleated cells and released during inflammation but are also involved in many physiological processes. Cytokines may have both pro- and anti-inflammatory effects. Normally there is a balance between these two effects to maintain homeostasis, but during inflammation there is typically a shift in cytokine production so that the normal balance is disrupted in favor of pro-inflammatory signaling. Interleukin 1 beta (IL-1β), TNF, IL-6, and IL-8 are pro-inflammatory cytokines that are implicated in the illness response (fever, fatigue, etc.) and produce hyperalgesia upon peripheral administration [14]. These pro-inflammatory cytokines are all produced in muscle tissue and released in response to exercise and tissue trauma. If overproduction of pro-inflammatory cytokines is part of the pathogenesis of jaw myalgia, one might assume that intramuscular levels of these cytokines would be increased as muscle levels of IL-6 are increased in delayed onset muscle soreness, i.e., myalgia that may develop after eccentric exercises [15].

6.4.9 Metabolites

Pyruvate and lactate are important end-products of glucose metabolism. Pyruvate is a key metabolite in many metabolic pathways and is involved in both aerobic and anaerobic metabolism. During aerobic metabolism, glucose is cleaved to pyruvate, which enters the Krebs cycle to produce additional energy. As part of anaerobic metabolism, pyruvate is converted by fermentation to lactate following glycolysis. This process

is reversible; when oxygen is present, lactate is reconverted to pyruvate, and through gluconeogenesis, pyruvate can be converted back to glucose. Pyruvate can also be converted to fatty acids and to the amino acid alanine.

It is well known that heavy exercise leads to metabolic changes within the muscle as lactate accumulates in the muscle and blood plasma. It has also been speculated that continuous low force exercise could alter local muscle metabolism since the same low-threshold motor units are activated during a longer time and with time may become overloaded. This, in turn, may alter the muscle pressure around the muscle fibers and impede blood flow, oxygen delivery, and removal of metabolites as ischemia develops [17]. Lactate itself does not seem to induce pain, but ischemia may cause the release of pain biomarkers and thus lead to pain.

6.5 Pain Evoked by Intramuscular Injection of Biomarkers in Humans

Intramuscular injection of glutamate into the jaw muscles of pain-free human volunteers was shown to evoke pain, pain referral, and mechanical allodynia, with stronger pain effects in women [18–21]. Both pain intensity and pain drawing area were greater in the temporalis muscle compared to the masseter [19]. Sensory-discriminative and affective-unpleasantness components to glutamate injections are similar to jaw myalgia, but the psychosocial features differ [21, 22]. Injection of glutamate into other muscles (splenius muscles, forearm) induces similar responses [23, 24]. Thus glutamate-evoked pain seems to be a valid model for myalgia. Pretreatment with NGF before glutamate injection increased masseter pain area and reduced PPTs [25], and pretreatment with glutamate before capsaicin injection enhanced pain variables [26]. However, pretreatment with capsaicin before glutamate injection diminished glutamate-induced pain [26, 27], which indicates that desensitization by capsaicin blocks the release of glutamate. Co-injection of the NMDA receptor antagonist ketamine (10 mmol/l) with glutamate in pain-free volunteers attenuated pain with a better effect in men [28], whereas co-injection of ketamine at higher dose (20 mmol/l) had similar effects in men and women [20]. However, injection of ketamine (10 mmol/l) did not alter pain and mechanical allodynia in jaw myalgia patients [29]. The results also suggest that local treatment with ketamine may not be clinically useful in jaw myalgia.

Injection of serotonin into human jaw muscles of healthy volunteers evoked pain and mechanical allodynia [30]. Similarly, injection of serotonin into the anterior tibialis muscle evoked pain but did not induce mechanical allodynia [31]. The lower dose of serotonin and the larger size of the muscle may explain these differences. Additionally, serotonin seems to enhance the effect of bradykinin, as combined injection of serotonin and bradykinin into the anterior tibialis and temporalis muscles increased pain responses and produced long-lasting secondary allodynia [32, 33]. Co-injection of serotonin and the 5-HT_3 receptor antagonist granisetron (1 mg/ml) attenuated the pain and hyperalgesia induced by serotonin [34]. Pretreatment with granisetron effectively attenuated hypertonic saline and acidic saline-induced masseter muscle pain [35, 36] with a greater increase of PPT in men [35]. Local and oral administration of granisetron increased muscle PPTs in pain-free subjects, with a greater effect in men [37, 38], indicating that serotonin may decrease the sensitivity of muscle mechanoreceptors. Moreover, local and systemic granisetron and other 5-HT_3 antagonists attenuate jaw myalgia [37] and chronic low back and trapezius muscle pain [39] and also have a positive effect on widespread pain in patients with fibromyalgia [40]. Thus, there is evidence from human studies that serotonin may activate and sensitize muscle nociceptors and that substances that block 5-HT_3 receptors may be of therapeutic value in jaw myalgia. However, more research is needed to establish this.

NGF injected into the masseter muscle of pain-free volunteers provoked mechanical allodynia that lasted up to 7 days with similar effects in men and women and pain upon chewing and yawning

[41, 42]. The injection also affected motor function. Injection of NGF into the tibialis anterior muscle induced long-lasting allodynia that also spread distally and proximally and was also apparent over the finger 1 day after injection, indicating signs of central sensitization [43]. Injection of hypertonic saline 1 day after NGF injection into the tibialis anterior muscle induced more pain in men than after isotonic saline injection on day 1. These results indicate that NGF injection may be a useful model for mechanical muscle allodynia.

Intramuscular injection of capsaicin into the masseter muscle of pain-free volunteers caused pain that lasted for 20 min and was described as "pressing" and "taut" and also pain referral, predominantly to the molar teeth as well as mechanical allodynia [44, 45]. It also decreased the electromyographic activity in the masseter muscle but increased the jaw stretch reflex amplitude [45, 46]. Experimental tooth grinding before capsaicin injection induced more long-lasting mechanical allodynia and reduced jaw opening compared to capsaicin alone [44]. Furthermore, capsaicin injection into the masseter increased local blood flow but also skin blood flow, which might indicate that it induces neurogenic inflammation [47].

Additionally, injections of a few other biomarkers have been investigated for potential effects on muscle pain. Injection of SP or NKA, or a combination of both, into the temporalis muscle of healthy subjects did not induce pain or mechanical hyperalgesia [48], while injection of potassium into the temporalis muscle did [49]. Injection of a combination of serotonin, histamine, bradykinin, PGE_2, and ATP into the trapezius muscle of healthy subjects and patients with tension-type headache induced prolonged pain and mechanical allodynia [50], but the combination has not been tested for jaw myalgia.

6.6 Tissue Levels of Biomarkers in Patients with Muscle Pain

So far few studies have investigated saliva or urine levels of pain biomarkers in patients with jaw myalgia. One study found no differences in saliva glutamate level compared to pain-free controls. Regarding urine, one study reported that the total amino acid excretion was positively correlated with pain intensity and that the urine levels of glutamine/glutamate correlated to pain duration in patients with jaw myalgia, which they interpreted as a depletion of metabolic reserves [51].

Studies employing intramuscular microdialysis to sample glutamate in female patients with jaw myalgia show contradictory results. One study did not show any difference in masseter glutamate levels between patients and controls [52], and another reported increased levels in female patients compared to age-matched pain-free controls [53]. Both studies found similar masseter levels of glutamate in the patients, but the controls in the latter study had lower glutamate levels, which may explain the contradictory results. The glutamate levels did not correlate with pain levels in either study. Muscle glutamate levels in the latter study were higher compared to plasma levels in the patients, but plasma levels did not differ between patients and controls [53]. The results from the latter study are corroborated by studies in patients with localized trapezius myalgia, which reported elevated glutamate levels in the trapezius muscle in patients with work-related trapezius myalgia and chronic widespread pain [54–56]. In the trapezius, high glutamate levels correlated to low PPT and high pain levels [54, 55]. However, other studies did not find any differences in trapezius levels of glutamate between patients with chronic trapezius myalgia (not specified), fibromyalgia, whiplash-associated disorders (WAD), and pain-free controls [57–59]. This might be due to methodological differences between studies, such as different flow-rates and a low number of subjects in certain studies. Neither did the levels of glutamate in trapezius tender points of chronic tension-type headache patients differ from pain-free controls [60].

Serum levels of serotonin did not differ between jaw myalgia and healthy controls [61]. However, the release of serotonin in the masseter, measured with microdialysis, was increased in patients with jaw myalgia [52, 62]. Additionally, the levels of serotonin correlated with pain and mechanical pain thresholds [62]. Similar results

are reported from the trapezius muscle, where levels of serotonin were elevated in patients with localized trapezius myalgia (work-related and WAD) as well as in fibromyalgia [54–56, 58, 63, 64]. These results also correlated with pain levels [54, 58]. Furthermore, recent results indicate that the frequency of sensory nerve fibers that express 5-HT_3 receptors in the masseter muscle is upregulated in female patients with jaw myalgia, when compared to pain-free matched controls [65]. However, the data need to be confirmed with a larger patient sample before any firm conclusions can be drawn. These results strengthen a role for serotonin in jaw myalgia.

Studies investigating tissue levels of other pain molecules in jaw myalgia are scarce. One study showed that the masseter levels of leukotriene B4 (LTB_4), but not PGE_2, were higher in patients with masseter myalgia than in healthy controls. However, PGE_2 levels were positively correlated to pain intensity [66]. No differences were found between patients with jaw myalgia and controls in levels of PGE_2, LTB_4, NGF, SP, or bradykinin in open biopsies obtained from the masseter muscle. However, the level of F(2) –isoprostane, a marker of oxidative stress, was lower in patients than in controls and correlated to muscle pain intensity and PPT [67]. In concordance with these studies, trapezius levels of PGE_2 in patients with chronic trapezius myalgia, and in tender points of patients with chronic tension-type headache, did not differ from healthy controls [57, 60], indicating that there is no detectable inflammation in myalgic/tender muscles. Increased levels of protons, bradykinin, SP, CGRP, and norepinephrine have been reported in active trigger points of the trapezius muscle [64].

A recent study reported no differences in masseter lactate or pyruvate levels in jaw myalgia as compared to healthy controls [52]. Muscle pyruvate and lactate levels in the trapezius muscle of patients with trapezius myalgia show varying results; most studies report increased levels [17, 55, 59, 68], but one found no differences to pain-free controls [58].

No study has investigated cytokines in myalgic jaw muscles. In patients with active trapezius muscle trigger points, the levels of IL-1β, TNF, IL-6, and IL-8 in the trapezius muscle [64], but also in the pain-free gastrocnemius muscle [69], were reported to be increased when compared to pain-free controls. Patients with WAD had increased levels of IL-6 in the trapezius muscle [58], but normal levels of IL-6 were found in patients with work-related trapezius myalgia [70]. Elevated blood levels of the pro-inflammatory cytokine monocyte chemotactic protein (MCP-1) and anti-inflammatory cytokine IL-1ra were recently reported in TMD patients [71], whereas increased blood and cerebrospinal fluid levels of pro-inflammatory (IL-8, TNF) and reduced blood levels of anti-inflammatory cytokines (IL-4, IL-10) were found in fibromyalgia patients [72]. Finally a recent study using positron emission tomography (PET) reported increased uptake of D-deprenyl, a marker of inflammation, in painful neck muscles in WAD [73]. Whether D-deprenyl uptake can be visualized in jaw myalgia patients is unknown but would be an interesting area for future research.

6.7 Muscle Biomarkers Tissue Levels After Exercise

Some studies have investigated the effect of muscle exercise on biomarker levels using microdialysis, but very few have been performed in the orofacial region. There were no alterations in masseter muscle levels of NPY in response to experimental tooth clenching in pain-free volunteers [74], and there were also no changes in the levels of serotonin, glutamate lactate, or pyruvate in patients with jaw myalgia, although clenching induced low levels of pain [52]. Similarly, serotonin levels did not change in the trapezius muscle of patients with work-related trapezius myalgia, WAD, and fibromyalgia or in pain-free controls after exercise (peg board) [56, 58, 63]. With regard to glutamate, lactate, and pyruvate, however, the results from the masseter muscle differ from those of the trapezius muscle, in which levels of these metabolites increased after exercise both in patients with work-related trapezius myalgia, WAD, fibromyalgia, and in pain-free controls

[17, 58, 75]. The difference between regions may be due to differences in exercise protocol: in the masseter muscle, a resting period of 1 min was alternated with clenching for 30 s, whereas in the trapezius, continuous repetitive arm movements were used. Glutamate and lactate levels, as well as the levels of PGE_2 and SP, were also increased in the calf muscle after eccentric exercise, which caused delayed-onset muscle soreness [16]. Pilot data from our group indicate that IL-6 and IL-8 levels in the masseter muscle of healthy subjects increase in response to tooth clenching (J. Goerlach et al., personal communication). Additionally, IL-6 levels in the trapezius muscle increased after exercise in patients with trapezius myalgia and healthy controls [58, 70].

6.8 Muscle Biomarker Levels in Experimental Myalgia

Human experimental pain models are important tools to bridge the gap between animal studies and the clinic. An often-used model to induce myalgia that mimics clinical situations is intramuscular injection of hypertonic saline. Recent studies show that hypertonic saline injection into the masseter cause the release of serotonin, glutamate, and glycerol but not lactate or pyruvate [76]. Similarly, hypertonic saline injection into the biceps muscle induced the release of glutamate but had no effect on lactate, PGE_2, or nitric oxide levels [15]. This indicates that, even as an acute pain model, injection of hypertonic saline shows similar clinical and molecular pain manifestations as clinical myalgia. Another experimental pain model that recently has gained attraction in human studies is intramuscular injections of acidic saline. In animals, two repeated injections of acidic saline 2–5 days apart induce long-lasting mechanical allodynia [77]. During ischemia, pH drops, and the released protons may activate acid-sensing ion channels that are thought to be the major channels involved in acid-induced pain [78]. Injection of acidic saline into the human anterior tibialis muscle evoked low-levels of pain and short-lasting mechanical allodynia [78]. However, mechanical allodynia in the masseter muscle was not induced with this model, although very low levels of pain were evoked [79]. The release of serotonin, glutamate, lactate, or pyruvate was not found to change in response to acidic saline-injection [80]. These results combined imply that the acidic saline model does not seem to be a useful experimental model of orofacial myalgia. Intramuscular infusion of a chemical mixture consisting of bradykinin, PGE_2, histamine, and serotonin into the trapezius muscle did not cause the release of glutamate, lactate, glucose, glycerol, pyruvate, and urea even as it evoked local pain and mechanical allodynia [81]. Collectively, these results show that only the hypertonic saline model seems to be a valid experimental model of clinical myalgia.

Summary

Table 6.2 summarizes the findings from human studies regarding the evidence for a role of potential biomarkers for jaw myalgia. Although there is evidence that glutamate and serotonin play a role in jaw myalgia, evidence for the role of other potential biomarkers in human muscle pain is scarce. To be useful to a clinician, a biomarker should be easily collected. This explains why blood or saliva samples would be preferable. Because of the lack of correlation between muscle and plasma levels of serotonin and glutamate, their usefulness as biomarkers for jaw myalgia is limited. In conclusion, no substance at present fulfills the role of a useful biomarker for jaw myalgia. Future studies with new and highly sensitive methods that can combine analyses of several biomarkers are therefore warranted to further advance the field.

Table 6.2 Summary of the effects of potential biomarkers for muscle pain in humans

Substance	Muscle levels		Muscle levels: other muscles		Muscle level correlation to pain variables	Receptor expression MMP	Effect of injection into masticatory muscles	Effect of receptor antagonist in MMP
	MMP	After exercise	MP	After exercise				
Glutamate	Increased	No change	Increased	Increased	Pain level, hyperalgesia	?	Pain, pain referral, hyperalgesia	Reduced pain and hyperalgesia
Serotonin	Increased	No change	Increased	Increased	Pain level, hyperalgesia	Increased (?)	Pain and hyperalgesia	Reduced pain and hyperalgesia
NGF	?	?	?	?	?	Normal	Hyperalgesia	?
Protons	?	?	Increased	?	?	?	Pain, hyperalgesia (capsaicin)	?
Bradykinin	?	?	Increased	?	?	Normal	Pain, hyperalgesia	?
PGE2	Normal	?	?	Increased	Pain level	Normal	No effect	?
LTB4	Increased	?	Increased	?	No	Normal	?	?
SP	?	?	Increased	?	?	Normal	No effect	?
CGRP	?	?	?	?	?	?	?	?
NPY	?	No change	Increased	Increased	No	?	?	?
IL-1beta	?	No change	Increased	?	?	?	?	?
TNF-alpha	?	No change	Increased	?	?	?	?	?
IL-6	?	Increased	Increased	?	?	?	?	?
IL-8	?	Increased	Increased	?	?	?	?	?
Lactate	Normal	No change	Increased	Increased	No	?	?	?
Pyruvate	Normal	No change	Increased	Increased	No	?	?	?

MMP masticatory muscle pain, *MP* muscle pain other regions (trapezius myalgia, fibromyalgia), *NGF* nerve growth factor, *PGE2* prostaglandin E2, *LTB4* leukotriene B4, *SP* substance P, *CGRP* calcitonin gene-related peptide, *NPY* neuropeptide Y, *IL* interleukin, *TNF* tumor necrosis factor, *?* no information available. See text for more information and references

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Molecular Temporomandibular Joint Pain Biomarkers

7

Per Alstergren

Abstract

This chapter covers immunological markers for inflammatory types of temporomandibular joint (TMJ) pain. The specific biological relevance or clinical value of biomarkers in TMJ pain is, however, so far insufficiently investigated. There are studies that indicate candidate biomarkers for diagnostic or prognostic purposes in diseases like rheumatoid arthritis and psoriatic arthritis. This chapter discusses available knowledge regarding cytokines, cytokine receptors, serotonin, prostaglandin E_2, and glutamate in relation to diagnosis, prognosis, and treatment of TMJ inflammatory pain.

7.1 Introduction

Much of the recent research regarding TMJ arthritis and molecular diagnostics aims to identify patients at risk for disease development and to enable early treatment to prevent chronification of the pain and tissue damage. The specific biological relevance or clinical value of biomarkers in temporomandibular joint (TMJ) pain is, however, so far insufficiently investigated. At the same time, there are numerous studies available that indicate highly interesting candidate biomarkers for diagnostic or prognostic purposes or to assess or monitor disease activity, especially in diseases like rheumatoid arthritis (RA) and psoriatic arthritis (PsA).

Inflammation of articular tissues, i.e., arthritis, frequently shows chronic pain as a major symptom. Chronic arthritis may also result in articular cartilage and bone tissue destruction. This is commonly seen, especially in systemic arthritides like RA and PsA. In these conditions, pain is the major factor for impaired daily activities and quality of life. Indeed, TMJ pain has a substantial negative impact on activities of daily living in RA [1].

The peripheral contribution to TMJ pain as well as cartilage and bone tissue destruction is locally and systemically modulated by a huge number of mediators, among them cytokines, serotonin, prostaglandins, and glutamate.

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7.2 Cytokines

Cytokines are small peptides with redundant effects that mediate potent stimulatory or inhibitory effects in immunity and inflammation. All nucleated cells are capable of synthesizing cytokines, produced de novo in response to immune stimuli, and most cell types respond to them. Cytokines generally act at very low concentrations during short periods of time in an autocrine or paracrine manner although additional endocrine effects have been described for some cytokines [2, 3].

It is now apparent that cytokines are involved in most physiological processes, including modulation of repair and remodeling of damaged tissue. For the most part, however, cytokines are produced and released during inflammation. Cytokines are often produced in a cascade, as one cytokine stimulates its target cells to make additional cytokines. The complex interconnectivity and dynamics of cytokine biology might better be visualized as a network within a cascade where cytokines can act independently, additively, or synergistically [3]. The central role of the cytokines tumor necrosis factor (TNF), interleukin-1β (IL-1β), and IL-6 within the proinflammatory cytokine network is now conclusively established [4].

Cytokine-mediated inflammation induces gene products usually not produced during a healthy state, i.e., other cytokines, phospholipase A2, and cyclooxygenase-2 (COX-2). TNF and IL-1 are particularly effective in initiating and stimulating the expression of these genes [5]. There is, on the other hand, a simultaneous production and release of anti-inflammatory cytokines like IL-4 and IL-10 and soluble cytokine receptors that block or suppress the intensity of this cascade as an endogenous control of the net cytokine effects [6]. In inflammatory conditions, a dramatic increase in cytokine production can be seen at the same time as the balance between the cytokine production and its control is disturbed [7]. Indeed, it appears that the balance of proinflammatory cytokines and their endogenous control mediators (anti-inflammatory cytokines, soluble or decoy receptors, and antagonists) is at least as important as the absolute levels of individual cytokines [8, 9].

Cytokines have been extensively studied in immune reactions and inflammation but less so regarding their specific and distinct roles in pain. The relation between cytokines and pain is probably better understood by placing it in a broader context as a part of an immune reaction or inflammation. In fact, most, if not all, experimental models to study hyperalgesia or pain facilitation induce release of proinflammatory cytokines [10].

7.2.1 Peripheral Cytokine Modulation of Pain

Locally released cytokines are believed to influence or modulate pain in a complex manner on several levels:

1. Direct effects on nociceptors via cell-surface receptors
2. Indirect effects on nociceptors via stimulation of local production of other cytokines and mediators with nociceptive effects
3. Pain/hyperalgesia as a part of the illness response
4. The cholinergic anti-inflammatory pathway

7.2.1.1 Direct Effects on Nociceptive Fibers via Cell-Surface Receptors

Peripheral nociceptive neurons express receptors for various cytokines. When stimulated, these receptors may influence nerve fiber depolarization and conductivity as well as apoptosis and gene expression of factors important for nociceptive signaling in the neuron [6]. There is also evidence for a role of proinflammatory cytokines not only in inflammatory pain but also in neuropathic pain [11, 12].

7.2.1.2 Indirect Effects on Nociceptors via Stimulation of Local Production of Other Cytokines and Mediators with Nociceptive Effects

In inflammation, release of hyperalgesic mediators seems to be secondary to the release of

proinflammatory cytokines. TNF and IL-1β rapidly induce synthesis and release of other nociceptive mediators such as IL-1, IL-6, bradykinin, serotonin, and prostaglandins as well as themselves. This has to be taken into account when discussing the contribution of these cytokines to pain, i.e., these cytokines exert both direct effects and indirect effects [11].

7.2.1.3 Pain/Hyperalgesia as a Part of the Illness Response

Besides local effects, cytokines also cause or modulate a wide array of changes called the "illness response," which follow immune activation by inflammation, injury, or infection. The illness response includes physiological, behavioral, endocrine, and neural changes like fever, increased sleep, decreased activity, social interaction, etc., changes that also form a part of chronic pain phenotype. Generalized reduction of pain thresholds and exaggerated pain responses, i.e., hyperalgesia, are also aspects of the illness response [9, 11, 13].

The illness response occurs due to peripheral release of proinflammatory cytokines, especially TNF, IL-1β, and IL-6, that spills over to the blood and stimulates the afferent portion of the vagus nerve in the liver. Once activated, the vagus nerve communicates with the brain to induce and maintain the illness response, including changes in the central pain processing resulting in pain and generalized sensitization [14].

7.2.1.4 The Cholinergic Anti-inflammatory Pathway

A neuronal cholinergic anti-inflammatory pathway exerting peripheral cytokine control has recently been demonstrated [5]. This pathway functions as a fast, reflex-like anti-inflammatory mechanism controlled by brain networks [5] and seems to be mediated by the vagus nerve but perhaps also by other parasympathetic efferents. Activation of this pathway reduces peripheral cytokine production by leukocytes in the reticuloendothelial system (e.g., liver and spleen) and redirection of leukocyte trafficking away from the periphery [15].

7.2.2 Cytokines as Clinical Biomarkers of Temporomandibular Joint Pain?

With relevance to cytokines, TMJ pain seems to be modulated by both local and systemic factors, as described above. For example, pressure-pain threshold over the TMJ in RA seems to be mainly modulated by systemic factors, whereas TMJ movement pain is mainly modulated by local inflammatory factors [16].

7.2.2.1 Tumor Necrosis Factor

Ligands The main physiological role of TNF is activation of the first-line reaction to microbial, viral, and mechanical stress. TNF exists in both a soluble (17 kD) and a cell-bound, transmembrane form (tmTNF, 26 kD), and it is primarily produced in response to various inflammatory stimuli [17]. TNF is synthesized by a variety of cell types like macrophages and monocytes, T cells, B cells, and fibroblasts. Interestingly, about 45% of small dorsal root ganglion neurons also express TNF, suggesting it may be released by peripheral afferent fibers as well [18].

Almost all stressful and inflammatory stimuli have been shown to induce TNF production and release. TNF can also upregulate its own synthesis. Several agents downregulate TNF expression, for example, inhibitors of prostaglandin synthesis (salicylate), glucocorticoids, and endogenous immunosuppressive cytokines like IL-4 and IL-10 [17]. TNF rapidly induces synthesis of other mediators such as IL-1, IL-6, and prostaglandins.

Receptors Biological responses to TNF are mediated by ligand-binding via two structurally distinct transmembrane receptors; the type I and II receptors (TNFRI; TNFRII). The TNFRI is present on all cell types except erythrocytes, whereas the TNFRII is mainly expressed by cells of the immune system [18]. The receptors primarily modulate activation of the transcription factor nuclear factor kappa B (NF-kB), which controls a large number of inflammatory genes [19].

During inflammatory conditions, the concentrations of both receptors increase dramatically. The TNF receptors are upregulated by a number of factors including glucocorticoids and IL-6 [19].

Both TNF receptors are subject to proteolytic cleavage by matrix metalloproteases. This cleavage is increased in response to inflammatory signals. These soluble receptors have been found in blood, urine, cerebrospinal fluid, and synovial fluid in patients, and the levels of these receptors increase during inflammation [20]. The soluble forms of both receptors, TNFsRI and TNFsRII, are primarily believed to be endogenous inhibitors of TNF bioactivity by binding to TNF and removing TNF from the site of its release [9, 20]. Indeed, a dimer of TNFsRII is today an approved pharmaceutical drug (etanercept, Enbrel®) in many countries for treatment of chronic and general inflammatory diseases like RA and psoriatic arthritis [21].

Experimental Findings Peripheral TNF signaling is involved in nociceptive responses including hyperalgesia [11, 22]. Local TNF administration evokes spontaneous activity in afferent C and Aδ nerve fibers that results in low-grade nociceptive input, contributing to central sensitization [22]. Indeed, peripheral TNF induces a mechanical hyperalgesia with rapid onset (< 30 min) when administered subcutaneously. It appears to result from sensitization of cutaneous C-fibers and to be associated with signs of local inflammation and increased levels of inflammatory mediators, e.g., prostaglandins [18]. For example, intraplantar injection of TNF in rats reduced mechanical nociceptive thresholds in a prostaglandin-dependent process [23].

TNF is involved in several animal models of arthritis [23]. Subcutaneous administration of the TNF inhibitor etanercept decreased mechanical hyperalgesia when administered prior to induction of arthritis by injection of complete Freund's adjuvant into the rat knee joint [24].

The effects of TNF associated with experimental hyperalgesia have been shown to be dependent on TNFRI [25], and it has been shown that non-neurally derived TNF directly acts via TNFRI on primary afferent neurons to produce hyperalgesia [26]. Indeed, TNFRI-neutralizing antibodies as well as antisense RNA against TNFRI, but not antibodies toward TNFRII, can reduce experimentally induced hyperalgesia [25].

TNFRI and TNFRII immunoreactivity has been found in the dorsal root ganglion [26]. The expression of TNFRI RNA in rat dorsal root ganglion seems to not be restricted to presumed nociceptive neurons in the dorsal root ganglion, which actually implies a broader role of TNF in primary sensory functions than nociception alone [26].

Clinical Findings TNF has been detected in the synovium and synovial fluid from patients with RA [27] and in patients with other inflammatory diseases such as PsA, pelvospondylitis, and reactive arthritis [28, 29]. TNF has also been found in the synovial fluid of patients with RA, PsA, and internal derangement of the TMJ [30] as well as in patients with unspecified TMJ disorders [31].

Synovial fluid TNF levels have been shown to be significantly higher in individuals with TMJ pain upon mandibular movement than in those without such pain [29]. In addition synovial fluid TNF levels were associated with tenderness to palpation of the TMJ, which corresponds to sensitization of afferent nerves in the synovial tissues and tissues surrounding the TMJ. As then can be expected, TNF levels in synovial fluid and plasma appear to be predictive for the treatment response to intra-articular administration of glucocorticoid into the TMJ. A high pretreatment level of TNF in the TMJ synovial fluid was found to be a positive predictor for TMJ pain relief after intra-articular administration of glucocorticoid [27]. The pain relief after treatment was associated with reduction of synovial fluid TNF. It is thus quite likely that TNF is involved in the modulation of joint pain.

Patients with RA have circulating levels of soluble TNF receptors that are higher than those observed in patients with osteoarthritis or non-RA inflammatory arthritis [32]. Insufficient systemic endogenous control of TNF, as estimated by the plasma level of the soluble receptor TNFsRII, contributes to TMJ pain in RA [9, 20].

7.2.2.2 Interleukin-1β

Ligands IL-1 has a molecular weight of 17 kDa and is mainly derived from macrophages and T cells. So far, three subtypes of IL-1 have been identified: two agonists with strong proinflammatory effects, IL-1α and IL-1β, as well as an endogenous IL-1 receptor antagonist, IL-1ra. Most of the IL-1α remains intracellularly or on the surface of the cell membrane where it functions as an autocrine messenger, while most IL-1β is transported out of the cell where it acts locally or enters the blood circulation [6]. Indeed, both have been shown to be involved in inflammatory reactions but only IL-1β has been found in synovial fluid from patients with RA [33]. IL-1ra competes with IL-1α and IL-1β for receptor binding and is produced in substantially higher concentrations than IL-1β during inflammation. IL-1ra does not elicit a biological response when coupled to an IL-1 receptor and has therefore an anti-inflammatory character [2]. Inflammation causes an increased local IL-1ra release but during active inflammation probably in insufficient amounts to inhibit the strong proinflammatory effects of IL-1β [2].

IL-1 induces several inflammatory events, i.e., activation of lymphocytes and stimulation of cytokine and prostaglandin and collagenase release from connective tissue cells, but it is also involved in hyperalgesia and pain. In addition, it has systemic effects by stimulating the production and release of C-reactive protein, eliciting fever and the illness response [34].

Receptors There are two IL-1 receptors identified: IL-1RI with low/high affinity for IL-1β/IL-1ra and IL-1RII with high/low affinity for IL-1β/IL-1ra. When stimulated, IL-1RI elicits a biological response in the cell, whereas IL-1RII causes no signal transduction and is therefore considered as a decoy receptor [6]. Lacking a second binding site, IL-1ra binds primarily to IL-1RI but does not trigger a biological response.

Stimulation of IL1-RI leads to activation of the transcription factor NF-kB, among others [35, 36]. In turn, genes encoding pro- and anti-inflammatory cytokines as well as enzymes involved in inflammation like COX-2, phospholipase A2, and nitric oxide synthase are upregulated [2].

There is also a soluble form of this receptor, IL-1sRI, which is released from the cell surface by proteolytic cleavage. It has anti-inflammatory effects by binding to and thereby blocking IL-1 from reaching cell-bound IL-1RI receptors.

IL-1RII is primarily expressed on monocytes, macrophages, neutrophils, and B lymphocytes. The IL-1sRII is a decoy receptor in that it lacks a cytoplasmic portion capable of signaling, and its primary ligand – IL-1β – binds to this receptor rather than to IL-1RI. Binding of IL-1β to IL-1RII is nearly irreversible [37, 38].

Similar to soluble receptors for TNF and IL-1RI, IL-1sRII has been demonstrated in the circulation and urine of healthy subjects and in inflammatory synovial and other pathologic body fluids [37, 39, 40]. The induction of release of the IL-1sRII decoy receptor is probably an early event in inflammation to limit the cascade [2].

Experimental Findings IL-1β is capable of decreasing nociceptive thresholds in peripheral tissues by direct excitatory and sensitizing action on nociceptive fibers [41]. Injection of IL-1β into one paw in rats evokes a dose-dependent hyperalgesia in both the ipsilateral and the contralateral paws, except for very low doses that solely produce hyperalgesia in the injected paw. This shows that IL-1β influences hyperalgesia both by local effect in the paw and by systemic effects. The hyperalgesia in the injected paw could be attenuated by COX inhibitors, suggesting that IL-1β evokes local hyperalgesia via stimulation of COX products as prostaglandins [42]. Intraperitoneal injection of IL-1β induces a generalized hyperalgesia via actions on the hepatic vagus nerve that elicits afferent signaling to the brain [11, 42].

In mice overexpressing TNF, a rheumatoid arthritis-like disease develops. Treatment with blocking antibodies to IL-1RI prevents the onset of disease [43], indicating a pathophysiologcal role for IL-1RI in an inflammatory disease with pain as one of its hallmarks.

Clinical Findings IL-1β is undetectable in TMJ synovial fluid from healthy individuals while patients with polyarthritides have significantly higher such concentrations [44]. The IL-1β found in

the synovial fluid of the TMJ from patients with inflammatory disorder seems to originate from local production since the correlation between plasma and synovial fluid levels is poor and the synovial fluid level is substantially higher than the plasma level. The synovial fluid level of IL-1β in human knees also shows a poor correlation to the plasma level and has accordingly been found to correlate more with local disease activity, for example, as measured by the Ritchie score (joint tenderness), than with systemic disease activity [45].

High level of IL-1β in the synovial fluid from the arthritic TMJ is associated with resting pain, tenderness to digital palpation, and a decreased pressure-pain tolerance [46]. Patients with high synovial fluid IL-1ra and low IL-1β concentrations show a more rapid resolution of arthritis, including pain variables, and the balance between synovial fluid IL-1β and IL-1ra concentrations seems to determine the progression of the inflammatory process [47]. Indeed, high TMJ synovial fluid level of IL-1ra in TMJ synovial fluid has been found to be associated with few or no painful mandibular movements [40]. High level of IL-1ra in TMJ synovial fluid was associated with few or no painful mandibular movements, perhaps due to receptor binding and inhibition of IL-1β [40].

IL-1sRI and IL-1sRII are present in the extracellular matrix and blood both in healthy individuals and in patients with inflammatory disorders. Elevated levels of especially IL-1sRII are found in plasma and synovial fluid of patients with inflammatory joint disease [9, 37]. However, RA patients seropositive for the rheumatoid factor seem to have lower plasma concentrations of IL-1sRII than seronegative patients, indicating a deficient systemic control of the effects of IL-1β [40]. Upregulation of these soluble receptors has anti-inflammatory effects per se [2], but these anti-inflammatory effects are often insufficient to completely inhibit the very strong proinflammatory effects of elevated IL-1β levels, especially during high inflammatory activity.

7.2.2.3 Interleukin-6

Ligand IL-6 is a protein of 186 amino acids with a molecular weight of 21–28 kDa. IL-6 can be produced and released by nearly all, if not all, nucleated cells, but the main sources are macrophages, fibroblasts, and endothelial cells [48]. IL-6 plays a pivotal role in chronic disease where it regulates both local inflammatory events and associated systemic symptoms such as fever, illness response, and induction of acute-phase reactants [49].

Receptor The IL-6 receptor (IL-6R) consists of two subunits: the extracellular portion, which binds IL-6 with low affinity, and the gp130, a transmembrane glycoprotein [48]. On target cells, IL-6 first binds to the IL-6R. The complex of IL-6 and IL-6R then associates with the gp130, thereby inducing signaling. gp130 is expressed by all cells in the body, whereas IL-6R is mainly expressed by hepatocytes, neutrophils, monocytes/macrophages, and some lymphocytes [50].

A naturally occurring soluble form of the IL-6R (IL-6sR) has been found in various body fluids. Besides IL-6 binding to cell-bound IL-6R to cause biological responses in target cells, an additional model of IL-6-IL-6R modulation of cell function has been described.

In addition, the IL-6 - IL-6sR complex may in fact bind to the cell-bound gp130 and thereby stimulate cells that express gp130 but not the cell-bound IL-6R. This mechanism is named IL-6 trans-signaling [48]. T cells, many neural cells, smooth muscle cells, and endothelial cells, among others, do not express cell-bound IL-6R, but they are remarkably responsive to IL-6 but only in the presence of IL-6sR [48, 50].

The agonistic properties of the sIL-6R are counteracted by a soluble form of gp130 (sgp130), which circulates at relatively high levels (100–300 ng/mL) in human sera [48, 50].

Experimental Findings Sensory neurons express receptors for cytokines including IL-6, and nociceptive effects of peripheral cytokines have been reported in behavioral studies [51]. IL-6 influences responses of unmyelinated knee joint afferents to mechanical stimulation in vivo. In the inflamed knee, local application of IL-6sR causes an increase in responses to mechanical stimuli. Thus, IL-6 and its receptor signaling are important factors in the generation of mechanical hypersensitivity under arthritic conditions [52].

Clinical Findings IL-6 is found more frequently in the synovial fluid from patients with TMJ pain than in healthy controls, and high IL-6 levels are associated with pain [53]. IL-6 is significantly raised in RA, and the plasma level of IL-6 was reduced after systemic administration of infliximab in parallel with a reduction of global joint pain intensity [54]. In an arthroscopic study of TMJ internal derangements, IL-6 showed the closest correlation with the degree of synovitis [30].

7.3 Serotonin

Serotonin (5-HT) is peripherally a mediator released during inflammation from platelets and perhaps also serotonergic neurons. For example, platelets activated by the rheumatoid factor in RA release serotonin and other mediators. Serotonin produces hyperalgesia by a direct action on the 5-HT_3 receptors of the primary afferent sensory neurons [55]. The 5-HT_3 receptor is peripherally located only on neurons [56]. Activation of 5-HT_3 receptors causes a long-lasting sensitization of high-threshold mechanosensitive afferents as well as a brief excitation of chemo- and mechanosensitive afferents in joints [57].

7.3.1 Serotonin as Clinical Biomarker of Temporomandibular Joint Pain?

Serotonin is undetectable in TMJ synovial fluid from healthy individuals [58], which is important from a diagnostic point of view. Elevated serotonin levels in the TMJ synovial fluid has been strongly associated with TMJ pain provoked during mandibular movements and reduced maximum voluntary mouth opening capacity [59]. Pain localized to the TMJ as a response to mandibular movement might thus be a useful clinical parameter for verification of inflammatory articular pain conditions of the TMJ [59]. Local and systemic serotonin predicts the effect of intra-articular glucocorticoid treatment on TMJ pain in patients with chronic TMJ arthritis of systemic nature, while change in pressure-pain threshold over the TMJ is influenced by systemic serotonin [60].

7.4 Prostaglandin E_2

Prostaglandin E2 (PGE_2) is locally synthesized and released during inflammation. The synthesis of prostaglandins is inhibited by glucocorticoids and by nonsteroidal anti-inflammatory drugs (NSAIDs) [61]. PGE_2 acts as a potent proinflammatory, immunoregulatory molecule via EP receptors and stimulates bone resorption, promotes sensitization of peripheral nociceptors, and elicits erythema as well as edema [62].

Ligands Prostaglandins are synthesized de novo from membrane-released arachidonic acid by most cells when these are activated by mechanical trauma or by specific cytokine, growth factor, and other stimuli. Prostaglandins exert their actions on target cells in close proximity, both as an inflammatory mediator released at the site of tissue inflammation and neuromodulator that alters neuronal excitability and synaptic processing [63].

Receptors There are at least nine known prostaglandin receptor forms in mouse and man, and some of these can be found in splice variants [64]. Most of the prostaglandin receptors are localized at the plasma membrane although some are situated at the nuclear envelope [65].

Clinical Findings PGE_2 has been found in TMJ synovial fluid from patients with internal derangement [66] and chronic TMJ inflammatory disorders but not in healthy individuals [67]. Alstergren and Kopp detected PGE_2 in 20 out of 30 TMJ in patients with chronic inflammatory joint disease, and the levels were found to be related to TMJ pain on mandibular movement [67].

7.5 Glutamate

Peripheral glutamate, which is a mediator not primarily associated with inflammation, and its receptors have been found to possess modulatory

roles in peripheral nociception and sensitization and are elevated in synovial fluid from joints with active arthritis [68]. Synovial tissue glutamate originates from inflammatory cells or nerve fibers in the inflamed synovial membrane but also from plasma extravasation into the synovial tissues [69].

Injection of glutamate activates the peripheral N-methyl-D-aspartate (NMDA) receptor. Glutamate injected in the healthy human TMJ evokes immediate pain that is partly mediated by peripheral NMDA receptors in the synovial tissues [70]. Peripheral block of the NMDA receptor reduces glutamate-induced TMJ resting pain but also reduces glutamate-induced palpation pain in women. However, peripheral NMDA receptors may play a minor role in the pathophysiology of TMJ arthralgia because ketamine, an NMDA antagonist, had little effect on TMJ pain in these patients [70]. The relevance of synovial fluid glutamate is thus still unclear and warrants further investigation.

7.6 Current or Potential Treatments of Temporomandibular Joint Pain

Targeting cytokine, serotonin, or prostaglandin receptors for specific treatment of TMJ pain is promising. However, this approach is not clinically available today and seems to require substantial development and trial before it may be considered an option.

7.6.1 Cytokine Receptors

There is today only one approved treatment with a specific cytokine receptor antagonist for clinical use: anakinra (IL-1ra). However, anakinra is approved for treatment of RA and not for specific treatment of pain, although pain is certainly an important aspect of RA. On the other hand, several anti-TNF drugs such as infliximab, etanercept, and adalimumab are now available for the treatment of RA [71]. The clinical efficacy profiles, including reduction of pain, for infliximab, etanercept, and adalimumab have been extensively reviewed in detail elsewhere (see, e.g., [72]).

Despite the revolutionary improvements in RA treatment with the introduction of anti-cytokine therapy, about a third of the patients do not respond to anti-cytokine treatment or still experience symptoms from a single or a few joints [73]. This suggests, in turn, a significant influence by non-TNF-modulated inflammatory mechanisms in the nonresponders. Accordingly, more than a third of patients with TMJ pain did not respond to systemic infliximab treatment within 24 weeks, and treatment failure was associated with high circulating levels of IL-1β or rheumatoid factor [74]. Since the major pathology takes place in the synovial tissues, intra-articular administration of cytokine blockers could be a future possibility to obtain remission of affected single joints. Indeed, there are some recent positive reports with single intra-articular infliximab injections in patients with RA or ankylosing spondylitis [75, 76]. There is also one case report of the use of multiple intra-articular infliximab injections. In that report, the clinical and radiographic course TMJ involvement in a patient with severe TMJ symptoms from psoriatic arthritis resistant to both systemic infliximab and intra-articular glucocorticoid was presented. The patient received multiple intra-articular infliximab injections every sixth week for 36 weeks, and the TMJ symptoms improved already after the first bilateral intra-articular infliximab injections but even more so after the second set of injections. A considerable improvement remained for the 36 weeks studied [16].

7.6.2 Serotonin Antagonists

Intra-articular TMJ injections of the serotonin 5-HT_3 receptor antagonist granisetron have been used in patients with RA. The injections caused a short-time reduction of TMJ movement pain, a reduction that was greater in patients with high serotonin levels in plasma [77].

Conclusion

There are potential candidate biomarkers for diagnostic or prognostic purposes in arthritis of the TMJ, especially in diseases like RA and PsA. The most promising candidates, today, probably belong to the cytokine family or any of their receptors. This is supported by the revolutionary effects seen by recent drugs in the treatment of RA, PsA, etc., where many of these drugs directly and specifically target some of the proinflammatory cytokines like TNF, IL-1, and IL-6. However, there is still a need for adequate studies aimed to establish diagnostic and prognostic value of these mediators.

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Genetic Biomarkers of Orofacial Pain Disorders

8

Ze'ev Seltzer and Scott R. Diehl

Abstract

Despite major advances in basic and translational research, pain medicine still offers no cure for persistent orofacial pain disorders, nor effective strategies for preventing their development. There is growing hope that knowledge garnered in pain genetics will identify novel target molecules for more effective drug treatments and "precision medicine" approaches that best fit each patient's genome. Combined with nongenetic information, knowledge of inherited variation may lead to development of algorithms that yield more precise biologically based diagnoses and prognoses for orofacial pain conditions. This chapter reviews the current status of pain genetics with a focus on persistent orofacial pain.

8.1 Introduction

Chronic pain conditions impact many aspects of life that can be dissected into spatiotemporal, emotive-aversive, and cognitive-evaluative parameters as well as an impact on functionality and participation at work, domestic, and social life. Each of these parameters is processed in dedicated peripheral and central nervous system (PNS, CNS, respectively) nodes of the pain network, involving specific mechanisms and unique neuronal and glial types and their cell-specific neurochemicals. Each pain disorder and functional parameter are likely to be encoded by a unique combination of genes, upregulated or downregulated at specific times following the etiological event in the different parts of the pain network in the PNS and CNS. A multitude of genes and sequence variants within them and throughout the large regions of the genome between the genes that we now know are important for gene regulation mean that persistent orofacial pain disorders (POPDs) are complex traits. In most cases, the overall heritable risk is the sum of the contributions of many genetic variants, each contributing a small effect size [1]. In addition to single nucleotide polymorphism (SNP) sequence variation, there are copy number

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polymorphisms, gene-environment interactions, epistatic (gene-gene) interactions, and epigenetic variation caused by factors other than changes in the DNA sequence. A POPD occurs when individuals, who inherited variations that increase risk for development of a certain type of pain disorder, are exposed to one or more environmental factors, often (though not always) comprising an acute injury. As described below, some pain disorders may develop spontaneously in carriers of genetic risk factors in genes such as *SCN9A* that encode the voltage-gated sodium channel NaV1.7 [2–4]. These genetic variants cause a specific POPD and/or its maintenance over time by producing certain peptides or proteins that are the building blocks of the orofacial pain network and structures that may be unique to the orofacial region or are shared with other parts of the body. Unfortunately, the identity of most POPD genes, their biologically important mutations, and the gene-gene, gene-environment, and epigenetic interactions at play are not yet known. This chapter provides an update on the limited number of confirmed or strongly suggested genes important for POPD and explores their potential utility as predictive biomarkers and as drug treatment targets.

8.2 Genetics of the Major Persistent Orofacial Pain Disorders

8.2.1 Temporomandibular Disorder (TMD)

The *COMT* gene encodes the enzyme catecholamine-O-methyltransferase that inactivates and catabolizes the neurotransmitters dopamine, norepinephrine (NE), and epinephrine, as well as caffeine and estrogens. Not surprisingly, COMT affects many neural functions including reward-motivated behavior, cognition, psychiatric disorders, arousal, motor control, and stress reactivity. It is expressed in several types of neurons and glia cells throughout the CNS and PNS. Since these neurotransmitters play important roles in processing nociceptive inputs, this gene has been the focus of a wide range of studies of diverse acute, inflammatory, and chronic pain conditions both in humans and rodent models [5]. As early as 1976, reduced COMT activity was reported in red blood cells with increased levels of catecholamine metabolites in the urine of POPD patients [6]. The past decade has seen a surge in research, reviewed in the following section, that provides an example of how genetic knowledge can be translated from mechanism to clinical applications using pharmacogenomic strategies.

COMT is located in chromosome 22 (at band q11.21). *COMT* has two alternative exon splicing patterns: one codes a soluble protein (S-COMT) and the other membrane-bound (MB-COMT) form is expressed more abundantly in the nervous system [7]. These two alternative transcripts are formed by differential mRNA splicing from the same DNA sequence on each of the two copies of each autosomal gene that every cell normally carries. Both forms of COMT methylate catechol neurotransmitters, and this inactivates them. But while S-COMT operates in the cytoplasm and the nucleus, the MB-COMT isoform is located in the endoplasmic reticulum and the outer membrane of the soma, axons, and dendrites of neurons and some glia cells. As the C-terminal catalytic domain of MB-COMT is located extracellularly, it is positioned to inactivate catechols in synaptic clefts and outside the synapse.

TMD is the most prevalent orofacial pain condition affecting mainly women, typically between 20 and 50 years of age. Based on a comparison of the prevalence of TMD in 1236 monozygotic (identical) versus 570 dizygotic (fraternal) female twin pairs, Plesh et al. estimated that TMD is 27% heritable [8], a value relatively low compared to other chronic pain conditions [9]. *COMT* has been assayed for six common SNPs throughout the gene, searching for association with TMD symptoms. These SNPs included *rs2097903* (located in the promoter of MB-COMT); *rs6269* (located in the promoter of S-COMT); *rs4633*, *rs4818*, and *rs4680* (located in exons shared by both isoforms); and *rs165599* (located in the 3′ untranslated region of *COMT*) [5, 10, 11]. Polymorphisms in *rs4633* and *rs4818* do not

change the protein sequence as they are synonymous SNPs, but were included as markers for other nearby potentially causative SNPs. *rs4680* is a non-synonymous SNP, where a nucleotide substitution between G--> A results in an amino acid change from valine (Val) to methionine (Met) at codon 158 (*Val*^{158}*Met*). The A (or *met*) allele is associated with lower enzymatic activity, due to thermal instability of the molecule at body core temperature. Thus, at normal body temperature, the enzyme becomes less catalytically effective, resulting in extended presence of catechol neurotransmitters in the synapse. Based on combinations of the genotypes of these SNPs, three haplotypes were identified: LPS, associated with low pain sensitivity to a diverse array of experimental noxious stimuli; APS, associated with average pain sensitivity; and HPS, associated with high pain sensitivity. Carriers of LPS had high COMT enzymatic activity, associated with rapid clearance of catechol neurotransmitters from synapses in pain pathways. Carriers of the APS haplotype had an average COMT enzymatic activity and average pain sensitivity to the same noxious stimuli, whereas carriers of the HPS haplotype had high pain sensitivity and low COMT enzymatic activity. Moreover, Diatchenko et al. (and others later on) showed that symptoms of TMD depended on the number of copies of these genotypes: carriers of LPS diplotypes (i.e., two copies of the LPS haplotype) had less TMD pain scores than the heterozygotes, and the latter had lower TMD pain score than those carrying no LPS haplotypes, suggesting that the LPS haplotype was associated with significant protection against TMD [5, 10, 11].

Inhibition of COMT activity in rats and mice has been found to be associated with increased nociception in several models of acute and inflammatory pain [12]. Compatible with this observation, *COMT* knockout mice were more sensitive to nociceptive stimuli and had reduced analgesic responses to opioids and stress, whereas mice overexpressing COMT showed decreased nociceptive sensitivity in such models. However, inhibition of COMT in rat models of neuropathic pain by nitecapone caused the unexpected antinociception and antiallodynia [13]. This finding makes sense if considering the reciprocal role that epinephrine and norepinephrine play in some types of neuropathic pain, operating in the CNS versus the PNS. Following nerve injury an abnormal excitatory link forms at the site of injury between postganglionic sympathetic efferents and some primary afferents. Persistence of pain in certain peripheral neuropathies is explained in part by ongoing and/or evoked sympathetic activity. This abnormal link is the product of upregulated expression of adrenoreceptor proteins in somata of injured afferents in dorsal root ganglia associated with the injured nerve. These proteins are shipped distally by axonal transport and assembled in the membrane of axonal sprouts of injured afferents in nerve end neuromas or neuromas in continuity [14]. NE, released from postganglionic sympathetic efferents, binds to adrenoreceptors on afferents causing suprathreshold depolarization and discharges of action potentials that contribute neuropathic pain [15]. *COMT* knockout mice and human carriers of the low enzymatic activity variant of MB-COMT are expected to have more NE present in neuromas, enhanced ectopic afferent firing, and sympathetically maintained neuropathic pain. This shows that *COMT* may have pleiotropic effects in some types of neuropathic pain, where the same gene may play two contrasting effects in two different targets along the same pain neuroaxis [16]. Thus, if confirmed by research, the impact of *COMT* will depend on the genotypes an individual carries and on the type of chronic pain.

Exogenous inhibitors of COMT have additional documented properties, including scavenging oxygen and nitrogen radicals, and these may also explain the antiallodynic effects found in some neuropathic pain models. Also, increased number of μ-opioid receptors in certain brain areas following nerve injury may be responsible for the enhanced opioid effects associated with low COMT activity. A low COMT activity also increases the availability of opioid receptors and may enhance opioid analgesia [17].

Gene-by-environment interaction between *COMT* haplotypes and orthodontic treatment was examined in view of the fact that this treatment is a risk factor in developing TMD. Carriers of the

TMD-protective *COMT* LPS haplotype, who had orthodontic treatment, were partially protected from developing TMD, whereas carriers of the APS and HPS haplotypes had a greater risk for TMD if undergoing such orthodontic treatment. This finding supports the model that TMD results from an interaction between heritable risk (here conferred by the APS and HPS *COMT* haplotypes) and exposure to an environmental effect [18].

Recent experiments in the rat show that acute pain sensitivity was reduced when blocking both adrenergic transmission and COMT activity [19]. This result has motivated an ongoing clinical trial where TMD pain patients were stratified by their LPS, APS, and HPS *COMT* haplotypes, addressing their TMD pain by blocking β-adrenergic neurotransmission with propranolol [20]. This ongoing study is one of the first to show the utility of pharmacogenetic approaches to the treatment of POPD.

Several other candidate POPD genes were additionally identified in the Orofacial Pain Prospective Evaluation and Risk Assessment (OPPERA) study [21]. The *ADRB2* (encoding the β2-adrenergic receptor) gene is located on human chromosome 5 (at band q31–32). It comprises about 5500 kb and harbors the following eight common SNPs that were genotyped to interrogate their possible association with TMD: *G-7027A*, *rs11948840*, *rs1432612*, *rs1432613*, and *rs2400696* are all located in the promoter region of this gene, whereas *rs1042703* ($Arg^{16}Gly$ – a non-synonymous polymorphism), *rs1042704* ($Gln^{27}Glu$ – a non-synonymous polymorphism), and *rs1042707* ($Leu^{83}Leu$ – a synonymous polymorphism) are in the coding regions. Three common haplotypes were identified, named H1, H2, and H3 [22], that play a role in TMD onset, somatization scores, and low blood pressure (known covariates of TMD) [23–25].

Two other interrogated TMD genes relate to the pivotal role that serotonin (5-HT) plays in processing nociceptive input. *HTR2A*, encoding the serotonin 5-HT_{2A} receptor, is located in chromosome 13 (band q14–21), harboring a synonymous $T^{102}C$ SNP where the less frequent allele (C) is associated with TMD [26] [24] (but not in a Japanese population [27]) and with fibromyalgia [28]. The second gene, *SLC6A4*, encodes the sodium-dependent serotonin transporter (also known as *5-HTTLPR*) that is located in chromosome 17 (at band q11.1–q12). It harbors a 44-base pair insertion/deletion "indel" variant in the promoter region. Two alleles of *SLC6A4* are known, a short allele "s" having a lower transcriptional activity and a long allele "l." Carrying the one allele results in more transporter molecules available for a faster clearance of serotoninergic synaptic clefts from serotonin or its clearance from non-synaptic peripheral targets of 5-HT on nociceptors. Thus, similar to COMT, the net effect of carrying either allele is complex, resulting from contrasting effects of 5-HT in the CNS and PNS. That net effect depends on which allele an individual carries, the number of such copies (i.e., ss, sl, or ll), and the pain phenotype in question: in the periphery, serotonin stimulates and sensitizes nociceptors and sprouts of neuroma afferents, whereas in the CNS there are pro-nociceptive effects and antinociceptive 5-HT effects depending on the type of receptors present on postsynaptic sites of serotoninergic synapses. *HTR2A* also harbors a common $T^{102}C$ polymorphism. This SNP (*rs6313*) is a synonymous substitution located in exon 1 where it codes the 34th amino acid as serine. It has been interrogated in a small cohort of 200 Brazilian men and women, of whom 100 were TMD cases. Carrying the *CC* genotype is associated with a higher risk for TMD at odds ratio (OR) of 2.25 (95% CI, 1.13–4.46) [28, 29]. This finding, however, did not replicate in a Turkish small cohort of women with fibromyalgia [30].

ERα (encoding estrogen receptor alpha) is another gene studied in the OPPERA project for an obvious reason: POPDs, including TMD-related pain, are significantly more prevalent in women than men. Moreover, estrogen targets tissues of the temporomandibular joint (TMJ) that are frequently involved in TMD pain disorders [31–33]. This gene is located on chromosome 6 (at band q25.1), harboring two SNPs that are seen frequently in the population: $T^{-396}C$ and $A^{-351}G$. Carriers of the substituted $T^{-396}C$ SNP have a tenfold increased transcription of *ERα* [34]. Brazilian women who carry the *CG* haplotype

had a 3.2-fold risk of developing TMD (95% CI, 1.6–6.2) [35], but not in Korean women [36].

Another biochemical pathway probed in the context of TMD is folate. Genes interrogated were *SHMT1* (encoding serine hydroxymethyltransferase-1), *MTHFD* (encoding methylenetetrahydrofolate dehydrogenase), and *MTRR* (encoding methionine synthase reductase) harboring the SNPs *rs1979277*, *rs638416*, *rs2236225*, and *rs1801394*. Aneiros-Guerrero et al. [37] reported on finding in a Spanish cohort significant associations between alleles of these SNPs and risk of developing TMD with ORs ranging from 2.35 to 3.99. However, since these findings reported on a small cohort comprising only 89 TMD cases (all women) versus 143 controls (lower 95% CI from 1.10 to 1.72, higher 95% CI from 5.00 to 9.25), replication in a larger cohort is needed. This group also reported that carrying the *GSTM1*-null polymorphism (a deletion of the *GSTM1* gene, encoding glutathione S-transferase μ1, an enzyme associated with inflammatory oxidative stress) was associated with TMD at an OR = 2.21 (95% CI, 1.24–4.36) [37]. This finding also needs replication.

8.2.2 Trigeminal Postherpetic Neuralgia (TPHN)

TPHN results from neural damage caused by reactivated varicella-zoster virus (VZV) in the trigeminal ganglion. Thus, genetic polymorphisms that influence the immune system and the inflammatory response to viral infections, particularly to VZV, are likely contributors to the heritable risk of TPHN. Likewise, polymorphisms predisposing trigeminal primary afferent nerve fibers to be sensitive to the damage caused by VZV particles are expected to render carriers to be more/less susceptible for TPHN, as do polymorphisms in genes associated with the response of CNS pain pathways to peripheral nerve injury. The extent of nerve injury and type of afferents injured will determine in part the likelihood of developing TPHN and its characteristics. A separate set of genetic polymorphisms determines the chances of deriving sufficient pain relief from certain analgesics and of developing adverse reactions to these analgesics.

About a million individuals in the USA develop shingles every year (http://www.cdc.gov/shingles/about/overview.html), but only about a fifth to a tenth will proceed to develop PHN [38]. Race also plays a role, e.g., African Americans are 50–75% less likely to develop PHN compared to Caucasians [39]. It is yet to be determined whether or not the same effect of race/ethnicity exists in TPHN. Age is another important covariate, as <10% of people who are younger than 60 years of age develop shingles, whereas in those who are older than 60 years, the incidence rises to about 40%. Sex is another covariate, as women are more susceptible to develop shingles than men [40].

Pain symptoms in TPHN vary highly across individuals, even when the shingles occupied the same size of skin and were of similar severity, suggesting that the pain symptoms are controlled genetically by a pool of genes different from the pool engaged in the disease itself. The median heritability value calculated for several chronic pain conditions, other than TPHN, is about 0.45, suggesting that approximately 45% of the variance in chronic pain levels is due to genetic factors [41, 42]. As TPHN has a late age of onset, it is difficult to estimate its heritability because a twin who did not develop the disease even at an advanced age cannot be considered a "control" because he/she may still be a latent "case" who could develop TPHN later.

Three candidate PHN genes were identified to date, however none as of yet for TPHN. Polymorphisms in chromosome 6 (band p23.1) were screened in a Japanese cohort of PHN. This genomic region harbors the human leukocyte antigen (HLA) system and, therefore, is implicated in the immune response to VZV reinfection. This study found a significant association between PHN and a haplotype comprising the *HLA-A*3303*, *B*4403*, and *DRB1*1302* alleles, but not with polymorphisms on the promoter of the *TNFA* gene (encoding tumor necrosis factor alpha, a cytokine that participates in inflammatory reactions) or *NCR3* (encoding natural cytotoxicity triggering receptor 3) also designated as

CD337, or cluster of differentiation 337, or NKp30 [43]. The second candidate gene was *APOE* that is mapped to human chromosome 19 [44]. *APOE* encodes for apolipoprotein E (APOE) that transports lipoproteins, fat-soluble vitamins, and cholesterol into the lymph system and then into the blood. APOE is mainly synthesized in the liver but also in the kidneys and spleen as well as in the nervous system. Astroglia and microglia are its primary producers in the CNS, whereas neurons express the receptors for APOE [44]. In the brain APOE is mostly known for its role in Alzheimer and Parkinson diseases and cognition, but the rationale for studying whether polymorphisms in APOE influence PHN was different, presuming that VZV particles bind to their cellular targets (i.e., afferents and their somata in the trigeminal ganglion) and enter them using the same sites in the cell surface to which APOE molecules bind. Thus, by way of competition with APOE, the damage caused by VZV particles could be limited. Another mechanism by which APOE could be involved in PHN is via its link to intraneuronal increase in calcium ion levels and apoptosis following injury [45]. Indeed, fewer females carrying the *e4* allele had PHN whereas carrying the *e3* allele conferred increased risk [43].

8.2.3 Trigeminal Neuralgia

Trigeminal neuralgia (TN) is said to be the most excruciating pain that humans suffer. It is typically triggered by low threshold inputs in the orofacial region and manifests as repetitive sharp paroxysmal attacks of pain that are limited to one or two divisions of the trigeminal nerve, usually unilaterally [46]. TN is rare, having an incidence of approximately 70/100,000, usually peaking in the fifth decade of life. It is so rare that the chances of having more than one individual with TN in the same family make it impractical to study heritability using pedigree analysis or twins. But several reports of TN cases occurring in multiple members of a family indicate that familial forms are likely to exist that may be caused by genes of major effect [47–49]. At this time, however, it is not known whether the same genetic polymorphisms confer risk for familial and nonfamilial TN.

Many, but not all, cases of TN develop following abnormal compression of the trigeminal root by an ectopic blood vessel. This implies that production, pathophysiology, and treatment of TN involve three largely independent sets of genetic variants. The first predisposes carriers to develop the etiological vascular deformity, which in itself is asymptomatic, but when they co-inherit the second set of genetic variants, TN occurs. The genetic variants causing neurovascular compression (NVC) are relatively common, since this condition is seen in about 16% of healthy adults, the vast majority never developing TN or related facial sensory abnormalities [50, 51]. Presumably, the first set of genes encode proteins that comprise the wall of blood vessel and/or neurons, glia, and connective tissue within the trigeminal root that interact mechanically and/or chemically with the pounding juxtaposed blood vessel or inflammatory cells responding to this injury.

The fact that only one in ~10,000 individuals who have NVC progresses to develop TN suggests that TN patients carry in addition to the first set of genetic variants another set of variants that is independent of the first. This second set predisposes carriers to develop TN pain symptoms given the presence of an NVC. These variants are likely in genes encoding proteins in pain pathways of the trigeminal system and/or inflammatory and immune cells that might be attracted to the site of root injury by NVC. As these genes are unknown as of yet, it is impossible at this stage to determine how unique are they to the trigeminal system or whether they play the same role in pain pathways elsewhere in the body. The fact that TN is only seen in the face and mouth may be due to the fact that the cause of TN does not occur elsewhere in the body. Nevertheless, if the second set of genetic variants is expressed throughout the somatosensory system, carbamazepine and oxcarbazepine, the drugs most often effective for treatment of TN, should be much more effective in treating paroxysmal neuropathic pain elsewhere in the body, but this is not the case [52]. These facts suggest that a preferential expression

of specific subtypes or combinations of sodium channels are expressed in the trigeminal system and lead to TN. While it is tempting to think of genes for sodium channels as the most likely candidates for TN (in light of the efficacy of carbamazepine and oxcarbazepine), this may not necessarily be so. The electrogenic properties of nociceptive primary afferents, and neurons in pain pathways in the CNS, depend on other ion channels as well, including potassium, calcium, and chloride, and on nonionic mechanisms, including glial properties.

The rarity of TN is compatible with it being a Mendelian trait governed by a gene of major effect with low penetrance that is expressed exclusively in the trigeminal system, perhaps modified by a few other genes as this would explain changes in TN over the years, variability in its type and severity of symptoms among different cases, and differences in response to medications. No less likely, however, TN may be a polygenic complex heritable trait, with risk determined by multiple rare and common genetic variants that only co-occur rarely in an individual who also inherits the NVC-causing genetic polymorphisms. Both scenarios equally well explain the wide range of phenotypic variation seen in TN, manifesting in the frequency, duration, intensity, and enormity of pain paroxysms, what stimulus provokes them, responses to treatments, as well as the impacts on functionality, sleep, and other aspects of the quality of life with TN.

One study analyzed gene expression in TN using reverse transcription polymerase chain reaction (RT-PCR) of gingival biopsies from TN pain-affected regions versus the same tissues from controls to determine the expressed levels of transcripts of the voltage-gated sodium channel types NaV1.7, NaV1.8, and NaV1. Regrettably, this study was severely underpowered, comparing only 10 TN patients versus 13 pain-free controls [53]. Patients with TN had reduced levels of NaV1.7 but increased levels of NaV1.3 (albeit at a weak significance level) and no significant difference from controls in gingivally expressed NaV1.8 levels. This study must be replicated. If confirmed, these findings could suggest that, in some cases, TN is a sodium channelopathy of primary afferents, compatible with the clinical experience of successfully treating TN patients with certain sodium channel blockers. Moreover, it would suggest that nociceptive afferents undergo phenotypic switch to become hyperexcitable to low-threshold mechanical stimuli, which are the typical triggers of TN pain paroxysms. But even if this is the case, this explanation is insufficient to explain how a localized, occasionally single mechanical stimulus applied to the gums could trigger repetitive TN attacks and their strong intensity.

In a recent press conference, the British pharmaceutical company Convergence reported promising results with a new drug currently called CNV1014802 in a placebo-controlled double-blind clinical trial of TN patients. This molecule is a state-dependent sodium channel blocker that is active against the NaV1.7 sodium channel. The trial demonstrated a consistent reduction of pain severity in 58% of patients and a 66% reduction in the number of paroxysms, at the cost of no serious adverse events [http://www.convergencepharma.com/index.asp?page_id=14].

A large-scale gene mapping study is currently underway, carried out by a group that includes the authors of this chapter and others. It is aimed at identifying the genetic underpinnings of TN using a genome-wide approach. Rather than interrogating candidate genes, a genome-wide approach is free of biases driven by preexisting etiological, mechanistic, or pharmacological information. Funded by the Facial Pain Research Foundation (FPRF, USA), its goal is to genotype in the discovery phase about 500 carefully diagnosed TN cases and replicate the results in a second cohort of 500 TN cases. The study includes a SNP array-based exome analysis of the DNA (see below) to identify SNPs segregating in patients having TN compared to the general population that serves as a control group. Since TN is so rare, it is safe to use genotypes drawn from the normal population as controls. An exome analysis can be carried out either by fully sequencing the coding regions (i.e., the exons) of all genes throughout the genome or with microarrays that carry hundreds of thousands of genetic probes developed to detect the presence of SNPs in these

exons. Exonic variants encode the amino acid sequence of all peptides and proteins comprising the trigeminal nervous system, including those that control the development and maintenance of TN. However, by focusing exclusively on the exome, this analysis ignores the introns (i.e., nucleotide bases in segments of the DNA that space between exons within genes). Introns harbor SNPs that may influence the transcription and stability of the mRNA. In addition, exome analysis also ignores SNPs in the vast spans of the DNA between the genes. Formerly referred to as "junk DNA," we now know that these regions contain innumerable sequence variations influencing gene expression that we are just now beginning to understand. Therefore, to complement the exome analysis, DNA samples of the same TN patients comprising the discovery cohort are also undergoing a genome-wide association study (GWAS) using an SNP microarray that contains more than 800,000 SNP targets, complementing the coverage of the exome array focusing on the coding regions that make up only around 3% of the human genome. The results of this analysis will undergo validation using the independent replication sample of 500 TN patients currently being recruited using the same patient selection criteria and pain phenotyping tools [http://www.facingfacialpain.org].

A third set of genetic variants may predispose carriers who are already TN patients to derive beneficial analgesic effects from membrane stabilizers, anticonvulsants, and antiarrhythmics and other analgesics with minimal adverse side effects. This means that those TN patients who do not carry these genetic variants may not benefit from these analgesics or they might be so sensitive to the medication's adverse side effects that preclude their use. It is likely that the analgesic efficacy of each medication type is controlled by unique genetic variants, with some overlap that may be related to shared transport mechanisms in the alimentary canal, catabolysis, or chaperoning to their targets in the trigeminal nervous system. A TN patient is likely to inherit a mixture of such genetic variants that reduce or enhance the efficacy of treatment outcomes. Until these variants are identified, enabling "precision medicine" where the most effective drug can be selected for each patient, clinicians have to proceed via a "trial-and-error" approach that for many patients is far from optimal.

8.2.4 Burning Mouth Syndrome (BMS)

BMS manifests as spontaneous burning painful sensation in the mouth, often worsening during the day and subsiding at night, typically associated with dysgeusia. BMS is comorbid with gastrointestinal, urogenital, psychiatric, neurologic, and metabolic disorders, as well as drug reactions. The estimated prevalence ranges widely from 0.7% (a value derived from self-reports of >45,000 American households [54]) up to 15% in a Finnish adult cohort (but half of whom had oral mucosal lesions) [55]. A prevalence of ~1% was documented in a retrospective study of >3000 Brazilians who were referred to an oral pathology service clinic [56]. The prevalence of BMS increases with age and is 2.5–7 times more common in women than men [56–58]. The vast majority of the women with BMS are peri- and postmenopausal [59].

The pathophysiology of BMS is far from being clear, and no genes have been identified as of yet for this condition. However, some abnormal sensory mechanisms associated with BMS may provide clues about its genetic underpinnings. For example, tongue biopsy from BMS patients showed reduced numbers of unmyelinated fibers associated with taste papillae and intraepithelial fibers [55, 58], suggesting that BMS is a small-fiber sensory neuropathy [60]. Increased nerve growth factor (NGF) in the saliva of patients with BMS [61] and increased densities of TRPV1 ion channels and P2×3 receptors on primary afferents of BMS patients were reported as well [62]. These abnormalities were previously linked to sensory hypersensitivity and neuropathic pain in several conditions of painful neuropathies in humans. CNS changes manifesting as "central sensitization" of trigeminal pain pathways have been proposed as well [63–65]. Likewise, functional magnetic resonance imaging

studies showed that, compared to controls, the brain of BMS patients abnormally processes noxious and thermal stimuli in the thalamus [66]. Dysregulation of the nigrostriatal dopaminergic system has also been demonstrated in the brain of BMS patients [67]. Interestingly, Parkinson patients are 5× more likely to have BMS than controls [68]. Some relief in BMS symptoms can be accomplished by consumption of alpha lipoic acid, clonazepam, capsaicin, and antidepressants as well as psychotherapy [69].

Although no study has yet identified genes directly associated with this enigmatic pain condition, some research has hinted at a genetic component based on the increased prevalence of supertasters (i.e., persons with enhanced abilities to detect bitter taste) among patients with BMS [70].

8.2.5 Paroxysmal Extreme Pain Disorder (PEPD)

PEPD is very rare, even less common than TN. Yet readers of this book may find it of interest to learn about this condition because of the genetic architecture that brings this condition about. Orofacial PEPD manifests as pain mainly in the mandibular and submandibular regions as well as around the ocular region, accompanied with cutaneous reddening, swelling, and increased warmth (flushing) [71]. These symptoms are identical to primary erythromelalgia, but unlike the latter that appears in the extremities, PEPD is associated with pain that begins at an early age (perhaps as early as in utero) in the rectal region and later on in life moves to the face. Like erythromelalgia, symptoms of PEPD persist throughout life, manifesting as strong spontaneous paroxysms or triggered by temperature changes (e.g., a cold breeze), emotional distress, and consuming spicy foods or drinking cold drinks.

The special interest in this pain condition arose when it was found that mutations in the very same gene (*SCN9A*, encoding the voltage-gated sodium channel NaV1.7*)* are associated with extremely contrasting phenotypes: congenital insensitivity to pain, erythromelalgia, and PEPD. Both of the latter two disorders are inherited in an autosomal dominant pattern so that a single copy of the mutated gene, inherited from one parent, is sufficient to cause them, while congenital insensitivity to pain is usually recessively transmitted.

SCN9A encodes the alpha subunit of the voltage-gated NaV1.7 sodium channel. The point mutations in some positions along the alpha subunit cause nociceptive primary afferents to be hyperexcitable by way of delaying the closing time of the NaV1.7 channels when action potentials are turned off, leading to the pain attacks. But this mechanism does not explain the regional nature of the pain in PEPD: why do carriers only express the extreme paroxysms first in the rectal region when they are young and what causes the syndrome as patients age to emerge in the trigeminal region (i.e., around the eyes and mandible)? [72].

In some PEPD cases, the duration of a typical pain paroxysm is a few seconds, whereas in others it may last up to hours at a time. Furthermore, in some cases the pain paroxysms are accompanied by epileptic seizures, while in others they may be associated with slow heartbeat or short epochs of apnea. These variations in pain and non-pain symptoms may suggest that in addition to mutations on *SCN9A*, carriers may co-inherit other genetic variants elsewhere in the genome that can modify the effect of the causative *SCN9A* mutations and/or bring about additional symptoms [72].

Summary

Orofacial pain medicine faces major challenges as available treatments neither cure nor prevent POPD. Current treatment approaches utilize the “trial-and-error” and “one-drug-fits-all” paradigms that are based on population average efficacy in treating different types of pain, ignoring individual variation. As a result, the average success of analgesic treatment of POPD is measured in a reduction of only about two points on a 0–11 numerical rating scale of pain severity. In many cases, even this modest effect is attained at the cost of unacceptable adverse side effects. Since a significant portion

of the phenotypic variation in POPD and other chronic pain syndromes is heritable, there is growing hope that a better understanding of human genome variation can lead to the development of improved analgesics and "precision medicine" approaches to treatments of POPD.

Pain genetics is still at an early stage of development, and much more work lies ahead. However, we strongly believe that the preliminary discoveries already made highlight the great potential of this approach. Among the major benefits of success will include ability to use genetic biomarkers to identify individuals at high risk of developing POPD (before symptoms emerge), thus offering potential for prevention, and a new era of individualized ("precision medicine") medications for more effective treatment of these pain disorders.

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9 Serum, Synovial, and Salivary Biomarkers for Orofacial Pain Conditions

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Abstract

Orofacial conditions such as temporomandibular disorders are often associated with extended bouts of debilitating chronic pain. Unfortunately, these and other similar pathologies are characterized by their inherent complexity and poorly understood etiologies making diagnoses and subsequent treatments exceedingly difficult. With a significant proportion of the population suffering from painful orofacial conditions, the development of new and accurate diagnostic procedures is essential to improve the current standard of care. Here, we overview the potential of serum, saliva, and synovial fluids as reservoirs of biochemical information capable of discerning specific disorders, including those correlated with orofacial pain. Determining the worth of these biofluids in the assessment of health status could expedite diagnoses and enhance pain management strategies while also enhancing our understanding of disease pathophysiology.

9.1 Introduction

With affected areas including the face, mouth, ears, eyes, nose, and neck, orofacial discomfort may result from a diverse range of conditions. Among these are temporomandibular disorders (TMDs), the second most common musculoskeletal condition after chronic lower back pain. TMDs are highly prevalent and frequently associated with debilitating chronic pain, a feature with devastating impact on patients' quality of life. This emphasizes the need for developing methodologies aimed at early diagnosis and effective management. However, the diagnosis of pain relies on patient reports, questionnaires, and semi-objective

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findings for diagnostics (see Chap. 1, 5, 6 and 7). As described in Chap. 6, the diagnosis of myalgia using the validated Diagnostic Criteria for TMD (DC/TMD], includes three pain subgroups: local myalgia, myofascial pain, and myofascial pain with referral. This current lack of specific validated diagnostic criteria for pain subgroups serves as a major barrier to achieving early diagnosis and effective management. Although much effort has been placed into establishing novel modes to accurately diagnose disorders of chronic pain, the most efficacious methods have yet to be determined.

One approach involves the evaluation of biofluids for molecular and microbial clues that may actually indicate the onset or progression of disease. While blood is considered the gold standard for these purposes, recent evidence suggests that saliva and synovial fluids could potentially be utilized as well. We begin our discussion here with an overview of these biofluids and go on to describe each of these as a prospective warehouse of biochemical indicators capable of determining individuals suffering from chronic oral facial pain.

9.2 Biofluids

A biofluid is defined as any aqueous solution produced by the body. With functions ranging from digestion to joint lubrication, these fluids may include serum, sweat, saliva, gastric acid, synovial fluid, tears, etc. Our focus will comprise an overview of serum, synovial, and oral fluids and the possible utilization of their respective molecular constituencies as diagnostic media. Elucidating disease-indicating entities within these fluids and exploring their etiology may facilitate a greater understanding of human pathophysiology and provide new insights regarding the diagnosis and management of chronic orofacial pain disorders. We begin our discussion here by detailing the use of blood serum as a diagnostic medium.

9.2.1 Blood Serum

Blood serum is the pale yellow liquid remaining when coagulated blood samples are centrifuged at high speeds. Not to be confused with blood plasma, which results from the forced separation of blood cells prior to clotting, serum is free of fibrinogens and is the predominant source of blood-based diagnostic analytes.

9.2.1.1 Function

The primary function of blood serum is to facilitate the transport of nutritive molecules and waste products throughout the body. It also plays a key role in regulating bodily fluids, controlling core body temperature, maintaining pH, and supporting immunological responses to pathogenic invasion.

9.2.1.2 Composition

Although serum is predominantly composed of water (95%), its molecular constituency consists of proteins and peptides (such as albumins, globulins, lipoproteins, enzymes, and hormones), amino acids, lipids, carbohydrates, electrolytes, and numerous additional entities present in minute concentrations [1]. As an easily accessible body fluid teeming with analytes, blood serum has been extensively investigated for decades. These efforts have resulted in the publication of comprehensive reference tables describing its biochemical composition. As an example, Psychogios and colleagues [1] developed a human serum metabolome database detailing over 4000 unique molecules found within blood samples (see Table 9.1). Aberrations in the respective concentrations of these analytes have routinely been associated with the presence or onset of distinct disease states. To this day, probing an individual's serum remains the most commonly employed technique for the analysis of biofluids and determination of health status.

9.2.2 Synovial Fluid

Similar to serum in appearance, synovial fluid is a viscous stress-bearing solution occupying the synovial cavities of highly mobile joints, such as the temporomandibular joint (TMJ) (see Chap. 7).

9.2.2.1 Function

While synovial fluid is considered multifunctional, its primary purpose is to lubricate cartilaginous tissues in regions where proximate bone structures

Table 9.1 Chemical classes in the serum metabolome database

Compound class	No.	Compound class	No.
Acyl glycines	10	Inorganic ions and gases	20
Acyl phosphates	10	Keto acids	8
Alcohol phosphates	2	Ketones	6
Alcohols and polyols	40	Leukotrienes	8
Aldehydes	3	Minerals and elements	40
Alkanes and alkenes	10	Miscellaneous	77
Amino acid phosphates	1	Nucleosides	24
Amino acids	114	Nucleotides	24
Amino alcohols	14	Peptides	21
Amino ketones	14	Phospholipids	2177
Aromatic acids	22	Polyamines	11
Bile acids	19	Polyphenols	22
Biotin and derivatives	2	Porphyrins	6
Carbohydrates	35	Prostanoids	23
Carnitines	22	Pterins	14
Catecholamines and derivatives	21	Purines and purine derivatives	11
Cobalamin derivatives	4	Pyridoxals and derivatives	7
Coenzyme A derivatives	1	Pyrimidines and pyrimidine derivatives	2
Cyclic amines	9	Quinones and derivatives	3
Dicarboxylic acids	17	Retinoids	11
Fatty acids	65	Sphingolipids	3
Glucuronides	8	Steroids and steroid derivatives	109
Glycerolipids	1070	Sugar phosphates	9
Glycolipids	15	Tricarboxylic acids	2
Hydroxy acids	129		
Indoles and indole derivatives	12		

Adapted from [1]

concertedly facilitate skeletal movements. In this milieu, synovial fluid serves to support coordinated physical actions via preserving and protecting adjacent bone tissues, thereby safeguarding against frictional wear and promoting the longevity of joints. Hence, synovial fluids are essential for the long-term utilization of complex skeletal structures.

9.2.2.2 Composition

The molecular composition of synovial fluid is derived from blood plasma as well as chondrocytes and other cell populations lining the synovial cavity. Interestingly, its biochemical makeup may actually be influenced by the synovium, a semipermeable membrane encompassing the non-cartilaginous surfaces of synovial joints. As a size-selective boundary, the synovium acts to inhibit the passage of high molecular weight compounds. Thus, substances such as hyaluronic acid, a compound secreted by synovial cell populations, are selectively retained inside the synovial space, while large blood-based molecules like fibrinogen are prohibited access. Consequently, synovial fluid is mainly a dialysate of blood plasma supplemented with locally produced constituents including hyaluronic acid, lubricin, and other joint-lubricating macromolecules [2].

The diverse nature of synovial-based compounds in combination with the intimate anatomical environment of the synovial space suggests the possibility that fluids bore from this region may contain a unique collection of biomarkers with the potential to reveal key information regarding joint health. Examining the molecular composition of synovial fluids could lead to the discovery of discriminatory factors indicative of disease pathogenesis with the capacity to preclude the occurrence of chronic pain (see Chap. 7).

9.2.3 Saliva

Saliva is produced by a number of salivary glands located within and around the oral cavity including the parotid, submandibular, sublingual, and minor salivary glands and posterior deep lingual glands (von Ebner's glands) (Fig. 9.1). Each gland is comprised of clustered acinar cells called acini, which concertedly produce about 500–1500 ml daily [3].

There are two categories of acinar cells: [1] serous cells (most commonly found in the parotid gland), which secrete a nonviscous watery product, and [2] mucous cells (predominant in the sublingual gland), which secrete a highly viscous mucous-like product. These cells produce a solution containing electrolytes, mucins, and enzymes, which subsequently flow into collecting tubes, where their composition can be further altered by the reabsorption of specific molecules before release into the mouth as saliva.

9.2.3.1 Function

Saliva lubricates and moistens the oral tissues to aid in speech, chewing, swallowing, and taste. Saliva also plays a key role in initiating and facilitating digestion. In addition, saliva's cleansing actions and intrinsic anti-pathogenic characteristics are crucial for maintenance of oral health.

9.2.3.2 Composition

Saliva is a continuously secreted, slightly acidic, clear, hypotonic fluid predominantly composed of water (99.5%). The remaining 0.5% is comprised of inorganic ions, including sodium, chloride, potassium, and calcium, along with organic components, such as amino acids, proteins, antibodies, hormones, enzymes, lipids, and cytokines, among many others.[4]. In addition, recent studies have shown that saliva actually contains a variety of genomic, transcriptomic, proteomic, microbiologic, and immunologic analytes [5–8] that may be capable of identifying both local and systemic disorders in afflicted individuals. Consequently, saliva is now the focal point of multiple investigations aimed at establishing oral fluids as the preferred diagnostic medium.

9.3 Serum, Synovial Fluid, and Saliva as Diagnostic Media

The orofacial region is anatomically complex and often presents with exclusive ailments and concomitant chronic pain not routinely experienced in other regions of the body. These include, but are not limited to, masticatory muscle and

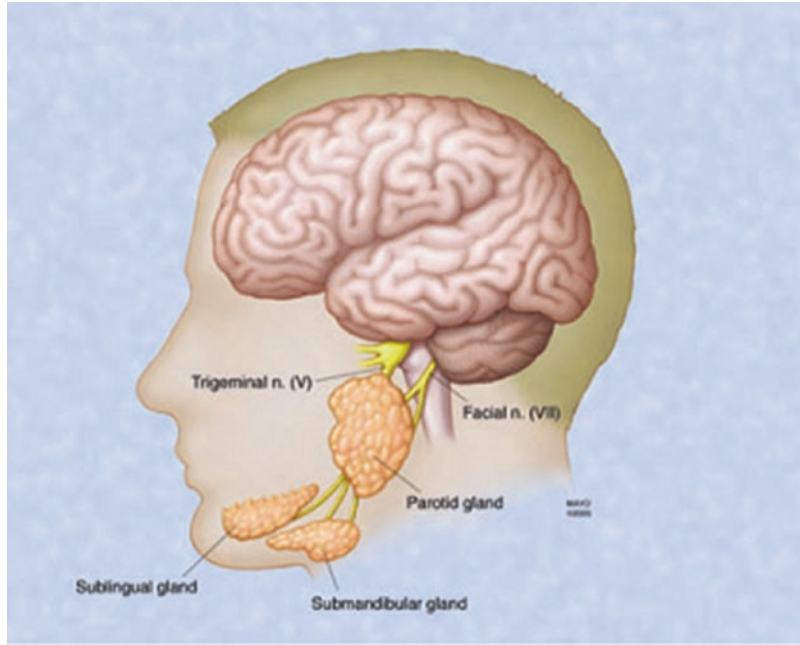

Fig. 9.1 Location of salivary glands: parotid, submandibular, and sublingual

temporomandibular joint disorders as well as burning mouth syndrome. Current diagnostic methodologies directed at the early identification of these conditions have thus far proved to be invasive and occasionally inaccurate. Developing new procedures designed to discriminate these and other maladies associated with chronic orofacial pain could help initiate the expeditious onset of corrective therapies and alleviate much of their associated enduring discomfort. The proceeding sections will describe the potential utilization of serum, synovial fluid, and saliva as diagnostic media with the power to discern patients suffering from orofacial disorders commonly presenting with chronic pain.

9.3.1 Biofluids in Disease Detection: Advantages and Disadvantages

9.3.1.1 Collection

Both serum and synovial samples are collected via insertion and retraction of hypodermic needles into specific anatomical locations. While this technique may allow for real-time analysis of disease-indicating molecules, its execution is not a straightforward task and carries a risk of iatrogenic infections. Moreover, these approaches require advanced training, a thorough understanding of anatomy, and patients willing to tolerate substantial discomfort despite the use of local anesthetic. Resulting anxiety may compel subjects to avoid or delay voluntary participation, leading to a lack of timely diagnosis and therapeutic intervention.

In contrast to serum and synovial fluids, salivary samples are collected painlessly and expeditiously with patients simply spitting into sterile tubes. Saliva-based analytes can be stabilized, stored, and even shipped without the need for specially trained staff or the inclusion of anticoagulants. In consideration, future measures could even include the possibility of collecting clinically invaluable salivary secretions at home.

While presented as simplistic, accumulating saliva is not without intermittent difficulties. For example, Sjögren's syndrome patients often present with dry mouth, while other individuals may manifest bleeding gums or mouth ulcers all of which can negatively affect the efficacy of the saliva sample. Despite these complications, saliva represents the most attractive technique aimed at procuring analyzable biofluids. Noninvasiveness and overall ease of collection highlight its potential as a diagnostic medium. Establishing the usefulness of saliva in this capacity could prove to be immensely useful in the field of molecular diagnostics.

In juxtaposing all three biomarker sources, synovial fluid may be the most limiting in terms of evaluating our physiologic state because of its small volume, especially in joints like TMJ. Saline aspirates from TMJs devised to overcome this barrier may lead to unknown dilution effects on target biomarkers [9] making it difficult to standardize measurements, thereby diminishing their diagnostic utility. Regardless, synovial fluid has the distinct advantage of presenting a concurrent portrait of joint health that may not be available by any other means [10]. This makes synovial fluid especially appealing as a tool for the early detection of joint-related conditions including TMDs.

Serum, while currently the standard in molecular diagnostics, is hindered, like synovial fluid, by the fact that its collection is invasive. Clearly, among these methodologies, saliva is the most favorable, although determining its value as a diagnostic medium comparable with that of serum is yet to be established.

9.3.1.2 Availability of Biomarkers

As mentioned previously, blood serum is currently the gold standard for discriminatory biomarker discovery and validation. Accordingly, serum has been the focal point of multiple investigations evaluating its constituents for indications of chronic pain conditions. For example, studies have shown that patients with TMD can be identified by a significant rise in serum 2,3-dihydroxybenzoic [11] as well as malondialdehyde and 8-hydroxydeoxyguanosine (8-OHdG) [12]. However, in recent years saliva and synovial fluids have piqued the interest of numerous researchers and clinicians as possible alternatives. Not surprisingly, this paradigm shift may be due to the noninvasiveness of saliva collection and the apparent tissue specificity of synovial fluid.

Despite the fact that its molecular community is partially derived from blood, synovial fluids are not an exact mirror image of plasma. Synovium, the semipermeable boundary lining the synovial joint, naturally restricts the passage of oversized molecules, thereby preventing select joint-specific biochemical markers from entering the bloodstream. Hence, synovial fluid analyses can uniquely pinpoint local conditions, a task not feasible through the evaluation of serum and saliva. These attributes distinguish the synovia as an attractive and perhaps optimal medium for the assessment of joint health.

Like serum, saliva is currently being pursued as a medium for biomarker development and disease detection [4]. Interestingly, most compounds found in blood are also present in saliva, albeit at a significantly lower concentration [13]. Even so, what is most interesting here is not that saliva-based molecular entities correspond with those of blood but that how this is even possible. In an effort to explain this phenomenon, it has been suggested that blood-borne molecules may act to induce salivary biomarkers by interacting with salivary glands and subsequently altering the molecular composition of oral fluids [14]. Exosome-like microvesicles are thought to have a key role in this process by encasing, protecting, and shuttling RNAs and proteins throughout the vasculature. In doing so, exosomes, shed from distant tissues, could deliver viable biochemical information to salivary glands, which in turn could be reflected in oral fluids [14–16]. Defining the mechanistic minutia of this long-range interaction may further our understanding of disease pathogenesis and extracellular communication while also establishing saliva as a credible diagnostic medium.

Summarily, under certain circumstances, molecular indicators housed within either blood or saliva may not be as informative as those found in synovial fluids. Nevertheless, blood and saliva should not be discounted as valuable in identifying the onset and progression of local and systemic disorders. Determining which biofluid to assess may highly depend on the pathophysiology of the disease in question as well as its tissue of origin [4].

9.3.2 Comparative Analysis

The overarching goal of molecular diagnosticians or clinicians is to identify disease prior to its genesis or at its earliest developmental stages. The preponderance of current protocols designed to address these needs utilize invasive blood tests or biopsies to determine the onset or advancement of pathologies, both local and distant. Nonetheless, recent research indicates this approach may not remain the status quo as the composition of saliva and synovial fluids may more accurately reflect orofacial physiological anomalies, including those associated with chronic pain [13, 17–19]. The following sections review selected research aimed at elucidating orofacial pain biomarkers within the biofluids described thus far.

9.3.2.1 Saliva Versus Serum

Both saliva and blood are complex bodily fluids containing a multitude of molecular and microbial analytes. Similarities in their respective constituencies have led to the idea that saliva may be an effective diagnostic alternative to blood, the most traditional and frequently accessed source of biochemical disease indicators.

Regardless, credentialing oral fluids as an acceptable diagnostic medium may be a difficult hurdle to overcome. Multiple studies suggest that while most blood-based analytes are also detected in saliva, they are substantially diminished or do not significantly correlate [13, 20–22]. Even so, a growing number of investigations conclude that saliva-based biomarkers are not only preferred but also accurate in discerning healthy subjects from those afflicted with periodontal disease or burning mouth syndrome [23–29]. Saliva has also been employed as an indicator of stress and chronic pain. For example, reports state that substance P, a neuropeptide associated with inflammation and pain, the stress hormone cortisol, and markers of oxidative stress can be repeatedly detected within salivary secretions [12, 30–32]. Suggesting a preference for saliva over serum in the detection of select markers, researchers go on to describe that substance P is actually more readily available in oral fluids than patient-matched blood samples.

Together, these findings support the idea that salivary secretions could supersede serum as the preferred biofluid for routine evaluation of our current physiologic state.

9.3.2.2 Synovial Fluid Versus Serum and Saliva

As a size-selective barrier, the synovium facilitates the confinement and concentration of distinct biomarkers capable of providing real-time information concerning joint health that may be unavailable in blood. This lack of correspondence suggests that the synovial fluids may be optimal in assessing TMDs. Expanding our understanding of synovial fluids and their potential role in biomarker development could not only enhance our ability to diagnose and treat joint-related disorders but also effectively manage related instances of chronic pain.

In considering the orofacial complex, disorders of the TMJs are often associated with substantial bouts of chronic pain. Identifying discriminatory biomarkers indicative of early disease onset may ameliorate patient discomfort by expediting the delivery of corrective therapies and pain management strategies. While saliva has also shown some promise in detecting potential temporomandibular biomarkers [12], researchers have also reported that it may be possible to reveal joint-related TMDs by evaluating the molecular content of synovial fluids (Chap. 7). In a recent study, researchers determined that insufficient hyaluronic acid [19] and enhanced concentrations of a hyperalgesic eicosanoid acid (15-HETE) within synovial fluid were highly correlated with TMD-positive patients [11]. Similar efforts describe a number of additional markers including nitric oxide [33], serotonin [34], aggrecanase [35, 36], chondroitin-4 and chondroitin-6 sulfate [37], cytokine receptors, and proteinases [38–45]. Although miRNAs (microRNAs), another novel biomarker, have also been identified within the synovial fluids of certain joints, there has been little to no correlation between their relative concentrations in either plasma and synovial fluid [46]. Furthermore, the mechanism for stability of synovial fluid miRNA remains to be determined. Similar to what has been observed in saliva, some miRNAs are thought to be transported inside of exosomes, but this idea is still in its infancy and other mechanisms may also exist. The elucidation of these analytes as biomarkers of joint-related disorders substantiates the significance of exploring synovial fluids as a reservoir of molecular information regarding the current state of joint health.

To expand upon the aforementioned, very few synovia-derived indicators have corresponding concentrations within the blood serum. In a study evaluating six distinct TMJ biomarkers, only one, bradykinin, was significantly correlated when comparing synovial fluid and serum [47]. Further studies support these findings, by determining that elevated synovial tumor necrosis factor alpha (TNF-alpha) was not observed in the serum of TMD patients [48]. These outcomes suggest that synovial compartments contain discriminatory biomarkers capable of identifying select pathologies with greater specificity and sensitivity than that of blood. Despite the compelling nature of this evidence, synovial fluids may not be an ideal matrix by which to determine the overall condition of our joints. As it turns out, a series of investigations comparing synovial- and serum-derived molecular analytes indicate a contrasting notion. Current data exists suggesting that evaluating serum-derived cell populations, peptides, miRNAs, and even neurotransmitters [31, 32, 49–51] can distinguish distinct manifestations of chronic pain disorders. While divisive, these investigations, along with the above statements, indicate that no one biofluid is an ideal diagnostic medium. Summarily, determining the most efficacious mode for rapid and accurate physiological assessment may be a function of each individual disease state.

9.3.2.3 Factors Affecting Biomarkers Present

Although theoretically simplistic, the collection and subsequent evaluation of biofluids is not without difficulty. Some common issues include subject's age, sleeping patterns [52–54], relevant comorbidities (see Chap. 2), pharmaceutical side effects [25, 55], physical activity [54], and

method of sample collection and processing [13, 56] (see Chap. 10). For example, Sjögren's syndrome patients receiving hydroxychloroquine treatments are commonly characterized by decreased salivary IL-6 and hyaluronic acid in comparison to serum [55]. In addition, substantial molecular discrepancies were noted [13, 56] in processed (centrifuged upon collection) versus unprocessed saliva samples, as well as in unstimulated drool versus filter paper sampling. Another important factor to consider is diurnal variation, a phenomenon defined by fluctuations in the concentrations of biofluid constituents throughout the day. Although this effect has not been assessed with regard to chronic orofacial pain disorders, it has been reported that levels of salivary cortisol and dehydroepiandrosterone (DHEA) can be influenced by time [52]. Similarly, diurnal concentrations of serum-based cartilage oligomeric matrix protein (COMP) levels in arthritic subjects have been shown to significantly vary with physical activity [53, 54].

Concertedly, these findings indicate that biomarker levels may be influenced by a number of confounding variables. With this in mind, researchers and clinicians should be cautious when utilizing biofluids to evaluate their patients' health status and pain levels. In any event, further investigation is required to not only determine the effectiveness of a particular biofluid as an indicator of chronic pain but also to establish biomarker diagnostics as the preferred mode of patient assessment, monitoring, and prognosis.

Summary

Chronic orofacial pain is a substantial medical corollary with mixed etiology from TMD to burning mouth syndrome. Left undiagnosed and without treatment, suffering patients can be subject to ongoing discomfort, loss of appetite, and lack of sleep. Unfortunately, most conditions presenting with chronic orofacial pain are difficult to differentiate, and establishing rapid and accurate methods of patient evaluation could allay a great deal of agony by identifying affected individuals at the earliest stages of pathogenesis.

Traditionally, blood serum has served as the most commonly accessed biofluid for the molecular diagnosis of systemic disease as well as certain orofacial conditions. However, recent efforts have determined that saliva could supplant blood in this capacity and optimize the processes by which physicians determine the onset of disease and monitor therapeutic progress. Employing oral fluids in this context not only utilizes a unique methodology of patient assessment; it also introduces the possibility of pain-free medicine, an idea long sought after by scientists, clinicians, and patients alike.

Along with saliva and serum, synovial fluid, a protective lubricant located in and around complex joints, could also prove to be an invaluable source of biochemical information. Although acquiring synovial samples is accompanied by a degree of invasiveness, its inimitable anatomical locale yields a real-time molecular overview of its immediate milieu. Information obtained from evaluating this fluid could be used to accurately discern localized physiological alterations, such as TMDs.

Finally, it can be inferred that at this time there is no one ideal biofluid capable of imparting an all-encompassing portrait of our current health status. While serum, saliva, and synovial fluids all contain biomarkers indicative of unique disease states, both local and systemic, none are considered comprehensive, hence necessitating the ongoing research for personalized diagnostics and therapeutics. At this time, determining the appropriate biofluid by which to appraise health status continues to be a function of the disease condition in question. Table 9.2 lists a series of orofacial disorders commonly associated with chronic pain along with their respective biomarkers and biofluid source.

9.4 Future Directions

The field of molecular diagnostics is an ever-expanding genre of basic and translational research. Newer methods, techniques, and ideas

are routinely introduced and explored as potential platforms for advancing our understanding of disease pathophysiology and early identification.

One area in particular that is gaining increasing popularity is the identification of DNA methylation biomarkers. Termed methylomics, this area of research focuses on evaluating the extent of methylation within a genome and determines its potential as a disease-specific biomarker. More specifically, these analyses identify the degree of DNA methylation for thousands of genes and relate that data to specific disease states, a truly impactful attribute. What's more is that these experiments can be performed utilizing extremely

Table 9.2 Orofacial conditions and their potential serum, synovial, and salivary biomarkers

Biofluid	Condition	Biomarker	Reference
Serum	Facial Arthromyalgia	15-HETE 2,3-dihydroxybenzoic acid	Aghabeigi et al. [11]
	Burning mouth sensation	IL-2 IL-6 Neurokinin A	Xia et al. [57] Boras et al. [58]
	TMD (serum and saliva)	8-OHdG Malondialdehyde MCP-1 IL-1ra IL-8 Serotonin P-IL-1sRII C-reactive protein	Rodriguez de Sotillo et al. [12] Slade et al. [51] Kopp and Alstergren [49] Voog et al. [59]
Saliva	Burning mouth syndrome	IL-2 IL-6 CGRP Chondroitin sulfate Kallikrein CD14 TLR-2 Magnesium	Simcic et al. [60] Zidverc-Trajkovic et al. [29] Srinivasan et al. [28] Loeb et al. [24] Pekiner et al. [26]
	Periodontal disease	8-OHdG 8-epi-PGF2alpha Carbonylated proteins Albumin Alkaline phosphatase Aspartate aminotransferase Calprotectin Cystatins Defensins Histatins IL-1alpha IL-1beta Immunoglobulin Lactate dehydrogenase Lactoferrin Lysozyme MMP-8 HSP70 Mucins Prostaglandin E2 Salivary amylase	Su et al. [61] Mirrielees et al. [25] Kibayashi et al. [23] Nishida et al. [62] Horst et al. (2011) Lee et al. [63] Sexton et al. [64]
	TMD	8-OHdG Malondialdehyde	Rodriguez de Sotillo et al. [12]

(continued)

Table 9.2 (continued)

Biofluid	Condition	Biomarker	Reference
Synovial fluid	TMD	Aggrecanase	Yoshida et al. [35, 36]
		Lubricin	Wei et al. [19]
		Hyaluronic acid	Takahashi et al. [33]
		Nitric oxide	Alstergren et al. [34]
		Serotonin	Murakami et al. [37]
		Chondroitin-4	Fredriksson et al. [48]
		Chondroitin-6	Kubota et al. [39]
		TNF-alpha	Kaneyama et al. [45]
		IL-1beta	Tominaga et al. [41]
		IL-1ra	Shafer et al. [44]
		P-IL-1sRII	Srinivas et al. [43]
		IL-6	Tanaka et al. [42]
		MMP-2	Snadler et al. [65]
		MMP-8	Guven et al. [66]
		MMP-9	Hajati et al. (2010)
		PGE2	Herr et al. [67]
		Cytokine receptors	
		EG-VEGF/PK1	
		Superoxide dismutase	
		Glutamate	

15-HETE 15-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid, *8-OHdG* 8-hydroxydeoxyguanosine, *CGRP* calcitonin gene-related peptide, *EG-VEGF/PK1* endocrine gland-derived vascular endothelial growth factor/prokineticin-1, *Hsp70* heat shock protein 70, *IL-1alpha* interleukin 1 alpha, *IL-1beta* interleukin 1 beta, *IL-1ra* IL-1 receptor antagonist, *IL-2* interleukin 2, *MCP-1* monocyte chemoattractant protein-1, *MMP-2* matrix metallopeptidase 2, *MMP-8* matrix metallopeptidase 8, *MMP-9* matrix metallopeptidase 9, *PGE2* prostaglandin E2, *P-IL-1sRII* interleukin-1 soluble receptor type II, *TLR-2* toll-like receptor-2, *8-epi-PGF2alpha* 8-epiprostagladin F2 alpha

small quantities of sample material suggesting that it may be possible to identify novel gene targets using minute volumes of select biofluids.

In line with this notion, a recent study of rheumatoid arthritis patients distinguished a number of hypermethylated and downregulated gene targets in synovial fibroblasts. The authors of this investigation further suggest that these genes may play an important role in TGF-beta signaling, a transduction pathway known to play a role in chronic pain and joint disorders [51]. Overall, these findings indicate that methylomic analysis may have a place in discerning the presence of orofacial disorders and therefore could be an interesting avenue to pursue in the continually growing arena of molecular diagnostics.

Conclusion

The development of discriminatory orofacial pain biomarkers could help alleviate a great deal of patient discomfort by facilitating and expediting the initiation of corrective treatments. Serum, salivary, and synovial fluids have all been shown to contain biochemical information that could serve to identify specific disease states associated with orofacial pain. Although substantial efforts have revealed their value as reservoirs of diagnostic analytes, continued research is necessary to establish their efficacy and comprehensive clinical acceptability. Credentialing these biofluids and their respective biomarkers could not only mitigate a great deal of patient discomfort but also support the formulation of novel preventive care, planning of therapeutic strategies, and furthering our understanding of the disease processes. However, much remains to be learned and achieved.

Competing Interests David Wong is co-founder of RNAmeTRIX Inc., a molecular diagnostic company. He holds equity in RNAmeTRIX, and serves as a company Director and Scientific Advisor. The University of California also holds

equity in RNAmeTRIX. Intellectual property that David Wong invented and which was patented by the University of California has been licensed to RNAmeTRIX. Additionally, he is a consultant to PeriRx.

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Part IV

Study Designs and Statistical Analysis for the Identification of Biomarkers, and Future Direction

Biomarkers in Epidemiologic Research: Definition, Classification, and Implication

10

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Abstract

The aim of this chapter is to give an introduction to biomarkers. It provides a definition for the term "biomarker" before describing their classification and the criteria applicable to each. This chapter also offers a general guideline for the development and assessment of biomarkers, including an insight into the various factors that need to be considered in order to advance biomarker discovery and validation. Finally, the chapter lists the possible contributions of biomarkers in clinical research.

10.1 Introduction

Chronic orofacial pain is a common condition and poses a significant global public health problem (Chaps. 1 and 2). Many studies have identified a series of risk factors for chronic orofacial pain. For instance, some of the most relevant and common risk factors are psychological and painful comorbidities (Chap. 2). Several randomized clinical trials (RCTs) investigated the effect of treatments for managing chronic orofacial pain. Two systematic reviews show that behavioral therapy [1] and the use of appliances [2] are effective in "alleviating" a type of orofacial pain call – "painful temporomandibular disorders" (TMD). However, the evidence for effectiveness of the appliances was weak [2]. These systematic reviews concluded that these treatments would not "cure" TMD.

The reasons for unsuccessful treatment outcomes are not clear. Ohrbach and Dworkin suggested that there is a very complex interaction between changes in physical and psychological

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factors responsible for chronic TMD pain, which could in part explain the weak effectiveness of the treatments [3].

The identification and validation of biomarkers that are indicators for pain chronicity and the effectiveness of pain management will undoubtedly lead to the development of optimal strategies for pain prevention and management. The next section defines and classifies biomarkers and describes the process for their development and analysis, as well as their contribution.

10.2 Biomarkers

Hulka and colleagues [4] defined biomarkers as "cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids." As defined by Strimbu and Tavel [5], "biomarker" is a term derived from "biological markers."

The National Institutes of Health Biomarkers Definitions Working Group defined biomarkers as "a characteristic that is *objectively* measured and evaluated as indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" [6]. The International Programme on Chemical Safety, under the World Health Organization (WHO), added that biomarkers are "any substance, structure, or process that can be measured in the body, or its products and influence, or predict the incidence of outcome or disease" [7]. Health Canada provided a specific definition to genomic biomarker as any "measurable characteristic that is an indicator of normal biological processes, pathogenic processes, and/or response to therapeutic or other interventions" [8].

10.3 Biomarkers Classification

Perera and Weinstein [9] classified biomarkers based on the disease pathway – from the etiology to the prognosis. Therefore, biomarkers are classified as (1) antecedent biomarkers to assess the risk of a disease, (2) screening biomarkers, (3) diagnostic biomarkers, (4) staging biomarkers to evaluate disease severity, and (5) prognostic biomarkers to predict the disease course [9, 10]. Biomarkers could indicate a variety of disease characteristics such as pain intensity, duration, and classification [11].

In addition, biomarkers are classified as exploratory, probable valid, or known valid biomarkers. "Exploratory" are "potential biomarkers tested in analytic studies where validity has not been demonstrated." A biomarker is defined as "probable" when "it is measured in an analytical test system with well-established performance characteristics and for which there is a scientific framework or body of evidence that appears to elucidate the physiologic, toxicologic, pharmacologic or clinical significance of the test results." A "known" biomarker is defined as "a biomarker that is measured in analytical test system with well-established performance characteristics and for which there is widespread agreement in the medical or scientific community about the physiologic, pharmacologic and or clinical significance of the results" [12].

The following criteria to validate a biomarker have been proposed [13]:

1. Content validity, assessing how much the biomarker reflects the study outcome (e.g., orofacial pain, pain intensity). This validity consists of the judgment by experts as to whether the exploratory biomarker seems suitable for the intended purpose (e.g., diagnosis, classification, surrogate of a risk factor, pain effectiveness).
2. Construct validity, evaluating the association between an exploratory biomarker and a specific "construct" (e.g., hypothesis). This description can be explained with the example of studies assessing the hypothesis that individuals with orofacial pain have higher levels of oxidative stress than individuals without this pain condition. Chapters 6, 7, 8, and 9 described numerous studies evaluating this validity.
3. Criterion validation, assessing how well the exploratory biomarker is in agreement with a specific "criterion" outcome. To assess this validity, it is necessary to identify the sensitivity, the specificity, and the predictive value of the exploratory biomarker. [4]. A biomarker must also be precise and reproducible [10].

Biomarkers may also be classified by pain mechanism. For example, Chaps. 6, 7, 8, and 9 classify biomarkers based on (1) direct activation of nociceptors (e.g., glutamate and protons), (2) inflammation (e.g., prostaglandins and cytokines), and (3) cell metabolism (e.g., lactate and pyruvate). Currently, none of the possible pain biomarkers may be classified as "validated" [14].

10.4 Biomarkers Development and Analysis

The development of biomarkers depends on the current knowledge of the biologic mechanism, the outcome, and the exploratory biomarker [15]. The analytical evaluation of the exploratory biomarker is primordial for success in the identification of a biomarker [10, 16].

Wagner and Ball [16] describe the following questions that should be addressed for a biomarker:

1. Is the biomarker measurement valid and reliable across instruments, laboratories, and clinical settings?
2. Is the biomarker associated with the clinical endpoint of interest?
3. What is the specific context of the proposed use?

The following protocol may assist in advancing the discovery and validation of biomarkers:

10.4.1 Rationale

It is vital to describe and justify the rationale of the evaluation of a specific biomarker [6]. We need to define the study outcome for which the assessed biomarker will be investigated (e.g., pain management, prediction of chronic pain, persistence of chronic pain). The rationale for the tested biomarker should be based on scientific evidence. As there is more than one mechanism and set of characteristics associated with chronic orofacial pain, several biomarkers may be investigated. In this case, investigators need to assess the crude effect of each biomarker and then evaluate how these influence one another in a multivariable analysis. For example, Rodrigues and colleagues found higher levels of DNA damage, and lipid peroxidation biomarkers were independently related to painful TMD [17]. In addition, investigators need to assess the best tissue or sample type from which to measure the biomarker: serum, plasma, saliva, synovial fluid, or muscle biopsy. The Rodrigues study [17] further found that DNA damage and lipid peroxidation biomarkers collected from saliva were more strongly related to TMD than from serum.

10.4.2 Study Population

A blinded investigator without knowledge of any candidate biomarker should perform the recruitment of the study population based on specific eligibility criteria [18].

10.4.3 Instrument to Assess Exploratory Biomarker

Valid and reliable instruments to assess exploratory biomarkers must be used to prevent or decrease the chance of misclassification. For example, enzyme-linked immunosorbent assays (ELISA) and flow cytometry such as Luminex have been employed to identify possible pain biomarkers in serum, plasma, or saliva. Other methods were described in Chaps. 6, 7, 8, and 9.

10.4.4 Assessment of Exploratory Biomarker

Each sample should be handled, processed, and stored consistently, as per the protocol, to prevent bias. A trained researcher should perform the assessment of the biomarker following the established standardized protocols. One relevant issue is the time of the sample collection. It is important to evaluate if there are considerable variations in the levels and/or concentration of possible biomarkers during the day. This is crucial since some exploratory biomarkers display this characteristic (e.g., cortisol [19], protein car-

bonyls [20]). If this evaluation is not possible, the investigators need to standardize the ideal time frame for sample collection (e.g., only mornings) or to stratify the analysis based on the time when the sample was collected. All measurement assays should be conducted in duplicate or triplicate.

10.4.5 Statistical Analysis

Appropriate statistical analyses need to be applied, taking into consideration the study design, putative confounders, and/or effect modifiers (e.g., age, sex, smoking status) (Chap. 11).

10.4.6 Results

The first clinical results should evaluate if the exploratory biomarker is reliable and if it is associated with the study outcome [18]. It is important to understand that a candidate biomarker "associated" with a condition (e.g., chronic orofacial pain) does not obligatorily indicate that it is a "cause" or part of the pathophysiological pathway of the specific condition (see [5] for more information). Moreover, it is important to evaluate not only if there is an association between the exploratory biomarker and the study outcome but also the strength and direction of this association. It is also relevant to verify other evidences of the relationship between the exploratory biomarker and study outcome (e.g., pain intensity) and which other factors could modify this association (e.g., sex, age).

We should assess the internal validity of the results [21] and its use in clinical practice [18]. Internal validity requires that "the index and comparison groups be compared in such a manner that the observed differences between them on the dependent variables under study may, apart from sampling error, be attributed only to the hypothesized effect under investigation" [22].

In this process, it is necessary to evaluate if there are already other scientific evidences for the results obtained in the study [23], any relationship between dose and response, and any bias or confounders that could have influenced the result. Bias is a process of inference at any stage that tends to produce results or conclusions that differ from the truth, leading to an incorrect estimate of the association between a putative risk factor and a disease [24]. This systematic error may occur when selecting the study population and/or collecting the information for the study (e.g., instruments to measure biomarkers or pain). Confounding is "a situation in which a measure of the effect of an exposure on risk is distorted because of the association of exposure with other factors(s) that influence the outcome under study" [22]. For example, gender is a potential confounder in the studies assessing potential biomarkers of orofacial pain. External validity of the results need also be appraised. External validity is met if a study "can produce unbiased inferences regarding a target population" [22].

It is relevant to recognize that biomarkers identified should be constantly evaluated, since new studies may identify new mechanisms, new outcomes, and/or patient characteristics that may explain the identified association [5].

10.5 Use of Valid Biomarkers in Clinical Studies

The potential contributions of biomarkers are to:

1. Serve as a diagnostic, screening, or prognostic tool. It can decrease the chance of misclassification of orofacial pain classification.
2. Assess the relationship between exposure and disease.
3. Evaluate pain mechanisms.
4. Serve as surrogate of risk factors. It can decrease the chance of misclassification of the risk factor.
5. Assess the efficacy of pain management in clinical trials, as well as dose-response relationships.

Conclusion

Biomarkers may assist in improving the screening and diagnosis of orofacial pain as well as its classification. They may also contribute to our understanding of disease patho-

genesis and their mechanisms, in addition to being used as endpoints for clinical trials assessing the effectiveness of treatment or prevention of chronic orofacial pain.

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Statistical Analysis in the Identification of Pain Biomarkers

11

Russell Steele

Abstract

The statistical analysis of data collected from orofacial pain biomarker studies presents challenges that go far beyond what is typically encountered in standard epidemiological studies. In this complex scientific context, it is critically important for researchers to properly identify the scientific question of interest. This chapter primarily focuses on the differentiation between finding predictive models for pain outcomes and identifying causal relationships between biological markers, potential treatments, and pain (typically measured by patient-reported instruments). The chapter begins by defining the scientific context and identifying two types of scientific questions. Next, I will introduce modern, but computationally accessible, techniques for biomarker prediction to be used when one wants simply to identify predictors of pain outcome. Subsequently, I will contrast predictive methods with approaches that are generally used to select causal models for quantifying the causal effects of intervention on pain outcomes. I will introduce the two complementary approaches that modern epidemiologists and statisticians use to assess causal relationships. In the next section, I will discuss the complications that result due to the fact that pain is a latent construct that can only noisily be observed via self-reported instruments. The chapter will end with a short overview of other statistical issues that often appear in the analysis of pain data.

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11.1 Introduction

The other chapters in this volume detail several scientific advances in orofacial pain research due to recent advances in biomarker measurement and understanding. However, these new scientific developments in methods of data collection and

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observation stretch the limits of classical statistical techniques to extract the greatest possible amount of information from the data collected.

In this chapter, I will provide an overview of how analyses of orofacial pain biomarker data could be conducted in a way to improve clinical decision-making and to better inform future decisions. The chapter begins by defining two basic axioms that must be kept in mind during any statistical analysis. The next section focuses on the most critical aspect of the analysis, definition of the scientific question. Depending on whether one wants to develop models for prediction or model for causal relationships, different statistical techniques will be appropriate. The next section then details the approaches that are generally used for determining appropriate causal models, with a particular focus on how to properly choose which variables should be included in the model. We then describe the advantages and disadvantages of various methods of statistical prediction for biomarker data and provide a guide for researchers as to what might be most appropriate for their problem. The latency of the pain construct aspect is often ignored in statistical analyses of pain data from studies. I will describe how this differentially impacts analyses when pain is both used as a covariate and when pain is the outcome that is being measured. Finally, I will briefly discuss other issues that occur in the analysis of pain data.

11.2 Two Basic Axioms for Modeling Pain Biomarker Data

The analysis of pain biomarker data is well suited to the idea of a *statistical model*. In this context, I will define a statistical model as the probability model that defines how the observed data were generated. The first key enabling idea for analyzing pain data applies to any statistical modeling problem.

11.3 Axiom 1: The True Model That Generates the Data Can Never Be Completely Correctly Specified, Even in the Presence of Large Numbers of Measurements

Axiom 1 holds in general for almost all statistical problems, but recognizing this limitation is very important in pain biomarker research. The biological systems which cause a subject to experience pain (chronic or otherwise) are complex, and the more that is learned, the more complex the system descriptions become. It is infeasible to measure every single aspect of the biological processes underlying patient pain, so at some point there will remain unmeasured aspects of the model that can't be identified from the observed data alone.

For example, Slade et al. [1] reported the analyses of levels of 22 cytokines taken from 344 patient blood serum samples in order to determine associations of these biomarkers with three types of case status (healthy control and temporomandibular disorder with [TMD+WPT] and without [TMD-WPT] widespread palpation tenderness). In the paper, they present two kinds of analyses. In one analysis, they present multivariate logistic regression coefficients for three cytokines (IL-8, MCP-1, and IL-1ra) in a model where case status (HC, TMD+WPT, TMD-WPT) is the response, where they have also adjusted for age and sex (not shown in the table). Obviously, this model is insufficient for completely describing patient pain. Rather, the model *approximates* the true model that underlies the generation of the data. It describes the association between case status and cytokine presence conditional on adjustments for age and gender. Implicitly, other patient characteristics have been averaged over in the sample or, more technically, *marginalized* in the analysis.

More particular to pain research, the pain a subject experiences is an inherently unmeasurable (or latent) characteristic. So in addition to the previous challenge, pain can never be directly observed, only through measured surrogates, which are generally either patient-reported

surrogates or other patient physiological characteristics (such as behavior or gait) thought to be related to pain. For example, one might choose to use pain instrument such as the McGill Pain Questionnaire (MPQ) or the Short Form-36 (SF-36) bodily pain scale or perhaps a blood serum biomarker that has been associated with patient pain in the past. These surrogates will never perfectly correlate with the target pain characteristic that the researcher is interested in, which again leads to imprecision in the inference from the statistical model. Therefore, we can state a second axiom of the analysis of pain data as:

11.4 Axiom 2: The Choice of Surrogate Measures for Patient Pain Will Inevitably Determine the Scientific Questions That One Can Ask of the Data

The impact of the choice of pain surrogate measures can often be lost in the interpretation of pain data results. One possible explanation for conflicting results can be subtle differences in the way that patient pain manifests itself differentially in the surrogate measures. For example, one may find that the most useful predictors of pain using the SF-36 pain scale will be different from those that predict the MPQ. This choice of surrogate (or surrogates) for response can impact inference as much or more than the associations that one is trying to assess.

Returning to Slade et al. [1] the first set of analyses use cytokine protein levels as the outcome and case status (control vs. two TMD disease groups) as the exposure of interest. However, the second set of analyses examines the associations between the cytokine levels and 16 intermediate pain phenotypes, some of which are patient-reported questionnaires (MPQ, SF-12v2) and others are physiological measurements (quantitative sensory testing). The observed values and statistical significance of associations of cytokines with the patient questionnaires are different from the physiological measurements, leading to potentially conflicting interpretations of answers to the question: "Which cytokines are associated with pain?" The conflicting interpretations result from the limitation to conclusions from the data regarding a different question, i.e., "Which cytokines are associated with which measured surrogates for pain?"

11.5 Defining the Scientific Question

The most critical step in any statistical analysis is a clear definition of the scientific question of interest. The stated goal of the analysis in Slade et al. [1] was to elucidate the "contributions of cytokines to TMD..." and other related phenotypes. The authors used standard methods, e.g., standard linear and multiple logistic regression, to analyze their data and were properly careful in their description of results as "associations." I will use their problem to illustrate how modern statistical methods would allow for one to go beyond just description of associations, but only by carefully stating the research question.

11.5.1 The Basic Conundrum: Prediction or Causation?

In introductory statistics education, students are taught that regression approaches should be used to answer questions about prediction and causation similarly and rarely is a distinction drawn. In many situations, e.g., in the analysis of clinical trials, the two kinds of questions produce somewhat similar answers. However, although the two axioms of the last section may seem overly formal, they are critically important for addressing the basic premise of the statistical investigation. We must accept that our models are limited in their ability to accurately reflect reality, and we also must accept that the surrogates that can be measured directly define the statistical reality. Therefore, our statistical models do not predict

patient pain; they predict patient responses to pain questionnaire items. Our models do not describe the exact causal relationships of biomarkers with pain, but instead describe the casual relationship of biomarkers with pain surrogates, marginalizing over other aspects of the patient that are known to be important for determining pain levels. Such distinctions may seem pedantic, but understanding the distinction can help researchers decide which methods should be used.

In a 2001 paper, Breiman [2] outlines the two broad classes of statistical analyses. Although much of the paper and discussion focus on the relative importance of the two classes, generally statisticians agree that they are focused on different objectives. In one class of statistical analysis, one assumes an underlying model that generates the data and the focus of estimation and inference is to estimate and interpret aspects of that model. In the second class, the actual underlying data-generating model is not relevant for decision-making and the focus is instead on prediction.

Neither class should be generally preferred to the other without considering the objective of the analysis. If the important scientific question requires knowledge of the underlying data model (or some aspect of the model), then it follows that statistical methods should be used that can allow one to obtain that knowledge. However, if one is merely interested in prediction and is content to allow for the data-generating model to be unknown, one can choose statistical methods that, by their design, provide little to no information about how the data came to be. One way to view this distinction is that requiring knowledge of the data-generating model places a constraint of the kinds of methods that could be used. In a setting where prediction is the primary goal, there is no such constraint and potentially better methods for prediction could be found outside of the previously restricted class.

11.5.2 Specifying the Question for Pain Biomarker Research

I return once again to the Slade et al. [1] analyses to illustrate the difference in motivation of the analyses. In Sect. 3.2 of the paper, they discuss the association of circulating cytokine protein levels with case status (TMD-WPT, TMD+WPT, and control). One could frame the objective of the analysis as one of *prediction*, i.e., to try to predict the case status of the individual on the basis of a particular cytokine profile. The interesting clinical question could be to try to build a clinical model, which distinguishes TMD WPT-positive patients from TMD WPT-negative patients. In contrast, one could argue that the interest lies in identifying the *causal* mechanisms, which underlie widespread palpitation tenderness in TMD patients. Both questions are equally valid and interesting, but the methods that would be appropriate will differ in both cases. The next two sections will outline the differences and give recommendations for analysis.

11.6 Identifying Causal Data-Generating Models

The notion that "correlation does not equal causation" has gone far beyond statisticians and permeated popular culture. However, asking questions about causal data-generating models from observational data requires subtle and sometimes nonintuitive choices for researchers. It is well understood in epidemiology that standard measures of observed association (e.g., t-statistics, regression coefficients, odds ratios) cannot automatically be interpreted as indicating a direct causal relationship between two variables due to the possibility of *confounding*. In its most basic form, we say that an observed association between two variables is confounded if there is at least one common cause of both variables that makes the observed association noncausal.

For example, if patient anxiety increases the levels of certain biomarkers and also increases the values to responses on a pain questionnaire, we may observe an association in the data between the biomarkers and pain that is not causal. An increase in biomarker level would be associated with an increase in reported pain, but changing the levels of the biomarker would not necessarily have an effect on the reported pain, as anxiety provides the real causal mechanism for

reported pain. The complexity of the general problem depends on the data collected, so we will divide our discussion into two kinds of data collection: cross-sectional and longitudinal.

11.6.1 Causal Models for Cross-Sectional Data

One way to visualize the relationships among covariate and outcome measurements is through the use of a directed acyclic graph (or DAG). Although DAGs cannot represent all aspects of a statistical model [3, 4], they do provide researchers with a visual tool for organizing their hypotheses regarding the causal structure of their model and their observed and unobserved quantities. Hernan et al. [5] provide a tutorial on the use of DAGs and their interpretation intended for clinical researchers. Shrier and Platt [6] describe a simple six-step process by which one can identify the most important potential confounders in an analysis.

Researchers collecting pain data would most benefit from following the instructions in Hernan et al. [5] and Shrier and Platt [6] at the study design stage, which would allow them to prioritize resources to measuring confounders and minimize resources allocated to the variables that will not confound the causal effect of interest. Rather than repeat the material contained in the other two papers, this chapter discusses some basic concepts that differentiate constructing DAGs for biomarker pain research from other clinical areas.

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Future Direction and Conclusion

12

Jean-Paul Goulet and Ana Miriam Velly

Our understanding of many aspects of chronic pain and more specifically of trigeminal pain has advanced substantially over the past 25 years (see Chap. 3). Among others are the processing of afferent inputs along the trigeminal path and at the brainstem trigeminal sensory complex, the peripheral as well as central mechanisms involved in sensitization that can contribute to the transition from acute to chronic pain, and the role played by non-neuronal cells and genetic and environmental factors. These progresses in our understanding of chronic pain also apply to chronic orofacial pain conditions even though physiologic studies on trigeminal pain point to several unique characteristics compared with the spinal nociceptive system in terms of differences in response patterns to tissue injury [1]. Despite the accumulation of new knowledge and insights into orofacial pain mechanisms, the advancement in management strategies has not kept the pace. That is reflected by the lack of significant changes seen in the treatment response for most chronic orofacial pain conditions over the past decade.

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The main alleged difference among the most common chronic orofacial pain conditions is that the predominant symptoms and physical manifestations arise from distinct anatomical location and target organs [2, 3]. For example, the masticatory muscles, temporomandibular joints, dentoalveolar process, tongue, and branches of the trigeminal nerve are all different structures or systems involved that push patients to seek care. When we dismiss the body region and target organ to focus on similarities, the most common chronic pain conditions share a number of important features. For one, clinical examination findings are less deviant than expected considering the number and extent of reported symptoms and associated suffering. In addition, the presence of comorbid conditions is more the norm than the exception, and most notably the etiology and pathogenesis of the pain remain unclear or at best speculative. This should make us wonder if we are really dealing with conditions that are unrelated and pain mechanisms that need different treatment strategies.

As new findings in orofacial pain unfold, our operationalized concept of chronic orofacial pain based on specific end organs is more than ever challenged [4]. At least for TMDs that feature persistent pain in the absence of organic substrate, the target organ identified as the source of the pain might not be where all the answers lie. While the view that peripheral inputs play a major role in chronic pain state and ongoing pathological processes occur within the end

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organ is frequently emphasized, the evidence remains equivocal for the cluster of chronic orofacial pain disorders that are commonly seen in clinic and more specifically for joint (arthralgia) and muscle pain (myalgia and its subtypes) related to TMD.

Recent studies have greatly advanced our understanding of biomarkers in orofacial pain, and so far, some putative biomarkers have been identified. The overview on masticatory muscle pain biomarkers in Chap. 6 indicates that glutamate and serotonin are implicated in jaw myalgia, although the exact pathological process is yet to be elucidated. Chapter 7 on molecular temporomandibular joint biomarkers underscores that a number of peripheral pain mediators are indeed elevated in the synovial fluid of TMJ arthritis patients with joint pain on mandibular movements. Significant correlation is reported for higher level of tumor necrosis factor, interleukin 6, serotonin, and prostaglandin E_2. Synovial fluid of arthritic TMJ with high level of interleukin-1β is associated with resting joint pain and tenderness to palpation. In addition, interleukin 6 is more frequently found in synovial fluid of patients with TMJ pain associated with cartilage destruction. These potential biomarkers are therefore good candidates for distinguishing TMJ arthritis from TMJ arthralgia, knowing that this distinction impacts on treatment decision and prognosis.

Arthralgia with masticatory muscle myalgia and its different subtypes are the most common TMDs featuring persistent pain in the absence of organic substrate. There is accumulating evidence that these conditions can be defined and understood through the appraisal of other painful symptoms and psychosocial factors as well (Chap. 2). This would be in line with the proposed hypotheses that biopsychosocial risk factors are appropriate predictors of distinct clusters of people with pain-related TMD in the absence of end-organ pathobiological substrate and that some manifestations result from the interplay of central and peripheral nociceptive mechanisms influenced by genes that regulate biological systems relevant to pain perception [5–7].

As pointed out in Chap. 8 (Seltzer and Diehl), a number of genes harboring single nucleotide polymorphisms (SNPs) can alter regulatory mechanisms of neurotransmitters involved in processing nociceptive input and contribute to the onset or put subjects at risk of developing chronic orofacial pain. Of particular interest is the catecholamine-O-methyltransferase (COMT) gene located on chromosome 22 that encodes the enzyme COMT responsible for the inactivation and catabolism of neurotransmitters such as dopamine and norepinephrine and the HTR2A gene that encodes one of the serotonin receptors (5-hydroxytryptamine receptor 2A). Dopamine and 5-hydroxytryptamine (5-HT) are neurotransmitters involved, respectively, in pain perception and pain transmission. Altered dopaminergic neurotransmission in the central nervous system has been reported in patients with burning mouth syndrome (BMS) and persistent idiopathic face pain (PIFP) [8, 9]. Moreover, patients with chronic masticatory muscle pain have elevated interstitial concentrations of 5-HT compared to healthy controls, and 5-HT levels are correlated with muscle pain and allodynia [10, 11]. More recently it has been shown that plasma dopamine level was elevated in muscle pain-related TMD and correlated with present pain intensity and perceived mental stress [12].

What is emphasized and discussed in Chap. 2 about the presence of other painful and non-painful comorbid symptoms that coexist with a chronic orofacial pain condition is the norm rather than the exception. Other comorbid pain disorders such as fibromyalgia, low back pain, and irritable bowel syndrome show similar patterns of clinical manifestations. This substantial overlap of physical symptoms related and unrelated to the end-organ conditions among different comorbid pain disorders raises the possibility of a common underlying substrate that needs full attention.

Thus, it is legitimate to consider unexplained chronic orofacial pain conditions as potential manifestations of general central nervous system dysregulation [7, 13]. Knowing the temporal relationship of these different clinical manifestations could uncover whether nonspecific symptoms

that occur frequently strongly influence and predict the onset of pain-related TMD [14]. The combined effect of genetic determinants and gene-environment interaction with psychosocial stress could represent a pathway giving rise not only to pain-related TMD but also to other chronic orofacial pain conditions such as burning mouth syndrome (BMS) and persistent idiopathic face pain (PIFP) that are unexplained by pathological processes involving the peripheral end organs.

This is conceivable as evidenced by data presented in Chap. 5 on neurophysiologic markers of orofacial pain attributed to a dysregulation or dysfunction of the trigeminal sensory system. Thermal hypoesthesia, a feature of small fiber system hypofunction as well as increased excitability within the trigeminal system evocative of a deficient top-down inhibition, has been reported in BMS and PIFP. Gain of function on the other hand was observed in subgroups of BMS, PIFP, and atypical odontalgia (AO) that represent three distinct end-organ chronic orofacial pain conditions commonly categorized as trigeminal neuropathic pain disorders.

From Chap. 9, saliva appears to represent an attractive biofluid for the analysis of potential biomarkers. The technique for the collection of saliva is noninvasive and can be done at specific time intervals in different environments with rather simple equipment. This offers the possibility of conducting longitudinal studies targeting the onset and temporal dimensions of somatic complaints related to comorbidities in unexplained chronic orofacial pain disorders while focusing on biomarkers of chronic activation of the body's stress system, the hypothalamic-pituitary-adrenal axis (HPA axis), and, more importantly, the sympathetic adrenomedullary (SAM) system.

Further advancement will only come with the improvement of our study protocols, and the need to better define prospectively the specific aims and the target population when studying biomarkers is well emphasized in Chaps. 10 and 11. Dismissing the importance of other subthreshold symptoms, the top-down influences of regulatory mechanisms, as well as the impact of psychosocial factors, represent a serious barrier to the future identification of meaningful orofacial pain biomarkers. Revisiting the conceptual framework of unexplained chronic orofacial pain disorders, and searching for biomarkers of autonomically mediated dysregulation as a generator of nonspecific symptoms in an apparent end-organ disorder, may provide answers regarding the natural history and the possibility of a common underlying substrate shared by the most common disorders. Therefore, it is crucial to identify a series of biomarkers indicative of diagnosis, classification, pain mechanism, prognosis, and orofacial pain management (Chap. 10).

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