International Journal of
Molecular Sciences

MDPI

Review

Fibromyalgia: Pathogenesis, Mechanisms, Diagnosis and Treatment Options Update

Rosalba Siracusa [1], Rosanna Di Paola [1,*], Salvatore Cuzzocrea [1,2,*] and Daniela Impellizzeri [1]

[1] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Ferdinando Stagno D'Alcontres, 31, 98166 Messina, Italy; rsiracusa@unime.it (R.S.); dimpellizzeri@unime.it (D.I.)
[2] Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, Saint Louis, MO 63104, USA
* Correspondence: dipaolar@unime.it (R.D.P.); salvator@unime.it (S.C.); Tel.: +39-90-676-5208 (S.C.)

Abstract: Fibromyalgia is a syndrome characterized by chronic and widespread musculoskeletal pain, often accompanied by other symptoms, such as fatigue, intestinal disorders and alterations in sleep and mood. It is estimated that two to eight percent of the world population is affected by fibromyalgia. From a medical point of view, this pathology still presents inexplicable aspects. It is known that fibromyalgia is caused by a central sensitization phenomenon characterized by the dysfunction of neuro-circuits, which involves the perception, transmission and processing of afferent nociceptive stimuli, with the prevalent manifestation of pain at the level of the locomotor system. In recent years, the pathogenesis of fibromyalgia has also been linked to other factors, such as inflammatory, immune, endocrine, genetic and psychosocial factors. A rheumatologist typically makes a diagnosis of fibromyalgia when the patient describes a history of pain spreading in all quadrants of the body for at least three months and when pain is caused by digital pressure in at least 11 out of 18 allogenic points, called tender points. Fibromyalgia does not involve organic damage, and several diagnostic approaches have been developed in recent years, including the analysis of genetic, epigenetic and serological biomarkers. Symptoms often begin after physical or emotional trauma, but in many cases, there appears to be no obvious trigger. Women are more prone to developing the disease than men. Unfortunately, the conventional medical therapies that target this pathology produce limited benefits. They remain largely pharmacological in nature and tend to treat the symptomatic aspects of various disorders reported by the patient. The statistics, however, highlight the fact that 90% of people with fibromyalgia also turn to complementary medicine to manage their symptoms.

Keywords: central sensitization; cognitive-emotional sensitization; biomarkers; genetic aspects; therapy

Citation: Siracusa, R.; Paola, R.D.; Cuzzocrea, S.; Impellizzeri, D. Fibromyalgia: Pathogenesis, Mechanisms, Diagnosis and Treatment Options Update. *Int. J. Mol. Sci.* **2021**, *22*, 3891. https://doi.org/10.3390/ijms22083891

Academic Editor: Emanuele Marzetti

Received: 27 January 2021
Accepted: 7 April 2021
Published: 9 April 2021

1. Fibromyalgia

Fibromyalgia (FM) is a syndrome characterized by chronic musculoskeletal pain. The main symptoms of this disease are muscle stiffness, joint stiffness, insomnia, fatigue, mood disorders, cognitive dysfunction, anxiety, depression, general sensitivity and the inability to carry out normal daily activities [1,2]. FM can also be associated with specific diseases, such as infections, diabetes, rheumatic diseases and psychiatric or neurological disorders [3]. FM was first described in the 19th century. In the 1970s and 1980s, an etiology of the disease involving the central nervous system was discovered [4]. In 1950, Graham introduced the concept of "pain syndrome" in the absence of a specific organic disease [5]. The term "fibromyalgia" was later coined by Smythe and Moldofsky following the identification of regions of extreme tenderness known as "pain points" [6]. These points are defined as areas of hyperalgesia/allodynia when a pressure of about 4 kg causes pain [7]. In 1990, the committee of the American College of Rheumatology (ACR) drew up diagnostic criteria, which have only recently been modified [8,9]. According to the ACR, the diagnosis of

FM includes two variables: (1) bilateral pain above and below the waist, characterized by centralized pain, and (2) chronic generalized pain that lasts for at least three months, characterized by pain on palpation in at least 11 of 18 specific body sites [10] (Figure 1). FM affects about 5% of the world population. The incidence is higher in women than in men, and the age range in which FM generally appears is between 30 and 35 years [11]. However, FM remains a poorly understood and difficult-to-diagnose condition.

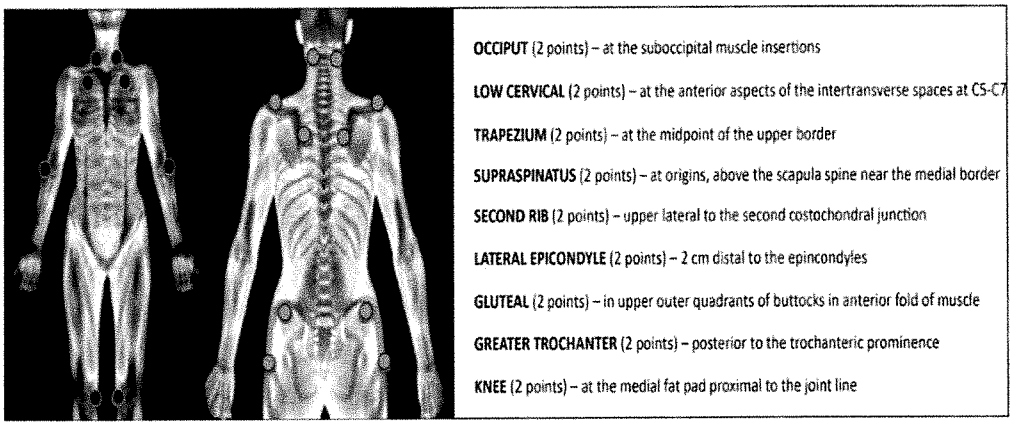

Figure 1. The dots indicate the 18 tenderness points important for the diagnosis of FM.

2. Pathophysiology

The pathophysiological factors of FM are not yet well known and continue to be the focus of much research. FM appears to be related to a pain-processing problem in the brain. In most cases, patients become hypersensitive to pain. The constant hypervigilance to pain can also be associated with psychological problems [12].

The main alterations observed in FM are dysfunctions in mono-aminergic neurotransmission, leading to elevated levels of excitatory neurotransmitters, such as glutamate and substance P, and decreased levels of serotonin and norepinephrine in the spinal cord at the level of descending anti-nociceptive pathways. Other anomalies observed are dopamine dysregulation and altered activity of endogenous cerebral opioids. Taken together, these phenomena seem to explain the central physiopathology of FM [13].

Over the years, peripheral pain generators have also been recognized as a possible cause of FM. In this case, patients manifest symptoms such as cognitive impairment, chronic fatigue, sleep disturbances, intestinal irritability, interstitial cystitis and mood disorders [14,15].

Peripheral abnormalities may contribute to increased nociceptive tonic supply in the spinal cord, which results in central sensitization. Other factors that appear to be involved in the pathophysiology of FM are neuroendocrine factors, genetic predisposition, oxidative stress and environmental and psychosocial changes [16,17].

FM appears to be more common in women than men for the following reasons: higher levels of anxiety and depression, altered behavior in response to pain, altered CNS input and hormonal effects related to the menstrual cycle [12].

2.1. Principal Processes Underlying FM

As mentioned above, FM is considered a central sensitivity syndrome. Central sensitization refers to a neuronal signal amplification mechanism within the central nervous system that leads to a greater perception of pain [18]. For this reason, patients with FM present an increase in the receptive field of pain, allodynia and hyperalgesia. Central sensitization is also implicated in persistent and chronic pain. Although central sensitization plays an important role in FM, it is even more important to understand the initial cause, that is, the persistent nociceptive input associated with tissue damage, including peripheral sensitization [19] (Figure 2). According to Vierck, if peripheral pain generators

can be blocked, the symptoms of FM should disappear or not even develop. Despite this, researchers focus more on central sensitization as the mechanism of pain sensitivity because there is less evidence to support the involvement of peripheral pain tissue abnormalities and nociceptive processes in FM [20]. However, sensitization is not a unitary phenomenon, and a distinction must be made between central, peripheral and psychosocial sensitization [21,22]. Brosschot et al. observed that FM patients were selectively attentive to information regarding the body and the environment in relation to pain. In this regard, they introduced the term "cognitive-emotional sensitization" to explain how selective attention to certain body pain can increase the perception of that pain. Pain sensitivity is also linked to social groups. It has been suggested that the mechanism that underlies "interpersonal sensitization" could be linked to the shared neuronal representation of the experience of pain. In other words, a feed-forward effect occurs in which a family, in an attempt to reduce painful behaviors in one of its members, actually creates a state of anxiety in the person concerned by increasing the perception of pain [23,24].

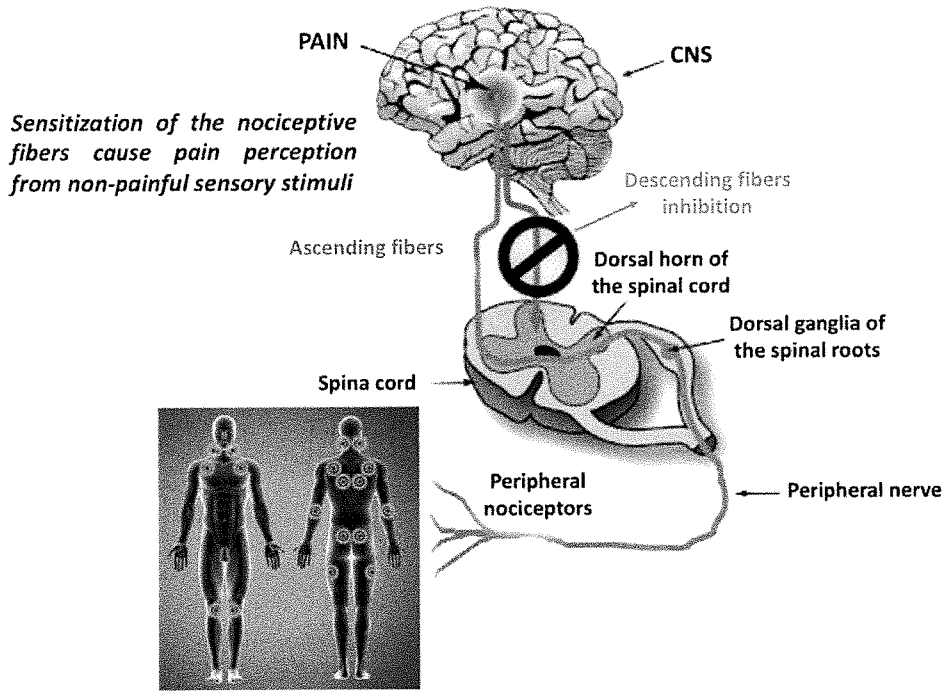

Figure 2. Ascending and descending pathways that influence pain sensitivity.

2.1.1. Peripheral and Central Sensitization

Patients with FM present a lower pain threshold that generates a condition of diffuse hyperalgesia and/or allodynia. This indicates that there may be a problem with the amplification of pain or with sensory processing in the CNS. These FM phenomena have been confirmed in clinical studies that used functional neuroimaging or measured alterations in neurotransmitter levels that influence sensory transmission and pain [25–27]. It was also observed that treatments with drugs aimed at increasing anti-nociceptive neurotransmitters in the CNS or at lowering the levels of pro-nociceptive excitatory neurotransmitters, such as glutamate, were able to improve these conditions in patients with FM. Exercise has also proved useful for increasing anti-nociceptive neurotransmitters and reducing glutamate [28,29]. In contrast, patients with FM do not respond to non-steroidal anti-inflammatory drug (NSAID) therapies aimed at resolving acute pain or pain induced by tissue damage or inflammation. Animal models of non-inflammatory pain, such as that induced by repeated injections of acidic saline (pH 4 or pH 5) into the gastrocnemius muscle, have been used to mimic the clinical signs and symptoms observed in FM [30–33]. These studies reported that widespread and lasting hyperalgesia was greater in female

mice than in males [31,32]. In addition, a pharmacological response similar to the clinical one was observed in animal models of non-inflammatory pain used for FM. In particular, a reduction in pain and hyperalgesia was observed following treatment with antidepressants, opioids, glutamate receptor antagonists and Na^+ channels, while no effect was observed with NSAIDs [34–36].

Clinical studies based on functional magnetic resonance imaging (fMRI) have confirmed a central neuronal alteration in nociceptive processes. In particular, following the same amount of pressure stimuli, patients with FM had greater neuronal activation in the pain-processing areas of the brain than control subjects [37,38]. In individuals with diffuse hyperalgesia or allodynia, the main brain regions in which greater neuronal activity is observed are the posterior insula and secondary somatosensory cortices [39,40]. The subtle differences in fMRI results in studies on FM or other chronic pain conditions are due to the fact that the stimulus to induce pain is not normalized and therefore may differ in intensity with each scan. fMRI studies have also been useful for determining the involvement of psychological factors in pain processing in FM [40–43].

The use of fMRI has also been useful for examining the degree of connection between the various brain regions. This analysis has been applied to both resting and active individuals. The advantage of the analysis at rest is that it provides data on brain changes associated with ongoing spontaneous chronic pain. With this technique, individuals with FM were shown to have greater connectivity between a network called "default mode" (active when the brain is at rest) and the insula (pro-nociceptive region) [44]. The degree of connectivity between these regions depends on the intensity of spontaneous and continuous pain [45]. It was later found that other brain regions may be hypo-connected in individuals with FM. For example, following a painful stimulus, a reduction in connectivity is observed between the anti-nociceptive areas in the brain stem in patients with FM. This suggests that there is a defect in the descending inhibitory systems in this condition [46]. Additionally, in clinical studies, an increase in glutamate levels has been observed in the brains of FM patients [47–49]. Using proton magnetic resonance spectroscopy, these levels were observed to increase in the main areas of pain processing, such as the insula [50–52].

Furthermore, pregabalin has been observed to reduce glutamatergic activity in the insula and functional connectivity between the default mode network and the insula in patients with FM [53]. Another important clinical observation is that some subgroups of patients with FM respond to treatment with N-methyl-D-aspartate (NMDA) glutamate receptor antagonists, suggesting an increase in glutamatergic activity. However, the use of these drugs is not always well tolerated and therefore may not always have a clinical use [54–56]. Alternatively, a low-glutamate diet has been shown to reduce FM symptoms [57]. Parallel to the clinical evidence, animal studies in the non-inflammatory pain model, which is used to induce the typical symptoms of FM, have shown increased glutamate release in the spinal and ventromedial rostral cords [58,59]. In particular, in non-inflammatory pain models that involve two injections (administered five days apart) of acidic saline into the gastrocnemius muscle, an increase in glutamate levels was observed after the second injection of acidic saline but not after the first. This increase corresponds to the development of prolonged diffuse hyperalgesia that occurs after the second injection. This suggests that greater excitability in the CNS induced by a single low-intensity muscle insult causes the same nervous system to mount an exaggerated response to a subsequent occurrence of the same stimulus. As in FM patients, experimental animals respond to the blockade of NMDA receptors [60–62]. An important role in the excitability of neurons is played by the NR1 subunit of the NMDA receptor, which is necessary for the formation of the receptor itself and its insertion into the synapse. In an animal study, recombinant lentiviruses were used to overexpress the NR1 subunit, and the nociceptive sensitivity to skin and muscle stimuli was measured to demonstrate that the increased expression of the NR1 subunit in the ventromedial rostral medulla is critical for the development of hypersensitivity. The results showed that NR1 overexpression in the rostral ventromedial medulla reduced the muscle and skin withdrawal threshold, leading to diffuse hyperal-

gesia similar to that observed in the non-inflammatory pain model. Furthermore, in the same study, the expression of NR1 was downregulated, and the hyperalgesia induced by repeated muscle injections of acid saline (pH 4.0) was measured. The results showed that downregulation of this subunit increased the muscle withdrawal threshold, preventing the onset of hyperalgesia [61,62]. Taken together, these data provide evidence that NMDA glutamate receptors in the CNS play a central role in the induction and maintenance of widespread hyperalgesia. Another fundamental role that has emerged from in vivo studies is that played by intracellular messengers, whose changes can produce lasting effects by altering neuronal excitability and improving gene transcription. In an FM animal model induced by restraint stress with intermittent cold stress, brain-derived neurotrophic factor (BDNF) and phospho-cAMP response element-binding protein (p-CREB) proteins were downregulated in the medial prefrontal cortex and hippocampus of animals with FM compared to the control group. Therefore, this animal model of FM could be used to investigate the BDNF–CREB pathway and pain [63]. Studies of acute paw inflammation induced by carrageenan [64] or subcutaneous formalin [65,66] and studies on neuropathic pain [67,68] have shown an increase in phosphorylated CREB due to the activation of the cAMP pathway. On the basis of these studies, a group of researchers aimed to evaluate the role of the cAMP pathway in mechanical hyperalgesia and in the phosphorylation of the transcription factor CREB in a model of chronic muscle pain (two injections of sterile saline at pH 4.0) which mimics the typical chronic muscle pain of patients with FM. The results of this study demonstrate that CREB phosphorylation is time-dependent and occurs in parallel to the cAMP-dependent phase of mechanical hyperalgesia. The increase in CREB phosphorylation is reversed by blocking the cAMP pathway and correlates with the mechanical withdrawal threshold. Therefore, the increase in phosphorylated CREB may contribute to mechanical hyperalgesia. These data have some clinical relevance, as they suggest that modulation of the cAMP pathway may be useful in the early stages of muscle hyperalgesia [69]. In addition, an increase in the phosphorylation of ERK, an intracellular signaling molecule, was also observed at the level of the paraventricular thalamus and in the amygdala [70,71]. This phenomenon and hyperalgesia can be prevented by the intracerebroventricular blockade of T-type Ca^{2+} channels [70]. This latter observation suggests that Ca^{2+} channels may mediate some of these changes. Therefore, the animal models studied show that alterations in central excitability occur throughout the nociceptive system, from the spinal cord to the cortex.

Although FM is thought to be a central pain disorder, many studies have shown peripheral nerve changes in patients with FM. In particular, people with FM have been reported to have a reduced number of epidermal nerve fibers in skin biopsies [72–75]. Clinical observations have also shown that FM patients score higher on neuropathic pain questionnaires and have altered heat, cold and pain thresholds [72,73]. By using microneurography on patients, some researchers have shown that mechanically insensitive C-fibers have higher spontaneous activity and increased sensitization to mechanical stimulation [76]. The notion that peripheral factors may underlie pain in FM is supported by the observation that the administration of lidocaine into the muscles of patients with FM significantly reduced hyperalgesia at the local site, and moreover, the pain perceived outside the injection site was reduced by 38% [77]. In a study investigating changes in muscle tissue, no differences were observed in the number of type I or II fibers or in the capillary density between individuals with FM and healthy subjects [78]. However, other clinical studies have shown that resistance to fatigue was closely related to the size of type I muscle fibers and the oxygenation of hemoglobin. FM patients who had a higher percentage of type I fibers were shown to recover strength more efficiently. According to these researchers, these measures may relate to the fatigue typical of FM. Although no differences were noted in measurements of performance or muscle fatigue between subjects with and without FM, individuals with FM, especially women, reported increased fatigue and pain during exercise [79,80]. Self-reported fatigue is generally thought to be caused by the CNS since

these symptoms often respond to centrally acting drugs, but according to the latest data, these symptoms are possibly due to changes in muscle tissue.

In humans, the intramuscular injection of an acid solution into the anterior tibial muscle induces muscle pain both at the site and in the ankle, producing primary and secondary hyperalgesia [81]. Therefore, a decrease in pH can contribute to hyperalgesia and referred pain. It has been reported that some acid-sensing ion channel (ASIC) members are activated following very slight acidification (from pH 7.4 to 7.2) and generate a depolarizing current at the peripheral terminals of nociceptors, leading to the detection of pain [82–84]. A greater number of acid-sensing ion channel 3 (ASIC3) receptors have been observed on sensory neurons innervating the skeletal muscle than on those innervating the skin. Furthermore, most of these afferents that innervate skeletal muscle and express ASIC3 also co-express calcitonin gene-related peptide (CGRP), a nociceptive marker [85,86]. The important role of ASIC3 activation on nociceptors that innervate the skeletal muscle in the induction of diffuse hyperalgesia was suggested by the results of an animal study, which demonstrated that blocking ASIC3 with APETx2 was capable of inhibiting the development of hyperalgesia [87–89]. Furthermore, the importance of ASIC3 activation was also observed in the repeated acid model or fatigue-induced model, as hyperalgesia was not detected in ASIC3 knockout mice [89,90].

However, it has been shown that an ASIC antagonist administered after the onset of hyperalgesia has no positive effect. This indicates that once hyperalgesia has developed, it is independent of the activation of nociceptors due to acid pH [87,91]. In contrast, in a reserpine-induced FM model, ASIC3 mRNA was highly expressed in the spinal dorsal root ganglion (DRG), and hyperalgesia was reversed by administering APETx2, thereby blocking ASICs. Furthermore, the mechanical reactivity of C fibers in this model was a greater in both the skin and the muscles [92]. These results therefore show that the ASIC3 present at the peripheral level could modulate widespread hyperalgesia. Indeed, the increased reactivity of C fibers at the skin level is similar to that observed in subjects with FM, and this suggests that ASIC3 may be involved in modulating altered peripheral sensitivity.

The release of substance P from nociceptive nerve fibers and the activation of its neurokinin 1 receptor (NK1), which is found post-synaptically in the spinal dorsal horn, as well as on immune cells, smooth muscle, blood vessels and other peripheral cells, seem to play an important role in the transmission of pain signals [93]. Substances that inhibit substance P signaling pathways usually produce anti-nociceptive effects in animal models [94,95]. Therefore, substance P is believed to promote pain. On the other hand, NK1 antagonists have failed to produce analgesic effects in many clinical studies; therefore, its function has yet to be clarified [96]. An unexpected anti-nociceptive role of substance P was observed in muscle-afferent DRG neurons. In a mouse model of chronic mechanical hyperalgesia induced by repeated intramuscular acid injections, the release of substance P in the muscle appeared to play a physiological role in nociceptive plasticity and limited the activation of muscle nociceptors induced by acid injection. This physiological mechanism could be of therapeutic interest, as the administration of a selective NK1 agonist prevents long-lasting hyperalgesia. In support of this hypothesis, animal studies have shown that a single intramuscular injection of acid in tachykinin precursor 1 gene (Tac1) knockout mice, which therefore lack substance P signaling, or the co-administration of NK1 receptor antagonists induces long-lasting rather than transient hyperalgesia, as occurs in controls. The anti-hyperalgesic effect of substance P was observed only in neurons expressing ASIC3, where substance P increases M-channel-like potassium currents through the NK1 receptor independently of protein G but in a tyrosine kinase-dependent manner. Thus, through these works, intramuscular substance P was confirmed to mediate an unconventional NK1 receptor signaling pathway that led to an unexpected anti-nociceptive effect against chronic mechanical hyperalgesia induced by repeated acid injections [97].

As previously mentioned, the chronic pain typical of FM is due to alterations in central and peripheral sensitization. Over the years, researchers have searched for biomarkers that are capable of detecting these changes. In particular, they focused on factors capable of

acting on the growth and survival of nerve cells, such as nerve growth factor (NGF). This factor is indeed involved in promoting the growth, proliferation and survival of sensory neurons that transmit pain, temperature and tactile sensations [98].

Data obtained in earlier studies showed an increase in NGF in the cerebrospinal fluid of FM patients [99]. However, these data disagree with those from a recent study in patients, in which plasma NGF levels were not found to differ between FM and control subjects. In this regard, different statistical methods were used, which nonetheless led to the same conclusions [100]. Further studies are therefore needed to understand the involvement of NGF in the pathophysiology of FM.

2.1.2. Inflammation and Immunity

Increasing evidence indicates that neurogenic-derived inflammatory processes occurring in the peripheral tissues, spinal cord and brain are also responsible for the pathophysiology of FM [101–103]. In fact, the release of biologically active agents, such as chemokines and cytokines, leads to the activation of the innate and adaptive immune system. All of this translates into many of the peripheral clinical features reported by patients with FM, such as swelling and dysesthesia, which can also affect central symptoms, including cognitive changes and fatigue. In addition, the physiological mechanisms related to stress and emotions are considered to be upstream drivers of neurogenic inflammation in FM [104].

Studies conducted in patients have confirmed that inflammation is involved in FM. Indeed, FM patients have been shown to have enhanced circulating inflammatory cytokines and inflammatory cytokines released by circulating immune cells [103,105].

Kadetoff et al. described an increased concentration of IL-8 in the cerebrospinal fluid of FM patients compared to healthy subjects [106]. This finding could be due to the activation of glial cells, which play an important role in the central sensitization process, as they are activated in response to excitatory synaptic signals (glutamate) [107]. Furthermore, since the synthesis of IL-8 is dependent on orthosympathetic activation, this could help explain the correlation between stress and fibromyalgia symptoms [108]. In addition to this, some studies have shown an increase in serum concentrations of IL-6, IL-8, IL-1β and TNF-α in individuals with FM, although no clear correlation with symptom severity has been identified, except, perhaps, for IL-6 [103,109–111]. It appears that immune cells such as mast cells, monocytes and neutrophils, as mediators of inflammation processes, may also have a function in defining an inflammatory substrate of fibromyalgia [112]. In animals, resident macrophages located in the muscle have been shown to contribute to the development of chronic widespread muscle pain. For example, the removal of macrophages at the acid injection site through a local injection of clodronate liposomes is capable of preventing the development of exercise-induced hyperalgesia [89]. Another observation is that pro-inflammatory cytokines, such as interleukins (IL-1β, IL-6) and tumor necrosis factor (TNFα), can activate and sensitize nociceptors, induce pain in humans and trigger hyperalgesia in animals. Another potential source of these cytokines is adipose tissue; many studies suggest that diffuse or multifocal pain is more common in obese individuals [113], and obese animals show enhanced nociceptive responses [114,115]. Therefore, pro-inflammatory cytokines could play a role in the generation of chronic muscle pain, including FM.

Finally, a study by Smart et al. described a subgroup of fibromyalgia patients characterized by ANA (anti-nuclear antibody) positivity, with the speckled pattern clearly predominating. The use of the Smart Index, which corrects the erythrocyte sedimentation rate value in relation to age, revealed that ANA-positive FM patients had a more pronounced inflammatory response profile than the ANA-negative subgroup, suggesting that autoimmunity potentially contributes to sub-inflammatory fibromyalgia [116].

2.1.3. Genetic Aspects

Over the years, studies have shown the potential involvement of genetic factors in the onset of FM [117,118]. Linkage studies have shown a correlation rate of 50% between

genetic variants and the development of chronic pain [119]. Currently, about 100 genes that regulate pain are believed to be relevant to pain sensitivity or analgesia. The main genes are those encoding for voltage-dependent sodium channels, GABAergic pathway proteins, mu-opioid receptors, catechol-O-methyltransferase and GTP cyclohydrolase 1 [120]. The small sample sizes did not allow the authors to confirm an association between single nucleotide polymorphisms and FM susceptibility. However, a genome-wide linkage scan study found that first-degree relatives had an increased risk of developing FM, reinforcing the genetic hypothesis. The serotonin transporter gene (*SLC64A4*) and the transient receptor 2 potential vanillic channel gene (*TRPV2*) are the major genes responsible for pain susceptibility in FM [121]. *SLC64A4* is characterized by a single nucleotide polymorphism and is associated with chronic pain conditions (for example, mandibular joint disorder), as well as increased levels of depression and psychological disorders related to an alteration in serotonin reuptake [122]. The *TRPV2* gene is expressed in mechano- and thermo-responsive neurons in the dorsal root and trigeminal ganglia and appears to be responsible for reducing the pain threshold in FM patients [123]. Other genetic polymorphisms that have been identified and associated with FM susceptibility are in the serotonin transporter (5-HTT), catechol-O-methyltransferase (COMT) and serotonin 2A (5-HT2A) genes. However, subsequent meta-analyses could only confirm that the 102T/C polymorphism in the 5-HT2A receptor is connected with FM [124]. Therefore, further studies are needed to understand the role of these genes in chronic pain conditions such as FM. A genome-wide association and copy number variant study in 952 FM cases and 644 controls revealed the existence of two variables associated with FM. One variable is the single nucleotide polymorphism *rs11127292* in a gene similar to myelin transcription factor 1 (MYT1L), which is responsible for neuronal differentiation and involved in cognitive alterations. The second is an intron copy number variable in the neurexin 3 (*NRXN3*) gene, which normally acts as a receptor and cell adhesion molecule in the nervous system, and variations in this gene are involved in autism spectrum disorder [125]. Other researchers analyzed 350 other genes that are specifically involved in pain treatment. Among these is the *TAAR1* gene, which mediates the availability of dopamine, whose reduction can increase the sensitivity to pain typical of FM [126]. Another widely studied gene is *RGS4*, which is expressed in the dorsal horn of the spinal cord, the locus coeruleus and the nuclei of the bed of the stria terminalis, and is responsible for modulating the descending inhibition of pain perception [127]. One gene studied and related to pain disorders is *CNR1*, which encodes the cannabinoid receptor CB-1 [128,129]. Another gene presumably involved in central sensitization is GRIA4, which mediates the rapid excitatory transmission of nociceptive signals in the central nervous system [130]. Taken together, these studies have increased the current knowledge on FM and support the genetic hypothesis as a potential factor in the pathogenesis of this disease. However, as FM remains a multifactorial disease, further studies are needed to examine haplotypes and combinations of different variants that could influence its development.

2.1.4. Endocrine Factors

The role of stress in the exacerbation of fibromyalgia symptoms has been widely described from an epidemiological point of view through both self-reports and clinical questionnaires. On the basis of these data, the hypothalamic–pituitary–adrenal axis, central to the stress response, was examined. Despite the discrepancy between different studies on possible alterations in plasma cortisol levels in FM patients, dysregulation of its circadian variation is frequently observed. In particular, flattening of the plasma cortisol concentration curve was observed during the day: this seems to manifest itself through a milder and more gradual descent compared to the morning peak of maximum concentration or through a lowering of the peak itself [131–135]. In addition to this, decreased cortisol secretion has also been described in response to adrenocorticotropic hormone (ACTH) tests [136]. The hypothalamic–pituitary–adrenal axis (HPA) comprises neurotransmitter and neuroendocrine response systems to stress and can be activated in FM [137]. This system may explain some of the symptoms seen in FM.

A patient study looked at levels of corticotropin-releasing factor (CRF) in cerebrospinal fluid (CSF), heart rate variability (HRV) and pain symptoms (e.g., fatigue and depression) in subjects with FM. The results obtained in this study showed that CRF levels were associated with sensory and affective pain symptoms but not with symptoms of fatigue. Furthermore, an increase in HRV was associated with an increase in CRF and pain in patients with FM. These results were subsequently adjusted for age, sex and depressive symptoms, and a correlation between CRF levels and sensory pain symptoms was confirmed. Another important finding was that women with FM and self-reported histories of physical or sexual abuse did not have increased levels of CRF in their CSF. This indicates that there may be subgroups of FM patients with different neurobiological characteristics. Therefore, further studies are needed to better understand the association between CRF and pain symptoms in FM [138]. In another study, the association between salivary cortisol levels and pain symptoms in patients with FM was assessed at different times of day. The results obtained in this study revealed a strong relationship between salivary cortisol and pain symptoms only at the time of awakening and the 1 h that followed in women with FM. Furthermore, no relationship was observed between the cortisol level and symptoms of fatigue or stress. These findings suggest that early-day pain symptoms are associated with changes in HPA function in women with FM.

However, to date, the results regarding the involvement of the HPA in the pathophysiology of FM have been conflicting, and new studies will be needed in the future to fully clarify this aspect [133]. Furthermore, there are indications that total and free cortisol levels are dissociated in FM patients. They have normal salivary and free plasma cortisol despite having reduced total cortisol levels. A possible explanation for this finding is a reduced concentration of glucocorticoid-binding globulin (CBG). Reduced levels of CBG have been reported in FM patients compared to healthy patients. It is of particular interest that chronic social stress can lead to reduced levels of CBG, while IL-6 and IL-1β, which can also inhibit CBG production, may contribute further [139].

The possible pathogenetic role of the growth hormone (GH)/insulin-like growth factor 1 (IGF-1) axis was also investigated. Several studies have found that about one-third of individuals with fibromyalgia have lower IGF-1 levels than control groups [140]. Serial measurements at 12 to 24 h also showed a reduction in GH secretion in patients with fibromyalgia, particularly at night. Since GH secretion occurs mainly during phase 3 of sleep and 80% of patients have sleep disturbances, it remains to be clarified whether the nature of this alteration is primary or secondary [141]. Given the higher prevalence of fibromyalgia in the female population, the role of estrogens in this pathology was investigated. However, the results of various studies suggest that this role is limited, and the only significant result is an increased serum concentration of the G protein-coupled estrogen receptor (GPER) in patients with fibromyalgia compared to healthy subjects [142]. Although a strong correlation with the disease allows us to hypothesize the possible use of this receptor as a potential diagnostic biomarker, the exact mechanism by which it fits into the pathophysiological cascade remains unclear [143].

2.1.5. Psychopathological Factors and Poor Sleep

As previously highlighted, psychiatric comorbidities in fibromyalgia constitute a relevant aspect of the disease, and a close correlation between stress and fibromyalgia symptoms has been described several times. According to several studies, the prevalence of psychiatric comorbidities, such as anxiety disorders and depression, among patients with this pathology reaches 60% in certain subpopulations [144]. The presence of depressive patterns has been shown to correlate with a worse prognosis: patients with comorbid symptoms of depression seem to report pain of greater severity and duration as well as a greater degree of hyperalgesia/allodynia than healthy controls. Furthermore, these psychiatric aspects seem to have a certain predictive value in relation to various somatic symptoms, including musculoskeletal pain and headaches [145]. The impact of depression symptoms on pain processing is still unclear. A study in FM patients attempted to eval-

uate this correlation by comparing the results of quantitative sensory tests and neuronal responses to pressure stimuli (assessed by functional magnetic resonance imaging (fMRI)) with the levels of symptoms of depression. The results showed that the symptom levels of depression were not associated with quantitative test results or with the extent of neuronal activation in brain areas, such as primary and secondary somatosensory cortices, that are associated with the sensory dimension of pain. However, symptoms of depression were observed to be associated with the extent of pain-evoked neuronal activation in the amygdala and contralateral anterior insula, which are brain areas associated with affective pain processing. Therefore, these findings suggest the existence of parallel, possibly independent, neuronal pain processing networks for sensory and affective pain elements [146]. Finally, the therapeutic aspect is an element that supports the pathogenetic overlap between depressive disorder and fibromyalgia. The efficacy of treatment with antidepressant drugs (e.g., serotonin-norepinephrine reuptake inhibitors (SNRIs) and tricyclics) has in fact been described by numerous studies on FM patients and constitutes one of the main therapeutic strategies in both FM and other chronic pain conditions, such as chronic headache and irritable bowel (IBD), which are often symptoms of FM [147–149]. The efficacy of SNRIs and other double-acting antidepressants, such as mirtazapine, suggests that neurotransmission dysfunction of both serotonin and norepinephrine exists in FM [150]. Similar to observations for the depressive pattern, stress also appears to be both a predictive and negative prognostic factor. It has been shown that stress can modulate pain sensitivity by inducing hyperalgesia or allodynia through alterations in the physiological circadian secretion of cholesterol, therefore indirectly inducing the release of pro-inflammatory cytokines and setting in motion the pathophysiological processes described above [151–153]. Animal studies have shown that stress induction (e.g., swimming stress and cold stress) can produce muscle and skin hyperalgesia that lasts for weeks after the stressor [154–156]. On the other hand, milder stressors (e.g., fatigue and acoustic stress), which do not produce hyperalgesia on their own, can cause an increase in and prolongation of the hyperalgesic response to a subthreshold or mild noxious stimulus [32,157,158]. Other studies have shown that animals exposed to stressors also exhibit changes in the spinal cord. In particular, animals showed a greater expression of c-fos in response to formalin, as well as a reduction in the basal and induced release of the inhibitory neurotransmitter GABA; a reduction in mu-opioid agonist antinociception enhanced the basal and evoked the release of glutamate [159,160], suggesting both increased central excitability and reduced central inhibition. In animals, stress-induced hyperalgesia was reduced by the spinal blockade of substance P, calcitonin gene-related peptide (CGRP), NMDA-glutamate receptors and neurokinin-1 receptors, all substances involved in the neurotransmission of pain [158]. At the supraspinal level, cold stress-induced alterations were observed in the serotonergic system, with reductions in both serotonin (5-HT) and 5-hydroxy indoleacetic acid (5-HIAA) levels in the supraspinal regions [161]. Thus, stress and psychological factors are involved in the development and severity of FM.

Sleep disorders are classically described within the symptomatic process of fibromyalgia. However, some recently reported data have generated the hypothesis that such disorders may be included among the causative factors of this pathology, rather than among its manifestations. Studies published in recent years have described a bidirectional correlation between sleep disturbances and widespread musculoskeletal pain, and it even seems that insomnia tends to precede the onset of pain and has predictive value regarding its onset and its persistence [162,163]. Studies carried out in healthy subjects also seem to show that total, partial and stage-specific sleep deprivation leads to hyperalgesia, an increased incidence of spontaneous pain and mood alterations, particularly anxiety and depression [164,165]. In a further study by Smith et al., the authors hypothesized that the development or aggravation of somatic and psychiatric symptoms is secondary to sleep discontinuity rather than sleep deprivation [166]. In addition to the number of awakenings, the cyclic alternating pattern (CAP) is a useful tool to analyze this discontinuity. It is represented by short cycles of periodic electroencephalographic activity of non-REM sleep,