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Fibromyalgia: Genetics and epigenetics insights may provide the basis for the development of diagnostic biomarkers

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Abstract

Fibromyalgia is a disease characterized by chronic widespread pain with additional symptoms, such as joint stiffness, fatigue, sleep disturbance, cognitive dysfunction, and depression. Currently, fibromyalgia diagnosis is based exclusively on a comprehensive clinical assessment, according to 2016 ACR criteria, but validated biological biomarkers associated with fibromyalgia have not yet been identified. Genome-wide association studies investigated genes potentially involved in fibromyalgia pathogenesis highlighting that genetic factors are possibly responsible for up to 50% of the disease susceptibility. Potential candidate genes found associated to fibromyalgia are *SLC64A4*, *TRPV2*, *MYT1L*, and *NRXN3*. Furthermore, a gene-environmental interaction has been proposed as triggering mechanism, through epigenetic alterations: In particular, fibromyalgia appears to be characterized by a hypomethylated DNA pattern, in genes implicated in stress response, DNA repair, autonomic system response, and subcortical neuronal abnormalities. Differences in the genome-wide expression profile of microRNAs were found among multiple tissues, indicating the involvement of distinct processes in fibromyalgia pathogenesis. Further studies should be dedicated to strength these preliminary findings, in larger multicenter cohorts, to identify reliable directions for biomarker research and clinical practice.

Keywords

Fibromyalgia, genetics, epigenetics, biomarkers, genome-wide association study, DNA methylation, miRNAs

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Introduction

Fibromyalgia (FM) is a common and complex chronic pain syndrome, affecting 1% to 5% of the population,[1] characterized by chronic widespread pain persisting for more than three months without any obvious organic lesion. Joint stiffness, fatigue, sleep disturbance, cognitive dysfunction, and depression are additional symptoms found associated with FM.[2,3]

The disease is more common in female than male,[4] with a ratio of 2:1 similarly to other chronic pain conditions, and it can occur at any age.[5] Since women show lower pain threshold and more severe symptoms than men,[6] the majority of researches focused on female subjects. However, the pathogenesis of FM is not fully understood, especially because compared to neuropathic conditions in FM, the source of sensory inputs is unknown;[7] some hypothesis on peripheral and central pathophysiological mechanisms have been proposed. Evidence support a central sensitization and a central dysregulation at a spinal and supra-spinal levels in FM patients compared to controls: FM patients showed an exaggerated pain response after sensory stimulation and

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an extended cutaneous silent period;[8,9] in healthy subjects, the application of an intense painful stimulus produces generalized whole-body analgesia, defined as *conditioned pain modulation*, while it is consistently reduced or even absent in FM subjects;[10,11] these observations lead to hypothesize a decreased serotonergic and noradrenergic activities.[12,13] The related neurotransmitters are involved in one of the principal descending monoaminergic pain control pathways[14] and thus play a fundamental role in the mechanism underlying acute and chronic pain.[15] Moreover, the reward/punishment circuit appears to be impaired in FM patients, consistently with the altered dopaminergic/GABAergic neurotransmission.[16] Even functional neuroimaging studies support the altered central neural processing in nociceptive pathways: Following pressure stimuli, a higher activation in brain pain-processing regions was observed in FM subjects compare to controls.[17]

The difficulty to identify a specific physiological pathway is also accompanied by difficulties in FM diagnosis, currently only based on a comprehensive clinical assessment; up to 2010, this was principally relying on the 1990 ACR criteria[18] of widespread pain, with at least 3 months consecutive pain and 11 painful "tender points" with digital palpation. Since 2010, new ACR criteria consider other two parameters: The widespread pain index, which locates pain or tenderness in specific body areas, and the symptom severity scale score, which considers both somatic and cognitive symptoms, as trouble thinking or remembering, fatigue, unrefreshed sleep, and depression.[19] Tender points and algometer measurement of pressure pain threshold are still fundamental steps for a comprehensive muscle-skeletal clinical examination and to exclude other diagnosis linked to widespread pain.[12] In 2016, previous criteria have been reviewed to minimize misclassification of other pain conditions, and FM diagnosis can now be made irrespective on other potential coexisting pathologies, if all the other key symptoms are present.[20] Nonetheless, the individual phenotypic variability and concomitant pathologies in the majority of patients lead to non-exhaustive clinical examinations for a precise diagnosis, making tough to define universal criteria for this condition. Furthermore, validated biological biomarkers have not yet been identified; research is thus oriented to discover possible new indicators for an objective diagnosis of affected individuals through the identification of genetic, environmental, and epigenetics factors underlying FM pathophysiology.[21]

Genetic contribution to FM development

Genetic variants and inheritance mechanisms in pain-related genes have been shown to contribute to 50% in the development of chronic pain, as shown by earlier linkage studies, illustrating the correlation between genetic variants and pain response.[22] At present, hundreds of pain-regulated genes potentially relevant to pain sensitivity or analgesia have been detected, among which genes encoding for voltage-gated sodium-channels, GTP cyclohydrolase 1, mu-opioid receptors, catechol-O-methyltransferase, and GABAergic pathway proteins.[23]

Even if many single nucleotide polymorphisms (SNPs) have been identified as potential candidates specifically associated to FM susceptibility (Table 1), the

Table 1. SNPs related to genes potentially involved in fibromyalgia's pathogenesis.

SNPs	Gene	Clinical relevance
5-HTTLPR[24]	*SLC6A4*	Temporal mandibular joint disorder
		Depression
		Psychological disorders
rs4680[28]	*COMT*	Depression
		Anxiety
		Disability
rs1048101[29]	*HTR2A*	FIQ disability
rs6313[30,31]	*HTR2A*	Fibromyalgia onset
rs11127292[32]	*MYT1L*	Cognitive disability
Intronic CNV[32]	*NRXN3*	Autism
rs8192619, rs4129256[33]	*TAAR1*	Impaired dopamine availability
		Enhanced pain sensitivity
rs10799897, rs2842003, rs2805050[33]	*RGS4*	Alteration in the descending inhibition of pain perception
rs6454674, rs1078602, rs10485171[33]	*CNR1*	Migraine
		Irritable bowel syndrome
		Post-traumatic stress disorder
rs642544, rs17104711, rs2510177, rs10895837[33]	*GRIA4*	Central sensitization

SNP: Single Nucleuotide Polymorphism; CNV: copy number variant.

low number of subjects involved did not often allow to confirm them in subsequent meta-analyses.

However, a genome-wide linkage scan study revealed a 13.6-fold increased risk of developing the syndrome in first-degree relatives[24] strengthening the genetic hypothesis. The research evidenced a linkage at markers *D17S2196* and *D17S1294* on chromosome 17p11.2–q11.2; two potential FM susceptibility candidate genes map on this region, the serotonin transporter gene (*SLC64A4*), and the transient receptor potential vanilloid channel 2 gene (*TRPV2*).[24] *SLC64A4* polymorphisms were already found associated with chronic pain conditions, like temporal mandibular joint disorder;[25] in addition, an alteration in serotonin reuptake was associated with high levels of depression and psychological disorders in the same patients.[26] Alterations in *TRPV2*, a gene expressed in mechano- and thermo-responsive neurons in the dorsal root and trigeminal ganglia,[27] could instead contribute to the impaired pain threshold in FM patients.

Candidate genes-associated studies report a correlation between *Val158Met* variant in *COMT* gene[28] and depression, anxiety and disability in FM women, *(1A)-AR-rs1383914* SNP and FM susceptibility, the *(1A)-AR-rs1048101* SNP and FIQ disability,[29] and *T102C* polymorphism of the *5-HT2A* receptor gene and FM onset.[30]

In order to clarify the potential association between gene polymorphisms in *5-HTT*, *COMT*, and *5-HT2A* genes and FM susceptibility, Lee et al.[31] have led a meta-analysis on FM genetic predisposition, highlighting the potential central role of *102T/C* polymorphism in 5-HT2A receptor; the significant associations of *5-HTTLPR* S/L allele and *COMT* Val158Met with FM were not confirmed.[31] More investigations need to understand the role of these genes in pain biology and in chronic pain diseases as FM.

Genome-wide association studies have contributed to sustain the possible involvement of central nervous system (CNS) dysfunction in FM. Recently, Docampo et al.[32] conducted a genome-wide association studies and copy number variant analyses in 952 FM cases and 644 controls. Their results showed two FM-associated variants, *rs11127292* SNP and an intronic copy number variant, belonging respectively to *MYT1L* (myelin transcription factor 1 like gene), which plays a key role in neuronal differentiation and it is involved in cognitive disability, and to *NRXN3* (neurexin 3 gene), which acts in the nervous system as receptor and cell adhesion molecule, and its genetic variants have been found involved in autism spectrum disorder.[32]

However, no SNPs have achieved the genome-wide significant threshold and, therefore, further analyses are needed to confirm these previous results. Smith et al.[33] evaluated 350 genes in particular including genes involved in pain treatment, as *TAAR1*, *RGS4*, *CNR1*, and *GRIA4*. In fact, impaired *TAAR*-mediated dopamine availability could enhance pain sensitivity, a typical symptom in FM subjects.[34] *RGS4* gene, expressed in the locus coeruleus, the bed nuclei of the stria terminalis, and in the dorsal horn of the spinal cord,[35] plays a modulatory role in the descending inhibition of pain perception. *CNR1* encodes to CB-1 cannabinoid receptor, and its variants have been shown related with other pain diseases, like migraine,[36] irritable bowel syndrome,[37] and post-traumatic stress disorder.[38] *GRIA4* encodes the AMPA sensitive, ionotropic glutamate receptor subunit *GluR4*, which mediates fast excitatory transmission of nociceptive signals in the CNS and it is presumably involved in the central sensitization.[39] These studies improved the knowledge about FM and supported the genetic hypothesis underlying its pathogenesis, suggesting potential genetic markers for FM susceptibility, even though universally validated SNPs have not yet been found. Potential explanations are the population specificity of genetic variants and, moreover, being FM a multifactorial condition, haplotypes, combinations of different variants, might affect the improved risk of FM development more than a single variant: A correlation of the disease and the "high pain sensitivity" haplotype (*ACCG*) belonged to *COMT* gene in a Spanish population[40] and the *B2-AR AC* haplotype in Mexican and Spanish populations were already identified.[41]

Environmental influences on the occurrence of FM

Beside a genetic predisposition to FM, environment may be involved in the development of the disease. In particular, early-life events, including both physical trauma and psychosocial stressors have been found to influence gene expression and thus contribute to the occurrence of FM.[42,43]

The evidence that physical trauma influence FM development in adulthood results from studies where the impact of early life pain experiences was evaluated: Early and childhood experiences have been associated with long-lasting changes in nociceptive circuitry and increases pain sensitivity in the older organism.[44] For example, adverse events during the neonatal and childhood life, like premature birth,[45] physical and sexual abuse,[46,47] have been shown to possibly contribute to an alteration of threshold pain in adulthood and the development of FM onset.[48] As result of stress events, an impairment of HPA (hypothalamic-pituitary-adrenal) axis could rise up, with a subsequent inefficient response to stress and enhanced sensitivity to pain and fatigue.[49]

In adulthood, repeated physical stressors have been demonstrated to be involved in the development of chronic widespread pain, particularly due to activities

like heavy lifting, repetitive motions, or squatting for extended periods of time.[50]

Among the researches on environmental triggers of FM, psychological and social stressors seem to represent the strong predictors of the disease, including chronic stress, emotional trauma,[51] with physical assault/abuse in women particularly associated with FM diagnoses.[52] Other environmental conditions recently discovered to affect FM are childhood maltreatment, as neglect, emotional abuse, and post-traumatic stress disorder. Interestingly, concomitant levels of depression and anxiety were significantly higher among these FM patients.[53]A bidirectional temporal association between depression and FM has also been demonstrated, with an increase risk to develop each other.[54] In support of this connection, altered gray and white matter morphometry including medial orbitofrontal cortex and cerebellum have been observed in FM patients, with the gray matter volume associated with the severity of depression and hyperalgesia.[55] This finding suggests a potential shared pathophysiological mechanism underlying FM and depression.

Stressful life events in FM patients persist even in spite of different cultures, demonstrating the transcultural soundness of the association between diagnoses of FM in adulthood, self-reported childhood maltreatments, and lifelong traumatic experiences.[56]

The physiological processes mediating the connection between experienced stress and the development of FM are still unknown.[57] The HPA axis failure has been proposed as potential responsible of this relationship;[58,59] the increased pain levels of FM patients, in fact, have been found related to decreased levels of hypothalamic corticotrophin-releasing hormone[58] and an increased levels of substance P and glutamate in cerebrospinal fluid (CSF).[57] Moreover, hypoactivity of dopaminergic, opioidergic, and serotoninergic systems have been evidenced in patients with FM, suggesting a complex derangement of psychobiological patterns.[39]

Based on this evidence, environmental factors, particularly chronic stress and traumatic experiences, can be hypothesized to influence neurophysiological responses through gene expression alteration, in turn interfering with peripheral and central pain perception.

Recent studies suggest that also environment and HPA axis reactions to stress have a great impact on gut microbial composition and balance, which in turn affect human brain health, auto-immune reactions, and encephalotoxic methabolites release. The correlation between host genetics and microbiome has already been explored in pathologies as diabetes and obesity.[60] Concerning FM, the observed mitochondrial dysfunction,[61] associated to pain sensitization and muscle pain, has been recently hypothesized to be potentially caused by a gastrointestinal microbial imbalance, revealing new possible research lines for FM understanding and treatment.[62]

The role of epigenetics: A new point of view

Previous studies demonstrated that early life experience and environmental factors in general could modulate genome function and the phenotype through epigenetic mechanisms, without altering the DNA sequence.[63] Main epigenetic mechanisms, supporting gene-environment interaction, are DNA methylation, covalent histone modifications, and non-coding RNAs. Epigenetic mechanisms have been observed to play an important role as mediators of long-term changes in central and peripheral nervous systems in chronic pain.[64] The environmental components observed in FM pathogenesis highlight a possible role of the gene-environment interaction in the development of this condition.

In particular, changes in methylation state, histone modifications, and miRNAs expression in pain-related regions appear to occur in the presence of peripheral inflammation and nerve injury.[65–67] Being chronic pain one of the main symptoms of FM, knowledge about how pain-related genes and environment interact may shed light on the etiological mechanism underlying this condition.

Studies on DNA methylation and FM

DNA methylation biochemical process involves the addition of a methyl group to the fifth carbon of DNA cytosine residues, leading to 5-methylcytosines. The process occurs mainly in cytosines and guanines rich regions, CpG islands, located in the 60% of human gene promoters,[68] and is mediated by a group of DNA methyltransferases (DNMTs): DNMT1, DNMT3a, and DNMT3b.[69]

A genome-wide DNA methylation study on healthy female monozygotic and dizygotic twins proved the implication of DNA methylation in thermal pain sensitivity.[70] In particular, a strong correlation of DNA methylation level in the promoter of *TRPA1* gene, expressed in peripheral nociceptors, and gate pain-related responses was identified.[71,72] Higher levels of *TRPA1* expression was related to lower DNA methylation state in its promoter and higher pain thresholds. A consistent link between level of DNA methylation state and heat pain sensitivity in healthy subjects was demonstrated.[72] DNA methylation alterations in FM patients have been also recently revealed[73,74] (Table 2).

The first study investigating epigenetic changes in FM women compared to controls was a genome-wide methylation pattern analysis that highlights 69 differentially methylated sites in cases against controls, and 91% of these sites were responsible of an increased micronuclei

Table 2. Genes differentially methylated in FM women.

Gene	Biological samples	Physiological function	Associations
BDNF[73]	Blood	Neuron Differentiation/nervous system development	Mood disorders Alzheimer Parkinson Huntington's disease
NAT15[73]	Blood	Histone acetyltransferase Chromatin compaction	Acetylation process Facilitation of transcription process
HDAC4[73]	Blood	Deacetylation of the core histones Muscle maturation	Deacetylation's process Gene silencing
PRKCA[73]	Blood	Cell signaling pathways	Post-traumatic stress syndrome Emotional memory formation Cancer
RTN1[73]	Blood	Secretion or membrane trafficking in neuroendocrine cells	Neurological diseases Cancer
PRKG1[73]	Blood	Regulation cardiovascular and neuronal functions Relax smooth muscle tone Prevent platelet aggregation Modulate cell growth	Aortic aneurysm Phosphoglycerate kinase deficiency
SLC17A9[74]	Blood	Regulation neuronal differentiation	Neuronal plasticity
TFAP2A[74]	Blood	Survival functions of sympathetic progenitors and noradrenergic neurons	Neuronal circuits

A general hypomethylated pattern in FM patients compared to healthy subjects seem to be revealed, considering the first studies on DNA methylation and FM.

frequency in FM women.[73] This correlation should be further investigate as useful tool evaluation and/or diagnosis. Genes mapped on differently methylated sites were *BDNF*, *NAT15*, *HDAC4*, *PRKCA*, *RTN1*, and *PRKG1*, suggesting the possible involvement of nervous system development, skeletal/organ system development, and chromatin compaction pathways in FM. More recently, Ciampi de Andrade et al.[74] have investigated DNA methylation state in blood samples from a cohort of 24 FM cases and 24 healthy controls. The results identified 1610 differentially methylated positions: 1042 (65%) were found hypomethylated and 568 (35%) hypermethylated in cases compared to controls. Most of the differentially methylated genes were related to signal transduction and calcium signaling, MAPK signaling pathway, regulation of actin cytoskeleton endocytosis, and neuroactive ligand-receptor interaction pathways.[74] In general, the differentially methylated sites identified associated with FM map on genes involved in biological processes as DNA repair, immune system, and membrane transport genes. The mechanisms behind FM may thus include pathways related to autonomic system response, subcortical neuronal abnormalities, and impaired cellular response to stress and to glutatione,[74] potentially explaining the significantly deregulated oxidative and antioxidative parameters observed in FM women.[75] However, these changes may not be specific to FM but due to concurrent conditions.

Cortical excitability parameters were also measured in both hemispheres of FM cases and controls, they resulted altered in parallel with methylation level changes in peripheral blood of FM patients.[74] This finding reveal the importance of DNA methylation research in peripheral blood to potentially develop biological markers of FM in the future.

MicroRNA profiles as new potential biomarkers

MicroRNAs are short non-coding RNA molecules approximately 20 to 22 nucleotides in length, highly evolutionary conserved; these factors have a fundamental role in the regulation of gene expression in disease processes and physiological pathways, since they are involved in cell growth, differentiation, stress response, and tissue remodeling; they exert several regulatory functions as mRNA cleavage, translational repression, or mRNAs deadenylation within cells where they were initially transcribed.[76] MicroRNAs regulate at least 30% of human genes,[77] and each microRNAs can repress hundreds of genes.[78] The presence of microRNAs in different cellular compartments and their stability in extracellular environment[79] make them attractive candidate biomarkers to better understand the etiology of complex disease like FM (Table 3).

They can be packaged with argonaute proteins or be transposed into biological fluids through exosomes. A fundamental role of miRNAs was observed in chronic pain conditions,[80] in which they alter and modulate the expression of signaling molecules, transmitters, ion channels, or structural proteins, contributing to develop

Table 3. MiRNAs differentially expressed in FM women compared with healthy controls.

miRNAs	Regulation in FM	Biological sample	Clinical symptoms
miR-145-5p[82]	Down	CSF	Pain and fatigue
miR-21-5p[82]	Down	CSF	Alteration of central circuits
miR-195-5p[82]	Down	CSF	Alteration in energy metabolism and growth Dementia
miR-223-3p[82]	Down	CSF	Inflammatory pain
miR-23a-3p[82]	Down	CSF	No correlation found
miR-23b[82]	Down	CSF	Alteration of μ-opioid receptor expression Alteration of outcome to long-term morphine treatment
miR-320a[82]	Up	Serum	Pain threshold
miR- 107 *miR-151a-5p* *miR-142-3p*[86]	Down	Serum	No correlation found
miR-30b-5p[86]	Down	Serum	Sleep quantity
miR-374b-5p[86]	Down	Serum	Pain threshold
miR-103a-3p *let-7a-5p*[86]	Down	Serum	Sleep quantity Pain
miR-451a *miR-338-3p* *miR-143-3p* ***miR-145-5p*** ***miR-223-3p***[89]	Down	PBMCs	No correlation found
miR-23a-3p[90]	Down	Serum	Maintenance of skeletal muscle integrity
miR-1 *miR-133a* *miR-346* *miR-139-5p* *miR-320b*[90]	Down	Serum Saliva	No correlation found

Some miRNAs (highlighted) are equally deregulated across different tissue like ***miR223-3p*** and ***miRNA-145-5p*** that have been found to be inhibited in both PBMCs and CSF of FM patients, and ***miR-23a-3p*** that has been found downregulated in both serum and CSF. CSF: cerebro spinal fluid; FM: fibromyalgia; PBMC: peripheral blood mononuclear cells.

long-term hyperexcitability in nociceptive neurons in the periphery and CNS.[81] A microRNAs genome-wide expression profile in FM women CSF, collected at rest by lumbar puncture thought the L3/l4 interspace, was assessed by Bjersing et al.;[82] the relation with peculiar FM symptoms including pain threshold,[83] levels of pain,[84] and fatigue[85] was also explored. The study was conducted on 10 women with FM compared to 8 age-matched healthy controls. Nine out of 742 human miRNAs total assayed were significantly differently expressed in CSF between FM and healthy controls;[82] the interaction with pain and fatigue was subsequently examined, and only *miR-145-5p* showed a significant correlation in FM patients. The same authors analyzed also circulating miRNAs in the serum of 20 FM patients[86] matched with healthy controls, identifying a different pattern from CSF micro-RNAs in FM.[82] Eight out of 374 total human miRNAs analyzed were differentially expressed: *miR-320a* expression was higher in FM patients than healthy controls, while the expression of the remaining seven microRNAs (*miR-103a-3p*, *miR-107*, *let-7a-5p*, *miR-30b-5p*, *miR-151a-5p*, *miR-142-3p*, and *miR-374b-5p*) was lower in FM cases compared to healthy subjects. Concerning the interaction with FM symptoms, *miR-30b-5p* correlated with sleep quantity in FM patients and *miR-374b-5p* was found inversely correlated with pain threshold; also *let-7a-5p* and *miR-103a-3p* tended to be associated with sleep quantity and pain. Lastly, *miR-320a*, higher expressed in FM, was inversely correlated with pain. These results seem to indicate a specificity of these processes in the periphery compared to the CNS: More researches should investigate this point since the study was conduct on restricted portion of the miRNAs sequenced available.[87,88] MiRNAs regulating genes related to the immune system have been potentially assumed involved in FM onset; to this purpose, miRNAs expression using 1212 probes in peripheral blood mononuclear cells were examined, and decreased expression of specific miRNAs was revealed.[89] In particular, 5 miRNAs, *miR-451a*, *miR-338-3p*, *miR-143-3p*, *miR-145-5p*, and *miR-223-3p*, showed a 6- to 13-fold inhibition in FM patients compared to controls. Even if no correlation with clinical criteria was found, *miR223-3p* and

miRNA-145-5p might be proposed as biomarkers of the disease since they were also found to be inhibited in CSF of FM patients.[82]

More recently, Masotti et al.[90] conducted a study on accurately selected FM patients, excluding drugs' use and thus avoiding variations of miRNA expression arising from analgesics.[91] The expression of six miRNAs has proved to be downregulated (*miR-23a-3p*, *miR-1*, *miR-133a*, *miR-346*, *miR-139-5p*, and *miR-320b*) in FM patients compared to controls and, interestingly, *miR-23a* was downregulated in both CSF[82] and serum of FM patients, although not significantly associated with FM symptoms.[82]

Interestingly, *mir-23a* is implicated in a cluster with *miR27a*|*24-2*, responsible of *MURF1* and *MAFbx* downregulation, two genes encoding ubiquitin ligases specific for muscle atrophy.[92] This evidence suggests a potential involvement of this miRNA in the maintenance of skeletal muscle integrity.[93] In general, miRNAs found dysregulated in FM patients appear to be involved in physical activity, pain, stress, mood disorders, and depressive symptoms; therefore, a good predictive model with high diagnostic power should probably include many of these traits-associated miRNAs. Further studies need to strengthen these preliminary findings in larger cohorts.

Histone modifications

Histone modifications are covalent post-translational modifications of histone proteins' N-terminal tails (H1, H2A, H2B, H3 e H4), in particular methylation, phosphorylation, acetylation, ubiquitylation, and sumoylation.[94] They alter chromatin structure and subsequently affect different biological processes, as DNA repair process,[95] gene transcription and translation,[96] and ageing process.[97] One of the most studied histone modifications in pain is acetylation/deacetylation, the addition or removal of acetyl groups on N-terminal lysine residues and on nucleosome surface. Acetylation mechanism, operated by histone acetyltransferase enzymes, mediates the shift from condensed to relaxed chromatin, more accessible to transcriptions factors; conversely, deacetylation, made by histone deacetylases (*HDACs*), closely condenses chromatin resulting in gene silencing.[98] *HDAC* inhibitors in pain conditions emerged to be potentially implicated in analgesia, in both inflammatory and neuropathic pain.[99,100] Their clinical effect is thought to be partially attributed to the reduced production of inflammatory cytokines such as *TNF-α* and *IL-1*.[101] However, histone modifications in FM patients have not yet been investigated.

Gene expression

Since epigenetic mechanisms modulate gene expression, studies investigated transcription changes comparing FM patients and controls: FM alterations in gene expression should be viewed considering that they might not be exclusively related to FM pathology because of FM concomitant diagnoses, as osteoarthritis, depression, and obesity.[102]

A recent analysis identified 482 differentially expressed genes between patients and healthy controls, shedding light on the relationship between FM status and upregulated inflammatory cytokines' genes (*IL10*, *IL25*, and *1L36A*).[102] *IL-10*, one of the most powerful anti-inflammatory cytokines,[103] has been shown to regulate substance P expression, thus probably increasing the pain threshold. *IL-25*[104] was found to upregulate the expression of pro-inflammatory cytokines, especially Th2 cytokines. Both these cytokines have been proposed as key mediators of Th2 cytokine response, linked to chronic fatigue syndrome. In addition, several solute carrier molecules' genes were found upregulated in FM subjects including *SLC1A5* and *SLC25A22*, which encode for glutamate transporters in the CNS.[105] The metabotropic glutamate receptor gene (*GRM6*), encoding for a group III G protein-coupled receptor linked to the inhibition of the cyclic AMP cascade and involved in neuropathic pain signaling in dorsal horn neurons, was also upregulated in FM subjects.[106]

A dysregulation of these pathways[105,106] may be relevant to the pathogenesis of FM and thus need to be validated in a large, multicenter, independent cohort of subjects with greater clinical heterogeneity. In addition, no studies investigated if epigenetic mechanisms reflect the observed changes in gene expression.

Conclusions

FM is a complex disorder characterized by chronic pain, joint stiffness, fatigue, sleep disturbance, cognitive dysfunction, and depression. Research on FM is becoming increasingly important because of patients impaired quality of life and for the economic burden placed on the medical care system. FM patients often show concomitant diagnoses, such as osteoarthritis, depression, and obesity, with the consequently high risk of misdiagnosis. Most of the studies have been thus focused on research of specific and measurable biomarkers to objectively identifying susceptible individuals, to confirm disease diagnosis, and to facilitate treatment.

To achieve these goals, many familial studies were conducted demonstrating an increased risk to develop FM in first-degree relatives; candidate gene studies highlighted potential mechanisms involved in FM pathogenesis, identifying associated SNPs to the disease:

central sensitization to pain and HPA axis impairment. Beside a genetic predisposition, environmental factors, like infant trauma, stress, and depression, play a fundamental role in the onset and development of FM, through epigenetic modulations. In particular, a hypomethylation state is shown in FM patients compare to healthy controls, especially in promoter of genes implicate in DNA repair, immune system, and membrane transport genes. Many studies investigated miRNAs expression in FM condition in a variety of biological samples, highlighting the involvement of both peripheral and central processes.

It should be noted that many of the epigenetics studies have been performed on blood samples. Despite DNA methylation patterns are tissue specific[107] and their study in chronic pain should be thus limited to the brain. Recently, a correspondence across different tissues emerged: Massart et al.[108] have found that 72% of the genes affected in T cells were also differentially methylated in prefrontal cortex post-nerve injury; other studies have identified a correspondence between 35% and 80% of known transcripts in both peripheral blood and brain tissues.[109] The observed correspondences identify blood samples as a reliable and more accessible source of FM biomarkers. This paper reviewed relevant FM studies in order to better understand the still unclear mechanisms underlying this complex disease. However, some of the results should be considered with caution in light of the following limitations, representing also important directions for future researches. Despite the relatively high prevalence of FM, many studies included small size sample and, because of FM comorbidities, enrolled patients with no precise exclusion criteria and attention for ongoing therapies. In addition, to clarify the temporal onset between FM and its additional symptoms, the use of longitudinal follow-ups could be considered. Details on patients' history could improve everyday clinical practice: Dietary and lifestyle that may lead to alteration in gastrointestinal microbiome, observed in this complex disease, should be also deepen.

If the obtained preliminary data will be confirmed, research in future could focus on the identification of more selective analgesics or new pharmacological approaches, as currently being testing against pain of diverse etiologies, including Postherpetic Neuralgia,[110] Inherited Erythromelalgia,[111] and Lumbosacral Radiculopathy,[112] with three phase II clinical trials on selective blockers drugs for sodium channels Nav1.7. In particular, since epigenetics plays a major role in regulating expression of pro- or antinociceptive genes, epigenetic drugs might potentially reverse aberrant gene expression profiles associated with FM states. First, preclinical data suggest chromatin-modifying drugs relevance for treating pain in particular in the context of inflammation[113]: 5-azacytidine administration in rats following tissue damage resulted in the inhibition of global DNA methylation increment and MeCP2 expression with subsequent decrease of painful behavior.[114]

Drugs targeting epigenetic mediators as histone deacetylase and acetylase or involving DNA methylation maintenance have been developed for different pathological conditions.[115] Similarly, improving FM biomarkers research, a new treatment scenario based on personalized medicine may be revealed, with major benefits and less side effects for FM patients and reduced cost for the national health-care systems.

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