Review

Exploring the abyss of fibromyalgia biomarkers

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ABSTRACT

Many researchers are studying fibromyalgia to identify objective, measurable biomarkers that may identify the patients, for the purpose of diagnosis or to measure the disease activity. The recent literature proposes instrumental or molecular biomarkers, but several of these are only useful for research purposes. Concentrated efforts to systematically evaluate additional objective measures in research trials will be vital for the ongoing progress in outcome research and translation into clinical practice. The aim of this review is a guided tour of the specific literature.

Fibromyalgia (FM) is a chronic condition characterised by widespread pain, tenderness and cognitive imbalance. Many patients have reported several symptoms, such as non restorative sleep and mood disorders. This disease has an incidence of 2% in the general population, but it is found more often in middle-aged women (3-4%) The etiopathogenesis of fibromyalgia has not been clarified yet. FM patients have a dysregulation of pain, which is mediated by neurotransmitters and by neurohormones, and is associated with irregularities in the physiology of sleep. The absence of anatomic-pathological lesions and of biohumoral abnormalities, demonstrated with classical instrumental methods, has led to considerable difficulties in diagnosis. Patients affected by primitive fibromyalgia do not have organ engagement or humoral alteration. Even though FM is not associated with evident organic damage, it is a highly debilitating disease. The diagnosis is a clinical one in accordance with the American College of Rheumatology (ACR) 1990 criteria (1). These are clinical parameters that lack specificity and do not allow one to evaluate the activity or the severity of the disease. These diagnostic criteria frequently overlap with those of other diseases. In fact, different patients with chronic fatigue syndrome (CFS) meet the criteria for FM, and the FM-like framework may be present even in non-rheumatic diseases. For example, patients affected by hypothyroidism have shown musculo-skeletal pain similar to that of FM. The diagnosis based on the ACR criteria must therefore be accompanied by the exclusion of diseases that have symptoms, but not the causes, common with FM, by the evaluation of markers and humoral scans (myositis, rheumatic polmyalgia, spondiloarthritis, etc.). Very often FM can be concurrent with other diseases as a confounding and aggravating factor (Sjögren, LES, AR, thyroid disease). Many authors report on the efforts to search for a biomarker in FM. This is necessary both for the diagnosis of the disease and to measure disease activity, which give an endpoint in clinical trials.

Genetics

Many articles support a genetic predisposition to FM. Polymorphisms in catechol-O-methyltransferase (COMT), of substance P receptors, dopamine transporters and alpha1-antitrypsin and serotonin transporters, are found in patients with FM. Notably, these polymorphisms all effect the metabolism or transport of monoamine, compounds that have a critical role in both the sensory processing (also nociception) and the human stress response. However, several polymorphisms associated with FM are also related with psychiatric comorbidities, suggesting that the above findings may be bound more closely with psychiatric conditions than inherent features of FM. The genetic alterations found in FM are shown in Table I.

Functional neural imaging

Functional imaging was used to study the response to pain in fibromyalgia pa-

tients. Functional magnetic resonance imagery (fMRI), which tracks local changes in blood flow, has a higher spatial and temporal resolution than other techniques such as positron emission tomography (PET) or single-photon emission tomography (SPECT). fMRI studies in fibromyalgia patients suggested that similar levels of subjective pain result in similar central nervous system (CNS) activation in both fibromyalgia patients and controls. However, for a similar stimulus, fibromyalgia patients have a greater subjective sensation of pain. This increased sensitivity is accompanied by a decreased activity in brain regions implicated in the descending pain inhibitory pathways. Table II shows the most representative studies of neuroimaging in FM. The limit of these interesting studies is the number of patients. However, the results obtained from the FM patients underlined the presence of several alterations in the brain region. These alterations are involved in the nociception and cognitive impairment of FM patients.

Table I. Genetics in fibromyalgia.

Reference	Year	n.	Findings
Cohen H *et al*.	2009	2	The authors observed a reduced frequency of COMT val(158) met polymorphism.
Tander B *et al*.	2008	3	The authors studied the polymorphisms of the serotonin-2A receptor and the catechol-O methyltransferase genes, and they suggested that results indicate that the investigated polymorphisms seem not to be the susceptibility factors in etiology of FMS.
Treister *et al*.	2009	4	The authors found an association between the dopamine transporter gene (DAT-1) polymorphism and cold pain tolerance. The results underlined the association between the DAT-1 polymorphism and a decrease in pain threshold.
Buskila D *et al*.	*2004*	*5*	The authors found a relationship between the dopamine D4 receptor exon III and fibromyalgia.
Ablin JN *et al*.	2009	6	No significant difference in substance P receptor (TACR1) 1354 G>C polymorphism in wide ethnicity FM patients.
Yunus *et al*.	1999	7	Linkage between HLA and FM
Potvin *et al*.	2010	8	No relationship between the polymorphism of the serotonin transporter promoter and pain perception in fibromyalgia patients and healthy controls.
Carvalho LS *et al*.	2008	9	The authors found several alterations in the promoter region of the serotonin transporter (5-HTTLPR).
Gürsoy S *et al*.	2008	10	The authors studied the polymorphism of MAO-A and MAO-B genes. They suggested a possible relation between the high-activated MAO-A, allele 3, in the occurrence of FS.

Table II. Neuroimaging and FM.

Reference	Year	n.	Findings
Fayed N *et al*.	2010	11	The authors found a higher level of glutamate + glutamine in the posterior gyrus of FM patients. They suggest a possible pathologic role of these aminoacids. Moreover, they found alterations in the Hippocampal region.
García-Campayo J *et al*.	2009	12	The authors found an alteration of the Hippocampal region, and they suggest that FM may be characterised as an aging process
Pujol J *et al*.	2009	13	FM showed significantly larger activation in the anterior insula-basal ganglia complex and the cingulate cortex after pain stimuli.
Burgmer M *et al*.	2009	14	Differences of activation in the fronto-cingulate cortex, the supplemental motor areas, and the thalamus were found between both groups with distinct differences in BOLD-signals changes over the time course of pain stimulation, even during the anticipation of pain.
Chen JJ *et al*.	2007	15	The authors used a single photon emission computed tomography (SPECT) to detect abnormal regional cerebral blood flow (rCBF) in FM patients. The authors found a reduced rCBF at the cortical regions, and in other areas such as the thalamus and the subcortical nucleus.

Autoantibodies

The search for specific autoantibodies is desirable for FM. Numerous studies were conducted to assess the presence of specific markers. Compared with healthy subjects or with patients affected by other diseases, FM patients present high anti-polymer antibodies (APA) and anti-serotonin antibody values, with contrasting results in literature. Other antibodies (anti-serotonin, antiganglioside and anti-phospholipids) were identified in FM patients compared to healthy subjects, but the sensitivity and specificity is not clear. Patients with fibromyalgia have a higher frequency of anti-thyroid antibodies, and their values seem to be correlated with the presence of certain symptoms. Moreover, FM is common in patients with autoimmune diseases and may be the source of many of the symptoms and much of the disability in these patients. In conclusion, the most interesting observations are linked to thyroid autoimmunity. Table III shows the studies on the presence of autoantibodies in FM patients.

Stress-response systems and sex hormones

The linkage between FM and stress response systems is supported by the studies underlining alteration of the hypothalamic-pituitary-adrenal axis and autonomic nervous system in FM. Several studies investigated the concentration of cortisol in FM patients, but none of these studies provide a link between FM and involvement of HPA-axis.

FM syndrome is more prevalent in women than in men, suggesting a role of sex hormones in the pathophysiology of FM. The reason for a female predominance in FM is complex and it requires further investigation.

Serologic and biochemical abnormalities

Some studies showed higher serum acid hyaluronic values in FM patients

Table III. presence of autoantibodies in FM patients.

Reference	Year	n.	Findings
Wilson RB *et al*.	2001	16	The authors found a higher prevalence of APA (67%) in FM patients in the USA population.
Bazzichi L *et al*.	2007	17	The authors found a lower percentage of APA seropositivity (23%), but they found a correlation between APA levels and FIQ score.
Werle A *et al*.	2001	18	The authors did not find a correlation between anti serotonin antibodies and prevalence of FM.
Pamuk ON *et al*.	2007	19	The authors did not find a difference in the percentage of thyroid autoimmunity in FM patients respect to RA patients, but a higher percentage than in controls.
Bazzichi L *et al*.	2007	20	The authors found a higher percentage of thyroid autoimmunity in FM patients. Moreover, they suggest an association between thyroid autoimmunity and some typical functional symptoms.
Bazzichi L and colleagues	2010	21	The authors suggest a possible role in thyroid autoimmunity and the development of FM.
Klein R *et al*.	1992	22	The authors found higher levels of Antiserotonin, antiganglioside and antiphospholipids autoantibodies in FM patients.
Klein R and Berg PA	1995	23	The authors found higher levels of Antiserotonin, antiganglioside and antiphospholipids autoantibodies in FM patients and patients with chronic fatigue syndrome.

Table IV. HPA-axis and sex hormones in FM.

Reference	Year	n.	Findings
Gur A *et al*.	2004	24	The authors found a reduction of plasmatic cortisol level in young patients with FM (<35 years).
Gur A *et al*.	2004	25	The authors observed a reduction of plasmatic cortisol level in FM patients with high BDI score (>17).
Malt *et al*.	2002	26	The authors found a normal value of plasmatic cortisol levels in a small cohort of patients. However, FM patients showed a reduction in reactivity in the central sympathetic system and perturbations in the sympathetic-parasympathetic balance.
Mc Lean SA *et al*.	2005	27	The author found normal values of the salivary cortisol level. Among women with FM, a strong relationship between cortisol levels and current pain symptoms were observed at the waking time point and 1 hour after waking. They suggest that the pain symptoms early in the day are associated with variations in function of the hypothalamic-pituitary-adrenal axis.
Crofford LJ and colleagues	1994	28	The authors found a reduction in a 24-hour urinary cortisol level in a small cohort of patients.
Samborski W *et al*.	2005	29	The results shown by the authors underlined that the sex hormone deficit does not appear to be part of the manifestations of FM.
Akkuş S *et al*.	2000	30	No differences of serum levels of oestradiol, FSH and LH in patients with FM and controls were found.

compared with healthy controls, but this data has not been confirmed (31, 32). Alterations of branched-chain amino acids (valine, leucine, isoleucine) and phenylalanine (33), collagen cross links and particularly a reduction in the ratio piridinolina/deoxypyridinoline, and decreased levels of hydroxipirolina (34) were also found. In a recent work we found an alteration of several aminoacids homeostasis in FM patients (35). In different studies low serum levels of 5-HT in FM patients were also found, compared with both healthy controls and with patients with rheumatic diseases as rheumatoid arthritis. These studies provided indirect evidence about the alterations of 5-HT metabolism in FM subjects. Instead, in a recent study of FM, patients exhibited a tendency to have lower serotonin levels than in patients with rheumatoid arthritis and healthy controls, but the variation of serotonin levels within the disease groups is too broad to differentiate FM from other conditions, especially depression (36). There is also evidence that suggests that FM patients may have alterations in the expression of the 5-HT transporters, due to a transcriptional polymorphism in the region that could lead to an increase of the same transcriptional region (37). An alteration of the growth hormone (GH) has also been suggested. The GH is an indirect modulator of the immune system that interacts with the hormonal system and which seems to protect the body from the immunosuppressive effects of glucocorticoids during a stress (38) and favours muscle repair IGF1-mediated. Altered serum cortisol and melatonin levels were found. This hormone secretion is closely associated with circadian rhythms. Alterations of 5-HT, somatomedin C, peptide-related to the calcitonin gene, calcitonin and cholecystokinin, and possible indicators of FM widespread pain, were also found (39). Studies conducted on FM patients showed an increase in plasma levels of IL-6, IL-8 compared with healthy controls, an increased production of IL-1 and TNF-alpha, and a reduced production of IL-2 and IFN-alpha, which together highlights an immune activation and a down-regulation of the HPA. In an our study (40), we showed an increase in plasma IL-10, IL-8 and TNF-alpha, in FM, independent of the presence of psychiatric comorbidity. This work supports the hypothesis of an activation of the immune system (41). Abnormal levels of ACTH, 5-HT, IGF-1 and FT4 were found, so there would seem to be an alteration of the endocrine system in this disease. The presence of free radicals in FM is controversial, but could also be indicative of an oxidative disorder. Furthermore, studies have shown high levels of malondialdeide, which are markers of oxidative damage, and low levels of superoxide dismutase, an intracellular antioxidant (42) in FM.

Neuropeptides

Several studies reported the alteration of various neuropeptides in FM patients.

Unfortunately, some of these studies were conducted on cerebrospinal fluid and are therefore difficult to repeat. Table V shows the most interesting papers about FM and neuropeptides.

Proteins

Recently, our group has published a study that investigated the alteration of salivary proteins in FM patients (48). The most relevant observation which emerged from the data analysis was the exclusive and significant over-expression of transaldolase and phosphoglycerate mutase I. These findings were validated by Western blot analysis, and the total optical density confirmed the significant up regulation of transaldolase and phosphoglycerate mutase I in FM samples with respect to healthy subjects. It was noteworthy that seven further salivary proteins resulted differentially expressed, namely: calgranulin A, calgranulin C, cyclophilin A, profilin 1, Rho GDP-dissociation inhibitor 2, proteasome subunit-a-type-2 and haptoglobin-related protein precursor. These preliminary results demonstrated the utility of salivary proteomic analysis in the identification of salivary biomarkers in FM patients and in clarifying some of the pathogenetic aspects of the disease.

Muscle abnormalities

Despite the major symptoms of FM involved the muscle, there are few papers about muscle abnormalities and FM. However, the abnormalities found in FM patients indicated an alteration of metabolism and some structural abnormalities. Table VI shown some articles on muscle abnormalities and FM.

Table V. neuropeptides and FM.

Reference	Year	n.	Findings
Di Franco M *et al*.	2009	43	FM patients showed higher serum levels of Neuropeptide Y. These results suggested that autonomic dysfunctions and NPY are crucial elements in the pathophysiology of FM.
McLean SA *et al*.	2006	44	In this study the authors showed higher levels of the corticotrophin-releasing factor, a mediator of stress response, in cerebrospinal fluid of FM patient. Moreover, this peptide is well correlated with pain.
Vaerøy H *et al*.	1988	45	The authors found higher levels of substance P in the cerebrospinal fluid of FM patients (especially in patients with Raynaud phenomena).
Russell IJ *et al*.	1994	46	Also, in this study, the authors found higher levels of substance P in the cerebrospinal fluid of FM patients.
Haas L. *et al*.	2010	47	The authors found increased levels plasma of Brain Derived Neurothropic Factor in FM patients.

Table VI. muscle abnormalities and FM.

Reference	Year	n.	Findings
Sprott *et al*.	2004	49	The authors found a disorganisation of myofibres; glycogen and lipid accumulation were also found. The number of mitochondria was lower in patients with FM than in controls and seemed to be morphologically altered, associated with nuclei DNA fragmentation.
Pongratz DE *et al*.	1998	50	The authors showed an increase in lipid droplets and a slight proliferation of mitochondria in type I muscle fibers. In some cases we could find some ragged red fibers (RRF) which histochemically show a pronounced accumulation of lipids and mitochondria and single fiber defects of cytochrome-c-oxidase.
Bazzichi L *et al*.	2009	51	The authors investigate the Surface Electromyography (SEMG) parameters in FM patients. They found some interesting muscle modifications in FM patients with respect to healthy controls. These results suggest that the patients might have a different fiber recruitment and muscle relaxation.
Gerdle B *et al*.	2010	52	The authors found higher interstitial concentrations of pyruvate and lactate in trapezius muscle of FM patients. These results indicate an alteration of metabolism in FM, especially hypo-oxygenation.

Conclusion

In spite of the alterations that we have found in these studies, the diagnosis of fibromyalgia is only clinical. The old and the new preliminary diagnostic and classifying criteria may not discriminate between other syndromes with similar symptoms to those of fibromyalgia, and they do not evaluate the functional symptoms associated with the disease.

At present, there are no specific markers of FM, and many of them are used only for studies. However, the presence of these factors helps to understand the pathogenetic mechanisms and to identify patient subgroups. Some of these factors could be used as indices of disease severity. The current literature is very lively in identifying and suggesting serological alterations or instrumental investigations as new markers specific to fibromyalgia. However, it is necessary to identify precise biomarkers of the disease according to feasibility and reproducibility criteria, for diagnostic and therapeutic purposes.

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References

1. WOLFE F, SMYTHE HA, YUNUS MD: The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. *Arthritis Rheum* 1990; 33: 160-72.
2. COHEN H, NEUMANN L, GLAZER Y, EBSTEIN RP, BUSKILA D: The relationship between a common catechol-O-methyltransferase (COMT) polymorphism val(158) met and fibromyalgia. *Clin Exp Rheumatol* 2009; 27 (Suppl. 56): S51-6.
3. TANDER B, GUNES S, BOKE O *et al*.: Polymorphisms of the serotonin-2A receptor and catechol-O-methyltransferase genes: a study on fibromyalgia susceptibility. *Rheumatol Int* 2008; 28: 685-91.
4. TREISTER R, PUD D, EBSTEIN RP *et al*.: Associations between polymorphisms in dopamine neurotransmitter pathway genes and pain response in healthy humans. *Pain* 2009; 147: 187-93.
5. BUSKILA D, COHEN H, NEUMANN L, EBSTEIN RP: An association between fibromyalgia and the dopamine D4 receptor exon

III repeat polymorphism and relationship to novelty seeking personality traits. *Mol Psychiatry* 2004; 9: 730-1.
6. ABLIN JN, BAR-SHIRA A, YARON M, ORR-URTREGER A: Candidate-gene approach in fibromyalgia syndrome: association analysis of the genes encoding substance P receptor, dopamine transporter and alpha1-antitrypsin. *Clin Exp Rheumatol* 2009; 27 (Suppl. 56): S33-8.
7. YUNUS MB, KHAN MA, RAWLINGS KK, GREEN JR, OLSON JM, SHAH S: Genetic linkage analysis of multicase families with fibromyalgia syndrome. *J Rheumatol* 1999; 26: 408-12.
8. POTVIN S, LAROUCHE A, NORMAND E *et al*.: No relationship between the polymorphism of the serotonin transporter promoter and pain perception in fibromyalgia patients and healthy controls. *Eur J Pain* 2010; 14: 742-6.
9. CARVALHO LS, CORREA H, SILVA GC *et al*.: May genetic factors in fibromyalgia help to identify patients with differentially altered frequencies of immune cells?. *Clin Exp Immunol* 2008; 154: 346-52.
10. GÜRSOY S, ERDAL E, SEZGIN M *et al*.: Which genotype of MAO gene that the patients have are likely to be most susceptible to the symptoms of fibromyalgia? *Rheumatol Int* 2008; 28: 307-11.
11. FAYED N, GARCIA-CAMPAYO J, MAGALLÓN R *et al*.: Localized 1H-NMR spectroscopy in patients with fibromyalgia: a controlled study of changes in cerebral glutamate/glutamine, inositol, choline, and N-acetylaspartate. *Arthritis Res Ther* 2010; 12: R134.
12. GARCÍA-CAMPAYO J, FAYED N, SERRANO-BLANCO A, ROCA M: Brain dysfunction behind functional symptoms: neuroimaging and somatoform, conversive, and dissociative disorders. *Curr Opin Psychiatry* 2009; 22: 224-31.
13. PUJOL J, LÓPEZ-SOLÀ M, ORTIZ H *et al*.: Mapping brain response to pain in fibromyalgia patients using temporal analysis of FMRI. *PLoS One* 2009; 4: e5224.
14. BURGMER M, POGATZKI-ZAHN E, GAUBITZ M, WESSOLECK E, HEUFT G, PFLEIDERER B: Altered brain activity during pain processing in fibromyalgia. *Neuroimage* 2009; 44: 502-8.
15. CHEN JJ, WANG JY, CHANG YM *et al*.: Regional cerebral blood flow between primary and concomitant fibromyalgia patients: a possible way to differentiate concomitant fibromyalgia from the primary disease. *Scand J Rheumatol* 2007; 36: 226-32.
16. WILSON RB, GLUCK OS, TESSER JR, RICE JC, MEYER A, BRIDGES AJ: Antipolymer antibody reactivity in a subset of patients with fibromyalgia correlates with severity. *J Rheumatol* 1999; 26: 402-7.
17. BAZZICHI L, GIACOMELLI C, DE FEO F *et al*.: Antipolymer antibody in Italian fibromyalgic patients. *Arthritis Res Ther* 2007; 9: R86.
18. WERLE A, FISCHER HP, MULLER A, FIEHN W, EICH W: Antibodies against serotonin have no diagnostic relevance in patients with fibromyalgia syndrome. *J Rheumatol* 2001; 28: 595-600.
19. PAMUK ON, CAKIR N: The frequency of thyroid antibodies in fibromyalgia patients and their relationship with symptoms. *Clin Rheumatol* 2007; 26: 55-9.
20. BAZZICHI L, ROSSI A, GIULIANO T *et al*.: Association between thyroid autoimmunity and fibromyalgic disease severity. *Clin Rheumatol* 2007; 26: 2115-20.
21. BAZZICHI L, ROSSI A, ZIRAFA C *et al*.: Thyroid autoimmunity may represent a predisposition for the development of fibromyalgia? *Rheumatol Int* 2010 Nov 18.
22. KLEIN R, BÄNSCH M, BERG PA: Clinical relevance of antibodies against serotonin and gangliosides in patients with primary fibromyalgia syndrome. *Psychoneuroendocrinology* 1992; 17: 593-8.
23. KLEIN R, BERG PA: High incidence of antibodies to 5-hydroxytryptamine, gangliosides and phospholipids in patients with chronic fatigue and fibromyalgia syndrome and their relatives: evidence for a clinical entity of both disorders. *Eur J Med Res* 1995; 1: 21-6.
24. GUR A, CEVIK R, SARAC AJ, COLPAN L, EM S: Hypothalamic-pituitary-gonadal axis and cortisol in young women with primary fibromyalgia: the potential roles of depression, fatigue, and sleep disturbance in the occurrence of hypocortisolism. *Ann Rheum Dis* 2004; 63: 1504-6.
25. GUR A, CEVIK R, NAS K, COLPAN L, SARAC S: Cortisol and hypothalamic-pituitary-gonadal axis hormones in follicular-phase women with fibromyalgia and chronic fatigue syndrome and effect of depressive symptoms on these hormones. *Arthritis Res Ther* 2004; 6: R232-8.
26. MALT EA, OLAFSSON S, LUND A, URSIN H: Factors explaining variance in perceived pain in women with fibromyalgia. *BMC Musculoskelet Disord* 2002; 3: 12.
27. MCLEAN SA, WILLIAMS DA, HARRIS RE *et al*.: Momentary relationship between cortisol secretion and symptoms in patients with fibromyalgia. *Arthritis Rheum* 2005; 52: 3660-9.
28. CROFFORD LJ, PILLEMER SR, KALOGERAS KT *et al*.: Hypothalamic-pituitary-adrenal axis perturbations in patients with fibromyalgia. *Arthritis Rheum* 1994; 37: 1583-92.
29. SAMBORSKI W, SOBIESKA M, PIETA P, DREWS K, BRZOSKO M: Normal profile of sex hormones in women with primary fibromyalgia. *Ann Acad Med Stetin* 2005; 51: 23-6.
30. AKKUŞ S, DELIBAŞ N, TAMER MN: Do sex hormones play a role in fibromyalgia? *Rheumatology* (Oxford). 2000; 39: 1161-3.
31. WERLE AND JAKEL HP, MULLER A, FISCHER HP, FIEHN W: Eich Serum Hyaluronic acid levels are elevated in arthritis patients, but normal and not associated with clinical data in patients with fibromyalgia syndrome. *Lab Clin* 2005; 51: 11-9.
32. WIGLEY RD, B, EM CHAMBERS: Hyaluronic acid serum levels in fibromyalgia, non-specific arm disorder, and controls. *J Rheumatol* 2001; 28: 2563.
33. MAES M, VERKERK R, DELMEIRE L, VAN GASTEL A, VAN HUNSEL F, SCHARPE S: Serotonergic markers and lowered plasme branched-chain amino-acid concentrations in fibromyalgia. *Psychiatry Res* 2000; 97: 11-20.
34. SPROTT H, A MULLER, H: Heine Collagen cross-links in fibromyalgia syndrome. *Z Rheumatol* 1998; 57: 52-5.
35. BAZZICHI L, PALEGO L, GIANNACCINI G *et al*.: Altered amino acid homeostasis in subjects affected by fibromyalgia. *Clin Biochem* 2009; 42: 1064-70.
36. JASCHKO G, HEPP U, BERKHOFF M *et al*.: Serum serotonin levels are not useful in diagnosing fibromyalgia. *Ann Rheum Dis* 2007; 66: 1267-68.
37. OFFENBAECHER M, BONDY B, DE JONGE ST *et al*.: Possible association of fibromyalgia with a polymorphism in the serotonin transporter gene regulatory region. *Arthritis Rheum* 1999; 42: 2482-8.
38. DORSHKIND K, HORSEMAN ND: The roles of prolactin, growth hormone, insulin-like growth factor-1, and thyroid hormones in lymphocyte development and function: insights from genetic models of hormone and hormone receptor deficiency. *Endocr Rev* 2000; 21: 292-312.
39. HOCHEREL K, FARBER L, LADENBURGER S *et al*.: Effect of tropisetron on circulating catecholamines and other putative biochemical markers in serum of patients with fibromyalgia. *Scand J Rheumatol* 2000; 113: 46-8.
40. BAZZICHI L, ROSSI A, MASSIMETTI G *et al*.: Cytokine patterns in fibromyalgia and their correlation with clinical manifestations. *Clin Exp Rheumatol* 2007; 25: 225-30.
41. TOGO F, NATELSON BH, ADLER GK *et al*.: Plasma cytokine fluctuations over time in healthy controls and patients with fibromyalgia. *Exp Biol Med* (Maywood). 2009; 234: 232-40.
42. BAGIS S, TAMER L, SAHIN G, BILGIN R H GULER, ERCAN B, ERDOGAN C: Free radicals and antioxidants in primary fibromyalgia: an oxidative stress disorder? *Rheumatol Int* 2005; 25: 188-90.
43. DI FRANCO M, IANNUCCELLI C, ALESSANDRI C *et al*.: Autonomic dysfunction and neuropeptide Y in fibromyalgia. *Clin Exp Rheumatol* 2009; 27 (Suppl. 56):S75-8.
44. MCLEAN SA, WILLIAMS DA, STEIN PK *et al*.: Cerebrospinal fluid corticotropin-releasing factor concentration is associated with pain but not fatigue symptoms in patients with fibromyalgia. *Neuropsychopharmacology*. 2006; 31: 2776-82.
45. VAERØY H, HELLE R, FØRRE O, KÅSS E, TERENIUS L: Elevated CSF levels of substance P and high incidence of Raynaud phenomenon in patients with fibromyalgia: new features for diagnosis. *Pain* 1988; 32: 21-6.
46. RUSSELL IJ, ORR MD, LITTMAN B *et al*.: Elevated cerebrospinal fluid levels of substance P in patients with the fibromyalgia syndrome. *Arthritis Rheum* 1994; 37: 1593-601.
47. HAAS L, PORTELA LV, BÖHMER AE, OSES JP, LARA DR: Increased plasma levels of brain derived neurotrophic factor (BDNF) in patients with fibromyalgia. *Neurochem Res* 2010; 35: 830-4.
48. BAZZICHI L, CIREGIA F, GIUSTI L *et al*.: Detection of potential markers of primary fibromyalgia syndrome in human saliva. *Pro-*

teomics Clin Appl 2009; 3: 1296-304.
49. SPROTT H, SALEMI S, GAY RE *et al*.: Increased DNA fragmentation and ultrastructural changes in fibromyalgic muscle fibres. *Ann Rheum Dis* 2004; 63: 245-51.
50. PONGRATZ DE, SPÄTH M: Morphologic aspects of fibromyalgia *Z Rheumatol* 1998; 57 (Suppl. 2): 47-51.
51. BAZZICHI L, DINI M, ROSSI A *et al*.: Modifications in fibromyalgic patients revealed by surface electromyography (SEMG) analysis. *BMC Musculoskelet Disord* 2009; 10: 36.
52. GERDLE B, SÖDERBERG K, SALVADOR PUIGVERT L, ROSENDAL L, LARSSON B: Increased interstitial concentrations of pyruvate and lactate in the trapezius muscle of patients with fibromyalgia: a microdialysis study. *J Rehabil Med* 2010; 42: 679-87.