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Diagnosis of Fibromyalgia Syndrome: Potential Biomarkers and Proteomic Approach

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1. Introduction

In 1990 the American College of Rheumatology (ACR) established criteria for the diagnosis of fibromyalgia (Wolfe et al., 1990), and more recently new criteria have been proposed (Wolfe et al., 2010, 2011). However, the absence of anatomic pathological lesions and of biohumoral abnormalities, demonstrated with classical instrumental methods, has led to considerable difficulties in diagnosis.

Until now, many attempts have been made to search for biomarkers in fibromyalgia, but at present no specific markers have been found (Bazzichi et al., 2010). The problem lies in the presence of too many data, often controversial, rather than in a lack of data.

We will discuss methods that are state of the art in searching for biomarkers in fibromyalgia. Furthermore, we will focus on the contribution that proteomics can give in the diagnosis of the disease on the basis of the study we carried out on human whole saliva of patients affected by fibromyalgia.

2. Genetic markers

The presence of fibromyalgia in family clusters and many studies support the idea that genetic factors may predispose to fibromyalgia in combination with environmental triggers such as trauma, infections or emotional stress. The principal gene polymorphisms supposed to be a risk factor for fibromyalgia are those implicated in mood disorders but results are often controversial.

These candidate genes include the serotonin transporter (5-HTT), the serotonin 2A (5-HT2A) receptor, catechol-O-methyltransferase (COMT) and the dopamine receptor.

Offenbaecher and colleagues (Offenbaecher et al., 1999) were the first to analyze the genotypes of the serotonin transporter promoter locus (5HTTLPR) in patients with fibromyalgia. They found that the S/S genotype of 5-HTT occurred more frequently in fibromyalgia patients than in healthy controls. This association is interesting considering that 5-HTT is involved in many conditions that are either risk factors for —or frequent concomitants to— fibromyalgia, such as anxiety, Bipolar Disorder, Psychosis, attention deficit hyperactivity disorder, and Major Depressive Disorder (Maletic & Raison, 2009).

Cohen and collaborators subsequently confirmed this association between fibromyalgia and 5-HTTLPR polymorphism (Cohen et al., 2002).

However, these data were not found in all studies. Recently, Lee and collaborators (Lee et al., 2010) conducted a meta-analysis, and from their results no association was found between 5-HTTLPR and fibromyalgia susceptibility. Potvin et al. found no evidence of a relationship between 5-HTTLPR and fibromyalgia-related psychiatric symptoms (Potvin et al., 2010). Thus, their results are inconsistent with previous reports from Offenbaecher and Cohen but in agreement with those of Gürsoy who concluded that neither 5-HTT nor its polymorphism is associated with fibromyalgia (Gürsoy, 2002).

5-HT2A is also a candidate for involvement in fibromyalgia. Bondy et al., investigated the T102C polymorphism of the 5-HT2A receptor gene and found a significantly different genotype distribution in fibromyalgia patients who had a decrease in T/T and an increase in both T/C and C/C genotypes as compared with the control population (Bondy et al., 1999). In contrast, the pain score was significantly higher for patients with the T/T genotype. It was suggested that the T102 allele might be involved in the complex circuits of nociception. Lee and collaborators using meta-analysis, provided evidence of an association of the 5-HT2A receptor with fibromyalgia susceptibility (Lee et al., 2010). On the contrary, Gürsoy, after studying T102C polymorphism in Turkish patients, concluded that it is not associated with the etiology of fibromyalgia (Gürsoy et al., 2001). Similar results were described by Matsuda et al. in the Brazilian population (Matsuda et al., 2010), and by Tander in a population of eighty patients (Tander et al., 2008).

Another area of investigation is the COMT gene. This gene has long been implicated to be involved in the pathogenesis of neuropsychiatric disorders, schizophrenia, bipolar affective disorder, migraine, and Parkinson's disease. In 2003, Zubieta et al. showed a positive relationship between the COMT gene polymorphism and the experience of pain (Zubieta et al., 2003). Their study focused on the val158met polymorphism, a single nucleotide polymorphism in codon 158 of the COMT gene, which substitutes valine for methionine and resulting in reduced enzyme activity. Individual homozygous for the met158 allele of this polymorphism showed a diminished mu-opioid system response to pain, compared with heterozygotes. Opposite effects on pain and negative effects have been found in val158 homozygotes. The conclusion was that COMT val158met polymorphism influences the human experience of pain and may underlie interindividual differences in the adaptation and responses to pain and other stressful stimuli (Zubieta et al., 2003). On the other hand, an extensive study on the association between this polymorphism and chronic widespread pain concluded that COMT pain sensitivity haplotypes do not play a role in disease susceptibility. The authors proposed that the genetics of chronic widespread pain may differ from that of other pain syndromes (Nicholl et al., 2010).

Cohen et al. observed an association between fibromyalgia and this polymorphism. Moreover, they found that unaffected relatives of fibromyalgia patients had a reduced percentage of the COMT met allele (Cohen et al., 2009). They suggested that this reduced frequency acts as a 'protective' allele in this group and prevents the development of clinical fibromyalgia (Cohen et al., 2009).

Gürsoy and collaborators assessed the significance of COMT polymorphism in fibromyalgia. There were three polymorphisms of the COMT gene: LL, LH, and HH. The analysis of COMT polymorphism was performed using a polymerase chain reaction. The LL and LH genotypes together were more highly represented in patients than in controls. In

addition, HH genotypes in patients were significantly lower than in the control group (Gürsoy et al., 2003). It was concluded that COMT polymorphism is of potential pharmacological importance concerning individual differences in the metabolism of catechol drugs. In addition, it may also be involved in the pathogenesis and treatment of fibromyalgia through adrenergic mechanisms as well as in the genetic predisposition to fibromyalgia (Gürsoy et al., 2003). Matsuda et al. confirmed these results in the Brazilian population (Matsuda et al., 2010) showing that the LL genotype was more frequently found among fibromyalgia patients.

On the other hand, Tander and subsequently Lee didn't find any associations between the COMT gene and fibromyalgia (Lee et al., 2010; Tander et al., 2008).

Finally, some studies have established a connection between fibromyalgia and the dopaminergic system.

Dopaminergic neurotransmission was found to be altered in fibromyalgia patients by Malt and colleagues. They suggested an increased sensitivity or density of dopamine D_2 receptors in fibromyalgia patients (Malt et al., 2003). Buskila and collaborators demonstrated an association between fibromyalgia and the 7-repeat allele in exon III of the D_4 receptor gene, specifically that the frequency of this polymorphism was significantly lower in people with fibromyalgia (Buskila et al., 2004). More recently, Treister et al. found an association between the dopamine transporter gene (DAT-1) polymorphism and cold pain tolerance. Their results, together with the known function of the investigated candidate gene polymorphisms, suggest that low dopaminergic activity can be associated with high pain sensitivity (Treister et al., 2009). However, Ablin and co-workers, investigating an association between fibromyalgia and DAT1, found no significant association between fibromyalgia and the genetic marker (Ablin et al., 2009).

All these findings are summarised in Table 1.

Reference	Year	Studied gene and findings in fibromyalgia	
Offenbaecher et al.	1999	**5-HTT**	The S/S genotype of 5-HTT occurred more frequently in fibromyalgia patients than in healthy controls.
Cohen et al.	2002		Association between fibromyalgia and serotonin transporter promoter region (5-HTTLPR) polymorphism.
Gürsoy	2002		no association
Lee et al.	2010		no association
Potvin et al.	2010		no association
Bondy et al.	1999	**5-HT2A**	They investigated the T102C polymorphism finding a significantly different genotype distribution in fibromyalgia patients with a decrease in T/T and an increase in both T/C and C/C genotypes as compared with the control population.

Lee et al.	2010		They found an association of the 5-HT2A receptor with fibromyalgia susceptibility.
Gürsoy et al.	2001		no association
Tander et al.	2008		no association
Matsuda et al.	2010		no association
Gürsoy et al.	2003	**COMT**	Authors assessed the significance of COMT polymorphism in fibromyalgia. The LL and LH genotypes together were more highly represented in patients than controls. In addition, HH genotypes in patients were significantly lower than in the control group.
Zubieta et al.	2003		COMT val158met polymorphism influences the experience of pain.
Cohen et al.	2009		They found an association between fibromyalgia and the val158met polymorphism of COMT gene.
Matsuda et al.	2010		The L/L genotype was more frequent among fibromyalgia patients.
Tander et al.	2008		no association
Lee et al.	2010		no association
Nicholl et al.	2010		No evidence of association between the COMT pain sensitivity haplotypes and chronic widespread pain.
Malt et al.	2003	**D_2 receptor**	The authors suggested an increased sensitivity or density of dopamine D2 receptors in fibromyalgia patients
Buskila et al.	2004	**D_4 receptor**	They have demonstrated an association between fibromyalgia and the 7 repeat allele in exon III of the D4 receptor gene. Specifically, the frequency of this polymorphism was significantly lower in patients with fibromyalgia.
Treister et al.	2009	**DAT1**	A significant association was found between the quantitative measure of cold tolerance and DAT-1 polymorphism.
Ablin et al.	2009		no association

Table 1. Genetics in fibromyalgia.

In conclusion, all these genetic studies support a role for the polymorphism of genes of the serotoninergic, catecholaminergic and dopaminergic systems in the etiopathogenesis of fibromyalgia.
These polymorphism are often associated with psychiatric disorders, thereby they could be related to psychiatric comorbidities rather than to sole fibromyalgia. Furthermore, genetic results are often controversial, and no specific candidate gene has been closely connected with fibromyalgia.

3. Serologic markers

There is a great interest in using a simple blood test to diagnose fibromyalgia. Thus, many attempts have been carried out in order to identify specific serologic markers. These results, as well as those of genetic studies, are often conflicting and, at present, no clinical test has been validated yet.

3.1 Autoantibodies

Autoimmunity has a central role in pathogenesis of many rheumatic diseases while fibromyalgia is generally considered to be a non-autoimmune disease. Nevertheless, a broad spectrum of autoantibodies has been widely investigated in the sera from patients. The studies have often generated controversial data, and the proposed antibodies don't have diagnostic relevance yet.
The association between the antipolymer antibody (APA) and fibromyalgia was evaluated because APAs were found in the sera of women with silicone breast implants presenting fibromyalgia-like symptoms (Wolfe, 1999). In addition, Wilson et al. found a higher prevalence of APAs (67%) in fibromyalgia patients in the USA population (Wilson et al., 1999). Furthermore, a Danish study showed that fibromyalgia patients tended to have slightly higher APA levels than controls when adjusted for symptom severity (Jensen et al., 2004). However, these results were not confirmed in the Italian population by Bazzichi and colleagues (Bazzichi et al., 2007). Instead, they found a lower percentage of APA seropositivity (23%) in fibromyalgia patients. A correlation between the APA and pain and fatigue severity in fibromyalgia was also proposed by Sarzi-Puttini et al. because the APA test did not distinguish this group from the controls, but instead a positive APA test prevalence increased with less severe pain or fatigue (Sarzi-Puttini et al., 2008). A more recent study evaluated the APA concentration in the serum of patients with fibromyalgia, patients with tension-type headache, and healthy controls. APAs were detected in only 17.6% of fibromyalgia patients confirming the lack of diagnostic relevance of the APA (Iannuccelli et al., 2010).
Two other autoantibodies, the anti-68/48 kDa and the anti-45 kDa, have been described as possible markers for certain clinical subsets of primary fibromyalgia and chronic fatigue syndrome, and for secondary fibromyalgia/psychiatric disorders, respectively (Nishikai et al., 2001).
Antiserotonin, antiganglioside and antiphospholipid antibodies have been shown to be higher in fibromyalgia patients as well as in those with chronic fatigue syndrome, supporting the concept that fibromyalgia and chronic fatigue syndrome may belong to the same clinical entity and may manifest themselves as 'psycho-neuro-endocrinological autoimmune diseases' (Klein & Berg, 1995). A different group, evaluating the prevalence

and potential diagnostic relevance of antiserotonin, antithromboplastin and antiganglioside in patients with fibromyalgia, confirmed an elevated prevalence of antibodies against serotonin and thromboplastin, but they concluded that the measurement of these antibodies had no diagnostic relevance (Werle et al., 2001).

Another important research field is thyroid autoimmunity. Bazzichi and collaborators found that autoimmune thyroiditis is present in an elevated percentage of fibromyalgia patients. In particular, 41% of these patients had at least one thyroid antibody, a percentage that was twice the value of the control group's (Bazzichi et al., 2007). More recently, they confirmed this significant association between thyroid autoimmunity and fibromyalgia (Bazzichi et al., 2010). Pamuk & Cakir also found a higher frequency of thyroid autoimmunity in fibromyalgia patients than in the control group, while the frequency was similar between fibromyalgia patients and the rheumatoid arthritis group (Pamuk & Cakir, 2007). Essentially, the association between rheumatic and thyroid disorders have long been known, the most common being the associations with rheumatoid arthritis, Sjögren's syndrome, and systemic lupus erythematosus.

Therefore, at present, the proposed antibodies have not been validated yet as being strong, useful diagnostic biomarkers for fibromyalgia and further studies are necessary to reach a research end point.

3.2 Neuropeptides

Neuropeptide Y is a neurotransmitter released mainly by sympathetic neurons in the autonomic nervous system, and it has a complex role in mediating analgesia and hyperalgesia. Thus, Neuropeptide Y has been shown, to both reduce and cause pain. Crofford and colleagues were the first to measure plasma Neuropeptide Y levels in fibromyalgia patients and they found that it was significantly lower in fibromyalgia patients than in normal subjects (Crofford et al., 1994). These results are inconsistent with subsequent findings from Anderberg who found significantly elevated plasma Neuropeptide Y levels in fibromyalgia patients compared with the healthy subjects (Anderberg et al, 1999). Furthermore, serum Neuropeptide Y levels have been shown to be significantly higher in fibromyalgia subjects, compared with healthy controls, by two different groups (Di Franco et al., 2009; Iannuccelli et al., 2010).

Substance P is another neuropeptide extensively studied in fibromyalgia patients. Substance P is released in the cerebral spinal fluid when axons are stimulated. An increase in substance P levels in the cerebral spinal fluid can be related to an increase of pain neurotransmitters in the spinal cord. Two studies from two different groups have demonstrated significantly elevated substance P levels in cerebral spinal fluid of fibromyalgia patients (Russell et al., 1994; Vaerøy et al., 1988) but levels of substance P determined in cerebral spinal fluid of patients with chronic fatigue syndrome were within normal range (Evengard et al., 1998). It was proposed that substance P in mice produces disturbances in sleep (Andersen et al., 2006). Subsequently, it is likely that the high levels of substance P found in fibromyalgia patients may be one of the causes of sleep dysfunction.

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family, which also includes the nerve growth factor. BDNF has a wide range of biological activities and is produced by different immune and structural cells (Iughetti et al., 2011). BDNF seems to have a role in Major Depressive Disorder, schizophrenia, and eating disorders such as bulimia and anorexia nervosa, Parkinson's and Alzheimer's diseases, and epileptic and psychogenic nonepileptic seizures (Iughetti et al., 2011). In addition, BDNF is a mediator of

pain in the peripheral nerve system, and some findings have also suggested an involvement of BDNF in pain syndrome (Laske et al., 2006). On the basis of this evidence, Laske and collaborators were the first to evaluate BDNF serum concentrations in fibromyalgia patients, and they found that levels significantly increased compared with healthy controls (Laske et al., 2006). Two different groups found significantly higher levels of BDNF in plasma and in the cerebral spinal fluid of fibromyalgia patients than in controls, indicating an involvement of BDNF in the pathophysiology of fibromyalgia (Haas et al., 2010; Sarchielli et al., 2007).

The excitatory neurotransmitter glutamate is known to function in pain neuropathways, and it is suspected to play a role in the pathophysiology of fibromyalgia. This hypothesis is supported by different studies. Harris and collaborators found a correlation between changing levels of insular glutamate and changes in pain in patients with fibromyalgia using proton magnetic resonance spectroscopy and functional magnetic resonance imaging (Harris et al., 2008). Subsequently, they extended these findings by investigating the relationship between insular glutamate and combined glutamate/glutamine in individuals with fibromyalgia and pain free controls (Harris et al., 2009). They concluded that enhanced glutamatergic neurotransmission within the posterior insula is a potential pathologic factor in fibromyalgia. In a pilot study, Fayed and colleagues compared three different magnetic resonance imaging examination methods for the diagnosis of fibromyalgia: magnetic resonance spectroscopy, diffusion-weighted imaging, and diffusion-tensor imaging. One of the principal findings was the increase in the combined glutamate+glutamine levels, and glutamate+glutamine/creatine ratio, in the posterior gyrus in patients with fibromyalgia compared with controls (Fayed et al., 2010). Another group used magnetic resonance spectroscopy techniques to study brain metabolites in the amygdala, thalami, and prefrontal cortex of these women. Patients with fibromyalgia showed higher levels of glutamate compounds in the right amygdala than did healthy controls, and pain was related to increased glutamate levels in the left thalamus (Valdés et al., 2010). These findings have implications for future therapies directed against glutamate receptors, but further studies are desirable to confirm whether these findings are observed in other functional pain syndromes.

3.3 Inflammation

Since fibromyalgia is characterised by widespread pain, and the origin of pain is inflammation, many studies have focused on the inflammatory hypothesis for fibromyalgia, although fibromyalgia is generally regarded as a non-inflammatory disease. Special attention has been paid on circulating pro-inflammatory cytokines as possible markers in fibromyalgia patients.

A connection between the pathogenesis of fibromyalgia and cytokines was suspected when in 1988, after being treated with IL-2 or IFN-α, patients with malignant melanoma, renal cell carcinoma, and hairy cell leukaemia showed fibromyalgia-like symptoms such as myalgias, arthralgias, cognitive impairment, and painful tender points (Wallace et al., 1988, as cited in Di Franco et al., 2010).

Therefore, cytokines have been suggested to be involved in the fibromyalgia syndrome, but results often appear to be controversial, especially because increases, reductions, and no significant changes have been reported. Levels of IL-10, an anti-inflammatory cytokine, were studied by four different groups with different findings. Wallace and Amel Kashipaz didn't find alterations in IL-10 levels, while Bazzichi and co-workers found an increase in plasma levels, and Uçeyler found a reduction (Amel Kashipaz et al., 2003; Bazzichi et al., 2007; Uçeyler et al., 2006; Wallace et al., 2001).

IL-8, a pro-inflammatory cytokine, has been consistently demonstrated to be increased in patients with fibromyalgia. Bearing in mind that IL-8 promotes sympathetic pain, it may play an important role in the occurrence of pain in fibromyalgia. If validated in further studies, the IL-8 expression pattern might help in the diagnosis of fibromyalgia and in the appropriate treatment approach.
Table 2 summarises the main findings for pro- and anti- inflammatory cytokines in fibromyalgia patients.

Reference	**n° of subjects**	**Samples**	**Cytokines**	**Findings in patients**
Wallace et al., 2001	56 fibromyalgia 36 controls	serum	IL-1β, IL-2, IL-10, serum IL-2 receptor (sIL-2R), IFN-γ, TNF-α, IL-8, IL-6, IL-1Ra	↑ IL-8, IL-1Ra
Gür et al., 2002	81 fibromyalgia 32 controls	serum	IL-I, sIL-2R, IL-6, IL-8	↑ IL-8, sIL-2R
Amel Kashipaz et al., 2003	22 chronic fatigue syndrome / fibromyalgia 19 controls	cells	TNF-α, IL-1α, IL-6, IL-10	no differences
Uçeyler et al., 2006	40 chronic fatigue syndrome (26 fibromyalgia) 40 controls	mRNA from blood	IL-2, IL-4, IL-8, IL-10, TNF-α, TGF-β1	↓ IL-4, IL-10
Bazzichi et al., 2007	80 fibromyalgia 45 controls	plasma	IL-1, IL-6, IL-8, IL-10, TNF-α,	↑ IL-8, IL-10, TNF-α
Wang et al., 2008	20 fibromyalgia 80 controls	serum	IL-4, IL-6, IL-8, IL-10, TNF-α,	↑ IL-8, TNF-α
Wang et al., 2009	20 fibromyalgia 80 controls	serum	IL-8	six months after therapy, IL-8 was still significantly higher
Kim et al., 2010	27 fibromyalgia 29 controls	serum	IL-1β, IL-6, IL-8	↑ IL-8

Table 2. Cytokines in Fibromyalgia.

4. Proteomic markers

In the last few years, it has become widely recognized that the genome represents only the first layer of complexity. Biological functions rely on a dynamic population of proteins, and the characterisation of the proteins can reveal posttranslational modifications (e.g., phosphorylation, glycosylation, and methylation), and give insight into protein-protein interactions and functions. For these reasons, there is an increasing interest in the field of proteomics wich is the identification of proteins contained in biological samples such as body fluids and tissue extracts.

In 2005 Baraniuk and collaborators analyzed the cerebral spinal fluid of patients with chronic fatigue syndrome by quadrupole-time-of flight mass spectrometry, compared chronic fatigue syndrome with Persian Gulf War Illness and fibromyalgia, and concluded that the three proteomes overlapped (Baraniuk et al., 2005). Recently, we carried out a study on human whole saliva of patients affected by fibromyalgia. The aim was to identify the protein content of whole saliva and to determine the quantitative or qualitative differences between fibromyalgia patients and healthy subjects. We expected to find multiple biomarkers rather than a single one because a panel of biomarkers may correlate more reliably with fibromyalgia than a single protein (Bazzichi et al., 2009).

In this work we used two-dimensional electrophoresis (2-DE) to obtain the whole saliva protein map of fibromyalgia patients. Figure 1 shows the typical protein pattern obtained from a healthy control subject (figure 1 A) and a fibromyalgia patient (figure 1 B).

Matrix-assisted laser desorption ionization time-of-flight/ time-of-flight mass spectrometry (MALDI-TOF/TOF MS) was used to identify proteins that we found differentially expressed from two analysed groups. In table 3, we report the most interesting proteins which emerged from the data analysis.

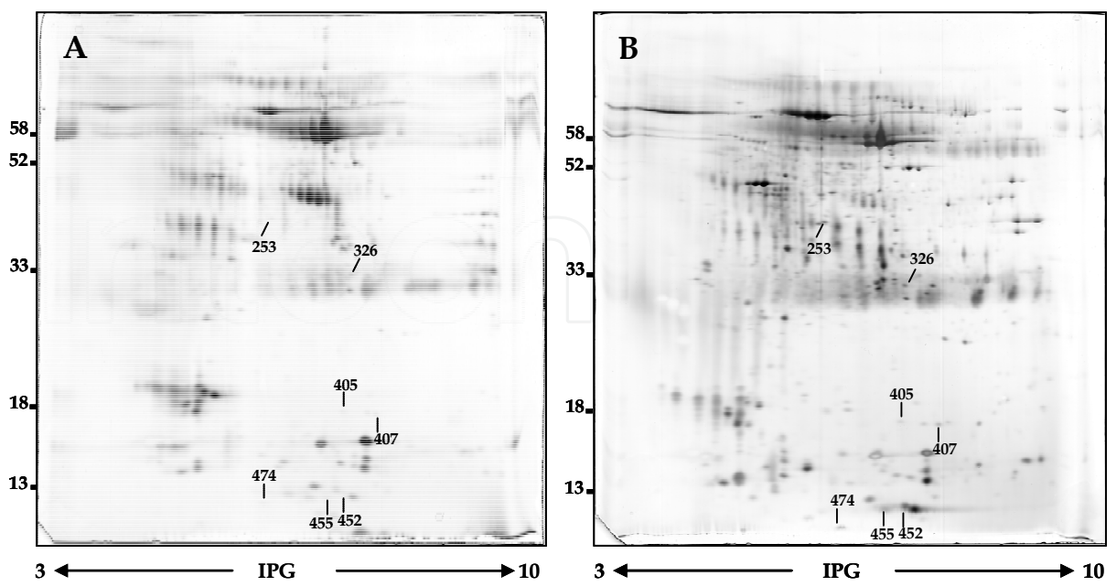

Fig. 1. Representative 2-DE gel map of salivary proteins. Control (A) and a fibromyalgia patient (B).

Spot n°	Accession number	Protein name	MW KDa	pI	score	coverage (%)	p-value
253	P37837	transaldolase	38	6.3	181	42	<0.0001
326	P18669	phosphoglycerate mutase 1	29	6.7	501	44	0.0011
405	P62937	cyclophilin A	18	7.7	242	40	0.05
407	P62937	cyclophilin A	18	7.7	450	58	0.0157
452	P05109	calgranulin A	11	6.5	313	47	0.0036
455	P05109	calgranulin A	11	6.5	187	37	0.0002
474	P80511	calgranulin C	11	5.8	68	7	0.001

Table 3. Protein identification of differentially expressed proteins in whole saliva of fibromyalgia patients by MS/MS

Figure 2 shows the enlarged images of these proteins, histograms show the percentage volumes of the proteins and each bar represents the mean±SD of the mean of each spot. Significant differences from control whole saliva are based on Mann-Whitney test; (*p<0.05, **p<0.01, ***p<0.001).

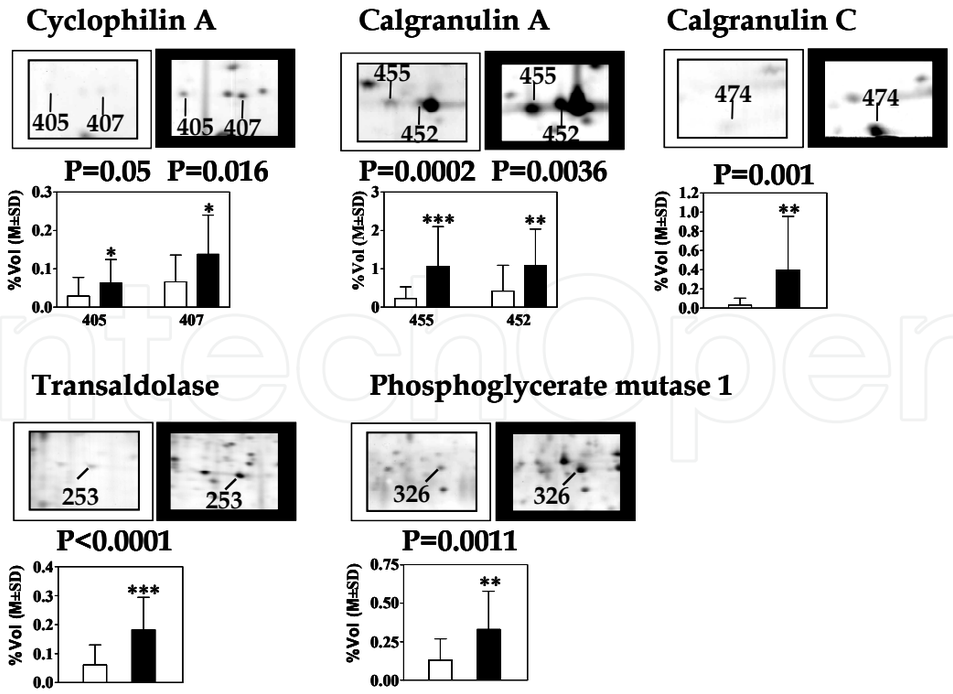

Fig. 2. Enlarged images of the 2-DE gels highlighting some differentially expressed proteins for two representative gels. White: controls, Black: fibromyalgia.

Cyclophilin A resulted over-expressed in fibromyalgia patients in comparison with healthy subjects. Cyclophilin A is a ubiquitously distributed protein belonging to the immunophilin family that can be secreted by cells in response to inflammatory stimuli (Arora et al., 2005). Secreted Cyclophilin A is a potent chemo attractant for monocytes, neutrophils, eosinophils and T cells in vitro. Satoh K and co-workers showed that Cyclophilin A is secreted from smooth muscle cells and macrophages also in response to oxidative stress (Satoh et al., 2008).

Other proteins found to be over expressed in fibromyalgia were calgranulins, belonging to the S100 multigene family implicated in a variety of intracellular activities such as cell proliferation and differentiation, cytoskeletal interactions, rearrangement and structural organization of membranes, intracellular Ca2+ homeostasis, cell migration, inflammation, and protection from oxidative cell damage (Yang et al., 2001). These proteins have already been found in autoinflammatory rheumatic diseases such as Sjögren Syndrome, Scleroderma, and Rheumatoid Arthritis (De Seny et al., 2008; Giusti et al., 2007a, 2007b, 2010). Considering that fibromyalgia is defined as a non-inflammatory disease, we suggested that the over-expression of calgranulins might be related to a sub-inflammatory process, as suggested by the cytokine alterations.

The main finding of our work was the significant over-expression of transaldolase and phosphoglycerate mutase 1 in fibromyalgia patients with respect to controls. The reliability of these potential biomarkers was assessed by receiver operating characteristic curves (figure 3). The sensitivity and the specificity of the transaldolase and phosphoglycerate mutase 1 were 77.3 and 84.6% and 95.5 and 50%, respectively.

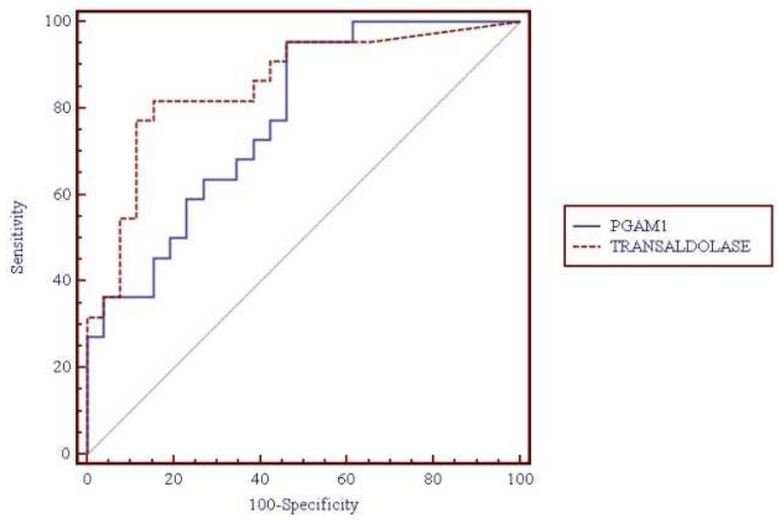

Fig. 3. ROC curve of transaldolase and phosphoglycerate mutase 1.

Furthermore, we validated the results by western blot analysis (figure 4). The statistical analysis of the optical density of specific detected bands confirmed the significant up-regulation of two enzymes in fibromyalgia patients with respect to controls.

Transaldolase is an enzyme of the non-oxidative phase of the pentose phosphate pathway, which is involved in the generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH). There is a lot of evidence have shown that oxidative stress and nitric oxide may play an important role in fibromyalgia pathophysiology (Ozgocmen et al., 2006).

The overexpression of transaldolase might be justified by the NADPH production, which could be involved in limiting oxidative damage to tissues. Moreover, transaldolase links the pentose phosphate pathway to glycolysis. From this point of view, it is interesting to note that phosphoglycerate mutase 1, an enzyme involved in glycolysis, was differently expressed in fibromyalgia patients.

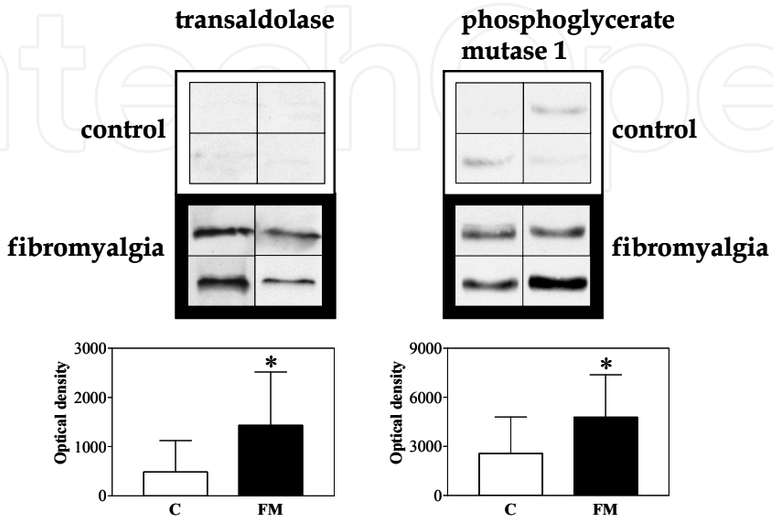

Fig. 4. Western Blot analysis of transaldolase and phosphoglycerate mutase 1 in whole saliva samples from healthy subjects and fibromyalgia patients. Densitometry of the blots is shown. Values that are significantly different ($p<0.05$) as determined by the Mann-Whitney test, are indicated.

In conclusion, our study has attested the potential usefulness of the proteomic characterization of human whole saliva in distinguishing fibromyalgia from healthy subjects. Moreover, the use of saliva may enable the easy characterization of non-invasively collected biological fluids, giving rise to a different approach in the diagnosis of fibromyalgia. The future focus of interest will be to validate the panel of biomarkers in different cohorts of pathological controls in order to identify biomarkers specific to fibromyalgia, and to exclude any interference of concomitant disorder (e.g. psychiatric comorbidities).
However, this study allows us to focus on some of the peculiar pathogenic aspects of fibromyalgia, especially on the oxidative stress which contradistinguishes this condition.

5. Conclusion

The pathogenesis of fibromyalgia is not entirely understood and, at present, the diagnosis is only clinical. Up to now, no objective measures have been determined to be reliable biomarkers, and these measures can only reflect a predisposition to fibromyalgia. However, the different studies offer us insights into the pathophysiology of fibromyalgia. The proteomic analysis, rather than the gene expression profile, might have potential applications as a new tool for the diagnosis of fibromyalgia because proteins are the final effectors that mediate disease pathogenesis. Therefore, proteomic seems to represent a necessary element in the advancement of disease diagnosis and therapeutic targets.

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7. References

Ablin, J.N., Bar-Shira, A., Yaron, M. & Orr-Urtreger, A. (2009). Candidate-gene approach in fibromyalgia syndrome: association analysis of the genes encoding substance P receptor, dopamine transporter and alpha1-antitrypsin. *Clinical and Experimental Rheumatology*, Vol. 27, No. 5 suppl 56, (September 2009), pp. S33-38, ISSN 0392-856X.

Amel Kashipaz, M.R., Swinden, D., Todd, I. & Powell, R.J. (2003). Normal production of inflammatory cytokines in chronic fatigue and fibromyalgia syndromes determined by intracellular cytokine staining in short-term cultured blood mononuclear cells. *Clinical & Experimental Immunology*, Vol. 132, No. 2, (May 2003), pp. 360-365, ISSN 0009-9104.

Anderberg, U.M., Liu, Z., Berglund, L. & Nyberg, F. (1999). Elevated plasma levels of neuropeptide Y in female fibromyalgia patients. *European Journal of Pain,* Vol. 3, No. 1, (March 1999), pp. 19-30, ISSN 1090-3801.

Andersen, M.L., Nascimento, D.C., Machado, R.B., Roizenblatt, S., Moldofsky, H. & Tufik, S. (2006). Sleep disturbance induced by substance P in mice. *Behavioural Brain Research,* Vol. 167, No. 2, (February 2006), pp. 212-218, ISSN 0166-4328.

Arora, K., Gwinn, W.M., Bower, M.A., Watson, A., Okwumabua, I., MacDonald, H.R., Bukrinsky, M.I. & Constant, S.L. Extracellular cyclophilins contribute to the regulation of inflammatory responses. *Journal of Rheumatology,* Vol. 175, No. 1, (July 2005), pp. 517-522, ISSN 0315-162X.

Baraniuk, J.N., Casado, B., Maibach, H., Clauw, D.J., Pannell, L.K. & Hess, S.S. (2005). A Chronic Fatigue Syndrome - related proteome in human cerebrospinal fluid. *BMC Neurology*, Vol. 5, No. 22, ISSN 1471-2377.

Bazzichi, L., Rossi, A., Massimetti, G., Giannaccini, G., Giuliano, T., De Feo, F., Ciapparelli, A., Dell'Osso, L. & Bombardieri, S. (2007). Cytokine patterns in fibromyalgia and their correlation with clinical manifestations. *Clinical and Experimental Rheumatology,* Vol. 25, No. 2, (March 2007), pp. 225-230, ISSN 0392-856X.

Bazzichi, L., Giacomelli, C., De Feo, F., Giuliano, T., Rossi, A., Doveri, M., Tani, C., Wilson, R.B. & Bombardieri, S. (2007). Antipolymer antibody in Italian fibromyalgic patients. *Arthritis Research & Therapy*, Vol. 9, No. 5, (September 2007), pp. R86, ISSN 1478-6354.

Bazzichi, L., Rossi, A., Giuliano, T., De Feo, F., Giacomelli, C., Consensi, A., Ciapparelli, A., Consoli, G., Dell'osso, L. & Bombardieri, S. (2007). Association between thyroid autoimmunity and fibromyalgic disease severity. *Clinical Rheumatology,* Vol. 26, No. 12, (December 2007), pp. 2115-2120, ISSN 0770-3198.

Bazzichi, L., Ciregia, F., Giusti, L., Baldini, C., Giannaccini, G., Giacomelli, C., Sernissi, F., Bombardieri, S. & Lucacchini, A. (2009). Detection of potential markers of primary fibromyalgia syndrome in human saliva. *Proteomics Clinical Application*, Vol. 3, No. 11, (November 2009), pp. 1296-1304, ISSN 1862-8346.

Bazzichi, L., Rossi, A., Zirafa, C., Monzani, F., Tognini, S., Dardano, A., Santini, F., Tonacchera, M., De Servi, M., Giacomelli, C., De Feo, F., Doveri, M., Massimetti, G. & Bombardieri, S. (2010). Thyroid autoimmunity may represent a predisposition for the development of fibromyalgia? *Rheumatology International*, DOI 10.1007/s00296-010-1620-1, ISSN 0172-8172.

Bazzichi, L., Rossi, A., Giacomelli, C. & Bombardieri, S. (2010). Exploring the abyss of fibromyalgia biomarkers. *Clinical and Experimental Rheumatology,* Vol. 28, No. 6 Suppl 63, (November-December 2010), pp. S125-130, ISSN 0392-856X.

Bondy, B., Spaeth, M., Offenbaecher, M., Glatzeder, K., Stratz, T., Schwarz, M., de Jonge, S., Krüger, M., Engel, R.R., Färber, L., Pongratz, D.E. & Ackenheil, M. (1999). The T102C polymorphism of the 5-HT2A-receptor gene in fibromyalgia. *Neurobiology of Disease,* Vol. 6, No. 5, (October 1999), pp. 433-439, ISSN 0969-9961.

Buskila, D., Cohen, H., Neumann, L. & Ebstein, R.P. (2004). An association between fibromyalgia and the dopamine D4 receptor exon III repeat polymorphism and relationship to novelty seeking personality traits. *Molecular Psychiatry*, Vol. 9, No. 8, (August 2004), pp. 730-731, ISSN 1359-4184.

Cohen, H., Buskila, D., Neumann, L. & Ebstein, R.P. (2002). Confirmation of an association between fibromyalgia and serotonin transporter promoter region (5-HTTLPR) polymorphism, and relationship to anxiety-related personality traits. *Arthritis and Rheumatism,* Vol. 46, No. 3, (March 2002), pp. 845-847, ISSN 0004-3591.

Cohen, H., Neumann, L., Glazer, Y., Ebstein, R.P. & Buskila, D. (2009). The relationship between a common catechol-O-methyltransferase (COMT) polymorphism val(158) met and fibromyalgia. *Clinical and Experimental Rheumatology,* Vol. 27, No. 5 suppl. 56, (September-October 2009), pp. S51-56, ISSN 0392-856X.

Crofford, L.J., Pillemer, S.R., Kalogeras, K.T., Cash, J.M., Michelson, D., Kling, M.A., Sternberg, E.M., Gold, P.W., Chrousos, G.P. & Wilder, R.L. (1994). Hypothalamic-pituitary-adrenal axis perturbations in patients with fibromyalgia. *Arthritis and Rheumatism,* Vol. 37, No.11, (November 1994), pp. 1583-1592, ISSN 0004-3591.

De Seny, D., Fillet, M., Ribbens, C., Marée, R., Meuwis, M.A., Lutteri, L., Chapelle, J.P., Wehenkel, L., Louis, E., Merville, M.P. & Malaise, M. (2008). Monomeric calgranulins measured by SELDI-TOF mass spectrometry and calprotectin measured by ELISA as biomarkers in arthritis. *Clinical Chemistry*, Vol. 54, No. 6, (June 2008), pp. 1066-1075, ISSN 0009-9147.

Di Franco, M., Iannuccelli, C., Alessandri, C., Paradiso, M., Riccieri, V., Libri, F. & Valesini, G. (2009). Autonomic dysfunction and neuropeptide Y in fibromyalgia. *Clinical and Experimental Rheumatology,* Vol. 27, No. 5 suppl 56, (September 2009), pp. S75-S78, ISSN 0392-856X.

Di Franco, M., Iannuccelli, C. & Valesini, G. (2010). Neuroendocrine immunology of fibromyalgia. *Annals of the New York Academy of Sciences*, Vol. 1193, (April 2010), pp. 84-90, ISSN 0077-8923.

Evengard, B., Nilsson, C.G., Lindh, G., Lindquist, L., Eneroth, P., Fredrikson, S., Terenius, L. & Henriksson, K.G. (1998). Chronic fatigue syndrome differs from fibromyalgia. No evidence for elevated substance P levels in cerebrospinal fluid of patients with chronic fatigue syndrome. *Pain*, Vol. 78, No. 2, (November 1998), pp. 153-155, ISSN 0304-3959.

Fayed, N., Garcia-Campayo, J., Magallón, R., Andrés-Bergareche, H., Luciano, J.V., Andres, E. & Beltrán, J. (2010). Localized 1H-NMR spectroscopy in patients with fibromyalgia: a controlled study of changes in cerebral glutamate/glutamine, inositol, choline, and N-acetylaspartate. *Arthritis Research & Therapy*, Vol. 12, No. 4, (July 2010), pp. R134, ISSN 1478-6354.

Giusti, L., Baldini, C., Bazzichi, L., Ciregia, F., Tonazzini, I., Mascia, G., Giannaccini, G., Bombardieri, S. & Lucacchini A. (2007). Proteome analysis of whole saliva: a new tool for rheumatic diseases--the example of Sjögren's syndrome. *Proteomics*, Vol. 7, No. 10, (May 2007), pp. 1634-1643, ISSN 1615-9853.

Giusti, L., Bazzichi, L., Baldini, C., Ciregia, F., Mascia, G., Giannaccini, G., Del Rosso, M., Bombardieri, S. & Lucacchini, A. (2007). Specific proteins identified in whole saliva from patients with diffuse systemic sclerosis. *Journal of Rheumatology*, Vol. 34, No. 10, (October 2007), pp. 2063-2069, ISSN 0315-162X.

Giusti, L., Baldini, C., Ciregia, F., Giannaccini, G., Giacomelli, C., De Feo, F., Delle Sedie, A., Riente, L., Lucacchini, A., Bazzichi, L. & Bombardieri, S. (2010). Is GRP78/BiP a potential salivary biomarker in patients with rheumatoid arthritis? *Proteomics Clinical applications,* Vol. 4, No. 3, (March 2010), pp. 315-324, ISSN 1862-8346.

Gür, A., Karakoç, M., Nas, K., Remzi, Cevik, Denli, A. & Saraç J. (2002). Cytokines and depression in cases with fibromyalgia. *Journal of Rheumatology,* Vol. 29, No. 2, (February 2002), pp. 358-361, ISSN 0315-162X.

Gürsoy, S., Erdal, E., Herken, H., Madenci, E. & Alaşehirli, B. (2001). Association of T102C polymorphism of the 5-HT2A receptor gene with psychiatric status in fibromyalgia syndrome. *Rheumatology International*, Vol. 21, No. 2, (October 2001), pp. 58-61, ISSN 0172-8172.

Gursoy, S. (2002). Absence of association of the serotonin transporter gene polymorphism with the mentally healthy subset of fibromyalgia patients. *Clinical Rheumatology*, Vol. 21, No. 3 (June 2002), pp. 194-197, ISSN 0770-3198.

Gürsoy, S., Erdal, E., Herken, H., Madenci, E., Alaşehirli, B. & Erdal, N. (2003). Significance of catechol-O-methyltransferase gene polymorphism in fibromyalgia syndrome. *Rheumatology International,* Vol. 23, No. 3, (May 2003), pp. 104-107, ISSN 0172-8172.

Haas, L., Portela, L.V., Böhmer, A.E., Oses, J.P. & Lara, D.R. (2007). Increased plasma levels of brain derived neurotrophic factor (BDNF) in patients with fibromyalgia. *Neurochemical Research*, Vol. 35, No. 5, (May 2010), pp. 830-834, ISSN 0364-3190.

Harris, R.E., Sundgren, P.C., Pang, Y., Hsu, M., Petrou, M., Kim, S.H., McLean, S.A., Gracely, R.H. & Clauw, D.J. (2008). Dynamic levels of glutamate within the insula are associated with improvements in multiple pain domains in fibromyalgia. *Arthritis and Rheumatism*, Vol. 58, No. 3, (March 2008), pp. 903-907, ISSN 0004-3591.

Harris, R.E., Sundgren, P.C., Craig, A.D., Kirshenbaum, E., Sen, A., Napadow, V. & Clauw, D.J. (2009). Elevated insular glutamate in fibromyalgia is associated with experimental pain. *Arthritis and Rheumatism,* Vol. 60, No. 10, (October 2009), pp. 3146-3152, ISSN 0004-3591.

Iannuccelli, C., Di Franco, M., Alessandri, C., Guzzo, M.P., Croia, C., Di Sabato, F., Foti, M. & Valesini, G. (2010). Pathophysiology of fibromyalgia: a comparison with the tension-type headache, a localized pain syndrome. *Annals of the New York Academy of Sciences*, Vol. 1193, (April 2010), pp. 78-83, ISSN 0077-8923.

Iughetti, L., Casarosa, E., Predieri, B., Patianna, V. & Luisi, S. (2011). Plasma brain-derived neurotrophic factor concentrations in children and adolescents. *Neuropeptides*, Vol. 45, No. 3, (June 2011), pp. 205-211, ISSN 0143-4179.

Jensen, B., Wittrup, I.H., Wiik, A., Bliddal, H., Friis, A.S., McLaughlin, J.K., Danneskiold-Samsøe, B. & Olsen, J.H. (2004). Antipolymer antibodies in Danish fibromyalgia patients. *Clinical and Experimental Rheumatology,* Vol. 22, No. 2, (March 2004), pp. 227-229, ISSN 0392-856X.

Kim, S.K., Kim, K.S., Lee, Y.S., Park, S.H. & Choe, J.Y. (2010). Arterial stiffness and proinflammatory cytokines in fibromyalgia syndrome. *Clinical and Experimental Rheumatology*, Vol. 28, No. 6 suppl 63, (November-December 2010), pp. S71-77, ISSN 0392-856X.

Klein, R. & Berg, P.A. (1995). 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. *European Journal of Medical Research*, Vol. 16, No. 1, (October 1995), pp. 21-26, ISSN 0949-2321.

Laske, C., Stransky, E., Eschweiler, G.W., Klein, R., Wittorf, A., Leyhe, T., Richartz, E., Köhler, N., Bartels, M., Buchkremer, G. & Schott, K. (2006). Increased BDNF serum concentration in fibromyalgia with or without depression or antidepressants. *Journal of Psychiatric Research*, Vol. 41, No. 7, (October 2006), pp. 600-605, ISSN 0022-3956.

Lee, Y.H., Choi, S.J., Ji, J.D. & Song, G.G. (2010). Candidate gene studies of fibromyalgia: a systematic review and meta-analysis. *Rheumatology International,* DOI 10.1007/s00296-010-1678-9, ISSN 0172-8172.

Maletic, V. & Raison, C.L. (2009). Neurobiology of depression, fibromyalgia and neuropathic pain. *Frontiers in Bioscience,* Vol. 1, No. 14, (June 2009), pp. 5291-5338, ISSN 1093-9946.

Malt, E.A., Olafsson, S., Aakvaag, A., Lund, A. & Ursin, H. (2003). Altered dopamine D2 receptor function in fibromyalgia patients: a neuroendocrine study with buspirone in women with fibromyalgia compared to female population based controls. *Journal of Affective Disorders*, Vol. 75, No. 1, (June 2003), pp. 77-82, ISSN 0165-0327.

Matsuda, J.B., Barbosa, F.R., Morel, L.J., França, S.deC., Zingaretti, S.M., da Silva, L.M., Pereira, A.M., Marins, M. & Fachin, AL. (2010). Serotonin receptor (5-HT 2A) and catechol-O-methyltransferase (COMT) gene polymorphisms: triggers of fibromyalgia? *Revista Brasileira de Reumatologia*, Vol. 50, No. 2, (April 2010), pp. 141-149, ISSN 0482-5004.

Nicholl, B.I., Holliday, K.L., Macfarlane, G.J., Thomson, W., Davies, K.A., O'Neill, T.W., Bartfai, G., Boonen, S., Casanueva, F., Finn, J.D., Forti, G., Giwercman, A., Huhtaniemi, I.T., Kula, K., Punab, M., Silman, A,J., Vanderschueren, D., Wu, F.C., McBeth, J. & European Male Ageing Study Group. (2010). No evidence for a role of the catechol-O-methyltransferase pain sensitivity haplotypes in chronic widespread pain. *Annals of the Rheumatic Diseases*, Vol. 69, No. 11, (November 2010), pp. 2009-2012, ISSN 0003-4967.

Nishikai, M., Tomomatsu, S., Hankins, R.W., Takagi, S., Miyachi, K., Kosaka, S. & Akiya, K. (2001). Autoantibodies to a 68/48 kDa protein in chronic fatigue syndrome and primary fibromyalgia: a possible marker for hypersomnia and cognitive disorders. *Rheumatology*, Vol. 40, No. 7, (July 2001), pp. 806-810, ISSN 1462-0324.

Offenbaecher, M., Bondy, B., de Jonge, S., Glatzeder, K., Krüger, M., Schoeps, P. & Ackenheil, M. (1999). Possible association of fibromyalgia with a polymorphism in the serotonin transporter gene regulatory region. *Arthritis and Rheumatism,* Vol. 42, No. 11, (November 1999), pp. 2482-2488, ISSN 0004-3591.

Ozgocmen, S., Ozyurt, H., Sogut, S. & Akyol, O. (2006). Current concepts in the pathophysiology of fibromyalgia: the potential role of oxidative stress and nitric oxide. *Rheumatology International*, Vol. 26, No. 7, (May 2006), pp. 585-597, ISSN 0172-8172.

Pamuk, O.N. & Cakir, N. (2007). The frequency of thyroid antibodies in fibromyalgia patients and their relationship with symptoms. *Clinical Rheumatology*, Vol. 26, No. 1, (January 2007), pp. 55-59, ISSN 0770-3198.

Potvin, S., Larouche, A., Normand, E., de Souza, J.B., Gaumond, I., Marchand, S. & Grignon, S. (2010). No relationship between the ins del polymorphism of the serotonin transporter promoter and pain perception in fibromyalgia patients and healthy controls. *European Journal of Pain*, Vol. 14, No. 7, (August 2010), pp. 742-746, ISSN 1090-3801.

Russell, I.J., Orr, M.D., Littman, B., Vipraio, G.A,, Alboukrek, D., Michalek, J.E., Lopez, Y. & MacKillip, F. (1994). Elevated cerebrospinal fluid levels of substance P in patients with the fibromyalgia syndrome. *Arthritis and Rheumatism*, Vol. 37, No. 11, (November 1994), pp. 1593-1601, ISSN 0004-3591.

Sarchielli, P., Mancini, M.L., Floridi, A., Coppola, F., Rossi, C., Nardi, K., Acciarresi, M., Pini, L.A. & Calabresi, P. (2007). Increased levels of neurotrophins are not specific for chronic migraine: evidence from primary fibromyalgia syndrome. *Journal of Pain*, Vol. 8, No. 9, (September 2007), pp. 737-745, ISSN 1526-5900.

Sarzi-Puttini, P., Atzeni, F., Di Franco, M., Lama, N., Batticciotto, A., Iannuccelli, C., Dell'Acqua, D., de Portu, S., Riccieri, V., Carrabba, M., Buskila, D., Doria, A. & Valesini, G. (2008). Anti-polymer antibodies are correlated with pain and fatigue severity in patients with fibromyalgia syndrome. *Autoimmunity*, Vol. 41, No. 1, (February 2008), pp. 74-9, ISSN 0891-6934.

Satoh, K., Matoba, T., Suzuki, J., O'Dell, M.R., Nigro, P., Cui, Z., Mohan, A., Pan, S., Li, L., Jin, Z.G., Yan, C., Abe, J. & Berk B.C. (2008). Cyclophilin A mediates vascular remodeling by promoting inflammation and vascular smooth muscle cell proliferation. *Circulation*, Vol. 117, No. 24, (June 2008), pp. 3088-3098, ISSN 0009-7322.

Tander, B., Gunes, S., Boke, O., Alayli, G., Kara, N., Bagci, H. & Canturk, F. (2008). Polymorphisms of the serotonin-2A receptor and catechol-O-methyltransferase genes: a study on fibromyalgia susceptibility. *Rheumatology International*, Vol. 28, No. 7, (May 2008), pp. 685-691, ISSN 0172-8172.

Treister, R., Pud, D., Ebstein, R.P., Laiba, E., Gershon, E., Haddad, M. & Eisenberg, E. (2009). Associations between polymorphisms in dopamine neurotransmitter pathway genes and pain response in healthy humans. *Pain,* Vol. 147, No. 1-3, (December 2009), pp. 187-193, ISSN 0304-3959.

Uçeyler, N., Valenza, R., Stock, M., Schedel, R., Sprotte, G. & Sommer, C. (2006). Reduced levels of antiinflammatory cytokines in patients with chronic widespread pain. *Arthritis and Rheumatism,* Vol. 54, No. 8, (August 2006), pp. 2656-2664, ISSN 0004-3591.

Vaerøy, H., Helle, R., Førre, O., Kåss, E. & Terenius, L. (1988). Elevated CSF levels of substance P and high incidence of Raynaud phenomenon in patients with fibromyalgia: new features for diagnosis. *Pain,* Vol. 32, No. 1, (January 1988), pp. 21-26, ISSN 0304-3959.

Valdés, M., Collado, A., Bargalló, N., Vázquez, M., Rami, L., Gómez, E. & Salamero, M. (2010). Increased glutamate/glutamine compounds in the brains of patients with fibromyalgia: a magnetic resonance spectroscopy study. *Arthritis and Rheumatism*, Vol. 62, No 6, (June 2010), pp. 1829-1836, ISSN 0004-3591.

Wallace, D.J., Linker-Israeli, M., Hallegua, D., Silverman, S., Silver, D. & Weisman, M.H. (2001). Cytokines play an aetiopathogenetic role in fibromyalgia: a hypothesis and pilot study. *Rheumatology*, Vol. 40, No. 7, (July 2001), pp. 743-749, ISSN ISSN 1462-0324.

Wang, H., Moser, M., Schiltenwolf, M. & Buchner, M. (2008). Circulating cytokine levels compared to pain in patients with fibromyalgia -- a prospective longitudinal study over 6 months. *Journal of Rheumatology,* Vol. 35, No. 7, (July 2008), pp. 1366-1370, ISSN 0315-162X.

Wang, H., Buchner, M., Moser, M.T., Daniel, V. & Schiltenwolf, M. (2009). The role of IL-8 in patients with fibromyalgia: a prospective longitudinal study of 6 months. *Clinical Journal of Pain*, Vol. 25, No. 1, (January 2009), pp. 1-4, ISSN 0749-8047.

Werle, E., Fischer, H.P., Müller, A., Fiehn, W. & Eich, W. (2001). Antibodies against serotonin have no diagnostic relevance in patients with fibromyalgia syndrome. *Journal of Rheumatology,* Vol. 28, No. 3, (March 2001), pp. 595-600, ISSN 0315-162X.

Wilson, R.B., Gluck, O.S., Tesser, J.R., Rice, J.C., Meyer, A. & Bridges, AJ. (1999). Antipolymer antibody reactivity in a subset of patients with fibromyalgia correlates with severity. *Journal of Rheumatology*, Vol. 26, No. 2, (February 1999), pp. 402-407, ISSN 0315-162X.

Wolfe, F., Smythe, H.A., Yunus, M.B., Bennett, R.M., Bombardier, C., Goldenberg, D.L., Tugwell, P., Campbell, S.M., Abeles, M., Clark, P., Fam, A.G., Farber, S.J., Fiechtner, J.J., Franklin, M., Gatter, R.A., Hamaty, D., Lessard, J., Lichtbroun, A.S., Masi, A.T., Mccain, G.A., Reynolds, W.J., Romano, T.J., Russell, J. & Sheon, R.P. (1990). The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. *Arthritis and Rheumatism*, Vol. 33, No. 2, (February 1990), pp. 160-172, ISSN 0004-3591.

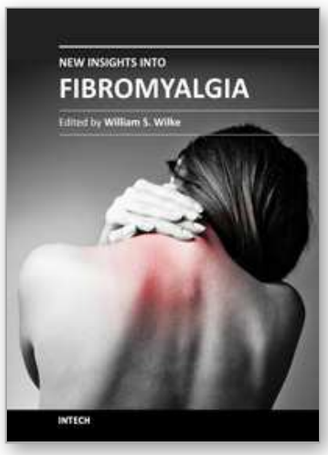
Wolfe, F. (1999). "Silicone related symptoms" are common in patients with fibromyalgia: no evidence for a new disease. *Journal of Rheumatology,* Vol. 26, No. 5, (May 1999), pp. 1172-1175, ISSN 0315-162X.

Wolfe, F., Clauw, D.J., Fitzcharles, M.A., Goldenberg, D.L., Katz, R.S., Mease, P., Russell, A.S., Russell, I.J., Winfield, J.B. & Yunus, M.B. (2010). The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arthritis Care & Research,* Vol.62, No 5, (May 2010), pp. 600-610, ISSN 2151-464X.

Wolfe, F., Clauw, D.J., Fitzcharles, M.A., Goldenberg, D.L., Häuser, W., Katz, R.S., Mease, P., Russell, A,S., Russell, I.J. & Winfield, J.B. (2011). Fibromyalgia Criteria and Severity Scales for Clinical and Epidemiological Studies: A Modification of the ACR Preliminary Diagnostic Criteria for Fibromyalgia. *Journal of Rheumatology,* Vol. 38, No. 6, (June 2011), pp. 1113-1122, ISSN 0315-162X.

Yang, Z., Tao, T., Raftery, M.J., Youssef, P., Di Girolamo, N. & Geczy, C.L. (2001) Proinflammatory properties of the human S100 protein S100A12. *Journal of Leukocyte Biology*, Vol. 69, No. 6, (June 2001), pp. 986-994, ISSN 0741-5400.

Zubieta, J.K., Heitzeg, M.M., Smith, Y.R., Bueller, J.A., Xu, K., Xu, Y., Koeppe, R.A., Stohler, C.S. & Goldman, D. (2003). COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. *Science,* Vol. 299, No. 5610, (February 2003), pp. 1240-1243, ISSN 0036-8075.



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Given the potential problems that can obscure any scientific enterprise, inconsistent results across studies are bound to occur. How are we to decide what is true? Let's turn to philosophy for a reasonable answer. The mathematician-philosopher Bertrand Russell approached a similar problem in his monograph The Problems of Philosophy (Russell B, 1912). He addressed the following question: How do we know that anything is "real"? Is the only reality subjective and simply in our minds, as Bishop Berkley challenged, or can we mostly believe the objective reality? His pragmatic answer: All possibilities may be true, but when the preponderance of evidence indicates that objective reality and knowledge are the most probable case, go with it. If the preponderance of all evidence about the clinical description of fibromyalgia and it's pathogenic mechanisms and treatment strategies indicate a highly probable interrelated hypothesis, go with it. The direction of the literature on the whole trumps the less likely tangents. At the same time, remember Bertrand Russell and his pragmatic answer, and keep an open mind.

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