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LETTER TO THE EDITOR

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Aging-Related Changes in Inflammatory and LKB1/AMPK Gene Expression in Fibromyalgia Patients

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Aging is characterized by a progressive loss of physiological integrity, leading to the impairment of numerous functions and increased vulnerability to death. This deterioration is the primary risk factor for aging-related major human pathologies, including cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases [1]. Several interconnected molecular events are involved in this process. Efficient control of energy metabolic homeostasis, enhanced stress resistance, and qualified cellular housekeeping are the hallmarks of improved health span and extended lifespan. Adenosine monophosphate-activated protein kinase (AMPK), the master regulator of cell energy levels, is activated by low adenosine triphosphate (ATP) levels that, in turn, increases glucose transport, fatty acid oxidation, and mitochondrial biogenesis. Furthermore, AMPK is also involved in the control of inflammatory processes [2]. Recent studies also indicate that the sensitivity of AMPK to cellular stress declines with aging, and this could impair downstream signaling and the maintenance of cellular energy balance and stress resistance. AMPK signaling decline can also decrease mitochondrial biogenesis, increase cellular stress, and induce inflammation, which are typical events of the aging process [3].

Fibromyalgia (FM) is a common chronic pain syndrome accompanied by other symptoms and a neuromuscular profile, the pathophysiological mechanisms of which have not been yet identified despite the fact it affects up to 5% of the general population worldwide. Recently, it has been observed that AMPK has a critical role in the pathophysiology of FM [4], accompanied by other important events such as reduced mitochondrial biogenesis [4] and NOD-like receptor family, pyrin domain containing 3 (NLRP3)-inflammasome activation [5]. It has also been suggested an acceleration changes of aging in FM such as increased brain gray matter loss [6] and short leukocyte telomere lengths [7]. To elucidate the possible involvement of aging in FM, we have studied the age-related changes in gene expression related to mitochondrial biogenesis, antioxidant defense and inflammation response in blood mononuclear cells (BMCs).

Patients and controls were distributed in three groups of age: 30–40 years (N = 16 patients and N = 13 controls); 41–50 years (N = 18 patients and N = 14 controls); 51–60 years (N = 16 patients and N = 13 controls). Mean of body mass index (BMI) in the FM group was 24.8 ± 3.3 and in the control group was 23.1 ± 2.2. Data in Table 1 are shown as mean ± SD. Data between different groups were analyzed statistically by using ANOVA on Ranks with Sigma Plot and Sigma Stat statistical software (SPSS for Windows, 19, 2010, SPSS, Chicago, IL, USA) using an all pairwise multiple comparison procedure (Tukey's test) for correction. A value of $P < 0.05$ was considered significant. Statistical analyses included Pearson's correlation coefficients between gene expression levels and age. Fisher's Z-transformation was used for the comparison of correlation coefficients.

BMCs from FM patients showed upregulation of several inflammation and inflammasome-related genes (IL-6, IL-8, IL-1β and NLRP3, $P < 0.001$) (Table 1), whereas genes linked to mitochondrial biogenesis (PGC-1a, TFAM) and antioxidant response (CuZnSOD and MnSOD) were down-regulated, ($P < 0.001$) (Table 1). Furthermore, AMPK and LKB1, one of the upstream activators of AMPK, gene expressions were down-regulated in FM BMCs compared with control BMCs (Table 1).

Table 1 Age-related gene expression level changes in FM patients

	Fibromyalgia		Healthy control	
	Gene expression Mean ± SD	Age r	Gene expression Mean ± SD	Age r
LKB1	82 ± 2.1	−0.71	176 ± 8.1	−0.30
AMPK	99.2 ± 5.1	−0.70*	160 ± 7.3	−0.31***
PGC-1α	91 ± 4.2	−0.62*	162 ± 4.9	0.16
Tfam	97.2 ± 8.1	−0.69*	155 ± 7.8	−0.25
SOD1	85.2 ± 7.4	−0.57*	152 ± 5.3	−0.24
SOD1	98.5 ± 5.2	−0.585**	149 ± 7.7	−0.12
IL-6	143 ± 7.2	0.42**	59.8 ± 3.2	−0.12
IL-8	113 ± 6.1	0.38**	64.3 ± 6.1	0.09
NLRP3	124 ± 4.8	0.60*	28.3 ± 3.7	0.17
IL-1β	124 ± 11.3	0.66*	47.1 ± 7	0.29

Values of gene expression are means ±SD; r, Pearson's Correlation Coefficient between gene expression and age. Values that are significantly different are indicated by asterisks, *$P < 0.001$, **$P < 0.005$, ***$P < 0.05$.

As shown in Table 1, we observed a significant correlation with age-related changes in FM patients which was absent in the healthy control group (except for AMPK). AMPK and LKB1 displayed a more notable decrease in relation to years in FM patients than in controls. Furthermore, we observed an important decrease of antioxidants (SOD1 and SOD2) and mitochondrial biogenesis gene expression levels (PGC-1α and TFAM) in FM patients. Both alterations have been previously associated with oxidative stress and mitochondrial dysfunction in FM [4] and aging [1]. On the other hand, levels of inflammatory gene expression levels were increased compared with age-matched healthy controls. Interestingly, FM patients also showed a very significant age-related increased expression of inflammasome genes, NLRP3 and IL-1β, compared with controls. Comparison of values of Pearson's correlation coefficients among gene expression levels and age range resulted in statistically significant differences in the 30–40, 41–50, and 51–60 years groups (Table 2).

After the correlation analysis among tender points, Fibromyalgia Impact Questionnaire (FIQ) and gene expression markers we did not find significant results with tender points. However, we observed significant correlations with FIQ (SOD1: -0.38, ($P < 0.05$); IL-6: 0.75, ($P < 0.001$); NLRP3: 0.31, ($P < 0.05$); IL-1β: 0.54, ($P < 0.005$); AMPK: −0.28, ($P < 0.05$); LKB1: −0.32, ($P < 0.05$).

This preliminary study suggests that age-related changes in gene expression appeared earlier in several FM patients related to accelerated aging, and this is consistent with previous reports [6,7]. FM meets all typical molecular alterations associated with aging such as, AMPK/PGC-1α axis decrease, mitochondrial dysfunction, inflammation, and telomere shortening, which are widely known hallmarks of aging [1]. Recent findings indicate a direct relationship between telomeres and mitochondria, connecting for the first time two major theories of aging [8]. Sahin and co-workers observed high correlation between pain and depression and telomere length. Consistent with these data, we have observed statistical significance between FIQ and several age-related gene expression levels. Therefore, we propose that age-related changes in gene expression in FM patients could affect several pathophysiological levels (inflammation, bioenergetics, etc.) which may have several implications over the years in the disease progression. Our data also show that comparisons of Pearson's correlation among age-related gene expression and age are statistically significant when the analysis was performed individually in the 41–50 and 51–60 years groups. The reduced statistical significance in the 30–40 years group may be due to the reduced number of patients and controls.

Aging also impairs AMPK activation and suppresses insulin-stimulated glucose uptake into rat skeletal muscles, which is held to enhance the development of metabolic syndrome [9]. In this respect, it has been suggested that insulin resistance is also involved in FM and may represent a risk factor for memory impairment in FM patients [10], and it has been reported that memory is an important marker of cognitive impairment in aging [11]. As FM has characteristics of a systemic disease because the same pattern of changes in inflammation markers, mitochondrial function and metabolism are found in different cells (BMCs,

Table 2 Correlation between age and gene expression of patients and controls to age range.

	Fibromyalgia			Healthy control			Fisher r-to-z transformation		
Range	30–40 r	41–50 r	51–60 r	30–40 r	41–50 r	51–60 r	30–40 Z	41–50 Z	51–60 Z
LKB1	−0.57	−0.68	−0.59	−0.24	−0.17	−0.15	−1.8	−3.03**	−2.44**
AMPK	−0.64	−0.68	−0.59	−0.27	0.11	−0.21	−2.19*	−4.3***	−2.11*
PGC-1α	−0.59	−0.75	−0.67	−0.21	−0.10	−0.25	−2.08*	−4***	−2.57**
Tfam	−0.59	−0.69	−0.66	−0.22	−0.03	−0.22	−2.1*	−3.79***	−2.58**
SOD1	−0.52	−0.71	−0.59	−0.19	−0.11	−0.20	−2*	−3.53***	−2.22*
SOD1	−0.49	−0.58	−0.57	−0.12	−0.07	−0.07	−1.96*	−2.67**	−2.62**
IL-6	0.46	0.59	0.47	0.05	0.08	0.12	2*	2.75**	1.55
IL-8	0.35	0.47	0.37	0.02	0.11	0.07	1.57	1.84*	1.49
NLRP3	0.45	0.62	0.53	0.05	0.12	0.05	1.98*	2.78**	2.5**
IL-1β	0.48	0.69	0.59	0.12	0.12	0.20	1.81*	3.32***	2.14*

Values of Gene expression are means ±SD; r, Pearson's Correlation Coefficient; Z, Fisher r-to-z transformation; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

platelets or muscle) from FM patients and central sensitization is typically seen in these patients [12], it could be interesting to study the same molecular changes (the LKB1/AMPK and inflammasome pathways) in the nervous system to confirm our results and a better understanding of FM.

However, we have to interpret these data with caution due to FM clinical heterogeneity and the fact that there are contradictory data in several parameters such as inflammation as a result of the existence of several subgroups of FM patients [13].

Given that chronic pain is a typical event in aging [14] like other aging-related disorders such as obesity, atherosclerosis or type 2 diabetes [1], we propose that, at least in a subgroup of patients, changes related to accelerated aging with special involvement of the LKB1/AMPK axis could be implicated in the pathophysiology of FM. However, we are aware of the limitation of our work, taking into account the reduced number of FM patients and control included in the study. Given the complexity of the disease, more studies will be necessary to confirm this hypothesis and to clarify whether the disease progression, the aging process, or both are driving age-related gene expression changes in FM.

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Conflict of Interest

All authors declare that there are no conflicts of interest.

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Supporting Information

The following supplementary material is available for this article:

Appendix S1. Patients.

