Association between environmental quality, stress and APOE gene variation in fibromyalgia susceptibility determination

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ABSTRACT

Introduction: Fibromyalgia is a multifactorial disease, of which etiology is based on interaction between genetic susceptibility and environment. However, few studies attempted to identify the risk factors. **Objective:** To investigate the genetic influence and its interaction with environmental quality and stress, as possible risk factors for fibromyalgia development**. Patients and Methods:** This cross-sectional study investigated two groups of women, of which 47 had a clinical diagnosis of fibromyalgia, and 41 women comprising thre control group, all from the town of Novo Hamburgo, RS. The apolipoprotein E (APOE) gene polymorphism was analyzed in DNA extracted from total blood, in both samples. Environmental factors were studied through Lipp's Inventory of Stress Symptoms for Adults and by applying the WHOQOL-100 domain V. **Results:** Among the patients, more women had high stress levels when compared to the control sample ($P < 0.001$); moreover, the average scores of the WHOQOL-100 domain V, which analyze environment quality, were lower in this group ($P < 0.001$). APOE genotypic and allelic frequencies were similar between the two groups. Multivariate analysis showed that low WHOQOL-100 scores increase the chance of disease development by 57.7 times ($P < 0.001$), and that high stress levels were related with the disease (OR = 197.2; $P < 0.001$). This approach pointed out an interaction between stress and presence of E*2 allele ($P = 0.028$). Fibromyalgia was much more frequent in patients with high stress levels that were E*2 non-carriers (estimated OR = 265.1), when compared to patients with the same stress level, but E*2 carriers (estimated OR = 1.06). **Conclusion:** E*2 allele presence could have a protective action regarding the association between fibromyalgia and stress.

Keywords: fibromyalgia, stress, environmental quality, gene x environment interaction, apolipoprotein E.

INTRODUCTION

Fibromyalgia (FM) is a disease characterized by a clinical history of generalized muscle pain for more than three months and by specific tender points. The diagnosis is basically clinical[1] with no evidence of laboratory or imaging abnormalities. According to Neumann and Buskilla,[2] the prevalence of fibromyalgia in different countries is estimated at 2% in general population. The syndrome prevalence increases with age (approximately 1% in women aged 18 to 29 years and 7% in women aged 70 to 79 years). To date, no scientific investigation seeking to determine FM prevalence has been published for Brazilian populations.

Studies carried out to date have demonstrated that FM is a multifactorial disease, with a possible genetic susceptibility

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being necessary, but not enough to trigger it.[2,3] This genetic predisposition, when associated with environmental factors, among which are stressor agents related to psychosocial and emotional aspects, seem to increase the risk for symptom triggering and aggravation. Wood[4] stated that fibromyalgia has been called a "stress-related disorder" due to the fact that its symptom onset and/or exacerbation occur in the context of stressor events. Van Houdenhove *et al.*[5] determined that stress was an etiopathogenic factor for FM, as long as it was associated with genetic predisposition factors.

In spite of this evidence, the genes involved with the disease are yet to be determined, as well as how the gene variability interacts with environmental factors. These investigations are mainly related to serotonergic system genes,[6,7] but no study has attempted to correlate the apolipoprotein E (APOE) gene with fibromyalgia.

The role of APOE on stress response and its association with chronic pain has been recorded by both clinical[8,9] and experimental studies.[10,11] These studies have demonstrated that APOE levels are related with stress response, induced pain in animal models and different types of chronic pain in humans. Still, no study had attempted to associate the variation of apo E protein type, codified by three different alleles called E*2, E*3 and E*4, with fibromyalgia susceptibility.

Therefore, the present study aims at investigating whether the environmental quality and stress levels are fibromyalgia-related factors and also whether any of the APOE gene alleles is associated with the risk of developing fibromyalgia. Additionally, the interaction among these three variables will be tested, in order to determine the association among them as well as their influence on fibromyalgia.

PATIENTS AND METHODS

Patients

The sample consisted of 41 adult female individuals, with a clinical diagnosis of fibromyalgia according to the criteria established by the American College of Rheumatology (ACR),[1] with a mean age of 47.93 ± 11.21 years. The exclusion criteria included the presence of cognitive deficit and motor incapacity. Patients underwent psychological evaluation aimed at determining stress levels through the Lipp Stress Symptom Inventory for Adults (ISSL)[12] and answered a questionnaire on environmental quality (WHOQOL-100 domain V),[13] which was applied retrospectively, i.e., patients were asked to answer the questions based on the time period before symptom onset.

The control sample consisted of 49 adult female individuals, with a mean age of 41.48 ± 10.78 years. All control individuals were evaluated regarding clinical symptoms and presence of tender points at palpation. The control sample only included individuals that did not meet the criteria for a clinical diagnosis of fibromyalgia, according to the ACR criteria.[1] Moreover, control individuals were submitted to a psychological assessment through the ISLL to determine stress levels and the volunteers also answered the questionnaire on environmental quality (WHOQOL-100 domain V).

All individuals included in the study live in the town of Novo Hamburgo, state of Rio Grande do Sul, Brazil and the FM patients are treated at the Physical Therapy Clinic and/or Extension Project that treats FM patients at Centro Universitário Feevale.

Genotyping methods

DNA from both samples was extracted from total blood, following the technique described by Lahiri and Nurnberger.[14] The *APOE* exon 4 polymorphism was genotyped through the PCR/RFLP technique, as described by de Andrade *et al.*,[15] at the Laboratory of Genetics and Molecular Biology of Centro Universitário Feevale. This genotyping method detects the presence of three possible alleles in the APOE gene, called E*2, E*3 E and*4, and their combination results in the possible existence of three homozygous genotypes (E*2/ E*2, E*3/ E*3 and E*4/ E*4), and three different heterozygous ones (E*2/ E*3, E*2/ E*4 and E*3/ E*4) in the sample.

Environment-related quality of life assessment method

The World Health Organization has developed a tool to evaluate quality of life through a multicentric project called WHOQOL-100. The Portuguese version of this tool has been developed at the Department of Psychiatry and Forensics of Universidade Federal do Rio Grande do Sul (UFRGS) and published by Fleck *et al.*[13]

The WHOQOL-100 consists of 6 domains, of which one – Domain V (Environment) – was selected for the present study. This domain evaluates the following aspects: physical safety and protection; home environment; financial resources; availability and quality of social and health care; opportunity to acquire new information and skills; participation in and opportunities of recreation and leisure activities; physical environment (pollution, noise, traffic, climate); and transportation.

Behavioral stress assessment method: the Lipp stress symptom inventory for adults (ISSL)

According to Lipp,[12] this inventory aims at objectively identifying whether the individual has stress symptoms and if they are predominantly physical or psychological. This inventory also establishes which stress phase the individual currently presents: alarm, resistance, near exhaustion and exhaustion. The alarm phase identifies the moment when the individual is getting ready to face the problem or flee from it, preserving his existence. At the second phase, resistance, the individual is trying to adapt in order to survive and find balance and sensations of weariness and fatigue appear. The third phase is that of almost exhaustion, when the individual cannot deal with unremitting stressor events and starts to get ill. When the situation becomes even worse, the fourth and last phase appears, exhaustion, in which all of the individual's adaptive energy has been spent and which determines the onset of the most severe diseases.

Statistical methods

The WHOQOL domain V score, as well as the separate assessment of each aspect, were carried out according to the test instructions (available at http://www.ufrgs.br/psiq/whoqol85.html). Thus, the final scores of each volunteer varied from 0 to 20 for each aspect and also in Domain V, in general.

To assess the genetic and environmental influence separately, *t* test and chi-square test were used. To assess genetic and environmental influence together, the multivariate logistic regression was used. The regression model used in the study was the "backward stepwise" model, after the removal of the least significant variable for each analysis, starting with the interactions. The simple variables included in the model were stress levels (codified as '1' - exhaustion or near exhaustion and '0' - no stress or resistance), WHOQOL domain V scores codified according to the 50th percentile, as '1' for values < 13.05, and '0' for values > 13.05) and APOE genotypes as two dummy variables (E*2 allele carriers and E*4 allele carriers). The tested interactions were all the possible ones among the simple variables included in the model (a total of five). All tests were carried out with the software SPSS for Windows, release 11.0.

Ethical implications

The present project was submitted to and approved by the Ethics Committee of Centro Universitário Feevale, # 2.02.02.06.346, and sample collection were started only after project approval. All volunteers signed the Free and Informed Consent Form before study enrollment.

RESULTS

Table 1 shows that stress levels differ significantly between FM patients and controls ($P < 0.001$). Most patients (62.5%) are at the "Exhaustion" or "Near Exhaustion" level, whereas this group corresponds to 8.6% of the control sample; moreover, almost half of the control individuals (48.9%) showed no stress, when compared to only 5% of the FM patients in the same category.

Table 1
Lipp Stress Symptom Inventory for Adults (ISSL): comparison of stress levels between patients and controls

Stress levels	Patients n = 40 (%)	Controls n = 47 (%)
No stress	2 (5)*	23 (48.9)
Alarm	5 (12.5)	1 (2.1)
Resistance	6 (15)*	19 (40.4)
Near exhaustion	22 (55)*	2 (4.3)
Exhaustion	5 (12.5)	2 (4.3)

$\chi^2 = 44.75$; $P < 0.001$. At the residual analysis, significant comparisons between patients and controls ($P < 0.01$)*.

The mean WHOQOL questionnaire scores for both groups are shown in Figure 1 and it is possible to observe that there was a statistically significant difference between patients and controls ($P < 0.001$, Figure 1), with controls presenting a better environmental domain quality (14.43 ± 1.96) when compared to patients (11.48 ± 2.15).

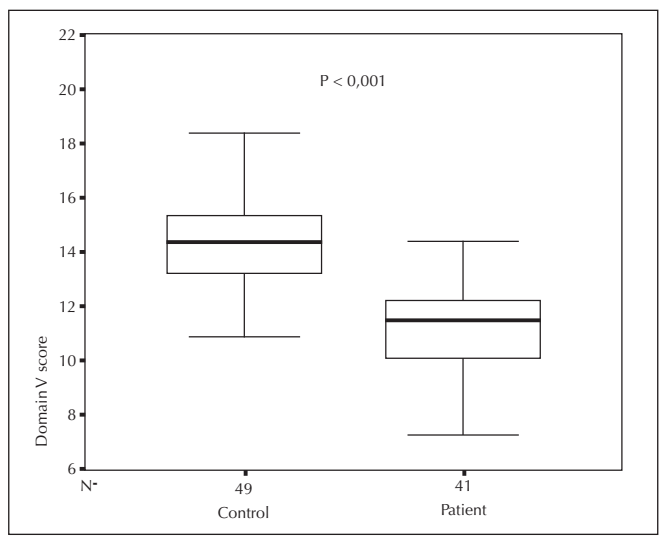

Figure 1
Boxplot of WHOQOL questionnaire Domain V scores in patients with FM (11.48 ± 2.15) and controls (14.43 ± 1.96), calculated from all means.

Table 2 shows the analysis of *APOE* polymorphism influence on FM susceptibility. Therefore, the frequencies of the three alleles (E*2, E*3 and E*4) and of four genotypes were compared between patient and control samples. This univariate analysis showed that, as there is no statistically significant difference, it was not possible to prove the isolated influence of this gene on the disease.

Aiming at evaluating whether the isolated factors investigated in the study (stress, environmental quality and *APOE* polymorphism) had any influence on FM when assessed together, a multivariate logistic regression was carried out, which included interaction variables. Table 3 shows the parameters of each model tested by the backward modeling technique. Based on these data, it is possible to observe that the parameters related to the different models are practically not modified after the removal of the interaction variables, and that all regressions performed are highly significant ($P < 0.001$). Therefore, the 5th model was chosen, as it contains the lowest confidence intervals for the odds ratio (OR) values. Thus, Table 4 demonstrates the most adequate regression model, with the interpretation of the identified interaction. These data confirmed the influence of the environment on the disease, as we identified individuals with the assessed domain scores < 13.05 (50th percentile), that is, individuals with bad environmental quality had a 57.7-higher chance of developing FM ($P = 0.001$). Similarly, the association between FM and stress was confirmed by this analysis (OR = 197.2; $P = 0.001$), but no interaction between stress levels and environment was identified. This analysis demonstrated that the presence of the two rare *APOE* alleles (E*2 and E*4) is not related, individually, with the chance of developing FM, but that there is a significant interaction between the presence of the E*2 allele and stress levels ($P = 0.028$). Therefore, the calculation of OR estimated in each group demonstrated that in the group of women who were *E*2*-allele carriers and had high stress levels, the chance of developing the disease was 1.06, whereas in the group of non-carriers, but who still had high stress levels, this chance increased to 265.1, thus demonstrating the protective role of the *E*2* allele presence.

Table 2
Comparison of *APOE* allelic and genotypic frequencies between patients and controls

	Patients (n = 38)	Controls (n = 40)	P
Alleles			
E*2	14.5%	12.5%	
E*3	84.2%	85%	0.82
E*4	1.3%	2.5%	
Genotypes			
E*2/E*2	-	1 (2.5%)	
E*2/E*3	11 (28.9%)	8 (20%)	0.59
E*3/E*3	26 (68.4%)	29 (72.5%)	
E*3/E*4	1 (2.7%)	2 (5%)	

DISCUSSION

The present study aimed at investigating the association between stress levels, environmental quality, APOE gene variation and how these variables interacted to influence FM susceptibility. As FM is a multifactorial etiology syndrome that remains poorly understood, the increase in the number of studies on its bases, physiological as well as genetic and environmental ones, is of utmost importance.

Table 3
Multiple logistic regression analysis: backward modeling technique

	Model 1	Model 2	Model 3	Model 4	Model 5
-2log Likelihood	33.89	33.89	33.89	34.54	36.44
χ^2	52.2 (P < 0.001)	52.2 (P < 0.001)	52.2 (P < 0.001)	51.5 (P < 0.001)	49.6 (P < 0.001)
$R^2x\ 100$	75.6	75.6	75.6	75.0	73.2
OR (95%CI)					
Whoqol[a]	53,473 (0-8.75 x 10^{43})	57,485 (0-3.15 x 10^{44})	54,108 (0-2.06 x 10^{43})	7,405,606 (0-3.95 x 10^{84})	57.7 (5.0-665.7)
Lipp[b]	133,683 (0-2.23 x 10^{44})	143,712 (0-8.01 x 10^{44})	135,270 (0-5.24 x 10^{43})	1.9 x 10^{7}(0-9.71 x 10^{84})	197.2 (8.6 – 4,507)
*E*2* carriers	11,140 (0-1.86 x 10^{43})	11,976 (0-6.66 x 10^{43})	11,272 (0-4.36 x 10^{42})	1,158.223 (0-6.24 x 10^{83})	5.5 (0.39-78.4)
*E*4* carriers	0.608 (0-4.13 x 10^{233})	0.653 (0-5.44 x 10^{233})	0 (0-8.86 x 10^{84})	0 (0-2.69 x 10^{51})	0.069 (0-23.0)
Lipp x *E*4*	14.67 (0-14.67)	-	-	-	-
Whoqol x *E*4*	0 (0-0)	0 (0- 1.08 x 10^{251})	-	-	-
Lipp x Whoqol	2,743 (0-7.41 x 10^{59})	3,282 (0-9.28 x 10^{61})	3,139 (0-5.1 x 10^{60})	-	-
Whoqol x *E*2*	0 (0-2.6 x 10^{35})	0 (0-8 x 10^{35})	0 (0-5.9 x 10^{34})	0 (0-1.1 x 10^{72})	-
Lipp x *E*2*	0 (0-3.48 10^{60})	0 (0-8.74 x 10^{59})	0 (0-2.69 x 10^{58})	0 (0-4.1 x 10^{70})	0.004 (0-0.55)

[a] Total scores of WHOQOL domain V were divided according with the 50th percentile as < 13.05 (1) and > 13.05 (0);
[b] Stress levels were recodified as exhaustion or near exhaustion (1) and no stress or resistance (0).

Table 4
Parameters of the multiple logistic regression analysis best model

	OR (95% CI)	P	beta
WHOQOL[a]	57.7 (5.0 – 665.7)	0.001	4.06
Lipp[b]	197.2 (8.6 – 4.507)	0.001	5.28
*E*2* carriers	5.5 (0.39 – 78.4)	0.205	1.71
*E*4* carriers	0.069 (0 – 23.0)	0.367	-2.68
Lipp[b] x *E*2*	**0.004 (0 – 0.55)**	**0.028**	**-5.50**

Interpretation of Lipp[a] x *E*2* interaction	**beta**	**OR**
- high stress levels (**beta Lipp = 1**) and E*2 carriers (**beta Lipp[a] x *E*2 = 1***) = 5.28 + (-5.50)	0.06	**1.06**
- high stress levels (**beta Lipp = 1**) and E*2 non-carriers (**beta Lipp[a] x *E*2 = 0***) = 5.28 + 0	5.58	**265.1**

[a] Total scores of WHOQOL domain V were divided according with the 50th percentile as < 13.05 (1) and > 13.05 (0);
[b] Stress levels were recodified as exhaustion or near exhaustion (1) and no stress or resistance (0).

The use of Lipp Stress Symptom Inventory for Adults (ISSL) showed a high stress level among patients. Although the association between fibromyalgia and stress has been pointed out in the literature[3,4], to date no study had investigated this association in Brazilian patients. The present study demonstrated a very high number (95%) of women with FM that presented some level of stress, when compared to 51.2% in the control sample. These figures are much higher than those identified in other Brazilian studies. Moreira *et al.*[16], when investigating infertile women, identified that almost 62% presented some level of stress. Santos and Alves Jr.,[17] when evaluating Master's Degree students in the state of Sergipe, Brazil, identified a value of 40.7%, although with a significant variation between the genders: 80% of the women and 18% of the men. Another study that demonstrated that stress is more frequently observed in women is the one performed by Calais *et al.*[18], in a sample of young individuals from the city of Campinas, state of Sao Paulo, Brazil, showing that 79% of young female individuals had stress, when compared to 52% of young male individuals. Therefore, the data demonstrated that the association between stress and FM is quite strong, as to date, the highest frequency of stress found in Brazilian studies was that observed in our sample.

However, some studies have shown that women are much more prone to stress and as our sample consists of women only, that could have been a confounding factor. To test this hypothesis, our sample was compared to a control sample that also consisted exclusively of women, whose identified stress frequency (51.2%) was within a variation range that had been previously identified by other studies with healthy women, of around 36%.[16,17]

Regarding stress levels, a disturbing proportion of our patient sample was at the two upper stress levels – exhaustion and near exhaustion 67.5%), when compared to 8.6% in our control sample. These figures are higher than all data found in the Brazilian literature: among infertile women, this frequency was 12.7%,[16] whereas in healthy women this frequency was zero or close to zero in young individuals,[17,18] 7.4% among adult women,[16] up to 15% and 23% in adult individuals living in Sao Paulo (capital and countryside, respectively).[19] Therefore, we realize that the stress data identified by the present analysis are within the variation range already identified for healthy individuals, but are much higher than any previously recorded value, when related to FM patients. This fact demonstrates the important association between the disease and stress, but unfortunately, it is not possible to suggest a cause-and-effect relation, as both possibilities are likely: FM can increase the chance of developing stress, as well as stress onset can increase the chance of developing FM. That is a hypothesis that must be further tested with longitudinal and prospective studies.

The existing literature demonstrates that FM is a multifactorial disease and thus, the WHOQOL domain V was chosen to assess the association between the environment and the chance of disease onset. As the type of question used in this tool allows it to be adapted for a past period of time, this method was used to perform a retrospective investigation and, therefore, the questionnaire was applied to patients based on the period prior to the disease development. This approach allowed the final results on the environment to be correlated with the disease onset, and not as a consequence of the disease. Hence, it was possible to detect a significantly lower environmental quality in patients, when compared to the control sample ($P < 0.001$). Evidently, as in any other retrospective study, there is a possibility that the patients were not capable of consistently answering the questionnaire, based on their healthy life period, but were influenced by the disease. On the other hand, a subjective analysis of the researchers that applied the tool demonstrated that the patients tended to be excessively optimistic regarding the period prior to symptom onset, as they compared their lives with the current period. Nevertheless, the data demonstrated a strong association between environment and

FM, and the values observed for patients and controls are within the variation range found in the literature.[20-23]

The association between the WHOQOL domain V scores and other diseases, although not assessed retrospectively, has been investigated by several studies carried out worldwide. However, few studies have identified a significant association between the domain V scores and some disease or characteristic. The association between decreased environmental quality and Parkinson's disease has been identified by Valeikiene *et al.*,[23] although Schestatsky *et al.*[24] did not identify any difference between patients and their caregivers. One investigation with automobile factory workers from Malaysia demonstrated that the domain V scores were positively correlated with work control, social support, physical health, psychological status and social relations, and negatively correlated with work demand, stress, anxiety and depression.[22] On the other hand, this domain scores were not significantly associated with treatment adherence for type 2 *diabetes mellitus*,[25] osteoarthritis,[23] successful aging process,[26] physical exercises in radiotherapy patients,[21] narcolepsy[27] and systemic lupus erythematosus.[28] Most of these studies determined significant associations between the investigated diseases and the scores of other WHOQOL domains, demonstrating that the tool is efficient, but that apparently, the environment is not associated with these characteristics. Thus, this analysis specifically demonstrated the importance of the association between the environment and FM, as we found a significant association that is not always found in the literature, and had not been investigated regarding this disease in any previous international study.

The last objective of the present study was to assess whether the variation in the apolipoprotein E gene could be associated with FM onset. This gene was chosen due to its role in stress response and also due to the pain threshold modification, demonstrated in both human studies and animal models.[8-11]

Additionally, a brief literature review showed that the polymorphism found in this gene is linked to the increased susceptibility to several diseases, such as Alzheimer's,[29,30] Parkinson's,[31] cholelithiasis,[32] atherosclerosis,[33] bone density,[34] rheumatoid arthritis,[35] depression in elderly,[36] schizophrenia and bipolar disorder,[37] among others. Still, to date, no study had tried to determine the association between the APOE gene and FM susceptibility in any population, although other genes have been associated with the disease, such as the 5HT2A serotonin receptor[6] and the HLA region.[38] The present study data did not demonstrate a significant association between the presence of the gene rare alleles (E*2 and E*4) and FM susceptibility (Table 3). However, when a multivariate analysis was performed, seeking to detect an association between FM, the APOE gene, environmental quality and stress levels, a significant interaction was identified between the presence of the E*2 allele and stress levels (Table 4).

There have been no studies in the literature that can be compared with this analysis. Moreover, caution is necessary when interpreting these data due to limitations inherent to the Lipp questionnaire and the low statistical power caused by the small sample size, which results in an excessive increase in identified OR values. Therefore, although our data suggest an interaction between stress and APOE gene, caution is necessary when interpreting them, especially regarding the magnitude of the estimated OR values.

Firstly, as the applied stress inventory is adequate to detect current symptoms, or at most, of the previous days, it has the limitation of not being able to be applied retrospectively. Therefore, it becomes impossible to establish a causal relation for the strong association between stress and fibromyalgia, so that either of the two characteristics can trigger the other. Therefore, the protective role of the E*2 allele presence can be interpreted as protection against the disease onset in women with high stress levels or as protection against high-stress level development in patients with fibromyalgia. The only way to test these hypotheses would be to carry out a prospective study, which must be greatly encouraged regarding future research on this subject. Additionally, there is obviously a possibility that the present data reflect a type I error, caused by the small sample size.

In spite of the discussed limitations, the importance of the present study lies mainly in the fact that it is the first one to investigate the association between the APOE gene and fibromyalgia. The data shown here have demonstrated a significant influence of the E*2 allele, of which presence acts as a protective factor for the association between stress and fibromyalgia. However, determining which of the two events was the causative agent was not the objective of the present study. Moreover, a review of apo E physiological role demonstrates that it has influence on stress physiology, at least in animal models, which supports the influence identified in our sample. However, we believe that interaction identified in the present study can be used as a basis for hypothesis investigation in larger samples, thus contributing to the increase in knowledge on fibromyalgia etiology.

REFERÊNCIAS
REFERENCES

1. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL. The American College of Rheumatology 1990 criteria for the classification of fibromyalgia: report of the multicenter criteria committee. Arthritis Rheum 1990; 33(2):160-72.
2. Neumann L, Buskila D. Epidemiology of Fibromyalgia. Current Pain and Headache Reports, 2003; 7:362-8.

3. Buskila D. Genetics of chronic pain states. Best Pract Res Clin Rheumatol 2007; 21(3):535-47.
4. Wood BP. Stress and dopamine: implications for the pathophysiology of chronic widespread pain. Med Hypotheses 2004; 62:420-4.
5. van Houdenhove B, Egle U, Luyten P. The Role of Life Stress in Fibromyalgia. Curr Rheumatol Rep 2005; 7:365-70.
6. Bondy B, Spaeth M, Offenbaecher M, Glatzeder K, Stratz T, Schwarz M *et al*. The T102C polymophism of the 5-HT2A-Receptor Gene in Fibromyalgia. Neurobiol Dis 1999; 6:433-9.
7. Frank B, Niesler B, Bondy B, Späth M, Pongratz DE, Ackenheil M *et al.* Mutational analysis of serotonin receptor genes: HTR3A and HTR3B in fibromyalgia patients. Clin Rheumatol 2004; 23:338-44.
8. VanderPutten DM, Cameron BM, Merril CR. Increased apolipoprotein-E concentrations in individuals suffering chronic low back syndrome identified by two-dimensional gel electrophoresis. Appl Theor Electrophor 1993; 3:247-52.
9. Cameron BM, VanderPutten DM, Merril CR. Preliminary study of an increase of a plasma apolipoprotein E variant associated with peripheral nerve damage. A finding in patients with chronic spinal pain. Spine 1995; 20:581-9.
10. Gordon I, Ben-Eliyahu S, Rosenne E, Sehayek E, Michaelson DM. Derangement in stress response of apolipoprotein E-deficient mice. Neurosci Letters 1996; 206:212-4.
11. Choudhuri R, Cui L, Yong C, Bowyer S, Klein RM, Welch KM *et al*. Cortical spreading depression and gene regulation: relevance to migraine. Ann Neurol 2002; 51:499-506.
12. Lipp MN. Manual do inventário de sintomas de stress de Lipp. São Paulo: Casa do Psicólogo, 2000.
13. Fleck M, Louzada S, Xavier M, Chachamovich E, Vieira G, Santos L *et al.* Desenvolvimento e aplicação da versão em português do instrumento de avaliação de qualidade de vida da Organização Mundial da Saúde (WHOQOL-100). Revista HCPA 1999; 19(1):9-24.
14. Lahiri DK, Nurnberger JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 1991; 19:5444.
15. de Andrade FM, Larrandaburu M, Callegari-Jacques SM, Gastaldo G, Hutz MH. Association of apolipoprotein E polymorphism with plasma lipids and Alzheimer´s disease in a Southern Brazilian population. Br J Med Biol Res 2000; 33:529-37.
16. Moreira SNT, Melo COM, Tomaz G, de Azevedo GD. Estresse e ansiedade em mulheres inférteis. Rev Bras Ginecol Obstet 2006; 28(6): 358-64.
17. Santos AF, Alves Jr A. Estresse e estratégias de enfrentamento em mestrandos de ciências da saúde. Psicologia: Reflexão e crítica 2007; 20 (1):104-13.
18. Calais SL, de Andrade LMB, Lipp MEN. Diferenças de Sexo e Escolaridade na Manifestação de Stress em Adultos Jovens. Psicologia: Reflexão e crítica, 2003; 16(2):257-63.
19. da Silva EAT, Martinez A. Diferença em nível de *stress* em duas amostras: capital e interior do estado de São Paulo. Estudos de Psicologia Campinas, 2005; 22(1):53-61.
20. dos Santos ECM, França Jr I, Lopes F. Quality of life of people living with HIV/AIDS in São Paulo, Brazil. Rev Saúde Pública 2007; 41(Supl. 2):1-7.
21. Hwang JH, Chang HJ, Shim YH, Park WH, Park W, Huh SJ *et al*. Effects of Supervised Exercise Therapy in Patients Receiving Radiotherapy for Breast Cancer. Yonsei Med J 2008; 49(3):443-50.
22. Rusli BN, Edimansyah BA, Naing L. Working conditions, self-perceived stress, anxiety, depression and quality of life: A structural equation modelling approach. BMC Public Health 2008; 8:48.
23. Valeikiene V, Ceremnych J, Alekna V, Juozulynas A. Differences in WHOQOL-100 domain scores in Parkinson´s disease and osteoarthritis. Med Sci Monit 2008; 14(4):CR221-7.
24. Schestatsky P, Zanatto VC, Margis R, Chachamovich E, Reche M, Batista RG *et al.* Quality of life in a Brazilian sample of patients with Parkinson's disease and their caregivers. Rev Bras Psiquiatr 2006; 28(3):209-11.
25. Martínez YV, Prado-Aguilar CA, Rascón-Pacheco RA, Valdivia-Martínez JJ. Quality of life associated with treatment adherence in patients with type 2 diabetes: a cross-sectional study. BMC Health Services Research 2008; 8:164.
26. de Moraes JFD, de Azevedo e Souza VB. Factors associated with the successful aging of the socially-active elderly in the metropolitan region of Porto Alegre. Rev Bras Psiquiatr 2005; 27(4):302-8.
27. Rovere H, Rossini S, Reimão R. Quality of life in patients with narcolepsy - a WHOQOL-Bref study. Arq Neuropsiquiatr 2008; 66(2-A):163-7.
28. Khanna S, Pal H, Pandey RM, Handa R. The relationship between disease activity and quality of life in systemic lupus erythematosus. Rheumatology 2004; 43:1536-40.
29. Laws SM, Hone E, Gandy S, Martins RN. Expanding the association between the APOE gene and the risk of Alzheimer´s disease: possible roles for APOE promoter polymorphisms and alterations in APOE transcription. J Neurochem 2003; 84:1215-36.
30. Bertram L, Mcqueen MB, Mullin K, Blacker D, Tanzi R. Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. Nat Genet 2007; 39:17-23.
31. Huang X, Chen PC, Poole C. APOE-[epsilon] 2 allele associated with higher prevalence of sporadic Parkinson disease. Neurology 2004; 62:2198-202.
32. Hasegawa K, Terada S, Kubota K, Itakura H, Imamura H, Ohnishi S *et al*. Effect of apolipoprotein E polymorphism on bile lipid composition and the formation of cholesterol gallstone. Am J Gastroenterol 2003; 98:1605-9.
33. Paternoster L, González NA, Lewis S, Sudlow C. Association between apolipoprotein E genotype and carotid intima-media thickness may suggest a specific effect on large artery atherothrombotic stroke. Stroke 2008; 39:48-54.
34. Lee SI, Lee SY, Yoo WH. Association of apolipoprotein E polymorphism with bone mineral density in postmenopausal women with rheumatoid arthritis. Rheumatology (Oxford) 2005; 44:1067-8.
35. Maury CP, Liljeström M, Tiitinen S, Laiho K, Kaarela K, Ehnholm C. Apolipoprotein E phenotypes in rheumatoid arthritis with or without amyloidosis. Amyloid 2001; 8:270-3.
36. Yen YC, Rebok GW, Gallo JJ, Yang MJ, Lung FW, Shih CH. ApoE4 allele is associated with late-life depression: a population-based study. Am J Geriatr Psychiatry 2007; 15:858-68.
37. Digney A, Keriakous D, Scarr E, Thomas E, Dean B. Differential changes in apolipoprotein E in schizophrenia and bipolar I disorder. Biol Psychiatry 2005; 57:711-5.
38. Yunus MB, Khan MA, Rawlings KK, Green JR, Oslon JM, Sahah S. Genetic linkage analysis of multicase families with fibromyalgia syndrome. J. Rheumatol.1999; 26:408-12.