

Does human serotonin-1A receptor polymorphism (rs6295) code for pain and associated symptoms in fibromyalgia syndrome?

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SUMMARY

Genetic predisposition may play an important role in the development of fibromyalgia syndrome (FMS). Serotonin is known to be involved in pain modulation and serotonin-1A receptor plays a considerable role in determining the central 5-HT tone. Consequently, variation in 5-HT_{1A} receptor gene (*HTR1A*) may be responsible for inter-individual variability in pain sensitivity and other clinical symptoms of FMS. Therefore, the objectives of this research work were to study the gene polymorphism of 5-*HTR1A* gene and to explore the correlation between rs6295 genotype (-1019C/G *HTR1A*) and duration of pain, pain intensity and pain related depression and anxiety, if any, in FMS.

5-*HTR1A* genotype for the C(-1019)G polymorphism was typed in 62 patients with FMS and 42 healthy subjects. Present pain intensity, components of pain and pain related depression and anxiety were assessed using the numerical pain rating scale, McGill pain questionnaire and Hamilton depression and anxiety rating scale respectively.

5-*HTR1A* gene was represented by three different genotypes, homozygous C/C, heterozygous C/G and homozygous G/G. Analysis of the 5-*HTR1A* gene showed a frequency of 58%, 31% and 11% for the C/C, C/G and G/G genotypes, respectively in FMS group. This proportion was 69%, 23% and 8% in healthy subjects. No significant correlation was observed between 5-*HTR1A* gene polymorphism and pain and related symptoms in FMS patients.

To the best of our knowledge this is the first study which investigated the correlation between the 5-*HTR1A* gene polymorphism and pain intensity, the affective component of pain, pain related depression and anxiety in FMS.

Key words: Fibromyalgia; serotonin-1A receptor polymorphism (rs6295); pain intensity, pain related depression and anxiety.

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■ INTRODUCTION

Fibromyalgia syndrome (FMS) is a severely debilitating chronic pain syndrome. It is defined by widespread musculoskeletal pain, for more than three months, and accompanied by auxiliary symptoms, like joint stiffness, chronic fatigue, unrefreshing sleep, depression, anxiety and cognitive malfunction (1). Genetic predisposition plays an important role in the progression of FMS (2). Previous studies on genetic vulnerability in FMS reported a dysregulation of central serotonergic metabolism, and reduced serotonin (5-HT)

metabolites in the cerebrospinal fluid (3). Several studies have also shown potential association between FMS and polymorphisms in serotonin-related genes, further corroborating the fact that the serotonergic system is markedly involved in the modulation of homeostatic, affective and pain-related mechanisms (4). A previous study has suggested that serotonin is essential in the regulation of anxiety, fear, and also in the tendency to commit suicide and other violent actions (5). These behaviours are mediated by at least 14 different serotonin receptors (6). Additionally, the serotonin-1A receptor (5-*HTR1A*) has been shown to

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help in detecting anxiety disorders as proven by the results of a clinical study showing that selective 5-HT1A receptor agonists are common tranquilizers (7).

5-HT1A receptors are vastly expressed in brain areas such as orbitofrontal cortex, amygdala, hippocampus, insula, cingulate cortex and midbrain raphe areas which are associated with affective and emotional processes (8). Altered 5-HT1A levels are also observed in major depressive disorder (9), bipolar disorder (10) and anxiety disorder (11).

Clinically, the partial 5-HT1A agonists and serotonin reuptake inhibitor are used to treat anxiety disorders (12) and depression (13).

Many genetic studies demonstrated that GG genotype or G allele carriers of the 5-HT1A gene promoter polymorphisms are associated with depression (5, 9) and may influence the response to antidepressant therapy (14, 15). Studies on major depression, anxiety, panic disorder, suicide and schizophrenia have also indicated the involvement of the G allele as a 'risk allele' (5, 14, 16).

There is considerable concomitance between chronic pain and affective disorders (17) suggesting interlinked neurobiological pathways, such as potentially serotonergic deregulation (18).

These findings suggest that the relationship between FMS and polymorphisms in serotonin-related genes needs further clarification and might be favoured by psychiatric comorbidity. Though many studies have been conducted to find the involvement of the genes related to serotonergic pathways related to pain modulation, limited work has been focused on 5-HT1A receptor gene polymorphism in FMS. In our literature search, no study has observed the effect of 5-HTR1A (rs6295) interaction on pain, its various affective components, pain-related depression and anxiety. Therefore, in this study, HTR1A gene polymorphism was compared between patients with FMS and healthy volunteers and correlated with pain intensity, the affective component of pain, pain-related depression and anxiety.

■ MATERIALS AND METHODS

Patient enrollment

The study was conducted in the Pain Research and TMS Laboratory, Department of Physiology, All India Institute of Medical Sciences (AIIMS), New Delhi and was approved by the Institute Human Ethics committee (Ref No: IESC/T-251/15.06.2013). The current data is a part of one of the objectives of a doctoral thesis which included the investigation on the effect of repetitive transcranial magnetic stimulation on pain and its modulation (which was performed with subjective, objective pain assessment tools and biochemical markers) in FMS patients (24). All procedures performed during the study involving patients were in accordance with the ethical standards of the Human Ethics committee of the AIIMS, New Delhi.

Female patients with FMS (age 18-50 years) having regular menstrual cycle were recruited from the Rheumatology Clinic, at the Department of Rheumatology. The enrollment was based on the American College of Rheumatology (ACR) 1990 criteria for the classification of FMS (19). Age and gender matched healthy subjects were recruited among patient relatives and Institute staff. Only the subjects who were able to give written informed consent were enrolled in the study. The FMS patients were excluded from the study, if they were:

- 1) pregnant or lactating;
- 2) having irregular menstrual cycle;
- 3) having chronic systemic disease, inflammatory joint diseases, secondary FMS, seizures, history of trauma;
- 4) with a concomitant diagnosis of chronic fatigue syndrome and/or major psychiatric disorder, currently undergoing psychotherapy.

Subjective assessment of pain and related symptoms:

The pain intensity was evaluated by Numerical Pain Rating Scale (NPRS) or NRS which is a 11-point numerical scale ranging from '0' meaning 'no pain' to '10' meaning 'pain as bad as you can imagine' or 'worst pain imaginable' (20). The multidimensional components of pain were

assessed by McGill pain questionnaire (MPQ), which is designed to measure the sensory, affective and evaluative aspects of pain in adults suffering from chronic pain. The scale consists of 78 descriptive items categorized into 20 subclasses evaluating sensory (question 1 to 10), affective (question 11 to 15), evaluative (question 16) and miscellaneous components of pain (question 17 to 20) (21). Pain-related anxiety was evaluated by Hamilton Anxiety Rating Scale (HARS). It contains 14 items, each defined by a series of symptoms, and measures both psychic anxiety (psychological distress and mental agitation) and somatic anxiety (physical complaints related to anxiety). Each item is scored on a scale of 0 (not present) to 4 (severe), with a total score range of 0-56, where <17 indicates mild anxiety, 18-24 mild to moderate anxiety and 25-30 moderate to severe anxiety (22). Pain-related depression was studied by utilizing Hamilton Depression Rating Scale (HDRS). Eight items are scored on a 5-point scale, ranging from 0, 'not present' to 4, 'severe'. Nine items are scored 0-2. First 17 items are added to obtain the total score (23).

Genotyping

Blood samples were collected in EDTA tubes and stored at -80°C until further use. DNA was extracted by using a DNA isolation kit (Qiagen) for the study of single nucleotide polymorphism. The DNA samples were stored at -80°C for further analysis. For genotyping of the rs6295 (-109C/G); A 544-bp fragment of the 5-HT1A gene was amplified using primers 5-HTR1A Forward: 5'-CACAAACGTAGTGGGAAGTGGC-3' and 5-HTR1A Reverse: 5'-GGAATTCCAACACTCCTTGCCT-3'. The reaction mixture contained 100 ng DNA, 2.5 pM of each primer, 100 mM dNTPs and 1 U Taq polymerase in a final reaction volume of 25- μ l. PCR amplification was carried out in a S1000TM thermal cycler under the following conditions: i. Initial denaturation: 95°C for 3 min, ii. 35 cycles of denaturation at 95°C for 30-s, iii. Annealing at 65°C for 40-s iv. Extension at 72°C for 60-s v. Final extension step at 72°C

for 5 min. vi. PCR products were checked for amplification on 1.5% agarose gel.

Restriction fragment length polymorphism (RFLP) analysis:

Amplified PCR products were digested with 2.5 U BtsCI (Fermentas, USA) and incubated at 37°C for 35 min and separated on 2.5% agarose gel. The 5-HTR1A-CC genotype was represented by fragments of 544bp, the 5-HTR1A-CG genotype by fragments of 544bp, 488bp and 56bp and 5-HTR1A-GG genotype by fragments of 488bp and 56bp.

Statistical analysis

All the analyses were performed using IBM SPSS Statistics version 22.0 (IBM Statistics for Windows, Chicago, IL, USA) and graphs were created using Graphpad Prism version 5.01 for Windows, (Graph-Pad Software, San Diego, California, USA). All data was checked for normality using D'Agostino and Pearson's omnibus normality test. The Baseline scores of the parameters between healthy subjects and FMS groups were compared using unpaired t test/ Mann-Whitney U test. Chi-Square test was applied to assess statistical difference in frequency of genotypes of 5-HT1A gene between healthy subjects and FMS patients. One Way ANOVA was applied to assess the statistical significance among the three genotypes with Tukey's Multiple Comparison Test. The p value of 0.05 or less was considered significant.

RESULTS

The subjective pain measures and blood samples were collected from 62 FMS patients and 42 healthy subjects [(n=37) non-relative controls; (n=5) relatives of FMS patients]. 5-HT1A gene was represented by three different genotypes, homozygous C/C, heterozygous C/G and homozygous G/G. In FMS, the analysis of the 5-HT1A gene showed a frequency of 58%, 31% and 11% for the C/C, C/G and G/G genotypes, respectively. This proportion was 69%, 23% and 8% in the healthy subjects. There was no significant difference (p=0.27) between the two groups. However, the analysis demonstrated that the wild type ho-

mozygous genotype is higher compared to the heterozygous genotype both in healthy subjects and FMS patients (Table I). There was a significant statistical difference in ratings of NPRS, HDRS and HARS between healthy subjects and FMS patients (Table I). We also compared the NPRS, MPQ, HDRS and HARS ratings among the three genotypes, *i.e.* C/C, C/G and G/G of FMS patients. Ratings for pain and asso-

Table I - Comparison of general characteristics and rs6295 genotype (-1019C/G HTR1A) of serotonin receptor gene (5-HTR1A) in fibromyalgia (FMS) patients and healthy controls.

Parameters	Units/Sub parameters	Healthy controls (n=42)	FMS patients (n=62)	*p
Age	Years	37.69±7.757	40.68±8.00	0.06
Height	cm	157.0±6.46	155.9±5.584	0.35
Weight	Kg	64.71±10.80	62.52±7.660	0.23
Systolic BP	mm/Hg	115.0±6.65	116.2±8.75	0.46
Diastolic BP	mm/Hg	77.69±7.01	76.98±6.94	0.53
Pulse	n/Min	65.0±7.86	67.27±8.39	0.16
Duration of pain	Year	-	8.435±5.585	-
Tender points	Number	0.93±1.31	14.0±2.76	0.0001
Ethnicity	-	Indo-Aryan	Indo-Aryan	-
Marital status	Unmarried	5%	67%	-
	Married	92%	31%	-
	Divorcee	3%	2%	-
Educational status	Illiterate	2%	15%	-
	Primary school	5%	24%	-
	High school	9%	18%	-
	Senior secondary	9%	9%	-
	Graduation	35%	29%	-
	Post-graduation	40%	5%	-
Employment status	Home-maker	15%	49%	-
	Student	41%	5%	-
	Employed	39%	35%	-
	Self employed	5%	11%	-
NPRS ratings	-	0.42±0.71	7.38±1.10	0.0001
	Sensory	-	23.19±4.91	-
	Affective	-	7.000±2.75	-
MPQ ratings	Evaluative	-	3.210±0.8896	-
	Miscellaneous	-	6.839±3.189	-
	Total pain ratings	-	40.24±6.201	-
	Present pain intensity	-	2.968±0.8291	-
HDRS ratings	-	8.60±3.12	20.58±5.32	0.0001
HARS ratings	-	12.55±4.01	22.31±6.24	0.0001
Genotype percentage	CC	69%	58%	0.27
	CG	23%	31%	
	GG	8%	11%	

Data is presented as Mean±SD or percent (%). p<0.05 was considered to be statistically significant.

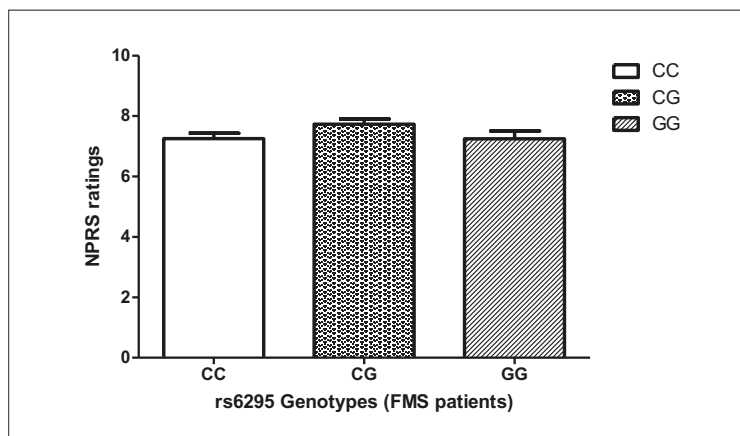


Figure 1 - NPRS ratings of rs6295 genotypes (CC, CG, GG) of the 5-HTR1A gene among FMS patients.

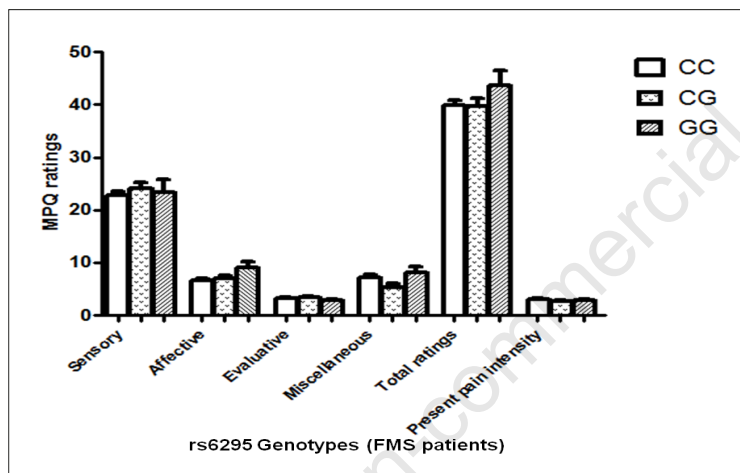


Figure 2 - McGill pain questionnaire (MPQ) ratings of rs6295 genotypes (CC, CG, GG) of the 5-HTR1A gene among FMS patients.

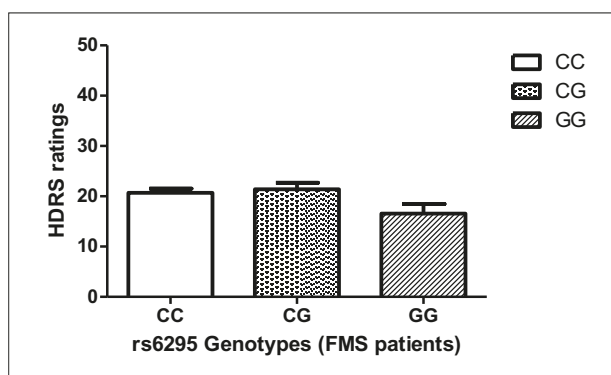


Figure 3 - Hamilton depression rating scale (HDRS) ratings of rs6295 genotypes (CC, CG, GG) of the 5-HTR1A gene among FMS patients.

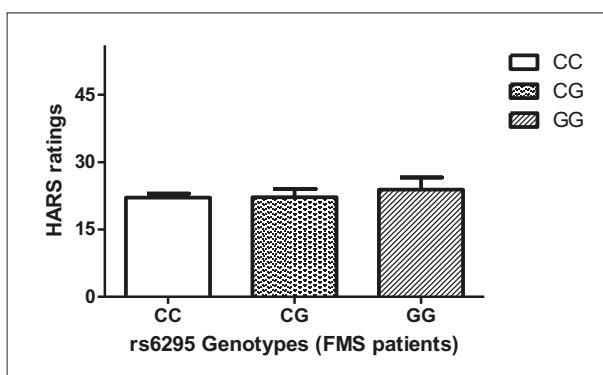


Figure 4 - Hamilton anxiety rating scale (HARS) ratings of rs6295 genotypes (CC, CG, GG) of the 5-HTR1A gene among FMS patients.

ciated symptoms were comparable among the C/C, C/G and G/G of FMS patients (Figures 1-4).

DISCUSSION

In the current study, rs6295 genotypes of 5-HTR1A gene were compared between healthy subjects (n=42) and FMS group (n=62) and no significant difference was observed between them. Numerical Pain Rating Scale, McGill Pain questionnaire, Hamilton Depression and anxiety scale ratings were also comparable among the three genotypes, i.e. C/C, C/G and G/G of FMS patients. Our findings are in line with previous research that could not observe an association between 5-HTR1A gene polymorphism and major depression (16) and anxiety in alcohol-dependent subjects (34). In contrast with our findings, a previous study reported a strong relationship between the G allele with anxiety-related features of neuroticism and harm avoidance in healthy participants (16). Previous research has shown that G-allele of HTR1A gene polymorphism was found to be over expressed in suicide (5), and was related to reduced sensitivity to drugs used for depression (14). Another study on the gene polymorphism of the substance P receptor (TACR1), dopamine transporter (DAT) and alpha1-antitrypsin (AAT) did not report any variations among the FMS patients suggesting that these genes may not be directly

linked to the etiopathology of FMS (25). A study on serotonin receptors (1A and 2A) has previously shown that gene promoter variations have gender-specific modulatory sequels on the depression of pain patients (26). These studies inferred that pain sensitivity, mood regulation and stress response can have common inherited-genetic factors; however, we could not find such a correlation. In a recent animal study with an intermittent psychological stress (IPS) model, the authors suggested that the lysophosphatidic acid receptor 1 (*LPA1*) gene plays a substantial role in IPS-induced fibromyalgia-like pain (27). These results suggest that the effect of 5-HTR1A on pain, depression and anxiety could be influenced by many other factors. The impact of 5-HTR1A on perception and/or its inhibition of pain is probably indirectly modulated by 5-HT1A gene polymorphism and also associated with other serotonin receptors genes. This may explain our negative results. However, the probable effect of 5-HT1A gene polymorphism on pain and pain-related depression and anxiety needs to be explored. Furthermore, serotonin affects pain modulation by genes controlling its metabolic enzymes/receptors, like serotonin2A or 5-HT3R, but not by genes managing its 5HT1A receptor (28). This has been indicated by the effectiveness of 5-HT3 antagonists in the treatment of FMS (29). Additionally, an earlier study (30) observed a relationship between the 5-HT2A receptor gene polymorphism and FMS though other authors could not identify such an association (31). A recent study evaluated the effects of mu-opioid receptor (*OPRM1*) gene, the serotonin transporter (*5-HTT*) gene (*5-HTTLPR*) and serotonin-1a receptor (*5-HT1a*) gene on exercise-induced hypoalgesia (EIH); and observed no specific impact on EIH in FMS patients, but rather an interaction between these genes and mu-opioid receptor signaling (32). Therefore, it can be inferred that serotonin does not produce elementary influences on pain, but causes various changes on pain depending on the downstream receptors (28).

Moreover, genetic factors are known to affect vulnerability to FMS, but no particular

genes have been convincingly associated with the pathogenesis of FMS (33). Since our study was conducted on a limited sample size, it is not possible to comment upon the correlation between genetic predisposition and FMS. Other probable reasons for these findings could be the inclusion of FMS patient relatives as controls and the lack of functional analysis of the gene studied. In addition, only a limited number of genetic studies with 5-HTR1A and FMS can be found in the literature. The correlation of serotonin transporter gene polymorphisms and other 5-HT receptor genes with pain, pain-related depression and anxiety should be explored in the future. We are the first to evaluate the correlation between 5-HTR1A gene polymorphism and pain intensity, the affective component of pain, pain-related depression and anxiety.

■ CONCLUSIONS

We could not observe a correlation between the 5-HTR1A gene polymorphism and the main features of FMS (chronic pain, pain-related depression/anxiety) as studied with NPRS, MPQ, HDRS and HARS. Moreover, the frequencies of 5-HTR1A genotypes between patients with FMS and healthy subjects were also comparable. We could not find a significant relationship between the 5-HTR1A polymorphism and FMS pain and associated symptoms. Our findings do not prove the hypothesis that pain and related symptoms of fibromyalgia syndrome are modulated by 5-HTR1A gene polymorphism. Therefore, more such studies should be undertaken in the future to draw firm conclusions.

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Conflict of interests

The authors declare no potential conflict of interests.

Authors' contributions

RB takes responsibility for the integrity of the work as a whole, from inception of idea to final manuscript. ST, UK, RD and RB made primary contributions in study design, data acquisition, analysis and interpretation of data and preparation of the final manuscript. BM made notable contributions in data acquisition, analysis, interpretation and preparation of the final manuscript along with its critical revision. All authors discussed the results and approved the final version to be submitted for publication.

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