ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com
Journal DOI: 10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

SNP-SNP interactions within catechol-O-methyltransferase (COMT) gene influence sleep quality in subjects having chronic musculoskeletal pain-A Genetic Exploration of Musculoskeletal Pain Study (GEMPS).

Harjot Dhillon[1], Shallu Khullar[1], Gurpreet Kaur[1], Ritu Sharma[1], Kanchan Mehta[1], JPS Walia[3], Monica Singh[1,2] and *Puneetpal Singh[1,2].

1. Department of Human Genetics, Punjabi University, Patiala.
2. Division of Pain Genetics, Molecular Genetics Lab, Department of Human Genetics, Punjabi University, Patiala.
3. Department of Orthopedics, Government Rajindra Medical College and Hospital, Patiala.

Manuscript Info

Manuscript History:

Received: 12 May 2016
Final Accepted: 19 June 2016
Published Online: July 2016

Key words:

Corresponding Author

Puneetpal Singh.

Abstract

Objective: To investigate the role and relevance of genetic variants within catechol-o-methyltransferase (COMT) gene as the genetic determinant of sleep quality in musculoskeletal pain, which remains to be examined in the population of Punjab, India.

Methods: The present cross sectional study included 493 subjects within age range of 35-65 years from tertiary health care hospitals of Punjab. Musculoskeletal disorders and associated chronic pain was assessed by Nordic Pain Questionnaire (NPQ) and Numerical rating Scale (NRS). Sleep quality was examined using Pittsburgh Sleep Quality Index (PSQI). Contribution of four relevant COMT single nucleotide polymorphisms (SNPs): rs4680 (exon4, A/G), rs4633 (exon3, C/T), rs4818 (exon4, C/G) and rs6269 (exon3, A/G) to sleep quality was investigated by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: Individually, SNPs rs4680 and rs4633 influenced sleep quality dominantly in the subjects having musculoskeletal pain ($P < 0.05$). In pair wise SNP-SNP interactive analysis, both these SNPs (rs4680 & rs4633) interacted to affect sleep in dominant x dominant mode. Whereas, SNP rs4680 paired up with rs4818 in additive x dominant mode, rs4633 interacted with rs4680 and rs6269 in interactive mode and rs4818 in concert with rs6269 in additive x additive mode.

Conclusion: The present study revealed that SNPs with in COMT gene collaborate and contribute to effect sleep quality in the subjects having musculoskeletal pain. G allele of rs4680 is the main culprit where it shows an epistatic effect on other three SNPs. Nonetheless, their effects are variable depending upon several factors which when revealed will surely comprise one important culprit and that being is COMT gene activity.

Introduction

Musculoskeletal pain is a highly complex sensation, influenced by physiological, social, psychological and genetic factors, hence highly variable among subjects. Scientific revelations have started exposing the intricate relationship of musculoskeletal pain and poor sleep. Chronic pain and sleep mutually interact and influence each other, hence have a bidirectional relationship. Follow-up studies have shown that poor sleep quality may predict onset of musculoskeletal complaints at later stages [1]. A retarded sleep worsens the pain condition in disease such as fibromyalgia by causing hyperalgesia. Moreover, poor sleep quality alters the drug efficacy of analgesics by interfering in opioidergic and serotonergic systems [2] and behavioral therapies aiming to improve sleep quality simultaneously decrease the pain sensitization in chronic pain conditions[3].

The incongruent subjective sensitivity to pain, coupled with co-existing, though unforeseen factors make it highly complex to understand. For instance, patients with musculoskeletal pain have higher chances of getting depression whereas, depressed subjects with chronic pain have severe symptoms of insomnia and sleep deprivation. All this milieu of perplexing pain conditions can be understood by investigating potential candidate genes and genetic variants that participate, contribute and collaborate with several unforeseen factors to cause pain.

To understand the genetic link between musculoskeletal pain and sleep, catechol-o-methyltransferase (COMT) is a potential candidate because of its imperative contribution in both pain modulation and sleep-wake regulation [4,5]. COMT is an enzyme which functions in metabolism of catecholamine involved in pain modulation, through the effect of endogenous μ-opioid system. The efficacy of this enzyme is controlled by COMT gene, polymorphisms within which may influence the over activity of μ-opioid system, decreasing its impact to control nociceptive input [6,7]. It has been well understood that dopamine has a crucial role in sleep-wake regulation and quality of sleep. Cerebral dopamine is regulated by COMT in the prefrontal cortex. Since the COMT enzyme function is regulated by COMT gene, hence common polymorphism due to adenine to guanine transition at codon 158, resulting in valine to methionine substitution shows higher COMT activity in valine (Val) homozygotes associated with lower dopaminergic signaling in prefrontal cortex than subjects homozygous for methionine (Met) allele [8].

Many studies have investigated the contribution of COMT gene SNPs in some disease conditions [7,9]. The role and relevance of individual COMT SNP on musculoskeletal pain may vary if other SNPs also contribute to it especially, when they are nearby and in linkage disequilibrium with the functional SNPs. In order to unwrinkle the interactive effects of various SNPs within COMT gene in relation to musculoskeletal pain and sleep quality, the present study explores individual SNPs and SNP-SNP interactions of four pertinent COMT SNPs (rs4680, rs4633, rs4818 and rs6269) as the genetic regulators of sleep in population of Punjab having musculoskeletal pain.

Subjects and Methods:-

This cross-sectional study involved 493 consenting patients who were suffering from chronic musculoskeletal pain and attended orthopedic outpatient departments (OPDs) and pain clinics of Government Medical Hospital, Patiala, Dayanand Medical College and Hospital (DMCH), Ludhiana, Orthonova Hospital, Jalandhar and Doctor Hardas Hospital and Advanced Research Centre, Amritsar. These hospitals are tertiary health care providers and cater to the referral patients of almost entire region of Punjab. Total 1147 subjects were screened and amongst them, 764 subjects were found eligible after preliminary exclusion criteria. These subjects were tested for musculoskeletal disorders by using Nordic Musculoskeletal Questionnaire (NMQ)[10]. Out of these 764 subjects, 114 subjects were excluded because they suffered from trauma, injury or surgical pain, cancer pain, neuropathic pain, phantom limb pain, migraine or headache and congenital analgesia. Subjects having congenital analgesia or suffering from any type of pain other than musculoskeletal and/or if its duration was less than three months were also excluded.157 subjects were excluded because of having neurological or psychiatric disorders other than depression, endocrinal disorders, post stroke pain, grieved with recent bereavement, hormone replacement therapy, calciotropic, corticosteroidal, heparin and anticonvulsant drugs, women with unusual gynecological history, unclear menopause status, irregular cycles or premature menopause before the age of 40 years, subjects having multiple disorderssuch as, complicated hypertension, cerebrovascular infarcts or angina. The final representative data comprised of 493 patients suffering from pain because of various musculoskeletal disorders (Figure 1).These patients were further categorized according to the severity of pain by usingNumeric rating scale (NRS), an 11 point numeric scale for deducing pain intensity. Patients were categorized on the basis of pain severity into three categories i.e. subjects with mild, moderate and severe pain[11].All these subjects were evaluated for co-existing sleep disturbances. Pattern and quality of sleep in the subjects was evaluated using Pittsburgh Sleep Quality Index (PSQI)[12]. It is comprised of nineteen questions generating seven components of sleep such as subjective sleep quality, sleep duration, sleep latency, habitual sleep efficiency, use of sleeping medications, sleep disturbances and daytime dysfunction during the last month. The sum of scores from these components generates global score, which if, <5 or >5 indicates good sleep or poor sleep respectively. A global PSQI >5 has been confirmed to deliver 89.6 percent sensitivity and 86.5 percent specificity in differentiating poor sleep from good sleep [12].All patients gave their written consent prior to participation and the study was approved by ethical committee of the institute.

Statistical Analysis:-

The difference between the groups was examined using chi-square test for categorical variables and student's t-test for continuous variables. All these tests were investigated between mild pain versus moderate pain and mild pain

versus severe pain (GLM procedure). SNP–SNP interactions in relation to sleep in musculoskeletal pain were observed from the genotype data implementing extended Kempthorne model by software epiSNP [13]. Single locus tests correspond to overall marker effect (M), dominant effect (D) and additive effect (A). Pairwise SNP analysis tests correspond to the interactive effect (I), allele x allele (Ax A), allele x genotype (Ax D), genotype x allele (D x A) and genotype x genotype (D x D) effect. On the basis of reconfirmation of sleep patterns and scores by another experienced physician blinded to case–control status, the enrollment procedure showed higher sensitivity and specificity (>90%). $P < 0.05$ was considered significant except for multiple comparisons where threshold of significance was adjusted to $P < 0.01$.

Results and Discussion:-

The present study explored the effects of individual SNPs and SNP-SNP interactions on poor sleep quality in the subjects suffering from chronic musculoskeletal pain. In individual SNP analysis (Table 1), rs4680 and rs4633 were observed to influence poor sleep in the subjects having mild, moderate and severe musculoskeletal pain ($P < 0.05$) in dominant mode however, rs4818 effects sleep quality through overall marker effect ($P < 0.05$). rs4633 impacts substantially to mild and moderate pain but not in severe pain. rs4680 was found to associate with severe and mild pain through additive and marker effect respectively whereas, rs4818 does it in severe pain through additive manner. It has been observed that rs6269 does not impact sleep quality in the subjects having musculoskeletal pain.

In pairwise SNP-SNP analysis (Table 2), it is evident that both rs4680 and rs4633 interact to influence poor sleep in dominant x dominant style. However, 4680 pairs with rs4818 in additive x dominant mode to impact moderate and severe pain respectively. rs4680 and rs6269 affect poor sleep in severe musculoskeletal pain only. rs4633 interacts with rs4818 and rs6269 influencing significantly to moderate and severe pain ($P < 0.05$) however, rs4818 pairs up with rs6269 in additive x additive mode impacting sleep quality in severe musculoskeletal pain ($P < 0.05$).

These SNP-SNP interactions in the present study suggest same unforeseen relationships of COMT gene and musculoskeletal pain within the realm of sleep quality. For instance, rs4680 A allele (Met) has been observed to be associated with low COMT enzymatic activity, higher dopamine levels, lower pain threshold and enhanced susceptibility to stress, whereas G allele (Val) is associated with high COMT activity causing lower dopamine levels, higher pain threshold, better stress resilience and less chances of cognitive impairment. Moreover, the worrier/warrior hypothesis [14] suggests that high COMT activity along with lower prefrontal dopamine release in G allele has advantage of processing of aversive stimuli (warrior strategy) in comparison to A allele. It is well understood that dopamine dictates the circadian rhythm in humans by interacting with norepinephrine receptors to release less melatonin [15]. Some more indirect relationships may help to clarify the role of these SNPs of COMT in sleep quality for instance, prolonged wakefulness and its consequence sleep deprivation effect cognitive function and *vice versa*. It has been revealed that rs4680 along with rs6269 and rs4818 affect cognitive function [16,17]. Hence, the interaction of rs4680 with rs4818 and rs6269 through additive x dominant mode influencing musculoskeletal pain in the present study may mirror upon their participation in poor sleep through cognitive derangements. Considering such interactive effects of SNPs within COMT gene, it may be implied that rs4680 G allele is the primary culprit and influence sleep quality in subjects having musculoskeletal pain which epistasises the effect of other SNPs to influence sleep quality. Moreover, rs4680 is in linkage disequilibrium with other 3 SNPs; rs4633, rs4818 and rs6269 [18] hence, their corroborative effect on sleep quality is variable depending upon many factors and the most important amongst them seems to be COMT gene activity.

Conflict of interest statement:-

The authors report no conflict of interest.

Acknowledgements:-

The grant for major research project sanctioned to PPS by University Grants Commission (F. No. 42-48/2013) is gratefully acknowledged. We are highly thankful to the University Grants Commission for granting UGC-BSR fellowships to GK, HD and KM. We are indebted to the guidance and support rendered by the Director and technical staff of Sophisticated Instrumentation Centre, Punjabi University, Patiala.

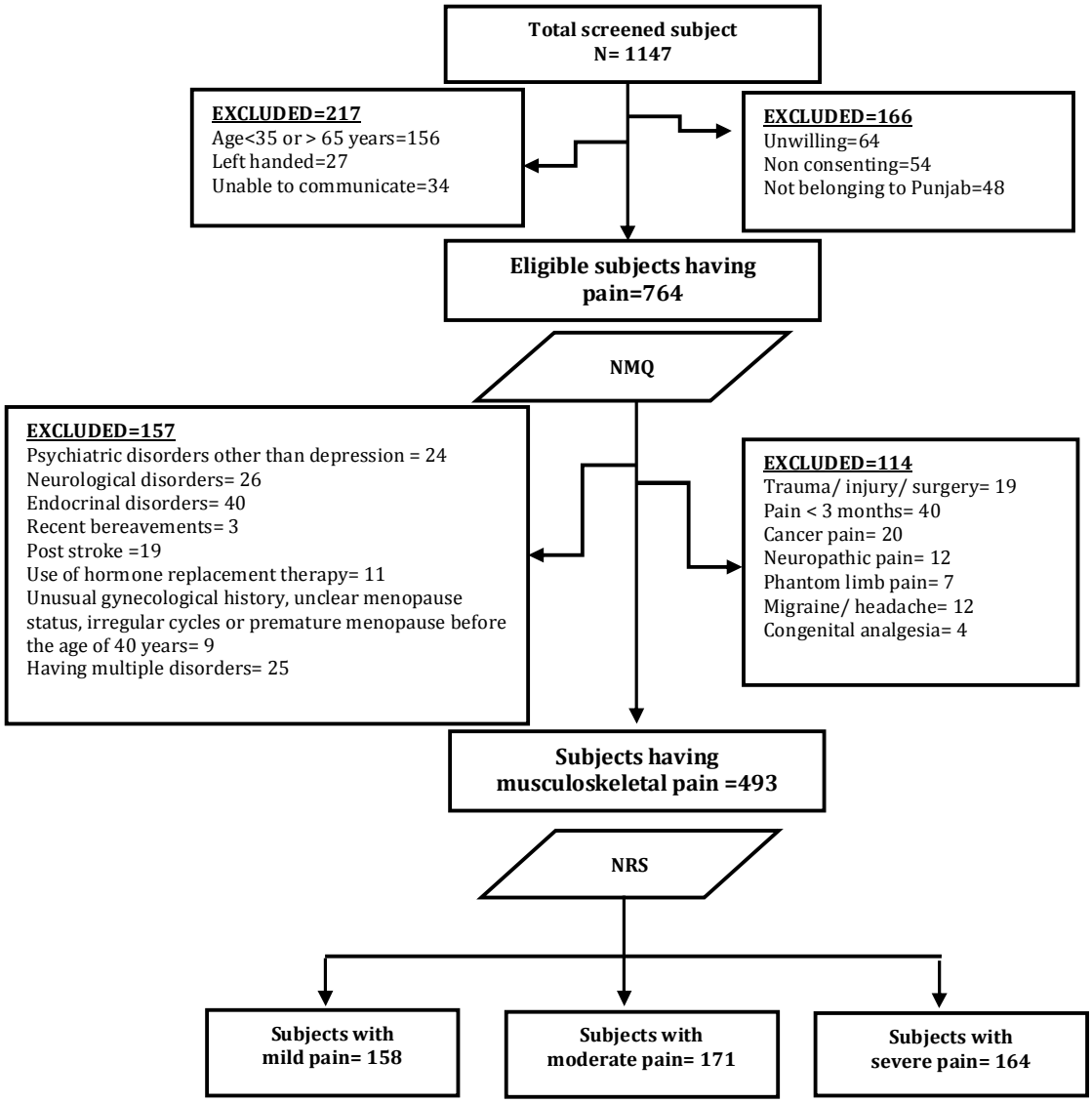

Figure 1:- Flow diagram showing split up of Study participants. NMQ- Nordic Musculoskeletal Questionnaire, NRS- Numeric Rating Scale

Table 1:- Significant effects of the individual COMT SNPs on poor sleep quality in the subjects having musculoskeletal pain.

dbSNPID	Locus	Trait	Test	P^a	P^b	P^c
rs4680	A>G	Poor sleep	D	**0.032**	**0.019**	**0.003**
rs4680	A>G	Poor sleep	A	0.171	0.189	**0.047**
rs4680	A>G	Poor sleep	M	**0.012**	1.133	2.441
rs4633	C>T	Poor sleep	A	**0.023**	**0.037**	0.091
rs4633	C>T	Poor sleep	D	**0.037**	**0.011**	**0.002**
rs4818	C>G	Poor sleep	A	2.192	0.073	**0.043**
rs4818	C>G	Poor sleep	M	**0.006**	**0.035**	**0.001**

dbSNP IDs from home at NCBI website (http://www.ncbi.nim.nih.gov/SNP). $P^{a,}$ P^b and P^c are P values of individual SNP effect on the traits in subjects having mild, moderate and severe chronic musculoskeletal pain respectively. Those values are presented only where statistical significance is evident in any of the group. M-marker overall effect, A-additive effect, D-dominant effect

Table 2:- Significant effects of SNP-SNP interactions of COMT SNP on poor sleep quality in the subjects having chronic musculoskeletal pain

SNP	SNP	Trait	Test	P^a	P^b	P^c
rs4680	rs4633	Poor sleep	DD	**0.03**	**0.001**	**<0.001**
rs4680	rs4818	Poor sleep	AD	2.43	**0.022**	**0.009**
rs4680	rs6269	Poor sleep	AD	0.37	0.26	**0.05**
rs4633	rs4818	Poor sleep	I	3.41	**0.02**	**0.015**
rs4633	rs6269	Poor sleep	I	0.08	**0.017**	**0.005**
rs4818	rs6269	Poor sleep	AA	3.69	1.66	**0.042**

Those values are shown where significance is evident in any one of the groups. $P^{a,}$ P^b and P^c are P values of individual SNP effect on the traits in subjects having mild, moderate and severe chronic musculoskeletal pain respectively. DD-dominance x dominance effect, AA-additive x additive effect, AD-additive x dominance effect, DA-dominance x additive effect and I-interactive effect between two SNPs

References:-

1. Bonvanie IJ, Oldehinkel AJ, Rosmalen JG, Janssens KA. Sleep problems and pain: a longitudinal cohort study in emerging adults. Pain. 2016; 157:957-963.
2. Kundermann B, Krieg JC, Schreiber W, Lautenbacher S. The effect of sleep deprivation on pain. Pain Res Manag. 2004;9:25-32.
3. Lami MJ, Martínez MP, Sánchez AI et al. Gender Differences in Patients with Fibromyalgia Undergoing Cognitive-Behavioral Therapy for Insomnia: Preliminary Data. Pain Pract. 2016;16:E23-34.
4. Valomon A, Holst SC, Bachmann V, Viola AU et al. Genetic polymorphisms of DAT1 and COMT differentially associate with actigraphy-derived sleep-wake cycles in young adults. Chronobiol Int. 2014;31:705-714.
5. Bakker N, Shahab S, Giacobbe P et al. rTMS of the dorsomedial prefrontal cortex for major depression: safety, tolerability, effectiveness, and outcome predictors for 10 Hz versus intermittent theta-burst stimulation. Brain Stimul. 2015;8:208-215.
6. Zubieta JK, Heitzeg MM, Smith YR et al. COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. Science. 2003;299:1240-1243.
7. Diatchenko L, Slade GD, Nackley AG et al. Genetic basis forindividual variations in pain perception and the development of a chronic pain condition. Hum Mol Genet. 2005; 14:135-143.
8. Dauvilliers Y, Tafti M, Landolt HP. Catechol-O-methyltransferase, dopamine, and sleep-wake regulation. Sleep Med Rev. 2015;22:47-53.
9. Hettema JM, An SS, Bukszar J, van den Oord EJ et al.COMT contributes to genetic susceptibility shared among anxiety spectrum phenotypes Biol Psychiatry. 2008; 64:302-310.
10. Kuorinka I, Jonsson B, Kilbom A et al. Standardised Nordic questionnaires for the analysis of musculoskeletal symptoms. ApplErgon 1987;18:233-237.
11. Jensen MP, Karoly P, O'Riordan EF, Bland F Jr, Burns RS. The subjective experience of acute pain. An assessment of the utility of 10 indices. Clin J Pain. 1989;5:153-159.
12. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989; 28:193-213.
13. Ma L, Runesha HB, Dvorkin D, garbe JR, Da Y. Parallel and serial computing tools for testing single-locus and epistatic SNP effects of quantitative traits in genome-wide association studies. BMC Bioinformatics 2008; 9:315.
14. Stein DJ, Newman TK, Savitz J, Ramesar R. Warriors versus worriers: the role of COMT gene variants. CNS Spectr. 2006; 11:745-748.
15. González S, Moreno-Delgado D, Moreno E et al. Circadian-related heteromerization of adrenergic and dopamine D_4 receptors modulates melatonin synthesis and release in the pineal gland. PLoS Biol. 2012; 10:e1001347.
16. Barnett JH, Heron J, Goldman D, Jones PB, Xu K. Effects of catechol-O-methyltransferase on normal variation in the cognitive function of children. Am J Psychiatry. 2009; 166:909-916.
17. Gaysina D, Xu MK, Barnett JH et al. LHA genetics group. The catechol-O-methyltransferase gene (COMT) and cognitive function from childhood through adolescence. Biol Psychol. 2013; 92:359-364.
18. Mukherjee N, Kidd KK, Pakstis AJ et al. The complex global pattern of genetic variation and linkage disequilibrium at catechol-O-methyltransferase. Mol Psychiatry. 2010; 15:216-225.