DISCUSSION

The present cross sectional study examined the role and relevance of Catechol-o-methyltransferase (COMT) and Apolipoprotein E (APOE) genes as the genetic determinants of musculoskeletal pain in 493 subjects of Punjab. There is lack of substantial information for the occurrence and associated risk factors of musculoskeletal disorders and its associated pain in India. First from this region, the present research reported that the prevalence of chronic musculoskeletal pain is 42.80 percent in men and 57.20 percent in women. Earlier reports have observed the prevalence of chronic pain to be 50.4 percent in Northwest Scotland (Elliott et al. 1999), 32.9 percent in USA (Cunningham et al. 1984) and 65.9 percent in Sweden (Brattberg et al. 1989). Although considerable variation of chronic musculoskeletal pain is evident in different regions of the world, but from the clinical chapters, it emerges clearly that the women subjects have higher odds of suffering from pain than men (Pueyo et al. 2012). Women in present study also have higher risk of both moderate and severe pain than men and it is not unexpected as advancing age coupled with menopausal decrements of bone health exacerbates pain. In India, every third woman is osteoporotic and consequently, osteoporosis related high fracture risk and declining bone mineral density (BMD) at forearm, neck and lumbar spine worsens the propensity of musculoskeletal pain. It has also been substantiated by other population based cross-sectional studies that women have often more musculoskeletal pain problems than men (Rollman et al. 2001, Picavet et al. 2002). In the present study, subjects having BMI ≥23-29.5 kg.m⁻² and ≥30 kg.m⁻² are at higher risk of suffering from moderate and severe musculoskeletal pain. This is endorsed by a meta-analysis comprising 33 studies which observed obesity to be a significant risk factor for chronic low back pain (Shiri et al. 2010). In another longitudinal study, the occurrence of chronic musculoskeletal pain has been examined in 30,000 subjects, which reveals that obese subjects have approximately 20 percent increased risk of chronic pain in
low back and neck shoulder regions than their normal counterparts (Nilsen et al. 2011).

Epidemiological studies have derived inverse relationship between socioeconomic status and musculoskeletal pain. British cohort study has reported that lowest social class has three fold increased risk of chronic widespread pain in comparison to highest social class (Macfarlane et al. 2009). Another study has reported that subjects living in less affluent areas have higher chances of widespread pain, physical disability, mental distress and low life satisfaction in comparison to subjects living in affluent areas (Brekke et al. 2002). A 15 months follow up study has revealed that subjects belonging to moderate and less affluent areas are more likely to have chronic widespread pain (CWP) (Davies et al. 2009). However, after adjusting the psychological factors in multivariate logistic regression, this impact is not evident suggesting that socioeconomic status which in many studies has been based upon home ownership, education level and employment status is not a risk predictor for musculoskeletal pain, unless residual confounding of other risk factors such as depression and sleep quality are not adjusted appropriately. Similar results have been observed in the present study as in logistic regression analysis; its impact is no longer evident, however in univariate testing socioeconomic status is observed to influence pain substantially.

Subjects who have sedentary lifestyle are at 3 and 4 fold higher risk of moderate and severe pain. Sedentary life style has substantial impact on musculoskeletal health and is independently associated with back problems (Vuori et al. 1995). Moreover, physical inactivity influences bone degradation owing to decreased synovial fluid release (Hootman et al. 2001). As a result, in acute back pain, orthopaedicians recommend light physical activity rather than complete bed rest (Liddle et al. 2007). However, a systematic review has also suggested that sedentary life style is not associated with low back pain (Chen et al. 2009). In the present study, the influence of sedentary life style attenuates substantially when adjusted for the effects of other variables in binary logistic regression analysis. It suggests that sedentary lifestyle is not an intransigent variable but influences by other co-existing risk variables.
In general population, statin use has been observed to confer adverse effect on musculoskeletal health and pain (Buettner et al. 2012). In the univariate testing, although statin use has been observed to be a risk factor for musculoskeletal pain, but it could not retain its predictability in multivariate model. The possible reason is that the anti-inflammatory effect of statin especially in arthritis may have counterbalanced the underlying physically active life style, good eating habits and less stressful lifestyle. Similarly, higher low density lipoprotein (LDL) (>100mg/dl) and triglyceride (TG) levels (>150mg/dl) have impacted musculoskeletal pain in univariate testing, however these factors do not emerge as independent predictors. In the present study, 48.07 percent subjects are statin users and 49.08 percent subjects were doing at least 1 hour aerobic exercise daily, both of which are likely to decrease LDL and TG levels. Moreover, BMI and menopausal status may also participate in reducing the effect of these lipid levels (Brenner et al. 2010). Alluding to this, it is not unreasonable to believe that LDL and TG may contribute to musculoskeletal pain but are not independent risk predictors for it.

Sleep deprivation, insomnia or insufficient sleep may coexist with other risk factors or independently influence the subjects for musculoskeletal pain especially low back pain (Van de Water et al. 2011). Other risk factors such as fatigue, cognitive disturbances, mood swings anxiety and depression may influence insomnia associated chronic pain (Neckelmann et al. 2007). In the present study, subjects who have been suffering from poor sleep are at approximately 1.7 to 2.2 fold higher risk of musculoskeletal pain in comparison to subjects having good sleep. The prevalence of depression in the present study is quite high amongst subjects having poor sleep in comparison to good sleepers. But in adjusted step wise model, impact of sleep on musculoskeletal pain exacerbates for both moderate to severe pain which is suggestive of its being an independent predictor for the risk of musculoskeletal pain.

The effect of pain on depression and vice versa is not easy to understand because of their usual co-existence and bidirectional relationship. It has been observed that pain threshold is reduced in subjects having depression, whereby somatic preoccupation may be the primary symptom (O'Sullivan et al. 2004). Almost 50 percent of the depressed patients suffering from depression, report
some kind of pain in their lifetime. It has also been proposed that chronic pain is a variant of depression (Blumer et al. 1982). In primary care setting the complex coexistence of pain and depression is largely overlooked and most of the times, depression is considered as an artifact of musculoskeletal pain, which may lead to poor prognosis, misdiagnosis and under treatment of existing pain (Bergbom et al. 2011). In the present study, each unit of depression in subjects having musculoskeletal disorders increases the risk of moderate and severe pain by a factor of 1.69 and 2.18 respectively.

Considering that genetics plays an inevitable part in the manifestation of chronic musculoskeletal pain, present study offers evidence in support of the fact that genetic variants within COMT and APOE genes contribute substantially towards chronic pain in the subjects having musculoskeletal disorders. COMT gene polymorphisms affect pain sensitivity in several musculoskeletal pain conditions such as fibromyalgia, temporo-mandibular disorder (TMD), chronic widespread pain, low back pain, and osteoarthritis. A relatively higher number of studies have been conducted to assess the genetic variation of COMT gene with fibromyalgia syndrome (FMS) associated pain and disease severity. COMT is one of the most extensively studied genes in relation to pain and nociception. It accelerates the transfer of a methyl group from S-adenosyl-methionine (SAM) to a hydroxyl group on the catecholamine derivatives such as dopamine, norepinephrine or catechol estrogen, hence causing their deactivation. Biochemical analysis has shown that COMT inhibition increases pain sensitivity towards noxious stimuli by producing mechanical and thermal hyperalgesia. The reduced COMT activity results in increased pain sensitivity by the upregulation of pro-inflammatory cytokine and nitric oxide production (Hartung et al. 2014). In brain, COMT and nuclear factor kappa B (NF-kB) receptors are co-localized in astrocytes. In nerve injury induced inflammatory pain models, increased levels of NF-kB in astrocytes reduce COMT expression retarding its transcription, hence increasing pain. Inhibition of NF-kB reduces pain by up re-establishing the blocked COMT expression (Hartung et al. 2015).

Four SNPs i.e. rs165599, rs4818, rs4633 and rs4680 within COMT gene have been investigated in the present study which reveals that SNP rs165599 does not correlate to musculoskeletal pain in the population of Punjab. However
some studies have shown that ‘G’ allele of this SNP influences fibromyalgia in Korean, Mexican and Spanish populations (Vargas-Alarcón G et al. 2007, Park et al. 2016). Another study has revealed that it influences pain after motor vehicle collision especially in males (Bortsov et al. 2014). Nonetheless, this SNP is not found to be associated with pain scores in patients with major depressive disease (MDD) in American population (Fijal et al. 2010). Another study by Omair et al. (2012) observed that ‘C’ allele carriers of SNP rs4633 respond better to the post-surgical treatment than the ‘T’ allele which suggests the protective effects of ‘C’ allele. Minor allele ‘T’ of this SNP was found to be associated with low back pain (LBP) whereas ‘C’ allele is found to be protective against the pain due to disc degeneration (Gruber et al. 2014). The findings in the present study are in line, that shows ‘C’ allele of rs4633 to be protective against moderate and severe musculoskeletal pain, whereas ‘T’ allele has been observed to be associated with increased risk of pain.

The present study has found association of rs4818 of COMT gene with moderate musculoskeletal pain. However, ‘G’ allele of this SNP was observed to be a significant risk marker for severe pain. This risk is exhibited through additive (GG), dominant and recessive genetic models. There are contrasting reports showing CC genotype, rather than GG genotype to be associated with pain in fibromyalgia syndrome (FMS) where patients with CC genotype are observed to have greater pain sensitivity (Barbosa et al. 2012). Another study reports GG genotype of this SNP to be a potent risk genotype for the occurrence of FMS and increased pain sensitivity (Park et al. 2016). There is no surprise in such contradictions, primarily because there is a huge variability in the pain thresholds which depends upon ethnic variability, different environmental gradients and indirect underlying genetic mechanisms. The nociceptive function of COMT has been reported to be modulated by indirect physiological mechanisms such as stress and inflammation (Kambur et al. 2008).

It has been observed that membrane bound COMT (MB-COMT) is capable of inactivating synaptic and extra synaptic dopamine on pre and post-synaptic neurons. Its inhibition induces apoptosis of neurons and cytotoxic mechanisms, which depends upon SNP rs4680 polymorphism (Chen et al. 2011). ‘G’ allele of this SNP is observed to be associated with low pain sensitivity in the haplotype.
based analysis (Diatchenko et al. 2005). Loggia et al. (2011) has inferred the AA genotype of this SNP has greater pain sensation in high intensity repeated pain stimuli. It has been further been endorsed that ‘A’ allele carriers of this SNP experience higher pain and pain related bone damage (Josep Garcia-Fructuoso et al. 2006). The results of the present study are in line with previous studies where ‘A’ allele was observed to influence moderate and severe pain substantially.

None of the study has examined the direct relationship of APOE gene within musculoskeletal pain so far. However, some studies have reported APOE polymorphism in relation to bone mineral density (BMD) (Long et al. 2004, Singh et al. 2010) and cognitive decline (Rantalainen et al. 2016). The role of APOE in stress response and its association has been demonstrated by both clinical (Cameron et al. 1995) and experimental studies (Choudhuri et al. 2002). These studies have concluded that APOE levels are related with stress response, induced pain in animal models and different types of chronic pain in humans. Moreover, nitric oxide activates the nociceptors of c-fibers and thereby causing the sensation of pain. The production of nitric oxide along with interleukins is influenced by APOE and this production is genotype specific (Czapiga and Colton, 2003). APOE is known to be regulating synaptic transmission as it increases the synaptic signaling strength by the process of long term potentiation (LTP) (Korwek et al. 2009). For the first time, present study has revealed that the carriers of ‘C’ allele of SNP rs440446 are at approximately 2 times higher risk of severe pain whereas, its effect on moderate pain is missing in the present analysis. Similarly, ‘T’ allele of another SNP rs7412 is observed to be associated with severe musculoskeletal pain but not with moderate pain. This influence is pronounced when ‘T’ allele is present in double dose (recessive model). It has also been brought out that ‘C’ allele of rs429358 within APOE gene confers substantial risk in the manifestation of moderate and severe musculoskeletal pain in the population of Punjab.

Single gene studies are liable to spurious and indistinct inferences especially, when the disease is multigenic and multifactorial. Therefore, it is appropriate to analyze the haplotypes where the cumulative effect of alleles can be discerned. In the present study ATGA haplotype within COMT gene is
observed to confer 4 times greater risk for the manifestation of moderate and severe pain. A study by Diatchenko et al. (2005) constructed haplotype based on 4 SNPs; rs6269, rs4818, rs4633 and rs4680 in order to understand the possible link between COMT gene and musculoskeletal pain. It has been revealed that ACCG haplotype predicts high pain sensitivity in chronic induced pain in experimental settings. Such inconsistency in the results entails skepticism possibly because of the presence of false negatives and false positives in the analysis. Considering this to be factual, changing the allele patterns in the haplotype would not change the risk of moderate or severe pain. To get the true picture statistical testing was designed by considering that subjects with severe pain and subjects with mild pain have same frequencies (Null hypothesis) and secondly that subjects carrying ATGA haplotype have higher frequency in subjects having severe pain than subjects having mild pain (Alternative hypothesis). Appropriate null was rejected when ‘G’ and ‘A’ alleles at the third and fourth SNPs of ATGA haplotype was replaced with ‘C’ and ‘G’ alleles, the risk of moderate and severe pain disappeared statistically in the haplotype ATCG (P>0.05).

Another haplotype CTT within APOE gene is found to influence both moderate and severe pain. The profound analysis has revealed that by replacing either ‘T’ allele of the second SNP or ‘C’ allele in the first SNP of haplotype CTT, forming CCT and GTT, their influence for the risk of moderate and severe pain vanished outrightly. It suggests that ‘T’ allele in the third SNP in the haplotype CTT showed epistatic effect on the other two alleles of the SNPs at first (rs440446) and second (rs7412) positions. The results in the present study could not be compared with other studies, which have analysed haplotype based association within these genes, mainly because of the contradicting difference in the selected SNPs and estimated haplotype.

In the scientific literature, scientists have consensus that gene-gene interactions exhibit accurate picture when analysed within the context of genetic modeling. Five types of gene-gene interactive effects of two candidate gene SNPs have been examined and these are; Additive x Additive, Additive x Dominance, Dominance x Additive, Dominance x Dominance and SNP x SNP. The epiSNP software used for the analysis of gene-gene interactions in the present
study has been documented to be extensively accurate because it analyses by removing the false negatives and false positives from the imputation files (Mao et al. 2006). Moreover, it displays the significant SNP-SNP interaction only with Bonferroni corrected significance levels. All the SNPs within COMT and APOE genes seem to be important genetic determinants of chronic musculoskeletal pain in the population of Punjab, as their interactions have shown all the possible five different effects. It has been observed that for the moderate musculoskeletal pain, SNP rs165599 of COMT gene interacts with rs440446 of APOE gene through dominance x dominance effect, which further interacts with SNPs rs4680, rs4633 of COMT gene through dominance x dominance and SNP x SNP effects respectively. Furthermore, SNP rs7412 of APOE gene interacts with rs4633, rs4818 and rs4680 of COMT gene conferring the risk of both moderate and severe pain. It has also been brought out in the present study that some gene-gene interactions also held significance for the mild pain, when analyzing the differences between subjects with mild pain and subjects with moderate or severe pain. More studies comprising large datasets will clear the overall picture regarding the extent and degree of such gene-gene interactions in the realm of musculoskeletal pain in the population of Punjab.

When the disease is multi-factorial, the impact of genetic value changes when some environmental variable also participates. Present study for the first time has revealed that rs16559 of COMT gene correlates positively with the duration of pain (DOP)(>7 years) through dominance effect for the risk of moderate pain, whereas, it interacted with higher levels of LDL (>100mg/dl) for the risk of moderate and severe pain. Other SNPs rs4633, rs4680 and rs4818 also interact with quantitative environmental traits for the risk of moderate and severe pain. Similarly, SNPs within APOE gene exhibit significant risk for both moderate and severe musculoskeletal pain while interacting with various environmental variables through marker, dominance and additive effects.

The present study offers the foremost evidence of SNP-SNP cross talks based on two loci epistatic effects. All the possible forms of epistasis were analysed including the collaborations between additive effects of two loci that are additive effect of the first locus and dominance effect of second locus, dominance effect of the first locus and additive effect at second locus and
dominance effect at two loci. All such forms of epistasis contribute differently to the overall genetic value of the two locus genotype. The present study has delineated that SNPs rs4633 of COMT gene and rs440446 of APOE gene have two way epistatic interactive effects for the risk of moderate musculoskeletal pain. The other epistatic effects observed are additive x additive between rs4818 (COMT)-rs7412 (APOE) and interactive effect between rs4680 (COMT)-rs429358 (APOE). Similarly, it has been revealed that interactions between SNPs rs4818 (COMT)-rs7412 (APOE) exist that worsen the risk of severe musculoskeletal pain in the population of Punjab. The indepth analysis of COMT and APOE genes as the genetic determinants of musculoskeletal pain makes a realization that to understand the modus operandi of these genes and environmental traits in the manifestations of musculoskeletal pain, more studies on different populations on large sample size are required to reach on unequivocal consensus that both of these genes are notorious culprits, which threaten the population of Punjab with the fangs of chronic musculoskeletal pain.
CONCLUSIONS

1. The present research revealed 42.80 and 57.20 percent prevalence of chronic musculoskeletal pain in men and women in the population of Punjab and observed that prevalence according to all main anatomical sites was also higher in women than men except pain at neck.

2. The present research exposed that sedentary life style, BMI ≥23 kg.m−², depression and poor sleep independently influenced the risk of musculoskeletal pain.

3. It has been affirmed that ‘T’ allele of rs4633 in COMT gene influenced moderate and severe pain in dominant model whereas, ‘G’ allele of rs4818 and ‘A’ allele of rs4680 showed their dominance for severe pain. Minor alleles ‘C’ of rs440446 and rs429358 within APOE influenced pain in both dominant and recessive modes whereas, ‘T’ allele of rs7412 exhibited its influence in recessive genetic model.

4. Haplotype ATGA within COMT gene was observed to be a susceptibility marker, carriers of which were at 4-4.5 times higher risk of musculoskeletal pain than those who did not possess this haplotype. Similarly, haplotype CTT within APOE gene was observed to be a risky haplotype which conferred 3.4-4 fold higher risk of pain in its carriers in comparison to those subjects who did not carry it.

5. Gene-Gene and Gene-Environment analysis showed that SNPs within COMT and APOE genes communicate with each other and with other risk variables to participate in the risk of moderate and severe musculoskeletal pain.

6. Furthermore, two way epistatic effects were observed between SNPs rs4633-rs440446, rs4818-rs7412 and rs4818-rs429358 for the development of moderate pain and rs4818-rs7412 for severe musculoskeletal pain in the population of Punjab.