Pain is a complex sensory modality often accompanying affective, motivational and cognitive aspects. It is generally narrated as a feeling of distress or discomfort. According to the International association for the study of pain (IASP), it is described as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (Merskey et al. 1994). Although an undesired entity, it is essential for the survival of an organism by acting as a warning system, thus alerting the body's defense mechanisms and reflexes about the potential injury. However, inspite of its immense utility, pain outlives its usefulness and becomes chronic and debilitating. Pain is generally associated with almost every disease as a co-morbid condition.

Musculoskeletal pain including bones, tendons, muscles and ligaments, is a forefront perpetrator in the toll of human sufferings. It is highly relevant as almost every individual has experienced it at least once in their lifetime. The recurrent or chronic pain as a manifestation of various musculoskeletal disorders is an extremely relevant health concern. It is a common problem prevalent in 30-40 percent of adult population in its global perspective (Woolf and Pfleger. 2003). Despite its formidable impact, pain management and convalescence of the patients are largely insufficient especially in the developing countries. It has trenched almost every nation in terms of health as well as monetary loss. A major share of world's economy is poured into pain management strategies every year. This condition is far more severe in developing countries like India where mortality rate is quite high due to the lack of adequate health care facilities.

Several risk factors which cooperate and contribute in the development of musculoskeletal pain vary greatly across humans because of different cultural, psychosocial, physiological, environmental and genetic factors. 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 or sleep deprivation. Therefore, it is possible that musculoskeletal pain may be inappropriately managed in the primary care setting because of the unidentified co-existing depression and its other inevitable concomitant poor sleep.

The prevalence of musculoskeletal pain among Indian population has not been reported so far, except a study conducted on residents of national capital region, Delhi (Bihari et al. 2011), however, few reports have analyzed the prevalence of musculoskeletal disorders in different occupational setting. In order to understand the impact of various risk factors, the present study aimed to ascertain the genetic and environmental associations of chronic pain in the population of Punjab suffering from musculoskeletal disorders.

In an attempt to unveil the mystery of pain, several scientists gave theories regarding the mechanism of pain modulation. The most primitive theory emerged in 1895 by Von Frey, who named it as ‘Pain control theory of specificity’. Since then, several new theories have been put forth to explain the mechanism of pain perception such as Strong’s theory, Central summation theory by Livestone (1943). Most widely accepted theories include ‘Gate control theory’ of pain modulation given by Melzack in 1965, Patterning theory (Goldschneider 1920), Central summation theory (1943) Neuromatrix theory (Wall and Melzack 1999) and Central biasing theory (Buxton 1999). Recently, a new theory named ‘Gain control theory’ was proposed in 2016 (Treede et al. 2016).

Classification of pain

Pain may be described verbally in several ways as sharp, needle prick like, burning and diffusing felt according to the site of origin, source of stimuli and pain mediators involved. On broader terms, a localized pain is defined as the one which involves a specific area where pain stimuli are conducted by rapid conducting A-delta fibers and often specific and non-spreading in nature. Whereas, a delocalized or diffuse pain is one which involves slow conducting C fibers and spread from the site of origin to other regions of the body.
Pain may also be classified on the basis of duration of pain such as acute and chronic pain. According to the guidelines of IASP, acute pain is defined as a pain lasting for less than three months and non-persistent in nature. It can be cured with mild analgesics. On the contrary, chronic pain is the one lasting for more than 8 weeks (3 months) and persistent in nature. It is often treated by a combination of non-steroidal anti-inflammatory drugs (NSAIDs).

On the basis of origin, pain may be divided into four categories; nociceptive, neuropathic, psychotic and inflammatory pain. Nociceptive pain is conducted by sensory nociceptors that relay pain signals to higher corticals of the brain via spinothalamic tracts. Nociceptive pain may further be categorized into superficial and deep pain. Neuropathic pain is often associated with neuropathy such as nerve injury in the peripheral or central nervous system (CNS). Several pathological conditions may cause neuropathic pain such as cancer pain. Psychotic or somatoform pain is a result of prolonged emotional distress and depression (Landa et al. 2012). Inflammatory pain is associated with increased sensitivity of sensory neurons by inflammatory mediators such as bradykinin and substance P (Petho et al. 2012).

Pain may be differentiated according to the systems involved in transmission of the noxious stimuli such as complex regional pain syndrome, involving sympathetic nervous system and central pain involving central nervous system (Blažeković et al. 2015).

Besides the well defined pain, there are several other subtypes also namely, functional pain which is associated with functional alterations in brain regions i.e. either hypersensitivity due to defects in higher brain regions or insensitivity to detect pain due to cortical or neuronal defects. Mixed pain accompanies mixed symptoms such as painful tonic spasm.

**Epidemiology of musculoskeletal pain**

**International status**

The prevalence of chronic pain was reported to be 22 percent by a WHO study (Gureje et al. 1998). The recurrent or chronic musculoskeletal pain problems are very common ranging from 30 to 40 percent of adult population
Prevalence of musculoskeletal pain has also been assessed in relation to specific disease or region such as low back pain or arthritic knee pain etc. depending on the anatomical site. The prevalence of pain has been reported in different populations according to the different occupational settings. It has been observed to be 92.8 percent in Turkish population (Kuru et al. 2011), 48.5 percent in Saudi Arabian construction workers (Alghadir et al. 2015), 45.7 percent in Ecuador (Guevara-Pacheco et al. 2016), 61 percent in UK (Macfarlane et al. 2015), 10.4 percent in Portuguese (Gouveia et al. 2016), 53.7 percent in Qom population of Argentina (Quintana et al. 2016) and 63.8 percent in Korean farmers (Min et al. 2016).

**National status**

There are few studies that reported prevalence of pain in India but some studies have been conducted according to different occupations. A prevalence of 25.9 percent of musculoskeletal pain was reported in national capital region Delhi (Bihari et al. 2011). Reddy et al. (2015) reported prevalence of musculoskeletal pain to be 70 percent in municipal solid waste workers, whereas it was found to be 78.5 percent among tea pluckers of Tamil Nadu (Vasanth et al. 2015).

**Regional status-Punjab**

There is no well defined study that has examined the prevalence of musculoskeletal pain in Punjab region except one study which reported prevalence of 68.3 percent in dentists (Bedi et al. 2015). However, some studies have reported the prevalence of musculoskeletal disorders in several occupational setting (Moon et al. 2015).

**Neurobiology of musculoskeletal pain**

Pain is a complex sensation where sensory, affective and cognitive dimensions of pain alongwith parallel neural networks in brain are associated with constellation of factors. Though pain occurs to show protective gesture, but when it surpasses threshold, exerts debilitating effect upon health and triggers concomitant physiological and psychological concerns of perilous ramifications.
Right from the activation of primary afferent nociceptors up to the cortical processing of the pain in the higher regions of the brain, pain trajectory can be dissected into transduction, conduction, synaptic transmission and modulation. Besides, environmental, behavioral and psychological risks involved, all these stages of pain sensitivity, severity and analgesic responses are mediated by different sets of genes and genetic variants.

1. TRANSDUCTION

Transduction marks the cardinal process for the pain realization. It involves detection of noxious stimuli by the peripheral nociceptors by sensing various substances such as substance P, bradykinin, etc. leading to the depolarization of the neurons causing generation of a receptor potential (Basbaum et al. 2009).

Heat pain

The nociceptors detect four major types of noxious stimuli namely; heat, cold, mechanical and chemical. The detection of these various types of stimuli is further regulated by numerous genes. Amongst the most studied one in relation to heat related sensitization are the transient receptor potential (TRP) family genes. TRP family is a calcium gated channel protein family comprising 6 subfamilies; TRPA, TRPV, TRPM, TRPML, TRPP and TRPL. While the majority of TRP gene family members detect specific types of noxious insults, some of them are polymodal in function. A class of TRP gene family; TRPV1 is activated by both heat and capsaicin found in hot chili pepper (Caterina et al. 1997). It is highly associated with detection of thermal, mechanical and pH shifts. The polymodal function of TRPV1 has been confirmed by various gene knockouts, siRNA knockout and inhibition studies (Tóth et al. 2011). In human, TRPV1 Met315Ile and Ile585Val polymorphism has been examined and the former is found to be associated with greater susceptibility to chronic neuropathic pain (Armero et al. 2012). TRPV1 acts in close amity with Protein kinase beta type II (PKCβII), as the activation of the latter by TRPV1 causes phosphorylation of Thr705, setting the pain and itch thresholds below normal ranges (Li et al. 2014). TRPV1 also regulates calcitonin gene related peptide (CGRP) functioning as TRPV1 knockouts show enhanced metabolism and longevity due to
suppression of CGRP activity (Riera et al. 2014). The TRPV1 knockout shows reduced thermal sensitivity while TRPV2 knockout shows normal function. Surprisingly, 1911A>G (rs8065080) polymorphism is found to be associated with cold hypoalgesia, less heat, pin prick and mechanical hyperalgesia, whereas, 1103C>G (rs2222747) is associated with reduced cold hypoesthesia. None of the TRPV polymorphisms give impaired functions, suggesting its indirect linkage to pain via somatosensory abnormalities than direct function in pain (Binder et al. 2011). In a complex manner, inflammatory mediator bradykinin enhances mechanical pain through Purinoreceptor 2X (P2X2,3) receptors mediation, and upregulates TRPV1 activation and Adenosine triphosphate (ATP) production via keratinocytes, Nerve growth factor (NGF) signaling etc, thus causing mechanical hyperalgesia (Murase et al. 2010). However, inflammation induced hyperalgesia involves several other inflammatory mediators besides bradykinin alone (de Oliveira Fusaro et al. 2010).

**Mechanical pain**

TRPV3 receptors mainly found in keratinocytes are activated by development of ATP by the cells. They are sensitized on moderate temperature ranges on repeated stimuli being found in traces in sensory neurons, as they are more associated with mechanical hyperalgesia. A polymorphic study shows the nominal association for TRPV3 gene SNP rs7217270 and TRPV1 gene SNP rs222741 with migraine related pain (Carreño et al. 2012), while no polymorphic study is found for TRPV2.

TRPV4 channels are found abundantly in blood vessels, kidney cells etc and act as sensors to mechanical, acidic and thermal pain. These channels promote inflammation and hyperalgesia on initiation as their murine knockouts show hypoalgesia in response to thermal, acidic and mechanical pain stimuli. Furthermore, TRPV4 knockout mice have longer latency to escape following hyperalgesia induced by inflammation in hot plate test (Todaka et al. 2004).
Cold pain

TRPA1 is sensitized at temperature below 18°C. Although being a cold sensor, due to their co-localization with TRPV1, cold stimuli related signals are misinterpreted as those from heat ones by a phenomenon named paradoxical cold (May et al. 2012). Carriers of ‘A’ allele in rs11988795 G>A SNP of TRPA1 glu/lys-179 polymorphism is associated with greater olfactory and cold related pain stimuli detection (Schütz et al. 2014). TRPA1 710G>A (rs920829) polymorphism is also found to be associated with greater paradoxical heat sensation (Binder et al. 2011). TRP channels induced cooling related pain signals are blocked by prostaglandin E2 receptors (PGE2) as shown in a cell culture study by decreasing cooling range from 32°C to 18°C causing reduced threshold for PKA values, thus, depicting an inhibitory effect of PGE2 on TRPM8 channel activation by cooling. In order to understand the genetic predictors of acute experimental cold and hot pain sensitivity in humans, Kim et al. (2006) investigated the role of TRPA1, TRPM8, TRPV1, opioid receptor delta 1 (OPRD1), catechol-o-methyltransferase (COMT) and Fatty acid amide hydrolase (FAAH) in four major ethnic populations. Haploblocks have been derived from the genes in European and American population, which are found to be associated more with females than males with respect to short duration cold pain sensitivity. A dopamine active transporter 1 gene (DAT-1) and solute carrier family 6 member 3 (SLC6A3) polymorphism study showed that carriers of homozygous 10-allele has enhanced cold associated pain tolerance than 9-allele carriers (Treister et al. 2013).

Chemical stimuli

For the detection of altered pH, TRPV1, acid sensing ion channel (ASIC) is found guilty (Sun et al. 2014). ASIC1 is found to be responsible for generating afferent potential in case of low pH as well as inflammatory conditions as interplanter acidic saline in mice and humans, cause pain, while ASIC3 is found to reduce inflammation in arthritic mice (Ikeuchi et al. 2009). ASIC genes are found to be the influencing agents in diseases like migraine (Rash et al. 2013). ASIC is blocked by oxytocin and opioids, so they may act as potential therapeutic target for analgesics (Qiu et al. 2014).
Besides the peripheral nociception, there are set of subcutaneous receptors namely purinergic receptor X and Y (P2X and P2Y genes). P2X and P2Y gene families have a complex involvement in transduction of pain. Their activation via ATP generation through keratinocytes enhances nociceptors. P2X3 receptors are found to be inducing inflammatory nociception by making primary afferent nociceptors more susceptible by interacting with TRPA1, 5-hydroxytryptamine (5-HT3) and 5-hydroxytryptamine 1 alpha (5-HT1A) receptors (Krimon et al. 2013). Furthermore, haplotype based association study confirmed rs1718125 G>A polymorphism of P2X7 gene to be related with cold pain and a cold pain sensitivity alongwith reduced analgesia (Ide et al. 2014).

Inflammation induced nociception is observed to be mediated via TRPA1, 5-HT3 and 5-HT1A receptors. Furthermore, TRPM8 forms a complex with 5-HT receptors and phospholipase D, thus reversing pain hypersensitivity (Vinuela–Fernandez et al. 2014). Astonishingly, 5-HT or serotonin was observed to be involved in enhancing nociception at peripheral level as it gives analgesic effects in pain at central level. It acts via an indirect mechanism to activate sensory neurons through neutrophil migration, causing local release of dopamine, norepinephrine and prostaglandin causing inflammation mediated nociception and hyperalgesia, which is blocked by non-selective inhibitors of cyclooxygenase 2 (COX), beta 1, 2 adrenergic receptors (ADRβ1,2) and dopamine proving involvement of multiple agents. However, 5-HT induced mechanical hyperalgesia is mediated by norepinephrine in dose dependent manner. A novel study on 5-HTRA1 SNP rs6295 polymorphism shows that carriers of minor ‘A’ allele have low susceptibility to thermal pain stimuli (Lindstedt et al. 2012).
2. CONDUCTION

Conduction involves conversion of numerous set of receptor potentials from the various sensory neurons, to form an action potential which transfers to the synaptic plate at the dorsal horn of spinal cord via secondary neurons. Once the receptor potential is generated, it requires a continuous and minimum threshold to propagate till synaptic level before submitting it ultimately to the higher brain regions.

Similar to ion channels present in transduction process, conduction also involves an array of ion channels. Hyperpolarization activated cyclic nucleotide gated cation channel (HCN) belongs to a class of channels that are permeable to both sodium and potassium ions, with four types; HCN1, 2, 3 and 4. They are essential for maintaining a continuous generation of action potential by acting as frequency modulators of continuous firing rate. It has been found that HCN2 channel influences firing rate in NaV1.8 expressing neurons (Emery et al. 2011). A class of potassium channels KCNQ contributes remarkably in pain conduction.
A member of KCNQ genes, potassium voltage gated channel family member 4 (kv3.4 or KCNC4) has been found to regulate action potential and firing rate following a spinal nerve injury in mice study. This has placed them in series of potential targets for drug delivery (Ritter et al. 2015). Another member of potassium channel sub-type is Kv9.1 channel encoded by KCNS1 gene that plays an anti-nociceptive role in pain conduction. It has been found that KCNS1 gene knockdowns demonstrate lowered pain thresholds and enhanced firing rates. Moreover, siRNA mediated knockdowns of kv9.1 gene demonstrate neuropathic pain behavior (Tsantoulas et al. 2012).

G-protein coupled inward rectifying potassium channels (GIRK) family is a potassium sensitive channel group with two subtypes; one expressing in brain and other in cardiac muscles. They are associated with opioid induced analgesia. A knockout study shows that GIRK channel activation, along with opioid receptor activation, inhibits nociceptive transmission thus leading to opioid induced analgesia (Bruehl et al. 2013). KCNJ3 or GIRK1 knockout or chemical inhibition leads to elevated thermal nociception and blunted opioid derived analgesia. GIRK2 is essential for opioid induced peripheral analgesia and signaling (Nockemann et al. 2013). Interestingly, GIRK1 and GIRK2 act at post synaptic level, quite different to all their siblings (Marker et al. 2005). Brain type GIRK1,2 are activated by opioids as well as ethanol, giving opioid and ethanol induced analgesia which was confirmed by GIRK2 knockout study using weaver mice (Kobayashi et al. 1999, Ikeda et al. 2002).

The opening of GIRK channels is also found to reduce hyperalgesia in rat mussator muscles (Chung et al. 2014). KCNJ6 or GIRK3 has been examined in several chronic pain conditions and it is inferred that ‘A’ allele of rs2070995 is a potential risk allele as individuals homozygous for ‘A’ allele require higher analgesic doses as compared to ‘G’ allele (Lötsch et al. 2010). A distinct set of receptors namely nociception/ orphanin receptors work in conjunction with GIRK channels. Their activation causes GIRK channel sensitization in periaqueductal grey (PAG) area of brain (Chiou et al. 2005). Sodium channels (SC) is a family of sodium conducting ion channels. There are several members in this class including: SCN1A, SCN2A, SCN3A, SCN5A, SCN8A, SCN9A, SCN10A and SCN11A. However, not all of them are found relevant in pain studies. SCN1A
or NaV1.1 is upregulated following a nerve injury, inducing neuropathic pain (Wang et al. 2011). Surprisingly, auditory induced pain is found to be associated with NaV1.7 while NaV1.6 is down regulated (Fryatt et al. 2011). It has been documented that long term nerve injury causes altered gene expression in prefrontal cortex region in function of receptors such as N-methyl-D-aspartic acid (NMDA), SCN1A etc. which is shown by reverse transcriptase-quantitative PCR (RT-QPCR) (Alvarado et al. 2013).

3. TRANSMISSION

Once the action potential has reached the presynaptic level, various ion channels enter the scene, as their opening or closing decides the fate of signal, whether the signal will be transmitted and with what amplitude. On reaching the presynaptic level, calcium channels open generating a presynaptic potential; leading to the release of various neurotransmitters especially acetylcholine in synaptic cleft. From there, the signal is transmitted to the higher corticals of the brain and spinal cord. However, several neurotransmitters such as Gamma-aminobutyric acid (GABA), dopamine, glutamate and acetylcholine alongwith a variety of receptors such as opioid receptor, cannaboid family members and many others also play significant role in transmission of pain signals.

Glutamate is an essential component for pain signal transmission. A class of glutamate receptor family gene GRIN1 has been studied in relation to pain and it is reported to be upregulated during inflammatory pain condition such as tendinitis along with N-methyl-D-aspartate (NMDA) receptors and substance P (Schizas et al. 2012). Acetylcholine is the most crucial component in synaptic transmission. Although acetylcholine is a neurotransmitter but acts in two distinct ways, either propagates or diminishes the pain signal. It has been found that acetylcholine injections are found to reduce pain. Contrarily, acetylcholine released at the site of injury tendinitis causes elevated levels of pain, leading to inflammation (Danielson et al. 2007). Genes coding for voltage dependent calcium channel subunit alpha-2/ delta-1 (CACNA2D1) controls the influx of ions to produce membrane depolarization. In a Swedish study, it has been inferred that presence of major allele ‘G’ is associated with higher opioid sensitivity (Rhodin et al. 2013). These channels are of high therapeutic
relevance as they are the targets of pregabalin and gabapentin (GABA inhibiting drugs) (Taylor et al. 2009). Pain has been described by neuroscientists as a subjective emotion. Hence, an ample share of pain modulation is accommodated by dopaminergic or catelcholenergic neurotransmitters. Dopamine has been found to be related to pain in a reverse manner (Treister et al. 2009). Lower dopamine levels are associated with pain related symptoms in neurodegenerative disease like Parkinson disease. Concordant to the existing thought, recently it has been found in a murine study that dopamine receptor D2 (DRD2) agonists could treat mechano-nociception, but fail to cure thermal nociception (Almanza et al. 2015). There are contradictory evidences in other pain conditions such as fibromyalgia, where no relation has been found in FM subjects showing an altered dopaminergic mechanism involved in fibromyalgia (Wood et al. 2007).

Adrenergic hormones mainly serotonin, adrenaline and nor-adrenaline and their respective receptors influence the pain at spinal and supraspinal levels. Their reuptake inhibitors attenuate pain by preventing their absorption at synapses, causing increased postsynaptic monoamines and sustained descending pathway activity (Zhuo et al. 1991). While the activity of dopamine and serotonin are variable, providing both nociceptive as well as anti-nociceptive behavior, the noradrenaline shows a purely anti-nociceptive effect. Therefore, selective noradrenaline reuptake inhibitors provide more robust anti-nociceptive effects than those of serotonin reuptake inhibitors. Several pain conditions such as osteoarthritis and rheumatoid arthritis involve disease worsening, with loss of noradrenalinergic fibers in synovial tissue (Miller et al. 2000, Miller et al. 2002). However, higher levels of noradrenaline in cerebrospinal fluid preoperatively are related to greater pain scores (Oehmke et al. 2008). A polymorphism has been studied in ADRA2C gene where del/del homozygotes have higher pain scores in response to cold than heterozygotes. Their reading improved after α-2 agonist proving more ADRA2C causes more pain relief (Kohli et al. 2010). Glucocorticoids work in synergy with adrenaline. Patients with rheumatoid arthritis have been tested with the hormone therapy for pain management. However, results vary due to individual cortisol sensitivity variation (Quax et al. 2012a, 2012b).
4. MODULATION

Modulation involves a huge range of effects on pain perception which may either be increasing or attenuating it. However, both the contrasting mechanisms involve descending pain pathway with distinct key players planning the fate of pain, i.e., whether to suppress it or to enhance it. Pain modulation may occur at three levels; peripheral level, dorsal level of spinal cord and at higher corticals of brain. At periphery, most of the TRPA, TRPV and ASIC ion channels are known to upregulate stimuli specific signals. The signals generated by them are eventually conducted by SCN sodium channels and HCN channels. Calcium channels further maintain the signal intensity presynaptically. On the contrary, potassium channels are known to work as anti nociceptive.

The activation of P2 family genes causes activation of various surface receptors, such as P2X4,7 which are responsible for upregulating several inflammatory mediators, leading to hyperalgesia. Specific polymorphisms found in these genes may upregulate the pain. P2X3 receptors show dual effect like opioid receptors as they act pre-nociceptive at peripheral level while anti-nociceptive at central level, thus reducing pain (Ding et al. 2000). Opioid receptors mediate pain in conjugation with P2X3 receptors via phospholipase C (PLC) dependent pathways (Molliver et al. 2011). Purinergic receptor (P2Y12) decreases inflammatory and neuropathic pain by blocking production of cytokines and thermal pain sensitization through IL-7 receptor mediation pathway. Suppression of these receptors has shown to give relief from pain (Horváth et al. 2014). KCNJ6 channel polymorphism is associated with post operative pain (Bruehl et al. 2013). Neuropeptide Y modulates pain at peripheral level via blocking signaling caused by substance P at dorsal horn level as well as causing an anti-hyperalgesic effect (Taylor et al. 2014). TRP1 secretes substance P via inflammatory mediator CGRP thus upregulating the pain. CGRP is also upregulated by prostaglandins during inflammatory pain conditions, hence administration of specific COX inhibitors wipe away pain (Lee et al. 2006). As per the universal norm, once the primary receptor potential is generated and if the whole is accompanied by inflammation, it will certainly end up regulating the pain. It has been inferred from several studies that in many
inflammatory pain conditions, the primary receptor potential is amplified by inflammatory mediators, thus causing generation of an enhanced pain receptor potential (de Oliveira Fusaro et al. 2010). Here variable forms of such agents like interleukins give a profound regulatory effect. Various other inflammatory mediators such as interleukins, cytokines and bradykinin come into scene, intensifying the whole scenario of pain realization. It involves activation of Protein kinase C (PKC), mitogen activated protein kinase (p38) and 9-Jun-N-terminal kinase (JNK) as well. Synthesis of IL-1β, IL-6 and TNF is associated with upregulation of bradykinin B1, B2 receptors (Meotti et al. 2012). Thus, selective bradykinin-1 receptor antagonists have shown to reverse inflammatory and mechanical hyperalgesia by desensitization of peripheral and spinal neurons.

There are major differences found in pain perception rating between male and females in both human and animal based studies. In humans, it is found that men are less prone to pain or have a greater endurance due to protective mechanism of male androgenic hormone testosterone than women. At cortical level, catechol-o-methyltransferase produced by COMT gene has emerged as a benchmark for pain perception in various populations across the globe. A specific nucleotide polymorphism has been examined and it is found that ‘A’ or Met allele of rs4680 SNP is associated with higher pain rating on various scales. Individuals with AA genotype had more pain, followed by AG and GG, in various diseases such as fibromyalgia, osteoarthritis and so on. In many studies, other SNPs of COMT gene such as rs4633, rs4818, and rs6269 constitute pain haplotypes (Rut et al. 2014). Besides neurotransmitters, there are other several notable transporter genes which are found to be associated with pain perception and modulation. The lipid transporter gene Apolipoprotein E (APOE) is a tri-allelic gene. Its ApoE-2 allele is found to be associated with higher disease susceptibility in migraine and fibromyalgia (Gupta et al. 2009, Becker et al. 2010, Reeser et al. 2011). Similarly, P-glycoprotein gene family member ABCB1 (3435 C>T) is associated with lower morphine requirements in severe pain conditions (Lötsch et al. 2009). The metabolic enzyme CYP2D6 also shows relevance to pain as reduced enzyme activity corresponds to reduced pain levels (Samer et al. 2010)
Diagnosis of pain

There are several methods which are employed for the diagnosis of pain depending upon the type, chronicity, region and sensitivity of pain. Biochemical analysis of inflammatory markers such as TNF-alpha and cytokines are utilized for inflammatory pain conditions such as rheumatoid arthritis. Brain imaging techniques such as fractional magnetic resonance imaging (fMRI) and computerized tomography (CT) scans are used to study neuro-anatomical and functional changes related to pain. They are of immense aid in placebo drug studies and to investigate the effect of neurotransmitter agonist and antagonist on the efficacy of brain.

Precise assessment of perceived pain is quite intrinsic and often prone to errors due to subjective variability of pain. Pain assessment techniques are based on the type of pain and clinical settings involved. In animal pain models, experimental pain is generated through various methods such as Freud’s agent, nerve ligation or injury and analysed using tail flick reflex.

In humans, under experimental pain settings, pain of various origins such as pressure-mechanical pain, thermal pain or chemically induced pain is administered and thresholds are recorded for the temperature and concentrations. Nerve specific pain may be examined by studying nerve conducting properties using various electro-diagnostic procedures such as electromyography (EMG), evoked potential studies (EP) and quantitative sensory testing. It may be accompanied by various self assessed questionnaires to quantify pain. Visual analogue scale (VAS) and Numerical rating scale (NRS) are widely used under experimental and clinical pain settings. Wong Becker pain scale is used for assessing pain in pediatric samples.

Specific questionnaires have been developed for pain associated with musculoskeletal disorders. Nordic musculoskeletal questionnaire (NMQ) is a generalized questionnaire for analyzing musculoskeletal pain related to any musculoskeletal disease. Many questionnaires assess disease specific pain such as Oswestry disability index (ODI) for assessing low back pain, Australian/Canadian hand osteoarthritis index (AUSCAN) and Western Ontario and McMaster Universities arthritis index (WOMAC) questionnaires for hand and knee osteoarthritis respectively. Several questionnaires have been developed to
Examine various aspects of pain such as disease associated disability, quality of life and so on. American college of Rheumatology (ACR) invented Fibromyalgia impact questionnaire for assessing pain and overall health in fibromyalgia. McGill health questionnaire discusses pain intensity, along with several other parameters affecting the final pain outcome such as depression and sleep quality.

**Molecular Genetics of pain**

Sensitivity towards pain is a subjective entity and varies greatly across humans. A growing body of evidences suggests that genetic factors play a significant role for this variability (Nielsen *et al.* 2008). Musculoskeletal pain comprises a huge genetic component as twin and family studies have revealed that it has a heritability content ranging from 40 to 60 percent depending upon distinct anatomical regions involved, type of pain and musculoskeletal disorders (Ritter and Bringel. 2009).

Only a limited number of genome wide association studies (GWAS) are available so far in relation to pain. A meta-analysis on GWAS on chronic widespread pain (CWP) in females of European descent confirmed the involvement of rs13361160 of TCP1-complex-5 gene (CCT5) and FAM173B on chromosome 5p15.2 to be associated with 30 percent greater risk of CWP (Peters *et al.* 2013). Another GWAS revealed SNP rs2562456 of long intergenic non protein coding RNA 680 (LOC400680), to be associated with analgesic onset of post surgical pain while rs6693882 of ZNF429 was found to be associated with maximum post operative pain ratings. A haplotypic analysis highlighted link of minor allele haplotypes of SNPs rs2650825, rs1879234, rs2562408 and rs2562466 of zinc finger protein 429 (ZNF429) gene with slow recovery rate (Kim *et al.* 2009). Several disease-based GWAS are available for low back pain, rheumatoid arthritis, fibromyalgia and osteoarthritis; however all of them showed overlapping SNPs and genes, hence suggestive but not conclusive.

The present study aims to examine the role of polymorphic variants within catechol-o-methyltransferase (COMT) and Apolipoprotein E (APOE) in relation to musculoskeletal pain in population of Punjab, suffering from
musculoskeletal disorders. While COMT has been extensively studied and thus a well established gene for its role in pain regulatory pathways, APOE also modulates various underlying processes such as stress, nitric oxide production, inflammation and other which in turn shape the final blueprint of pain perception.

**Catechol-o-methyltransferase (COMT)**

Catechol-o-methyltransferase is a catecholamine degrading enzyme discovered by Axelrod and Tomchick in 1958 (Axelrod et al. 1958). COMT enzyme is coded by COMT gene, located on chromosome 22q11.2. COMT is found abundantly in prefrontal cortex and responsible for dopamine degradation postsynaptically (Karoum et al. 1994). COMT is associated with numerous functions such as cognition, memory, emotional processing and pain modulation. Several studies worldwide have confirmed its association with diseases such as schizophrenia, anorexia nervosa, Down syndrome and obsessive compulsive disorder.

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-adenosylmethionine (SAM) to a hydroxyl group on the catecholamine derivatives such as dopamine, norepinephrine or catechol estrogen, hence causing their deactivation. Biochemical analysis showed 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 reestablishing the blocked COMT expression (Hartung et al. 2015).

COMT gene produces two distinct proteins i.e. soluble-COMT (S-COMT) and membrane bound-COMT (MB-COMT) using single promoter and translation initiation sites, which play distinct roles in the synaptic transmission and in cortical neurons. Animal models suggest that S-COMT deficient mice possess
lower levels of dopamine in prefrontal cortex and altered spinal pain reflex in a sex dependent manner whereby male having far reduced values (Tammimäki et al. 2010). MB-COMT is found abundantly in cortical neurons and dendrites. MB-COMT is capable of inactivating synaptic and extra synaptic dopamine on pre and post synaptic neurons.

COMT is also known to be regulating stress related behavior by dopaminergic systems in prefrontal cortex. COMT knockout mice has demonstrated increased anxiety related higher production of corticosterone especially in females, but better performance in cognitive tasks (Desbonnet et al. 2012). While over expressed valine knocked in mice shows a diminished pain and stress response but attenuated attention and cognitive ability which is mediated by prefrontal cortex calcium/calmodulin-dependant protein kinase II (CaMKII) levels (Papaleo et al. 2008). Age related grey and white matter volumes in females are also thought to be governed by COMT genotypes (Zinkstok et al. 2006). Moreover, COMT inhibition induces apoptosis and the cytotoxic mechanism is dependent on val/met (A/G) genotypes (Chen et al. 2011).

Furthermore, COMT also modulates the opioid based analgesic drug efficacy as it is a key enzyme of adrenergic system, which degrades catecholamines including epinephrine, nor-epinephrine and dopamine. Thus, it regulates pain both directly and indirectly via effective endogenous μ-opioid function. COMT performs a differential effect on modulation of pain depending upon the site of action. Enhanced catecholamine transmission in the spinal cord gives analgesic effect while increased transmission at the periphery afferent neurons produces hyperalgesia when stimulated, in conjunction to beta 2, 3-adrenergic receptors (Nackley et al. 2007). Various polymorphisms within COMT gene resulting in reduced COMT enzyme production, lead to chronic over-activity of μ-opioid systems, which decreases its ability to modulate nociceptive input (Kowarik et al. 2012). There is convincible amount of evidence in post operative pain conditions, suggesting role of COMT in determining the dosage requirements to combat pain.

Polymorphisms within COMT gene have been studied in relation to pain perception in both clinical and experimental pain settings. Variants of COMT
gene have been found to be associated with pain sensitivity (Zubieta et al. 2003), vulnerability to chronic pain (Diatchenko et al. 2005) and anxiety disorders (Hettema et al. 2008). Most widely examined amongst others is 158Val/Met (A/G) polymorphism of rs4680 producing methionine and valine amino acids in substitution to each other. Methionine variant produces more thermostable form of enzyme and reduced enzyme activity than valine, thus produces greater pain sensitivity (Diatchenko et al. 2006). Besides these, rs4680, rs4633, rs488 and rs6269 have been studied extensively in relation to pain and low pain sensitivity (LPS), average pain sensitivity (APS) and high pain sensitivity (HPS). Haplotypes have been derived on the basis of COMT production. They are found to be associated with musculoskeletal pain in various diseases such as TMD, osteoarthritis, low back pain and fibromyalgia. Being a candidate gene for depression, COMT polymorphisms are also found to be related to disease related depression and disease worsening.

**Apolipoprotein E (APOE)**

Although some studies have suggested a link of APOE with pain, yet a huge association of APOE with the underlying process of pain hints the indirect association of APOE with pain. Apolipoprotein E is one of the lipoproteins, found in chylomicrons and intermediate density lipoproteins (IDLs). Peripherally, it is produced by liver and macrophages, however in brain it is produced by astrocytes. The major function of APOE is transport of cholesterol to the neurons via APOE receptors and ligand mediated lipid metabolism. Apolipoprotein E (APOE) gene is localized on 19cen-q13.2 in an approximately 45kb gene cluster containing genes for APOC-I, C-II, C-IV and C-I pseudo-gene alongwith elements controlling their tissue specific transcription (Allan et al. 1995). APOE exists in three polymorphic forms; ApoE-2, ApoE-3 and ApoE-4. ApoE-3 is the wild type allele and is associated with normal lipid levels where ApoE-2 is related with hypodyslipidemia and ApoE-4 is related with hyperdyslipidemia. There are three relatively common allelic variants of ApoE, as defined by two SNPs, rs429358 and rs7412 known as ApoE-2, ApoE-3, and ApoE-4.
APOE is the major lipid transporter and cholesterol regulating gene. It is responsible for atherogenic dyslipidemia which is characterized by reduced HDL levels along with elevated levels of TC and TG serving as the disease hallmarks. It also involves chronic endotoxemia and systemic inflammation. Abnormal lipid levels pose risk of atherosclerosis, which in turn promotes the progression of disc degeneration and low back pain (Kauppila et al. 2009). Elevated lipid levels have been found in tendonitis, where lipid lowering drugs reduce inflammation and other manifestations such as pain (Klemp et al. 1993).

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 potentisation (LTP) (Korwek et al. 2009). APOE knockout mice showed decreased synapse/neuron ratio, along with increase in synaptic size in a trend where the reduction was far drastic in ApoE-4 than ApoE-2 knockout mice. Moreover, genetic analysis have reported that ApoE-4 allele is associated with accelerated neuro-degeneration in brain, as ApoE-4 has less dopamine in prefrontal cortex thus having reduced task efficiency, reduced cortical strength, especially C/C women being more prone to damage which is accelerated by higher fatty acid diet (Tolonen et al. 2011).

Proteomic studies have confirmed that APOE is upregulated following a nerve injury and participate in nerve regeneration, hence an intrinsic factor (Melemedjian et al. 2013). Evident to the fact, murine studies have shown that APOE knockout mice, fed on high fat diet showed increased oxidized LDL levels in tendons that had accelerated its degeneration (Grewal et al. 2014). Elevated levels of ApoE protein were found in chronic lumbar pain and spinal nerve injury (Cameron et al. 1995). The upregulation of APOE is influenced by increased inflammation (VanderPutten et al. 1993). Polymorphic studies have
inferred that ApoE-4 is also found to be related to increased lipid levels, pro-inflammatory and oxidative stress (Jofre-Monseny et al. 2008).

APOE participates in vitamin K metabolism. Vitamin K is essential for carboxylating osteocalcin, thus higher levels of uncarboxylated osteocalcin are co-related with increased risk of hip fractures. ApoE-4 has far lower plasma levels of vitamin K causing a reduced transport, which is found in osteoarthritic women (Olson et al. 2000).

Several genetic polymorphism studies have been conducted in humans in relation to their genotypic susceptibility to various pain related disorders. APOE is studied in relation to migraine, where ApoE-2 emerged as a risk factor for having migraine and ApoE-4 being the protective one (Gupta et al. 2009). Surprisingly, the scenario is reversed in terms of musculoskeletal disorder related pain. Presence of E-2 allele has been found to be protective factor for fibromyalgia and disease related stress (Becker et al. 2010). While ApoE-4 allele of APOE gene is considered as a risk factor of having post traumatic fibromyalgia syndrome (FMS) (Reeser et al. 2011). ApoE-4 is also considered as a potential risk marker for development of amyloids and inflammation in rheumatoid arthritis patients especially E-3/4 genotype (Toms et al. 2012).

**Role of hypothalamic pituitary axis in pain**

Hormonal regulation of pain is governed by the hypothalamic pituitary axis (HPA). There is satisfying amount of evidence for stress induced analgesia. Periaqueductal gray matter (PAGM) in midbrain is involved in stress induced analgesia in corticotrophin-releasing factor (CRF) and glucocorticoids induced somatic pain sensitivity (Yarushkina et al. 2015). A hyporesponsive HPA axis has been found to be associated with musculoskeletal pain at age early age (Paananen et al. 2015). Genetic variants in corticotropin releasing hormone such as corticotropin releasing hormone binding protein (CRHBP), nuclear receptor subfamily 3group C member 1 (NR3C1) and corticotropin releasing hormone receptor R1 (CRHR1) are associated with musculoskeletal pain (Linnstaedt et al. 2016). Physical stress also activates the beta endorphin production by activating opioid receptors, thus activating an endogenous analgesic system.
Significant risk factors associated with musculoskeletal pain

Pain is a complex entity which is influenced by milieu of physiological, environmental, psychosocial and genetic factors such as age, gender, ethnicity, smoking, alcohol drinking, body mass index (BMI), socioeconomic status, duration of disease, co-existing morbid disease, depression, sleep, diet, bereavement of the loved one, high cytokine levels and dyslipidemia, however, some of these are discussed here.

Age

Advancing age after 40 years is generally considered as a risk factor for some illness. It is believed that pain perception increases as the age advances, nonetheless, exceptions do prevail as it is not the rule. One of the reasons behind it is that older people usually suffer from more chronic diseases than younger individuals due to weakened immune system, slow DNA repair and sluggish neuronal regeneration. However, it is also not untrue that with age, there is reduced sensitization of pain and greater coping up skills. Murine studies have shown that younger mice showed greater sensitization to mechanical pain with increased firing rates of C and A fibers in acute inflammation. The firing rates were drastically reduced to half in chronic inflammation. No such increase in pain sensitization is seen in aged mice either in acute or chronic inflammation (Weyer et al. 2016).

Duration of disease

Intensity of pain increases with the prolonged duration of chronic and degenerative disease such as low back pain and rheumatoid arthritis. With prolonged duration of musculoskeletal disorders, there is an increase in anatomical sites and tender points having pain (Pollard et al. 2012).

Being a woman

Many murine and human studies have concluded that women are more prone to pain in comparison to males. It has been inferred that testosterone gives an overall anti-nociceptive effect while estradiol gives pro-nociceptive effects (Bartley et al. 2015). Women are found to be more predisposed to the
risk of developing diseases such as osteoporosis, osteoarthritis, low back pain and Rheumatoid arthritis (Pueyo et al. 2012). Many studies investigating gender role expectation of pain (GREP) found women being more prone to pain than men although they failed to highlight any ethnic differences in pain (Defrin et al. 2009, Alabas et al. 2013).

**Menopausal age**

The odds of occurrence of musculoskeletal pain and disorders in women rise during perimenopause and menopause (Dugan et al. 2006). Menarche at an early age is associated with an early outburst of painful conditions such as low back pain, upper extremity pain and chronic widespread pain (Kvalheim et al. 2016). The potential reason might be an exponential decline in female hormones during menopause, leading to accelerated bone calcium loss, making them more prone to musculoskeletal disease and resulting pain.

**Diet**

Deficiency of certain elements in diet such as vitamin D, calcium or over consumption of saturated fatty acids may worsen the pain. Immune cells have vitamin D receptors, thus vitamin D plays an active role in inflammatory diseases such as rheumatoid arthritis (Guillot et al. 2010). Recently, a Brazilian study has shown that chronic musculoskeletal pain is independently associated with malnutrition (Barbara Pereira Costa et al. 2016). The European male ageing study (EMAS) in a follow up of 3369 men has concluded that lack of sufficient vitamin D is responsible for developing chronic widespread pain (McCabe et al. 2016).

**Marital status**

Several studies examining the relationship of marital status with pain have obtained mixed results. One view suggests that married people experience lesser pain as it has been observed that happy partners show less pain and cognition than singles (Taylor et al. 2013). Being a single, divorced or separated poses a two fold higher risk of developing chronic pain (McBeth et al. 2015). A second far contrary perception suggests that in comparison to married subjects,
widows have reduced psychiatric symptoms associated with pain. They experience less depression than divorced or separated depicting an emotional resilience to pain (Wade et al. 2013).

**Education**

Education has a profound impact on the overall health of an individual as it encourages one to understand the basis of disease condition and developing personal coping up strategies to tackle pain. A Norwegian study predicted five fold increased risk of developing musculoskeletal disorders in subjects having primary education than higher education group (Valset et al. 2007). Reddy et al. (2015) have reported that illiterate women are found to have a higher prevalence of musculoskeletal disorders.

**Socioeconomic status**

It has been observed that individuals belonging to lower socioeconomic group of the society tend to have a higher vulnerability for musculoskeletal disorders and the consequent pain due to reduced sources of medical care and hygiene. Having a sound socioeconomic background is predicted to be a protective factor for persistent low back pain (Hestbaek et al. 2008).

**Body mass index/Obesity**

A body mass index (BMI) value is related with obesity. Studies have shown that higher BMI or being obese is a risk factor for various musculoskeletal diseases such as low back pain, osteoarthritis and Rheumatoid arthritis (Heuch et al. 2015). Contrarily, obesity is found to improve areal bone mineral density and bone in certain diseases such as osteoporosis (Maïmoun et al. 2015). A few studies have given a controversial inference where obesity is associated with reduced nociceptive pathways due to dampening of noxious stimuli signals through the insulation of underlying adipose tissue. Summarizing the facts, most of the studies predict obesity to be a risk factor for degenerative and inflammatory pain conditions.
**Blood pressure**

Blood pressure influences pain modulation in a dramatic manner. Non-steroidal anti-inflammatory drugs (NSAIDs) used to suppress chronic pain are observed to increase hypertension, which suggests that alternate medications can be used (Chawla et al. 1999). A Brazilian study has concluded that both men and women with uncontrolled hypertension have a higher prevalence of musculoskeletal complaints (Kerkhoff et al. 2012). Several other studies have reported reverse relationship, whereby high blood pressure is inversely related to the prevalence of musculoskeletal and other pain disorders, probably due to hypertension associated hypoalgesia (Stovner et al. 2009). Individuals with high systolic and diastolic blood pressure have low prevalence of chronic musculoskeletal complaints (Hagen et al. 2005). Overall, the relationship of hypertension with chronic pain presents a complex and controversial picture, where it is still to be classified that whether hypertension is causal to pain or just a manifestation of it.

**Lipid levels**

Lipids levels are found to be in discordance than their normal levels in several musculoskeletal pain conditions especially the ones which involve an inflammatory state such as rheumatoid arthritis (Liu et al. 2015). In vivo studies have reported reduced HDL levels in inflammatory pain conditions through atherogenic dyslipidemia and reverse cholesterol transport (RCT) (de la Llera Moya et al. 2012). Moreover, atherosclerosis resulting from abnormal lipid levels also causes a predisposition for low back pain and disc degeneration (Kauppila et al. 2009). Higher levels of high density lipoprotein (HDL) is reported to be inversely related to pain while higher triglyceride (TG) levels are directly associated with risk of chronic low back pain (Heuch et al. 2014).

**Use of statins**

Statins or 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitors are lipid lowering drugs, generally prescribed to patients having hyperlipidemia and hypercholesterolemia. Use of statins is associated with muscle injury on prolonged use even in asymptomatic individuals (Parker et al. 2013) and often
results in statin induced myopathy (Rodine et al. 2010). It has been observed that in adult population having age above 40 years, the risk of pain increase by 1.5 to 2 fold in subjects using statins than non users (Buettner et al. 2008). This effect is observed to be enhanced by a coexisting vitamin D deficiency (Morioka et al. 2015).

**Alcohol drinking**

Observational, epidemiological and clinical studies have shown that alcohol consumption gives an anti-nociceptive effect in a dose dependent manner, however prolonged use is associated with reduced pain thresholds and withdrawal associated hyperalgesia (Zhang et al. 2015). The subjects having familial history of alcoholism are found to be more prone to greater pain sensitivity irrespective of their drinking status (Stewart et al. 1995). Conversely, individuals having a pain condition are more likely to develop alcohol dependence (Goldberg et al. 1999). Ethanol modulates the dopamine mediated reward mechanism in pain as both ethanol and dopamine governs through common neuronal pathway. Hence, pain relief serves as a reward through alcohol intake. Alcohol being a gamma-amino butyric acid (GABA) agonist, also acts as analgesic in pain conditions having lower levels of GABA such as fibromyalgia (Kim et al. 2013).

**Smoking**

Smoking has been observed to confer an adverse effect on both respiratory and nociceptive functions of the body. Although taking anti-nociceptive in acute quantities, prolonged smoking or even passive starts decreasing pain perception due to neuronal loss in hippocampus region (Csabai et al. 2016). Concordant to this, prolonged emotional pain stress (PEPS) causes reduction in neuronal density in hippocampus region CA3 (Shiryaeva et al. 2008). Contrarily, some studies have reported that prolonged cigarette or tobacco smoking is associated with increased pain perceptions. The precise mechanism behind this phenomenon is probably due to reduced immune system and accelerated neuronal degeneration. A Meta-analysis highlighted that tobacco use, being the major cause of addiction owing to its acute nicotine
induced analgesic effects; which is more prevalent in chronic pain conditions, thus worsening the situation (Ditre et al. 2016). Smoking is also a risk factor for sciatica, lumbar radicular pain and cessation of smoking reduces it (Shiri et al. 2016).

**Depression**

Depression has a bi-directional relationship with pain. Pre-existing anxiety and/or depression is significantly correlated with incident musculoskeletal pain (Del Campo et al. 2016) and patients who suffer from musculoskeletal pain are found to be more prone to anxiety and depression (Gureje et al. 1998). Thus, it has been seen that an improvement in depression and anxiety reduces the pain intensity and number of sites bearing pain (Scott et al. 2016).

**Exercise**

Exercise is one of the most effective non invasive clinical interventions for managing chronic pain. Exercise promotes bone growth by increasing bone mass and muscle strength. Secondly, the release of beta endorphins is induced by exercise which gives antidepressant and endogenous analgesic effects by activating supraspinal inhibitory pathway (Nijs et al. 2012). Thirdly, the levels of inflammatory mediators and neurotransmitters are observed to be reduced post exercise, giving a hypoalgesic effect in chronic pain conditions (Kawi et al. 2016). There is an exponential increase in anti-inflammatory cytokines such as (interleukins) IL-6, IL-10, IL-1RA and C-reactive protein (CRP) following exercise (Petersen et al. 2005). Fourth, exercise promotes attainment of greater peak bone mineral density higher than sedentary subjects, independent of calcium intake, which in later stages serves a protective factor for osteoporosis (Anderson et al. 2000). A therapy involving different types of exercises, according to the anatomical site of pain is proven to reduce pain in most effective way (Mansi et al. 2014). Last but not the least, regular exercise is found to be efficient in reducing work related musculoskeletal pain conditions such as cervical spondylosis, neck pain, and low back pain (Rodrigues et al. 2014). It is
also found to be effective in reduced analgesic requirement for pain relief (Juhl et al. 2014).

**Sleep**

It has been revealed that both chronic pain and sleep influence each other. Follow up studies have shown that poor sleep quality may predict onset of musculoskeletal complaints at later stages (Bonvanie et al. 2016). A retarded sleep worsens the pain condition in several diseases such as fibromyalgia by causing hyperalgesia. It alters the drug efficacy of analgesics by interfering in opioidergic and serotonergic systems (Kundermann et al. 2004). Behavioral therapies which aim to improve sleep quality simultaneously decrease pain sensitization in chronic pain conditions (Lami et al. 2016).

The genetic contributions and consequences of musculoskeletal pain have not been explored in the population of Punjab hitherto. So the present cross sectional study was designed with the following objectives.

**Objectives:**

1. To investigate the effect of COMT and APOE genes as the genetic determinants of pain in the population of Punjab having musculoskeletal disorders.
2. To assess their gene-gene, gene-environment interactions both individually and at haplotype level.
3. SNP-SNP cross talks will be analysed for their epistatic and hypostatic effect of musculoskeletal pain.