REVIEW OF LITRATURE

Every stimulus that evokes pain has been investigated thoroughly from physical, physiological and psychological perspectives. In the last 20 years various gene association studies have heralded constellations of genes and genetic variants in the galaxy of human molecular genetics of musculoskeletal pain. Animal studies employing selectively bred line and inbred strain panels elucidated the underlying genetic correlation of musculoskeletal pain. These studies have exposed that there is a unique inheritance pattern of different pain conditions and there is an inverse relationship between pain sensitivity and analgesic response. Some monogenic conditions of pain are also known where the pain sensitivity depends upon the single gene. It is well understood that musculoskeletal pain is a multigenic, multifactorial complex problem, where innumerable factors interact with each other for sensing the initial noxious signal, interpreting it and then collaborate with the natural internal analgesia of the body to announce the final verdict of pain. All this complex combinations within the brain engage physical, physiological and psychological triggers, but growing body of evidence suggests that sensory input is first and foremost realized, processed and mediated through an individual’s genetic profile. Moreover, even the endogenous effect and efficacy of anti-nociceptive drugs depends upon the genes one carries. Although several genes have been observed to be associated with pain in different clinical settings, the present study employed catechol-o-methyltransferase (COMT) and Apolipoprotein E (APOE) genes as the genetic determinants of musculoskeletal pain in the population of Punjab.

Catechol-o-methyltransferase (COMT)

Catechol-o-methyltransferase (COMT) is a major mammalian enzyme responsible for metabolic degradation of catecholamines by catalyzing the transfer of a methyl group from the S-adenosyl-methionine (SAM) to a hydroxyl
group on catechol nucleus thus destabilizing the structure. It was first discovered by Julius Axelrod in 1957. Catechol-o-methyltransferase is coded by COMT gene, located on chromosome 22q11.21 in humans and contains 6 exons (Salminen et al. 1990).

COMT is the key regulator of dopaminergic system as it degrades the catecholamines such as epinephrine, norepinephrine and dopamine, hence modulates the final outcome of pain, cognitive and stress related behaviors. COMT gene produces two distinct proteins i.e. soluble COMT (S-COMT) and membrane bound COMT (MB-COMT) using single promoter. They have their distinct role in the synaptic transmission, and cortical neurons. S-COMT is found in nucleus and plasma membranes. S-COMT has a greater efficacy of degrading dopamine than MB-COMT as concluded in study kinetics of the two isoforms (Lotta et al. 1995). Animal models have suggested that S-COMT deficient mice possess lower levels of dopamine in prefrontal cortex and altered spinal pain reflex in a sex dependent manner, where males have 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. Moreover, COMT inhibition induces apoptosis of neurons and the cytotoxic mechanism which is dependent on Val/Met genotypes of COMT single nucleotide polymorphism (SNP) rs4680 (Chen et al. 2011).

**Relationship of COMT with pain:**

Several genetic studies on humans as well as animals have confirmed the role of COMT gene in modulating pain and related stress. However, there is a huge variability in the results due to genetic, ethnic, gender and other environmental differences. The nociceptive function of COMT is modulated in an intricate way, which in turn, is mediated through various physiological mechanisms such as stress and inflammation. The preliminary experiments conducted on COMT gene knockout mice demonstrated that inhibiting COMT activity enhances the pain sensations. However, during stress conditions, COMT knockout mice produce far enhanced morphine induced anti-nociception due to greater availability of opioid receptors in the absence of competitive binding by COMT (Kambur et al. 2008). Kambur et al. (2010) further reported the anti-
nociceptive action of COMT using COMT inhibitors, where administration of it subsequently lowers the thermal and mechanical pain threshold values in animals. The pro-nociceptive effects of COMT inhibitors retained their effect when given in heavy doses. During acute stress conditions, COMT inhibition restrains stress induced analgesic response by the endogenous system of the body. In a murine study by Desbonnet et al. (2012), COMT knockout mice have shown an increase in corticosterone levels owing to anxiety in acute pain but not in chronic pain with a modified cytokine profile in chronic stress. Female COMT knockout mice showed elevated levels of anxiety and corticosterone than males, suggesting the role of COMT in stress related hormonal and immune system control in sex dependent manner. Inhibition of COMT increases the production of nitric oxide derivatives and other inflammatory mediators such as tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) which is mediated through adrenergic receptors. Furthermore, inhibition of nitric oxide synthases enzyme and inflammatory markers cease the COMT activity dependent pain sensation (Hartung et al. 2014).

**COMT polymorphisms in nociceptive and experimental pain**

Genetic polymorphism within COMT gene has been extensively studied and is found to be associated with nociceptive pain thresholds in various clinical settings. Most extensively studied one is val158met polymorphism which is a non-synonymous functional polymorphism. A single nucleotide substitution from A to G at 158 codon results in the production of methionine and valine amino acids respectively. Thus a genotype AA is a homozygous for methionine amino acid depicted by met/met while GG is valine homozygous shown by val/val and AG shows the heterozygous genotype abbreviated as val/met. The methionine variant is quite thermostable than valine variant, thus accounting for three to four fold reduced enzyme activity than valine which results in greater pain sensitivity. Diatchenko et al. (2005) in an attempt to dissect the possible link between COMT polymorphisms and pain sensitivity in chronic induced pain in experimental settings, examined seven COMT SNPs in normal human subjects. Haplotypes were constructed on the basis of four SNPs, rs6269, rs4633, rs4818 and rs4680 where GCCG was termed as low pain sensitivity
(LPS), ATCA as average pain sensitivity (APS) and ACCG as high pain sensitivity (HPS) markers. The presence of single copy of LPS haplotype was not found to be associated with lowered pain tolerance. The same were also beneficial in predicting the risk of developing pain in various musculoskeletal regions of the body (Diatchenko et al. 2005). Another genetic study by Diatchenko et al. (2006) confirmed the haplotype specific association of COMT SNPs rs6269, rs4633, rs4818 and rs4680 with experimental pain in determining the resting pain thresholds. In this replication sample, GCCG, ATCA and ACCG were confirmed as HPS, APS and LPS respectively. The LPS/LPS diplotype was associated with least pain tolerance, APS/APS with average pain and APS/HPS had the highest values. However, haplotypes showed no considerable effect on temporal summation of pain during multiple pain evoking stimuli, which in turn, was found to be influenced by COMT val158met polymorphism. Expanding the concept of haplotypes related to pain perception, Nackley et al. (2009) reported some novel SNPs that play supplementary role in building pain sensitive and pain protective haplotypes. They added more SNPs in the previously established pain sensitivity haplotypes. The statistical analysis revealed that rs769224 was associated with APS haplotypes while rs6267, rs740602 and rs8192488 were associated with HPS haplotypes, nevertheless they did not affect mRNA expression, thus might be neutral to particular haplotype. Far lesser variations were reported in LPS haplotype. Further, mRNA analysis of COMT supported the hypothesis that coexistence of alleles in coding regions of COMT gene affects the secondary structure of corresponding mRNA transcripts. Hence, SNPs in promoter region of COMT affect the production and efficacy of the enzyme (Nackley et al. 2010). A study conducted on motor vehicle collision accident survivors found that patients with met/met genotype were suffering from moderate to severe neck pain in comparison to subjects with val/met who had mild to moderate pain rating and val/val genotype showed the least on pain scorings (McLean et al. 2011). mRNA analysis of COMT supported the hypothesis that coexistence of alleles in coding regions of COMT gene affects the secondary structure of corresponding mRNA transcripts (Nackley et al. 2010).
COMT is found to be influencing pain by involving prefrontal cortex and dopaminergic mechanisms of the brain. It has been confirmed by fractional magnetic resonance imaging (fMRI) based analysis while testing the hypothesis that COMT mediated analgesic action is activated when brain is adequately challenged by repeated pain stimuli. It was inferred that COMT individuals who had met/met genotypes of COMT gene polymorphism of SNP rs4680 had greater sensation depicted by illumination in associated brain regions. These changes were putatively seen only by high intensity repeated pain stimuli (Loggia et al. 2011).

On examining the relationship between pain, gender and genetic variations, no noteworthy haplotypic associations were derived in response to capsaicin induced pain except the fact that LPS/LPS were related with greater sensitivity for pain than HPS/HPS haplotype in humans (Belfer et al. 2013). Furthermore, COMT ancestral allele in the absence of short interspersed nuclear element B2 (B2 SINE) transposonal insertion was found to be related with higher capsaicin induced pain perception, which was considerable only in female mice, whereas the values remained insignificant in males (Belfer et al. 2013). A review by Sprangers et al. (2014) evaluated the effect of various components such as pain, emotional and social functioning on the quality of life. In a multigenic association analysis COMT gene was found to be associated with musculoskeletal, neuropathic and experimental pain conditions and dopaminergic mediated opioid drug efficacy, thus influencing the overall quality of life of an individual. In another set of experiments, pain rating was also analyzed in patients who survived motor vehicle collision. In that sample, ten COMT SNPs were examined and haplotypes were analyzed accordingly. Haploblock-1 included rs2020917, rs737865 and rs1544325, haploblock-2 included rs4633, rs4818, rs4680 and rs165774 and haploblock-3 included rs174697 and rs165599 SNPs. Haplotype TCG of haploblock-1 was found to be associated with decreased pain interference, whereas haplotype CGGG of haploblock-2 was considered to be pain protective, but it was effective only in the presence of at least one copy of TCG haplotype of haploblock-1. Haplotype AG from haploblock-3 was observed to be associated with pain only in men as values were insignificant in women (Bortsov et al. 2014). A study aimed to
investigate genetic association of pain in multi-modal multi-tissue of healthy subjects, was comprised of multiple SNPs of COMT, opioid receptor delta (OPRD), opioid receptor kappa (OPRK) and opioid receptor mu (OPRM) genes. The findings of the study concluded that ‘A’ allele carriers of rs4680 SNP of COMT reported higher bone pressure pain tolerance thresholds, whereas ‘C’ allele of rs6473799 of OPRK gene was observed to be associated with mechanical pain tolerance (Nielsen et al. 2016). COMT was reported to modulate pain in gender specific manner. The response to stress with HPS haplotype was found to be associated with increased pain sensitivity in non-stressed subjects of both genders markedly. In post motor collision, male subjects with HPS had greater pain sensitivity in response to stress while no such association was found in female subjects (Meloto et al. 2016).

**COMT polymorphisms in musculoskeletal pain conditions**

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 rs4680 polymorphism was found to be associated with increased disease severity of fibromyalgia in Spanish individuals where met/met and val/val genotypes were associated with highest and lowest disease related damage respectively (Josep García-Fructuoso et al. 2006). In another study on Spanish population examining the role of COMT polymorphisms in relation to FMS related pain, it was found that haplotype ACCG of rs6269, rs4633, rs4818 and rs4680 was related to higher pain intensity and disease severity (Vargas-Alarcon et al. 2007). Cohen et al. (2009), in a case control study found the same dose dependent effect of met/val alleles on pain sensitivity where met/met genotype carriers of rs4860 SNP reported enhanced pain sensitivity than met/val or val/val subjects, making it a potential risk factor for FMS. A genetic study involving serotonin receptor gene 5-hydroxytrptamine type 2 (5-HT2A) and COMT genes found that the number of individuals with met/met genotype of COMT rs4680 polymorphism was significantly high in
fibromyalgia patients than normal subjects, whereas CC genotype of 5-HT2A gene was more prevalent in subjects having FMS, thus showing a greater association of met/met and CC genotypes of COMT and 5-HT2A genes with FMS (Matsuda et al. 2010). On studying the effect of pain on daily routine with respect to COMT and opioid receptor mu-subunit 1 (OPRM1) genes, rs4680 met/met genotype in COMT was significantly associated with higher pain perception and greater reduction in positive effect on pain days in fibromyalgia related pain. The genotype asn/asn of rs1799971 in opioid receptor mu-1 (OPRM1) gene was related only with reduced positive days inspite of pain, probably due to its role reward mechanism (Finan et al. 2010). Extending the previous work, Finan et al. (2011) analyzed effect of COMT variant rs4680 with respect to attention and pain coping in women. They found that fibromyalgia patients with met/met genotype reported higher pain sensitization, catastrophizing and maladaptive pain related coping skills. Similar results were published by Barbosa et al. (2012), where they concluded that CC genotype of rs4818 and AA genotype of rs4680 in COMT were quite frequent in FMS patients and consequently were related to higher fibromyalgia impact scores with greater pain sensitivity than the heterozygote individuals (Barbosa et al. 2012). COMT was also found to influence psychological distress in FMS as the patients with met/met genotype had the highest anxiety, depression, catastrophizing and analgesic intake than other two genotypes. Moreover, the frequency of met allele was much higher in FMS cases than control subjects (Desmeules et al. 2012). In order to study the effect of COMT polymorphisms on pain sensitivity in fibromyalgia in Spanish population, haplotypes were derived for rs6269, rs4633, rs4818 and rs4680 where GCGG, ATCA and ACCG were recognized as LPS, APS and HPS haplotypes respectively. The FMS patients having HPS/APS diplotypes showed higher pain sensitivity for both thermal and pressure pain stimuli than patients with HPS/HPS diplotypes. Additionally, FMS patients who had met/met genotype of rs4680 reported increased pain sensitivity than controls (Martínez-Jauand et al. 2013). Desmeules et al. (2014) analysed the central pain sensitization in fibromyalgia with respect to COMT rs4680 polymorphism. They measured the spinal nociceptive flexion reflexes, where met/met patients were found to be experiencing greater pain and central
sensitization than other two genotypic groups (Desmeules et al. 2014). COMT val158met polymorphism was found to be associated with pain sensitivity in fibromyalgia, where it was inferred that patients having met/met genotypes had greater pain affinity and fibromyalgia related disability (Inanir et al. 2014). In Korean population, SNPs rs165599, rs4818 and rs4680 of COMT were examined and haplotypes were derived. The results of their study showed the presence of GG genotype of rs4818 and ACG haplotype as potent risk factors for the occurrence of FMS and increased pain sensitivity, suggesting an ethnic content in genetic variation (Park et al. 2016).

There is sufficient amount of scientific evidence available that supports the role of COMT in temporo-mandibular disorders (TMD). In a clinical drug trial, COMT enzyme activity based haplotypes were assessed in relation to pain rating in response to propranolol treatment. Haplotypes were analyzed as already described by Diatchenko et al. (2005). It was observed that subjects lacking LPS haplotype gave the best drug response, followed by an intermediate response in LPS/APS heterozygotes and least in LPS/LPS diplotypes (Tchivileva et al. 2010). A novel non synonymous SNP at 58 codon causing arginine to serine transition in COMT gene was found to be related to increased pain sensitivity in TMD (D’Antò et al. 2010). A genetic study was conducted to analyze 358 genes for their possible involvement in pain processing. The results verified that SNP rs174697 of COMT gene was found to be independently associated with chronic TMD (Smith et al. 2011). In another genetic study, effects of COMT polymorphisms were associated with coexisting depression on pain intensity in TMD related pain. SNP rs5993882 of COMT was identified as most prevalent and potent risk factor for TMD pain in non depressed subjects. Similarly, rs1544325 of COMT was found to be more frequent in depressed subjects and correlated significantly to higher TMD associated pain (Schwahn et al. 2012). COMT polymorphisms within 40 SNPs were studied in Italian population with respect to TMD susceptibility where rs165656 and rs4646310 SNPs were found to be the main culprits (Michelotti et al. 2014).

Osteoarthritis related pain as a result of genetic polymorphism within COMT gene has been investigated by several researchers. Many studies hinted the involvement of COMT in osteoarthritis related pain. Similar to the
association found in FMS, osteoarthritis patients who had met/met genotype of rs4680 SNP experienced greater pain in hip. This genotype specific association with pain was found to be significant only in women (van Meurs et al. 2009). Contrary to the previous findings, AA or val/val genotype of rs4680 was found to be associated with exacerbation of pain after daily physical activity than other genotypes (Martire et al. 2016).

Consistent with the results from other musculoskeletal pain conditions, COMT polymorphism affected pain sensitivity in low back pain (LBP). Dai et al. (2010) studied COMT polymorphism in patients undergoing surgery who were suffering from disc degeneration disease (DDD). They constructed haplotype by the combination of SNPs rs6269, rs4633, rs4818 and rs4680 similar to those by Diatchenko et al. (2005). The LPS haplotype ATCA was found to be significantly associated with greater pain sensitivity and disability in low back pain, which in turn was far elevated in ATCA/ATCA diplotype individuals. Moreover 'T' allele of rs4633 was also observed to be related to greater improvement in pain scores following the surgery (Dai et al. 2010). In a genetic case control study including subjects with low back pain, discogenic pain and sciatica, it was revealed that patients having met/met genotype experienced greater pain in lower back and showed slow recovery than patients with val/met or val/val genotypes following lumbar disc herniation (Jacobsen et al. 2012). Polymorphisms within four SNPs of COMT were studied in low back pain patients who underwent lumbar fusion surgery, where presence of ‘C’ allele in rs4633 and AG (met/val) genotype of rs4680 showed the greatest reduction in pain ratings post treatment (Omair et al. 2012). Gruber et al. (2014) reported that ‘T’ allele of rs4633 and ‘C’ allele of rs165656 SNPs within COMT gene were associated with greater LBP due to disc degeneration (Gruber et al. 2014). The ‘A’ (met) allele of rs4680 SNP was observed to be significantly associated with greater disease associated disability in low back pain patients (Omair et al. 2015).

A few studies have been performed highlighting the association of COMT gene variants with shoulder pain. George et al. (2008) reported that individuals with APS/HPS diplotypes of SNPs rs6269, rs4633, rs4818 and rs4860 reported
greater pain catastrophizing along with increased pain sensitivity in patients suffering from shoulder pain than other diplotypes (George et al. 2015).

COMT gene polymorphisms influence the pain perception in both neuropathic and nociceptive pain, however COMT follows quite diverse mode of action in these two processes (Segall et al. 2012). It influences pain thresholds in various neuropathic pain conditions such as migraine, tension type headache and cancer pain. Hagen et al. (2006) reported that COMT rs4680 polymorphism was associated with migraine, where migraine patients with met allele were estimated to be more prone to pain, especially men while majority of controls carried val/val genotype.

**COMT polymorphism in analgesic functioning**

COMT gene is one of the key players in dopaminergic system. It affects the functioning of opioid system, as COMT competes with beta-2, 3 adrenergic derivatives for binding with receptors. Polymorphisms within COMT are also known to be involved in regulating analgesic requirement and drug efficacy mechanism in post-operative pain. Individuals with met/met genotype of rs4680 within COMT gene were found to be exhibiting a diminished regional μ-opioid system while in pain, as compared to individuals having other genotypes of same allele (Zubieta et al. 2003). COMT gene works in conjunction with opioid and beta-2,3-adrenalinergic receptors. In a clinical trial studying COMT rs4680 polymorphism, presence of met allele was significantly associated with better recovery by giving intrathecal morphine to cure pain in chronic low back pain. Conversely, migraine subjects having homozygous set of met allele showed poor analgesic outcome by frovatriptan (Cargnin et al. 2013). Henker et al. (2013) investigated polymorphic association of OPRM and COMT genes in subjects having post operative pain with opioid consumption and opioid induced sedation. They inferred that GCGG haplotype of SNPs rs6269, rs4633, rs4818 and rs4680 within COMT gene was related to higher pain scale within 15 minutes of post anesthetic dose. Individually, met or ‘A’ allele of rs4680 and ‘T’ allele of rs4633 were independently associated with higher pain scores and greater analgesic dose requirements. Additionally, the ‘G’ allele of rs179971 of OPRM1 was also related with reduced sedation scores (Henker et al. 2013).
Same haplotype GCGG for the four COMT SNPs was observed to be associated with greater post operative pain ratings in children who were undergoing adeno-tonsillectomy, thus requiring a greater dose of pain relieving drugs (Sadhasivam et al. 2014). In a follow up study, investigating the effect of COMT polymorphism on pain intensity in lumbar spine surgery for one level symptomatic disc disease, genotypes AA of rs6269, TT of rs4633, CC of rs4818 and AA of rs4680 were related to lowest preoperative and post operative pain intensity scores. Moreover, haplotype analysis revealed that HPS haplotype ACCG for SNPs rs6269, rs4633, rs4818 and rs4680 was related with reduced pain and disability scores one year after surgery (Rut et al. 2014). Similarly, the post operative pain subjects with GG genotypes of rs4818 and val/val genotype of rs4860 demanded greater opioid analgesic doses than individuals with other genotypes (Candiottii et al. 2014). COMT, in association with GCH1 polymorphism was found to be related with post operative pain. Individually, ‘G’ allele of rs6269 and ‘C’ allele of rs4633 within COMT gene and ‘A’ allele of rs3783641 and ‘T’ allele of rs8007267 of GCH1 gene were associated with less pain related impairment. Haplotype analysis revealed that GCG haplotype of rs4680, rs4633, rs6269 was associated with reduced pain and related impairment in post herniotomy. Within GCH1 gene, the AT haplotype showed protective effect against pain and impairment postoperatively (Belfer et al. 2015).

COMT gene is also found to be a critical factor for other psychiatric conditions that influence pain such as depression. It has emerged that women are more likely to suffer from pain and depression than men. It was also evident from the study where haplotype for LPS comprising of rs6269, rs4848, rs4633 and rs4680 SNPs within COMT gene was found to be posing greater pain in women with major depressive disorder (MDD) but not in men (Fijal et al. 2010).

**Refutations and contradictions of COMT gene in pain conditions:**

In spite of numerous studies confirming the association of COMT gene polymorphism with pain, many studies have failed to replicate the results in some other populations. A study by Kim et al. (2004) could not find any significant genetic association of COMT gene polymorphism with reference to
ethnic, gender and psychological variations. The British birth cohort study inferred that none of the SNP out of 11 SNPs of COMT gene influenced chronic widespread pain (Hocking et al. 2010). Another study by Nicholl et al. (2010), constituted data of EPIFUND and European Male Ageing Study (EMAS) analyzed the role of rs4680, rs4633, rs4818 and rs6269, but were not able to pin down relevance of these COMT gene variants in chronic widespread pain, neither individually nor at haplotype level. A genetic study in Chinese Han population showed no significant correlation between various COMT SNPs except rs4633 in relation to thermal pain thresholds (Xiang et al. 2012). Similarly, no association of COMT gene in relation to pain intensity, disability or analgesic demand was inferred in women suffering from FMS. However, patients carrying met/met genotype of rs4680 were having higher IgA concentration, greater disturbed activity of sympathetic nervous system and humoral immune system than control subjects (Fernández-de-las-Peñas et al. 2014).

In conclusion, it is summarized that inspite of some genetic studies refuting the role of COMT polymorphism with pain phenotypes, large number of studies validate the role of COMT variants in nociceptive, musculoskeletal and neuropathic pain. It also influences the analgesic efficacy of opioid drugs, which is mediated by dopaminergic pathway. Hence, its role in pain modulation is well established and cannot be overlooked.

Apolipoprotein E (APOE)

Apolipoprotein E or ApoE is a type of apolipoprotein found in peripheral and brain tissues. The gene coding for ApoE is located on chromosome 19q13.2 with 4 exons. APOE is a tri-allelic gene comprising of ApoE-2, ApoE-3 and ApoE-4 alleles as defined by preliminary iso-electric focusing studies (Uttermann et al. 1980). It is the key transporter of cholesterol, thus regulating lipid metabolism through endogenous, exogenous and reverse cholesterol transport mechanisms. It was first examined by Havekes et al. (1980) in cultured fibroblasts, where suppression of cholesterol occurred on inhibiting the APOE activity.

Although, the studies depicting a well defined role of APOE gene in musculoskeletal pain are scarce, yet several animal and clinical studies give an idea about its role in primary mechanisms in nociception. APOE is found to be
regulating inflammation, stress, cognition, depression, muscle degeneration, apoptosis, nitric oxide synthesis, vitamin K mediated bone growth and neuronal signaling. These processes, indirectly, modulate the final outcome of pain perception. In a murine study, APOE gene double knockout mice who were fed on high fat diet showed increased levels of oxidized low density lipoprotein (LDL) levels in tendons, thus accelerating its degradation and causing increased pain in tendons (Grewal et al. 2014). Polymorphisms within APOE gene have a differential effect on the same processes, thus suggesting association of genotypic variation within APOE gene on musculoskeletal pain.

**APOE influencing brain function mediated pain:**

APOE, being the key transporter of cholesterol in brain, also influences the gray and white matter volumes. APOE was observed to be associated with traumatic brain as ApoE-4 mice had shown less dopamine in the frontal lobe and reduced accuracy in cognitive tasks (Reverte et al. 2016). Furthermore, ApoE-4 was found to be damaging white matter tracks, hence associated with cognitive impairment attention (Zhang et al. 2015). More evidences emerged from fMRI studies, where ApoE-4 carriers showed a reduced fractional anisotropy and increased mean diffusivity values. ApoE-4 allele is also responsible for hippocampus atrophy, a hallmark for depression and depressive symptomatology. Presence of ApoE-4 allele is a confirmed risk factor for Alzheimer’s disease (Limon-Sztencel et al. 2016). ApoE-4 also delays recycling in hepatocyte and neuronal cells (Ye et al. 2005). While ApoE-2 and ApoE-3 uses low density lipoprotein receptor related protein 1 (LRP1) and very low density lipoprotein receptor (VLDLR) to cross blood brain barrier (BBB), causing a faster amyloid beta protein (Aβ) efflux, ApoE-4 uses only VLDLR for Aβ transport, thus a retarded Aβ efflux results in generation of amyloid plaque in Alzheimer’s disease (Deane et al. 2008).

APOE is intensely related to stress induced corticosterone levels. Corticosterone is released in response to stress response via hypothalamic pituitary (HP) axis. Higher glucocorticoid levels, if remain increased due to longer stress session, may lead to hippocampus degradation and memory loss. This degradation is more prominent in ApoE-4 allele carriers. Prolonged stress
is associated with higher cortisol levels in cerebrospinal fluid. Surprisingly, sudden increase in stress levels causes anti nociception in tail flick tests of mice, mediated by higher cortisols, suggesting stress induced analgesic effect. ApoE-2 allele was found to be a risk factor for post traumatic stress disorder (PTSD) as the mice carrying ApoE-2 allele showed impairment in fear extinction, behavioral, cognitive and neuro-endocrine alterations following trauma (Johnson et al. 2015).

APOE regulates CNS synaptogenesis in central nervous system, which is a process of synapse formation between neurons of nervous system (Mauch et al. 2001). APOE gene knockout mice showed significant increase in synaptic protein called synaptotagmin. ApoE-4 allele reduces the N-methyl-D-aspartate (NMDA) receptor function by impairing the APOE receptor recycling. Thus ApoE-4 showed an enhanced impairment in synaptic plasticity by reelin protein mediated suppression of long term potentisation (LTP) (Chen et al. 2010). Cortical cell culture analysis revealed that debilitating effects of ApoE-4 induced neurotoxicity were suppressed by calcium channel blockers, suggesting the role of calcium sensor proteins in mediation (Veinbergs et al. 2002). During aging, ApoE-4 mice showed decreased synapses per neuron ratio along with an increase in synaptic size. This reduction was observed to be highest in ApoE-4 allele, followed by ApoE-2 and least in APOE gene knockout conditions. Murine studies revealed that ApoE-4 allele enhances long term potentization (Korwek et al. 2009).

**APOE in relation to apoptosis of neurons:**

ApoE protein is also known to affect apoptosis in neurons as it gives protective effect from apoptosis. APOE gene expression is upregulated following apoptosis in both neuronal and non neuronal cells. Cell culture analysis showed an upto to 6 to 8 fold increase in APOE protein levels in cells once apoptosis was induced. These findings speculated the role of APOE in clearing apoptotic debris through APOE-receptor interactions, making way for new cells (Elliott et al. 2007). Hayashi et al. (2007) reported that APOE protein binds to the low density lipoprotein receptor protein 1 (LDLR-1), which in turn activates protein kinase C-delta (PKC) and inhibits the pro-apoptotic glycogen synthase kinase-3
beta (GSK3B) enzyme activity, thus preventing neuronal apoptosis. This anti-apoptotic effect was found to be the largest in ApoE-3 allele, whereas presence of ApoE-4 allele was observed to be a risk factor for neuronal degeneration (Hayashi et al. 2007). The APOE gene polymorphic variations analysis revealed that the anti-apoptotic effect of ApoE was most efficiently executed by ApoE-3 allele and worst by ApoE-4 allele (Hayashi et al. 2009). Detailed analysis revealed that PKC is regulated by peptide hormone ghrelin, which enhances neuronal excitability by inhibiting neural potassium channel Kv7/KCNQ. Blockage of Kv7/KCNQ channels demolishes its anti-nociceptive effects by upregulating the synaptic transmission of nociceptive signals by ghrelin via phospholipase C–protein kinase C (PLC-PKC) pathway (Shi et al. 2013). ApoE protein is found to promote nerve regeneration after a nerve injury. Proteomic studies validated that ApoE protein levels are elevated following a nerve injury and participate in nerve regeneration (Melemedjian et al. 2013). ApoE-4 allele is found to cause an increased influx of calcium ion levels and apoptosis in neurons following mechanical nerve injury by reducing potassium inwardly rectifying channel subunit 3 (KIR3) function. The opening of these KIR3 channels gives an anti-nociceptive effect (Nakamura et al. 2014). The function of KIR3 channel is least in the presence of ApoE-4 allele (Jiang L et al. 2015).

**APOE in relation to inflammation through nitric oxide pathway**

Inflammatory conditions are known to cause upregulation of pain sensation. Murine studies have confirmed the role of APOE regulating the anti-inflammatory effects through lipids. However, ApoE-4 is associated with reduced C-reactive protein (CRP) levels but not white blood cell (WBC) count thus suggesting involvement of a non-inflammatory pathway (Yun et al. 2015).

Nitric oxide (NO) affects the inflammatory pathway, which in turn, upregulates the pain perception. NO synthesis is dependent and mediated through APOE expression. Several murine studies have highlighted that nitric oxide gives an anti-nociceptive effect by activating NO/cyclic GMP-ATP sensitive potassium channel cascade by activating ATP sensitive potassium channels (Déciga-Campos et al. 2004).
When APOE knockout mice were fed on western diet, they showed reduced oxidized LDL and macrophage levels in atherosclerotic plaques. APOE double knockout mice showed reduced production in the presence of normal nitric oxide function. However, these beneficiary effects eloped on inhibiting nitric oxide function. Activation of endothelial nitric oxide synthase (eNOS) in endothelial cells inhibits the LDL oxidation. Angiopoetin-2 (Ang2) induced nitric oxide release in growth factor angiopoietin surface receptor which is mediated by Tie-2 pathway thus giving an arthero-protective role of Ang-2 without causing inflammation, thus bypassing the inflammation related muscular pain (Ahmed et al. 2009).

Role of APOE in vitamin K mediated risk of pain

APOE regulates the vitamin K pathway, which in turn mediates the bone growth through osteocalcin pathway. Depletion in bone mineral density (BMD) poses the risk of developing musculoskeletal disorder and related pain. Polymorphic variations within APOE gene influence BMD differentially. A follow up study hinted that individuals with ApoE3-4 and ApoE4-4 genotypes developed more bone fractures than any other groups (Kohlmeier et al. 1998). Johnston et al. (1999) reported ApoE-4 to be a risk factor for hip fracture independent from the effect of dementia and falling, thus suggesting the involvement of vitamin K mediated bone resorption pathways. Similarly, Cauley et al. (1999) highlighted that women with ApoE-4 allele were at potential risk for developing hip and wrist fractures. In the initial studies, there was conclusive association reported when ApoE2-2 and ApoE4-4 genotype subjects were compared on the basis of BMD variations (Stulc et al. 2000). Vitamin K enriched diet was associated with reduced hip fracture risk but no effect on bone mineral density (Booth et al. 2000). Olson et al. (2000) proposed a hypothesis suggesting the relation between vitamin K1 and K2 intake with the risk of osteoporosis. Later in 2003, in post menopausal women ApoE-4 was found to be significantly associated with lower values of BMD (Zajícková et al. 2003). APOE polymorphisms were studied in relation to BMD values in Caucasian population. Haplotypes were derived for SNPs rs440446, rs769450, rs429358 and rs7412. Haplotypes CGTC, GGTT and GATC were found to be
associated with spinal BMD and GATC for total hip BMD in men only. No
genotype or haplotype based significant associations were inferred in women
(Long et al. 2004). A meta-analysis concluded ApoE-4 to be associated with
reduced BMD values than ApoE-3 or ApoE-2 especially in lumbar spine region
(Peter et al. 2011). In a study which examined patients who were undergoing
hip replacement surgery due to fragility fracture and osteoporosis, total and
uncarboxylated osteocalcin levels were measured. The subjects with ApoE-4
allele had significantly lower levels of bone osteocalcin/collagen activity in hip
fracture and osteoporosis (Rodrigues et al. 2012).

Thus it can be inferred from the above mentioned studies that APOE
mediates the pain in musculoskeletal pain conditions not through cholesterol
transport and dyslipidemia but through vitamin K pathway which regulated
BMD and any alteration in normal levels poses risk for developing
musculoskeletal pain conditions such as rheumatoid arthritis and osteoarthritis.

**APOE polymorphism in musculoskeletal pain conditions**

APOE is studied in relation to its role in various musculoskeletal and
other related pain conditions such as rheumatoid arthritis (RA), osteoarthritis
(OA), osteoporosis and fibromyalgia. In preliminary studies searching for a
possible link between APOE polymorphism with rheumatoid arthritis
susceptibility, ApoE-4 allele was found to be a potential risk factor for
development of amyloids in RA patients. This manifestation was most
prominent in patients with ApoE3-4 genotype (Hasegawa et al. 1996). Similar
results were obtained by Maury et al. (2001) who reported that ApoE3-4
genotype was more frequent in RA patients with amyloid. However, ApoE-4
allele did not emerge out to be associated with the risk of developing RA (Maury
et al. 2001). APOE gene knockout arthritic mice developed resistance to
occurrence of collagen induced arthritis (Asquith et al. 2010). In a case control
study, APOE was found to affect inflammation in rheumatoid arthritis, where
ApoE-4 allele was associated with increased inflammation and dyslipidemia in
rheumatoid arthritis patients (Toms et al. 2012). Recently, a murine study
reported that higher HDL levels cause accelerated synovial inflammation and
ectopic bone formation in experimentally induced osteoarthritis in animals,
which was found to be quite prominent in APOE double knockout mice (de Munter et al. 2016).

APOE is considered as a potent risk factor for osteoporosis because of its role in the regulation of BMD. Genetic variants within APOE gene were analyzed in relation to BMD in osteoporotic women where haplotypes were derived for SNPS rs440446, rs769450, rs429358 and rs7412. It was inferred that presence of CGTC haplotype predisposes women to a greater risk of developing osteoporosis and reduced BMD values (Singh et al. 2010).

Becker et al. (2010) studied the association of fibromyalgia susceptibility with environmental factors such as stress in relation to APOE polymorphism, where it was concluded that subjects with ApoE-2 allele were less susceptible to stress and fibromyalgia, thus giving a protective effect for both, in a statistically significant manner (Becker et al. 2010). Later, in 2011 another study documented that the survivors of motor vehicle collision, who had at least one copy of ApoE-4 allele were more prone to developing posttraumatic fibromyalgia. However, ApoE-4 allele was not observed to regulate the fibromyalgia related disease severity (Reeser et al. 2011).

Scarce literature is available with respect to association of APOE in neuropathic pain conditions. Only a few studies have summed up to add the pieces of the puzzle regarding the relationship of APOE and pain which includes mainly migraine and tension type headache. ApoE-4 allele emerged as a protective factor in subjects having migraine and tension type headache, while ApoE-2 was found to be associated with increased risk for these painful conditions (Gupta et al. 2009). Individuals with ApoE3-4 and ApoE2-3 genotypes were conferring risk for total migraine and migraine with aura (Joshi et al. 2011). Miao et al. (2015) concluded in their meta-analysis that ApoE-4 allele was associated with headache in migraine, although ApoE-4 was not found to be responsible for migraine susceptibility.

**Refutations and contradictions of the role of APOE gene in pain conditions:**

None of the studies found the clear association of APOE polymorphism in low back pain condition, yet ApoE protein levels were found to be raised by 2 to 5 fold in cases than normal subjects having low back syndrome (VanderPutten
et al. 1993). Cameron et al. (1995) also found that elevated levels of ApoE were found in lumbar pain, spinal pain and peripheral nerve damage patients.

In one of the preliminary studies analyzing the relationship between APOE and migraine, Rainero et al. (2002) failed to reveal any significant inference between APOE polymorphism and migraine. In another study evaluating the association of APOE, no relation of APOE alleles was found in Australian Caucasian population with the susceptibility of migraine (Stuart et al. 2013). Similarly, in Finnish population suffering from Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL), no association was found among APOE, angiotensin (AGT) and neurogenic locus notch homolog protein 3 (NOTCH3) genes in relation to disease related migraine (Siitonen et al. 2015).