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PROGRESS IN GENETIC PAIN STUDIES REGARDING ANALGESICS THERAPY - *A Systemic Review*

PART I - GENETIC MODULATION OF PAIN from genotype-phenotype

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Abstract: The intervention of the genetic factor in pain have a decisive importance not only for the effectiveness of the therapeutic strategy but also for avoiding the adverse (unwanted) effects of the drug molecules. The human genome assures by coding and synthesizing the functional protein structures participating in the mechanisms of receiving, conducting and projection of pain sensitivity in superior nerve centers as perception and interpretation of pro-nociceptive and anti-nociceptive molecules as well as modulation of pharmacokinetics and pharmacodynamics of analgesics. Genes with an indirect pain impact are a large number of genes, each having a little contribution to the interindividual variability of pain parameters in humans and to response type to analgesic therapy reflected by the required dose, administration time and efficiency. Epigenetic factors with effects on the mechanisms of pain and the patient's analgesic responses types are numerous and of wide diversity.

1.INTRODUCTION

Association between genetics and pain is not surprising, as it is to be expected, given that few are the fields of human pathology without the involvement of heredity as a predisposition, modulation, or causal determination. This is also the case for painful sensitivity, and for the last two decades substantial contributions have been made to the genetics of pain phenotypes. Pain is the most common symptom in the pathology, either in acute form associated with an underlying disease or as a self-standing disease - chronic pain. In cancer, for example, on average over 70% of patients accuse of continuous pain, being a major cause of healthcare and opioid treatment, given that the dose is of crucial importance, and the establishment of an ideal therapy (the "magic bullet ") is likely to become possible in a number of cases by genotyping. At the base of the inter-individual variability of the perception of painful stimuli as well as the different response to analgesics, there is a complex sum of contributing factors on which the pain phenotype profile is grouped into genetic factors of the individual (allelic variations in genomic DNA sequences) and environmental epigenetic factors.
This study bring into attention the latest achievements of pain genes involvement into pain treatment. A better understanding of the specific role of these genes in pain mechanism will give the possibility to made a genetic profile of the patients in order to improve the actual pharmacological treatment to an individualized one.

2. GENETIC MECHANISMS OF PAIN

Although acquisitions in medical genetics over the last 40 years (starting with DNA and proteins\sequencing - Sanger, 1975-double Laureate Nobel for Chemistry) are impressive, consistent data on gene contributions to pain mechanisms are recent and they deserve to be highlighted and known. In the same time, with all the performances currently achieved by the new generations of analgesics reaching the patients, they are still not in line with expectations, therapeutic satisfaction remains behind of speed of the emergence of new pharmacological agents, and despite intense studies regarding the genetic component of pain (only between 2005 and 2016 there are 846 studies in the field according to data published by the Human Pain Genes Database-HPGD, 2017). Difficulties encountered come from the heterogeneity of the studied human populations and from the "silent" way of the pressure of environmental epigenetic factors on genotypes that alter the phenotypic pain architecture, from differences in applied molecular technologies and some reproductive failures of experimental studies conducted by various research groups (Klepstad, 2011). Knowing in detail the intervention of the genetic factor in pain is of decisive importance not only for the effectiveness of the therapeutic strategy but also for avoiding the adverse (unwanted) effects of the drug molecules, the effects of which usually reach a significant value of 6,7%. This finding is particularly important in the treatment of major, long-term or high-dose analgesics, especially for pain types such as persistent pain (chronic pain), intense (tumor) and post-surgical (acute). The prevalence of chronic pain (the most

expensive in analgesic therapy) represents 15-20% of the adult population (Wilson, 2006), characterized by marked individuality, modest therapeutic efficiency, severe intensity and particular and important affective-emotional reactions which profoundly disturbs the professional, social and family relationships of the patient. Under these conditions, have become inevitable the concerning of the specialized clinics/centers in the therapy of chronic pain (especially oncological, rheumatological) about the need to determine the genetic profile of some patients in order to assess the risk of inappropriate therapy with opioids or other classes of analgesics, considered by many specialists as becoming in the near future routine stages (LaCroix-Fralisch, 2009; Trescot, 2014). Diagnosis and etiological pharmacology of pain by genotyping (gene therapy of pain) is extremely useful even though the proportion of genomic protein coding regions with important functional roles in pain mechanisms appears to be of minor importance, accounting for only 2% of the human genome, and the impact of deviation of gene polymorphisms has a contribution of only about 1% for painful phenotypic variants in general pathology. In addition, recent research shows the existence of genetic changes that influence pain that also occurs in RNA regions that do not encode proteins, which increases the size and importance of the impact of genetics involvement in painful suffering (Muralidharan, 2011).

The attempts to determine the causal relationship between genetic factors/epigenetic pain factors do not yet have a definite answer. In experimental animal studies, the contribution of genetic factors to the variability of nociception, hypo- and hypersensitivity to pain in different species (in mice, for example) varies between 25-60% or even 76% (Lariviere, 2002). In man, the contribution of genetic factors from all the factors involved in the pain phenomenon is variable, but some permanent and widespread diseases among the population have a strong hereditary character: 39-58% migraine, 21-67 % low back pain and cervical pain 50% shoulder and elbow pain, 55% menstrual pain (Kim, 2005).

The human genome assures by coding and synthesizing the functional protein structures participating in the mechanisms of receiving, conducting and projection of pain sensitivity in superior nerve centers as perception and interpretation of pro-nociceptive and anti-nociceptive molecules as well as modulation of pharmacokinetics and pharmacodynamics of analgesics. Epigenetic factors exert small but added pressures on environmental factors that act on the same sphere of patient pain and variable effectiveness of analgesic therapy plus age, gender, lifestyle, integrity of hepatic-renal functions, co-morbidities and associated medication (eg. co-analgesics and para-analgesics). The complete decode of the human genome within the well-known international project "The Human Genome" (1990-2003) was the time when molecular biology studies, implicitly those of pharmacogenetics, pharmacokinetics, pharmacodynamics, and pain-targeted pharmacotherapy, get soar on completion of the determination of all genomic DNA sequences (over 3.7 billion nucleotides) and their assembling modes in each chromosome, all human genes being identified physically and functionally (total number of human genes = 22,333 plus / minus 1000 genes after Data from the National Center for Biotechnological Information (NCBI-USA) (Pertea and Salzberg, 2010) of which about 19,000 genes are protein coding (Ezkurdia, 2014). For more genes, there is evidence that they are responsible for the mechanisms of different types of pain (HPGD, 2016), of which over 25 genes are confirmed to be directly involved in painful sensory mechanisms and associated with clinical systemically manifestations proven experimentally and clinically on homozygous and heterozygous, while other genes have the status of indirect participation in pain modulation. Genes with direct involvement in painful pathology, few in number, are responsible for congenital familial diseases with severe impairment of pain perception. Genes with an indirect pain impact are a large number of genes, each having a little contribution to the interindividual variability of pain parameters in humans and to response type to analgesic therapy reflected by the required dose, administration time and efficiency, gene modulation of pain being cumulative in this case, as a sum derived from the interactions of several genes, and on the other hand, from the association of these interactions with the pressure of the epigenetic factors. In analgesic therapy, the genome responds by its nucleotide sequences of gene expression, transcription and translation, with implications in both the enzymatic metabolism of the substrate (represented by the administered analgesic and the absorption, transport, distribution, synthesis of pharmacological receptor populations, binding on the receptor of the active drug molecule and the kinetics of the excretion of the resulting metabolites. The transport and processing of a drug substance through the body is accomplished with genetically engineered functional proteins. The least common genetic disorders of painful sensitivity (but also the most severe) are eredo-familial, and the most widespread types of gene changes among the population with less severe influence on pain are polymorphisms. The latter are represented by small variations in some individuals in the nucleotide structure of the DNA / RNA sequences, resulting in changes in the structure of the synthesized functional proteins. The most common allelic polymorphic modification is uninucleotidic polymorphism type (SNP- single nucleotide polymorphism) given by gene mutations by substitutions, duplications / replications, deletions or nucleotide insertions (e.g., substitution of an A, T, C or G nitrogen base from a single nucleotide, each nucleotide having 2 possible alleles). SNP variants represent 90% of all variations in the human genome (one variation per 1000 base pairs) (James, 2013), which can be found in exons (coding regions) or introns (non-coding regions) of the genes without phenotypic effect or with phenotypic effect (which is pathogenic). Existence of polymorphisms amplifies to the carrier individuals the risk of

developing pathogenic abnormal phenotypes by altering the transcription and translation of amino acids by mRNA in newly formed functional protein molecules. Depending on frequence, SNP polymorphisms may be common (present in some common diseases) or rare (in some rare diseases). The notions of *polymorphism* and *mutation* are used to have the same meaning, but generally the mutation refers to allelic variants with a frequency below 1% in the population, and the polymorphisms refer to allelic variations with a frequency above 1%.
SNP polymorphisms in the field of physiological and pathological pain can induce (not-necessary) coding modifications of neuro-functional proteins that are phenotypically finalized by affecting pain sensitivity and pharmacokinetic and pharmacodynamic modulations of the therapeutically administered analgesic (**Figure 1**).

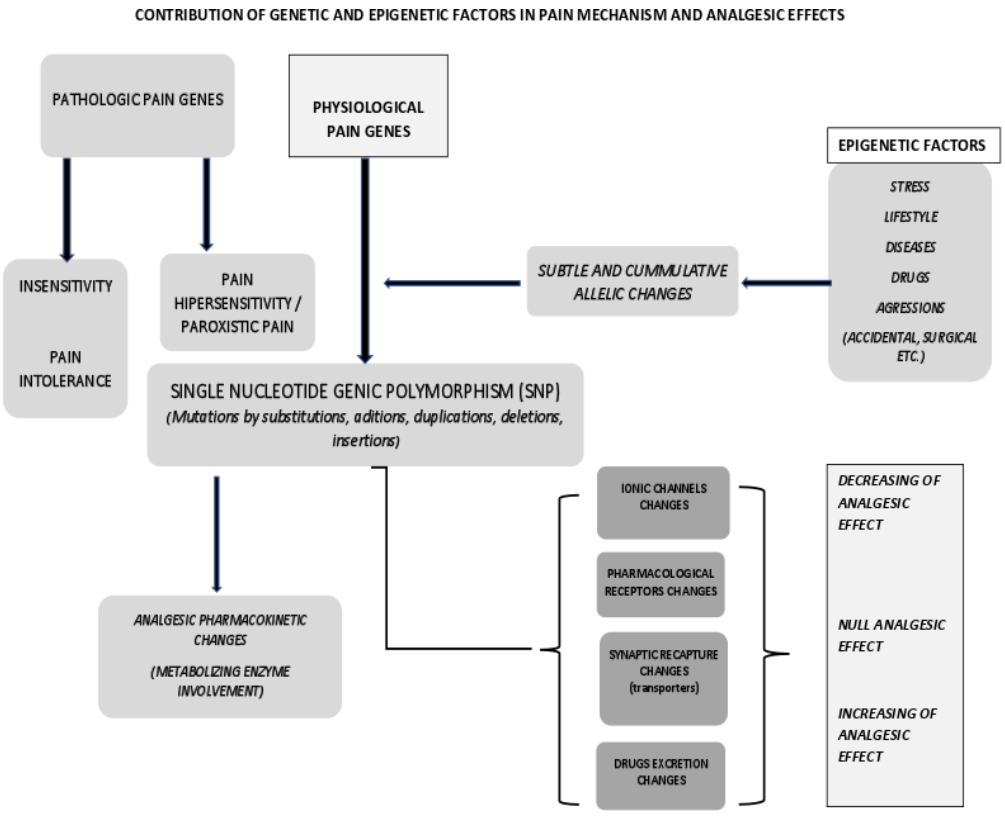

Figure 1

These changes outline the variability of interindividual response to therapy as efficacy, inducing to the carriers the susceptibility to respond unsatisfactorily (requiring an increase in the analgesic dose) or rather high (requiring lowering the dose), the variability ranging from treatment ineffectiveness to excessive toxicity manifested to a small part of the population carrier of a particular SNP.

2.1. **Monogenic modulation of pain**

Pain genes

Over 25 functional genes are decisively involved in altering painful perceptions, of which at least 12 genes are responsible for either pain insensitivity or paroxysmal pain, hereditary transmitted. Each of the 25 genes listed below represents the genomic support of some clinical manifestations related to the pain phenomenon in man or animal (experimental pain) (HPGD, 2016):

- Genes with hereditary transmission of *pain insensitivity*: *SCN9A, SPTLC1, HSN2, IBKAP, NTRK1, NGFB;*

- Genes with hereditary transmission of *paroxysmal pain* (*pathological pain*): *CACNA1A* (familial hemiplegic migraine-MHF type I), *ATP1A2* (MHFtip II), *SCN1A* (MHF type III), *SCN9A;*
- SNP genes that *amplify pain*: *COMT* (low back pain, fibromyalgia, nociception), *KCNS1* and *SC9A* (sciatica, ghost pain), *HTR2A* and *SLC6A4* (fibromyalgia), *CACNG2* (post-operative pain), *CYP19A* (migraine), *GCH1* (pain, fibromyalgia, nociception), *TRPA1* (neuropathic pain), *TRPV1* (arthritic pain);
- Pain-reducing SNP genes: in human - *COMT, OPRM1, MC1R, GCH1, CYP2D6, TRPV1* and experimental animal-*TRPA1* (experimental pain).

Some of the above genes are part of both the group that amplifies the pain perception and the group that decrease this perception, depending on the allele/genotype variant and/or the SNP variant (*rs*) (for example, the allelic variant: the COMT gene *SNP rs 4680* genotype G induces analgesia, and G/G genotype low back pain). There are also individuals carrying genes that do not associate with clinical expressions of painful perception, but which in turn highlight an increased risk for the induction of pain (*SCN9A*-coding gene of Na^{2+} channels, *KCNS1*-coding gene of K^{+}, *CACNG2* - gamma 2 subunit coding gene from Ca^{2+} dependent channel) or a low risk of pain appearance (*CACNA2D3*-delta 2 encoded gene from the Ca^{2+} dependent channel) (Diatchenko, 2007).

Severe monogenic disorders of painful sensitivity

In humans there are two major serious impairment of the pain sensitivity generated by gene mutations, phenotypically represented either by the loss of sensitive function (pain insensitivity) or the increase in the activity of this function (painful hypersensitivity), both of which are rare but serious.

Congenital insensitivity to pain through non-functional gene mutations

The most pronounced genetic damage to painful sensitivity is highlighted in individuals with inherited insensitivity to pain involving several types of genes that have undergone mutations. The transmission of nociceptive signals is altered by disrupting the synthesis of proteins from a wide bio-functional range: enzymes, transcription factors, neurotrophins or ion channels of Na^{+} and Ca^{2+} (canalopathies). All clinical forms are hereditary transmitted and show, among other signs, the inability to sense pain or indifference to pain. These mutations induce sensitive vegetative neuropathies (*Hereditary Sensory and Autonomic Neuropathy* - HSAN), due to aberrant protein encoding, thus becoming ineffective in the mechanisms of nerve influx conduction (LaCroix-Fralisch, 2009). Thus, the carriers of polymorphisms of the *SPTLC1* and *WNK1/HSN2* genes which affect the encoding of the key enzyme required for the synthesis of neuronal axonal sphinomyelin (serine-palmitoyl transferase and lysinkinase-1 respectively) transmit HSAN type I disease (autosomal dominant) and type II (autosomal recessive) characterized by decreased thermo-algesic sensitivity, acropathies and mutilations of the hands and feet. Mutations of the *IKBKAP* gene encoding the proteinic complex -1k B kinase which inhibit *kappa* opioid receptors which transmit HSAN type III neuropathy (*Autosomal Recessive Riley-Day* syndrome) manifested by weak reaction to painful and thermal stimulation, vegetative disorders, hyperhidrosis and alacrimia, and the *NRTK1* gene that modulates the structure of the neurotrophic tyrosine kinase receptor transduces HSAN type IV neuropathy (autosomal recessive) to descendants defined by the absence of response to painful stimuli, anhydrosis, cutaneous/corneal lesions and average mental retardation. Modification of nerve growth coding in *NGFB* gene carriers induces HSAN V disease (autosomal recessive) clinically evidenced by pain insensitivity and joint deformity. Ion channels for sodium types Na_v 1.3, 1.7, 1.8. and 1.9. type are also known as important structures involved in the transmission of pain. Individuals carrying SNPs with non-sense mutations in the SCN9A gene are insensitive to pain, with the exception of the Na_v 1.7 channel (Ahn, 2010). Instead, patients with pain indifference recognize the painful sensation, but have a marked decrease in the affective-motivational component, and the withdrawal reaction to painful excitatory application is absent. Pain-insensitivity frequently causes the death of these individuals in childhood, as they are not able to grasp the dangers of the external or internal environment of the body related to pain.

Hypersensitivity to pain (pathological pain) through hyperfunctional gene mutations

In individuals with manifestations of exacerbation of hereditary transmitted sensitive, have been identified genes that have undergone mutations that phenotypic induce powerful decreasing in pain threshold and marked increase in excitability by abnormal intensification of the activity of ion channels in nociceptive neurons of the spinal ganglia. The SNPs variant of the SCN9A gene (rs 6746030 allele A) coding the modification of the sodium channel protein structure (Nav1.7.) and the strong activation of the pain conducting C fibers (Reimann, 2010) induces rare familial pain syndromes evidenced by two disorders with intense pain: Hereditary erythromelalgia and Paroxysmal pain syndrome, the first showing signs of burning intermittent pain, and the second paroxysmal rectal, mandibular and ocular pains. Another condition characterized by painful episodes with genetic substrate is Familial Hemiplegic Migraine (MHF) with three forms of clinical manifestations. MHF type I is due to the *CACNA1A* mutant gene and the affected protein belongs to the Ca^{2+} P/Q channel being represented by the alpha 1 subunit from the Ca_v2.1 ionic channel. The condition is characterized by migraine attacks with aura,

hemiplegia and epilepsy. Type II MHF involves the *ATP1A2* gene with affinity of the Na^+ -K^+ -ATPase enzyme alpha 1 subunit, and in the Type III MHF mutation of the *SCN1A* gene that alters the alpha subunit protein structure belonging to Na_v1 dependent voltage Na^+ channels. The MHF type II and III MHF clinical signs are similar to MHF type I but without neurological associations.

Monogenic polymorphisms at risk for analgesic therapy

Therapeutical risk studies associated with genetic analysis have shown the existence of an appreciable potential in establishing linkages between certain gene polymorphisms and the variability of clinical efficacy of analgesics. This is the case for morphine, the first-line painkiller that is recommended as efficacy (World Health Organization, 1996), which explains that many studies target the mechanism of variability of response to this substance. The possibility of predicting optimal morphine doses (for example in cancerous pain) based on genetic testing has become of fundamental importance for adequate pain management treatment. The most important target of morphine is the *mu* receptor whose coding responds to the *OPRM1* gene located in the chromosome *bq24-q25*, the gene considered to be the first candidate to influence the analgesic efficacy of opioids. The *OPRM1* gene has a large number of polymorphisms (SNPs) identified in the promoter, but only some have relevance to opioid analgesia. The most common and investigated SNPs of this gene is substitution in nucleotide of adenine with guanine 118A> G (A118G) SNP in exon 1 which will induce the replacement of the asparagine amino acid with aspartic acid at position 40 (N40D) in the OPRM1 receptor protein with a frequency between 8-48% in population depending of ethnicity and geographical area (Tegeder, 2009). In the individuals with the mutant variant decrease the response to opioids, the morphine dose needs to be increased, for example in the GG variant with 93% in the therapy of these patients. Moreover, homozygous patients carrying two 118GG alleles decrease the potency of the most potent analgesic metabolite of morphine (morphine-6-glucuronide-M6G) compared to single-altered (heterozygous) alleles or wild-type 118AA alleles (unmodified) (Lotsch-2007). If in patients with renal insufficiency which take morphine, the M6G metabolite can accumulate up to the risk of opioid toxicity, in contrast G118-carrier patients (decrease in morphine metabolism) have no adverse effects (sedation, somnolence, decreased alertness) states which are present in morphine treated carriers of wild alleles and are at risk from this point of view (Stamer, 2007).

Another modulating gene of the *mu*-opioid system is the COMT gene encoding the catechol-oxymethyl transferase enzyme that catabolizes dopamine, adrenaline and noradrenaline neurotransmitters with key roles in modulating nociception, analgesia, and pain behavior. The COMT gene has a poorly functional polymorphic nucleotide (G472A) encoded variant (G472A) identified by Zubieta (2003) in which the *valine* amino acid of the COMT structure is replaced by *methionine* at position 158 (COMT Val158Met), which reduces COMT activity by 3-4 fold. Homozygous individuals carrier of the 158Met type exhibit an increased sensitivity to associated pain and a high rate of affective living of pain. Increasing levels of the dopamine neurotransmitter by chronic activation (due to its low metabolism / inactivation due to the COMT enzyme inefficiency) induces the reduction of endogenous opioid activity (evidenced by enkephalin depletion - a situation that is counteracted by activation of increasing of opioid receptors population *mu* active (*up-regulation*) in different regions of the brain. This makes that heterozygotes having only one SNP 158Met variant, but especially homozygotes with two 158Met variants, require much lower doses of morphine in the case of long-term analgesia (e.g. In the cancerous disease), while patients carrying wild alleles need daily high doses of morphine. The existence of the SNPs Val118Met polymorphic variant in the COMT gene has thus become a significant predictor of the required morphine dose in cancer pain therapy (Raakvag, 2005).

The synthesis of a major mediator of pain perception, nitrogen monoxide (NO), in the presence of the enzyme GTP cyclohydrolase (GCH1), is also genetically determined. GCH1 regulates the production of dihydroneopterin (BH2) and further of tetrahydrobiopterin (BH4), the latter molecule being an enzymatic cofactor essential for the synthesis of NO, serotonin and catecholamines. Excessive BH4 growth following various axonal aggressions contributes to neuropathic pain, but the existence of the SNP mutation that confronts the inactive, nonfunctional GCH1 enzyme gene is associated with a decrease in pain under conditions of BH2 and BH4 depletion and NO synthesis reduction. Batch studies on cancer patients carrying mutant polymorphism haplotypes showed that the time elapsed from the diagnosis of cancer to opioid treatment is double in homozygotes carrying the mutant alleles compared to the heterozygous carrier of mutant genes but no effect, and almost triple to non-carrier heterozygotes (Lotsch, 2010), the decrease of the GCH1 enzyme synthesis, determining the reducing of the duration of opioid therapy under the same conditions of therapeutic efficacy, hence resulting the prophylactic role of inhibiting the enzyme GCH1 in neuropathic pain therapy.

2.2**. Polygenic modulation of pain (combined mechanisms)**

A common and widespread situation in population regarding the genetic contribution to pain mechanisms is polygenic modulation, as pain represent a complex phenotypic traitfeature that involves the intervention of several genes, each bringing a small individual effect and influencing pain behavior more than one single gene. The concept of polygenics

explains the influence of genes on the variability of the analgesic response of individuals carrying altered alleles. For example, the A118G polymorphism of the *mu* receptor synthesizing OPRM1 gene has interrelated effects with the SNP polymorphism of the *COMT Val158Met* gene so that for the carriers of non-functional homozygous SNPs alleles of both *OPRM1* G/G genotype and *COMT* Val /Val genotype, are required high doses of morphine to treat pain, in contrast to *OPRM1* A/A and *COMT* Met/Met genotypes where pain is suspended at doses reduced in half, which is a tremendous advantage (Reyes-Gibby, 2007).

A receptor known as an analgesic modulator is also the melanocortin receptor MC1R (encoded by the gene *MC1R* from the distal end of chromosome 8 ($68_c/16_q24.3$), a specific receptor for coupling melanocito-stimulating adenohypophysis hormone (MSH). The MC1R receptor modulates analgesia by *kappa*-opioids, but only in females (sexually analgesic dimorphism) (Stamer, 2010). Normally, MC1R coupling with MSH have as a result in both sexes, synthesis, diffusion of melanin pigment and darkening of the skin and hair, that people with non-functional gene mutations for MC1R synthesis show reddish hair and light skin, a 75% of these individuals carrying two or more inactive variants of the *MC1R* gene. The most important such variants are 29*ins*A, 451C> T (coding the R151C MC1 receptors), *478C>T* (coding for R160w MC1 receptors) and *880G> C* (D294H receptors), all those listed being *variants* of MC1R receptors with low-function due to impairment of their coupling to G protein. In female patients carrying poorly functional variants, effective *kappa*-opioid analgesia is obtained with low therapeutic doses of *kappa* agonists (eg. Pentazocin) , aspect also proved in animals with *e/e* experimental deletions of the gene responsible for MC1R synthesis (Mogil, 2005). In individuals without *MC1R* mutations, *kappa* opioids are fixed in males only on *kappa* receptors and in women on both receptors (*kappa* and MC1R) activating, but the coupling with the functional MC1R receptor (in women) has anti-opioid effect. In contrast, in women carrying non-functional mutations of the *MC1R* gene, coupling of *kappa* agonists occurs exclusively on these latter receptors (present mainly in central nerve structures that play a major role in suppressing of pain - for example, cerebrospinal gray matter in the brainstem), that the effect is a robust analgesia, identical behavior to female mice with *e/e* provoked deletions. This latter finding is of particular importance not only to the need for a genetic test in certain groups of patients before analgesic therapy but also to the fact that, in some instances, the translation of animal-to-human experimental results is identical, operable and useful. The cause of this type of response only in women is due to the circulating estrogenic hormone, in experimental animal studies the phenomenon disappeared after ovariectomy and reinstalled after estrogen therapy (Rees, 1999).

There is another link involved in pain, this time sex-independent, between MC1R receptors and opioid *mu* receptors. Thus, the codeine metabolite (morphine-6-glucuronide) that selectively couples opioid *mu* receptors induces a strong analgesic effect in patients with mutant gene not functional for the MC1R receptor in both men and women, which is in contrast to the effect of *kappa* opioids (e.g., pentazocine), which produce strong analgesia, as shown above, exclusively in women carrying the mutant gene for MC1R receptors (Mogil, 2005). Researches has shown that not only the *kappa* receptors but also the active population of opioid receptor population may increase in the presence of high estrogen concentrations, situation in which women perceive less pain. The explanation seems to be due to increased secretion of endogenous opioid in conditions of increased estrogen, which activates multiple opioid receptors (*up-regulation*). Thus in the menstrual phase when estrogens are low, the risk of migraine is high. Estrogen administration blocks headache, but not bleeding, and progesterone blocks bleeding, but not headache. The existence of sexual dimorphism (as a totality of differentiation phenotypes between the two sexes) regarding the pain phenomenon and the effectiveness of analgesics is also supported by clinical evidence. For example, *kappa* agonists, such as nalbufine, reduce pain at birth more effectively than morphine (mu agonist), and in men effective morphine doses are 30-40% higher than in women for the same type of general pain pain, which adds to these findings of a high efficacy in severe pain, so the therapeutic recommendations are different (Wilson, 2006). These data require that when restrictions on specific treatments with *kappa* opioids (sex-independent) or *mu* (sex-dependent) are required, genetic testing is required.

Stress-induced analgesia (SIA) is another example of polygenic modulation of pain characterized by sexual dimorphism. SIA occurs in both sexes, but with different genetic mechanisms, mediation being made in males through the couple: opioid receptor *kappa*- NMDA receptor (N-methyl-D-Aspartate), involved in pain transmission, while women have a SIA system represented by the couple: *kappa* receptors and non-NMDA receptors that have been shown to be potentiated/activated by circulating estrogenic hormone (in female animal experiments, they are also insensitive to NMDA receptor antagonist therapy) (Butler, 2009). Determination of genic location by QTL technique (Quantitative Trait Locus-mapping) has revealed that DNA gene sequence which respond to SIA in women are transmitted in conjunction with the distal region of chromosome 8 - "*Si1fq1*" (absent linkage in males), place which also corresponds to the gene coding of the MC1R melanocortin receptor with a modulatory role along with plasma estrogen levels ("68_c *M"/16q24.3*), as mentioned previously (animal and human studies obtained by *mapping, linking* but also pharmacologically and clinically). In other words, although in both men and women stress analgesia is mediated through the *kappa*-opioid system, sexual dimorphism

on *kappa*-opioid analgesia mediation and stress analgesia has in women a neurochemical substrate similar to that of the MC1R receptor gene for the MSH hormone, a gene also present at the distal end of chromosome 8. Clinical consequences are important because women with normal genetic equipment on chromosome 8 require higher doses of *kappa* agonists, while women with mutant *MC1R* variants null functionally or with the pharmacological blockade of MC1R benefits by a strong analgesic effect of *kappa*-opioid agonists. It should be underlined that the problem of genetic sex-dependent differences in pain perception targets not only receptors, but also the existence of separate nerve circuits, neuro-secretions and neuro-processing distinct and separate in the brain in men and women.

A polygenic intervention is also present in low back pain syndrome, a widespread disease in the population due to disc degeneration or lumbar disc herniation or osteoporosis and which is estimated to be inherited in the proportion of 30-45%, while in the rest of cases intervening structural, psychosocial and occupational factors. Once known the genes encoding the extracellular matrix proteins in the bone and cartilages (*genome mapping*) have been identified and SNP polymorphisms associated with discopathies which frequently generating low back pain, the genetic variants being consistent with MRI and clinical data (Tegeder, 2009). Thus, polymorphisms of genes *COL9A2* and *COL9A3* encoding the alpha 2 and alpha 3 chains of heterotrimeric collagen IX (major component of the intervertebral disc) are associated with premature alteration of the mechanical properties of the disc and contribute to the predisposition of lumbar disc herniation, root compression and of low back pain especially in Finnish, Japanese and Chinese (Aladin, 2007) which present TRP2 alleles (Glu326Trp-exchange in the amino acid alpha 2 chain, glycine with tryptophan, the most hydrophobic amino acid). In German and Greek, *TRP2* was not detected, but the rate of relapse of discopathy after discectomy was high in the carriers of the SNP variant with the Glu326Arg gene of the COL9A2 gene (Kales, 2004). *Sp1* variant carriers of the *COL1A1* alpha1 chain have, in addition to the risk of disc degeneration and osteoporosis, at the postmenopausal women (Sp1 variant is a G/T polymorphism promoter of the *COL1A1* gene, carriers having reduced expression of this collagen) but other genes SNP may also contribute, such as beta-transforming growth factor, estrogen receptor and for vitamin D3, or the *ACAN* gene that synthesizes the aggrecan protein (Ralston, 2006).

Studies on genetically modulated pain pathways have indicated that the increased number of genetic variants corresponding to clinical pain phenotypes is confirmed by their multifactorial nature. There is no response yet on the cause of genetic abnormalities generating disturbances in pain sensitivity in the population and their elimination by natural selection. However, the explanations will be different for the two different cases of the genetic contribution to pain described above: rare disease/rare genetic variant (serious conditions such as insensitivity and hypersensitivity) and common disease/common gene variant (common monogenic and polygenic pain modulation) (Diatchenko, 2007). In the first case, of rare diseases with low population extent are involved rare genes encoding crucial elements for pain transmission but with high deletional penetration of familial genes and a minor contribution of environmental factors (not known yet if the genes causing the rare and extreme painful pathology are involved in a subtle form also to the pain associated with various common diseases). Instead, in the case of genetic modulation of pain from common diseases (for example, the variability of opioid receptor populations or their response patterns, the metabolism of analgesics or neurotransmitters transporters etc.) based on combinations of polymorphic gene variants (SNP) subtle, with no dramatic allelic deviations, with wider expansion in the population, but with low penetration, the phenotypic expression requires both intergenic and epigenetic factors interactions (favoring external and internal environmental factors).

3. EPIGENETIC FACTORS INVOLVED IN THE PAIN MECHANISM

For 60 years, the gene term is synonymous with the region of the genome encoding mRNAs that is translated into proteins. After coding of polymorphic mARNs (2% of the human genome) a large amount of RNA remains largely transcribed into non-coding protein RNAs (ncARNs – without translation in protein) consisting of short or long molecules of RNAs. Recently, extensive human genome studies have shown that it is transcriptible everywhere and has the ability to produce thousands of ncARNs (microARNs small interfering RNAs, and various long classes of RNAs) (Taft, 2010). It has now become clear that the types of ncARNs fulfill by such forms key roles of transcriptional and post-transcriptional regulators and guides chromatin-modifying complexes by facilitating development and physiological processes. One of the important functions of these RNAs is to be an *epigenetic regulator* of functional protein coding genes, implicitly those participating essentially in pain mechanisms such as receptors, ion channels or metabolic enzymes. Specific SNPs alleles present in microARNs (miARNs-variety of ncARNs) facilitate interactions between the *transcriptome - environment - epigenome*, so that genetic information can be modified in response to environmental changes. In this way, ncARNs regulate thousands of genes, perturbations of miARNs being involved in many diseases. From current research it follows that regulation of

gene-environment communication through silent RNA editing appears as a possible mechanism for fine post-transcriptional control of gene expression and represents a field of great interest for bio-medical pathology (Vaiman, 2016).
Epigenetic factors with effects on the mechanisms of pain and the patient's analgesic responses types are numerous and of wide diversity (environmental conditions, dietary structure and calorie intake in 24h, co-morbidity and various applied pharmacological therapies etc.) involves modifications of histone proteins associated with DNA sequences in the chromatin structure resulting in activation of the individual genetic patrimony (Moore, 2015). The phenotypic changes produced by this can be inherited without changes in subordinate DNA sequences (*"silent"* modifications), but they appear to play a key role, for example, in neuronal plasticity due to aggressions applied to peripheral nerves (Uchida, 2010). Such common epigenetic mechanisms involving functional proteins with a crucial role in pain modulation through their presence in opioid receptors (*mu*) and the Na^+ ($Na_v1.8$) and K^+ ($K_v4.3$) channels are reduced as long-term phenotypic expression due to the involvement of a intragenic *neuron restrictive silencer factor* acting as a repressor of *OPRM1*, *SCN10A* and *KCND3* gene transcription from dorsal spinal nerve neurons participating in the pathways of painful sensitivity (Zhang, 2015).

CONCLUSIONS

The analgesic suppression of acute and chronic pain represents one of the main goals of therapeutic management. The analgesic efficacy depends on the interindividual variability in differential pain sensitivity and also on the different response to analgesic medication.
The action of a single gene or more frequently the interaction of multiple genes, each of them with a small effect shows that pain more frequently is a complex phenotypic feature *involving intervention of multiple genes*.
Now it is possible to individualize the therapy for few categories of patients and the recommended dose is evaluated by genotyping. This allows both optimal therapeutic results and the prevention of adverse and/or side effects from inappropriate doses.

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