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Genetic addiction risk score (GARS)™, a predictor of vulnerability to opioid dependence

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1. ABSTRACT

The interaction of neurotransmitters and genes that control the release of dopamine is the Brain Reward Cascade (BRC). Variations within the BRC, whether genetic or epigenetic, may predispose individuals to addictive behaviors and altered pain tolerance. This discussion authored by a group of concerned scientists and clinicians examines the Genetic Addiction Risk Score (GARS), the first test to accurately predict vulnerability to pain, addiction, and other compulsive behaviors, defined as Reward Deficiency Syndrome (RDS). Innovative strategies to combat epidemic opioid, iatrogenic prescription drug abuse and death, based on the role of dopaminergic tone in pain pathways, are proposed. Sensitivity to pain may reside in the mesolimbic projection system, where genetic polymorphisms associate with a predisposition to pain vulnerability or tolerance. They provide unique therapeutic targets that could assist in the treatment of pain, and identify risk for subsequent addiction. Pharmacogenomic testing of candidate genes like CB1, mu receptors, and PENK might result in pharmacogenomic, personalized solutions, and improved clinical outcomes. Genetically identifying risk for all RDS behaviors, especially in compromised populations, may be a frontline tool to assist municipalities to provide better resource allocation.

2. INTRODUCTION

The treatment of non-cancerous pain has become a major challenge for primary care medicine. In the United States alone we are faced with an iatrogenically induced opiate /opioid epidemic killing thousands every year with at least 110 dying daily from a narcotic overdose. The devastation caused by overdose deaths continues. Regarding prescription drug abuse, in 2007, there was one unintentional (Iatrogenic) drug overdose death in the United States every 19 minutes, (1,2) . The increase in drug overdose mortality rates has been driven by greater use of prescription opioid analgesics. Although, more overdose deaths involved opioid analgesics than heroin and cocaine combined in 2003 (3,4) (recently, the availability on the street of cheap heroin has driven heroin dependence (5). In 2014 for every opioid overdose death, nine individuals were admitted for treatment of substance use disorder (SUD), 35 visited emergency departments, 161 reported drug dependence, and 461 reported nonmedical opioid use (3). Currently, in 2016 thousands of innocent people are dying from opiate/opioid overdose. This alarming problem is concerning to Republicans and Democrats alike. This consensus paper has been developed by a multidisciplinary group of scientists and clinicians including neuropsychopharmacologists, psychiatrists, psychologists, emergency medicine physicians, geneticists, neuroscientists, sports medicine physicians, behavioral scientists, clinical addiction specialists, addiction medicine physicians, and chemists. The recommendation that genetic testing should be mandated before treatment of pain with synthetic opioids is an attempt to reduce this problem. This hypothesis involves ~~the~~ understanding ~~of~~ the neurochemical interactions of cannabinergic- endorphinergic- glutaminergic – dopaminergic systems.

Regarding both toxicity and treatment efficacy individuals respond differently to medications and certain nutraceuticals (6). Clinical variables in drug (nutrient) effects include the person's age, nutritional status~~;~~, kidney and liver function~~;~~ concomitant illnesses, and the pathogenesis and severity of the disease being treated. However, inherited genetic variants (polymorphisms), can also result in alterations in metabolism, and influence the efficacy and toxicity of medications and nutraceuticals (7) . Clinical observations of inherited differences in drug effects were initially documented in the 1950s when an inherited deficiency in the genes

that encode cholinesterase, the enzyme responsible for the breakdown of drug suxamethonium, caused prolonged muscle relaxation (8) . The next gene-based drug response was observed when some patients who carried a gene variant that lowered the activity of blood cell glucose 6-phosphate-dehydrogenase, bled to death after they were treated with an anti-malarial therapy (9) . These observed differences in drug response gave rise to the field of 'pharmacogenetics'.

Several genes determine individual differences in response to drugs and/or nutrients that encode proteins~~,~~ ,like receptors, transporters, and enzymes~~,~~ which are involved in multiple pathways of drug/nutrient metabolism, and these individual differences are not due to single gene variants (10). Within this evolutionary era of pharmacogenetics, individual differences, an individual's inherited genotype~~,~~ that governs response to drugs~~,~~ and/or nutrients will be used to improve the efficacy of medications or nutrients. Indeed, understanding the structure/function of normal molecular biology, as well as, certain observable dysfunctions, may lead to promising nutrient-based targets. Knowledge afforded by accurate DNA-based prescreening (genotyping), will make it possible to design accurate~~ly~~, effective nutraceuticals by initiating ongoing research and development that incorporates pharmacogenomics.

Out of the three million unshared DNA bases, individuals could carry gene variants (polymorphisms) that might result in either an increase or a decrease of certain essential drug/nutrient response-related proteins. These proteins form the molecular basis of cell cycle control, and the synthesis or catabolism of structures like receptors, enzymes, and chemical messengers. Many molecular studies show genes that encode drug targets have genetic polymorphisms~~,~~ that can change their sensitivity to specific medications, and that might offer specific targets for therapy. However, molecular studies that involve a genome-based response to nutrients are needed.

Pharmacogenetics investigates the role of genetics in inter-individual variability in responses to drugs and therapy~~,~~ . Research concerning pharmacogenetic studies of opioid drugs are often reported (11) . Opioid analgesics are used widely for pain management, and information on genetic polymorphisms and inter-patient variability with opioid therapy are documented involving enzymes, receptors, and transporters related to opioid disposition (pharmacokinetics) and pharmacology (pharmacodynamics) (12) . Some examples are the pharmacogenetics of enzymes, including the cytochrome P450s and uridine diphospho-glucuronosyl-transferases, the ABC family of transporters, and opioid receptors.

3. PAIN TREATMENT WITH OPIATES AND ADDICTION RISK

Due to inconsistent criteria for addiction and pain, there is a paucity of ~~the~~ medical literature in the treatment of pain with opioids in patients with active addiction or who are in recovery~~,~~. ~~f~~For example, inconsistent criteria were used to define addiction and chronic pain typology (13). There are clear differences between dependence, tolerance, and addiction. Patients with a history of drug or alcohol addiction are known to present to physicians with pain complaints. Importantly, the drug-seeking behavior may be seen with either active addiction or pseudoaddiction or as part of deviant behavior such as "drug diversion." The highest rates of opioid analgesic misuse and overdose death are among non-Hispanic whites, men aged 20-64 years, and poor and rural populations. Individuals with a comorbid mental illness who are prescribed opioids are also at high risk for overdose. Nine million people who report the long-term medical use of opioids and about 5 million individuals who report nonmedical use, in the past five months are the two largest US populations at risk for prescription drug overdose (5). Identification of individuals from these high risk~~s~~ populations, as well as~~,~~ those with genetic risk, should be part of a prescreening in the pain field to reduce pseudo iatrogenic addiction. Prescripton of medications with abuse potential can be safe with interventions like setting medication goals with the patient, achieving adequate pain control, monitored pill counts and drug screens and careful documentation (13). Many medications can be used for pain control in the place of short and long-term opioids or as adjunctives to opiate analgesics.

3.1. Prescription Opiate Predicament

Opiate prescriptions to treat chronic pain have increased significantly with the equivalent of 96 mg of morphine equivalents per person being distributed in 1997, increasing to about 700 mg per person in 2007. Clinicians struggle to treat pain patients without overprescribing these drugs. Patients who abuse opioids have learned to exploit clinicians, encouraged by a culture of increased practitioner sensitivity to treating pain. Relatively low doses, less than 100 mg morphine equivalent dose per day, are prescribed to about 80 percent of pain patients, and they account for an estimated 20 percent of all prescription drug overdoses. Higher doses of greater than100 mg morphine equivalents per day are prescribed for ten percent of patients; they account for an estimated forty percent of prescription opioid overdoses. These patients are usually seen by one by prescribing physician; the remaining ten percent of patients who seek care from multiple clinicians are of greatest concern and are prescribed high daily doses ~~and~~ of opioid on numerous occasions. They are at high risk for overdose themselves, accounting for the other 40 percent of overdoses, however, they are likely diverting drugs to others who use them without prescriptions (4, 6, 7).

Without a prior history of addiction or genetic vulnerability to addiction, short-term (1-2 week), use of a therapeutic dose of opiates does not lead to long-term abuse. Opiates are the most commonly prescribed medication for pain and many patients receive them for several years and decades. Conditions that require long-term treatment of pain include low back pain and trigeminal pain and other forms of neuralgia and neuropathies. In these patients, opioid agents start losing analgesic efficacy because of the development of tolerance. Tolerance puts the patients and clinicians in a bind and requires them to increase the dose to achieve the same level of analgesia. Eventually, the dose cannot be increased due to respiratory depression, and other adverse effects and pain control is inadequate and made worse by opiate induced increased pain sensitivity. Chronic users of opiates also become dependent experiencing both physical and mental withdrawal symptoms. Opiates are also known to cause depression leading to suicidality and many patients in this situation overdose. The overdose is not merely an attempt to alleviate pain and frustration but also to act on active or passive suicidal ideations.

Opiates are, therefore, unsafe for the treatment for chronic pain. There is enough information to develop non-pharmacological techniques of controlling chronic pain even though understanding of the brain mechanisms of pain control and pain perception is limited at this time. These techniques are particularly important for individuals with greater genetic vulnerability to opiate dependence.

4. PAIN, ADDICTION AND GENETICS

Drug addiction is a severe worldwide problem with both environmental and genetic influences. It appears that although all genes associated with pain mechanisms are not common to a predisposition of addictive behavior there are similar antecedents. Thus, similar pharmacogenomic treatment solutions are indeed primary therapeutic targets. As we have hypothesized, reward circuitry impinges on pain control and associated mechanisms. Thus, to be successful in the treatment of pain the clinician should be cognizant that central reward mechanisms and the genes associated with these mechanisms are crucial to understanding pain therapeutics (14-19).

Blum and Nobles' groups discovered the first genetic association, with severe alcoholism (16)). Despite controversy from the scientific community in the 1990s, the Dopamine D2 receptor gene (located on chromosome 11 q22-q23 (15) was the first culprit to identify the dopaminergic reward system as central to all addictive behaviors. The subsequent development of the field "Psychiatric Genetics" was based on early studies of genetic ass~~i~~ociations of addictive behaviors including addiction to alcohol and other psychoactive substances like opiates (14-19. Following these earlier studies the term Reward Deficiency Syndrome (RDS) was coined to help define substance, and behavioral addictions like gambling, sex, and othe obsessive and

compulsive behaviors and to understand the genetic risk for these reward behaviors (16). As expected, we now know, following thousands (17, 20 Pubmed 4-22-16) of peer reviewed articles, that all addictive behaviors involve polygenic variants including many SNPs and point-mutations such as the GABA (A) receptor subtypes (21).

Various technologies have uncovered other genes and pathways that underly addiction. Li *et al.* (19) integrated 2343 items of evidence from peer-reviewed publications between 1976 and 2006 linking genes and chromosome regions to addiction by single gene strategies, microarray, proteomics, or genetic studies. They identified 1500 human addiction-related genes and developed KARG (http://karg.cbi.pku.edu.cn), the first molecular database for addiction-related genes with extensive annotations and a friendly Web interface. In a meta-analysis of 396 genes, each gene was supported in the studies by at least two pieces of evidence. Li *et al.* (19) found 18 molecular pathways (both downstream effects and upstream signaling events) that were statistically significant. Five common molecular pathways were found to be significant for four different types of addictive drugs. Two new pathways were also identified, the GnRH signaling pathway, and the gap junction. They mapped the common pathways into a hypothetical common molecular network for addiction. They also observed that fast and slow positive feedback loops were interlinked through CAMKII, which may provide clues to explain some of the irreversible features of addiction. Interestingly, the common thread involves dopaminergic and glutaminergic genes.

4.1. Neurogenetic Support of Opioid Pain Mechanisms

Differences in human responses to opioids have been well known for some time~~,~~. For example, a particular type of opioid may provide better analgesia than other opioids ~~for~~ in any one individual patient. Differences in individual responses are not unique to analgesic effect, they can also be seen with other opioid effects such as interactions, side effects and toxicities. As research gained from databases on knockout rodents, pharmacogenetics, and gene polymorphisms unravels the various genetics~~,~~ receptor interactions, and biochemical differences of opioid responses in humans, some of the differences may be exploited to provide better care. Testing will become more readily available and cost-effective as an aid to clinicians. Instead of having to rely solely on patient feedback, clinical judgment and trial and error, clinicians will be able to predict patient responses to doses of specific opioids, individualize opioid analgesic therapy, and devise optimal opioid rotation strategies. In the future, information of this type may translate into improved patient care, as clinicians become adept at tailoring appropriate opioid therapy. Although presently perfect candidate genes for gene-directed opioid therapy are not obvious (20), certain candidate genes have been studied, and associations with analgesic requirements for acute and chronic pain states, as well as with sensitivity to pain, have been found (22). These associations were a consequence of an intense investigation of the candidate genes for catechol-O-methyl-transferase, melanocortin-1 receptor, guanosine triphosphate glycohydrolase, and mu-opioid receptor. The genetic variants of drug-metabolizing enzymes, in contrast, have well known and described impacts on responses to pharmacotherapy. The analgesic efficacy of codeine, tramadol, nonsteroidal anti-inflammatory drugs and tricyclic antidepressants are influenced by polymorphisms of the cytochrome P450 enzymes. For example, genetically caused cytochrome P450 (CYP) 2D6 inactivity, renders codeine ineffective due to lack of morphine formation, slightly decreases the clearance of methadone, and the efficacy of tramadol due to lack of formation of the active O-desmethyl-tramadol (23-25).

In an animal genetic experiment Mogil *et al.* investigated sensitivity and tolerance to morphine. They used two strains of mice and C57BL/6By and BALB/cBy, and seven recombinant inbred strains of their reciprocal F1 hybrids. Following the administration of 20 mg/kg of morphine hydrochloride or saline, sensitivity was measured using a locomotive activity. The ‘hot plate’ method was used to measure tolerance following the single or repeated administration of 20 mg/kg of morphine hydrochloride or saline. Results indicated that both sensitivity and tolerance to morphine were found to be dependent on genotype, with inheritance characterized by dominance or partial dominance (22). Ongoing research will target other candidate gene polymorphisms

and drug metabolizing enzyme genetic variants searching for associations between drug response and an individual's genetic profile (pharmacogenetics).

The mu opioid receptor gene encodes the receptor targets for some endogenous opioids and studies of mu-opioid receptor polymorphisms, have contributed substantially to knowledge of genetic influences on cocaine and opiate (including heroin, morphine, and synthetic opioids) addiction. Monoaminergic system genes and other genes of the endogenous opioid system, particularly genes encoding the dopamine, serotonin, and norepinephrine transporters, and dopamine β-hydroxylase, have also been studied (24).

Methadone is an opiate used in substitution therapy to treat opioid dependence. Variability in individualized responses to methadone dosage, affects program retention rates, due, in part to withdrawal symptoms and further heroin craving and use, caused by low, non-optimal dosing. Methadone is a substrate for the P-glycoprotein transporter, encoded by the ABCB1 gene, which regulates central nervous system exposure. Coller *et al.* demonstrated that that ABCB1 genetic variability influenced daily methadone dose requirements. They found that subjects who are carrying two copies of the wild-type haplotype when compared with those with carriers of one or no copies, required higher methadone doses (98.3. ± 10.4., 58.6. ± 20.9., and 55.4. ± 26.1. mg/d, respectively; P = 0.0.29). They also found that doses that are significantly lower are required by carriers rather than non-carriers of the AGCTT haplotype (38.0. ± 16.8. and 61.3. ± 24.6. mg/d, respectively; P = 0.0.4). Thus, ABCB1 genetic variability may offer help for clinical methadone dosage individualization (23). Opioids are among the P-glycoprotein substrates. Opioid pharmacology may be affected by Multi-Drug Resistance Gene (MDR1) mutations. Higher fentanyl doses are required by carriers of the mutated G118 allele. The G118 allele has been associated with decreased analgesic effects including decreased potency of morphine and morphine-6-glucuronide (26). Clinical response to opioid therapy can be altered by genetic variations which may trigger or modify drug interactions. Another example is the inhibition of CYP2D6 paroxetine which in extensive metabolizers of debrisoquine/sparteine but not in poor metabolizers increases the steady-state plasma concentrations of (R)-methadone (25).

The clinical consequences of opioid Pharmacogenetics have, so far been limited. Genetically precipitated drug interactions that might cause standard opioid doses to be toxic require caution and codeine should not be administered to poor metabolizers of debrisoquine/sparteine. The on-demand administration of opioids may limit the utility of understanding the effects of mutations on opioid receptors, pain perception and pain processing, to merely explaining why some patients require higher opioid doses. However, the adverse effects profile of patients may indeed be modified by these mutations. An example is labor analgesia; women with the muOR 304G variant demonstrate more responsiveness to opioids and require significantly reduced intrathecal fentanyl ED (50). These findings for intrathecal fentanyl Pharmacogenetics may have implications for patients receiving opioids in other settings (25-27). Thus, Pharmacogenetics can be expected to facilitate individualized opioid therapy.

The following sampling of the genes involved in the addictive process can also be indicative of which genes are engaged in pain mechanisms, pain sensitivity, and opiate addiction. The list includes the mu opioid receptor, a δ-opioid receptor, metabolotropic receptors mGluR6 and mGluR8, nuclear receptor NR4A2, and photolyase-like cryptochrome 1. The dopamine receptor genes 1 to 5, dopamine transporter gene DAT1, Dopamine Beta-Hydroxylase (DBH), proenkephalin (PENK) and prodynorphin (PDYN) genes are implicated. The CAMKII enzyme, Gonadotrophin-releasing hormone (GnRH), and the CYP2D6, CYP2B6, CYP2C19, and CYP2C9 genes members of the cytochrome P450 superfamily of enzymes have a role. Brain-derived neutropenic factor (BDNF), and Neurotrophin-3 NT-3 are neurotrophic genes, and GABA receptor subunit genes on 5q33, GABA (A)gamma2, OPRM1, G-protein alpha subunits, and OPRK1, alpha2-adrenoceptor are involved. The TTC12, ANKK1, NCAM1, and TTC12 are important for drug exposure in heroin dependence (28) , and morphine stimulates zinc finger CCHC-type, RNA-binding motif 1 (ZCRB1) (29) and RGS-R7 (30) .Other genes involved include Interleukin-2, Gbeta5, MAO-A, 287 A/G polymorphism of catechol-

O-methyltransferase, serotonin transporter, Ca2+/cAMP responsive element binding protein, CNR1, ABCB1, P-glycoprotein, UGT2B7, and CREB. Some genes are involved in pain mechanisms and the healing process, the following tables represent a sampling (see Tables 1 and 2).

5. GENES AND PAIN

Certainly, some genes and associated polymorphisms and epigenetics are believed to impact pain tolerance and sensitivity. A test to identify candidate gene polymorphism can provide unique therapeutic targets to assist in the treatment of pain. Hypothetically, pharmacogenetic testing of candidate genes like mu receptors and PENK will result in personalized pharmacogenomic solutions, with the potential to improve clinical outcome (31), especially in those patients who carry risk alleles known to impart vulnerability to addiction as identified by the Genetic Addiction Risk Score (GARS) test. In addition to the determination of vulnerability to the development of addictive behaviors ~~and~~ the test can also determine the likely addiction severity of each person. This information could help in the individualized selection of the type and duration of pain treatment, and therapy and could in the future be used to formulate gene therapy (31). Understanding the role of gene polymorphisms in pain mechanisms is essential regarding the neurochemistry of pain control.

Clark *et al.* (31) analyzed the role of rs1076560 in opioid dependence by genotyping 1,325 opioid addicts. The single nucleotide polymorphism (SNP), 1076560 of the DRD2, was associated with increased risk for drug dependence (p = 0.0.038, OR = 1.2.9), significant when combined opioid-addicted ancestral samples were examined. Other examples include ~~T~~the work of David's group (32, 33), and Lerman *et al.* (18) are other examples that show the association of both the dopamine D2 transporter and DRD2 gene polymorphisms with nicotine addiction amongst other dopaminergic genes (34-38). Gilbert *et al.* (39) and Spitz *et al.* (40) found dopaminergic gene polymorphisms associated with abstinence from smoking and other addictive behaviors (41-44). .

More recently, Hau *et al.* (45) revealed that persistent pain maintains –morphine –seeking behavior after morphine withdrawal via attenuated methyl CpG-binding protein 2 (MeCP2) expression of GluA1at the level of the central nucleus of the amygdala (CeA). The CeA is a limbic structure critically involved in the affective dimension of pain. Proteins of GluA1 subunits of glutamate AMPA receptors were upregulated during morphine withdrawal, and the morphine-seeking behavior was eliminated in withdrawn rats of the pain group by the viral knockdown of CeA GluA1. The authors suggest that according to these results, after protracted morphine withdrawal, direct MeCp2 repression of the GluA1 function is the mechanism that likely maintained morphine-seeking behavior when affective pain becomes chronic.

While there are many neurotransmitters and even second messengers involved in the very complex interaction of pain control mechanisms, it is important to realize that dopamine tone is linked to tolerance and sensitivity to pain. Emery *et al.* (46) showed in animal experiments that the baseline activation levels of signaling molecules are modulated differently by various opioids and responses to a D2/D3 dopamine receptor agonist are ligand-selective. They stated that the notion that various opioids carry differential risks to the dopamine reward system is supported the complexity of this interplay and should be considered to balance opioid effectiveness with minimal risk. This recommendation serves as the basis for this hypothesis concerning mandated genetic testing. The following is a brief review of how neurotransmitter systems interact and regulate signaling molecules (like cannabinoids-opioids-glutamine and dopamine) to eaffect pain, reward, and addiction, which further supports this view.

5.1. Focusing on cannabinergic systems and pain

Chronic opiate therapy in the non-cancer population has been brought into question by recent studies theof safety and efficacy. Other modalities that can be used instead of opioids like nonsteroidal anti-inflammatory medications, antidepressants, anticonvulsants, topical agents, cannabinoids, and botulinum have been

supported in the literature (47). The promise of cannabinoids as therapeutic agents has driven slowly increasing research into endogenous cannabinoid systems and potential cannabinoid pharmaceuticals. The development and clinical use of cannabis have been complicated by recognition of their botanical source and prohibition. Intense public interest exists in making cannabis available for recreational and medicinal use. Twenty-five states currently allow the use of medical (plant) marijuana and three allow recreational use. There have been problems with the misuse of basic research in the development of synthetic cannabinoids (48) There is political pressure for Federal reclassifying marijuana as a Schedule II drug and facilitation of increased research. Clinical trials are limited due to U.S. regulatory hurdles. Expanded research on cannabis is required, the individual and public health effects of increasing use of herbal cannabis need to be identified, and work is needed to advance understanding of the pharmaceutical potential of cannabinoids as medications (49).

For millennia, preparations of the Cannabis sativa plant have been used for analgesic effect and patients will elect to use cannabis. Despite legal issues and limited evidence to guide care, clinicians need to be prepared to advise them (49). Cannabis strains contain more than one hundred different cannabinoids. Cannabinoid compounds including phytocannabinoids, delta-9-tetrahydrocannabinol (THC)~~;~~, endocannabinoids, and more recently ~~and~~ cannabidiol (CBD) and synthetics have been widely used. Cannabidiol may be anti-inflammatory, anxiolytic, and anti-seizure with no euphoria and some studies have thus far ~~have~~ shown evidence to support the use of cannabinoids for some cancer, neuropathic, spasticity, acute, and chronic pain conditions (49). The bioavailability and metabolism of cannabinoid compounds are very different depending on the route of delivery, inhalation versus oral/sublingual routes.

The endocannabinoid (EC) system is the endogenous system responsible for regulation of pain sensation, and modulation of the pain processing pathways.CB1 receptors are found at presynaptic sites in the central and peripheral nervous system (49), and CB2 receptors are found principally in the immune and hematopoietic systems. The EC system has two main classes of short-acting lipid neurotransmitter endogenous ligands (endocannabinoids). The endocannabinoid ligands are either the N-acyl ethanolamine (NAEs) class, for example, anandamide (AEA)~~;~~, or the monoacylglycerol class, for example, 2-arachidonoyl glycerol (2-AG). Both classes are synthesized on demand, then signaling is rapidly terminated by specific enzymes. The EC's act at CB1to negatively regulate neurotransmission throughout the nervous system, while those acting at CB2 regulate the activity of CNS immune cells. Signaling through both of these receptor subtypes has a role in normal nociceptive processing and also in the development resolution of acute pain states (49).

It is of interest that the cannabinoid system plays a major role in the control of pain as well as in mood regulation, reward processing and the development of addiction in less than 10% of nonmedical users (49) Opioid and cannabinoid receptors are both coupled to G-proteins and are expressed throughout the brain reinforcement circuitry. To better understand opioid-cannabinoid interactions researchers used genetically modified mice to help identify some of the specific contributions of each component of these endogenous systems to reward processing. This *in vivo* research may provide novel strategies for therapies in addicted individuals, (50).

Li *et al.* (51) demonstrate that when compared to early postnatal rats, adult rats γ-aminobutyric acid (GABA) release is lower and opioid effects are more evident. In mature, but not in immature rostral ventromedial medulla (RVM) neurons, GABA release was significantly increased by a cannabinoid receptor antagonist, suggesting the presence of local endocannabinoid tone in mature RVM neurons. Neurons in RVM play critical and complex roles in pain modulation. Studies of pain behaviors have shown that electrical stimulation of the RVM produces pain facilitation in young animals (postnatal (PN) day < 21) but predominantly inhibits pain in adults rats. GABAergic neurotransmission and several other neurotransmitter systems, undergo developmental changes that mature by PN day 21. Li *et al.* (51) show that the probability of GABA release, is

lower, and that opioid and that endocannabinoid effects are more evident in (mature) adult rats compared to (immature) early postnatal rats. It is noteworthy that these differences in these properties of RVM neurons may contribute to the developmental changes in descending control of pain from the RVM to the spinal cord. The ~~of~~ studies, Li *et al.* (51), are in agreement with earlier reports by Lau & Vaughan (52) who hypothesized that through an indirect process of 'GABA disinhibition'-suppression of inhibitory GABAergic inputs onto output neurons along the descending analgesic pathway, opioids and cannabinoids activate descending analgesia. According to Daigle *et al.* (53), results from their study~~,~~ suggest that dopamine D1 receptors and N-methyl-D-aspartate receptors act in an opposite manner to regulate striatal CB1 cannabinoid receptor signal transduction, thereby affecting pain.

Opioids and cannabinoids and are distinct drug classes that have been used to treat a variety of pain states either separately or in combination. Indeed, it is widely known that antinociceptive properties in different pain models can be produced by either the opioid or cannabinoid systems. The existence of reciprocal interactions between both systems, suggest a common underlying mechanism which is supported by several biochemical, molecular and pharmacological studies (54). Studies have demonstrated that the endogenous opioid system could be involved in cannabinoid antinociception, and recent data have also provided evidence for a role of the endogenous cannabinoid system in opioid antinociception. It is well known that certain neurons in the periaqueductal gray have co-localization and expression of both CB1 and mu opiate receptors providing antinociception (55). In fact, Haller *et al.* (56) revealed that if you protect the natural cannabinoid receptor agonist arachidonoylethanolamide from degradation, due to fatty acid amide hydrolase (FAAH), this endogenous agonist of the CB (1) receptor interacts with kappa opioid receptor systems in opioid analgesia. Moreover, inhibition of FAAH produces analgesia (57).

It is very interesting that the endogenous opioids met- and leu-enkephalin are inactivated by peptidases preventing the activation of opioid receptors. Inhibition of enzymes that degrade enkephalin and increase endogenous enkephalin levels stimulate robust behavioral effects. In animal studies, RB101, an inhibitor of enkephalin-degrading enzymes produces antinociceptive, anxiolytic and antidepressant effects without negative side effects typical of opioids. Although enkephalins are not selective endogenous ligands, enkephalins increased by RB101 can produce selective, robust behavioral effects in preclinical models. RB101 induces the antinociceptive effects through either the mu-opioid receptor alone or through activation of both mu- and delta-opioid receptors. The antidepressant-like and anxiolytic effects, however, are mediated only through the delta-opioid receptor suggesting endogenous opioid peptides (58). RB101 induces these behaviors through receptor-selective activity although enkephalins are not selective endogenous ligands. These findings suggest an important role for other inhibitors of enkephalin-degrading enzymes like D-Phenylalanine (DPA) for the treatment of pain, depression, and anxiety. This result has been shown for thiorphan another enkephalinase inhibitor potentiating delta 9-tetrahydrocannabinol-induced antinociception in mice (59).

5.2. Focusing on glutaminergic systems and pain

The main excitatory and inhibitory neurotransmitters in the adult central nervous system are glutamates, and gamma-amino butyric acid (GABA), and they exert their action through ionotropic and metabotropic receptors respectively. The Ionotropic receptors are ligand-gated ion channels involved in fast synaptic transmission; Metabotropic receptors belong to the superfamily of G-protein-coupled receptors (GPCRs), they are responsible for the neuromodulatory effect of glutamate and GABA (60). Zhuo (61) suggested that chronic anxiety triggered by injury or chronic pain is mediated through presynaptic long-term potentiation (LTP) in the anterior cingulate cortex (ACC), a key cortical region the of perception for pain. Conversely, NMDA receptor-dependent postsynaptic LTP is involved in the behavioral sensitization of and decreased levels of dopamine likely contribute to the painful symptoms that frequently occur in Parkinson's disease. Zugaib *et al.* (62) pointed out that ACC can modulate the motivational-affective component of pain and elucidated specific

neurotransmitter interactions. Following eloquent research, in guinea pigs, they suggest that activation of NMDA receptors or blockade of GABAergic neurotransmission promotes pronociception.

However, it is now well established that triggering the release of glutamate that ultimately via activation of NMDA receptors enhances the release of dopamine from dopaminergic nerve terminals in the NAc and as such influences pain perception (63). Abnormalities in dopaminergic neurotransmission like decreased levels of dopamine likely contribute to the painful symptoms that frequently occur in Parkinson's disease and have been demonstrated in other painful clinical conditions like fibromyalgia, burning mouth syndrome and restless legs syndrome. Indirect evidence from pharmaceutical trials and evidence from animal models also suggest a role for dopamine in chronic regional pain syndrome and painful diabetic neuropathy. Several novel classes of medication with analgesic properties have bearing on dopaminergic activity as evident in the capacity of dopamine antagonists to attenuate their analgesic capacity (64).

6. DOPAMINERGIC SYSTEMS AND PAIN

The principal ascending pathways for pain (e.g., the spinothalamic tract) originate mainly in the dorsal horn of the spinal cord and in the medulla, wherein second order neurons receive synaptic input from primary afferent neurons that supply nociceptors in tissue. The second order neurons of origin are within layer I as well as deep layers (IV–VI) of the dorsal horn (8). Second order neurons of origin of pain-related pathways are mainly wide-dynamic-range neurons or nociceptive-specific neurons, and these two types of neurons process both exteroceptive and interoceptive information associated with pain. Our cutaneous nociceptive system clearly serves as an exteroreceptive role in signaling potentially dangerous stimuli impinging upon our bodies, so that we can respond appropriately, depending upon the situational context. Our interoreceptive nociceptive system signals tissue disorders (e.g., rheumatoid) that are essentially inescapable, and it calls for responses more obviously in the homeostatic domain.

6.1. Mesolimbic dopamine in the suppression of tonic pain

There is little information to date concerning the identity of the endogenous pain systems that serve to inhibit tonic pain. The suppression of tonic pain involves systems in addition to those known to suppress phasic pain, and ~~that~~ these systems appear to involve forebrain sites, rostral to the brainstem. A clue to this problem is that both opioids and psychostimulants reduce tonic pain and increase transmission in mesocorticolimbic dopamine neurons known to be activated by natural rewards such as food and sex. These neurons arise from dopamine cell bodies that lie in the ventral tegmental area (VTA), and project to various forebrain sites such as the nucleus accumbens (Nacc), amygdala, and prefrontal cortex. Opioids cause the release of dopamine from these neurons through their indirect activation, whereas psychostimulant drugs such as amphetamine and cocaine increase dopamine extracellularly by decreasing reuptake and/or inducing release. Moreover, opioids and psychostimulants have both rewarding and analgesic effects in the clinical setting, suggesting that their effects might share common neural substrates (65)~~,~~It was Morgan *et al* (65) who found that dopamine–depleting 6-hydroxydopamine lesions of the ventral midbrain, which contains the cell bodies of the neurons that give rise to ascending forebrain projections, block the analgesic effects of systemic morphine and amphetamine in the formalin, but not the tail flick test. Their findings provided the first evidence that mesolimbic dopamine neurons play a role in the suppression of tonic, but not in the phasic pain. In the recent studies, Taylor *et al.* (66) found that while the D1-selective agonist SKF38393 was without effect at a dose of 0.5. nmol/side, the D2-selective agonist quinpirole, dose dependency (0.0.5–5.0. nmol/side, bilateral), inhibited the persistent phase of formalin-induced nociception. This was blocked by pre-administration of a selective D2-dopaminergic antagonist raclopride. These results indicate that dopamine agonists that activate D2 receptors in the Nacc inhibit inflammatory pain.

Plastic changes in synaptic neurotransmission in the brain are thought to play a role in chronic pain. Animal

studies suggest that striatal and cortical dopaminergic systems participate in pain transmission or modulation. Dopamine D2 receptors have been reported to mediate the inhibitory role of dopamine in animal models for persistent pain (67). Hagelberg *et al.* (68) showed in healthy volunteers that high D2 receptor availability in the putamen is associated with low cold pain threshold and a high pain modulation capacity induced by conditioning stimulation. This effect involves mu receptor interaction (69). Furthermore, decreased (18F) FDOPA uptake and increased D2 receptor availability have been demonstrated in the putamen in a chronic orofacial pain state, the burning mouth syndrome (70).

Moreover, it was found that the increase in D2 receptor availability in the left putamen and the decrease in D1/D2 ratio imply that alterations in the striatal dopamineric system, as evaluated by PET, may be involved in chronic orofacial pain conditions. In essence, we hypothesize that low or hypodopaminergic function in the brain may predispose individuals to low pain tolerance. Current research would support this concept, and thus carriers of the D2 TaqA1 allele as observed in reward deficiency syndrome (RDS) (71) behaviors may be good candidates for nutrients or bioactive substances designed to enhance dopamine release in the brain.

Catechol-O-methyltransferase (COMT) metabolizes catecholaminergic neurotransmitters. Numerous studies have linked COMT to pivotal brain functions such as mood, cognition, response to stress, and pain. Both nociception and risk of clinical pain have been associated with COMT genetic variants (a functional marker, rs165774, situated in the 3' untranslated region of a newfound splice variant, (a)-COMT), and this association was shown to be mediated through adrenergic pathways. Recently Meloto *et al* (72) found that the pain-protective A allele of rs165774 coincides with lower COMT activity, suggesting contribution to decreased pain sensitivity through increased dopaminergic rather than decreased adrenergic tone. Their results provide evidence for an essential role of the (a)-COMT isoform in nociceptive signaling and suggest that genetic variations in (a)-COMT isoforms may contribute to individual variability in pain phenotypes.

7. STRESS AND PAIN

The effects of excessive stress in modern life lead to chronic states of fatigue-related depression. According to the American Academy of Family Physicians, about 2/3 of all office visits are related to stress and depression. Therefore, it is important to understand that it is our position that in an individual with chronic pain, the subject is definitely in a stressful condition, and has increased neuronal firing. There are numerous examples in the literature to support this contention. Furthermore, if an individual has the DRD2A1 variant, numerous studies have shown that resultant low dopamine D2 receptors caused an inability to cope with stress in the family, and as an individual (73-75). In this regard, it is known that stress could even reduce the D2 receptor mRNA message in the substantia nigra, the lateral part of the VTA, the basal ganglia, and especially in the nucleus accumbens (76). This polymorphism, as well as others, could ~~not only~~ affect the ability to cope with stress and alter one's pain sensitivity.

7.1. Nutrients and anti-stress

This work supports the concept that forebrain dopamine systems are involved in mediating the behavioral effects of chronic mild stress. It further supports the view that in subjects with pain (with chronic mild to moderate stress) with a compromised number of D2 receptor sites and reduced mRNA message, the firing frequency of a catecholaminergic neuron is enhanced and would be quite receptive to l-tyrosine, a dopamine precursor. Moreover, it is also known that neuronal depletion of dopamine could also induce an independent end-product inhibitory state for tyrosine–hydroxylase, which will also respond to l-tyrosine supplementation. In this regard, in order to provide an up-regulation in D2 receptors, we proposed a slow release, personalized designed natural solution, providing a constant dopamine release because of the effect of enhanced opioidergic activity 'via d-phenylalanine (a known enkephalinase inhibitor) on substania nigra GABA neurons. The main point here is that pharmacological manipulation of up-regulation of dopaminergic pathways will

ultimately lead to the reduction of stress, since it is well known that the dopamine molecule is considered as the endogenous anti-stress substance.

7.2. Stress and dopamine and chronic pain

The relationship between stress, endorphins and hypothalmic-pituitary-adrenal (HPA) axis is well researched (77). Certainly in the world of addiction stress plays a critical role in both the acquisition and relapse. It is known that certain genetic and environmental elements play significant roles in drug dependency and dysregulation of brain reward pathways. In fact, dopamine D2 receptor polymorphisms have been associated with stress-coping mechanisms and posttraumatic stress disorder (78). Interestingly, either stress can induce a painful condition or it can exacerbate the pain. Exposure to stress also activates dopamine transmission in mesocorticolimbic dopamine neurons (79), and this effect appears to involve opioid mechanisms in the VTA. More specifically, intra-VTA infusions of the opioid receptor antagonist Naltrexone, prevent the stress-induced activation of dopamine metabolism in the NAcc and prefrontal cortex, and exposure to stress causes the release of met-enkephalin into the VTA (80). These findings combined with those indicating that exposure to stress can inhibit tonic pain and that intra-VTA morphine induces analgesia in the formalin test suggest that the endogenous release of opioids in the VTA might be a mechanism underlying the stress-induced inhibition of tonic pain. This has been supported by the finding that intra-VTA infusions of the opioid receptor antagonist, naltrexone, block stress-induced analgesia in the formalin test (81). In addition, it has been proposed that release of the tachykinin neuropeptide, substance P (SP), in the VTA might play a similar role in the stress-induced suppression of tonic pain. Moreover, it has been found that activation of midbrain dopamine neurons by SP did indeed inhibit tonic pain in the formalin test (82,83). The current data suggest that exposure to stress induces analgesia by causing a release of SP in the VTA, which in turn activates mesocorticolimbic dopamine neurons. Finally, opioids, anphetamine, and SP all share the ability to increase dopamine release in the NAcc. Moreover, opioids administered systemically or into the VTA augment dopamine metabolism and extracellular levels of dopamine in the NAc.

With that background, it becomes increasingly clear that tonic pain maybe attenuated by dopamine D2 activation. It follows then that in this hypothesis we embrace the concept that supportive research in the area of developing a natural method to cause a preferential release of dopamine in mesocorticolimbic pathways seems warranted. Thus, support of an attenuation of stress has been found with a variant of a complex with dopaminergic activation properties shown in one double-blind placebo-controlled study (84). We further hypothesize herein that unless there is a way of increasing endogenous opioids, which in turn inhibit GABA causing dopamine release in the NAc, simple neurotransmitter precursors will not be as effective in reducing tonic pain.

8. PHARMACOLOGICAL ASPECTS OF PAIN CONTROL

Opioids such as morphine and heroin and psychostimulant drugs such as amphetamine and cocaine are effective pharmacological tools against chronic pain. Interestingly, amphetamine and related drugs relieve cancer pain and sometimes administered as an adjuvant analgesic in the clinical situation, because they potentiate opioid analgesia and counter opioid-related sedation and cognitive disturbances. In support of these clinical findings, studies have shown that, in rats, psychostimulants potentiate the analgesic effect of morphine in an animal model of persistent pain (85). There is increasing evidence that sites rostral to the brainstem play a critical role in the analgesic effects of opioid and psychostimulant drugs.

It is well known that opioids can inhibit pain by acting at spinal sites and at sites in the brainstem, where they modulate activity in descending brainstem pathways projecting to the spinal cord. A primary site of action is the periacqueductal gray of the brainstem where stimulation of opioid receptors activates, through direct projections, serotonin-containing cells in the nucleus raphe magnus. In turn, the latter cells activate neurons

that project, via the dorsolateral funiculus, to the dorsal horns of the spinal cord where they inhibit cells that transmit information about noxious painful stimulation from the periphery to supraspinal sites. The brainstem descending pain-suppression system, however, plays a more important role in the suppression of brief, rapidly rising, transient, and well-localized (i.e., phasic) pain than it does in the suppression of injury-produced persistent (i.e., tonic) and inescapable pain. However, several lines of evidence suggest that the inhibition of the tonic pain requires the activation of neural systems (missing word) to those required to inhibit phasic pain (86).

In terms of molecular genetic testing there are three types of current interest. These include: Pharmacogenetics (primarily evaluating metabolizing enzymes for high and low metabolizers with for example opiates); Genetic Addiction Risk Score (to determine through a panel of reward gene polymorphisms stratification risk or vulnerability to all RDS behaviors including pain tolerance); and Pharmacogenomics (personalized addiction medicine based on genotyping an individual and targeting specific gene loci).

9. PHARMACOGENETIC TESTING

The importance of pharmacogenetic testing of the above-mentioned genes will provide information related to potential genetic antecedents for a predisposition to not only aberrant pain sensitivity but to an inability to heal properly. This genetic information will ultimately lead to a DNA-directed development of a personalized treatment regimen including a pharmacogenomic resolution. Genetic testing will provide medical evidence for rationale treatment protocols.

Based on the findings reviewed herein, we hypothesize that the subsequent coupling of the identified genes as described in this paper, as well as other genes relative to polymorphisms, would allow for additional pharmacologically active substances-based pharmacogenomic mapping. The combination will provide a map that will serve as a platform to derive novel DNA targeted areas, which will link bioactive substances with potential anti-craving actions and pain relief mechanisms. In essence, the linking of known reward genes and other physiological-based endogenous opioid receptors and or other signaling substrates will ensure successful personalized medical treatments for individuals with aberrant inborn pain sensitivity.

Various alleles in the P450 system are currently utilized in pain medicine clinics to evaluate metabolic concerns to help identify high and low metabolizers. For the most part this has not translated to significant clinical utility, but may have some relevance in terms of buprenorphine/naloxone treatments (87).

10. GENETIC ADDICTION RISK SCORE

It is now known that in terms of nature (genes) and nurture (environment) and behavioral outcome in homo sapiens the contribution is 50% genes and 50% epigenetics. Thus molecular genetic or DNA testing is very important especially linking aberrant behaviors to any individual.

Blum's laboratory proposed (88) that any disturbance along this brain reward cascade due to either gene variations (polymorphisms) or environment (epigenetics) will result in aberrant addictive behaviors or RDS. In spite of a global search to uncover specific or candidate genes or even clusters of genes characterized from high-density SNP arrays, it is well-known that many attempts have not been replicated or have been inconclusive. However, Palmer *et al.* (89) recently showed that between 25–36 percent of the variance in the generalized vulnerability to substance dependence is attributable to common single nucleotide polymorphisms. Moreover, the additive effect of common single nucleotide polymorphisms is shared across important indicators of comorbid drug problems. Furthermore, as a result of these studies more recent evidence has revealed that specific candidate gene variants account for risk prediction.

Adopting a Bayesian approach, earlier studies from Blum's laboratory determined a Positive Predictive Value

(PPV) for the DRD2 A1 variant (low number of D2 receptors) of 74%, indicating that if a child is born with this polymorphism they have a very high risk of becoming addicted to either drugs, food, or aberrant behaviors at some point in their future (90, 91). Over the many years to come since the 1990 finding, laboratories all across the globe including NIDA and NIAAA not only confirmed this early work especially in heroin dependence (92) but extended the magnitude of many other candidate genes, especially genes and second messengers located in the reward circuitry of the brain.

Examples include, Moeller *et al.* (93) who suggested that drug cues contribute to relapse, and their neurogenetic results have identified the DAT1R 9R-allele as a vulnerability allele for relapse especially during early abstinence (e.g., detoxification). The DAT1 9 allele influences the fast acting transport of dopamine sequestered from the synapse leading to a hypodopaminergic trait.

It seems prudent to embrace genetic testing to reveal reward circuitry gene polymorphisms especially those related to dopaminergic pathways as well as opioid receptor (s) as a way of improving treatment outcomes. Understanding the interaction of reward circuitry involvement in buprenorphine effects and respective genotypes provide a novel framework to augment a patient's clinical experience and benefits during opioid replacement therapy (87).

GARS patented (patents pending and issued) genetic risk score represents a panel of known reward genes and associated risk polymorphisms providing genetic risk for addiction and other behaviors including medical monitoring and clinical outcome response.

10.1. Pharmacogenomics – customized addiction medicine

Along these lines Blum and Kozlowski developed the "Brain Reward Cascade" (BRC) (94). This concept served as a blue print for how neurotransmitters interact in the reward system of the brain. In addition, it has been firmly established that respective reward genes that regulate these chemical messengers ultimately control the amount of dopamine released into not only the reward site but other regions of the brain.

Moreover, it is well established that resting state functional connectivity integrity is important for normal homeostatic functioning. Zhang *et al.* (95) recently showed that in heroin addicts there is reduced connectivity between dorsal anterior cingulate cortex (dACC) and rostral (rACC), as well as reduced connectivity between subcallosal (sACC) and dACC. Their findings of variations of functional connectivity in three sub-regions of ACC in heroin addicts implied that these sub-regions of the ACC together with other key brain areas (such as dorsal striatum, putamen, orbital frontal cortex, dorsal striatum, cerebellum, amygdala, etc.) potentially play important roles in heroin addiction. Most recently Blum's laboratory along with Zhang's group (96) showed in abstinent heroin addicts that KB220Z™ a complex putative dopamine D2 agonist, induced an increase in BOLD activation in caudate-accumbens-dopaminergic pathways, compared to placebo, following one-hour acute administration. Furthermore, KB220Z™ also reduced resting state activity in the putamen of abstinent heroin addicts. In the second phase of this pilot study ~~of~~ for all ten abstinent heroin-dependent subjects, three brain regions of interest (ROIs) were observed to be significantly activated from resting state by KB220Z™ compared to placebo ($P < 0.0.5$). Increased functional connectivity was observed in a putative network that included the dorsal anterior cingulate, medial frontal gyrus, nucleus accumbens, posterior cingulate, occipital cortical areas and cerebellum. Utilizing DNA based testing successful development of polymorphic gene testing enabled customized (personalized) anti-obesity compounds. This serves as the basis of futuristic personalized addiction medicine utilizing Dominion's Genetic Addiction Risk Score.

While there is a plethora of very positive experiments involving thousands of studies for many candidate gene associations with all RDS behaviors including pain, there are also negative results (97-100). Currently, a number of companies have entered the genetic testing arena in the addiction and pain industrial space claiming "personalized care". However, we believe these companies have not done their homework in a

scientific manner. These issues include exaggerated claims such as using Blum's original work (41,43) stating that their genetic test is 74% predictive. This is indeed false because they use one gene (DRD2) to back their claim and commercialize a full panel of other candidate genes and never carried out any outcome studies with their panel. Additionally, they make other false claims suggesting that patient's results are compared to population controls. Review of their so called «disease free» controls reveal significant flaws especially in light of not controlling for a remarkable list of RDS behaviors (101). They would have to utilize what has been termed "Super-Controls." Simply stated population controls may carry many invisible RDS behaviors that must be identified so that the control would be RDS free.

Otherwise utilization "Super-Controls." will lead to spurious and false results (102). Another issue is that these companies have selected genes that may be involved in risky behavior but they do not utilize the correct variant in their tests or use very rare variants that do not truly prove addiction risk. Specifically, Mayer and Höllt (103) correctly proposed that the vast number of non-coding, intronic or promoter polymorphisms in the opioid receptors may influence addictive behavior, but these polymorphisms are far less studied, and their physiological significance remains to be demonstrated.» Most importantly, these companies have never performed research to show whether their genetic full-panel test significantly predicts anything l*et al*one addiction risk or any associated behaviors.

While we, the authors, may have a personal bias because over the many years that Blum's laboratory has dedicated work to develop an accurate genetic test to predict true liability/risk for RDS and associated behaviors, we will attempt to explain why our current laboratory testing site has successfully developed the first GARS™ in conjunction with the Institute of Behavioral Genetics, University of Colorado, Boulder.

To develop GARS we first selected ten reward candidate genes (DRD1, 2, 3, 4; DAT1; serotonin transporter, COMT, MAO, GABA, Mu opiate receptor) and a number of SNPs and point mutations that influence the net release of dopamine at the brain reward site. The variants or SNPs, including point-mutations, were chosen to reflect a hypodopaminergic trait. In terms of validation we partnered with the developers of the Addiction Severity Index- Media Version (ASI-MV), a test mandated in 13 states, for both alcohol and drug severity risk scores (104) We contacted eight very diverse treatment centers across the United States resulting in a total of 393 subjects that were genotyped using the selected GARS panel. All the data was genotyped and analyzed at the Institute for Behavioral Genetics (IBG) at the University of Colorado Boulder. Without going into specifics we found a significant association between a summed score of all GARS panel risk alleles (variant forms) and both the ASI-MV alcohol ($p<0.0.04$) and drug ($P<0.0.5$) severity indices in a total of 273 subjects.

In fact, the higher the number of risk alleles the stronger the prediction of alcohol or drug use severity. It was also found that family problems, psychological issues and medicalization significantly correlated as well. One important caveat was that if we changed any specific SNP the significance was lost. This strongly suggests how important the selected GARS panel is and any deviation will produce false results that may occur with other commercial tests that have no research to validate their tests.

11. CONCLUSION

While it is well established that the principal ascending pathways for pain originate in the dorsal horn of the spinal cord and in the medulla, the control and sensitivity to pain may reside in additional neurological loci, especially in the mesolimbic system of the brain (i.e., a reward center), and a number of genes and associated polymorphisms may indeed impact pain tolerance and or sensitivity (105). It is hypothesized that these polymorphisms associate with a predisposition to intolerance or tolerance to pain. It is further hypothesized that identification of certain gene polymorphisms provides a unique therapeutic target to assist in the treatment of pain (105). It is hereby hypothesized that pharmacogenetic testing of certain candidate genes (i.e., CB1, mu receptors, PENK etc.) will result in pharmacogenomic solutions personalized to the individual

patient, with potential improvement in clinical outcomes (106). However, the equal or even more important message herein, is that we the authors believe that with continuing iatrogenic induced opioid epidemic with so many lives young and old dying, not only in America but across the globe, it is incumbent upon the government to carefully assess the situation. One path to victory is that genetic testing for not only pharmacogenetics (metabolism that could affect dosage) but genetic vulnerability or predisposition of genetic risk to all RDS behaviors (107), substance and non-substance related including cannabis (108,109), become mandated, especially in all pain clinics prior to treatment of both acute and chronic, non-cancerous pain to prevent addiction.

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