

NEUROGENETIC IMPAIRMENTS OF BRAIN REWARD CIRCUITRY LINKS TO REWARD DEFICIENCY SYNDROME (RDS) AS EVIDENCED BY GENETIC ADDICTION RISK SCORE (GARS): A CASE STUDY

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ABSTRACT

Importantly, research from our laboratory in both in-patient and outpatient facilities utilizing the Comprehensive Analysis of Reported Drugs (CARD)TM found a significant lack of compliance to prescribed treatment medications and a lack of abstinence from drugs of abuse during active recovery. This unpublished, ongoing research provides an impetus to develop accurate genetic diagnosis and holistic approaches that will safely activate brain reward circuitry in the mesolimbic dopamine system. Our laboratory has extensively published the neurogenetics of brain reward systems with particular reference to genes related to dopaminergic function. In 1996, we coined "Reward Deficiency Syndrome" (RDS), used to describe behaviors found to have an association with gene-based hypodopaminergic function. Many subsequent studies have embraced RDS as a useful concept to help expand our understanding of Substance Use Disorder (SUD), process addictions, and other obsessive, compulsive and impulsive behaviors. Here, we illustrate the usefulness of the genetic testing of a panel of reward-related genes, the Genetic Addiction Risk Score (GARS) in only one case study. Interestingly, we were able to describe lifetime RDS behaviors in a recovery addict (17 years sober) blindly by just assessing resultant GARS data. We encourage further required studies in this important emerging field.

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KEY WORDS

Genetic Addiction Risk Score (GARS), Dopaminergic System, Reward Genes; Reward Deficiency Syndrome (RDS)

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[1] INTRODUCTION

The brain's mesolimbic reward system is a critical site for experiences of well-being. The reward center is where chemical messengers including serotonin, enkephalin, γ -aminobutyric acid (GABA), dopamine (DA), acetylcholine (ACH) and many second messenger proteins act in concert to provide a net release of DA in the nucleus accumbens (NAc). The idea that the synthesis, vesicular storage, metabolism, receptor formation, and catabolism of neurotransmitters are controlled by genes is well documented [1-3].

Most importantly, polymorphisms of reward genes can disrupt the neurochemical events that culminate in neuronal release of DA within the mesolimbic reward circuitry. A breakdown of these neuronal events in the "The Brain Reward Cascade" [4]

will eventually lead to DA dysfunction. DA neurotransmission is essential for an individual to experience of pleasure (reward) and the reduction of stress. DA dysfunction then can result in a deficiency in reward and a predisposition to substance-seeking in an attempt to ameliorate hypodopaminergic function [5].

1.1. Neurogenetic considerations

Homo sapiens have a biological predisposition to drink, eat, reproduce, and desire pleasurable experiences. DNA polymorphisms, together with epigenetic and/or environmental factors can result in multiple impulsive, compulsive, and addictive behaviors by impairment of the normal flow of

neurotransmitter activity in the reward center of the brain. From the many genes known to predispose individuals to excessive cravings and result in substance use disorders (SUDs), some of the most prominent genes with known polymorphisms make up the provisional GARS panel they include: the serotonergic 2A receptor (5-HTT2a); serotonin transporter (5HTTLPR); DA D1

receptor (DRD1); DA D2 receptor (DRD2); DA D3 receptor (DRD3); DA D4 receptor (DRD4); DA transporter (DAT1), and the catechol-O-methyltransferase (COMT), monoamine oxidase (MOA); Mu-opiate receptor (MOR); GABA β -3; Gamma 2 subunit genes; as well as the PENK Cytochrome P450 gene [5-7][Table-1].

Table: 1. Proposed Genetic Addiction Risk Score (GARS)

Dopamine D1 Receptor Gene
 Dopamine D2 Receptor Gene
 Dopamine D3 Receptor Gene
 Dopamine D4 Receptor Gene
 Dopamine D4 Receptor Gene
 Serotonin 2a Receptor Gene
 Serotonin Transporter Gene
 Mu-opiate Receptor Gene
 GABA β -3 Receptor Gene
 PENK Gene
 Mono-Amine β -Oxidase A Gene
 Catecholamine β -Methyl-Transferase Gene
 Cytochrome P450 Gene

The first controversial study on the association of polymorphisms of the DRD2 A1 allele and severe alcoholism [4] started, the explosive field known as “Psychiatric Genetics”. Since then an association has been identified between common genetic variants of the DA D2 receptor gene (DRD2) polymorphisms [8, 9] and other reward genes and polymorphisms [5, 6, 7] that result in hypodopaminergic function. An association between hypodopaminergic function and impulsive, compulsive, and addictive behaviors and has also been identified [5, 6, 10].

Individuals are predisposed to self-medicate with substances and behaviors that will trigger the release of DA. For example, an increased rate of mitochondrial DA breakdown due to increased MOA activity or an increased rate of synaptic DA breakdown due to having high catabolic genotype of the COMT gene lead to a “hypodopaminergic” trait. On the other hand, slower breakdown of DA due to polymorphisms in both the MOA and or COMT may lead to hyperactivity as seen in Attention Deficit Hyperactivity Disorder (ADHD).

Addictions, including alcohol, opiates, psychostimulants (cocaine, methamphetamine), nicotine, glucose, gambling, sex addiction, excessive spending, and even uncontrolled internet gaming are associated with the release of DA in the mesocorticolimbic system or reward pathway of the brain [4, 5, 11-14]. While activation of this dopaminergic system results in feelings of reward and pleasure [12-16], reduced activity of this system (hypodopaminergic functioning) can trigger drug-seeking behavior [17-21].

Hypodopaminergic functioning including reduced DA receptor density, blunted response to DA, or enhanced DA catabolism in

the reward pathway, which can be induced by variant alleles or defined polymorphisms have been identified over at least two decades [22]. Cessations of chronic drug use also can produce a hypodopaminergic state that prompts drug-seeking behaviors in an attempt to address the unwanted withdrawal-induced state [23].

1.2. Neurotransmitter mechanisms

Well-being can be produced by acute use of psychoactive substances, however, sustained and prolonged abuse results in tolerance and discomfort [24]. Opioid desensitization/tolerance mechanisms have focused on adaptations that include receptor phosphorylation, internalization, and sub-cellular trafficking on the level of the mu-opioid receptor (MOR). Recent research has revealed augmented isoform-specific synthesis of adenylyl cyclase and their phosphorylation and augmented phosphorylation of the G(beta) subunit of G(beta gamma). These changes result in a shift of mu-opioid receptor-coupled signaling to inhibitory (G(i)-derived) G(beta gamma) stimulatory adenylyl cyclase signaling, from predominantly G(i alpha) [25]. It is noteworthy, that polymorphisms related to MOR have been associated with excessive drug (ethanol) seeking behavior that interacts with dopaminergic pathways in the NAc [26].

A PUBMED (10-24-12) search revealed at least 197 articles dedicated to the role of Dopamine D2 receptor gene and excessive cravings caused by carrying the DRD2 A1 allelic genotype. While a deficit in DA receptors, is compounded by consequential drug seeking behavior, conversely, normal densities of DA receptors result in reduced craving behaviors [18].

Attenuation of craving to prevent or treat Substance Use Disorder (SUD) could result from proliferation of DA D2 receptors in genetically predisposed individuals [27- 29] and those with hypodopaminergic function, secondary to stress or the toxic effects of the abused substances [30] would also benefit from proliferation of DA D2 receptors.. Boundy et al. [27, 30] have shown, in-vitro, that constant stimulation of the DA D2 receptor system with low doses of a D2 agonist results in significant proliferation of D2 receptors, in spite of genetic antecedents [31]. Proliferation of D2 receptors caused by messenger RNA expression is induced by negative feedback mechanisms in the mesolimbic system, signaled by moderate chronic D2 receptor stimulation [27, 30]. Thus, stimulating rather than blocking dopaminergic receptor sites may be a worthwhile solution to the hypodopaminergic state or trait [32-37]. In nonhuman animals DNA-directed overexpression of the DRD2 receptors induces a significant reduction, in both alcohol and cocaine craving and drug seeking [34-36].

Most recently our laboratory embarked on an unpublished scientific investigation using GARS to assess clients attending two treatment facilities in the United States: Malibu Beach Recovery Center, Malibu Beach, California and G&G Holistic Addiction Treatment Center, North Miami Beach, Florida. It is noteworthy that subsequent to the development of an algorithm based stratification of risk assessment of 70 tested patients 100% carried at least one risk allele for RDS behaviors; 5% carried high risk; 81% moderate risk and 14% low risk.

[II] METHODS

Utilizing this genetic test we describe herein one case of a recovering addict's (17 years sobriety) life-history especially as it relates to RDS behaviors and her GARS results. The exercise was to blindly predict clinically relevant information about the person's past behavioral history by identifying individual polymorphic risk alleles [Table-2]. The behaviors that associate with them were recorded and blindly compared to the clinical history (Case Study) provided later by the subject.

Table: 2. shows the resultant analysis on EW's (AKA) GARS and individual genotypes

Genes/ Alleles	Results
Caspi MAOA uVNTR	3R
Caspi MAOA uVNTR	4R
DRD4	2R
DRD4	4R
DAT	10R
DAT	10R
5HTTLR diallelic	S/S
COMT	A/G
DRD2 Taq1	A2/A2
DRD3 C=Gly	C/T
OPRM1 A=Asn G=Asp	G/G
GABRA3	181
GABRA3	197
Allele #	10
Score	0.56
Severity	Moderate

DNA extraction was obtained by saliva collection and genotyped at the Colorado University Institute of Behavioral Genetics utilizing standard techniques [38].

[III] RESULTS

EW has 10 alleles out of the 9 genes with a GARS score of 0.56 which is rather high but fits within the modest Risk. There are 18 alleles for females and 17 alleles for males. Interestingly, EW is not positive for the DRD2 A1, this could have helped her recovery process, whereby the DRD2 A1 has been associated with relapse (Dahlgren et al., 2011) [39]. However, she is positive for MOA gene but is heterozygote 3R/4R, which may result because of the 3R, in a slower breakdown of mitochondrial DA when it is brought back into the presynaptic neuron. Interestingly, EW is polymorphic for the dopamine transporter gene having 10R/10R. This suggests that she may have impulsive tendencies and hyperactivity and possibly ADHD. One noteworthy finding is that EW possesses S/S for the serotonin transporter gene which has been linked to excessive alcohol intake. In terms of the enzyme COMT which breaks down Dopamine in the synapse she carries the AG genotype. The G allele called VAL has been associated with opiate abuse. However, it is clear that she also carries the homozygote of the mu opiate receptor MOR identified as G/G which has been found to endorse drinking to enhance positive affect (liking). She also carries the C/T genotype for the Dopamine D3 gene which has been associated with substance abuse. EW also carries the heterozygote 183 allele of GABA receptor subunit and as such also may like alcohol to relieve her anxiety due to low GABA receptors sensitivity.

[IV] CASE STUDY

EW (AKA) is a 54 year old Caucasian female with a long standing history of polysubstance abuse. Her first use was alcohol at age 13. Over the next few years, she progressed to regular use of benzodiazepines, prescription stimulants, and LSD. At age 19, she began using heroin and cocaine intravenously and quickly became addicted. During this period, she also drank intermittently. She would become violently ill every time she used any opiates or alcohol, but continued to use to modify her feelings. Over the next several years, EW "detoxed" multiple times on methadone, but repeatedly returned to drug use. At the age of 27, she began attending AA. She was able to stay sober for the majority of that time, with three very brief relapses on alcohol, methamphetamine, and benzodiazepines. She currently has 17 years of uninterrupted sobriety.

In sobriety, EW was diagnosed with ADHD and has had constant problems with impulse control. She believes that this played a large part in her relapse history; as well as affecting her personal relationships, social functioning, and overall well-being. EW does have a family history of addiction. Her father, deceased, was a recovering alcoholic. She also reports

alcoholism in her maternal great-grandfather. Both EW's mother and grandmother used prescription opiates and sedatives to excess. There is a family history of depression and suicide.

[V] DISCUSSION

Homo sapiens in evolutionary terms are changing very slowly, and certain genetic traits such as genes that regulate pleasure seeking may be the exception [32, 33]. Interestingly, the DNA analysis of the discovered Iceman (Ötzi), for the most part, with the exception of the genes responsible for lactose intolerance, atherosclerosis, and having *Borrelia burgdorferi* making him the earliest known human with Lyme disease, matches to some extent modern day humans. His autosomal DNA is most closely related to southern Europeans, from geographically isolated populations in Sardinia and Corsica but he seems to be closer to Neanderthal ancestry [40]. However, we do not know whether the DRD2 A1 allele is an older gene allele or if it is newer than the DRD2 A2 allele. Identifying this will help clarify the nature of the relationship humans have with pleasure-seeking and perhaps how it benefits our survival. For example, carriers of the DRD2 A1 allele are more aggressive than carriers of the DRD2 A2 allele [41-43].

The work of Blum et al. [4] and others including brain imaging studies [44] have helped us explain molecular mechanisms of addiction. One component of all this serious investigation suggests that hypodopaminergic function stimulates cravings, which in turn affects attention to goals. Maintenance of cognitive control is required to override compulsions to use drugs. Cognitive control involves the ability to generate action plans and then monitor actions/behaviors to attain goals [45]. The steady influx of DA that occurs with drug abuse becomes the sole focus of attention. The central goal, is obtaining more drugs. Motivated by cravings for drugs, even though the drugs have long stopped providing pleasure, victims of SUDs and process addictions are caught in a spiral of physical brain changes and the psychological consequences of those changes that lead to further physical and psychological changes and consequences [46, 47].

DA is a key genetically induced deficient neurotransmitter causing in abnormal craving behavior and excessive pleasure seeking. Finding ways to increase DA D2 density, instead of blocking dopaminergic function, may be the best strategy to unlock the elusive addiction riddle and attenuate abuse [32, 46, 48].

[VI] CONCLUSION

New treatment and genetic diagnostic approaches are required in view of our most recent unpublished work derived from studies with CARD™. Specifically, studies from our laboratory in both in-patient and outpatient facilities utilizing the Comprehensive Analysis of Reported Drugs (CARD)™ found a significant lack

of compliance to prescribed treatment medications and a lack of abstinence from drugs of abuse during active recovery [49].

We are proposing a paradigm shift a solution for RDS that embraces the coupling of (1) genotyping of individuals for candidate reward genes to determine stratification of genetic risk for all RDS behaviors (GARS)™ [48,50], (2) the use of slow acting D2 agonist therapy (e.g. KB220Z™) to activate dopaminergic pathways in the NAc (affecting abnormal craving) and other brain regions (affecting decision –making) and (3) the use of CARD™ during active recovery to assess compliance to prescribed treatment medications and abstinence from drugs of abuse.

Potential utilization of these tools may provide the clinician the means to generate better diagnosis and recovery rates. Further research, in terms of reinforcement experiments in nonhuman animal models [51] and human trials, will assist in promotion of these novel strategies for the early diagnosis, prevention, treatment and attenuation of relapse in RDS [52,53] including process addictions [54, 55].

CONFLICT OF INTERESTS

Kenneth Blum, Mary Hauser, B. William Downs, Margaret A. Madigan, and John Giordano have a conflict of interest due to the commercial development of the GARS test co -marketed by LifeGen, Inc and Dominion LLC.

FINANCIAL DISCLOSURE

The work was carried out without any financial support from Dominion Diagnostic. LLC.

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