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Genetic Addiction Risk Score (GARS): Testing For Polygenetic Predisposition and Risk to Reward Deficiency Syndrome (RDS)

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1. Introduction

Dopamine is a neurotransmitter in the brain, which controls feelings of wellbeing. This sense of wellbeing results from the interaction of dopamine and neurotransmitters such as serotonin, the opioids, and other brain chemicals. Low serotonin levels are associated with depression. High levels of the opioids (the brain's opium) are also associated with a sense of wellbeing. (Blum et al., 2010).

Dopamine has been called the "anti-stress" and/or "pleasure" molecule. When released into the synapse, dopamine stimulates a number of receptors (D1 – D5), which results in increased feelings of wellbeing and stress reduction (Kreek and Koob, 2007). The mesocorticolimbic dopaminergic pathway plays an important role in mediating reinforcement of natural rewards such as food and sex, as well as unnatural rewards such as drugs of abuse (Bruijnzeel et al., 2007). Natural rewards include satisfaction of physiological drives (e.g. hunger and reproduction) and unnatural rewards are learned and involve satisfaction of acquired pleasures such as hedonic sensations derived from alcohol and other drugs, as well as from gambling and other risk-taking behaviors. (Olsen, 2011).

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The DRD2 gene is responsible for the synthesis of dopamine D2 receptors (Grady et al., 1989). Further depending on the genotype (allelic form A1 versus A2), the DRD2 gene dictates the number of these receptors at post-junctional sites (Blum et al., 1990; Noble et al. 1991). A low number of D2 receptors leads to hypodopaminergic function. When there is a paucity of dopamine receptors, the person is more prone to seek any substance or behavior that stimulates the dopaminergic system (Conrad et al., 2010). The D2 receptor has been associated with pleasure, and the DRD2 gene has been referred to as a reward gene (Heber & Carpenter, 2011).

Although the DRD2 gene, and especially the Taq1 A1 allele, has been most associated with neuropsychiatric disorders in general and in alcoholism in particular, it is likely involved in other addictions (e.g., carbohydrate). It may also be involved in co-morbid antisocial personality disorder symptoms, especially in children and adults with attention deficit hyperactivity disorder (ADHD), or Tourettes Syndrome, and high novelty seeking behaviors (Noble, 2003).

Reward Deficiency Syndrome was first defined in 1996 as a putative predictor of impulsive and addictive behaviors (Blum et al., 1996). Herein, "Reward Deficiency Syndrome" or "RDS" refers to a group of related addictive behaviors. Dopamine is a major component in the mechanisms related to RDS and brain function. Specifically, RDS involves dopamine resistance, a form of sensory deprivation of the brain's reward or pleasure mechanisms (Bowirrat & Oscar-Berman, 2005). The syndrome can be manifested in relatively mild or severe forms that follow as a consequence of an individual's biochemical inability to derive reward from ordinary, everyday activities. The DRD2 A1 genetic variant is also associated with a spectrum of impulsive, compulsive, and addictive behaviors (Gardner, 2011). RDS or anti-reward pathways unite these disorders and explain how certain genetic anomalies give rise to complex aberrant behavior.

In discussing RDS, specific reference is made to an insensitivity and inefficiency in the brain's reward system. There may be a common neurocircuitry, neuroanatomy, and neurobiology for multiple addictions and for a number of psychiatric disorders. Due to specific genetic antecedents and environmental influences, a deficiency of for example, the D2 receptors may predispose individuals to a high risk for multiple addictive, impulsive, and compulsive behaviors. It is well known that alcohol and other drugs of abuse, as well as most positive reinforcements (e.g., sex, food, gambling, aggressive thrills, etc.), cause activation and neuronal release of brain dopamine, which can decrease negative feelings and satisfy abnormal cravings for substances such as alcohol, cocaine, heroin, and nicotine, which, among others, are linked to low dopamine function (Blum et al., 2008).

In individuals possessing an abnormality in the DRD2 gene, the brain lacks enough dopamine receptor sites to achieve adequate dopamine sensitivity and function from the "normal" dopamine produced in the Reward Center of the brain. Carriers of the A1 DRD2 gene variant may have unhealthy appetites, abuse cocaine, indulge in overeating (which can lead to obesity) or, on the other extreme, be anorexic and/or suffer greater consequences of chronic stress (Bau et al., 2000). In these individuals, their addictive brains lead to generalized craving behavior. In essence, they seek substances including alcohol, opiates, cocaine, nicotine, and/or glucose (all substances known to cause preferential release of dopamine at the Nucleus accumbens (NAc)) to activate dopaminergic pathways in order to offset their low D2 receptors, which are caused by the dopamine D2 receptor gene Taq1 A1 allele antecedents (Sullivan et al., 2011). However, evidence is emerging that anterior cingulate cortex rather than the NAc may be involved in operant effort-based decision

making (Walton et al., 2009). In addition, the DRD2 A1 polymorphism is also associated with abnormally aggressive behavior, which also stimulates the brain's use of dopamine (Chen et al., 2005).

2. Candidate genes and predisposition to reward dependence behaviors

RDS is linked to flawed dopamine metabolism, and especially to low D2 receptor density. Moreover, RDS results from a dysfunction in the mesolimbic system of the brain, which directly links abnormal craving behavior with a defect in the Dopamine D2 Receptor Gene (DRD2) as well as other dopaminergic genes (D1, D3, D4, and D5, DATA1, MAO, COMT), including many genes associated with the brain reward function (Pinto & Ansseau, 2009), as listed in Table 1, below.

REWARD -DEPENDENCE- PATH WAY	CANDIDATE GENES
Signal Transduction	**ADCY7**
Signal Transduction	**AVPR1A**
Signal Transduction	**AVPR1B**
Signal Transduction	**CDK5R1**
Signal Transduction	**CREB1**
Signal Transduction	**CSNKLE**
Signal Transduction	**FEV**
Signal Transduction	**FDS**
Signal Transduction	**FOSL1**
Signal Transduction	**FOSL2**
Signal Transduction	**GSK3B**
Signal Transduction	**JUN**
Signal Transduction	**MAPK1**
Signal Transduction	**MAPK3**
Signal Transduction	**MAPK14**
Signal Transduction	**MPD2**
Signal Transduction	**MGFB**
Signal Transduction	**NTRK2**
Signal Transduction	**NTSR1**
Signal Transduction	**NTSR2**
Signal Transduction	**PPP1R1B**
Signal Transduction	**PRKCE**
_____	_____
Serotonin	**HTRIA**
Serotonin	**HTRIB**
Serotonin	**HTR2A**
Serotonin	**HTR2C**
Serotonin	**HTR3A**
Serotonin	**HTR3B**
Serotonin	**MAOA**
Serotonin	**MAOB**

REWARD -DEPENDENCE- PATHWAY	CANDIDATE GENES
Serotonin	SLC64A
Serotonin	TPH1
Serotonin	TPH2
Opioid	OPRMI
Opioid	OPRKI
Opioid	PDYN
Opioid	PMOC
Opioid	PRD1
Opioid	OPRL1
Opioid	PENK
Opioid	PNOC
GABA	GABRA2
GABA	GABRA3
GABA	GABRA4
GABA	GABRA6
GABA	GABRB1
GABA	GABRB2
GABA	GABRB3
GABA	GABRD
GABA	GABRE
GABA	GABRG2
GABA	GABRG3
GABA	GABRQ
GABA	SLC6A7
GABA	SL6A11
GABA	SLC32A1
GABA	GAD1
GABA	GAD2
GABA	DB1
Dopamine	COMT
Dopamine	DDC
Dopamine	DRD1
Dopamine	DRD2
Dopamine	DRD3
Dopamine	DRD4
Dopamine	DRD5
Dopamine	SLC18A2
Dopamine	SLC6A3
Dopamine	TH
Cannabinoid	CNR1

REWARD -DEPENDENCE- PATHWAY	CANDIDATE GENES
Cannabinoid	**FAAH**
Cholinergic	**CHRMI**
Cholinergic	**CHRM2**
Cholinergic	**CHRM3**
Cholinergic	**CHRM5**
Cholinergic	**CHRNA4**
Cholinergic	**CHRNB2**
Adrenergic	**ADRA1A**
Adrenergic	**ADRA2B**
Adrenergic	**ADRB2**
Adrenergic	**SLC6A2**
Adrenergic	**DRA2A**
Adrenergic	**DRA2C**
Adrenergic	**ARRB2**
Adrenergic	**DBH**
Glycine	**GLRA1**
Glycine	**GLRA2**
Glycine	**GLRB**
Glycine	**GPHN**
NDMA	**GR1K1**
NDMA	**GRINI**
NDMA	**GRIN2A**
NDMA	**GRIN2B**
NDMA	**GRIN2C**
NDMA	**GRM1**
Stress	**CRH**
Stress	**CRHEP**
Stress	**CRHR1**
Stress	**CRHR2**
Stress	**GAL**
Stress	**NPY**
Stress	**NPY1R**
Stress	**NPY2R**
Stress	**NPY5R**
Drug Metabolizing	ALDH1
Drug Metabolizing	ALDH2
Drug Metabolizing	CAT

REWARD -DEPENDENCE- PATH WAY	CANDIDATE GENES
Drug Metabolizing	CYPZE1
Drug Metabolizing	ADH1A
Drug Metabolizing	ADH1B
Drug Metabolizing	ADH1C
Drug Metabolizing	ADH4
Drug Metabolizing	ADH5
Drug Metabolizing	ADH6
Drug Metabolizing	ADH6
Drug Metabolizing	ADH7
Others	BDNF
Others	CART
Others	CCK
Others	CCKAR
Others	CLOCK
Others	HCRT
Others	LEP
Others	NR3C1
Others	SLC29A1
Others	TAC

Table 1. Genes involved in various Reward Dependence Pathways.

The genesis of all behavior, be it "normal" (socially acceptable) or "abnormal" (socially unacceptable), derives from an individual's genetic makeup at birth. This genetic predisposition, due to multiple gene combinations and polymorphisms, is expressed differently based on numerous environmental factors including family, friends, educational and socioeconomic status, environmental contaminant exposure, and the availability of psychoactive drugs, including food. The core of predisposition to these behaviors is a set of genes interacting with the environment , which promote a feeling of wellbeing via neurotransmitter interaction at the "reward center" of the brain (located in the meso-limbic system), leading to normal dopamine release (Kendler et al 2011).

Subjects afflicted with RDS carry polymorphic genes in dopaminergic pathways that result in hypo-dopominergic function caused by a reduced number of dopamine D2 receptors, reduced synthesis of dopamine (by dopamine beta –hydroxylase), reduced net release of pre-synaptic dopamine (from, e.g., the dopamine D1 receptor), increased synaptic clearance due to a high number of dopamine transporter sites (dopamine transporter), and low D2 receptor densities (dopamine D2 receptor), making such people more vulnerable to addictive behaviors (Comings and Blum, 2000). The RDS concept involves shared genes and their mRNA expressions and behavioral tendencies, including dependence on alcohol, psycho-stimulants, marijuana, nicotine (smoking), and opiates, altered opiate receptor function, carbohydrate issues (e.g., sugar-binging), obesity, pathological gambling, sex addiction, premeditated aggression, stress, pathological aggression, and certain personality disorders, including novelty-seeking and sex addiction. The common theme across all of these substances and behaviors is that they induce pre-synaptic dopamine release (Dreyer,

2010). Spectrum disorders such as ADHD, Tourettes Syndrome, and Autism are also included due to dopamine dysregulation. As well as other rare mutations (Sundaram et al., 2010) have been associated with Tourettes and Autism. One example includes the association with Neuroligin 4 (NLGN4) is a member of a cell adhesion protein family that appears to play a role in the maturation and function of neuronal synapses (Lawson-Yuen et al., 2008).

3. Neurogenetics of reward deficiency syndrome (RDS)

Since the discovery of the double helix, explorations of brain function in terms of both physiology and behavioral traits have resulted in a plethora of studies linking these activities to neurotransmitter functions having a genetic basis. The mechanisms underlining gene expression and the potential impairments due to polygenic inheritance -- and as such, predisposition to addiction and self-destructive behaviors -- have been studied. Our studies have shown that the prevalence of the DRD2 A1 allele in Cocaine dependent (CD) subjects (n = 53) was 50.9%. It was significantly higher than either the 16.0% prevalence ($P < 10(-4)$) in non-substance abusing controls (n = 100) or the 30.9% prevalence ($P < 10(-2)$) in population controls (n = 265) wherein substance abusers were not excluded. Logistic regression analysis of CD subjects identified potent routes of cocaine use and the interaction of early deviant behaviors and parental alcoholism as significant risk factors associated with the DRD2 A1 allele. The cumulative number of these risk factors in CD subjects was positively and significantly ($P < 10^{-3}$) related to DRD2 A1 allelic prevalence. The data showing a strong association of the minor alleles (A1 and B1) of the DRD2 with CD indicates that a gene, located on the q22-q23 region of chromosome 11, confers susceptibility to this drug disorder (Noble et al.,1993)

Over half a century of dedicated and rigorous scientific research on the mesolimbic system, has provided insight into the addictive brain and the neurogenetic mechanisms involved in man's quest for happiness (Blum et al., 2009). In brief, the site of the brain where one experiences feelings of wellbeing is the mesolimbic system. This part of the brain has been termed the "reward center". Chemical messages including serotonin, enkephalins, GABA, and dopamine (DA) work in concert to provide a net release of DA at the nucleus accumbens (NAc), a region in the mesolimbic system. It is well known that genes control the synthesis, vesicular storage, metabolism, receptor formation, and neurotransmitter catabolism. The polymorphic versions of these genes have certain variations that could lead to an impairment of the neurochemical events involved in the neuronal release of DA. The cascade of these neuronal events has been termed "Brain Reward Cascade". See Figure 1. A breakdown of this cascade ultimately leads to a dysregulation and dysfunction of DA. Since DA has been established as the "pleasure molecule" and the "anti-stress molecule," any reduction in its function could lead to reward deficiency and resultant aberrant substance seeking behavior and a lack of wellness (Blum & Kozlowski, 1989).

It is well –known that humans are biologically predisposed to drink, eat, reproduce, and desire pleasurable experiences (Tindell et al. , 2006, Peciña et al., 2006). Impairment in the mechanisms involved in these natural processes lead to multiple impulsive, compulsive, and addictive behaviors governed by genetic polymorphisms. While there are a plethora of genetic variations at the level of mesolimbic activity, polymorphisms of the serotonergic-2A receptor (5-HTT2a), serotonergic transporter (5HTTLPR**),** dopamine D2 receptor (DRD2), dopamine D4 receptor (DRD4), dopamine transporter (DAT1), and Catechol-o-methyl–

transferase (COMT), and monoamine–oxidase (MOA) genes, as well as other genes, predispose individuals to excessive cravings and resultant aberrant behaviors.

As stated earlier, an umbrella term to describe the common genetic antecedents of multiple impulsive, compulsive, and addictive behaviors is RDS. Individuals possessing a paucity of serotonergic and/or dopaminergic receptors and an increased rate of synaptic DA catabolism, due to high catabolic genotype of the COMT gene, or high MOA activity are predisposed to self-medicating with any substance or behavior that will activate DA release including alcohol, opiates, psychostimulants, nicotine, glucose, gambling, sex, and even excessive internet gaming, among others. Use of most drugs of abuse, including alcohol, is associated with release of dopamine in the mesocorticolimbic system or "reward pathway" of the brain (Di Chiara, 1995). Activation of this dopaminergic system induces feelings of reward and pleasure. However, reduced activity of the dopamine system (hypodopaminergic functioning) can trigger drug-seeking behavior. Variant alleles can induce hypodopaminergic functioning through reduced dopamine receptor density, blunted response to dopamine, or enhanced dopamine catabolism in the reward pathway. Cessation of chronic drug use induces a hypodopaminergic state that prompts drug-seeking behavior in an attempt to address the withdrawal–induced state (Berridge, 2009).

Acute utilization of these substances can induce a feeling of wellbeing. But, unfortunately sustained and prolonged abuse leads to a toxic pseudo feeling of well being resulting in tolerance and disease or discomfort. Thus, low DA receptors due to carrying the DRD2 A1 allelic genotype results in excessive cravings and consequential behavior, whereas normal or high DA receptors results in low craving induced behavior. In terms of preventing substance abuse, or excessive glucose craving, one goal is to induce a proliferation of DA D2 receptors in genetically prone individuals. Experiments in vitro have shown that constant stimulation of the DA receptor system via a known D2 agonist in low doses results in significant proliferation of D2 receptors in spite of genetic antecedents. In essence (Boundry et al., 1996) D2 receptor stimulation signals negative feedback mechanisms in the mesolimbic system to induce mRNA expression causing proliferation of D2 receptors. This molecular finding serves as the basis to naturally induce DA release to also cause the same induction of D2-directed mRNA and thus proliferation of D2 receptors in the human. This proliferation of D2 receptors in turn, will induce the attenuation of craving behavior. In fact this has been proven with work showing DNA–directed over-expression (a form of gene therapy) of the DRD2 receptors and significant reduction in both alcohol and cocaine craving-induced behavior in animals (Filtz et al.,1994). These observations are the basis for the development of a functional hypothesis of drug–seeking and drug use. The hypothesis is that the presence of a hypodopaminergic state, regardless of the source, is a primary cause of drug–seeking behavior. Thus, genetic polymorphisms that induce hypodopaminergic functioning may be the causal mechanism of a genetic predisposition to chronic drug use and relapse. Finally, utilizing the long term dopaminergic activation approach will ultimately lead to a common safe and effective modality to treat RDS behaviors including Substance Use Disorders (SUD), Attention Deficit Hyperactivity Disorder (ADHD), and obesity among other reward deficient aberrant behaviors (Rothman & Glowa, 1995; Peng et al., 2010).

Support for the impulsive nature of individuals possessing dopaminergic gene variants is derived from a number of important studies illustrating the genetic risk for drug-seeking behaviors based on association and linkage studies implicating these alleles as risk antecedents having impact in the mesocorticolimbic system (see Figure 1).

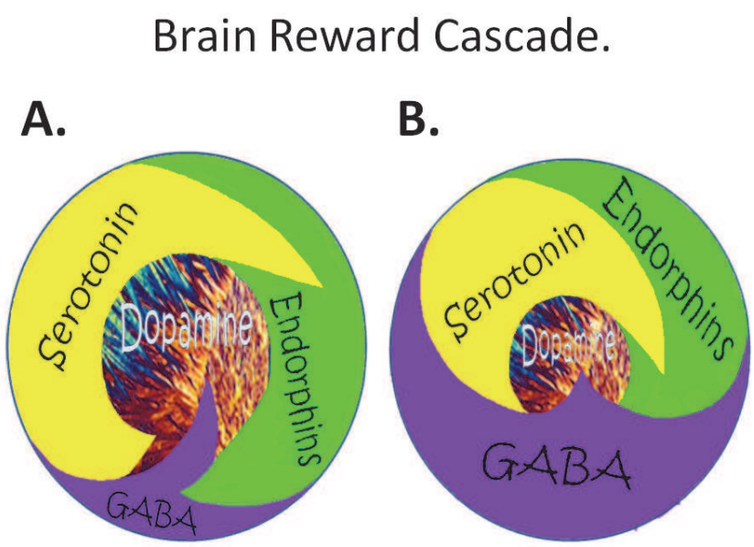

Fig. 1. Schematic of Brain reward Cascade Normal and abnormal representation. (A) represents the normal physiologic state of the neurotransmitter interaction at the mesolimbic region of the brain. Briefly, serotonin in the hypothalamus stimulates neuronal projections of methionine enkephalin in the hypothalamus that, in turn, inhibits the release of GABA in the substania nigra, thereby allowing for the normal amount of Dopamine to be released at the Nucleus Accumbens (NAc; reward site of the brain). (B) Represents hypodopaminergic function of the mesolimbic region of the brain. The hypodopaminergic state is due to gene polymorphisms as well as environmental elements, including both stress and neurotoxicity from aberrant abuse of psychoactive drugs (*i.e. alcohol, heroin, cocaine etc).* Genetic variables include serotonergic genes (serotonergic receptors [5HT2a]; serotonin transporter 5HTlPR); endorphinergic genes (the mu OPRM1 gene; proenkephalin (PENK); PENK polymorphic 3' UTR dinucleotide (CA) repeats}; GABergic genes (GABRB3); and dopaminergic genes (including ANKKI Taq A; DRD2 C957T, DRD4 7R, COMT Val/met substitution, MAO-A uVNTR, and SLC6A3 9 or 10R). Any of these genetic and or environmental impairments could result in reduced release of dopamine and or reduced number of dopaminergic receptors. Taken from Blum et al IIOAB 1(2) 2010.

In doing association studies that require a representative control sample for a single RDS psychiatric diagnosis or for potential subsets of RDS, one limitation relates to controls poorly screened for multiple RDS behaviors and other related psychiatric disorders (Neiswanger et al.,1995). Missing behaviors that are part of the RDS subset may be the reason for spurious results when genotyping for single subsets of RDS behaviors. For example, in our unpublished study, an individual may not drink alcohol or use drugs but may have other RDS behaviors such as overeating or intensive video-gaming. In support of this, a very strong association of the dopamine D2 receptor A1 allele (100%) was found in one family (Family A) studied over five generations. In addition, every individual in another family, Family B, also had at least one dopaminergic high risk allele (100%) (48% carried the DRD2 A1 allele). Moreover, in Family B only three adult individuals exhibited no addictive behavior. When compared to results in which 55 RDS subjects carried the

DRD2 A1 allele at a frequency of 78.2% and the results of a study in which 597 severe alcoholics carried the A1 allele at a frequency of 49.3%, there was a significant difference between these two groups (X^2=16.9, $p<0.001$). This demonstrated that the A1 allele's prevalence increases with multiple RDS behaviors. The results from these experiments show that multifaceted non-specific RDS behaviors should be considered as the true "reward" phenotype (endophenotype) instead of a single subset RDS behavior such as alcoholism.

4. The Genetic Addiction Risk Score (GARS) test

Very few behaviors depend upon a single gene. Complexes of genes (polygenic) drive most of heredity-based human actions, and RDS is no exception (Greven et al., 2011). For example, DRD2 and DAT1 gene polymorphisms are significantly associated with reward–dependent traits such as cocaine dependence (Brousse et al., 2010). As a polygenic disorder involving multiple genes and many polymorphisms, RDS likely requires a threshold number of RDS-associated polymorphisms in order to manifest in a particular subject. Unaffected individuals in the population carry some of these alleles. For example, the dopamine D2 receptor gene A1 allele is present in about one-third of unscreened Americans (Noble, 2003).

The GARS test has direct implications for both the diagnosis and targeted treatment of RDS behaviors by analyzing the association of dopaminergic genetic polymorphisms. Without being bound to a particular theory, in keeping with the concept of common neurogenetic mechanisms we believe that RDS is a basic phenotype covering many reward behaviors and psychiatric disorders, including spectrum disorders (Blum & Gold, 2011). The test provides for the first time a polygenic diagnosis of RDS predisposition. Such tests can be used, for example, to determine stratified genetic risk of patients with addictive behaviors upon their entry into a treatment facility, as information about an individual's genetic predisposition to RDS based on the analysis of a number of RDS-associated alleles will be very useful.

4.1 RDS-associated SNPs

RDS-associated SNPs can be identified by any suitable method, including DNA sequencing of patients diagnosed with one or more RDS behaviors. After validation, newly identified RDS-associated SNPs can be used in the test. As will be appreciated, once identified and validated, the presence, if any, of one or more RDS-associated SNPs in the nucleic acids derived from a biological sample taken from a patient can be determined using any suitable now known or later-developed assay, including those that rely on site-specific hybridization, restriction enzyme analysis, or DNA sequencing. Table 2 below, lists a number of particularly preferred RDS- associated SNPs, whereby, the detection of which can be used for the GARS test.

GENE	SNP
DRD2	CTGGACGTCCAGCTGGGCGCCTGCCT[C/T]GACCAGCACTTTGAGG ATGGCTGTG
	AGGAACTCCTTGGCCTAGCCCACCCT[G/T]CTGCCTTCTGACGGCCC TGCAATGT
	CTGCTTCCCACCTCCCTGCCCAGGCC[A/G]GCCAGCCTCACCCTTGC GAACCGTG
	GTCACTATTATGGTTTTTATTACTAT[G/T]GCTCTTTTTGAGGAATTG GGAAATT

GENE	SNP
	CTGACTCTCCCCGACCCGTCCCACCA[C/T]GGTCTCCACAGCACTCCCCGACAGCC
	CCACCAGCTGACTCTCCCCGACCCGT[C/G]CCACCACGGTCTCCACAGCACTCCC
	ACCCATCTCACTGGCCCCTCCCTTTC[A/C]CCCTCTGAAGACTCCTGCAAACACC
	TTTTGCTGAGTGACCTTAGGCAAGTT[G/T]CTTACCTTCTATGAGCCTGTTTCCT
	TGTGATGAATGGGTGCCAAATACACA[A/G]ATACAGAATCTAAGAAAACACATGG
	CTAGAGGAAGTGATGTTCAACAGACA[A/G]ACAACTGAAGGATGTGTAGGAATTA
	TGGAAGTCATGTGCTTTGTATGAAAC[A/G]CCTTGGAATGCTGATAAGTTTAATT
	GTCTAAAGCAAATGGAACCTTTAGGG[A/G]GAGAGATTTGTGTTTGCTGTGTCCC
	GAGGGGACTGGGGTCAGGCCTCATTC[A/G]GGTTCCCTAGAGTGGAAAGGATTGG
	GTATCAGACAGATCTAGGCTCAAATA[A/C]CAGCTTCAGTTCTCACCACCTGTGT
	CCTGAGTGCACAGGATGCTGGAGCCT[C/T]CCAGTTTCTCTGGCTTTCATCTCGT
	AATCCCCCAACCCCTCCTACCCGTT[-/C]CAGGCCGGGGATCGCCGAGGAGGTA
	GAGGACCCAGCCTGCAATCACAGCTT[A/G]TTACTCTGGGTGTGGGTGGGAGCGC
5HT2A	GCTCAATGGTTGCTCTAGGAAAGCAG[C/T]ATTCTGAAGAGGCTTCTAAAGACAA
	CTGTGAACTCAGGAGCAAGTGCACAC[A/G]TTGCTTATCACTTACCAGAAGCATT
	CTCTGGTTTTAAGCAAGTCATTTAAT[-/C/T]GGAGTTTTTTTTCTCCCATAAAATG
	AAATGGTCCTACCATCTATCCAGATA[C/T]ACAGCTTAAAAACTTAGGAGTCTCT
	TGCTATTTGTAATGCTGCTTATTAGA[G/T]ACATCGCTGATCCTCCTGTCAACTC
	ATGAACCAAATTGCATGAGCTCTATT[A/G]TGTGCCCCTCTTGTAATATAAAAAT
	CAGGCAGAATTTCCACAAATGAAATG[C/G]AAATTCAGATATATATCTCTTAATC
	TCACTCATAACTGAAGATCATTTCAC[C/T]TTTGAATGAGAATTTGTCTCTGAAG
	TGGGCAGAGGAGGGGAAGGGTCACTG[C/T]ACTCAGGGACAAGAGAAGGGGTGGG
	ATCAGTGTGGTCACTTCACTGCTTGC[C/G]AAGGATTCCATCTAATTCTGAGGAA
	AGGCTCTACAGTAATGACTTTAACTC[C/T]GGAGAAGCTAACACTTCTGATGCAT
	TATGTCCTCGGAGTGCTGTGAGTGTC[C/T]GGCACTTCCATCCAAAGCCAACAGT
ANKK1	GGAGGGGGGTCTTGCCCTCAGCCTCA[C/T]GCAGGTTGGGGTCAGCCTGACGGGA

GENE	SNP
	GGAGGGGGGTCTTGCCCTCAGCCTCA[C/T]GCAGGTTGGGGTCAGC CTGACGGGA
	CCTGCAAGCTGTCGCTGCGCCAGCCC[A/G]GGGAGGTGAGTGTGTG GGCTGGGCA
	ACTTTGCAGCCCAGAATGGGGATGAC[C/G]GCACTGCGCGCCTGCT CCTGGACCA
OPRK1	AATTTCCCAAAAACTACAGTTTTTTT[- /T]TCTTAGCATGCTATTCAGGTAAACA
	TTTGCTAGGTAAGGTTCAGCACCCAT[C/T]TGCTGTGGCCTTCCTAT GAAACGTA
	ATGACTAGTCGTGGAGATGTCTTCGT[A/C]CAGTTCTTCGGGAAGA GAGGAGTTC
	GAAAACACAAGTGTGATCAAATGCCA[C/T]GGACCCACAGGAAGC TGGTGGCTCT
	AAGAAATTGTCTGCATATAAACAAAT[A/G]CATCACATTTCCACAA AAGACTTTG
	TGAGAGCTAATGTTTCAAAGAAACTT[G/T]AAATTCCCAAGATTAA AATTATTGT
	CCCAGTAAGTGAATTAAATACTTTCA[C/T]AGACACTCTCCATCTAG TAGAACAA
	TGATAGGCACTGGTTCTACAGTGAGA[C/T]ATATCTCTCCTAAGTCT GGTGACAA
	ACAGTGGCACGATCTCGGCTCACTGC[A/C]ACCTCCACCTCCCGGG TTTAAGTGA
	TTACCTGGCTAACAGTTTTCTATCTC[C/T]CACACGAGCCTGGTGGG AGGCAGTG
	AGCTCTTGTTATCTTACCATTCCCAC[A/G]TTGATTCTCATTTTTATC CCTCTCC
	TCAAGATAGCTAATTGAGAACAAGCA[C/T]GAGACTCCACTCCTGG TCCCCAAGC
	CCATTTTCTTTTCTTCTTTGCTTGTC[G/T]TTTTTTTCTGTTTGTTTTTCT TTTC
	AGAAAATAACTTTTGCTAGATTCACC[A/G]TTGGTTATAGACCTGC ATGATCTAA
	GTGATGTTACCAGCCTGAGGGAAGGA[A/G]GGTTCACAGCCTGATA TGTTGGTGA
	AGTTAGCTCTGGTCAAGGCTAAAAAT[C/G]AATGAGCAAAATGGC AGTATTAACA
	AATTTTATTAGATTAAACAATTTTTA[A/G]CAGACCTCATGCTTGTT GGAGATAA
	GGTCCAGGGTACACAACCAAGCAGCC[A/T]TGCTCTAGAGCCCAG CAAGACAGGG
	ACTGAAGAATAATCATGCTTAACTCA[A/G]GAGAAATGCTCCACCA GACGGGCTG
	GCACATTTACTGTTTTGTCTAACCTG[C/T]CTAGCCATTTCAGTCAA GCTGATTG
	GCAATCAGAAAGAAATTCAGTTATTA[C/T]AGTATATGCAAGTCAC ACTGCAAGC
	AATGAAACACAAATCATAATCTCTGA[A/G]GCAAATAAGAATGGA AGGACTCCTG
	TTTAGGGCAAGTCAGAAAGTCCAAAA[A/G]TGCCTCAGATATTCTG TGTGAGTGA
	AAAAACTGGGCCTGAGCTCAGATGAA[C/T]TGGAGAACTGAACTTT GGCTTAGAA

GENE	SNP
	GAGTCATCAGCTCCCAAGGTTTTCTG[C/T]ATGGCTCTGTTTTTATGATTTCTGT
	GTGTGTACTGCAGTCTGGTCCCATCG[C/T]ATTGCCTTGTGGGATTTGGGAGTAG
COMT	GCTCCTACGGTCCCTCAGGCTTGGAG[A/G]GTCACTTTAAACAATAAAAGCAAC
	TGTGGTTACTTTCTGGAGAGAGCATG[C/T]GGCATGCAGGAGCTGGAGGGGGGGT
	AAAAGTTACGCTTAATAATGAATGTT[G/T]CAGCACTTTCTTCTCTTCAGGTATT
	CTGTGAGGCACTGAGGATGCCCTCAC[A/G]CGTGCATCTGCATGTGGCGTGCATG
	CTGGTTTGTGTATGTTCTTGGTAAAC[C/T]AGCCCTTGGTCTTACACATCATTTC
	GCTTCCCTGTTCTCTTCTGCTCTGTC[C/T]TCTGGTGCCCTGAGGCTGGCCTCCA
	GGCATTTCTGAACCTTGCCCCTCTGC[A/G]AACACAAGGGGGCGATGGTGGCACT
	ATAAGTAACTGTCGAGAAGATTCTCA[C/T]AGGAGACCACGTGGGTTGCCTGAAG
SLC6A3	AATGTCCTCAGCTGGTTCTTCCCCCA[A/G]TGCCCTGATCCTGGGCTCACATGTG
	GAGACGAAGACCCCAGGAAGTCATCC[C/T]GCAATGGGAGAGACACGAACAAACC
	AAAATCAAGTAATGATTGATTTGTAG[A/G]AGTTTGAGTGAGGCATCGGATCCCC
	ACCGTGCCCAGCCCTGTGTGGGCATC[A/G]GAGGTGGTTCCCTCTGGTCCTGTCG
	GTCCAGGCCCCAGGAGCTGCCGCAGC[A/G]GGCAGTGGAAGGAAGGCACGTTCAG
	CAGCTTCCCCTCCCAACACAGAGGCG[A/C]GGCCCAAGTGCAGGACTCACAACGG
	AAGACACAGTGACGGTATACTCATGA[C/T]GGAATATGATTCGGCCTTAAAACAA
	AAGGCGAAGCCGGCGATGGTACGTAC[A/G]TTGGTGACGCAGAACAGGGACAGGA
	GCCATCGCCACGCTCCCTCTGTCCTC[A/G]GCCTGGGCCGTGGTCTTCTTCATCA
	TGCTTCCTGCTACCAGCAGGCAGACT[C/T]GGATGGAGGTGGAGGGGACGAGAGT
	CACGGTAAAAATACAAGGACAGTGTG[A/T]GCAGCAGAATGGCCAGGCAGACCAC
	AGGGTTATTAGGATGCTGTGGTCATG[C/T]CGTGTGTGGATGAGTCCATGCTGTT
	GCCAGGCAGGGGCTGGTGGAGGTGCA[C/G]GGCCTGGAGGAACACAGAGCCCAGC
	AGGAGAGGACGTTTGCGCGATTCTCC[C/G]CAGATCCAGTGTTTCCCGTCAGCCA
	GGCTCGTGGCCCTGCGGGCGGATCTT[G/T]GGAAGAGCTTGTTCACACTCACCTA
	TCGAGGCAGGGCCACCGGGGACGTCC[A/G]AGAACATTGGTGATCCCTTCCCAGG
	AATGCAGGCGTGGGACAAGGCAGCTC[C/T]GAGTCCTGCTCAATGGTTTTGTGAC

GENE	SNP
	GAGCTCATCCTTGTCAAGGAGCAGAA[C/T]GGAGTGCAGCTCACCA GCTCCACCC
	GTGGGGAGGGGTGCAGGGGAAGGAGG[A/G]GCAAACCAGAGTGT CTGTCTTGAGG
	AAACACGCTGCTGCTGGATCCAAATG[A/C]CAGAAGTCGCCCTGGC TGGGGCGGT
	CTGCGCGCTGGTGCTCTGGGCAGGGC[G/T]GGGAGGCCGGGCGAG GACTCGCCAG
	GGAGCCAGGACGCGAGGGCGACCCCG[A/T]CGGCGGGAGGGCGG GGCGGGGCGGA
HTR3B	CCTTTACAGCCTTTACCTAAGGCAGT[A/G]CTCTTGCTGACATTCAG GACACTAA
	TTTGGCCTTCTCTCTTGGGCCAAGGA[A/G]TTTCTGCTCTATTGCATG TTCTCAT
	GAGAGCTCCTTGGAGATGGAATAGGC[C/T]CCAAGGTTAGCCTGTA ATTGCCTCC
	CCTTAGCACCTGTGTGTCTATCATTC[C/T]GGGCAGGAAAACTTGCA CAATTAAA
PPARG	AAACTCTGGGAGATTCTCCTATTGAC[C/G]CAGAAAGCGATTCCTT CACTGATAC
	AGGATTTTCTTACATTTAAAGCAGAA[C/T]GACACTACTGATACAC AAAAGTAAA
	GAGAAATCTTCGGAGGGCTCACCAGC[A/G]TCACAAGTAGGTAGA CCAGAAGAGG
	GTTTACAGACCTTGTCAGAGTTGGTA[C/G]TAATTCCAGAATATAAT CATTTCAA
	TGGTTGACACAGAGATGCCATTCTGG[C/G]CCACCAACTTTGGGAT CAGCTCCGT
	GATTTATTTAAATCATCTCTAATTCT[C/T]ACAACTCCGAAAAGATA AGAAAACA
	AGGATTTTCTTACATTTAAAGCAGAA[C/T]GACACTACTGATACAC AAAAGTAAA
	GTTGGGGATCCAGTTGGCCTCATTCT[A/G]AGCTGGCTGTGGATTCA CAGAAGAA
	AAGATACGGGGGAGGAAATTCACTGG[A/G]TTTTACAATATATTTT TCAAGGCAA
	GAGAAATCTTCGGAGGGCTCACCAGC[A/G]TCACAAGTAGGTAGA CCAGAAGAGG
ChREBP	GACAAAAAGCAATTGAGGTCCAGGAG[C/G]TGCCGCCCACCCGGC TCCTCCTCTG
FTO	GCTGTCAGCACCTGGTACAAATACCA[A/G]GATAGGGTTTTTGGGG CCACATTTT
	GCATGCCAGTTGCCCACTGTGGCAAT[A/C]AATATCTGAGCCTGTG GTTTTTGCC
	AGGTTCCTTGCGACTGCTGTGAATTT[A/T]GTGATGCACTTGGATAG TCTCTGTT
	GATGACAACATGCAAACTTTATGGCC[A/G]GAAACCAAAGAGTCA GGCAAAATAT
	AAAAGAAAGTAAACATATTTAAGGTC[A/G]TAAATAAGGCCATGT CTAATAGTGA
	AGGAATGTTCTGATGGCTTGGCCCAG[G/T]TGGTGACTGTGCAGAT AGACTGAAG
	TCAGCACCCAAGGGACCATCAAAGAG[A/G]CTGTTGTGGAGAGGG AATCCGAAGG

GENE	SNP
	TGGAGTGTTTTTCCTTCACCTTTTCC[A/G]GTCTCTGGGTTGCATCGC CAGACTG
	TGTCTAGCCCTGTGGGTTTACATTAG[A/T]TAGGGTAGGTTATTGCT GCAACGTA
	CTATCCAGGATGGCTCTAAAGGGACT[C/T]CGCTATAGGTTGGGGC TATGATAGA
	GCTTATATTCAAAGCTCCAGGTAAAT[A/G]TAAGATGTTGCTATAAT TACCTAAG
	GGTAGGCAGGTGGATCTGAAATCTCA[C/T]ATAGTACCAAGACACG TGACTAGGA
	TTGATTCTTATACTTTTTTGTTTAGT[C/T]GTTGAAATATGTTGTTTTG GTTGAA
	AATTAGGAAGATTTGAGTAGCTAAAA[A/G]TTCCAAGAGTGGAAT AATAGTTTTA
	TTTGGTGCACTCCCAATTTACTCTAA[A/T]CTTCTACGGGCTTCCTTG GAGAAAC
	GACCTGAAAATAGGTGAGCTGTCAAG[G/T]TGTTGGCAGGGAGAG GCTCCTCTGG
	TGGTTCACTGCATATTCCCAGTAATT[C/G]GAACAATGCCTGACATG AAGTAGAC
	TTAGAATGTCTGAATTATTATTCTAG[A/G]TTCCTTGCGACTGCTGTG AATTTTG
	TTGATTTCGGTAGTCATAACACCACC[C/T]TGGAAGGCACCCTAGA TAGAGGTCA
	TTCATTCTACCTGTCTTTAGTATCAT[A/G]GGGGTAGTTACCTCAGC GGGGGTAG
	TTGCTCAAGGTCACACAGTAACCTTA[A/G]GTAGGCAGGATAAGCT CTGGTTCTG
	TATGATGGTTAGGTTAGGTTGCAAGT[C/T]TTGGAATATATGCAGAG GAATAACT
	TTATAAACCTCTAAAATAGTTACTAA[A/G]TAAGTTATTCTTTTAGG TATTTTTC
	TTTTATTTCCGCAATCACTCCCTAAT[C/G]TTTATTTCTTTTTTGCTTC GCATCA
	TAGCATTTTTCTGGAGCGTAATTTCA[C/T]AATGTGAATCAGAAGTC TTAATAGT
	GAGCACAGGTGGAGAGAAAGGGGAGT[A/G]AGAGAAGCAAAGAA GAAAAGCCTTT
	TAGGGACACAAAAAGGGACATACTAC[A/G]TGAATTACTAATATCT AAGAAAATA
	ATGAATTACTAATATCTAAGAAAATA[C/T]GATACATTTGAGAACT TAGATGAAG
	GAAATGTGGTGTAGACGTGACCCAGG[A/G]GGAAATGAGTTTTGTT GGACAGATT
	CTACATCTCCTACTTAGCCGAGGTCT[C/T]TTCACTCTCTGGGCAAG TCTCCTCA
	ACACGGCTGAAGAGTCAGGAGTGGGA[C/T]GAAAAATACACTTCA TTTGTAGGTG
	GCACATTTATGCCTTTTATATGCCAC[A/G]TACACACGAAAACTccat atattct
	AGAGTGAATAAAATTATTTCTAAATT[C/T]ATGCTTCATACCGTGTG TAATTTAG
	TGTTGCAACAGAGATGATGGCAGTTT[C/T]GGCCACGGTGTAAGAA GCAGAGGTG

GENE	SNP
	ACATCTGCCTTCCCAGAGAAAGGAAA[A/G]TCAATGTTTAAAGTCT ATTTAAAAA
TNF Alpha	GGGAAGCAAAGGAGAAGCTGAGAAGA[C/T]GAAGGAAAAGTCAG GGTCTGGAGGG
	GGAGGCAATAGGTTTTGAGGGGCATG[A/G]GGACGGGGTTCAGCC TCCAGGGTCC
	TGGCCCAGAAGACCCCCCTCGGAATC[A/G]GAGCAGGGAGGATGG GGAGTGTGAG
	TCTTTCTGCATCCCCGTCTTTCTCCA[C/T]GTTTTTTTCTCTCCATCCC TCCCTA
	GTTGAATGCCTGGAAGGTGAATACAC[A/G]GATGAATGGAGAGAG AAAACCAGAC
MANEA	CATTTTACAATAGATAAATGCTTGTG[C/T]TACCTAAAGCACTTAGC ACACAGTT
Leptin OB	GCTCTGGGAATGTCTATCCTATGCAA[C/T]GGAGATAAGGACTGAG ATACGCCCT
	ATGCAATGGAGATAAGGACTGAGATA[C/T]GCCCTGGTCTCCTGCA GTACCCTCA
	GGAGCCCCGTAGGAATCGCAGCGCCA[A/G]CGGTTGCAAGGTAAG GCCCCGGCGC
	AAGTTCCTGACCTCTGAATGAGAGGG[A/G]CTGTGTAAGGCCAATG CCTGGGAGG
	AATAAAAATAAATGTTCTTCCTTGCA[A/T]TGAAGTTAAATATGTAA ATTCTCAA
	ACTTAGGTATTAGAGGGTGGCCATTA[C/T]TTGAGAGTGACTATGA CCACAGTTA
	TGGGTGAATGTGTTATGCTCTCTCCC[A/G]CCACCATGTCTTTATAC CCCCTGAT
	CTCCCAGTGGGTGGGAGAGAAAGGAC[A/G]TAAGGAAGCAAGTGG TAAAGGCCCT
PEMT	ATCCCTTCACCAGAGTGATTTCCTCG[A/C]GGCAGGTGCCTGGGGT AGCCACTGG
	GGACTGCCTGGTTGTGCTTCGGACCC[A/G]GAGGCAGACAGAGGA GGCCTTTGAA
MAO-A	CCCACTAGGCAAGCCTCCTAAAAGCA[A/G]TATGGTTGTAGATCAC TGGAAAATA
	GTAAACATGCAAACTGAAACATTAGC[A/G]CCCATTTATTCAGCAT CTTAGAAGA
	GAGTGAAGGCCAGGTACAGAGGAAAT[A/G]AAGCATTCCAAATAA TGCCAGGTAA
	CCAAAGTTAACTTGTGAACCCTTCTA[A/G]TAAACTGCTCCAAGAT ATGACAAAA
	GTTTGCCATGGATGAACCACCAGGAT[A/G]GTGGGGGAGACAGAA AAGGTTGATG
	GGAAAATTCCCCTTCCCCTAAGACAT[C/T]CACCCTTCTGGTTTGGG TAATTCCT
	GCAGAGAGAAACCAGTTAATTCAGCG[G/T]CTTCCAATGGGAGCTG TCATTAAGT
	GTGCATGATGTATTACAAGGAGGCC[G/T]TCTGGAAGAAGAAGGGT AGGCTGCT
	AGAGAAGGAAGTGGTGTCCCCACAAA[G/T]GAATTGCTAAGGAGT TCCACAGCCT
	AAGAGAAAACAAAGCTGAAATGCTGC[A/G]AGTCAATAATATCGT TGCTTTAACA

GENE	SNP
	TTTGACAACTATTTCTAGAATTTGCA[C/T]TGAACTCTGCTTTTCCTTTTAAATT
	GGTCTCGGGAAGGTGACCGAGAAAGA[C/T]ATCTGGGTACAAGAACCTGAATCAA
CRH	CTGTCCCACAACATGGGGTCTTACAG[C/T]TCTTTGATGTATCCCCCCACAGGGG
	GCCTCTGGGGTCACCAGGTACATCTT[C/T]GATCTTGGCCACACTGGAGAGTCAA
	TTTCTAAACACAGAGGACTGGTGTTG[C/T]GTTATGCAAAGAAAAATGCTTCTTA
	AAGACACTCAGGTGCAGGGACCCTCT[A/C]CATTTTTGCCCAGCAGCAGCCATGC
	AGGGCCAGGAACCATGAACCAGCGCG[G/T]GTGGGGGCAGCCTCTTCAGGCCTGG
	GGCACACCAGTCCTTTTGAGCCCCAG[C/T]GTCCCCAGGTTAATAACCTAGAATT
	TGAACACGGAGGCCACACAAGAGTGG[A/G]TTCCAAGTGAAGGAGTGACCAACTC
	TCCTTTCCTGGGATCACAGAGGGAAG[C/T]GCGGGGGAGCCTAGAGAGCACCACA
	TACAGGTGAAGGAAAGTGATTCTTTC[C/T]CCGTTAACTTTGTTTCACGCCAGAT
	CCCCCAACCAGAGATGATGATGGGGG[A/G]CAGGGGAGGCACCAAACCCTGGGCC
	AGCAGCATACCCCTAGGGACCTAGGA[A/G]CAGGGAGGGAGAGAGGCAGCCCTGG
	CAGCTGGCACTGACAGCCTGGGGGGG[-/C/G]CGCTCTCCCCCTGCAGCCGTGCAGG
	GAGCACAAGAAGGCCAGCCCACTGGG[C/G]CCTGGGGCTGCCCTCGGCAACCGTG
	CTGCTTCCCACCAATCAGCACAGCTC[A/C]TGCCTGGGGCTGGGACACACTCCCG
ADIPOQ	ATCAGAATGTGTGGCTTGCAAGAACC[A/G]GCTCAGATCCTGCCCTTCAAAAACA
	GTTCTACTGCTATTAGCTCTGCCCGG[G/T]CATGACCAGGAAACCACGACTCAAG
STS	GATGACAAGCCAGGCAGGGAGGAATG[A/G]ACCTGGATTCCTGGTGAAGGACGTG
VDR	GTCAGCGATTCTTAATATAAGAAAAA[A/G]TGGTGAAATGTGTTTAGAGTGTGCT
	CCTGGGGTGCAGGACGCCGCGCTGAT[C/T]GAGGCCATCCAGGACCGCCTGTCCA
	GTTCCTGGGGCCACAGACAGGCCTGC[A/G]CATTCCCAATACTCAGGCTCTGCTC
	CATAAGACCTACGACCCCACCTACTC[C/T]GACTTCTGCCAGTTCCGGCCTCCAG
	TGGCCTGCTTGCTGTTCTTACAGGGA[C/T]GGAGGCAATGGCGGCCAGCACTTCC
	TGTGGGGGTGGGCCAGCCCAGCTTAG[A/G]TTATCTTGGCTCATTGTCCACTAGT
DBI	TCTGTCCTCAGGCCAGGGCTTCGCTG[A/C]AGCCCCGGCCACTCCCTAGTGCCTG
	TACGAACTCACTGTAAAACTCACCTT[C/T]GCCATAAGACCTTCTTCAACTAAGT

GENE	SNP
	ACAGAGTTTACGAACTCACTGTAAAA[C/T]TCACCTTCGCCATAAG ACCTTCTTC
	GGAGAGAAAACAAAGTCAATGGGGCA[C/T]GTGTGGGAAACCAGC CTGACCTGTG
	TTACAGGGACTTCCAAGGAAGATGCC[A/G]TGAAAGCTTACATCAA CAAAGTAGA
GABRA6	TTGGGAAAGGAGAGTCTGAAGGGACA[A/G]TGCATGGTCGGAGAG CAGTGACAAT
	AAATTGGAAATCTGTAACGCAGCTTC[C/T]GTAAGCATGTGTGGGC AAAAAAGCA
	TTCTTTCCATCTGGCACCTATTTATT[C/G]ACTATTTATGCATTCGTT GAATTAT
	CTCTTTCACCATTGACAAATATTTAT[G/T]GACGACTTACTTTCTATG TAAGGTC
GABRB3	CGTTCAGTTTAGTAAGCAAAGGCTTC[C/T]TGGCTTCTCTGGTGATG GGGTTTGT
	AGCTTACCATTTAAGTAGAACTGTTT[A/G]AGATGCTGGACATTCTA ATACAATC
	CCAAATCTGAAATTTACTTGTCACTT[C/T]AGAGTTGTCTTTGAACG GAAAGATT
	TCTGTTGAGTGATAATCTTTCTCGCA[A/G]ATAACTCACAATATTTA AAAATTGT
	AAGAACTCTTCCATGATTGAAATGGT[A/C]GCACATGGAATAACAT CGATAAGTT
	ACAGCAGGTTGGAGCACAGGGCCTAA[A/G]TGGGAGGCCAGGGAG GTGGGCAGAG
	ATTGCTGATTTTCAGGCAAACTATGT[A/T]ACATGGCTTTCAATGGG TGCTTGGC
MTHFR	GAAGCAGTTAGTTCTGACACCAACAA[A/G]TGGTGATAAGAGGTTG ATAGCCTAG
	GTGGGGGGAGGAGCTGACCAGTGAAG[A/C]AAGTGTCTTTGAAGT CTTTGTTCTT
	CTTGAAGGAGAAGGTGTCTGCGGGAG[C/T]CGATTTCATCATCACG CAGCTTTTC
	AGATGTTCCACCCCGGGCCTGGACCC[C/T]GAGCGGCATGAGA
MLXIPL	GACAAAAAGCAATTGAGGTCCAGGAG[C/G]TGCCGCCCACCCGGC TCCTCCTCTG
	CAGGTAACTGACCCTTCACACATTTA[C/T]GGTGCCCATCTGACATT CATAGCAT
VEGF	GCGCGCGGGCGTGCGAGCAGCGAAAG[C/G]GACAGGGGCAAAGT GAGTGACCTGC
	AGACATGTCCCATTTGTGGGAACTGT[A/G]ACCCTTCCTGTGTGAGC TGGAGGCA
	AGACATGTCCCATTTGTGGGAACTGT[A/G]ACCCTTCCTGTGTGAGC TGGAGGCA
	ACATCCTGAGGTGTGTTCTCTTGGGC[C/T]TGGCAGGCATGGAGAG CTCTGGTTC
	AGCATTCCCGGGCGGGTGACCCAGCA[C/T]GGTCCCTCTTGGAATT GGATTCGCC
	ATCCTCTTCCTGCTCCCCTTCCTGGG[A/G]TGCAGCCTAAAAGGACC TATGTCCT

Table 2. RDS-associated SNPs.

4.2 Testing parameters and assay requirements: a note on methodology

A common class of experiments, known as a multiplexed assay or multiplexed biochemical experiment, comprises of reacting a sample known or suspected to contain one more target analyte species with a set of "probe" molecules. Multiplexing allows two or more, often many more (e.g., 10, 50, 100, 1,000 or more), target analyte species to be probed simultaneously (i.e., in parallel). Genome Wide scans have now been used to identify gene clusters in alcohol and drug addiction (Agrawal et al., 2010). For example, in a gene expression assay, each species of target analyte, usually a nucleic acid (i.e., DNA or RNA) of known nucleotide sequence but whose presence in a particular sample is suspected but is not known with certainty, can be detected using a short probe nucleic acid (e.g., a synthetically produced oligonucleotide) having a nucleotide sequence at least a portion of which is sufficiently complementary to the target sequence in the particular target analyte to allow stable hybridization under the various assay conditions used (including hybridization and stringent washing conditions) so that probe/target hybrids can later be detected using a desired detection scheme. As those in the art appreciate, such assays can involve those wherein target analyte species are labeled (or not) with a detectable label or wherein the various target analyte-specific probe species are labeled (directly or indirectly) with any suitable label that can be detected by the detector used with the particular assay format (e.g., bead-based formats, gene arrays, etc.). Labels include fluorescent molecules.

For example, in many known DNA/genomic bead-based multiplex assays, each probe species includes a DNA molecule of a predetermined nucleotide sequence and length attached to an encoded or otherwise identifiable bead or particle. When a labeled "target" analyte (in this case, a detectably labeled (e.g., fluorescently labeled) DNA molecule containing a target nucleotide sequence) is mixed with the probes, segments of the labeled target analyte selectively bind to complementary segments of the DNA sequence of one of the bead-bound probe species. The probes are then spatially separated and examined for fluorescence. The beads that fluoresce indicate that molecules of the target analyte have attached or hybridized to complementary probe molecules on that bead. The DNA sequence of the target analyte can then be determined, as the complementary nucleotide sequence of the particular probe species hybridized to the labeled target is known, and identification of the encoded bead indicates which probe species was bound to that bead. In addition, in such assays the level of fluorescence is indicative of how many of the target molecules hybridized (or attached) to the probe molecules for a given bead. As is known, similar bead-based assays may be performed with any set of known and unknown molecules, analytes, or ligands.

In such bead-based assays, the bead-bound probes are allowed to mix with samples that may contain the target analytes without any specific spatial position; as such, such assays are commonly called "random bead assays". In addition, because the bead-bound probes are free to move (usually in a liquid medium), the probe molecules and target analytes have a better opportunity to interact than in other assay techniques, such as in a conventional planar microarray assay format.

There are many bead/substrate types that can be used for tagging or otherwise uniquely identifying individual beads with attached probes. Known methods include using polystyrene latex spheres that are colored or fluorescently labeled. Other methods include using small plastic particles with a conventional bar code applied, or a small container having a solid support material and a radio-frequency (RF) tag. Still other bead-based approaches involve vary small encoded beads, particles, or substrates capable of providing a large number of unique codes (e.g., greater than 1 million codes) are known.

In multiplex assays designed to simultaneously examine 2-25 or so different target analyte species, other assay formats can be adapted for use in the GARS test. Indeed, any format suited for analysis of multiple genes, either simultaneously in one or more parallel reactions or in different reactions carried out in series or at different times, can readily be adapted for use.

5. Brief description of risk alleles in a number of reward genes

5.1 Dopamine D2 receptor gene (DRD2)

The dopamine D2 receptor gene (DRD2) first associated with severe alcoholism is the most widely studied gene in psychiatric genetics (Blum et a l., 1990). The *Taq*1 A is a single nucleotide polymorphism (SNP rs: 1800497) originally thought to be located in the 3'-untranslated region of the DRD2 but has since been shown to be located within exon 8 of an adjacent gene, the ankyrin repeat and kinase domain containing 1 (ANKK1). Importantly, while there may be distinct differences in function, the mis-location of the *Taq*1 A allele may be attributable to the ANKKI and the DRD2 being on the same haplotype or the ANKKI being involved in reward processing through a signal transduction pathway (Neville et al.,2004). The ANKKI and the DRD2 gene polymorphisms may have distinct, different actions with regard to brain function (Huang et al, 2009). Presence of the $A1^+$ genotype (A1/A1, A1/A2) compared to the A^- genotype (A2/A2) is associated with reduced receptor density. (Noble et al, 1991; Montag et al., 2010; Jönsson et al., 1999). This reduction causes hypodopaminergic functioning in the dopamine reward pathway. Other DRD2 polymorphisms such as the C (57T, A SNP (rs: 6277) at exon 7 also associates with a number of RDS behaviors including drug use (Duan et al., 2003). Compared to the T^- genotype (C/C), the T^+ genotype (T/T, T/C) is associated with reduced translation of DRD2 mRNA and diminished DRD2 mRNA, leading to reduced DRD2 density and a predisposition to alcohol dependence (Hirvonen et al.,2004) The *Taq*1 A allele is a predictive risk allele in families (Hill et al., 2008).

More recently, the DRD2 haplotypes I-C-G-A2 and I-C-A-A1 have been found to occur with a higher frequency in alcoholics [P=0.026, odds ratio (OR): 1.340; P=0.010, OR: 1.521, respectively]. The rare haplotype I-C-A-A2 occurred less often in alcoholics (P=0.010, OR: 0.507), and was also less often transmitted from parents to their affected children (1 vs. 7). Among the subgroups, I-C-G-A2 and I-C-A-A1 had a higher frequency in Cloninger 1 alcoholics (P=0.083 and 0.001, OR: 1.917, respectively) and in alcoholics with a positive family history (P=0.031, OR: 1.478; P=0.073, respectively). Cloninger 2 alcoholics had a higher frequency of the rare haplotype D-T-A-A2 ($P<0.001$, OR: 4.614) always compared with controls. In patients with positive family history, haplotype I-C-A-A2 (P=0.004, OR: 0.209) and in Cloninger 1 alcoholics, haplotype I-T-A-A1 (P=0.045 OR: 0.460) was less often present, confirming that haplotypes, which are supposed to induce a low DRD2 expression, are associated with alcohol dependence. Furthermore, supposedly high-expressing haplotypes weakened or neutralized the action of low-expressing haplotypes (Kraschewski et al 2009).

5.2 D4 dopamine receptor (DRD4)

There is evidence that the length of the D4 dopamine receptor (DRD4) exon 3 variable number of tandem repeats (VNTR) affects DRD4 functioning by modulating the expression and efficiency of maturation of the receptor. (Schoots & Van Tol 2003). The 7 repeat (7R) VNTR requires significantly higher amounts of dopamine to produce a response of the same

magnitude as other size VNTRs (Oak et al., 2000). This reduced sensitivity or "dopamine resistance" leads to hypodopaminergic functioning. Thus 7R VNTR has been associated with substance–seeking behavior (McGeary et al., 2007). Survival analysis has revealed that by 25 years of age 76% of subjects with a DRD4 7-repeat allele have significantly more persistent ADHD compared with 66% of subjects without the risk allele. In contrast, there were no significant associations between the course of ADHD and the DAT1 10-repeat allele (P=0.94) and 5HTTLPR long allele, suggesting that the DRD4 7-repeat allele is associated with a more persistent course of ADHD (Biederman et al., 2009). This is consistent with the finding of the presence of the 7R DAT genotype in the heroin addict. Moreover, in a study evaluating the role of dopamine D4 receptor (DRD4) exon 3 polymorphisms (48 bp VNTR) in the pathogenesis of alcoholism, significant differences in the short alleles (2-5 VNTR) frequencies were found between controls and patients with a history of delirium tremors and/or alcohol seizures (p = 0.043) (Grzywacz et al., 2009). A trend was also observed in the higher frequency of short alleles amongst individuals with an early age of onset of alcoholism (p = 0.063). These results indicate that inherited short variants of DRD4 alleles (3R) may play a role in pathogenesis of alcohol dependence and carriers of the 4R may have a protective effect for alcoholism risk behaviors. It is of further note that the DRD4 7-repeat allele is significantly over-represented in the opioid-dependent cohort and confers a relative risk of 2.46 (Kotler et al., 1997).

5.3 Dopamine transporter gene (DAT1)

The dopamine transporter protein regulates dopamine –mediated neurotransmission by rapidly accumulating dopamine that has been released into the synapse (Vandenbergh, 1998). The dopamine transporter gene (SLC6A3 or DAT1) is localized to chromosome 5p15.3. Moreover, there is a VNTR polymorphism within the 3' non-coding region of DAT1 (Michelhaugh et al., 2001). There are two important alleles that may independently increase risk for RDS behaviors. The 9 repeat (9R) VNTR has been shown to influence gene expression and to augment transcription of the dopamine transporter protein, resulting in an enhanced clearance of synaptic dopamine, yielding reduced levels of dopamine to activate postsynaptic neurons. Presence of the 9R VNTR has also been linked to Substance Use Disorder (SUD) (Guindalini et al., 2006). Moreover, in terms of RDS behaviors, tandem repeats of the dopamine transporter gene (DAT) have been associated with high risk for ADHD in children and in adults alike (Vandenbergh et al., 2002; Cook et al., 1995). The 10-repeat allele is significant for hyperactivity-impulsivity (HI) symptoms (Lee et al., 2007).

5.4 Catechol-O-methyltransferase (COMT)

The catechol-O-methyltransferase (COMT) is an enzyme involved in the metabolism of dopamine, adrenaline and noradrenaline. The Val158Met polymorphism of the COMT gene has been previously associated with a variability of the COMT activity, and alcoholism. Serý (2006) found a relationship between the Val158Met polymorphism of the COMT gene and alcoholism in male subjects. Serý (2006) found the significant difference between male alcoholics and male controls in allele and genotype frequencies (p<0,007; and p<0, 04 respectively. Interestingly in one of the subjects genotyped herein, who battles with heroin as an addiction while carrying the DRD2 A1 allele also carried the low enzyme COMT activity genotype (A/A). This is agreement with the work of Cao *et al.* (2003) who did not find an association with the high G/G and heroin addiction. No differences in genotype and

allele frequencies of 108 val/met polymorphism of COMT gene were observed between heroin-dependent subjects and normal controls (genotype-wise: chi-square=1.67, P=0.43; allele-wise: chi-square=1.23, P=0.27). No differences in genotype and allele frequencies of 900 Ins C/Del C polymorphism of COMT gene were observed between heroin-dependent subjects and normal controls (genotype-wise: chi-square=3.73, P=0.16; allele-wise: chi-square=0.76, P=0.38). While there is still some controversy regarding the COMT association with heroin addiction it was also interesting that the A allele of the val/met polymorphisms (-287 A/G) found by Cao *et al*. (2003) was found to be much higher in heroin addicts than controls. Faster metabolism results in reduced dopamine availability at the synapse, which reduces postsynaptic activation, inducing hypodopaminergic functioning. Generally Vanderbergh et al. (1997) and Wang et al. (2001) support an association with the Val allele and SUD but others do not (Samochowise et al. 2006).

5.5 Monoamine – Oxidase A

Monoamine oxidase-A (MAOA) is a mitochondrial enzyme that degrades the neurotransmitters serotonin, norepinephrine, and dopamine. This system is involved with both psychological and physical functioning. The gene that encodes MAOA is found on the X chromosome and contains a polymorphism (MAOA-uVNTR) located 1.2 kb upstream of the MAOA coding sequences (Shih, 1991). In this polymorphism, consisting of a 30-base pair repeated sequence, six allele variants containing either 2-, 3-, 3.5-, 4-, 5-, or 6-repeat copies have been identified (Zhu & Shih, 1997). Functional studies indicate that certain alleles may confer lower transcriptional efficiency than others. The 3-repeat variant conveys lower efficiency, whereas 3.5- and 4-repeat alleles result in higher efficiency (Brummett et al., 2007). The 3- and 4-repeat alleles are the most common, and to date there is less consensus regarding the transcriptional efficiency of the other less commonly occurring alleles (e.g., 2-, 5-, and 6-repeat). The primary role of MAOA in regulating monoamine turnover, and hence ultimately influencing levels of norepinephrine, dopamine, and serotonin, indicates that its gene is a highly plausible candidate for affecting individual differences in the manifestation of psychological traits and psychiatric disorders (Shih et al., 1999). For example, recent evidence indicates that the MAOA gene may be associated with depression (Brummett et al., 2008) and stress (Lee et al., 2009). However, evidence regarding whether higher or lower MAOA gene transcriptional efficiency is positively associated with psychological pathology as been mixed. The low-activity 3-repeat allele of the MAOA-uVNTR polymorphism has been positively related to symptoms of antisocial personality (Ponce et al., 2009) and cluster B personality disorders. Other studies, however, suggest that alleles associated with higher transcriptional efficiency are related to unhealthy psychological characteristics such as trait aggressiveness and impulsivity. High MAO activity and low levels of dopamine are 2 important factors in the development of alcohol dependence. MAO is an important enzyme associated with the metabolism of biogenic amines. Therefore, Huang *et al.* (2007) investigated whether the association between the dopamine D2 receptor (DRD2) gene and alcoholism is affected by different polymorphisms of the MAO type A (MAOA) gene. The genetic variant of the DRD2 gene was only associated with the anxiety, depression (ANX/DEP) ALC phenotype, and the genetic variant of the MAOA gene was associated with ALC. Subjects carrying the MAOA 3-repeat allele and genotype A1/A1 of the DRD2 were 3.48 times (95% confidence interval = 1.47-8.25) more likely to be ANX/DEP ALC than the subjects carrying the MAOA 3-repeat allele and DRD2 A2/A2 genotype. The MAOA gene may modify the association between the DRD2 gene and ANX/DEP ALC phenotype.

Overall, Vanyukov et al (2004) suggested that, although not definitive, variants in MAOA account for a small portion of the variance of SUD risk, possibly mediated by liability to early onset behavioral problems (Vanyukov et al., 2004).

5.6 Serotonin transporter gene

The human serotonin (5-hydroxytryptamine) transporter, encoded by the SLC6A4 gene on chromosome 17q11.1-q12, is the cellular reuptake site for serotonin and a site of action for several drugs with central nervous system effects, including both therapeutic agents (e.g. antidepressants) and drugs of abuse (e.g. cocaine). It is known that the serotonin transporter plays an important role in the metabolic cycle of a broad range of antidepressants, antipsychotics, anxiolytics, antiemetics, and anti-migraine drugs. Salz *et al.* (2009) found an excess of -1438G and 5-HTTLPR L carriers in alcoholic patients in comparison to the heroin dependent group [OR (95% CI)=1.98 (1.13-3.45) and 1.92 (1.07-3.44), respectively]. The A-1438G and 5-HTTLPR polymorphisms also interacted in distinguishing alcohol from heroin dependent patients (df) =10.21 (4), p=0.037). The association of -1438A/G with alcohol dependence was especially pronounced in the presence of 5-HTTLPR S/S, less evident with 5-HTTLPR L/S and not present with 5-HTTLPR.

5.7 Gamma-aminobutyric acid (GABA) receptor genes

Gamma-aminobutyric acid (GABA) receptor genes have also received some attention as candidates for drug use disorders. One reason for this is that the dopamine and GABA systems are functionally interrelated (White, 1996). Research suggests that dopamine neurons projecting from the anterior ventral tegmental area to the NAc are tonically inhibited by GABA through its actions at the GABAA receptor (Ikemoto, Kohl, & McBride, 1997). Moreover, it has been shown that alcohol (Theile, Morikawa, Gonzales, & Morrisett, 2008) or opioid (Johnson & North, 1992) enhancement of GABAergic (through GABAA receptor) transmission inhibits the release of dopamine in the mesocorticolimbic system. Thus, a hyperactive GABA system, by inhibiting dopamine release, could also lead to hypodopaminergic functioning. Because of this, GABA genes are of interest in the search for causes of drug use disorders. A dinucleotide repeat (DNR) polymorphism of the GABA receptor β3 subunit gene (GABRB3) results in either the presence of the 181-bp G1 or 11 other repeats designated as non-G1 (NG1). Research indicates that the NG1 is more prevalent in children of alcoholics (COAs; Namkoong, Cheon, Kim, Jun, & Lee, 2008). Presence of the NG1 has been associated with alcohol dependence (Noble et al., 1998; Song et al., 2003). In addition, other GABA receptor genes have also been associated with this disorder (Edenberg et al., 2004).

6. Multiplex analysis and GARS

There is a need to classify patients at genetic risk for drug seeking behavior prior to or upon entry to residential and or non-residential chemical dependency programs. Instead of continuing to evaluate single gene associations to predict future drug abuse, it seems parsimonious to evaluate multiple genes involved in the brain reward cascade and hypodopaminergic antecedents. As described in this example, such methods employ RDS-associated gene panels to stratify or classify patients entering a treatment facility as having low, moderate, or high genetic predictive risk based on a number of now known and/or

later discovered RDS risk alleles. Our laboratory developed a Genetic Addiction Risk Score (GARS) for this purpose. This example describes genetic studies for seven RDS-associated alleles for six candidate genes in a patient population (n=26) of recovering poly-drug abusers (Blum et al., 2010).

To determine RDS risk severity for each of these 26 patients, the percentage of prevalence of the risk alleles was calculated and a severity score based on the percentage of these alleles present in a given patient was developed. Subjects carry the following risk alleles: DRD2=A1; SLC6A3 (DAT) =10R; DRD4=3R or 7R; 5HTTlRP = L or $L_{A;}$ MAO= 3R; and COMT=G. As depicted in Tables 5 and 6, below, Low Severity (LS) = 1-36%, Moderate Severity (MS) =37-50%, and High Severity (HS) = 51-100%, scores were assigned. Two distinct ethnic populations among the 26 patients were studied. Group 1 consisted of 16 male Caucasian psycho-stimulant addicts and Group 2 consisted of 10 Chinese heroin-addicted males. Based on this analysis, the 16 Caucasian 100% of subjects had at least one risk allele (see tables 5 and 6). Therefore, using this approach it was found that 81% of the patients were at moderate to high risk for addictive behavior. Of particular interest was the discovery that 56% of the subjects carried the DRD2 A1 allele (9/16).

Out of the nine Chinese heroin addicts (Group 2), it was found that 11% (1) were HS, 56% (5) were MS, and 33% (3) were LS. These scores were then converted to a fraction and represented as GARS, whereby the average GARS was found to be: 0.28 Low Severity; 0.43 moderate severity; and 0.54 high severity, respectively. Therefore, using GARS it was discovered that 67% of the Group 2 patients were at moderate to high risk for addictive behavior. As with Group 1, 56% of the Group 2 subjects carried the DRD2 A1 allele (5/9). Statistical analysis revealed that the two groups did not differ in terms of overall severity (67% vs. 81%) in these two distinct populations. Combining these two independent study populations reveals that subjects entering a residential treatment facility for poly-drug abuse carry at least one risk allele (100%). Moreover, 74% of the combined 25 subjects who were genotyped by SNP analysis had a moderate-to-high GARS.

The 16 patients of Group 1 were interviewed and evaluated for chemical dependence using a standard battery of diagnostic tests and questionnaires. The tests included the following: a drug history questionnaire; a physical assessment, urine drug tests; a breathalyzer; complete CBC blood test; and a symptom severity questionnaire. The patients were determined to be substance dependent according to Diagnostic and Statistical Manual (DSM-IV) criteria. All patients were residential in-patients enrolled in 30-90 day chemical dependence rehabilitation programs at either of two treatment centers in the U.S.

Table 3 shows the demographics of the 16 patients, including gender, race, age, and length of abstinence. The median age was 29.5 ± 8.8 SD years. The population breakdown was as follows: 87.5 % Caucasian and 12.5% Hispanic. The average number of months abstinent for the entire population was 9.5 ±23.3. There were 3 pure cocaine-only addicts; 4 cocaine crack addicts; and 9 cocaine plus other drugs of abuse (alcohol, opiates and marijuana).

Table 4 includes genotype data from a functional MRI (fMRI) study in China evaluating involving 10 heroin-addicted Chinese males with a median age of 33 ± 7.6 SD years. Diagnosis of heroin dependence was also determined in this group using DSM-1 V criteria and other behavioral instruments. The average number of months abstinent for the entire population was 16 ± 7.9.

	Median ±st.dev.	**(min, max)**	***N (total = 16)***
Age	29.5 ±8.80	(19, 48)	16
Clean time (months)	9.5 ±23.33	(2, 101)	16
Race = Caucasian			14
Race = Hispanic			2
Sex = Male			16
Primary Substance = Cocaine only			3
Primary Substance = Crack cocaine			4
Primary Substance = Cocaine + Other*			9

Table 3. Demographics of all Caucasian subjects combined.

	Median ±st.dev.	**(min, max)**	***N (total = 10)***
Age	33 ± 7.57	(20, 44)	10
Clean time (months)	16 ± 7.91	(1, 24)	10
Race = Chinese			10
Sex = Male			10
Primary Substance = Heroin only			10
Primary Substance = Heroin + other			0

*One sample was eliminated because genotyping could not be performed. Source Blum et al 2010.

Table 4. Demographics of all Chinese subjects combined*.

6.1 Genotyping

Genotyping (Blum et al., 2010) was performed as follows. Each patient was also genotyped for the following gene polymorphisms: MAOA-VNTR, 5HTTLPR, SLC6A3, DRD4, ANKKI, DRD2 TaqIA (rs1800497), and the COMT val^{158}met SNP (rs4680). Genotypes were scored independently by two investigators.

The dopamine transporter (DAT1, locus symbol SLC6A3, which maps to 5p15.3, contains a 40 base-pair Variable Number Tandem Repeat (VNTR) element consisting of 3-11 copies in the 3' untranslated region (UTR) of the gene.

The dopamine D4 receptor (DRD4), which maps to 11p15.5, contains a 48 bp VNTR polymorphism in the third exon, which consists of 2-11 repeats.

Monoamine Oxidase A upstream VNTR (MAOA-uVNTR). The MAOA gene, which maps to Xp11.3-11.4, contains a 30 bp VNTR in the 5′ regulatory region of the gene that has been shown to affect expression. A genotype by environment interaction has been reported for this polymorphism.

Serotonin Transporter-Linked Polymorphic region (5HTTLPR). The serotonin transporter (5HTT, Locus Symbol SLC6A4), which maps to 17q11.1-17q12, contains a 43 bp insertion/deletion (ins/del) polymorphism in the 5′ regulatory region of the gene. A SNP (rs25531, A/G) in the Long form of 5HTTLPR has functional significance: The more common L_A allele is associated with the reported higher basal activity, whereas the less common L_G allele has transcriptional activity no greater than the S. The SNP rs25531 is assayed by incubating the full length PCR product with the restriction endonuclease MspI.

For all of the above VNTR and ins/del polymorphisms, PCR reactions contained approximately 20 ng of DNA, 10% DMSO, 1.8 mM MgCl2, 200 μM deoxynucleotides, with 7′-deaza-2′-deoxyGTP substituted for one half of the dGTP, 400 nM of appropriate forward

and reverse amplification primers, and 1 unit of AmpliTaq Gold® polymerase, in a total volume of 20 µl. Amplification was performed using touchdown PCR. After amplification, an aliquot of PCR product was combined with loading buffer containing size standard (Genescan 1200 Liz) and analyzed with an ABI PRISM® 3130 Genetic Analyzer.
DRD2 TaqIA (rs1800497). The gene encoding the dopamine D2 receptor maps to 11q23, and contains a polymorphic TaqI restriction endonuclease site located within exon of the adjacent ANKKI gene that was originally thought to be located in the 3' untranslated region of the gene. The A1 allele has been reported to reduce the amount of receptor protein. This SNP was assayed using a Taqman (5'Nuclease) assay.
COMT val^{158}met SNP (rs4680). The gene encoding COMT maps to 22q11.21, and codes for both the membrane-bound and soluble forms of the enzyme that metabolizes dopamine to 3-methoxy-4-hydroxyphenylethylamine. An A→G mutation results in a valine to methionine substitution at codons 158/108, respectively. This amino acid substitution has been associated with a four-fold reduction in enzymatic activity. The COMT SNP was assayed with a Taqman method.

6.2 Genetic Addiction Risk Score (GARS)

In terms of genotyping data it has been determined that when multiple RDS-associated genes are analyzed, such as the genes for serotonergic- 2A receptor (5-HTT2a), serotonergic transportor (5HTTLPR), (dopamine D2 receptor (DRD2), Dopamine D4 receptor (DRD4), Dopamine transporter (DAT1), Catechol-o-methyl –transferase (COMT), and monoamine–oxidase (MOA), 100% of all subjects carried at least one risk allele. To our knowledge this is the first reported attempt to stratify or classify addiction risk by incorporating an algorithm formulation that combines genotyping results for a number of RDS-associated risk alleles by pre-assigning an allele as a risk allele having predictive value for drug use. Previously, Blum et al (1996) using Bayesian statistics it was shown that the DRD2 A1 allele had a predictive value of 74.4% for all Reward Deficiency Syndrome (RDS). Here, the subjects studied in this investigation had multiple drug abuse relapses and presented to in-patient residential treatment programs.

Sub	MAOAuVNTR	5HTTLPR	5HTTLPR	SLC6A3	DRD4	DRD2	COMT	Any risk allele	SEVERITY* GARS
1	3R	S/L	S/L_G	9R/10R	4R/4R	A1/A2	G/G	POSITIVE	0.46–MS
2	3R	S/L	S/L_A	10R/10R	4R/7R	A2/A2	G/G	POSITIVE	0.62 –HS
3	3R	L/L	L_A/L_G	9R/9R	3R/4R	A1/A2	A/G	POSITIVE	0.57-HS
4	4R	S/L	S/L_A	10R/10R	3R/7R	A2/A2	G/G	POSITIVE	0.46-MS
5	4R	L/L	L_A/L_A	10R/10R	4R/7R	A2/A2	A/G	POSITIVE	0.62 –HS
6	3R	S/S	S/S	9R/10R	4R/7R	A2/A2	A/G	POSITIVE	0.30 –LS
7	4R	S/L	S/L_G	10R/10R	4R/4R	A1/A1	A/A	POSITIVE	0.38 –MS
8	4R	S/L	S/L_A	9R/10R	3R/4R	A2/A2	A/A	POSITIVE	0.23-LS
9	3R	L/L	L_A//L_A	9R/9R	4R/7R	A2/A2	A/G	POSITIVE	0.54-HS
10	4R	L/L	L_A/L_A	9R/10R	4R/4R	A2/A2	G/G	POSITIVE	0.54 –HS
11	3R	S/L	S/ L_A	9R/10R	4R/4R	A1/A2	G/G	POSITIVE	0.54-HS
12	4R	L/L	L_A/L_A	9R/10R	4R/4R	A1/A2	A/G	POSITIVE	0.54-HS
13	4R	S/L	S/ L_A	10R/10R	4R/4R	A1/A2	A/G	POSITIVE	0.46 –MS
14	4R	S/S	S/S	9R/10R	4R/4R	A1/A2	G/G	POSITIVE	0.30-LS
15	3R	L/L	L_A / L_A	10R/10R	4R/4R	A1/A2	A/G	POSITIVE	0.69 –HS
16	4R	S/L	S/L_A	10R/10R	4R/7R	A1/A2	A/A	POSITIVE	0.46-MS

Table 5. Group 1 genotyping data for each Caucasian patient. Data taken from Blum *et al.* 2010.

The finding that 75% of these individuals have moderate-to-high GARS, whereas only 25% had low GARS, indicates that pre-screening patients prior enrolling in a treatment program could be beneficial. Clinically, this will be important for understanding expectations of future success and the need for intensive treatment involving genomic solutions coupled with medical therapies, including bio-holistic therapies. It will also reduce quilt and denial of the entering patient.

Subject	MAOAuVNTR	5HTTLPR	5HTTLPR	SLC6A3	DRD4	*DRD2*	*COMT*	Any risk allele	SEVERITY* GARS
1	4R	S/L	S/L_A	10R/10R	4R/4R	A2/A2	A/A	POSITIVE	0.30–LS
2	3R	S/S	S/S	10R/10R	2R/4R	A1/A2	GAG	POSITIVE	0.38–MS
3	4R	S/S	S/S	10R/10R	3R/4R	A1/A2	G/G	POSITIVE	0.46-MS
4	3R	S/S	S/S	10R/10R	4R/6R	A2/A2	G/G	POSITIVE	0.38-MS
5	4R	S/S	S/S	10R/10R	4R/4R	A1/A2	A/G	POSITIVE	0.30–LS
6	3R	L/L	S/ L_G	10R/10R	4R/4R	A1/A2	ND	POSITIVE	0.45 –MS
7	4R	L/L	L_A/L_G	10R/10R	4R/4R	A1/A2	A/G	POSITIVE	0.54 –HS
8	4R	S/S	S/S	10R/10R	4R/5R	A2/A2	A/G	POSITIVE	0.23-LS
9	3R	S/L	S/L_A	10R/10R	2R/4R	A2/A2	A/G	POSITIVE	0.46-MS

Table 6. Group 2 genotyping data for each Chinese patient. Data taken from Blum et al 2010.

The GARS study supports the understanding that identifying hypodopaminergic genotypes may be the best predictor of adult and adolescent drug abuse or other SUD behavior. These results are also consistent with a number of functional MRI studies that show that the hypodopaminergic DRD2 A1 genotype leads to blunted responses that can could lead to aberrant drug and/or food seeking behavior (Stice et al., 2008), while the hyperdopaminergic A2 genotype serves as a protective factor against the development of drug disorders (Volkow et al 2006).

A further strength of this study is that only male subjects were used. Males with hypodopaminegic functioning are more likely to abuse drugs that stimulate the mesocorticolimbic system than those with normal dopaminergic functioning. In contrast, females living in a negative environment are at increased risk (possibly not due to their genotypes) for using more drugs and even more types of drug that increase their risk for SUD.

Another strength of this study is that it is in agreement with the work of Conner *et al.* [2010] confirming the importance of the cumulative effect of multiple genotypes coding for hypodopaminergic functioning, regardless of their genomic location, as a predictive method of drug use in males. Moreover, it extends the current literature, by suggesting for the first time a simple method using genetic testing to classify risk behavior in male patients seeking in-patient residential treatment (see table 7).

6.3 A representative RDS gene panel

Table 7, describes a RDS-associated gene/polymorphism panel that can be used in accordance with the potential development of the GARS test.

Gene	Significance	Comment
ALDH2	P= 5 X 10^{-37}	With alcoholism and alcohol-induced medical diseases
ADH1B	P= 2 X 10^{-21}	With alcoholism and alcohol-induced medical diseases
ADH1C	P = 4 X 10^{-33}	With alcoholism and alcohol-induced medical diseases
DRD2	P =1 X 10^{-8}	With alcohol and drug abuse
DRD4	P= 1 X 10^{-2}	With alcohol and drug abuse
SLC6A4	P= 2 X 10^{-3}	With alcohol , heroin, cocaine, methamphetamine dependence
HTRIB	P= 5 X 10^{-1}	With alcohol and drug abuse
HTRI2A	P= 5 X 10^{-1}	With alcohol and drug abuse
TPH	P = 2 X 10^{-3}	With alcohol and drug abuse
MAOA	P = 9 X 10^{-5}	With alcohol and drug abuse
OPRD1	P= 5 X 10^{-1}	With alcohol and drug abuse
GABRG2	P= 5 X 10^{-4}	With alcohol and drug abuse
GABRA2	P= 7 X 10^{-4}	With alcohol and drug abuse
GABRA6	P= 6 X 10^{-4}	With alcohol and drug abuse
COMT*	P= 5 X 10^{-1}	With alcohol and drug abuse in Asians
DAT1	P= 5 X 10^{-1}	With alcohol and drug abuse in Asians
CNR1	P= 5 X 10^{-1}	With alcohol and drug abuse
CYP2E1*	P =7 X10^{-2}	With alcohol LIVER DISEASE

Table 7. An RDS gene panel. Chen et al. Journal of Psychoactive drugs. June 2011.

7. Conclusion

The need to genetically test individuals, especially at entry into a residential or even non-residential chemical dependency program, has long been recognized. In the GARS study, a high percentage (75%) of subjects were found to carry a moderate to high GARS, and 100% of individuals tested possessed at least one of the RDS risk alleles tested. It is of some interest that in the Group 2 population only rare DRD4 alleles such as 2R, 5R, and 6R were found. This study supports our understanding that hypodopaminergic state is due to gene polymorphisms, as well as environmental elements including both stress and neurotoxicity from aberrant abuse of psychoactive drugs (e.g., alcohol, heroin, cocaine etc). This study demonstrates that useful genetic variables include serotonergic genes (e.g., serotonergic receptors [5HT2a], serotonin transporter 5HTlPR, etc.), endorphinergic genes (e.g., mu OPRM1 gene, proenkephalin (PENK) [PENK polymorphic 3' UTR dinucleotide (CA) repeats]), and dopaminergic genes (e.g., ANKKI Taq A; DRD2 C957T, DRD4 7R, COMT Val/met substation, MAO-A uVNTR, and SLC3 9 or 10R). Future studies could add other genes (e.g., GABARB3) and D3 dopamine receptor.

Any of these genetic and/or environmental impairments could result in reduced release of dopamine and or reduced number of dopaminergic receptors. The use of GARS will have prevention and treatment benefits in those patients afflicted with genetic antecedents to RDS-seeking behaviors.

8. Conflict of interest

Kenneth Blum, B.William Downs. and Roger Waite owns stock in LifeGen, Inc. the world wide distributors of the GARS test based on US and foreign patents. Frank Fornari is an consultant and owns interest in Dominion Diagnostics Lab the commercial developers of the GARS test. John Giordano is a LifeGen, Inc. partner.

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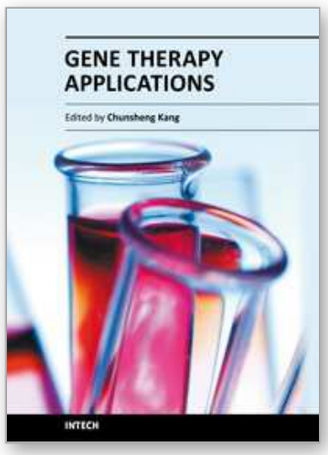

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