Blum *et al. J Transl Genet Genom.* 2025;9:76-100
**DOI:** 10.20517/jtgg.2024.56

**Journal of Translational Genetics and Genomics**

**Original Article**

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# Criterion validity of the genetic addiction risk severity (GARS) as a marker of reward deficiency in polydrug addiction: a multi-center study

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**How to cite this article:** Blum K, Haberstick BC, Smolen A, Han D, Elman I, Dennen CA, Oscar-Berman M, Smith DE, Simpatico T, Giordano J, Braverman ER, Hauser M, Butler S, III AKR, Thanos PK, Inaba D, Fried L, Ceccanti M, Zeine F, Jafari N, Sunder K, Bowirrat A, Pinhasov A, Lewandrowski KU, Sharafshah A, Murphy KT, Makale M, Khalsa J, Baron DD, Badgaiyan R, Modestino EJ, Gilley E, McLaughlin T, Gold MS. Criterion validity of the genetic addiction risk severity (GARS) as a marker of reward deficiency in polydrug addiction: a multi-center study. *J Transl Genet Genom.* 2025;9:76-100. https://dx.doi.org/10.20517/jtgg.2024.56

**Received:** 26 Aug 2024 **First Decision:** 28 Oct 2024 **Revised:** 5 Feb 2025 **Accepted:** 3 Mar 2025 **Published:** 24 Mar 2025

**Academic Editor:** Ramón Cacabelos **Copy Editor:** Ping Zhang **Production Editor:** Ping Zhang

## Abstract

**Aim**: This study evaluated the Genetic Addiction Risk Severity (GARS) panel, which assesses genetic predisposition to addictive disorders by examining eleven polymorphisms in ten genes associated with dopaminergic reward system functioning.

**Methods**: The GARS registered mark instead panel includes six single-nucleotide polymorphisms [*DRD1, DRD2, DRD3, DRD4, OPRM1,* and *catechol-O-methyltransferase* (*COMT*)], four simple sequence repeats (*5HTT, DAT1, DRD4,* and *MAOA*), and one dinucleotide repeat (*GABRA3*). Criterion validity was tested in 393 polydrug abusers by correlating GARS scores with Addiction Severity Index-Multimedia Version (ASI-MV) alcohol and drug severity scores.

**Results**: We identified a significant correlation between GARS and the ASI-MV alcohol severity score. While individuals with elevated drug severity also exhibited increased GARS, the relationship did not follow a strictly linear pattern. Variations in multiple genes involved in dopaminergic signaling contributed to risk in an additive manner, with age serving as a significant covariate. A greater number (≥ 7) of reward gene polymorphisms associated with moderate reductions in dopamine signaling demonstrated a significant association with higher ASI-MV alcohol severity scores. In contrast, individuals possessing four or more reward gene polymorphisms associated with moderate reductions in dopamine signaling exhibited significantly elevated ASI-MV drug severity scores.

**Conclusion:** Our findings align with previous research implicating dopaminergic pathways in the progression of alcoholism and substance abuse. Additionally, they build upon prior work by identifying a potential pre-existing polygenic risk factor, as defined by the GARS panel, that may be influenced by age-related physiological changes and environmental factors. Further research is warranted to explore associated endophenotypes, with particular emphasis on the role of Reward Deficiency Syndrome linked to dysfunction within the dopaminergic reward system.

**Keywords:** Genetic addiction risk score (GARS), dopaminergic system, polymorphisms, reward deficiency syndrome, reward gene, brain reward circuitry

## INTRODUCTION

The United States (US) is currently facing a significant public health crisis due to addiction and overdoses. According to the Centers for Disease Control and Prevention (CDC) in 2022, the US experienced a staggering 107,941 drug overdose deaths[1]. This represents a dramatic increase in the rate of drug overdose deaths, which has nearly quadrupled since 2002[1]. Additionally, approximately 70.3 million people over the age of 12 were found to have used illicit drugs in 2022, according to the Substance Abuse and Mental Health Services Administration (SAMHSA)[2]. The most commonly abused illicit drug was marijuana (61.9 million), followed by hallucinogens (8.5 million), pain relievers (8.5 million), cocaine (5.3 million), sedative/tranquilizer (4.8 million), prescription stimulants (4.3 million), methamphetamines (2.7 million), inhalants (2.3 million), and heroin (1 million)[2]. Addiction and substance use often culminate in substance use disorders (SUDs) and 48.7 million individuals over the age of 12 were diagnosed with a SUD in 2022[2].

While illicit drug use garners significant attention, the consumption of non-illicit substances, such as alcohol, tobacco, or vaping, poses equally pressing public health concerns. SAMHSA reported that 168.7 million people, over the age of 12, were found to have used tobacco, alcohol, or an illicit drug in the past month[2]. Specifically, 46.6 million people had used illicit drugs, 50.9 million had used a tobacco product, 23.5 million had vaped nicotine, and 137.4 million people had consumed alcohol in the past month[2]. When analyzing alcohol use, it was also found that, in individuals over the age of 12, 61.2 million individuals had engaged in binge drinking and 16.1 million people in heavy drinking within the past month[2]. Finally, it was reported that approximately 29.5 million individuals had been diagnosed with an alcohol use disorder (AUD) in 2022[2].

According to data from the National Institute on Drug Abuse (NIDA)[3], the annual cost of substance abuse in the US-including illicit drugs, alcohol, and tobacco exceeds $740 billion and continues to increase. The ongoing rise in addiction to chemical substances[4,5] underscores the necessity for innovative neurobiological insights to enhance prevention, diagnosis, and treatment strategies[6]. Studies in animals[7] and neuroimaging human research[8,9] attribute a pivotal role to the mesolimbic dopaminergic circuits subserving reward and motivation[10,11] at various stages of alcohol/drug addiction ranging from the acquisition of self-administration[12,13] to craving[14] and relapsing after a period of abstinence[15]. Alcohol and other addictive substances initially stimulate dopaminergic neurotransmission[16] within the brain's reward network. This network includes dopamine-releasing neurons in the ventral tegmental area and their terminal projections in the nucleus accumbens, orbitofrontal cortex, the ventral striatum, and several nuclei of the amygdala[17]. Over time, however, alcohol/drugs produce a counter-adaptive response, characterized by a hypodopaminergic state[18,19], clinically associated with negative affect, reduced motivation and a diminished ability to experience pleasure, collectively referred to as reward deficiency syndrome (RDS)[20-23], driving further alcohol consumption that provides temporary comfort but eventually exacerbating aversive feelings and intensifying craving[24]. RDS may not only be a consequence of chronic substance use, but also a pre-existing condition of genetic origin[22,25]. Thus, individuals with RDS often perceive themselves as being perpetually "several drinks behind" the rest of the world[26] and possess a personality trait characterized by high novelty seeking[27,28], driving their consumption of alcohol and drugs[29]. Moreover, in the form of emotional numbing[30,31], anhedonia, or affective flattening[32,33], RDS is a common element of other neuropsychiatric conditions that are highly comorbid with alcoholism and drug abuse, to name a few, major depression[34], schizophrenia[35], sugar-binging[36], obesity[37], gambling disorder[38], sex addiction[39], post-traumatic stress disorder (PTSD)[30], aggression[40-43], and suicidality[44]. Hence, it is crucial to find the genetic factors underlying the connection between alcohol/drug addiction and RDS so that they may be harnessed for preventive, diagnostic, and therapeutic efforts. Such an investigation faces a first major question: how to define and operationalize reduced dopaminergic function? Although RDS has been defined from clinical[45], behavioral[46], pathophysiological[30,47], neuroimaging[48], and neurochemical[49,50]

standpoints, understanding its genetic basis is challenging in part due to its multifactorial nature involving polymorphic genes implicated in dopaminergic pathways[51]. Likewise, RDS may be caused by a reduced number of dopamine receptors[52], especially D2[53], reduced dopamine synthesis[54] and release[55], and increased synaptic dopamine clearance due to a high number of dopamine transporter sites[56] or combination of these underlying mechanisms.

Even though it is generally accepted that RDS is linked to mesolimbic circuitry, the debate about the roles of specific candidate genes is still ongoing. One extant panel[57] is comprised of comprehensive haplotype information for candidate genes in alcoholism, other addictions, and mood and anxiety disorders; it is comprised of 130 genes that were tagged and genotyped in 51 reference populations and 7 case/control populations using Illumina Golden Gate single-nucleotide polymorphism (SNP) genotyping technology. A comprehensive analysis[58] that synthesized data from 2,343 peer-reviewed studies, utilizing methodologies such as single-gene strategies, microarray analyses, proteomics, and genetic approaches to examine associations between genes or chromosomal regions and addiction, identified 1,500 human genes implicated in addictive behaviors. The resultant "Knowledgebase for Addiction Related Genes" (http://karg.cbi.pku.edu.cn) is the first molecular database for addiction-related genes with extensive annotations and a Web interface. The same authors also performed a meta-analysis of 396 genes supported by two or more independent items of evidence to define a putative common molecular network for addiction[58]. Nonetheless, it remains unclear what specific genes out of these 396 candidates subserve RDS and related neuropsychopathology.

As with complexities inherent in defining a single subtype of RDS, such as alcoholism or PTSD[17,47,59-61], the exigencies of an accurate control sample recruitment that is representative of the population of interest could confound genetic study results. According to the Epidemiological Catchment Area survey, close to one-third of the general population meets the criteria for common psychiatric disorders at some point in their lives. RDS is a "polygenic disorder" that involves multiple genes with various polymorphisms[62]. Expression of RDS requires reaching a threshold number of these polygenes and associated variants. Consequently, individuals may carry some of these polymorphic genes but do not exhibit RDS behaviors because the threshold has not been reached.

To that end, we balanced the recruiting efforts with pragmatics by removing confounding cases from the control group and by using stratified (weighting) samples to design the Genetic Addiction Risk Score (GARS) test, capturing distinct aspects of dopaminergic function that predicts liability for addiction and RDS[63,64]. Reward candidate genes were selected based on a thorough literature review to yield the rationale utilized for each listed risk allele. The unifying concept guiding the selection of specific alleles included in the panel was based on their significant contributions to diminished dopaminergic function, including genes encoding dopamine receptors (*DRD1*, *DRD2*, *DRD3*, *DRD4*), the dopamine transporter (*DAT1*), the serotonin transporter, catechol-O-methyltransferase (COMT), monoamine oxidase (MAO), gamma-aminobutyric acid (GABA) receptors, the μ-opioid receptor (OPRM1), and cytochrome P450. In addition, specific single nucleotide polymorphisms (SNPs) and point mutations that influence dopamine release in the brain's reward centers were incorporated. These genetic variants were selected to represent a hypodopaminergic phenotype, a choice substantiated by thousands of association studies that have provided compelling evidence linking these risk alleles to various addictive behaviors[65].

The ten genes (encompassing eleven polymorphisms) examined in this study encode proteins that are abundantly present in both cortical and subcortical neural structures. Dysregulation of these genes in the prefrontal cortex and limbic system may result in RDS[66-67]. A prior study[68] proposed a five dopamine-

related gene panel including the *DRD2*/*ANKKI*, *DRD3*, and *DAT* to predict depression. Similarly, the Convergent Functional Genomics project[69] identified eleven candidate genes separating alcoholics from non-alcoholics. Even a single gene such as synuclein alpha (SNCA) regulating dopamine signaling showed a strong association with severe alcoholism ($P$ = 0.0001). While it might be appealing to adopt such a parsimonious approach, it would be unreasonable to limit the testing to SNCA alone due to concerns regarding specificity and sensitivity[68]. Therefore, we have chosen to avoid an overly reductionistic approach to GARS by utilizing a small number of genes, e.g., *DRD2*/*ANKKI, DRD1*, and *DAT1*.

Although GARS' construct validity can be ascertained via existing theoretical and empirical considerations[70-72], it is also important to juxtapose GARS against validated benchmarks, i.e., to assess its criterion validity.

The Addiction Severity Index (ASI) exemplifies a well-established tool for collecting comprehensive data, providing detailed information on the quantity, frequency, recency, and duration of alcohol and drug use[73]. This commonly utilized semi-structured interview also elicits information about life areas that are affected by alcohol/drug addiction. As both a psychodiagnostic and psychometric tool, ASI assesses addiction as a continuous measure of symptom severity c.f., categorical (present or absent) variables enabling the performance of more powerful correlational analyses.

The primary hypothesis pertained to an association between GARS and the ASI alcohol/drug scores. An a priori emphasis was given to the assumption that alcoholism, as well as addiction to other addictive substances, develops via negative reinforcement, i.e., alcohol/drugs are used to ameliorate the discomfort associated with the RDS[74]. However, an alternative explanation may be surmised in the form of the positive reinforcement idea - explicitly, that RDS, manifested via depressive symptomatology and related negative affective states, enhances alcohol/drug use by amplifying their rewarding and reinforcing properties[75]. This concept is reinforced by studies demonstrating a positive correlation between drug-induced euphoria and depressive symptoms, as reported by other research groups[76,77] and confirmed in our own investigations[78]. Furthermore, alcohol consumption may paradoxically offer a form of harm reduction in individuals with RDS by enhancing the sensitivity of previously under-responsive reward circuitry. This cross-sensitization effect not only augments responsiveness to drugs but also increases sensitivity to natural rewards, including those associated with social functioning[79]. Among varied drugs that are abused concurrently with alcohol, we could not a priori predict the net result. Therefore, exploratory analyses were employed to examine directionality and even the presence of potential interactions.

## METHODS

### Subjects

Study subjects were enrolled from nine addiction treatment centers geographically dispersed throughout the United States, namely Inflexxion, Addiction Recovery Resource, Catholic Charities of Maine, Center for Psychiatric Medicine, G & G Holistic Addiction Treatment Center, Integrative Life Center, Malibu Beach Recovery Center, Meadows Edge Recovery Center, and Tennessee Treatment Center. All study protocols were reviewed and approved by the PATH FOUNDATION IRB and received an exemption notification. The participants provided written informed consent after the study procedures were thoroughly explained to them. The genotyping data collection process conformed to the standards defined by the Health Insurance Portability and Accountability- and Genetic Information Non-Discrimination Acts.

### Exclusion/Inclusion criteria

Since this paper primarily involved genotyping whereby the subjects volunteered to provide cheek cells for DNA analysis, whereby DNA is not altered by environmental factors including disease, the exclusion criteria included exposure to radiation or pollution toxicity. The second criterion involved English language comprehension, as each participant had to complete an online ASI Media V questionnaire.

### Biopsychosocial assessments

Patients were interviewed and evaluated by Addiction Specialists using a standard battery of psychometric and -diagnostic questionnaires. Alcohol and drug use data were obtained using the ASI-Multi-Media (ASI-MV) version, which includes questions about lifetime alcohol/drug use and use in the last 30 days. Severity scores, determined via the algorithm generated by Inflexxion, range from "no real problem" (0) to "extreme problem" (9)[80]. Clinically, severity scores are utilized for disease staging and treatment planning purposes. Complementary psychometric tools included the Drug History Questionnaire[81] and Symptom Severity Questionnaires[82]. All subjects were evaluated for acute intoxication in each treatment center. Patients in each treatment center were also screened for standard biochemical tests including: urine toxicology screening, breathalyzer, and complete blood count.

### Sample collection and chain of custody

Participants were instructed to provide approximately 2 mL of saliva into a collection tube provided by the Institute for Behavioral Genetics (IBG) at the University of Colorado Boulder, Boulder, CO. Each sample was assigned a predetermined identification number and barcoded by Dominion Diagnostics. The saliva was stabilized using a buffer solution composed of Tris-EDTA, sodium dodecyl sulfate, and proteinase K at pH 8.0. Specimens were stored at room temperature at the collection sites and subsequently shipped to Dominion Diagnostics for transfer to Andrew Smollen at IBG for DNA extraction and isolation using standardized protocols[83].

### Genotyping

An index of the genes included in the GARS panel and their associated polymorphisms are provided in Table 1. Each genetic variant or polymorphism was selected based on its well-established association with RDS, specifically its role in contribution to hypodopaminergic functioning within the brain's reward circuitry. The allele and genotype frequencies observed for each variant align closely with those reported previously and in publicly available databases, suggesting that genotyping error (i.e., a ready source of bias that can reduce the power to detect a true effect) did not contribute to the observed frequencies.

Because we did not have RDS-free controls, we decided to count the number of alleles instead of developing a weighted power analysis (utilizing Odds Ratios). To further understand the relationship between ten genes and eleven polymorphisms employed in this study and the power of counting compared to weighing the power of each gene polymorphism, a subsequent test was statistically utilized, whereby each allele was provided with a higher power score (without actual ORs each allele was multiplied by a number above 1 to provide a differential power). In doing so, this test resulted in non-significance. Furthermore, switching an allele, for example, 10 R rather than 9R for *DAT1*, similarly canceled any statistical significance. Such manipulations suggest that utilizing the cluster of candidate genes and strict polymorphisms of the reward genes as represented by GARS provides discriminatory validity for addiction features defined via ASI clinical scores.

Assays for Amelogenin, MAOA-uVNTR, 5HTTLPR, DRD4, and DAT1[71] were performed using a multiplex polymerase chain reactiom (PCR) approach. In each 20 μL reaction, 2 μL of DNA (20 ng or less) was combined with 1.8 mM $MgCl_2$, 180 μM of each deoxynucleotide triphosphate (dNTP, NEB), and 10%

**Table 1. GARS panel: genetic variants and risk alleles**

| Polymorphisms/Repeat | Gene | Variants | Risk allele |
|---|---|---|---|
| Single nucleotide polymorphisms (SNPs) | | | |
| rs4532 | *Dopamine D1 Receptor (DRD1)* | A/G | G |
| rs1800497 | *Dopamine D2 Receptor (DRD2)* | A1 (A)/A2 (G) | A1 |
| rs6280 | *Dopamine D3 Receptor (DRD3)* | C/T | C |
| rs1800955 | *Dopamine D4 Receptor (DRD4)* | C/T | C |
| rs4680 | *Catechol-O-Methyltransferase (COMT)* | A (Met)/G (Val) | G |
| rs1799971 | *Mu-Opioid Receptor (OPRM1)* | A (Asn40)/G (Asp40) | G |
| Simple sequence repeats (variable number tandem repeats & insertion/deletions) | | | |
| 3′ 40 base-pair repeat | *Dopamine Transporter Receptor (DAT1)* | 9 repeat (R) | 9R |
| Intron 3, 48 base-pair repeat | *Dopamine D4 Receptor (DRD4)* | 7, 8, 9, 10, 11 repeats (R) | 7R |
| 3′ 30 base-pair Repeat | *Monoamine Oxidase A* | 3.5, 4, 5 repeats (R) | 4R |
| 43 base-pair 5′ insertion/deletion + rs25531 (5HTTLPR) | *Serotonin Transporter Receptor (5HTT)* | S (short) or Lg | S′ |
| Dinucleotide repeats | | | |
| CA-repeat | *Gamma-Aminobutyric Acid (GABA) A Receptor, beta 3 (GABRA3)* | 171 - 201 | 181 |

DMSO, with 7'-deazadeoxyGTP (deaza-GTP, Roche Applied Science, Indianapolis, IN) substituting for half of the dGTP concentration. Fluorescently labeled forward primers and the corresponding reverse primers were included, as detailed in Table 2. The reaction mixture also contained one unit of AmpliTaq Gold® polymerase (Life Technologies, Grand Island, NY) and 1 × PCR buffer II, resulting in a final volume of 20 µL[71].

The analysis of the dinucleotide repeat in GABRB3 was performed in a reaction containing 2 µL of DNA (20 ng or less), 200 µM of each deoxynucleotide triphosphate (dNTP, NEB), 2.5 mM MgCl2_22, fluorescently labeled forward and reverse primers (details provided in Table 2), 1 unit of AmpliTaq Gold® polymerase, and 1× PCR buffer II, resulting in a total reaction volume of 20 µL[84]. Amplifications utilized a modified touchdown PCR protocol[85,86].

The PCR cycling protocol commenced with an initial denaturation at 95 °C for 10 min. This was followed by two cycles consisting of 95 °C for 30 s, 72 °C for 60 s, and 65 °C for 30 s. Subsequently, the annealing temperature was decreased by 2 °C every two cycles from 65 °C to 57 °C across 10 cycles. This phase was then succeeded by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s. The reaction concluded with a final extension at 72 °C for 30 min, followed by a hold at 4 °C. To ensure assay accuracy, each 96-well plate incorporated non-template controls and DNA standards with established genotypes.

The assay for rs25531 (A/G), located within the long form of the 5HTTLPR region, has been described in detail elsewhere. This SNP facilitates the distinction between the LA and LG alleles. Amplification of the 5HTTLPR site containing the SNP was performed using a single PCR reaction composed as previously described, with primers referenced in prior studies. The thermal cycling conditions included an initial denaturation at 95 °C for 10 min, followed by two cycles of 95 °C for 30 s, 65 °C for 30 s, and 72 °C for 60 s. This was followed by two cycles at 95 °C for 30 s, 63 °C for 30 s, and 72 °C for 60 s, then 30 cycles at 95 °C for 30 s, 61 °C for 30 s, and 72 °C for 60 s. The process concluded with a final extension at 72 °C for 30 min

**Table 2. Marker, primer, and resulting size ranges of characterized polymorphisms**

| Primer | Sequence (5′ → 3′) | Concentration (nM) | Size range (bp) |
|---|---|---|---|
| Amelogenin-F | NED-CCC TGG GCT CTG TAA AGA ATA GTG | 300 | 103,109 |
| Amelogenin-R | ATC AGA GCT TAA ACT GGG AAG CTG | 300 | (X, Y) |
| MAO-uVNTR-F | 6FAM-ACA GCC TGA CCG TGG AGA AG | 200 | 291-381 |
| MAO-uVNTR-R | GAA CGG ACG CTC CAT TCG GA | 200 | (2R-5R) |
| DAT1-F | 6FAM-TGT GGT GTA GGG AAC GGC CTG AG | 300 | 200-600 |
| DAT1-R | CTT CCT GGA GGT CAC GGC TCA AGG | 300 | (3R-13R) |
| DRD4-F | VIC-GCT CAT GCT GCT GCT CTA CTG GGC | 600 | 279-711 |
| DRD4-R | CTG CGG GTC TGC GGT GGA GTC TGG | 600 | (2R-11R) |
| 5HTTLPR-F | NED-ATG CCA GCA CCT AAC CCC TAA TGT | 600 | 376, 419-549 |
| 5HTTLPR-R | GGA CCG CAA GGT GGG CGG GA | 600 | (S, L-XL) |
| 5HTTLPR-Hu-F | 6FAM-GCA ACC TCC CAG CAA CTC CCT GT | 500 | 138, 181 |
| 5HTTLPR-Hu-R | GAG GTG CAG GGG GAT GCT GGA A | 500 | (S, L) |
| GABRB3-F | 6FAM-CTC TTG TTC CTG TTG CTT TCA ATA CAC | 500 | 171 - 201 |
| GABRB3-R | CAC TGT GCT AGT AGA TTC AGC TC | 500 | |

and a hold at 4 °C.

Following PCR amplification, the products were incubated with five units of MspI (NEB, Ipswitch, MA) at 37 °C for 90 min[87]. The presence of a 97 bp restriction fragment indicated the LG allele. For the GARS panel, two combined alleles were reported: S', which includes the S and LG alleles, and L', which comprises the LA and extra-long alleles. These categories represent activity bins rather than individual alleles. Each 96-well plate included non-template controls and DNA standards with known genotypes. Following amplification, the PCR products and MspI digests were purified using Zymo Research ZR-96 DNA Sequencing Clean-up Kits in strict adherence to the manufacturer's protocol. The purified samples were then combined with a loading buffer containing a size standard (Rox1000, Gel Company, San Francisco, CA) and analyzed on an ABI PRISM® 3130xl Genetic Analyzer (Life Technologies) according to the manufacturer's guidelines. Data processing was performed using GeneMapper software, with all results independently validated by two investigators.

Genotyping of the SNPs TaqIA (rs1800497), COMT val158met (rs4680), DRD1 (rs4532), DRD3 (rs6280), DRD4-521C/T (rs1800955), and OPRM1 (rs1799971) was performed using the fluorogenic 5' nuclease assay (TaqMan®, ABI, Foster City, CA)[88] on an ABI Prism® 7000 Sequence Detection System in allelic discrimination mode[89,90]. Each reaction was conducted in a total volume of 15 μL, containing 20 ng of genomic DNA, TaqMan® Universal PCR Master Mix, and primers and probes at final concentrations of 900 nM and 200 nM, respectively. A standardized cycling protocol was employed, with primer and probe sequences for TaqIA and COMT detailed in Table 3. Assays for the remaining SNPs were obtained directly from Life Technologies. To ensure genotyping accuracy, each 96-well plate included non-template controls and DNA standards with verified genotypes.

### Statistical analysis

Mean, standard deviations, regression tests, and chi-square were executed using SPSS (Version 21.0). Significant differences between dichotomized alcohol and drug severity scores were assessed using a Fischer Exact Test. Scores above and below the means of 4.65 for alcohol and 4.00 for drugs were respectively determined to be "high" (1) and "low" (0). Regression analyses were executed to determine the relationship between the severity risk score and genetic risk score, adjusting for age (continuous) and sex (dichotomous)

**Table 3. Probe and primer sequences for *COMT* val158met and *DRD2* Taq1A polymorphisms**

| Gene | Probe/Primer | Sequence (5′ → 3′) |
|---|---|---|
| *COMT* | T (met) probe | VIC-ACCTTGTCCTTCATGCCAGCGAAAT-NFGMGB |
| | C (Val) probe | FAM-CCTTGTCCTTCACGCCAGCGA-NFQMGB |
| | Forward primer | TCGAGATCAACCCCGACTGT |
| | Reverse primer | AACGGGTCAGGCATGCA |
| *DRD2* | T (A1) probe | VIC-CCTGCCTTGACCAGC-NFQMGB |
| | C (A2) probe | FAM-CTGCCTCGACCAGC-NFQMGB |
| | Forward primer | GTGCAGCTCACTCCATCCT |
| | Reverse primer | GCAACACAGCCATCCTCAAAG |

COMT: Catechol-O-methyltransferase.

variables. The distribution of scores on the alcohol and drug severity risk scales exhibited a slight skew toward lower values. To assess potential normalization, the effects of square-root and log-transformations were examined. In accordance with predefined criteria, each identified risk allele was assigned a value of 1. An individual's overall genetic risk score was then determined by summing the total number of risk alleles present within their genotype.

A dichotomous addiction risk scale was created by classifying those at or below the mean of 7.97 as having a "low" number of risk alleles and those above the mean as having a "high" number of addiction risk alleles. GARS severity scores were computed by dividing the summed GARS score by the total possible number of alleles (22 alleles from 11 genes). Scores below 0.30 were binned together in the "low" severity range, scores between 0.31 and 0.69 into the "moderate" severity range, and 0.70 to 0.99 into the "high" severity range. Hardy Weinberg equilibrium (HWE) was confirmed for each gene using chi-square tests.

## RESULTS

### Sample characteristics

The mean age of this sample was 35.3± standard deviation (SD) = 13.1 years; 57.8% ($n$ = 160) of the sample were males and 88.1% ($n$ = 244) reported their race as White [Table 4]. The "consented" subjects at seven different treatment centers were similar in terms of their demographics, except for younger males at the G & C Holistic Addiction Treatment Centers. One center failed to provide adequate ASI-MV data, thus bringing the number of subjects with both genetic and ASI data to 273. Furthermore, 50 additional subjects were removed from the analyses due to zero alcohol severity scores. Among the remaining 223 patients, the mean alcohol severity rating score ±SD was 4.65±2.55 and it did not differ significantly between males and females or as a function of the treatment center. The mean ±SD for the drug severity ratings ($n$ = 244) was 5.81±2.45.

### Allele and genotype frequencies

Among the discovery sample ($n$ = 393), 17.6% scored in the "low severity" range, 80.7% in the "moderate" severity range, and 1.5% in the "high" severity range. No deviations from HWE were detected for any of the genotypes generated [Tables 5 and 6].

### Alcohol and drug severity scores

ASI alcohol and drug severity scores were slightly skewed to the left (Alcohol: skewness = -0.211, kurtosis = -1.473; Drugs: skewness = -0.922, kurtosis = -0.483), suggesting a mild-moderate range of addictive problems related to chemical substances. Patients with high alcohol severity ratings had increased psychiatric ($\chi^2$ = 10.26, $P$ = 0.001), family- and medical- ($\chi^2$ = 8.20, $P$ < 0.004), but not economic- or legal

**Table 4. Demographics by treatment center (*N* = 273)**

| Treatment center | N (%) | Gender | Age (years) mean (standard deviation, N) | Ethnicity (% white) |
|---|---|---|---|---|
| Addiction recovery resource | 39 (14.1) | Males | 32.9 (12.7, 29) | 96.6 |
| | | Females | 35.5 (15.1, 10) | 80.0 |
| Catholic charities - maine | 41 (14.8) | Males | 33.7 (9.5, 34) | 88.2 |
| | | Females | 40.4 (10.7, 1) | 85.7 |
| Center for psychiatric medicine | 1 (0.4) | Males | 34.0 (0, 1) | 100 |
| | | Females | -- | -- |
| G & G holistic addiction treatment centers | 108 (39.0) | Males | **30.9 (12.0, 59)** | 88.1 |
| | | Females | **37.1 (14.2, 49)** | 91.8 |
| Integrative life center | 2 (0.9) | Males | 22 (0, 1) | 100 |
| | | Females | 22 (0, 1) | 100 |
| Malibu beach recovery center | 65 (23.5) | Males | 38.1 (13.8, 28) | 85.7 |
| | | Females | 39.8 (14.3, 37) | 86.5 |
| Meadows edge recovery center | 17 (6.1) | Males | 36.9 (15.4, 8) | 100 |
| | | Females | 37.3 (11.3, 9) | 88.0 |

N: Sample size; Bolded: indicates significant mean differences between males and females.

problems ($\chi^2 > 0.1$). Higher GARS was associated with higher alcohol severity scores ($\chi^2 = 8.84$, $P = 0.004$); this association survived gender and age adjustment. When dichotomized alcohol severity scores (low and high) were employed, there was a trend ($\chi^2 = 3.37$, $P = 0.07$) association. Patients with "high" drug severity scores had increased psychological- ($\chi^2 = 8.26$, $P = 0.004$) and family- ($\chi^2 = 11.7$, $P = 0.001$), but not medical-, economic-, or legal problems ($\chi^2 > 0.1$). Those with high drug severity had more addiction risk alleles, i.e., heightened GARS ($P = 0.05$); this did not seem to be a linear association evidenced in an insignificant result when continuous drug severity values were considered ($\chi^2 = 1.43$, $P = 0.71$); the contributions of age and gender were significant (See Figures 1-4 and Tables 7-9).

## DISCUSSION

This study was carried out with a medium-sized cohort of addicted patients recruited from seven diverse chemical dependence programs. Besides gender at one center, there were no significant variations in any of the tested parameters across the cohort. Consequently, the dataset meets the Hardy-Weinberg Equilibrium criteria[91] for gene polymorphism distribution, thus enhancing our findings' validity. An important aspect of the present multi-locus approach[92,93] study is that it captures the polymorphisms affecting the following fundamental processes of the dopaminergic system: (a) dopamine transport across the neuron; (b) synaptic metabolism of dopamine; (c) dopamine binding at the primary receptor subtypes; (d) endorphinergic sites determining the synaptic content of dopamine that are influenced by serotonin transporters, and e) GABA receptors that are inhibited via opiate receptor influencing the neuronal release of dopamine. A higher number (≥ 7) of reward-gene-polymorphisms linked to reduced dopamine signaling were significantly associated with increased ASI-MV alcohol severity scores. Similarly, a high number (≥ 4) of such polymorphisms were significantly correlated with higher ASI-MV drug severity scores.

Interestingly, the importance of a hypodopaminergic trait was demonstrated when significance was improved by exchanging specific SNPs of the DAT 10 allele with reduced dopaminergic function (9 alleles). Another key finding from this study is that these genetic influences are additive. We also found that age, but not gender, was a predictor of the ASI alcohol and drug severity scores. This finding may have molecular underpinnings given the well-described age-related D2 density reduction[94]. Since age is associated with

**Table 5. Allele and genotype frequencies of specific genes**

| Gene | *N* | Allele | | Genotype | | | HWE (*P*-value) | Risk allele |
|---|---|---|---|---|---|---|---|---|
| *DRD1* | 368 | T | C | TT | CT | CC | 0.95 | C |
| | | 0.66 (488) | 0.34 (246) | 0.44 (162) | 0.45 (164) | 0.11 (42) | | |
| *DRD2 (rs1800497, Taq1A)* | 381 | A1 | A2 | A1A1 | A1A2 | A2A2 | 0.99 | A1 |
| | | 0.25 (188) | 0.75 (574) | 0.06 (23) | 0.37 (141) | 0.57 (216) | | |
| *DRD3 (rs6280)* | 383 | T | C | TT | CT | CC | 0.97 | C |
| | | 0.62 (472) | 0.38 (294) | 0.38 (145) | 0.47 (181) | 0.15 (56) | | |
| *DRD4 (rs180095)* | 350 | T | C | TT | CT | CC | 0.99 | C |
| | | 0.51 (360) | 0.49 (340) | 0.26 (92) | 0.50 (174) | 0.24 (82) | | |
| *COMT (rs4680)* | 380 | A | G | AA | AG | GG | 0.99 | G |
| | | 0.45 (345) | 0.55 (415) | 0.21 (78) | 0.50 (188) | 0.30 (113) | | |
| *OPMR1* | 385 | A | G | AA | AG | GG | 0.86 | G |
| | | 0.87 (670) | 0.13 (100) | 0.76 (291) | 0.23 (87) | 0.02 (6) | | |
| *DAT1 VNTR* | 372 | 9R | 10R | 9R/9R | 9R/10R | 10R/10R | 0.97 | 9R |
| | | 0.25 (187) | 0.75 (557) | 0.06 (23) | 0.38 (139) | 0.56 (208) | | |
| *DRD4 VNTR* | 372 | 4R | 7R | 4R/4R | 4R/7R | 7R/7R | 0.98 | 9R |
| | | 0.78 (587) | 0.22 (165) | 0.61 (229) | 0.34 (128) | 0.05 (18) | | |
| *MAOA-uVNTR VNTR** | 375 (males + females) | 3R | 4R | 3R/3R | 3R/4R | 4R/4R | | 4R |
| | | 0.37 (274) | 0.63 (476) | 0.13 (50) | 0.46 (173) | 0.40 (151) | | |
| *MAOA-uVNTR VNTR* | 169 (females only) | 3R | 4R | 3R/3R | 3R/4R | 4R/4R | 0.96 | 4R |
| | | 0.37 (124) | 0.63 (214) | 0.13 (22) | 0.46 (78) | 0.40 (68) | | |
| *DRD1 (rs 4531)* | 274 | T | C | TT | CT | CC | 0.96 | C |
| | | 0.66 (359) | 0.34 (189) | 0.43 (117) | 0.45 (123) | 0.12 (32) | | |
| *DRD2 (rs1800497, Taq1A)* | 278 | A1 | A2 | A1A1 | A1A2 | A2A2 | 0.97 | A1 |
| | | 0.24 (135) | 0.76 (421) | 0.06 (16) | 0.37 (102) | 0.57 (159) | | |
| *DRD3 (rs6280)* | 277 | T | C | TT | CT | CC | 0.96 | C |
| | | 0.62 (345) | 0.38 (209) | 0.39 (107) | 0.47 (130) | 0.14 (39) | | |
| *DRD4 (rs180095)* | 271 | T | C | TT | CT | CC | 0.99 | C |
| | | 0.51 (278) | 0.49 (264) | 0.26 (71) | 0.50 (135) | 0.24 (64) | | |
| *COMT (rs4680)* | 271 | A | G | AA | AG | GG | 0.99 | G |
| | | 0.49 (278) | 0.51 (264) | 0.24 (64) | 0.50 (135) | 0.36 (71) | | |
| *OPMR1 (rs1799971)* | 278 | A | G | AA | AG | GG | 0.97 | G |
| | | 0.88 (490) | 0.12 (66) | 0.78 (215) | 0.21 (58) | 0.01 (4) | | |
| *DAT1 VNTR* | 277 | 9R | 10R | 9R/9R | 9R/10R | 10R/10R | 0.93 | 9R |
| | | 0.24 (135) | 0.76 (419) | 0.06 (16) | 0.37 (102) | 0.57 (158) | | |
| *DRD4 VNTR* | 275 | 4R | 7R | 4R/4R | 4R/7R | 7R/7R | 0.95 | 9R |
| | | 0.76 (420) | 0.24 (130) | 0.58 (160) | 0.36 (99) | 0.06 (15) | | |

VNTR: MAOA-uVNTR repeat; *: Males and females were included together in this population (N). HWE could not be calculated as MAOA-uVNTR males are hemizygous for this polymorphism. For females, only a HWE p-value was listed; however, as noted under each table, HWE could not be calculated, i.e., "HWE cannot be calculated as MAOA-uVNTR is on the X-Chromosome."

hypodopaminergic state due to a reduced number of D2 receptors[95], it may drive substance-seeking behaviors[96]. In short, a combination of age and high GARS should have clinical relevance and may even serve as the basis for enhanced RDS spectrum behaviors[97], e.g., alcoholism in the elderly[98].

The present results extend our earlier report on the allelic association of the dopamine D2 receptor gene in alcoholism, in which we suggested that the presence of a specific allele predicted 77% of alcoholics whereas

**Table 6. Repeat number and genotype frequencies of specific genes**

| Gene | *N* | Repeat number | | Genotype | | | HWE (*P*-value) | Risk allele |
|---|---|---|---|---|---|---|---|---|
| *GABRA3* dinucleotide repeat | 362 | 179* | 0.71 (512) | 179/179 | 179/181 | 181/181 | 0.97 | 181 |
| | | 181 | 0.29 (212) | 0.50 (181) | 0.41 (149) | 0.09 (31) | | |
| *5HTTLPR* insertion/deletion and rs25531 SNP** | 371 | S′ | 0.51 (377) | S′/S′ | S′/L′ | L′/L′ | 0.96 | S′ |
| | | L′ | 0.49 (365) | 0.26 (96) | 0.50 (185) | 0.24 (90) | | |
| *GABRA3* dinucleotide repeat allele | 274 | 179* | 0.70 (382) | 179/179 | 179/181 | 181/181 | 0.98 | 181 |
| | | 181 | 0.30 (166) | 0.49 (133) | 0.42 (115) | 0.09 (25) | | |
| *5HTTLPR* insertion/deletion and rs25531 SNP** | 271 | S′ | 0.49 (264) | S′/S′ | S′/L′ | L′/L′ | 0.96 | S′ |
| | | L′ | 0.51 (278) | 0.23 (64) | 0.50 (135) | 0.26 (71) | | |

HEW: Hardy weinberg equilibrium; N: population sample; VNTR: variable number tandem repeat; SNP: single-nucleotide polymorphism; *179: denotes all repeats except for 181. **5HTTLPR includes Short (S) and Long (L) variants; rs25531 includes A and G alleles; S′/S′ genotype includes S/S, L-G/S, L-G/L-G; S′/L′ genotype includes L-A/S, L-A/L-G; L′/L′ genotype includes L-A/L-A.

**Table 7. Mean, standard deviation, sample size for alcohol & drug risk severity scores and genetic addiction risk scale by treatment center and gender**

| Treatment center | Gender | Alcohol risk severity score Mean (standard deviation, N) | Drugs risk severity score Mean (standard deviation, N) | GARS Mean (standard deviation, N) |
|---|---|---|---|---|
| Addiction Recovery Resource | Males | 5.15 (2.65, 26) | 6.64 (1.85, 28) | **7.90 (1.88, 29)** |
| | Females | 6.11 (2.52 9) | 6.00 (2.16, 7) | **9.80 (3.49, 10)** |
| Catholic Charities - Maine | Males | 3.33 (2.23, 30) | 5.75 (2.14, 32) | 7.62 (2.39, 34) |
| | Females | 4.00 (2.94, 4) | 6.60 (1.52, 5) | 7.71 (2.87. 7) |
| Center for Psychiatric Medicine | Males | 2.00 (0, 1) | 4.00 (0, 1) | 7.00 (0, 1) |
| | Females | -- | -- | -- |
| G & G Holistic Addiction Treatment Centers | Males | 5.46 (2.22, 39) | **5.24 (2.73, 42)** | 8.08 (2.18, 49) |
| | Females | 4.71 (2.74, 42) | **6.47 (1.95, 59)** | 7.71 (2.40, 59) |
| Integrative Life Center | Males | 1.00 (0, 1) | 5.00 (0, 1) | 7.00 (0, 1) |
| | Females | 4.00 (0, 1) | 5.00 (0, 1) | 7.00 (0, 1) |
| Malibu Beach Recovery Center | Males | 4.65 (2.65, 23) | 5.34 (2.91, 26) | 7.96 (1.97, 28) |
| | Females | 4.44 (2.96, 34) | 5.33 (3.10, 27) | 8.38 (2.25, 37) |
| Meadows Edge Recovery Center | Males | 5.67 (2.94, 6) | 4.38 (2.50, 8) | 7.88 (2.14, 8) |
| | Females | 3.29 (2.63, 7) | 5.43, 3.04, 7) | 7.67 (3.32, 9) |

GARS: Genetic addiction risk score; N: sample size; S.D.: standard deviation; Bolded: indicates significant mean differences between males and females.

**Table 8. Parameter estimates and summary statistics for ASI alcohol risk severity score**

| Variable | B | S.E. | Wald Chi-Square | Df |
|---|---|---|---|---|
| Intercept | -1.93 | 0.52 | 13.84 | 1 |
| Sex | -0.19 | 0.29 | 0.42 | 1 |
| Age | 0.05 | 0.11 | 15.29 | 1 |
| GARS | 0.74 | 0.29 | 6.39 | 1 |

B: regression parameter estimates; S.E.: standard error; df: degrees of freedom; GARS: genetic addiction risk score; ASI: addiction severity index.

**Table 9. Parameter estimates and summary statistics for ASI drug risk severity score**

| Variable | Non-standardized beta | Unstandardized S.E. | Standardized beta | T | Significance |
|---|---|---|---|---|---|
| Intercept | 0.92 | 0.09 | - | 10.50 | < 0.001 |
| Sex | 0.07 | 0.04 | 0.12 | 1.87 | 0.06 |
| Age | -0.01 | 0.01 | -0.21 | -3.43 | 0.001 |
| GARS | -0.13 | 0.01 | -0.10 | -1.59 | 0.11 |

S.E.: standard error; GARS: genetic addiction risk score; ASI: addiction severity index.

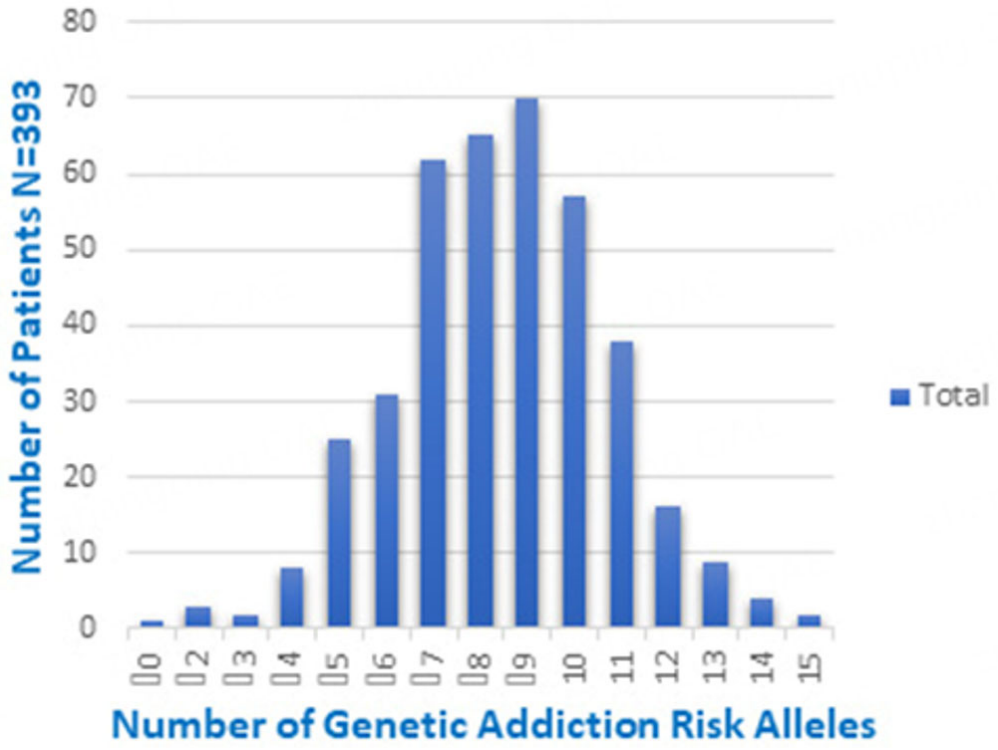


**Figure 1.** The allelic distribution of the total 393 cohort.

lack thereof predicted 72% of non-alcoholics[99]. Although there were conceptual similarities between the latter and present study (e.g., alcoholic subjects and genotyping techniques), there were also important differences, including the comprehensive panel measuring reward genes and polymorphisms, a larger sample size, as well as the focus on a clinical population rather than on post-mortem samples. Thus, the present data demonstrate a creation validity of GARS against the established criterion, that is to say, the ASI, which is the most commonly used psychometric tool in substance use disorders (https://www.sciencedirect.com/topics/medicine-and-dentistry/addiction-severity-index).

The observed pattern in ASI drug severity scores (i.e., higher GARS in patients with high *vs.* low drug severity) is consistent with prior observations[100], including the association of the DRD2/AAKKI haplotype with comorbid alcohol- and drug addiction[101]. However, a lack of linear relationship between GARS and ASI drug severity scores calls for further inquiry into the type of this relationship. Several hypotheses could explain this non-linear relationship. One possibility is that at a certain severity level, individuals might substitute drugs with alcohol consumption, which could temporarily improve some aspects of drug-related

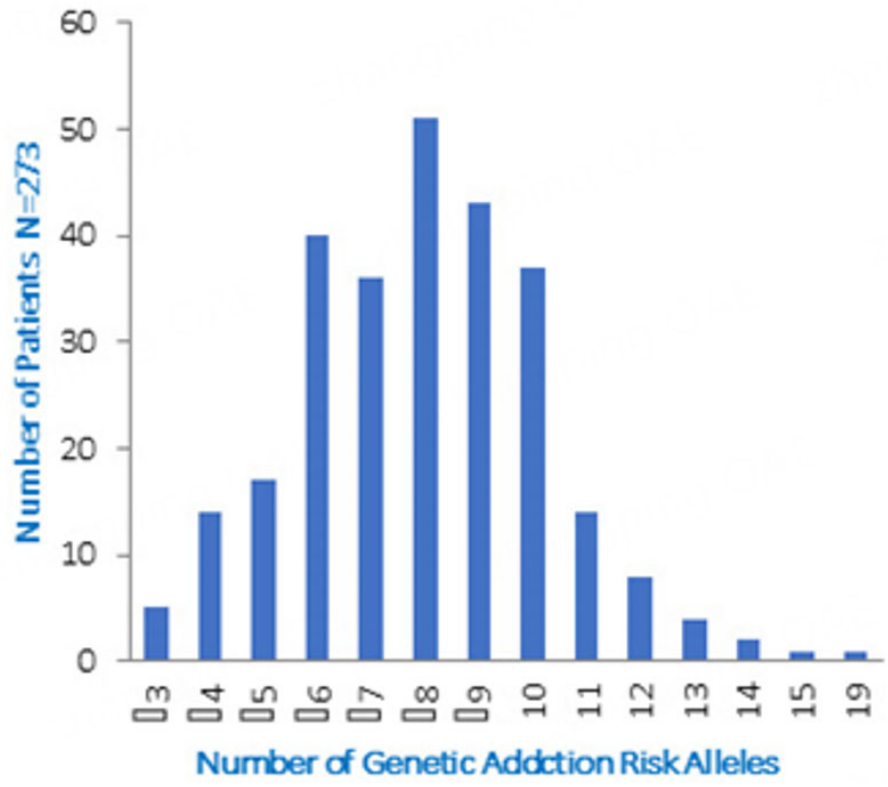


**Figure 2.** The allelic distribution of the cohort that completed the ASI questionnaire (*n* = 273). ASI: Addiction severity index.

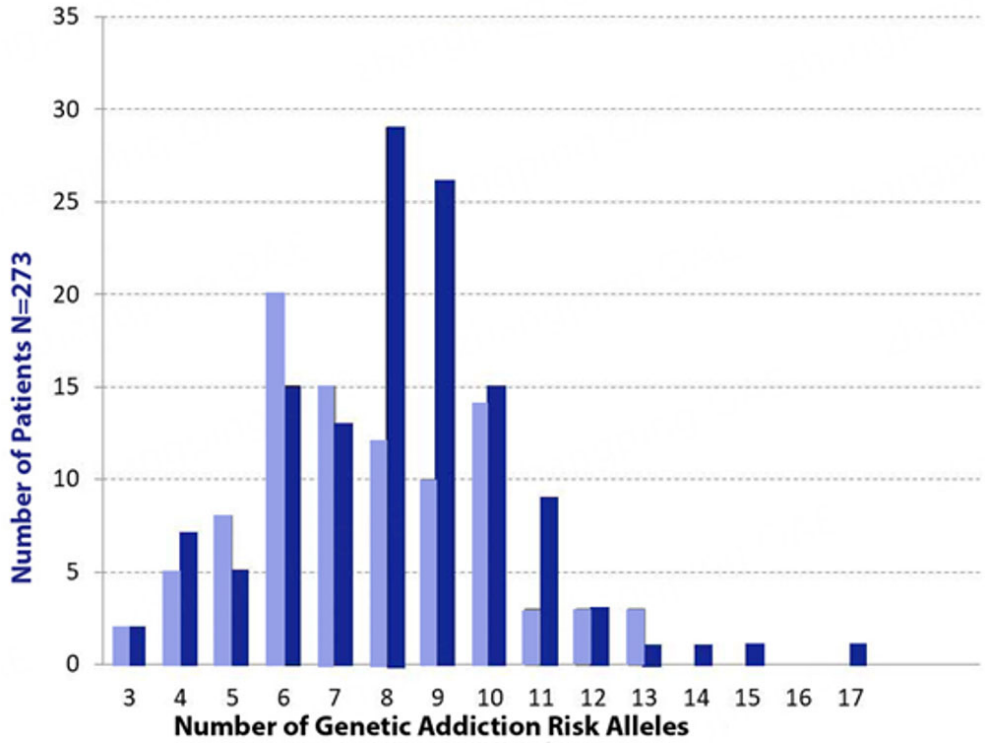


**Figure 3.** Distribution of genetic addiction risk alleles as a function of "low" and "high" alcohol severity ratings scores.

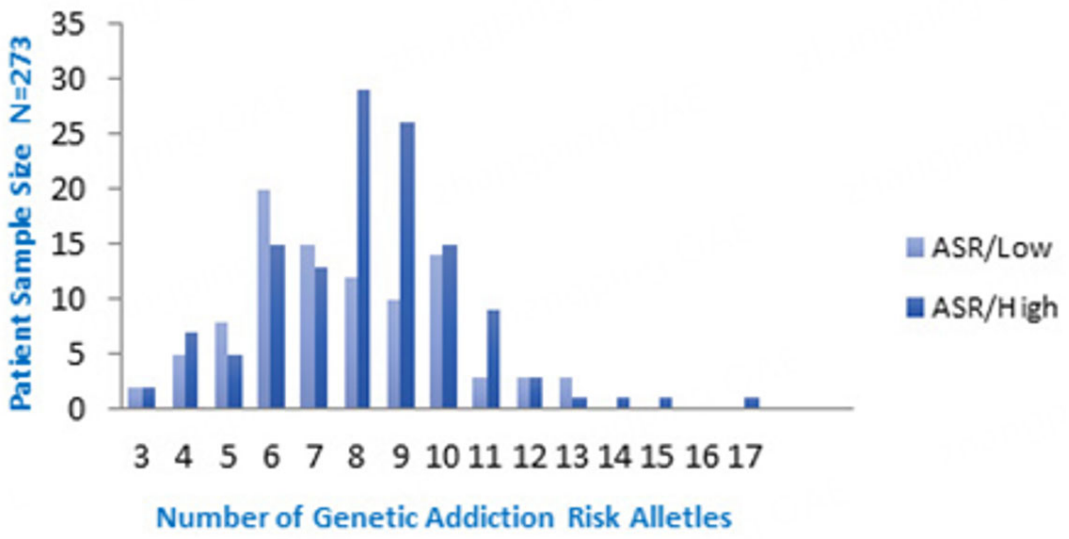


**Figure 4.** Distribution of genetic addiction risk alleles as a function of 'low" and "high" drug severity ratings scores.

severity[102]. Alternatively, a more complex independent pattern may emerge particularly given the heterogeneous nature of addictive substances and their varying effects on the dopaminergic system[103]. It is also possible that the obtained results are a mere reflection of the underreporting of illegal drug consumption as compared to a legal substance, alcohol. Regardless of the underlying factors, it is critical for clinical assessments of addicted patients to account for all classes of addictive substances for comprehensive psychopathology characterization and for formulation of optimal treatment plans. Importantly, patients with "high" alcohol severity ratings also had increased psychological, family, and medical but not economic or legal problems. A similar pattern was recognized for those scoring in the "high" range of drug severity ratings, that is to say, increased psychological and familial but not medical, economic, or legal problems. In contrast, we did not detect a significant difference in the diagnosed psychiatric disorders. Taken together, these observations support further inquiry into additional GARS' validity criteria along with disentanglement of inherited phenotypic traits vs. the ones that are acquired prior- or during addictive disorders.

## Caveats

Overall, our results support the contribution of a hypodopaminergic trait to the pathogenesis of alcohol and drug use disorders, with potential implications for other non-substance-related RDS behaviors. Nonetheless, in addition to the aforementioned heterogeneous drug consumption patterns, several other caveats are usefully stated in advance, including a relatively modest sample size, lack of complementary psychodiagnostic assessments alongside the ASI, such as the Structured Clinical Interview for DSM-5, or the lack of additional psychometric assessments, such as the RDS Questionnaire or a uniform and validated RDS severity score index[104]. Additionally, while the results are robust, uncontrolled environmental factors could have affected dopamine and other monoamine metabolism and function, including storage, catabolism, synthesis, transport, cellular distribution and concentration, neuronal release, synaptic clearance, and catabolism, as well as other intracellular gene and environmental interactions.

As with the identification of substance-related diagnoses, patient characterization factors such as comorbidities, medications, demographics, personality traits, and overall health status could also potentially confound the outcomes of this sort of genetic study. For example, the presence of depression, even if it

occurred long before the study, may alter the brain's reward system[105], as might the concurrent use of psychotropic medicines[106,107].

Given the substantial prevalence of depression[108], however, implementing this as an exclusion factor would likely rule out such a high percentage of patient candidates as to make clinical genetic studies unfeasible. An alternative way for evaluating the origin of the hyporesponsive reward system in alcohol and drug addiction is through examination of monozygotic twins discordant for alcohol/drug exposure or via prospective studies.

The ideal technique for risk stratification is still being debated. The utility of a specific test in identifying a risk factor within a population is often conveyed through measures such as the odds ratio or relative risk for complex behaviors, such as RDS, in individuals with positive versus negative test results. However, the ability of current tests, including the GARS panel, to predict RDS on an individual level remains limited. In general, odds ratios exceeding 15 to 20 are typically required to meaningfully impact individual risk prediction[109]. Such high odds ratios are rarely observed for individual dichotomous predictors or continuous covariates, even when there are significant differences between affected and unaffected groups.

A comprehensive evaluation of a risk marker or risk score requires analyzing multiple parameters that reflect distinct performance characteristics. Essential metrics include sensitivity, specificity, area under the receiver operating characteristic (ROC) curve (AUC) or C-statistic, informativeness of the criteria, clinical likelihood ratios, model calibration, and reclassification. Although ROC curves and AUC were initially developed for assessing diagnostic tests, they serve as valuable tools for determining the discriminative power of a risk estimator. In terms of the GARS test, we will continue our research utilizing "super controls" to allow for appropriately weighted genes and apply logistic regression analysis to determine odds ratios and ROC. However, the use of trichomization is informative and has been standard in the field of medicine as utilized by pencil and paper questionnaires like the ASI.

### Future directions

Our findings indicate that incorporating genetic models into routine clinical practice has the potential to enhance the personalization of preventive, diagnostic, and therapeutic interventions. However, a significant challenge lies in selecting appropriate tests, validating their clinical relevance, and identifying disorders that can be effectively applied to real-world patients. Since patient well-being cannot be adequately addressed through simplistic definitions, it is crucial to define suitable targets for RDS models. These targets should account for factors such as environmental, lifestyle, psychological, and neuroendocrine influences. Conversely, the use of inappropriate methodologies could lead to counterproductive outcomes, including inconclusive or irrelevant results. Furthermore, expanding the GARS panel to include additional candidate genes, such as those involved in the P450 enzyme system[110], FGF21[111], and APOE, could significantly enhance its clinical relevance and broaden its applicability.

### Understanding genetic testing

If early diagnosis leads to better treatment outcomes, then at what point should the proposed paradigm shift using genotyping be adopted? Federal and state mandates already require gene testing at birth for some conditions. Unlike rare diseases with limited treatment options (e.g., sickle cell anemia, Huntington's disease, congenital hypothyroidism, phenylketonuria, and galactosemia), RDS has effective therapeutic strategies[112]. Although RDS is not instantaneously life-threatening, early genetic testing could allow for timely diagnosis and non-pharmacologic interventions. It may transpire that exercise, diet, psychotherapy, behavioral interventions, or a safe and non-stimulant nutraceutical dopaminergic agonist therapy reduces the gene abnormality's impact[113]. For instance, attention deficit hyperactivity disorder (ADHD), a subtype

of RDS[114], is diagnosed in children and extends into adulthood. If not treated in a timely fashion, ADHD may constitute a risk factor for the development of substance use disorders[115]. Accordingly, even lay media (Bill Moyers of PBS) has suggested the heuristic value of early ADHD diagnosis for the prevention of the ensuing comorbid disorders. We propose that GARS be considered as a pre-diagnostic tool for early identification of RDS in our young people, including those with ADHD.

### Limitations

A common in psychiatric genetics, including this article, is the inadequate or incomplete screening of controls. While our results are promising, we encourage future studies involving a larger population. If indeed others perform genome-wide association study (GWAS) studies directed at all addictive substance and non-substance behaviors (RDS), we caution that all controls are extensively screened for hidden RDS behaviors like gaming, overeating, hoarding, *etc*. The lack of employing highly screened controls free of RDS behaviors, in our opinion, may be a significant flaw in current and previous studies. Understanding this caveat could significantly reduce spurious results.

### Statistical validation of GARS

The initial statistical evaluation concentrated on reward-related genes and their polymorphisms, predominantly associated with reduced dopamine activity within the mesolimbic brain reward circuitry. The GARS panel was assessed for its ability to predict risk for AUD using data from 74,566 case-control subjects[116]. Analysis of the identified risk alleles demonstrated significant odds ratios (OR) with 95% confidence intervals (CI), alongside an estimated population prevalence of alcoholism at 8%. Genes encoding dopamine receptors, including *DRD1*, *DRD2*, *DRD3*, and *DRD4*, as well as *DAT1*, *COMT*, *OPRM1*, and *SLC6A4*, exhibited significant associations with AUD risk compared to non-AUD controls, based on meta-analytic OR data. However, polymorphisms in GABRB3 and MAOA did not reach statistical significance, likely due to insufficient sample sizes.

Ongoing investigations into dopaminergic dysregulation underlying a hypothesized "pre-addiction" phenotype are currently under review. This research involves an extensive in silico analysis of GWAS data encompassing 88,788,381 samples from 1,373 studies, identifying 18 significant genes. Among these, APOE (*P*-value = 1.0E-126) was associated with pathways linked to opioid signaling, pain regulation, aging, and apoptosis. These findings are being integrated with the GARS test, highlighting key genes such as *MAOA*, *COMT*, *APOE,* and *SLC4A6* as central components of these pathways, as identified through STRING-based interaction modeling. Additionally, the analysis revealed interactions with microRNAs hsa-miR-16-5p and hsa-miR-24-3p, while expanding the role of SLC6A4 to include interactions with 27 unique genes. Pharmacogenomic analyses identified 1,173 variant annotations for these genes, corroborated by enrichment and meta-analytic studies.

Collectively, these findings emphasize the critical role of dopaminergic pathways in linking addictive behaviors with depressive symptoms. They further propose RDS as a foundational "pre-addiction" phenotype, with pain, opioid dependency, aging, and apoptosis emerging as significant endophenotypic contributors.

### Application of GARS in preaddiction screening

There is a critical need for the development of new therapies and reliable, safe, and effective screening methods for SUDs. Despite significant federal investment in research and treatment innovation over the past decades, treatment penetration rates remain below 25%[2]. A comparable situation occurred in the diabetes field, where early identification and intervention for prediabetes led to a substantial improvement in treatment penetration and outcomes[117,118]. The prediabetes model, which facilitates early detection and

timely intervention, has proven successful in slowing the progression of the disease[117,118]. Similarly, the emerging concept of "preaddiction" has been proposed for inclusion in the DSM. Preaddiction may reflect a disruption in hedonistic homeostatic regulation, such as hypodopaminergia in the mesolimbic reward system, and is linked to a range of neurochemical imbalances involving opioidergic, serotonergic, cannabinergic, GABAergic, glutaminergic, and cholinergic pathways[60,116,119]. Therefore, Genetic testing, such as the GARS test, could be employed to screen for individuals at risk of preaddiction, as it identifies genetic predispositions to addictive behaviors and has been shown to predict the severity of alcohol and drug use.

### Summary

Our study found a significant association between a ten-gene panel with eleven SNPs linked to a hypodopaminergic trait and ASI-MV alcohol and Drug severity scores. Seven or more reward-gene-polymorphisms were correlated with higher ASI-MV alcohol severity, while four or more were linked to higher ASI-MV drug severity scores. Replacing DAT 10 allele SNPs with DAT 9 allele SNPs, indicative of greater hypodopaminergic function, led to significant improvement. These genetic effects are additive and influenced by age, but gender was not a predictor of severity scores.

While the candidate gene approach may still be controversial[120], to our knowledge, this study is the first to correlate a panel of genes with polymorphisms reflecting the "Brain Reward Cascade"[121]. Future research should extend the GARS test to include more genes and polymorphisms linked to hypodopaminergic traits. The GARS test can be used to improve clinical interactions and decision making by reducing denial, validating family genetic patterns, and guiding assessment of addiction/relapse risk and treatment plans. Larger studies are needed for confirmation, and the inclusion of RDS "free controls" would undoubtedly strengthen the results and assist in determining accurate odds ratios for each allele.

Since our 1990 JAMA report linking the DRD2 Taq A1 allele to severe alcoholism, genetic candidate association studies, including GWAS, have proliferated. To identify individuals at risk for at least AUD, Blum's group developed a GARS test, which includes ten genes and eleven associated risk alleles, as described herein. We validated these risk alleles by analyzing studies from 1990 until 2021 involving 74,566 case-controls related to AUD. This analysis calculated the Hardy-Weinberg Equilibrium of each polymorphism in cases and controls and used Pearson's $\chi^2$or Fisher's exact tests for gender, genotype, and allele distribution. The analyses found the OR, 95%CI for OR, and a post-risk estimation of 8% for the population's alcoholism prevalence revealed a significant detection. The OR results showed significance for DAT1, DRD2, DRD3, DRD4, COMT, OPRM1, and 5HTT at 5%. While most GARS research is derived from our laboratory, we encourage more independent research to confirm our findings[71,120-144].

### Conclusion

This study identified a significant association between the GARS panel and the ASI-MV alcohol severity score, reinforcing previous findings that link dopaminergic function to alcoholism and substance abuse. The results also suggest that polygenic risk factors defined by GARS may be modulated by age-related pathophysiological and environmental factors. Future research, including GWAS, is warranted to explore the endophenotypes associated with RDS, which arise from a hypofunctional dopaminergic system.

## DECLARATIONS

### Acknowledgments

The authors appreciate the staff at Western University Health Sciences, Pomona, CA., USA and a number of individuals that contributed to this investigation: Carolee Lindsey, Joan Borsten, Elizabeth Winchel, Holly Cook, Adi Jaffe, Colin Hanna, Luis Llanos Gomez, Ashim Gupta, Stephanie Bickley, Aryeh R Pollack, Albert Villapiano, Lee McCormick, Edward Chapman, Marjorie C. Gondre-Lewis, and John Femino.

### Authors' contributions

This work was accomplished at Colorado University Bouler -Institute of Behavioral Genetics, under the direction of: Smolen A, Haberstick BC, Blum K
The first draft was written: Blum K, Smolen A, Haberstick BC
The statistics were accomplished: Haberstick BC, Han D
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All data were collected and housed at Dominion: Hauser M
The ASI Data were produced at INFEXXION: Bowirrat A
The figures were developed: Gilley G
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Following a number of draft revisions, each author contributed comments and edits as well.

### Availability of data and materials

The data involved in the study have been included in the main text. Additional raw data will be made available by the corresponding author upon reasonable request.

### Financial support and sponsorship

Dr. Blum K is one of NIH recipients of R41 MD012318/MD/NIMHD NIH HHS/United States.

### Conflicts of interest

The primary conflict of interest is that of the lead author Blum K. It is to be noted that Blum K is credited with an issued USA-GARS Patent and other patents on genetic testing for pre-addiction domestically and worldwide. Blum K is also credited with 22 GARS-issued European patents. Blum K has assigned these patents toTranspliceGen Holdings LLC and licensed them to TranspliceGen Therapeutics Inc. There are no other conflicts to report. Blum K is an Editorial Board member of *Journal of Translational Genetics and Genomics*. Blum K was not involved in any steps of the editorial process, including reviewer selection, manuscript handling, and decision making. The other authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Since the requested procedure does not involve invasive techniques on the patients in the investigation but only consists of a simple cheek cell swabbing and a pencil-paper questionnaire, it is considered exempt. All study protocols were reviewed and approved by the PATH FOUNDATION IRB, which issued an exemption notification. The participants provided written informed consent after the study procedures were thoroughly explained to them.

### Consent for publication

Not applicable.

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