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Genome-wide Association Study of a Quantitative Disordered Gambling Trait

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Abstract

Disordered gambling is a moderately heritable trait, but the underlying genetic basis is largely unknown. We performed a genome-wide association study (GWAS) for disordered gambling using a quantitative factor score in 1,312 twins from 894 Australian families. Association was conducted for 2,381,914 single nucleotide polymorphisms (SNPs) using the family-based association test in Merlin followed by gene and pathway enrichment analyses. Although no SNP reached genome-wide significance, six achieved P-values $< 1 \times 10^{-5}$ with variants in three genes (*MT1X*, *ATXN1* and *VLDLR*) implicated in disordered gambling. Secondary case-control analyses found two SNPs on chromosome 9 (rs1106076 and rs12305135 near *VLDLR*) and rs10812227 near *FZD10* on chromosome 12 to be significantly associated with lifetime DSM-IV pathological gambling and SOGS classified probable pathological gambling status. Furthermore, several addiction-related pathways were enriched for SNPs associated with disordered gambling. Finally, gene-based analysis of 24 candidate genes for dopamine agonist induced gambling in individuals with Parkinson's disease suggested an enrichment of SNPs associated with disordered gambling. We report the first GWAS of disordered gambling. While further replication is required, the identification of susceptibility loci and biological pathways will be important in characterizing the biological mechanisms that underpin disordered gambling.

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Author Contributions

WSS and NGM were responsible for the study concept. WSS provided phenotypic expertise. NGM, ACH, PAFM and GWM contributed to the genotypic data acquisition. PAL, GZ and NGM provided genotypic analysis expertise. Data were analyzed by PAL. PAL, WSS and NGM assisted with interpretation of findings. PAL and WSS drafted the manuscript. All authors approved final version for publication.

Keywords

association; disordered gambling; genomewide; MERLIN; quantitative

Introduction

There is emerging evidence suggesting that the propensity to gamble is heritable (Slutske et al., 2009b). Whether it be purchasing a lottery ticket, betting on the outcome of a horse race, or playing a slot machine, about half of the variation between people in the propensity to engage in a specific gambling activity can be attributed to genetic differences (Slutske et al., 2009b). The same is true of how much time or money one spends on gambling (Slutske et al., 2009b) as well as whether one develops problems as a consequence of one's gambling (Eisen et al., 1998; Slutske et al., 2010) -- that is, disordered gambling.

Disordered gambling (DG) refers to the full continuum of problems related to excessive gambling that include pathological gambling disorder, for example, as defined by the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 1994). There have been only two major twin studies of DG. In the all-male United States Vietnam Era Twin (VET) Registry, the lifetime rates of diagnosed DG disorder were significantly elevated among the monozygotic (23%) and dizygotic (10%) co-twins of men with DG, compared to the lifetime prevalence in the full sample (1.4%) (Eisen et al., 1998). Depending on the DG symptom cut-off imposed (from one or more to four or more symptoms), the heritability estimate ranged from 40–54% (Eisen et al., 1998). The other major twin study of DG was conducted with male, female, and opposite-sex twin pairs recruited from the Australian Twin Registry (ATR) (Slutske et al., 2010). The heritability estimate of DG in the ATR study ranged from 40–58% depending on the symptom cut-off imposed and did not significantly differ for men and women. Additionally, there was no evidence to suggest that there were sex differences in the specific genetic risk factors for DG. This study extended the findings of the previous VET registry study of men in the United States studied in the early 1990's to women and the heavier gambling culture of Australia.

There have been only a handful of published molecular genetic studies of DG to date (for a recent review, see Lobo and Kennedy, 2009). All of the studies have been candidate gene association studies -- there has not yet been a genome-wide linkage or association study of DG. The focus of most of the association studies has been one or more of the dopamine receptor genes (including *DRD1, DRD2, DRD3, DRD4, DRD5*) and the dopamine transporter gene (*DAT*), with at least one positive finding reported for *DRD1, DRD2*, and *DRD4*. Other candidate genes that have been the focus of association studies of DG are the serotonin transporter gene, the monoamine oxidase A and B genes, and the tyrosine hydroxylase gene, with at least one positive finding reported for the serotonin transporter and monoamine oxidase A genes. Although the positive findings are intriguing there have been no replicated results. It is difficult to draw any firm conclusions from so few molecular genetic studies of DG.

The association studies of DG to date have used a case-control design comparing patients that were affected with diagnosed DG disorder to unaffected controls (who were not diagnosed with DG disorder). The present investigation differs from the previous studies in four important ways: (1) participants were drawn from a community-based sample rather than from DG treatment programs, (2) the phenotype of interest was a quantitative continuous trait rather than a binary categorical DG diagnosis, (3) the use of single nucleotide polymorphism (SNP) chips enabled us to search for loci across the entire genome

rather than focusing on a selected number of candidate genes or a restricted set of markers, and (4) a genome-wide association analysis (GWAS) was supplemented with a follow-up analysis that focused on genes involved in putative biological pathways.

Use of a community-based sample may allow for greater generalizability of the results. Treatment-ascertained samples of individuals with DG are likely to be unrepresentative given the low rate of treatment-seeking for gambling disorders (~10–20% in the United States and Australia; Slutske et al., 2009a). Furthermore, the most effective use of the information that can be provided by a community-based sample is through the use of a continuous, quantitative DG phenotype. This is because even though very few individuals in a community-based sample will exceed the diagnostic threshold for a DG diagnosis many will provide valuable information about individual differences along the broader DG continuum. Previous studies that treated all individuals unaffected with DG disorder as being equivalent may have had considerable heterogeneity in their samples of unaffected controls. Recognizing the variation below the diagnostic threshold can turn this potential liability into an asset. The use of a quantitative DG phenotype is also consistent with the increasing appreciation for the idea of DG, along with many other psychiatric disorders as reflecting a continuum of pathology (Slutske et al., 2011). Finally, the use of a quantitative DG phenotype has the advantage of increased statistical power to detect genetic associations (Evans, 2009).

There have been a series of reports on the incidence of DG among individuals with Parkinson's disease and restless legs syndrome (for review, see Dagher and Robbins, 2009). These individuals were being treated with a dopamine agonist medication (that typically demonstrate relative selectivity for dopamine D_3 receptors) in combination with or without levodopa (an amino acid precursor of dopamine that shows greater selectivity for dopamine D_1 and D_2 receptors), and whose DG usually resolved with the discontinuation of the dopamine agonist therapy. These correlational findings are supported by experimental evidence from studies of rats and humans demonstrating that administration of a dopamine D_2/D_3 selective receptor agonist (Johnson et al., 2011) or the administration of levodopa in the presence of the 4/7 *DRD4* genotype (Eisenegger et al., 2010) increases gambling-like behaviors in the laboratory. These pharmacologic findings provide important clues to a potential neurobiological pathway to DG and might prove to be useful in gene identification; this information was incorporated into the follow-up gene enrichment analyses in the present study.

The aim of the present study was to present the results of the first genome-wide association study of a quantitative DG trait using data collected from a large community-based cohort of 1,312 Australian twins. We also report biological pathways associated with DG, which may provide new insights into the etiology of DG.

Methods

Participants

The participants for this study were members of the national community-based Australian Twin Registry (ATR) Cohort II (Slutske et al., 2009b). A structured telephone interview containing a thorough assessment of gambling behaviors was completed with 4,764 ATR Cohort II members (individual response rate of 80%). The mean age of the participants was 37.7 years (range = 32–43) and 57.2% of the sample was female. For more details about sample characteristics, participation rates, potential sampling biases, and zygosity determination, see Slutske et al (2009b). Re-interviews were conducted with 166 participants who had completed the baseline interview (follow-up interval M = 3.4 months, SD = 1.4 months, range = 1.2 – 9.5 months), with an oversampling of participants who

reported symptoms of DG, for the purpose of obtaining estimates of reliability of the study measures. Genotypic and phenotypic data were available for 1,312 of the 4,764 individuals who had completed the gambling assessment. Those individuals comprised 201 monozygotic (MZ) twin pairs (117 female and 84 male), 214 dizygotic (DZ) pairs (78 female, 49 male and 87 opposite sex), 50 unpaired MZ twins and 432 unpaired DZ twins from 894 families.

Genotyping and Imputation

DNA samples used in the current study were part of several different projects focused primarily on the genetics of nicotine and alcohol addiction, collected in accordance with standard protocols and were drawn from 5 of 10 Illumina GWAS subsamples (N = 19,257 individuals) genotyped from the Genetic Epidemiology laboratory at QIMR. DNA samples were genotyped using Illumina HumanCNV370-Quadv3 (N = 966) or Human610-Quadv1 (N = 346) single nucleotide polymorphism (SNP) BeadChips. Standard QC filters were applied and any SNP was removed unless it satisfied all of the following conditions: mean Illumina Beadstudio GenCall scores ≥ 0.7, SNP call rate > 0.95, minor allele frequencies (MAF) ≥ 1%, deviation from Hardy-Weinberg equilibrium (HWE) > 10^{-6}. Samples were also screened for ancestry outliers by using principal-component analysis. As described elsewhere (Medland et al., 2009), a consensus SNP set (N=271,069) common to all BeadChips was imputed HapMap CEU Phase I+II data (Release 22, Build 36) using MACH (Li and Abecasis, 2006), resulting in a dataset of 2,373,249 SNPs. Imputed SNPs were dropped if they had an Rsq imputation quality score < 0.3, MAF < 1% or showed significant deviations from HWE < 10^{-6} in the overall sample. Genotypes with Mendelian errors were set to missing. When only one individual from a monozygotic twin pair had been genotyped, the available genotype was used for both twins.

Measures

A quantitative disordered gambling phenotype was derived from four different indexes of non-disordered gambling involvement and two different inventories of DG that were contained in the structured interview. Indicators of non-disordered gambling involvement were included to provide a better characterization of the full DG continuum (i.e. non-diagnostic items might provide information about lower levels of the DG continuum that are not well differentiated by items used to establish a DG diagnosis) and to therefore obtain a more normally-distributed DG continuum.

Gambling involvement—Participants reported whether they had ever engaged in 11 different gambling activities (lottery, electronic gambling machines, instant scratch tickets, betting on horse or dog races, playing casino table games, keno, bingo, card games, betting on a sporting event, betting on games of skill, and internet casino gambling) in their lifetime. Gambling versatility was a composite continuous indicator of the number of different activities in which the respondent had ever been engaged, which reflects the extensiveness or diversity of the respondent's gambling involvement. The internal consistency and test-retest reliabilities of the gambling versatility index were 0.68 and 0.73, respectively (Slutske et al., 2009b). Three categorical indices of the frequency of gambling involvement were also included in this study: ever gambled at least once a month for at least six months in a row (test-retest reliability, $\kappa = 0.69$), ever gambled at least one a week for at least six months in a row (test-retest reliability, $\kappa = 0.77$), and ever gambled at least daily for at least two weeks in a row (test-retest reliability, $\kappa = 0.46$).

Disordered gambling—Symptoms of DG were assessed using two different measures: the National Opinion Research Center DSM-IV Screen for Gambling Problems(DSM-IV; Gerstein et al., 1999) and the South Oaks Gambling Screen (SOGS; Lesieur and Blume,

1987). The test-retest and internal consistency reliabilities of the 10-item DSM-IV lifetime symptom count (test-retest r = 0.86; coefficient alpha = 0.85) and the 20-item SOGS lifetime symptom count (test-retest r = 0.86; coefficient alpha = 0.81) were all high. Exploratory factor analyses provided strong and convincing evidence consistent with a single-factor model of DG for both the DSM-IV and the SOGS symptom sets (Slutske et al., 2011).

Data analysis

Development of a quantitative disordered gambling trait—Using the entire sample of 4,764 respondents, a single factor was extracted from the four indexes of non-disordered gambling involvement, the 10 items from the DSM-IV symptom set and the 20 items from the SOGS using Mplus (Muthén and Muthén, 1998–2004). The factor score derived from this analysis was used as the quantitative DG phenotype used in the genetic analyses. In the entire sample, the mean, standard deviation, skewness, and kurtosis of the factor score were 0.06, 0.78, 0.38, and −0.31, respectively; in the subsample included in the GWAS analyses, they were 0.13, 0.78, 0.32, and −0.21, respectively. The quantitative DG trait was approximately normally distributed, and the mean scores of the genotyped subsample were slightly higher than those of the full twin sample. The heritability of the quantitative DG trait was 0.52 (95% confidence interval 0.36 – 0.69).

SNP-based genome-wide association analyses—The modal genotype at each SNP was tested for association with the quantitative DG trait using the family-based association test in Merlin (Chen and Abecasis, 2007) (fastassoc) that accounts for family relationships and zygosity. The additive genetic effect was computed by modeling the genotypic mean of the heterozygote (Aa) as the average of the two homozygotes (AA, aa). Correction for sex, age, age^2, sex*age and sex*age^2 was performed by fitting covariates in the regression model. Phenotypes were also adjusted for possible effects of population stratification in our sample by fitting the first ten eigenvectors (PC1-PC10) from European-only principal components analysis of ancestry in the regression model (McEvoy et al., 2009). Association analyses of genotyped markers on the X-chromosome were conducted using Minx (as implemented in Merlin). Because the imputation software did not support sex chromosomes, SNPs at the X-chromosome are not imputed; the association analyses only included those SNPs that have been genotyped for at least 85% of the sample (N= 8,666). We adopted a genome-wide significance level for the association between SNP and phenotype of 7.2×10^{-8} or smaller to correct for the total number of independent tests (Dudbridge and Gusnanto, 2008).

Gene-based analyses—A gene-based test (VEGAS), feasible for use with GWA data with related individuals (Liu et al., 2010), was conducted to determine whether any genes harbored an excess of SNPs with small P-values. In brief, this test explores association on a per-gene basis taking the p-values of all SNPs within 50kb of each gene, as well as linkage disequilibrium (LD) and number of SNPs per gene into account. A p-value below $\alpha = 2.8 \times 10^{-6}$ was considered to be significant as the gene-based association test included 17,687 autosomal genes (0.05/17,687).

Enrichment analysis—We explored canonical pathway enrichment using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). The top 10,000 SNPs from the GWAS were mapped to genes and then assigned to canonical pathways. For each pathway, a P-value (the likelihood that the association between the genes in the dataset and the canonical pathway is explained by chance alone using a right-tailed Fisher's exact test) and ratio (the number of molecules from the data set that map to the pathway divided by the total number of molecules that map to the canonical pathway) were calculated. In WebGestalt (Duncan et al., 2010), the top 10,000 GWAS SNPs were compared to the

human genome reference set and assigned to Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment evaluation analysis using the hypergeometric test with the Benjamini-Hochberg test for multiple test adjustment. The significance cut-off was P = 0.05 and a minimum of 2 proteins were accepted per category. The resulting data are displayed as a "Ratio of Enrichment" (the number of genes in the GWAS dataset present in the KEGG pathway divided by the expected number of genes in the KEGG pathway) compared to the entire human genome.

Results

We conducted a genome-wide association study of a quantitative DG trait in 1,312 individuals from 894 Australian families. The average age of the genotyped sample was 37.6 years (SD = 2.3) for males and 37.9 years (SD = 2.3) for females. Males obtained a slightly higher DG factor score; the average (± SD) and range of DG scores for males was 0.304 ± 0.794 (−1.343 to 3.204) and for females was 0.048 ± 0.728 (−1.343 to 2.753). The distribution of the quantitative disordered gambling factor score by sex and lifetime DSM-IV disordered gambling status is provided in Supplementary Figure S1.

The Manhattan plot of association p-values for 2,381,914 autosomal and X chromosome SNPs is shown in Figure 1. While no SNP achieved genomewide significance ($P < 7.2 \times 10^{-8}$), regions of suggestive association ($P < 1 \times 10^{-5}$) were observed on chromosome 6p23, 9p24, 12q24 and 16q13. The quantile-quantile plot (Q-Q plot) of the observed versus expected $-\log_{10}$(P-value) from the association analysis is presented in Figure 2. The genomic control λ (0.997) was close to 1.0, indicating that there was no evidence for inflation of the test statistics or a bias due to possible population stratification in the results (Bacanu et al., 2000) and that the family based association model had correctly accounted for relatedness.

The most significant SNPs are presented in Table 1, at a threshold of $P < 1 \times 10^{-5}$ after excluding redundant SNPs that are in high LD ($r^2 > 0.8$) with more significant SNPs. Three SNPs were located in two genes, *FLJ35024* and *ATXN1,* but none were exonic. The remaining SNPs were located downstream of *MT1X* and nearby (within 50 Kb) to *VLDLR* and *FZD10*. Regional association plots for the 6 SNPs are provided in the Supplementary material (Figures S2–S7). While these SNPs almost explain 11% of the variance in DG, this result is most likely inflated because the estimate of the genetic effect explained by top SNPs in smaller samples, such as our study of 1,312 twins, is significantly overestimated (the 'winner's curse') (Zollner and Pritchard, 2007). Secondary case-control analyses for the six SNPs were performed in a sub-sample of unrelated twins comprising (a) 31 lifetime DSM-IV pathological gamblers and 863 controls; (b) 44 SOGS classified probable pathological gamblers and 850 controls; (c) 101 SOGS classified problem gamblers or probable pathological gamblers and 793 controls; and (d) 425 individuals that met criteria for ≥ 1 (out of 30) DSM-IV or SOGS symptom and 469 individuals that scored 0 symptoms (see Supplemental Table S1). The results for the first three analyses were similar where SNPs on chromosome 9 (rs1106076 and rs12305135 near *VLDLR*) and 12 (rs10812227 near *FZD10*) remained significant following 10,000 permutations. For example, DSM-IV pathological gamblers were ~2.5 times more likely to carry rs1106076 and rs12305135 C-alleles than controls (empirical P = 0.036 and 0.027, respectively) and 5.6-fold less likely to carry the rs10812227 T-allele (OR: 0.18, 95% CI: 0.04–0.75, empirical P = 0.0019). A different pattern of association was observed for the '≥1 DSM-IV/SOGS symptom' phenotype, where three SNPs (rs9383153 in *ATXN1*, rs8064100 downstream of *MT1X* and rs12305135 near *VLDLR*) remained significant following permutation analysis. The strongest evidence of association was with rs9383153 where cases were 2.4-fold less likely to carry the rs9383153 G-allele (OR: 0.42, 95% CI: 0.26–0.67, empirical P = 0.0007).

To complement our genome-wide association analysis, we performed genome-wide gene-based tests using VEGAS. The entire GWAS SNP dataset was assigned to genes according to their positions on the UCSC Genome Browser hg18 assembly, with gene boundaries defined as ±50 Kb beyond the 5′ and 3′UTRs. The gene-based analysis did not reveal significant results that survived correction for multiple testing ($P < 2.8 \times 10^{-6}$), with the smallest empirical P-value being 0.00028 for *PNMA1*. We list the 50 genes most associated with the quantitative DG factor score in Supplemental Table S2. Gene names, ranks and P-values, as well as the top SNP for each gene, are provided. The most notable result was the third ranked *CDKRAP2*, a gene associated with three traits measuring an individual's level of response to alcohol (an endophenotype for alcohol use disorders) (Joslyn et al., 2010).

We then tested a candidate gene set derived from a candidate gene study for pathological gambling by Comings et al. (2001) and literature on dopamine agonist induced disordered gambling. Supplemental Figure S8 illustrates the interactions between dopamine, dopamine agonists (cabergoline, levodopa, pergolide and pramipexole) and the proteins encoded by the 24 candidate genes. Gene-based results are shown in Table 2, with *ADRA2C* and *CREB1* achieving P-values < 0.05 but no gene was associated with P < 0.0021 (0.05/24). However, the average gene ranking for the 24 genes was 6645. In R, we randomly sampled sets of 24 genes 10,000 times and empirically observed that an average ranking of 6645 was higher than expected by chance (P = 0.0165). As shown in the Q-Q plot in Supplementary Figure S9, the distributions of P-values for this candidate gene set (2,160 SNPs) was enriched for association with DG. First, the shape of the Q-Q plot suggests that there is an inflation of weak associations with SNPs of small effect that do not reach genome-wide significance in the full GWAS SNP because of power constraints and second, enrichment is reflected by the high λ value of 1.503 compared to 0.997 in the full GWAS analysis.

Next we tested whether genes with the strongest association signals were enriched within canonical or biological pathways. We performed canonical pathway analyses including the top 10,000 SNPs (P-values < 0.0047) using Ingenuity Pathway Analysis. The most significant pathways (P < 0.01) and the dopamine receptor signaling and Parkinson's signaling pathways are summarized in Table 3. Three pathways (synaptic long term potentiation, synaptic long term depression, gonadotrophin releasing hormone [GNRH] signaling) had been previously identified by Li and Wei (Li et al., 2008) to be enriched for substance addiction-related genes, with the synaptic long term depression and GNRH signaling pathways common to cocaine, alcohol, opioid and nicotine addiction. It should be noted that many of the genes in these three pathways overlap (see Supplemental Table S3). Supplemental Table S4 shows the results of secondary KEGG pathway enrichment analyses, respectively, using WebGestalt. Five of the ten most enriched KEGG pathways listed overlapped the canonical pathways summarized in Table 3. A sixth KEGG pathway (gap junction) was common to all four addictive substances investigated by Li and Wei (Li et al., 2008).

Discussion

We performed the first genome-wide association analysis of disordered gambling (DG) reported to date in a community-based sample of 1,312 Australian twins from 894 families using a quantitative factor score derived from four indexes of non-disordered gambling involvement and symptoms of DG assessed by the National Opinion Research Center DSM-IV Screen for Gambling Problems and South Oaks Gambling Screen. We did not detect genome-wide significant SNPs and the quantile-quantile plot (Figure 2) shows that the distribution of observed associations closely follows that expected under the null hypothesis of no association. Our finding is consistent with the majority of GWAS of addictive behavior in larger European ancestry samples that have failed to identify genome-wide

significant variants of large effect for alcoholism (Heath et al., 2011; for review, see Treutlein and Rietschel, 2011), cigarette smoking behaviors (Liu et al., 2009; Uhl et al., 2010; Vink et al., 2009), nicotine dependence (Bierut et al., 2007) and heroin dependence (for review, see Treutlein and Rietschel, 2011).

Four of six independent SNPs showing suggestive association with the quantitative DG score ($P < 1 \times 10^{-5}$) were located in or nearby genes that appear to be theoretically relevant to disordered gambling. The top GWAS hit (rs8064100) is located downstream of metallothionein 1X (*MT1X*) and nearby to a cluster of five metallothionein 1 (MT1) gene family members. *MT1* expression occurs primarily in astrocytes and to a lesser degree in neurons (Xie et al., 2004). *MT1X* has been previously reported to play a role in addiction, including alcohol and opioid dependence (Li et al., 2008), while *MT1* expression is associated with anxiety (Czibere et al., 2011) and a neuroprotective role in MDMA-induced toxicity to dopamine neurons in mice (Xie et al., 2004). Furthermore, *MT1* expression in rats is induced throughout the brain by physical stress and by dopamine in neurons (Gasull et al., 1994). In our gene-based test *MT1X* gene had a P-value of 0.0046 (ranked 77).

The variant rs9383153 was located in the seventh intron of *ATXN1* (ataxin 1), previously known as *SCA1* (spinocerebellar ataxia type 1 protein), on chromosome 6p23. *ATXN1* plays a key role in spinocerebellar ataxia, type 1, a neurodegenerative disease characterized by loss of motor coordination (ataxia), dysarthria and mild cognitive impairment (Zoghbi and Orr, 2009). There is also evidence that *ATXN1* is involved in alcohol dependence where *ATXN1* expression is down-regulated in post-mortem frontal and motor cortices of human alcoholics (Mayfield et al., 2002) and in successful smoking cessation (Uhl et al., 2009). ATXN1 has also been shown to occupy the dopamine 2 receptor (DRD2) promoter *in vivo* and to regulate *DRD2* gene expression in Purkinje cells of the cerebellum in mice (Hearst et al., 2010). In our gene-based test *ATXN1* gene had a P-value of 0.182 (ranked 2,910).

Two SNPs (rs12237653 and rs10812227) were located upstream within 50 Kb of *VLDLR*, the very low density lipoprotein receptor gene on chromosome 9p24. VLDLR is a receptor for Reelin and the Reelin-VLDLR/ApoER2 signaling pathway controls cortical neuronal migration in early development and modulates synaptic plasticity, memory and learning in the adult brain (Herz and Chen, 2006). Furthermore, the signaling pathway has been postulated to contribute to the pathophysiology of schizophrenia (Suzuki et al., 2008), bipolar and unipolar depression (Barr et al., 2007).

We next analyzed the GWAS data using a systems biology strategy. First we performed the VEGAS gene-based test to determine whether any genes harboured an excess of variants with weak associations. The gene enrichment analysis did not show any study-wide significant gene hits for DG. We therefore proceeded to the pathway analysis, which is able to mine a wider range of association results by testing for over-representation of genes within pre-defined pathways without restricting the search to ‘significant’ SNPs. This led to identification of 11 enriched canonical pathways listed in Table 3 for DG. Three pathways (synaptic long term potentiation, synaptic long term depression and GNRH signaling) have been previously implicated in aspects of alcohol and nicotine dependence and addiction to opioids and cocaine. The enriched KEGG gap junction pathway has been identified as a common molecular pathway for all four drugs of abuse (Supplemental Table S4).

The observation that DG and substance dependence share enriched pathways is supported by a proposed change to the fifth revision of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) where pathological gambling will be included as a non-substance (or behavioural) addiction in a section labelled ‘Addiction and Related Disorders’ (O’Brien, 2011). Brain imaging and neurochemical studies have shown that gambling activates the

same reward system in the brain as addictive drugs with problem casino gamblers showing increases in dopamine, a key 'reward' neurotransmitter in the brain (Holden, 2010). It is hypothesized that different substances of abuse (and hypothetically by extension, behavioral addictions) act on different receptors yet activate common downstream signaling cascades and events (Ron and Jurd, 2005) and that there may be common neurochemical substrates and neuronal circuits for pathological gambling and addiction (Mutschler et al., 2010).

Synaptic long term depression (LTD) and long term potentiation (LTP) are the activity-dependent weakening and strengthening of synaptic transmission respectively, and are essential for synaptic plasticity. Synaptic plasticity underlies neural adaptation to substances of abuse and is thought to be important in the development of maintenance of addictions (for review, see Kauer and Malenka, 2007). Gonadotropin-releasing hormone (GnRH) controls the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Elevated levels of gonadotropins (LH and FSH) are reported in male chronic alcoholics (Heinz et al., 1995).

Turning to other enriched pathways, the axonal guidance signaling pathway is critical for neurodevelopment and has also been implicated in neuroadaptive responses elicited by addictive drugs (Jassen et al., 2006). Guanine nucleotide-binding protein (G-protein) coupled receptors (GPCRs) are a large protein family that regulate physiological responses to a spectrum of biologically active substances such as dopamine and glutamate (Gainetdinov et al., 2004). The glutamate receptor signaling pathway has been shown to play a role in pathological gambling. Two small pharmacotherapy studies of glutamate-modulating agents (N-acetyl cysteine and memantine) resulted in treated pathological gamblers showing reduced Yale Brown Obsessive Compulsive Scale Modified for Pathological Gambling scores (Grant et al., 2007) as well as reduced hours spent gambling per week and money spent gambling (Grant et al., 2010). Interestingly, while there seems to be no relationship between our most enriched pathway, neuropathic pain signaling in dorsal horn neurons, and addiction, another study also identified this pathway as significantly overrepresented (ranked 6th) among addiction-related genes (Sun and Zhao, 2010).

A number of limitations must be kept in mind in considering the present results. First is the comparatively small sample size. Second, it is clear that larger sample sizes are needed to achieve the power required to detect common variants of smaller effect and that the reported size of effects for the six top GWAS SNPs are most certainly overestimates. Power simulations in Merlin showed that our sample had 80% power and 92% power to detect genome-wide significant SNPs explaining 3% and 3.5% of variance in DG respectively. Third, the gene and pathway enrichment analyses are gene centric and do not include markers located in intergenic regions of the genome. A final feature, which could be considered either a strength or a limitation, is recruitment of subjects from the general population rather than a clinical source. The severity of disordered gambling may well be less among a population-based sample, but most gambling-related problems occur in the large number of people who are only moderately affected.

In summary, we report results from the first GWAS of DG using a quantitative factor score. We identified three loci (*ATXN1* and two intergenic loci located near *MT1X* and *VLDLR*) for DG with highly significant evidence of association ($P < 1 \times 10^{-5}$). These loci are novel with respect to DG and will complement the findings of candidate gene studies in the literature. We also report biological pathways enriched in DG that have been previously associated with substance addiction. Our findings offer the potential for new insights into the etiology of DG and will serve as a resource for replication in other studies to clarify the potential role of variants in these genes and pathways with DG.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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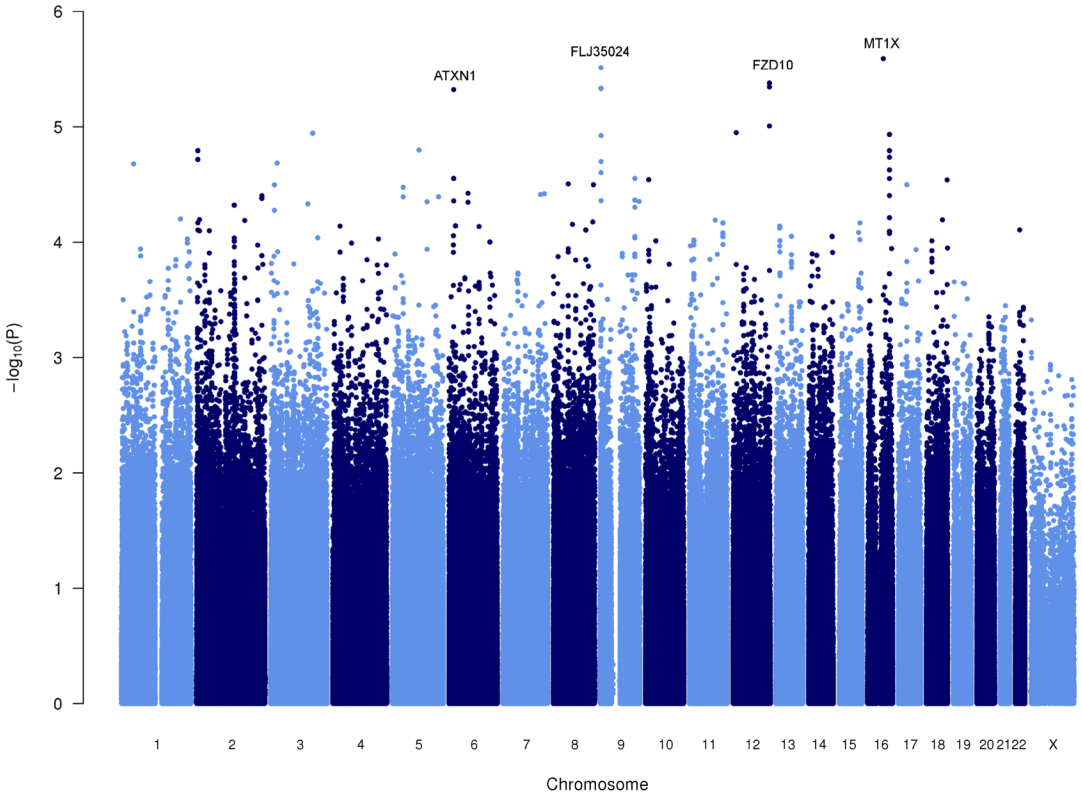

Figure 1.
Manhatten plot for the quantitative disordered gambling factor score (DG). The vertical axis shows the $-\log_{10}$ of the association P-values and the horizontal axis shows the whole autosomal genome divided into 22 autosomes and the X chromosome.

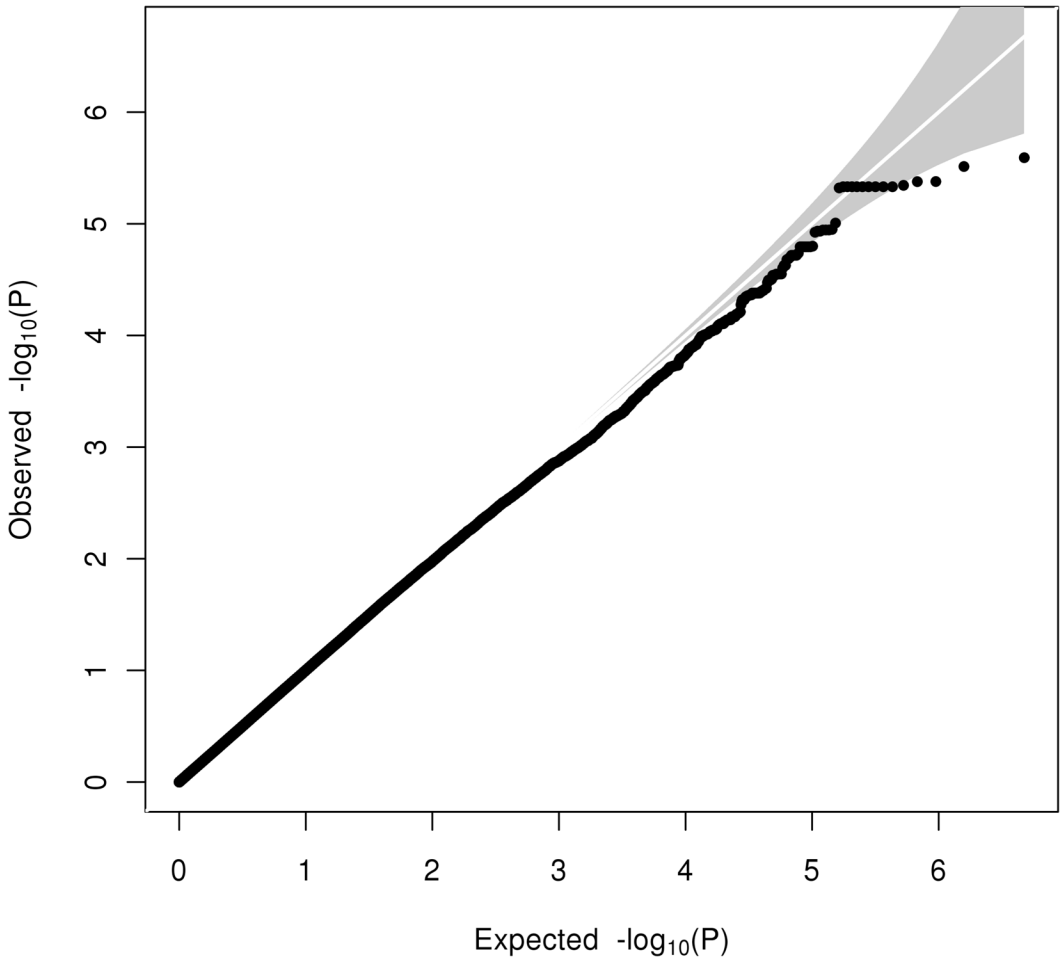

Figure 2.
Quantile-Quantile (Q-Q) plot for the quantitative disordered gambling factor score (genomic inflation $\lambda = 0.997$). The horizontal axis shows the $-\log_{10}$ of expected P-values of association from a 1 d.f. chi-square distribution and the vertical axis shows the $-\log_{10}$ of P-values from the observed chi-square distribution. The shaded region represents the 95% confidence interval of the expected chi-square statistics under the null hypothesis of no association.

Table 1

Variants showing the strongest association with the quantitative disordered gambling factor score (independent markers with $P < 1 \times 10^{-5}$).

Chr	SNP	Position	SNPs in LD	Gene	Location	Nearby Gene	A1	Freq	β	SE	R2 (%)	Pcorr
16q13	rs8064100	55,282,674		*MT1X*	Downstream	*MT1IP, MT1H, MT1G, MT1F, MT1B, NUP93*	A	0.582	−0.148	0.031	1.89	2.57E-06
9p24	rs12237653	2,541,654		*FLJ35024*	Intron	*VLDLR*	T	0.868	0.225	0.048	2.06	3.08E-06
12q24	rs11060736	129,196,888	2			*FZD10, FLJ31485*	T	0.941	−0.285	0.062	1.60	4.19E-06
9p24	rs10812227	2,538,556	8	*FLJ35024*	Intron	*VLDLR*	C	0.868	0.221	0.048	1.98	4.67E-06
6p23	rs9383153	16,451,035		*ATXN1*	Intron	*GMPR*	A	0.948	0.331	0.072	1.91	4.79E-06
12q24	rs12305135	129,201,158				*FZD10, FLJ31485*	T	0.933	−0.259	0.059	1.49	9.87E-06

Note: The linkage disequilibrium (LD) between independent markers is $r^2 < 0.8$. *SNPs* in *LD* is the number of correlated SNPs with P-values $< 1 \times 10^{-5}$. *Location* is the physical location of the SNP within the gene. *Nearby Gene* is the gene(s) closest to the SNP (cells are empty if no gene is within ±50 kb). *A1* is the reference allele, and *Freq* is the frequency of the reference allele, *β* shows the mean increase in the disordered gambling factor score per added reference allele controlling for sex, age, age^2, sex*age, sex*age^2 and the first ten eigenvectors (PC1–PC10) from European-only principal components analysis of ancestry, *SE* gives the standard error of the β-coefficient, *R2* represents the percentage of phenotypic variance explained by the SNP, and *Pcorr* gives the P-value of association controlling for genomic inflation ($\lambda = 0.9972$).

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Table 2

VEGAS gene rankings and P-values of 24 candidate genes for disordered gambling.

Gene Rank	Test statistics for Disordered Gambling candidate genes						Test statistics for the SNP most strongly associated within each candidate gene				
	Position	Symbol	Gene	Size (bp)	# SNPs	P-value	SNP	P	Allele		SE
8456	22q11.23	*ADORA2A*	Adenosine A2a receptor	14,796	77	0.532	rs8141793	0.05375	G	0.133	0.069
257	**4p16.3**	***ADRA2C***	**Adrenergic, alpha-2C-, receptor**	**1,958**	**29**	**0.015**	rs11725040	**0.03658**	C	−0.068	0.032
4993	22q11.21	*COMT*	Catechol-O-methyltransferase	27,222	130	0.305	rs2531716	0.01827	T	−0.087	0.037
370	**2q34**	***CREB1***	**cAMP responsive element binding protein 1**	**68,885**	**91**	**0.022**	rs12998817	**0.00233**	C	−0.096	0.032
9588	7p11	*DDC*	Dopa decarboxylase (aromatic L-amino acid decarboxylase)	107,021	251	0.596	rs10235371	0.01231	C	0.14	0.056
4869	5q34	*DRD1*	Dopamine receptor D1	3,489	136	0.309	rs251937	0.0132	T	0.086	0.035
3309	11q22	*DRD2*	Dopamine receptor D2	65,685	197	0.244	rs17529477	0.00546	G	0.103	0.037
6526	3q13.3	*DRD3*	Dopamine receptor D3	50,343	143	0.387	rs7620955	0.03214	C	−0.074	0.034
17202	11p15.5	*DRD4*	Dopamine receptor D4	3,399	46	0.982	rs6598007	0.09252	C	0.232	0.138
8007	4p16.1	*DRD5*	Dopamine receptor D5	2,376	88	0.516	rs1519094	0.05932	C	0.15	0.079
8020	14q24.3	*FOS*	FBJ murine osteosarcoma viral oncogene homolog	3,382	53	0.521	rs6574222	0.05985	G	−0.072	0.038
8522	9q34.3	*GRIN1*	Glutamate receptor, ionotropic, N-methyl D-aspartate 1	29,600	18	0.519	rs12238250	0.05361	A	0.159	0.082
5052	12p12	*GRIN2B*	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B	418,613	659	0.312	rs10772723	0.00169	C	−0.1	0.032
11842	5q11.2	*HTR1A*	5-hydroxytryptamine (serotonin) receptor 1A	1,269	41	0.693	rs13361335	0.1444	T	0.073	0.05
7210	13q14	*HTR2A*	5-hydroxytryptamine (serotonin) receptor 2A	62,663	212	0.451	rs2094591	0.0405	A	0.071	0.035
1408	2q36.3	*HTR2B*	5-hydroxytryptamine (serotonin) receptor 2B	16,870	45	0.087	rs13424110	0.03594	C	0.122	0.058
7891	9q34.11	*NCS1*	Neuronal calcium sensor 1	64,727	160	0.481	rs2240913	0.01547	A	0.081	0.033
7830	14q24.3	*PSEN1*	Presenilin 1	83,931	135	0.477	rs362353	0.04441	A	0.144	0.071
15404	8p21.3	*SLC18A1*	Solute carrier family 18 (vesicular monoamine), member 1	38,346	147	0.173	rs2410639	0.01301	G	−0.202	0.081
2765	10q25	*SLC18A2*	Solute carrier family 18 (vesicular monoamine), member 2	36,380	158	0.477	rs363241	0.01835	T	0.079	0.033
10164	5p15.3	*SLC6A3*	Solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	52,629	114	0.617	rs7732456	0.02884	A	0.254	0.116
7034	17q11.2	*SLC6A4*	Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	37,810	75	0.464	rs2020941	0.02856	C	−0.32	0.146

Gene Rank	Test statistics for Disordered Gambling candidate genes						Test statistics for the SNP most strongly associated within each candidate gene				
	Position	Symbol	Gene	Size (bp)	# SNPs	P-value	SNP	P	Allele		SE
1905	11p15.5	*TH*	Tyrosine hydroxylase	7,877	111	0.116	rs2070762	0.00121	G	−0.104	0.032
859	12q15	*TPH2*	Tryptophan hydroxylase 2	93,596	215	0.053	rs11179002	0.00698	C	−0.099	0.037

#*SNPs* is the number of SNPs in each gene (± 50 Kb beyond the 5′ and 3′UTRs), *P* gives the P-value of association for the SNP, *Allele* is the reference allele, β shows the mean increase in the disordered gambling factor score per added reference allele controlling for sex, age, age^2, sex*age, sex*age^2 and the first ten eigenvectors (PC1–PC10) from European-only principal components analysis of ancestry, *SE* gives the standard error of the β-coefficient.

Table 3

Enrichment of Ingenuity canonical pathways for disordered gambling.

Rank	Canonical Pathway	P-value	Ratio	Genes
1	Neuropathic Pain Signaling In Dorsal Horn Neurons	0.000023	0.18	*GRIN2B,CAMK1D,GRM1,ITPR2,ITPR1,GRIA4,PRKAG1,GRM5,TACR1,GRM7,CAMK2D,PRKAR2B,PRKAG2,PLCB1,KCNQ3,PRKCH,PRKD1,PRKCA,PRKCB*
2	Synaptic Long Term Potentiation	0.000023	0.17	*PPP1R14C,GRIN2B,GRM1,PPP1R1A,ITPR2,CACNA1C,ITPR1,GRIA4,PRKAG1,GRM5,GRM7,CAMK2D,PRKAR2B,PRKAG2,PLCB1,PRKCH,PRKD1,PRKCA,PRKCB*
3	Synaptic Long Term Depression	0.000076	0.14	*GUCY1A3,GRM1,ITPR2,GRID2,RYR2,GNAI1,ITPR1,GRIA4,GRM7,GRM5,PRKG1,LCAT,PPP2R2B,RYR3,IGF1R,PLCB1,PRKCH,PRKD1,PRKCA,PRKCB*
4	CREB Signaling in Neurons	0.0021	0.10	*GRIN2B,ADCY2,GRM1,ITPR2,GRID2,GNAI1,ITPR1,GRIA4,GNG5,PRKAG1,GRM7,GRM5,PRKAR2B,CAMK2D,PRKAG2,PLCB1,PRKCH,PRKD1,GRIK1,PRKCA,PRKCB*
5	Breast Cancer Regulation by Stathmin1	0.0030	0.11	*PPP1R14C,ADCY2,CAMK1D,ITPR2,ARHGEF7,GNAI1,ITPR1,TUBA1B,PRKAG1,GNG5,PRKAR2B,CAMK2D,TUBA1A,ARHGEF10,PPP2R2B,PRKAG2,PLCB1,PRKCH,ARHGEF3,PRKD1,PRKCA,PRKCB*
6	GNRH S ignaling	0.0049	0.11	*ADCY2,ITPR2,MAP3K1,GNAI1,ITPR1,MAP3K4,PRKAG1,CAMK2D,PRKAR2B,PRKAG2,PLCB1,PRKCH,PRKD1,PRKCB,EGFR,PRKCA*
7	α-Adrenergic Signaling	0.0049	0.12	*ADCY2,ITPR2,GNAI1,ITPR1,GNG5,PRKAG1,PRKAR2B,PRKAG2,PRKCH,ADRA1A,PRKD1,PRKCA,PRKCB*
8	Hepatic Cholestasis	0.0049	0.10	*ADCY2,MAP3K4,IL1R1,PRKAG1,IL1F9,PRKAR2B,IL1RL2,ABCC1,PRKAG2,PRKCH,INSR,SLCO1B1,HNF4A,IRAK4,PRKD1,PRKCB,PRKCA*
9	Axonal Guidance Signaling	0.0056	0.08	*LRRC4C,ADAM22,EPHB2,NFATC3,ARHGEF7,ROBO1,PRKAG1,EFNA5,ABLIM3,DCC,UNC5D,PLCB1,PRKD1,GLIS1,PRKCA,SEMA5A,GNAI1,NFATC1,SLIT2,TUBA1B,BMP5,PLXND1,GNG5,DOCK1,PRKAR2B,TUBA1A,GLIS2,NTRK3,SEMA6D,PRKAG2,PRKCH,PDGFD,BMP6,PRKCB*
10	Glutamate Receptor Signaling	0.0058	0.15	*GRM5,GRM7,GRIN2B,SLC1A6,GRM1,GRID2,SLC1A2,GRIA4,GNG5,GRIK1*
11	Melatonin Signaling	0.0062	0.14	*PRKAR2B,CAMK2D,RORA,GNAI1,PRKAG2,PLCB1,PRKCH,PRKAG1,PRKD1,PRKCA,PRKCB*
64	Dopamine Receptor Signaling	0.275	0.08	*PPP1R14C,TH,ADCY2,PRKAR2B,PPP2R2B,PRKAG2,PRKAG1*
164	Parkinson's Signaling	0.951	0.06	*PARK2*

Enrichment analysis was performed in Ingenuity Pathway Analysis using the top 10,000 SNPs from the GWAS. The 15 most enriched canonical pathways are listed, as well as the dopamine receptor signaling and Parkinson's signaling candidate pathways. *P-value* is the P-value of enrichment adjusted for multiple testing using the Benjamini-Hochberg test, *Ratio* is the number of genes in the GWAS dataset compared to the total number of genes in the pathway, *Genes* is the list of pathway genes present in the GWAS dataset.