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Genome-wide association study of pathological gambling

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ABSTRACT

Background: Pathological gambling is a behavioural addiction with negative economic, social, and psychological consequences. Identification of contributing genes and pathways may improve understanding of aetiology and facilitate therapy and prevention. Here, we report the first genome-wide association study of pathological gambling. Our aims were to identify pathways involved in pathological gambling, and examine whether there is a genetic overlap between pathological gambling and alcohol dependence.
Methods: Four hundred and forty-five individuals with a diagnosis of pathological gambling according to the Diagnostic and Statistical Manual of Mental Disorders were recruited in Germany, and 986 controls were drawn from a German general population sample. A genome-wide association study of pathological gambling comprising single marker, gene-based, and pathway analyses, was performed. Polygenic risk scores were generated using data from a German genome-wide association study of alcohol dependence.
Results: No genome-wide significant association with pathological gambling was found for single markers or genes. Pathways for Huntington's disease (P-value = 6.63×10^{-3}); 5′-adenosine monophosphate-activated protein kinase signalling (P-value = 9.57×10^{-3}); and apoptosis (P-value = 1.75×10^{-2}) were significant. Polygenic risk score analysis of the alcohol dependence dataset yielded a one-sided nominal

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significant *P*-value in subjects with pathological gambling, irrespective of comorbid alcohol dependence status.
Conclusions: The present results accord with previous quantitative formal genetic studies which showed genetic overlap between non-substance- and substance-related addictions. Furthermore, pathway analysis suggests shared pathology between Huntington's disease and pathological gambling. This finding is consistent with previous imaging studies.
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1. Introduction

Although gambling is common to most cultures, only a proportion of individuals develop pathological gambling (PG) resulting in negative psychological, social, and economic consequences for the affected individuals and their social network [1]. Among adults, reported prevalence rates for PG range between 0.02 and 2.0% [2]. In the German population, the prevalence of PG has been estimated to be 0.3% [3]. Identifying the biological causes of PG may facilitate prevention and treatment.

The precise diagnostic classification of PG is still evolving. In previous decades, PG was classified as an impulse control disorder. However, accumulating research evidence suggests that PG resembles substance-related addictions in many domains [4]. As a result, PG (renamed as gambling disorder) is now classified in the 5th edition of the American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders* as a non-substance-related behavioural addiction [5].

PG is more prevalent in males compared to females [6]. Risk factors are low socioeconomic status [7], immigration [8], and high impulsivity combined with emotional instability [9]. PG shows comorbidity with other mental health disorders, including mood and anxiety disorders, personality disorders, alcohol dependence (AD), and substance use [10–12]. The reasons for this comorbidity are unclear. Twin studies of PG have generated insights into comorbidity with various alcohol use disorders [13–15]. However, the focus of these studies was not to investigate patients with PG but rather to determine correlations between the full continuum of gambling related problems and alcohol use disorders. These studies were performed in the general population [13,15], and in a male sample from the United States Vietnam Era Twin Registry [14]. Disordered gambling (DG) was defined as the presence of one or more gambling related problems, including PG. Genetic correlation between DG and alcohol use disorders ranged between 30 and 45%, indicating the strength of the correlation between the genetic liabilities [13–15].

Twin studies have also investigated the genetic mechanisms underlying PG [14,16–20]. Research has shown that genes are of equal importance in the aetiology of DG in men and women [16,17]. Their findings also suggest that genetic factors contribute around 50% to the risk for DG [17,19] and that the same genes are involved in the spectrum from less severe DG to PG [14,17]. Although samples sizes were large (up to 6744 individuals [14]), only 104 ($<$ 2.2% [18]) or fewer individuals fulfilled the DSM criteria for PG, which reflects the estimated prevalence of PG in the general population. There have been no genome-wide association analyses (GWAS) of PG per se; to date, only a GWAS of a quantitative disordered gambling trait has been reported [20].

So far, most molecular genetic studies of PG have applied candidate gene approaches, primarily reporting an involvement of the dopaminergic and serotonergic neurochemical systems [21–26]. However, multiple neurotransmitter systems have been implicated in PG [27–29].

To our knowledge, the present study is the first case-control GWAS of PG, with all cases being assigned a diagnosis of PG. Due to the small sample size (445 cases and 986 controls), genome-wide significant single marker findings would only be expected for strong effects, such as those reported for the aldehyde dehydrogenase 2 in alcohol consumption [30]. However, pathway analyses and polygenic risk score tests were used to investigate whether the analysis of pathways may uncover hidden effects, and whether polygenic risk score tests may provide information concerning a common genetic background for AD and PG.

Besides limited sample sizes, a further challenge in this research field is the fact that the PG phenotype can be influenced by many factors. Potential confounders include sex, age, and socioeconomic status. As it is not possible to control for all of these possible confounders, in a first step, we controlled for principal components (PC) only. In a second step, the analyses were also controlled for age and sex.

2. Subjects and methods

2.1. Participants

The study was approved by the respective local ethics committees, and all subjects provided written informed consent prior to inclusion.

The sample comprised 445 cases and 986 controls. The PG cases were recruited from inpatient and outpatient treatment centres following presentation for treatment of PG ("Baden-Württemberg Study: assessing psychological, neurobiological, and genetic mechanisms of pathological gambling in Baden-Württemberg", a federal state of Germany). A smaller part of the sample was recruited via a nationwide telephone survey ('Pathologisches Glücksspielen und Epidemiologie' (PAGE), an epidemiological study for pathological gambling in Germany).

All patients were diagnosed according to DSM-III or -IV diagnostic criteria for PG. These criteria were assessed using two different tools. In the Baden-Württemberg study, these criteria were assessed using the South Oaks Gambling Screen (SOGS; cut-off $\geq$ 5 for PG [31]) based on DSM-III diagnostic criteria. In the Page study, the Composite Diagnostic Interview (CIDI; Version 3.0 of the World Health Organisation) was used to assess the criteria for DSM-IV diagnosis. Comorbid AD was assessed using the German version of the Structural Clinical Interview for DSM-IV (SCID-I; [32]), and diagnoses were assigned according to DSM-IV criteria.

For controls, genome-wide genotype data for population-based individuals were obtained from the SHIP-TREND study, which is a longitudinal population-based investigation of individuals from West Pomerania, Germany [33]. This study investigates the prevalence, incidence, and complex associations of common risk factors, subclinical disorders, and clinical diseases. The sample was randomly stratified for age, sex, and county of residence.

2.2. Genotyping

DNA was extracted from whole blood using chemagic MSMI (PerkinElmer Chemagen Technologie GmbH; Rodgau, Germany) or saliva, collected with Oragene Self-Collection Kit OG-500, and

extracted with Oragene prepIT, L2P (DNA Genotek Inc, Ontario, Canada). For all controls, whole blood, extracted with Gentra Puregene Blood Kit (Qiagen; Hilden, Germany) was used. For cases, 147 saliva and 298 blood samples were used. Cases and controls were genotyped using Illumina's HumanOmniExpress (n = 730,525 markers), and HumanOmni2.5 BeadChips (n = 2,450,000 markers), respectively.

2.3. Quality control

Stringent quality control filtering criteria were applied. Detailed information on these criteria is provided in the supplementary text. In brief, single nucleotide polymorphisms (SNPs) with the following characteristics were removed: call rate < 0.98; minor allele frequency < 0.01 in cases or controls; or deviation from Hardy–Weinberg equilibrium of $< 10^{-6}$ (cases) or $< 10^{-4}$ (controls). Individuals with the following characteristics were removed: call rates < 0.97; duplicated or cryptic related samples; or outlier status. A consensus SNP set common to both Illumina genotyping platforms (n = 595,867) was used for further analysis.

2.4. Single marker analysis

A logistic regression approach was used for the single marker association tests for autosomal SNPs. PG was used as binary trait. Correction for population stratification was performed using the first five PCs from a principal component analysis across independent autosomal markers (see supplementary text). An additional analysis also included age and sex as covariates.

2.5. Gene-based analysis

A gene-based test was performed using the Versatile Gene-based Association Study programme 2 [34]. A P-value below $\alpha = 2.1 \times 10^{-6}$ (0.05/23,804) was considered to be significant, as the gene-based test included 23,804 autosomal genes.

2.6. Pathway/gene-set based analyses

The global test was used to determine whether groups of genes were significantly related to the outcome of interest [35]. This was applied to the dataset using three pathway and gene-set databases: the Kyoto Encyclopaedia of Genes and Genomes (KEGG; [36,37]); Reactome [38,39]; and Gene ontology (GO; [40]). Details of the procedures used for obtaining gene-sets and mapping SNPs to genes and corresponding pathways, and the methods used to account for possible bias, are described in the supplementary text.

2.7. Polygenic risk scores for DG and AD

Polygenic risk scores were calculated to summarise the genetic effects of markers for DG and AD. These polygenic risk scores were calculated using the method introduced by Purcell et al. [41]. Marker weights for AD and DG were based on association results obtained in a German GWAS of AD [42], and summary data of a quantitative gambling trait in an Australian sample reported by Lind et al. [20]. A detailed description of the calculation is provided in the supplementary text.

3. Results

Most of the pathological gamblers (n = 338) were recruited from inpatient and outpatient treatment centres within the context of the "Baden-Württemberg Study". The remaining 107 cases were drawn from the PAGE sample. Following quality control, the sample comprised 1362 individuals: 396 cases and 966 controls. The 396 PG cases ($n_{males} = 358$, $n_{females} = 38$) included 280 inpatients, 83 outpatients, and 33 currently non-treated cases. The mean age of these cases was 40.18 years (range 16–75, standard deviation (SD) = 11.00).

For controls, genome-wide genotype data for 966 population-based individuals ($n_{males} = 427$, $n_{females} = 539$) were obtained from the SHIP-TREND study. The mean age of the controls was 50.16 years (range 20–81, SD 13.65).

Of the PG cases, 149 had a comorbid diagnosis of AD, and 222 were not alcohol-dependent. For the remaining cases, no information on AD status was available.

A total of 595,867 autosomal SNPs were available for analysis.

The Manhattan plots of the GWAS for the two analyses are shown in Figs. 1 and 2. These analyses use "PC 1 to 5", and "PC 1 to 5, age and sex" as covariates, respectively. The corresponding Quantile–Quantile plots of the observed vs expected $-\log_{10}$ P-values of the association analysis are shown in Figs. 3 and 4.

3.1. Single marker analysis

No SNP achieved genome-wide significance. The top SNPs (16), with P-values of $< 5 \times 10^{-5}$ in the analysis that included PC 1 to 5 only, are listed in Table 1a. The first three top SNPs (P-values of $< 10^{-5}$) are located on 16p12.3 and 20q13.12. The SNP rs6065904 (P-value = 1.48×10^{-6}, odds ratio (OR): 0.53; confidence interval (CI) = [0.41, 0.69]) is located in an intron of phospholipid transfer protein (*PLTP*). The SNP rs4810479 (P-value = 4.67×10^{-6}, OR: 0.57; CI = [0.44, 0.72]) is located nearby and in strong linkage disequilibrium with rs6065904 ($r^2 = 0.79$) in the upstream region of *PLTP*. The SNP rs3943418 (P-value = 6.61×10^{-6}, OR: 1.71; CI = [1.36, 2.16]) is located in an intron of Xylosyltransferase 1 (*XYLT1*). All three top SNPs had P-values in the range of 4.6 to 6.9×10^{-5} following the inclusion of age and sex. Top hits corrected for sex and age with P-values of $< 5 \times 10^{-5}$ are shown in Table 1b. Here, the top two SNPs $< 10^{-5}$ were: (i) rs7591351 (P-value = 5.88×10^{-6}, OR: 1.67; CI = [1.34, 2.09], only PC 1 to 5 corrected, 2.94×10^{-4}); and (ii) rs6738409 (P-value = 7.39×10^{-6}, OR: 0.60; CI = [0.48, 0.75], PC 1 to 5 corrected 1.44×10^{-4}). Both SNPs are located in the protein kinase C gene (*PRKCE*). All further top SNPs $< 10^{-4}$ are listed in the supplementary tables S1a and b. Please also find a comparison with the SNP top hits of Lind et al. [20] in supplementary text.

3.2. Gene-based analysis

In the gene-based analysis, no nominal significant finding survived correction for multiple testing (P-value $< 2.1 \times 10^{-6}$). The top hits ($< 10^{-3}$) are listed in Tables 2a and 2b. The lowest empirical P-value for the first approach was 3.8×10^{-5} for *PCIF1*, which encodes PDX1 C-terminal inhibiting factor 1. Nine genes out of 19 shared one signal: for these nine genes, the top SNP was rs6065904. The top hit for the age and sex corrected version was *RBM33*, with a P-value of 7.6×10^{-5}. Here, *PCIF1* retained a P-value of 4.88×10^{-4}. Descriptions of genes as well as a comparison with results of previous studies are listed in supplementary text and supplementary table S2a and b.

3.3. Pathway/gene-set based analyses

In KEGG, 35 out of 257 pathways achieved nominal significance (P-value < 0.05). Of these, 13 had P-values of < 0.01, including three pathways with a corrected P-value of < 0.05 in the analysis that controlled for PC 1 to 5. The SNP- and case-control permutation tests suggested that these three best pathways are reliable, as all achieved P-values of < 0.003. These pathways

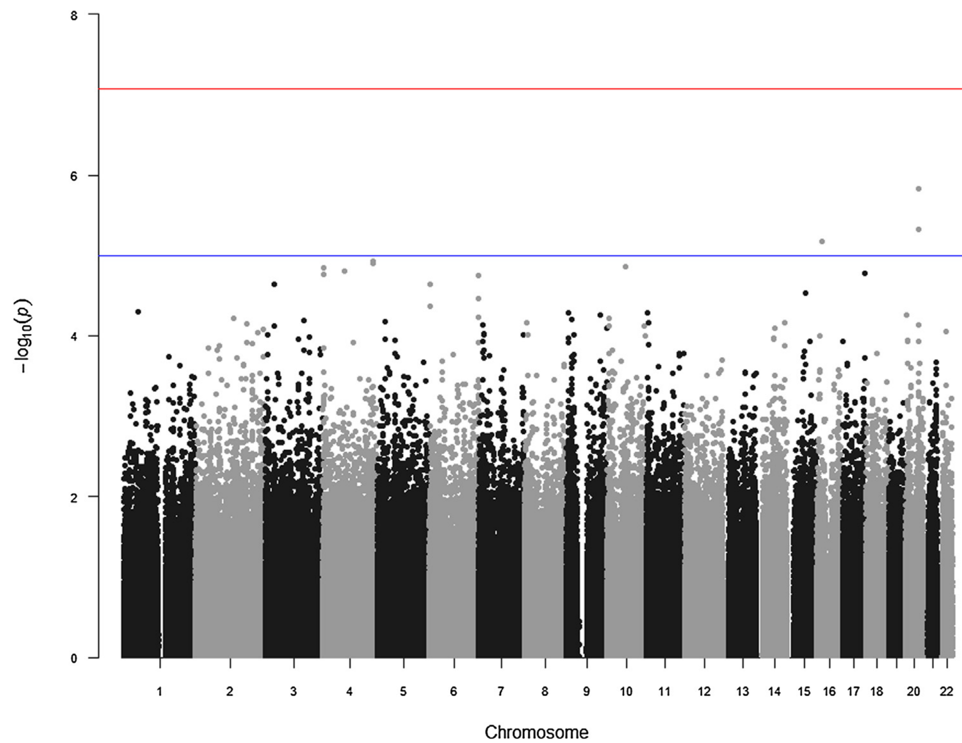

Fig. 1. Manhattan plot of the association *P*-values for pathological gamblers vs. controls. The horizontal axis represents the genome, which is divided into its autosomes; the vertical axis shows the $-\log_{10}$ values of the association *P*-values. The red line shows the genome-wide significance threshold. The blue line shows the threshold for "suggestive" associations. The figure shows results for the analysis including PC 1 to 5.

(hsa05016, Huntington's disease; hsa04152, 5′-adenosine monophosphate-activated protein kinase [AMPK] signalling pathway; and hsa04210, Apoptosis) remained significant after Benjamini-Hochberg correction for the 257 pathways of the KEGG database (see Table 3a). In the sex and age corrected analysis (see Table 3b), no pathway remained significant after correction: 43 had *P*-values of < 0.05, and 12 had *P*-values of < 0.01. The top-ranking pathway was AMPK signalling, with a *P*-value of 5.36×10^{-4}. All three

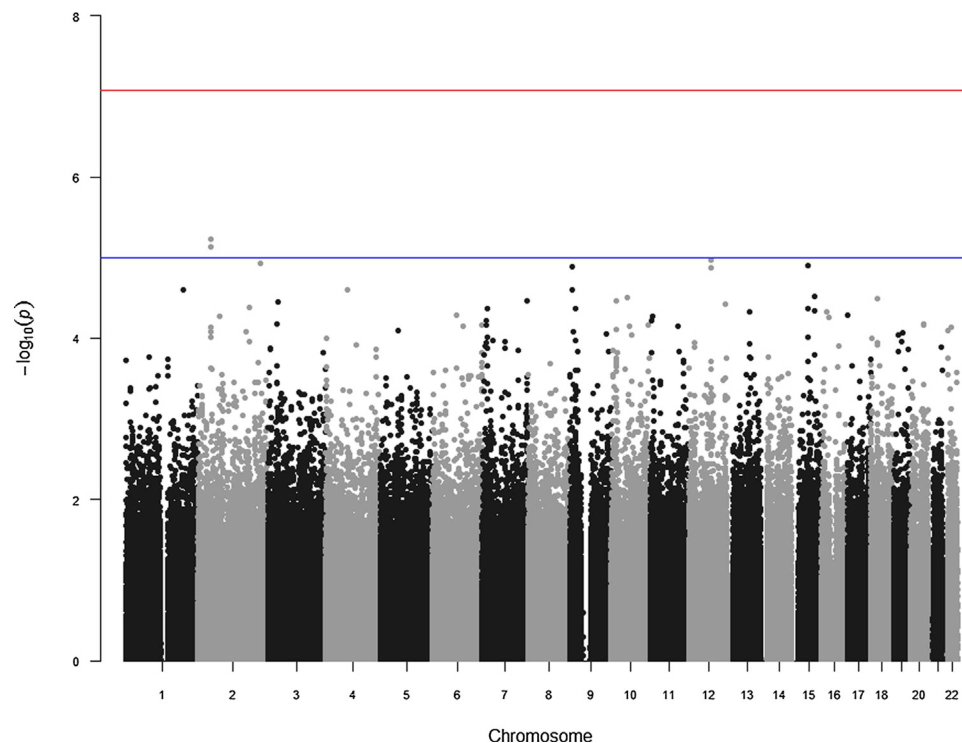

Fig. 2. Manhattan plot of the association *P*-values for pathological gamblers vs. controls. The horizontal axis represents the genome, which is divided into its autosomes; the vertical axis shows the $-\log_{10}$ values of the association *P*-values. The red line shows the genome-wide significance threshold. The blue line shows the threshold for "suggestive" associations. The figure shows results for the analysis including PC 1 to 5, sex and age.

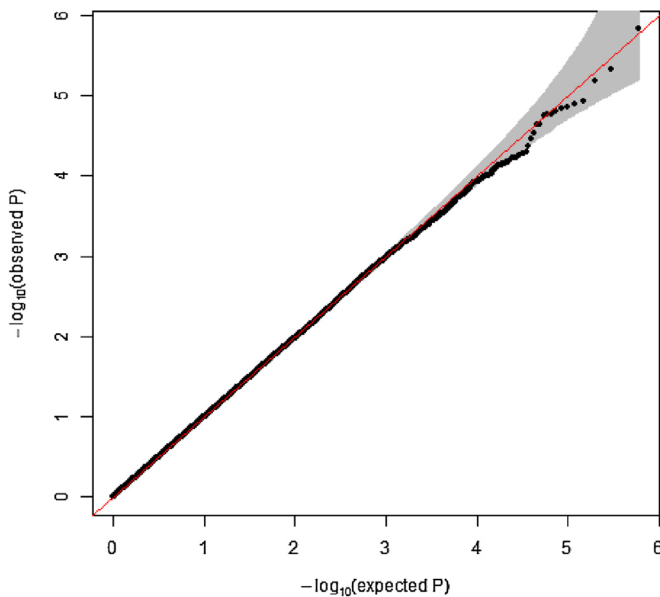

Fig. 3. Quantile–Quantile plot of association for pathological gamblers vs. controls. The horizontal axis represents the $-\log_{10}$ of expected association test *P*-values, and the vertical axis shows the $-\log_{10}$ of *P*-values from the *P*-values. The shaded region shows the 95% confidence bands of expected values under the null hypothesis of no association. Fig. 1 shows results for the analysis that included PC 1 to 5 as covariates. The Lambda was 1 for this analysis.

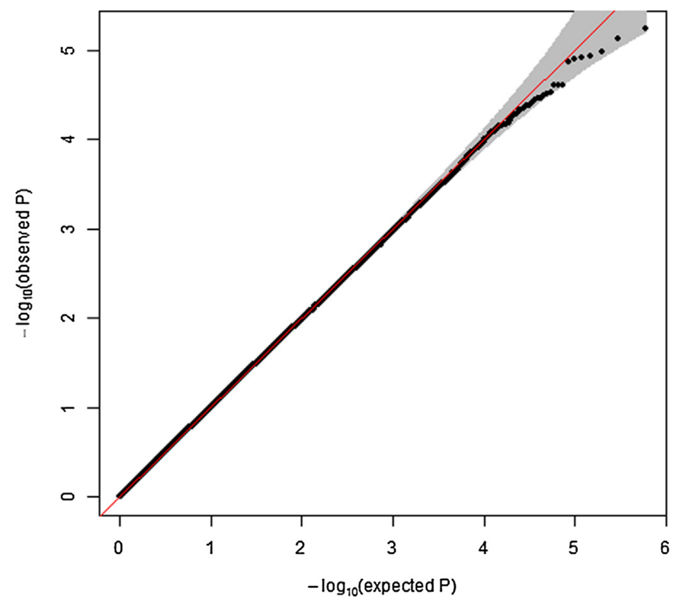

Fig. 4. Quantile–Quantile plot of association for pathological gamblers vs. controls. The horizontal axis represents the $-\log_{10}$ of expected association test *P*-values, and the vertical axis shows the $-\log_{10}$ of *P*-values from the *P*-values. The shaded region shows the 95% confidence bands of expected values under the null hypothesis of no association. The figure shows results for the analysis that included PC 1 to 5, sex and age as covariates. The Lambda was 1.0046 for this analysis.

previously significant pathways from the first analysis (PC 1 to 5) remained among the top hits $P < 0.01$ hits. All KEGG pathways with nominally significant *P*-values of < 0.01 and detailed results are shown in supplementary tables S3a and b as well as in the supplementary text. Tables S3c and d indicate the proportions of genes with overlap between the top pathways. Table S3b shows additional interesting pathways with *P*-values of < 0.05.

In Reactome and GO, no gene-sets remained significant (i.e. *P*-value < 0.05) after Benjamini-Hochberg correction for all gene-sets in the databases. Of 1180 pathways, a total of 23 in the analysis correcting for PC 1 to 5, and 18 in the analysis correcting for age and sex, had *P*-values of < 0.01 (uncorrected) in Reactome. These are listed in supplementary table S4a and b. Of 8474 pathways in GO, a total of 32 (PC 1 to 5 corrected) and 14 (age and sex corrected analysis) had a *P*-value of $< 10^{-3}$. These pathways are described in supplementary tables S5a and b as well as in the supplementary text.

3.4. Polygenic risk scores for DG and AD

The association between risk score for AD and PG status was nominally significant (*P*-value = 0.047, one-sided test, see Table 4a). This value improved after the inclusion of sex and age, yielding a *P*-value of 0.024 for a one-sided test, see Table 4b. No association was found between risk scores for DG and PG status in either approach (see Tables 5a and 5b). Quartile plots of polygenic risk scores are shown in supplementary figs. S1 and S2.

4. Discussion

To our knowledge, the present study represents the first GWAS of PG. No genome-wide significant association was found with any SNP or gene. This was unsurprising, given the small sample size. However, although well below the significance threshold, the three top SNPs (*P*-values $< 10^{-5}$) are of potential interest. The SNPs

Table 1a
Top SNP findings (*P*-value $< 5 \times 10^{-5}$) for the logistic regression including PC 1 to 5 as covariates.

CHR	SNP	Position (hg19)	Gene	Minor allele	OR	L95	U95	*P*-value
20	rs6065904	44534651	*PLTP* (intron variant)	A	0.53	0.41	0.69	1.48×10^{-6}
20	rs4810479	44545048	Intergenic	C	0.57	0.44	0.72	4.67×10^{-6}
16	rs3943418	17337724	*XYLT1* (intron variant)	A	1.71	1.36	2.16	6.61×10^{-6}
4	rs11723785	178136407	Intergenic	T	1.71	1.34	2.17	1.19×10^{-5}
4	rs4690502	178141976	*LOC105377557* (intron variant)	A	1.7	1.34	2.15	1.25×10^{-5}
10	rs10995114	64074412	Intergenic	T	3.05	1.84	5.03	1.37×10^{-5}
4	rs10031235	5324465	*STK32B* (intron variant)	C	1.86	1.41	2.46	1.44×10^{-5}
4	rs6853653	77725242	*LOC105377289* (upstream variant 2KB)	C	1.7	1.34	2.16	1.56×10^{-5}
17	rs8078855	78225055	*SLC26A11* (intron variant)	T	1.56	1.28	1.92	1.69×10^{-5}
4	rs6853980	5324579	*STK32B* (intron variant)	A	1.76	1.36	2.28	1.72×10^{-5}
6	rs9396970	169966644	*WDR27* (intron variant)	C	2.36	1.6	3.49	1.76×10^{-5}
3	rs1868488	29964567	*RBMS3* (intron variant)	C	1.85	1.39	2.46	2.29×10^{-5}
6	rs2745599	1613686	*FOXC1* (UTR variant 3')	G	1.54	1.26	1.88	2.3×10^{-5}
15	rs3803497	63053858	*TLN2* (intron variant)	C	2.40	1.59	3.62	2.92×10^{-5}
6	rs2860492	169930402	*WDR27* (intron variant)	T	2.2	1.51	3.19	3.41×10^{-5}
6	rs2745596	1606031	*FOXCUT* (nc transcript variant)	T	0.66	0.54	0.81	4.23×10^{-5}

CHR: chromosome; OR: odds ratio; L95 and U95: lower and upper 95% confidence intervals; SNP: single nucleotide polymorphism.

Table 1b
Top SNP findings (P-value $< 5 \times 10^{-5}$) of the logistic regression including PC 1 to 5, age and sex as covariates.

CHR	SNP	Position (hg19)	Gene	Minor allele	OR	L95	U95	P-value
2	rs7591351	46063406	*PRKCE* (protein kinase C, epsilon; intron variant)	T	1.67	1.34	2.09	5.88×10^{-6}
2	rs6738409	46062550	*PRKCE* (protein kinase C, epsilon; intron variant)	C	0.60	0.48	0.75	7.39×10^{-6}
12	rs6582294	76034992	intergenic	A	1.69	1.34	2.14	1.07×10^{-5}
2	rs13021421	216811307	*MREG* (downstream variant 500B, intron variant)	T	2.19	1.54	3.11	1.16×10^{-5}
15	rs17255585	54107802	intergenic	C	0.26	0.14	0.48	1.24×10^{-5}
9	rs10815757	8097977	intergenic	C	1.70	1.34	2.15	1.28×10^{-5}
12	rs3898937	75947644	intergenic	G	1.67	1.33	2.11	1.35×10^{-5}
1	rs2359854	198561100	intergenic	A	0.60	0.47	0.76	2.49×10^{-5}
4	rs6853653	77725242	*LOC105377289* (upstream variant 2KB)	C	1.81	1.37	2.38	2.52×10^{-5}
9	rs10815753	8093954	intergenic	G	1.66	1.31	2.10	2.53×10^{-5}
15	rs8036417	78419476	CIB2	G	1.71	1.33	2.21	2.99×10^{-5}
10	rs10825357	56323564	PCDH15	T	0.51	0.37	0.70	3.17×10^{-5}
18	rs190166	24499317	intron variant (*AQP4-AS1* and *CHST9*)	A	1.62	1.29	2.04	3.25×10^{-5}
7	rs579864	154539863	*DPP6* (intron variant)	A	0.60	0.47	0.76	3.43×10^{-5}
10	rs1411823	20077441	PLXDC2	A	0.62	0.49	0.77	3.49×10^{-5}
3	rs6550215	33277828	intergenic	G	1.71	1.33	2.21	3.57×10^{-5}
12	rs7965173	127897824	intergenic	T	1.61	1.29	2.03	3.76×10^{-5}
2	rs10497460	177691497	intergenic	G	0.60	0.48	0.77	4.12×10^{-5}
9	rs768703	18070475	*ADAMTSL1* (intron variant)	A	1.62	1.29	2.05	4.23×10^{-5}
15	rs4776181	54122875	intergenic	C	0.22	0.10	0.45	4.25×10^{-5}
7	rs17351688	19193072	intergenic	G	1.66	1.30	2.12	4.32×10^{-5}
15	rs2289524	78390414	*SH2D7* (missense)	C	1.59	1.27	1.99	4.50×10^{-5}
16	rs3943418	17337724	*XYLT1* (intron variant)	A	1.75	1.34	2.30	4.65×10^{-5}
13	rs1465661	75906289	*TBC1D4* (intron variant)	C	2.129	1.48	3.07	4.76×10^{-5}

CHR: chromosome; OR: odds ratio; L95 and u95: lower and upper 95% confidence intervals; SNP: single nucleotide polymorphism.

rs6065904 and rs4810479 are located in close proximity to each other within and near the gene *PLTP*. The product of *PLTP* is the phospholipid transfer protein. This protein is involved in the phospholipid metabolism, and has been reported to show significantly higher activity in individuals who abuse alcohol [43] and in individuals who drink heavily [44]. Meta-analyses have shown strong association between these two SNPs and various lipid metabolism phenotypes, with reported P-values as low as 4×10^{-40} [45] and 2×10^{-42} [46], respectively. The SNP rs3943418 is located in an intron region of *XYLT*. This gene encodes xylosyltransferase 1, an enzyme that is involved in the proteoglycan metabolism. A potential role in PG is not apparent.

Unsurprisingly, results changed after correction for age and sex. However, the values for the top three hits changed only slightly. Following correction for age and sex, the two top hits were rs7591351 and rs6738409, P-value $< 10^{-5}$. Both SNPs are in *PRKCE*, encoding the protein kinase c epsilon. Research has shown that *PRKCE* influences ethanol and nicotine self-administration in mice, and is associated with alterations in the cholinergic modulation of dopamine release in the nucleus accumbens [47].

Formal genetic studies in twins have reported a genetic overlap between PG and AD, and between PG and DG [14,15]. Here, we attempted to demonstrate these overlaps on a molecular level using polygenic score analyses. While nominal significance was found for an overlap between PG and AD, no significant overlap was observed for PG and DG. This discrepancy could be due to the fact that the training sample for AD comprised patients with severe AD [42], whereas the training sample for DG, which had been recruited from the general population, included only 31 individuals with a PG diagnosis [20]. This sample may thus have lacked sufficient statistical power for the detection of an overlap. Nonetheless, given the very small sample sizes, the present finding for PG and AD appears to provide convincing support for a genetic overlap between these two disorders.

Table 2a
Gene-wide associations with P-value $< \times 10^{-3}$ of the gene-based analysis including PC 1 to 5 as covariates.

Gene	Chromosome	Start (hg19)	Stop (hg19)	P-value	Top SNP
PCIF1	20	44513316	44626662	3.80×10^{-5}	rs6065904*
PLTP	20	44477258	44591003	8.03×10^{-5}	rs6065904*
CTSA	20	44469590	44577458	1.08×10^{-4}	rs6065904*
NEURL2	20	44467110	44569926	1.80×10^{-4}	rs6065904*
SPATA25	20	44465129	44566238	1.90×10^{-4}	rs6065904*
MIR3926-2	8	12534745	12634808	2.18×10^{-4}	rs6989065#
ZSWIM1	20	44459847	44563905	2.22×10^{-4}	rs6065904*
MIR3926-1	8	12534740	12634813	2.23×10^{-4}	rs6989065#
ZNF335	20	44527291	44650833	2.41×10^{-4}	rs6065904*
ZSWIM3	20	44436219	44557769	2.55×10^{-4}	rs6065904*
LONRF1	8	12529405	12662992	3.02×10^{-4}	rs6989065
GAPVD1	9	127974072	128177290	3.67×10^{-4}	rs10760397
ACOT8	20	44420359	44536048	4.82×10^{-4}	rs6065904*
DNAI2	17	72220385	72361023	4.96×10^{-4}	rs11652975
DNAH7	2	196552426	196983536	5.84×10^{-4}	rs16841018
FERD3L	7	19134404	19235044	6.15×10^{-4}	rs7780145**
HSPA5	9	127947126	128053666	6.81×10^{-4}	rs393721
TWIST1	7	19105090	19207295	7.14×10^{-4}	rs7780145**
KIF19	17	72272350	72401959	7.85×10^{-4}	rs11652975

*, **, #, ## same SNP drives finding for different genes.

Table 2b
Gene-wide associations with *P*-value $< \times 10^{-3}$ of the gene-based analysis including PC 1 to 5, sex, and age as covariates.

Gene	Chromosome	Start (hg19)	Stop (hg19)	*P*-value	Top SNP
RBM33	7	155387202	155624179	7.60×10^{-5}	rs872723
MIR3926-1	8	12534740	12634813	1.37×10^{-4}	rs11784167*
LONRF1	8	12529405	12662992	1.38×10^{-4}	rs11784167*
MIR3926-2	8	12534745	12634808	1.41×10^{-4}	rs11784167*
PPY	17	41968171	42069833	2.23×10^{-4}	rs1642598
MIR5003	15	78325874	78425901	3.25×10^{-4}	rs8036417***
SH2D7	15	78334926	78446393	4.20×10^{-4}	rs8036417***
FAM215A	17	41944575	42045355	4.40×10^{-4}	rs1642598
CNST	1	246679638	246881884	4.57×10^{-4}	rs7518651
PCIF1	20	44513316	44626662	4.88×10^{-4}	rs4810479#
CTSA	20	44469590	44577458	5.51×10^{-4}	rs4810479#
PLTP	20	44477258	44591003	5.57×10^{-4}	rs4810479#
FERD3L	7	19134404	19235044	6.22×10^{-4}	rs17351688##
MAFB	20	39264487	39367880	7.23×10^{-4}	rs1078571
TFB2 M	1	246653862	246779565	7.97×10^{-4}	rs4494115
ZSWIM1	20	44459847	44563905	8.11×10^{-4}	rs4810479#
SPATA25	20	44465129	44566238	8.36×10^{-4}	rs4810479#
NEURL2	20	44467110	44569926	8.52×10^{-4}	rs4810479#
ACTG1	17	79426996	79529892	8.65×10^{-4}	rs7342974#
CIB2	15	78346947	78473877	8.91×10^{-4}	rs8036417*
TWIST1	7	19105090	19207295	9.60×10^{-4}	rs17351688##

*, **, ***, #, ## same SNP drives finding for different genes.

The findings of the pathway analyses were promising. The first analysis yielded significant results for Huntington's disease, the AMPK signalling pathway, and apoptosis.

Huntington's disease (HD) is an autosomal dominant inherited degenerative disorder, and is characterised by progressive motor, cognitive, and behavioural deterioration. Research has shown that patients with HD are at increased risk of addiction if they engage in gambling behaviour [48]. However, the reasons for this remain contentious. In a simulated gambling task, decision-making deficits- in the form of an increased choice of disadvantageous decks were observed in both HD [49,50] and PG [51]. In PG, poorer performance has been linked to motivational processes [51]. In HD, Campbell et al. [49] attributed a poorer performance to reduced autonomic responsiveness to gambling task losses. In contrast, Busemeyer et al. [50] concluded that poorer performance in HD was due in part to cognitive processes, but also to altered choice response mechanisms (resulting from recklessness or impulsiveness).

Table 3a
KEGG global test results with *P*-values < 0.01 of the analysis including PC 1 to 5 as covariates.

Pathway ID	Pathway	*P*-value	*P*-value*
hsa05016	**Huntington's disease**	$\mathbf{2.58 \times 10^{-5}}$	$\mathbf{6.63 \times 10^{-3}}$
hsa04152	**AMPK signalling pathway**	$\mathbf{7.45 \times 10^{-5}}$	$\mathbf{9.57 \times 10^{-3}}$
hsa04210	**Apoptosis**	$\mathbf{2.05 \times 10^{-4}}$	$\mathbf{1.75 \times 10^{-2}}$
hsa04920	Adipocytokine signalling pathway	1.13×10^{-3}	5.83×10^{-2}
hsa04668	TNF signalling pathway	1.13×10^{-3}	5.83×10^{-2}
hsa00051	Fructose and mannose metabolism	1.45×10^{-3}	6.23×10^{-2}
hsa04910	Insulin signalling pathway	2.98×10^{-3}	1.10×10^{-1}
hsa00410	beta-Alanine metabolism	3.44×10^{-3}	1.10×10^{-1}
hsa04915	Estrogen signalling pathway	5.23×10^{-3}	1.49×10^{-1}
hsa04350	TGF-beta signalling pathway	8.23×10^{-3}	1.76×10^{-1}
hsa05010	Alzheimer's disease	7.56×10^{-3}	1.76×10^{-1}
hsa04024	cAMP signalling pathway	7.40×10^{-3}	1.76×10^{-1}
hsa05030	Cocaine addiction	9.39×10^{-3}	1.86×10^{-1}

Values remaining significant after correction are shown in bold.
* Benjamini-Hochberg corrected.

Table 3b
KEGG global test results with *P*-values < 0.01 of the analysis including PC 1 to 5, sex, and age as covariates.

Pathway ID	Pathway	*P*-value	*P*-value*
hsa04152	**AMPK signalling pathway**	5.36×10^{-4}	1.38×10^{-1}
hsa04340	Hedgehog signalling pathway	1.66×10^{-3}	1.64×10^{-1}
hsa05030	Cocaine addiction	1.94×10^{-3}	1.64×10^{-1}
hsa05410	Hypertrophic cardiomyopathy (HCM)	5.26×10^{-3}	1.64×10^{-1}
hsa04920	Adipocytokine signalling pathway	5.77×10^{-3}	1.64×10^{-1}
hsa05031	Amphetamine addiction	5.84×10^{-3}	1.64×10^{-1}
hsa05414	Dilated cardiomyopathy	6.13×10^{-3}	1.64×10^{-1}
hsa04910	Insulin signalling pathway	6.24×10^{-3}	1.64×10^{-1}
hsa05016	**Huntington disease (HD)**	6.64×10^{-3}	1.64×10^{-1}
hsa04932	Non-alcoholic fatty liver disease (NAFLD)	6.66×10^{-3}	1.64×10^{-1}
hsa04210	**Apoptosis**	7.01×10^{-3}	1.64×10^{-1}
hsa04921	Oxytocin signalling pathway	8.88×10^{-3}	1.90×10^{-1}

* Previously significant pathways (in the first analysis) shown in bold.

Table 4a
Polygenic risk score results of the AD GWAS by Frank et al. [42] as applied to the PG data including covariates PC 1 to 5.

P-values included	One-tailed *P*-values
$P < 0.01$	0.469
$P < 0.05$	0.421
$P < 0.1$	0.381
$P < 0.2$	0.135
$P < 0.3$	0.109
$P < 0.4$	0.056
$P < 0.5$	0.047

AD: alcohol dependence; GWAS: genome-wide association study; PG: pathological gambling.

Table 4b
Polygenic risk score results of the AD GWAS by Frank et al. [42] as applied to the PG data including PC 1 to 5, age and sex as covariates.

P-values included	One-tailed *P*-values
$P < 0.01$	0.425
$P < 0.05$	0.372
$P < 0.1$	0.337
$P < 0.2$	0.141
$P < 0.3$	0.067
$P < 0.4$	0.032
$P < 0.5$	0.024

AD: alcohol dependence; GWAS: genome-wide association study; PG: pathological gambling.

Table 5a
Polygenic risk score results of the GWAS of DG by Lind et al. [20] as applied to the PG data including covariates PC 1 to 5.

P-values included	One-tailed *P*-values
$P < 0.01$	0.068
$P < 0.05$	0.182
$P < 0.1$	0.315
$P < 0.2$	0.129
$P < 0.3$	0.079
$P < 0.4$	0.144
$P < 0.5$	0.188

GWAS: genome-wide association study; PG: pathological gambling; DG: disordered gambling.

Table 5b
Polygenic risk score results of the GWAS of DG by Lind et al. [20] as applied to the PG data including PC 1 to 5, age and sex as covariates.

P-values included	One-tailed *P*-values
$P < 0.01$	0.066
$P < 0.05$	0.232
$P < 0.1$	0.399
$P < 0.2$	0.197
$P < 0.3$	0.144
$P < 0.4$	0.204
$P < 0.5$	0.225

GWAS: genome-wide association study; PG: pathological gambling; DG: disordered gambling.

Even though it is still a matter of debate, it is known that symptoms in Huntington are caused by progressive striatal atrophy. The cortico-striatal circuits that are affected in Huntington's disease are also involved in the predisposition to PG and other addictions comprising disinhibition-related symptoms, such as altered impulsivity, sensitivity to reward, and the inability to consider long-term advantage over short-term reward [48].

The second top hit of the pathway analyses, and which ranked first in the analysis that included age and sex, was AMPK signalling. AMPK is a sensor of energy status, and acts both as a key regulator of cellular energy homeostasis [52], and as a central regulator of both lipid and glucose metabolism [53]. In vivo and in vitro studies have shown that AMPK limits anabolic pathways and activates catabolic reactions [54]. AMPK activation is repressed by glucose withdrawal, and is inhibited by chronic ethanol exposure [54].

The third pathway that remained significant after correction is apoptosis. Apoptosis refers to the controlled and regulated process of cell death, which maintains a healthy balance between cellular death and survival [55]. Apoptotic signals guard genomic integrity and are regulated at several levels [55]. It is not clear in what way these far-reaching processes might play a role in PG. Previous research suggests that cocaine abuse alters processes related to apoptosis [56].

A limitation of the present study is the small sample size, which provided limited power to detect risk alleles. As a result, we could not control for all potential confounders. However, to account for some important potential confounders, we used two approaches: one including PC 1 to 5 only; and one that also included age and sex.

A further limitation is that patients were heterogeneous in terms of assessment instruments and DSM classification. However, there is evidence for a common etiologic structure between the SOGS and the DSM-IV [57]. The most likely effect of a heterogeneous patient population in GWAS is a reduction in power. However, this will lead to missing true effects rather than to false positive findings.

Furthermore, polygenic risk score calculations of AD were based on GWAS data from our German GWAS of AD only in order to increase homogeneity and comparability between the samples. Although a larger sample might have yielded more power, this would have been at the cost of greater heterogeneity. Another limitation of the present study was that the results are limited to a population of Caucasian ethnicity. Further studies are required to determine whether, and how, the identified pathways – or the genes that contribute to them – are involved in the aetiology of PG.

In summary, this first GWAS of PG identified pathways and points to genes with possible involvement in the aetiology of PG. The results are consistent with previous formal genetic studies, which showed an overlap between PG and AD on a molecular genetic level. A number of the higher ranked markers, genes, and pathways appear plausible candidates for the PG phenotype and warrant further investigation. Compared to recent GWAS of schizophrenia and other psychiatric disorders, the power of the present sample was low. However, the results are promising, and warrant a future collaborative research effort to uncover the genetic variants that predispose to PG.

Disclosure of interest

The authors declare that they have no competing interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eurpsy.2016.04.001.

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