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SPOCK3, a risk gene for adult ADHD and personality disorders

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Abstract Attention-deficit/hyperactivity disorder (ADHD) is the most frequent psychiatric disorder in children, where it displays a global prevalence of 5 %. In up to 50 % of the cases, ADHD may persist into adulthood (aADHD), where it is often comorbid with personality disorders. Due to a potentially heritable nature of this comorbidity, we hypothesized that their genetic framework may contain common risk-modifying genes. *SPOCK3*, a poorly characterized, putatively Ca(2+)-binding extracellular heparan/chondroitin sulfate proteoglycan gene encoded by the human chromosomal region 4q32.3, was found to be associated with polymorphisms among the top ranks in a genome-wide association study (GWAS) on ADHD and a pooled GWAS on personality disorder (PD). We therefore genotyped 48 single nucleotide polymorphisms (SNPs) representative of the *SPOCK3* gene region in 1,790 individuals ($n_{aADHD} = 624$, $n_{PD} = 630$, $n_{controls} = 536$). In this analysis, we found two SNPs to be nominally associated with aADHD (rs7689440, rs897511) and four PD-associated SNPs (rs7689440, rs897511, rs17052671 and rs1485318); the latter even reached marginal significance after rigorous Bonferroni correction. Bioinformatics tools predicted a possible influence of rs1485318 on transcription factor binding, whereas the other candidate SNPs may have effects on alternative splicing. Our results suggest that *SPOCK3* may modify the genetic risk for ADHD and PD; further studies are, however, needed to identify the underlying mechanisms.

Keywords Association · GWAS · Personality disorder · Adult ADHD · Testican3

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Introduction

With a global prevalence of ~5 % [43], attention-deficit/hyperactivity disorder (ADHD) belongs to the most frequent mental disorder in children, characterized primarily by the coexistence of attention problems, impulsivity and hyperactivity [9]. In 30 to 50 % of all diagnosed children, the symptoms persist into adulthood (adult ADHD, aADHD; [4]), where frequently comorbid disorders, like substance abuse (45 %), mood (57 %), anxiety (27 %) and personality disorders (e. g. histrionic personality disorder: 35 %), modulate or even dominate the clinical phenotype [27]. The etiology of ADHD remains largely unclear, but it is obvious that its genetic component with an expected heritability of 0.8 plays an essential role [18, 32, 50]. To date, only a limited number of genes was found to be associated with ADHD, but similar to other complex

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genetic disorders in psychiatry, they only explain a small part of the genetic contribution; moreover, specific ADHD susceptibility genes are still missing [3, 18, 32]. To overcome limitations of candidate gene studies and to obtain a more detailed picture of this complex disease, a first genome-wide association (GWA) study was performed in 2008. Although the investigation of 958 ADHD-affected family trios [39] did not result in a genome-wide significant single nucleotide polymorphism (SNP) association, the study yielded promising findings. Among them was rs7657608, an intronic SNP of *SPOCK3* [sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 3], which was ranked no. 10 among the results ($p = 0.00003$). Most interestingly, several SNPs from this gene were also among the nominally significant associations found in a pooling GWAS on Cluster B and C personality disorders [58], which are frequent comorbidities of ADHD: The downstream SNP rs12502996 displayed the sixth strongest association with Cluster B, while Cluster C was nominally associated with SNPs rs6854438 (rank 109) rs6552224 (rank 335), rs9996171 (rank 483), rs11940700 (rank 568) and rs9994664 (rank 594) [58].

The human *SPOCK3* gene, located on chromosome 4q32.3, is coding for a poorly characterized, putative Ca(2+)-binding extracellular heparan/chondroitin sulfate proteoglycan [34]. Proteoglycanes are constituents of the extracellular matrix (ECM) and present in nearly all tissues, but of unusual high diversity and most abundant in the adult brain [5, 40]. In vitro studies showed that proteoglycanes play essential roles in development and interconnection of the neuronal system [5], as well as in synaptic plasticity [16, 24]. In addition to most other proteoglycanes, members of the SPOCK family enclose a thyroglobulin domain with a rare cystein-rich pattern similarity to genes which are specific for growth factor binding [52] or cell–cell and cell–matrix interactions [37], suggesting that they may have similar activities [2]. SPOCK1, the best characterized family member which might functionally be very similar to SPOCK3 [49], has been reported to inhibit adhesion processes and neurite outgrowth in cell culture and is expected to be involved in axonal regeneration after injury [26, 35, 51]. Likewise, *SPOCK3* expression is downregulated in rat brains after experimentally induced stroke [11]. During embryonic development of mice, *SPOCK3* mRNA can be detected in the vascular system, liver, inner ear and neurons of the central nervous system. In adult mice, expression of *SPOCK3,* however, is restricted to the brain and is therefore likely to play a necessary role in the central nervous system. Immunohistochemically detected *SPOCK*3 displayed the highest protein levels in brains of adult mice, particularly in the bulbus olfactorius, cortex, thalamus, hippocampus and striatum [10, 24, 34, 48]. Notably, the latter three regions are well known for their involvement in mental disorders [17, 22, 23, 30, 33, 42]. This prompted us to design a case–control study with 1160 ($n = 624$ cases; $n = 536$ controls) individuals to investigate the genetic relevance of *SPOCK3* in adult attention-deficit/hyperactivity disorder (aADHD). As adult ADHD goes along with significant (up to 35 %) rates of comorbid Cluster B and C personality disorders [27], and as several SNPs of the *SPOCK3* locus interestingly were found to be associated with these disorders in a pooling GWAS [58], our second step was to analyze further 630 individuals affected by personality disorders. This was aimed to clarify the role and the specificity of human *SPOCK3* in the pathogenesis of these, often comorbid disorders.

Materials and methods

Samples

The aADHD study sample has previously been described in greater detail [20, 27]; briefly, it comprises 624 (mean age 40.2 ± 10.1 years, 45 % female) in- and outpatients of German origin, which were recruited at the Department of Psychiatry, University of Würzburg. All patients completed a semi-structured interview on the basis of DSM-IV (Diagnostic and Statistical Manual for Mental Disorders) criteria, where age of onset under 7 years, lifelong persistence, current diagnosis and age of recruitment between 18 and 65 years were determined for diagnosis of aADHD. Additionally, information about comorbid affection with other psychiatric disorders was documented. Further 630 (mean age 35.2 ± 10.1 years, 46 % female) unrelated patients of German origin formed the personality disorder (PD) sample and were ascertained also at the Department of Psychiatry, University of Würzburg, by trained psychiatrists according to the DSM-IV criteria on the basis of structured interviews. Patients with schizoaffective or other psychotic disorders, comorbid substance abuse disorders, mental retardation, neurological or neurodegenerative disorders impairing psychiatric evaluation were not included in this analysis. Patients with substance-induced disorders were excluded as well. Information about comorbidity with other psychiatric disorders was not documented. Further details on this sample can be obtained from previous publications [47]. The control sample consisted of 536 (mean age 30.7 ± 9.8 years, 52 % female) healthy subjects and was composed of blood donors, staff members and volunteers all originating from the Lower Franconia region. Only subjects who gave written informed consent were enrolled in the study, which complied with the Declaration of Helsinki and was approved by the Ethic Committee of the University Hospital Würzburg.

SNP selection and genotyping

The *SPOCK3* gene is located on the minus strand of chromosome 4q32.3. Due to alternative transcription start and end sites, between four and twelve exons are transcribed, generating primary transcripts of lengths between 234 and 501 kb. In order to capture the common allelic variation in the 523 kb region spanning the *SPOCK3* gene including putative regulatory sites with a minimal genotyping effort, the Haploview [6] version 4.2 (http://www.broad.mit.edu/mpg/haploview) *Tagger* function (default settings) was used to choose 48 tag SNPs from HapMap CEU data [21]. SNP genotyping was performed using the Sequenom MassArray® system according to the manufacturer's instructions. All PCR reactions were done with the iPlex® chemistry following the MassArray® iPlex® standard operation procedure. Primer sequences can be found in Supplementary Table 1.

Statistical analysis and power calculations

Statistical analysis of genotype data was performed with PLINK V1.07 [45] and Haploview V4. 2 [6]. To check the quality of genotyping data, all 48 variants had to pass several quality criteria. At first, the minimal call rate threshold in all three samples was set to 85 %; rs7675734 yielded in both samples a call rate below the threshold and was thus excluded from further analysis. Also monomorphic SNPs as well as those with a minor allele frequency (MAF) of smaller than 0.01 (rs9990922, rs12650927, rs12648509) were not further analyzed. Finally, genotype frequencies were ascertained for overall Hardy–Weinberg equilibrium (HWE; χ^2 HWE $p \geq 0.05$); four SNPs (rs7682844, rs1427633, rs1485325, rs4992204) showed deviations from HWE in both samples and rs1346376 only in the aADHD sample. In summary, of the 48 genotyped tag SNPs, 40 passed the quality control in the PD and 39 SNPs in the aADHD sample. Single marker associations were calculated by comparison of allele counts in 1-degree-of-freedom χ^2 tests. For multi-marker association tests, haplotype blocks were defined according to the four gamete rule [56]; inferred haplotype counts in groups were compared also with 1-degree-of-freedom χ^2 tests. Both, single marker and haplotype analyses were adjusted for multiple testing using the conservative Bonferroni correction, i.e., within each sample nominal results were corrected for 85 tests (40 SNPs + 45 haplotypes). With an estimated prevalence of 4.4 % for both disorders (aADHD: [29]; PD: [14]), our sample provides a power of 51 (13) and 52 % (14 %) to detect nominally (Bonferroni) significant SNPs and haplotypes, respectively, conveying a relative risk of 1.4 to develop the disorder, assuming a codominant model and a MAF of 0.05 [36].

Assessment of SNP function

Analyses of SNPs were performed with tools that are contained in the GenEpi toolbox [13] and the SNP function prediction tool on the National Institute of Environmental Health Sciences (NIEHS; http://manticore.niehs.nih.gov/snpfunc.htm). Annotation of SNPs in linkage disequilibrium (LD) with associated SNPs in a distance of 500 kb was retrieved from the SNAP website version 2.2 [28]. Differential transcription factor binding site (TFBS) predictions were made using the web-based tool MatInspector version 2.1 [12]. To indicate a SNP's possible influence on splice junctions, predictions of splice sites and binding sites for splicing regulatory elements (SREs) like intronic splicing enhancer (IES) and intronic splicing silencer (ISS), were made with the Human Splicing Finder software (HSF) version 2.4.1 [15]. Allele-specific binding of miRNAs were assessed with the Patrocles database [25]. To further corroborate the predicted influence of significantly associated promoter SNPs on gene expression, we examined the *SPOCK3* expression and genotypes of the unrelated CEU population (available from the GSE6536 series dataset at http://www.ncbi.nlm.nih.gov/geo and http://hapmap.ncbi.nlm.nih.gov). Linear models were used to analyze the additive effect of the minor alleles on gene expression in log scale.

Results

To further characterize the role of *SPOCK3* in psychiatric disorders, we genotyped 48 tag SNPs representing the gene's common allelic variation in one control and two disorder (aADHD and PD) samples. After a stringent quality control, 40 SNPs were subjected to case–control analysis in the PD sample, while 39 SNPs remained for association analysis in the aADHD sample (see Table 1).

Single marker associations

Analysis of individual polymorphisms revealed four SNPs that were associated with at least one of the both examined phenotypes at the nominal level (Table 1). Thereof, the minor alleles of the intronic SNPs rs7689440 (i.e., T; $OR_{ADHD} = 1.257$, $p_{aADHD} = 0.029$; $OR_{PD} = 1.234$, $p_{PD} = 0.039$) and rs897511 (i.e., C; $OR_{ADHD} = 1.294$, $p_{ADHD} = 0.008$; $OR_{PD} = 1.234$, $p_{PD} = 0.033$) both displayed a concordant and nominally significant enrichment in aADHD and PD, thus conveying genetic risk. Furthermore, the rarer variant of rs17052671 (i.e., T) was found more frequently in PD patients ($OR = 1.231$, $p_{nominal} = 0.039$). Overall, the most significant difference between affected and unaffected individuals was found for the promoter SNP

Table 1 Association results in the ADHD and the PD sample

SNP	($n = 536$)		ADHD Sample ($n = 624$)			PD Sample ($n = 630$)		
	Alleles (d/D)	Controls n d/D	Cases n d/D	Nominal p value	Corrected p value	Cases n d/D	Nominal p value	Corrected p value
rs11943562	C/T	149/647	226/920	0.582	1	173/891	0.165	1
rs2318483	G/C	45/855	73/1089	0.214	1	45/971	0.556	1
rs4602517	G/A	266/536	350/808	0.168	1	359/709	0.839	1
rs7689440	**T/C**	**238/660**	**363/801**	**0.020**	**1**	**311/699**	**0.039**	**1**
rs17052591	C/T	169/711	234/894	0.393	1	186/810	0.770	1
rs897511	**C/A**	**265/493**	**466/670**	**0.008**	**0.675**	**429/647**	**0.033**	**1**
rs17598103	T/A	61/823	89/1087	0.564	1	80/1014	0.723	1
rs17696409	G/C	147/651	189/951	0.292	1	193/885	0.774	1
rs17052602	G/A	12/878	13/1133	0.664	1	21/949	0.183	1
rs10018183	A/C	78/726	121/1027	0.547	1	107/979	0.913	1
rs9637685	C/G	203/613	276/870	0.687	1	287/811	0.532	1
rs897514	T/C	274/620	334/816	0.431	1	280/718	0.216	1
rs7660401	T/C	132/766	185/1003	0.582	1	142/978	0.188	1
rs7440269	G/A	66/844	101/1059	0.228	1	72/938	0.916	1
rs17702109	A/G	103/793	119/1061	0.303	1	142/974	0.402	1
rs17520441	C/T	30/872	47/1115	0.393	1	37/965	0.665	1
rs13113012	G/T	253/575	400/756	0.059	1	347/753	0.642	1
rs10025945	A/C	89/817	118/1064	0.904	1	109/1003	0.987	1
rs6553415	C/T	47/861	74/1096	0.267	1	44/974	0.378	1
rs17520763	C/T	440/462	569/601	0.947	1	461/531	0.315	1
rs17052671	**T/C**	**240/544**	**382/746**	**0.135**	**1**	**373/687**	**0.039**	**1**
rs11725742	C/A	271/541	415/727	0.176	1	353/713	0.906	1
rs13114933	A/G	92/734	120/1026	0.637	1	102/984	0.211	1
rs13102367	A/T	137/765	191/975	0.462	1	138/874	0.334	1
rs1346376	T/C	103/671	–	–	–	149/929	0.750	1
rs1834833	A/G	13/891	26/1138	0.187	1	16/1004	0.815	1
rs17702475	G/A	63/843	92/1096	0.494	1	78/1046	0.990	1
rs1593770	T/C	111/709	156/992	0.973	1	174/920	0.150	1
rs6857340	A/G	37/867	67/1119	0.105	1	49/1055	0.704	1
rs11722292	C/T	290/548	408/776	0.946	1	390/710	0.698	1
rs7660050	T/C	183/715	261/907	0.281	1	211/895	0.466	1
rs13128738	A/G	39/851	39/1123	0.229	1	51/1053	0.800	1
rs1427635	C/T	89/809	140/1034	0.147	1	118/992	0.598	1
rs13117458	T/C	134/768	182/972	0.568	1	138/884	0.395	1
rs7698061	T/G	173/733	243/943	0.429	1	212/910	0.909	1
rs6822214	T/A	81/813	97/1055	0.610	1	94/914	0.842	1
rs7683298	G/A	26/798	31/1127	0.530	1	26/1066	0.302	1
rs17053121	C/T	26/864	33/1147	0.866	1	33/1081	0.957	1
rs1485318	**T/C**	**184/726**	**221/935**	**0.531**	**1**	**147/873**	**0.001**	**0.062**
rs7694278	A/C	290/612	374/808	0.805	1	353/665	0.242	1

Data shown correspond to examined SNPs along with their minor (d)/major (D) alleles (converted to the coding strand), genotype and allele counts for cases and controls, and the nominal and the Bonferroni-corrected (corrected for 26 tests) p values. Bold indicates $p < 0.05$ in at least one of the samples. All p values were rounded to the third decimal place

rs1485318, whose association with PD was marginally significant after Bonferroni correction (OR = 0.664, $p_{corrected} = 0.062$). In contrast to all other associated SNPs, in this case, the major allele (i.e., C) was more enriched in individuals affected by PD (see Table 1). Analyses of PD subtypes showed that the intronic variants rs7689440,

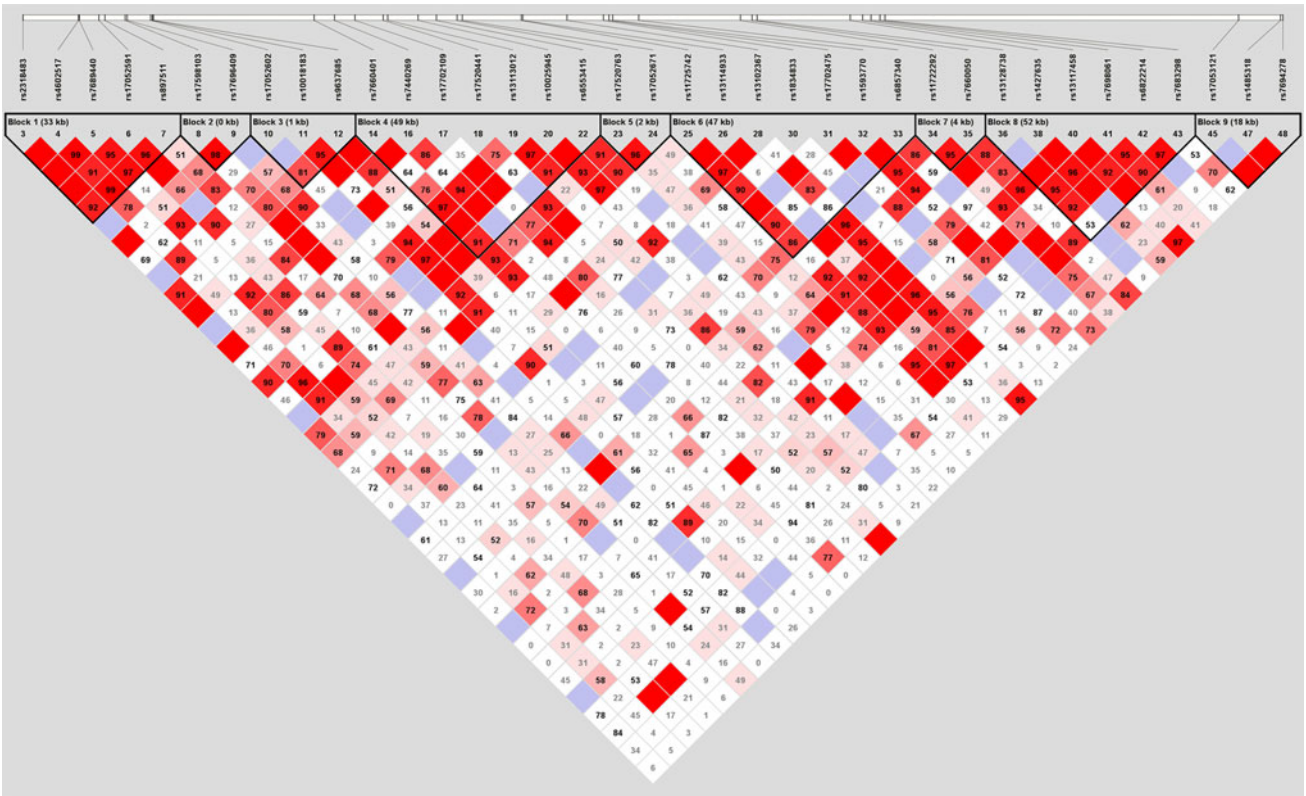

Fig. 1 Linkage disequilibrium (LD) plot of *SPOCK3*. LD is displayed as D'; haplotype blocks were generated according to the four gamete rule

rs897511 and rs17052671 were mainly associated with the impulsive Cluster B subtype ($p_{rs7689440} = 0.063$; $p_{rs897511} = 0.039$; $p_{rs17052671} = 0.059$), whereas the promoter SNP rs1485318 showed the strongest significance in the Cluster C PD subsample ($p = 0.005$; Supplementary Table 2). To exclude the possibility that observed cross-disorder effects are due to comorbidly affected individuals with ADHD and PD, we examined the pure ADHD and the ADHD with comorbid PD subsamples. The results (Supplementary Table 3) of the pure ADHD subsample confirmed the associations found in the total ADHD sample (i.e., cross-disorder-relevant SNPs), while the ADHD with comorbid PD subsample confirmed one ADHD/cross-disorder SNP, but none of the PD-specific ones were confirmed.

Haplotype analysis

The tag SNPs were found to be in considerable LD, allowing the definition of nine haplotype blocks (see Fig. 1; Table 2). Three blocks (namely 1, 5 and 9) contained haplotypes that were nominally associated with at least one of the both analyzed disorders. The association pattern of haplotypes generally matched to that of enclosed SNPs (see Table 2). In haploblock 1, enclosing two associated SNPs, the genetic risk was conveyed specifically by the haplotype containing both risk (i.e., minor) alleles. Moreover, the haplotype with both associated major alleles was found to have a significantly protective effect in aADHD (see Table 2). Finally, at the haplotype level, 5′-TTC-3′ in block 9 displayed the strongest association protecting from PD, reflecting the single marker association of rs1485318 (see Table 2).

Assessment of SNP function

For functional prediction of the four associated SNPs, high LD proxies ($r^2 \geq 0.8$ and $D' = 1$) were searched within a distance of 500 kb (Table 3). This yielded 30 high LD proxies of which eight were found in *SPOCK3*'s promoter region, 21 are located in intronic regions and one is synonymously coding (rs1057377). For the promoter SNP rs1485318 which was found to be most strongly associated with PD, no function was predicted, but the major allele has predicted TFBS for RUNX2, NANOG, OTX2, GFI1 and OC2 that overlap with four of rs1485318's eight proxies. The minor allele lacks these TFBS, but creates another for the universal transcription factor ATATA which overlaps with one proxy. However, in the unrelated CEU dataset, no significant influence of the rs1485318 genotype on *SPOCK3* gene expression was found.

Table 2 Association of Haplotypes in the ADHD and PD sample

rs2318483	rs4602517	rs7689440	rs17052591	rs897511			ADHD Sample ($n = 624$)			PD Sample ($n = 630$)		
							Case/control frequencies	Nominal p value	Permutation p value	Case/control frequencies	Nominal p value	Permutation p value
C	**G**	**C**	**T**	**A**			**0.291/0.342**	**0.014**	**1**	**0.333/0.346**	**0.546**	**1**
C	**A**	**T**	**T**	**C**			**0.298/0.254**	**0.033**	**1**	**0.298/0.255**	**0.039**	**1**
C	A	C	C	A			0.134/0.141	0.645	1	0.134/0.141	0.640	1
C	A	C	T	C			0.104/0.105	0.940	1	0.095/0.104	0.529	1
C	A	C	T	A			0.093/0.094	0.887	1	0.083/0.092	0.488	1
G	A	C	C	A			0.058/0.049	0.403	1	0.045/0.050	0.671	1
rs17598103	**rs17696409**											
A	C						0.833/0.820	0.412	1	0.820/0.818	0.904	1
A	G						0.091/0.108	0.177	1	0.106/0.110	0.763	1
T	G						0.076/0.072	0.727	1	0.074/0.072	0.856	1
rs17052602	**rs10018183**	**rs9637685**										
A	C	G					0.759/0.751	0.663	1	0.736/0.749	0.519	1
A	C	C					0.127/0.140	0.358	1	0.146/0.143	0.842	1
A	A	C					0.102/0.093	0.476	1	0.092/0.092	0.969	1
G	C	C					0.010/0.013	0.488	1	0.019/0.013	0.288	1
rs7660401	**rs7440269**	**rs17702109**	**rs17520441**	**rs13113012**	**rs10025945**	**rs6553415**						
C	A	G	T	T	C	T	0.481/0.488	0.740	1	0.492/0.486	0.794	1
C	A	A	T	T	C	T	0.091/0.110	0.155	1	0.114/0.110	0.784	1
C	A	G	T	G	A	T	0.096/0.097	0.921	1	0.099/0.097	0.890	1
C	A	G	T	G	C	T	0.086/0.080	0.626	1	0.084/0.079	0.698	1
C	G	G	T	T	C	T	0.081/0.073	0.460	1	0.066/0.072	0.577	1
T	A	G	T	G	C	T	0.053/0.062	0.376	1	0.062/0.065	0.778	1
T	A	G	T	G	C	C	0.063/0.049	0.194	1	0.038/0.048	0.254	1
T	A	G	C	G	C	T	0.037/0.032	0.560	1	0.024/0.032	0.282	1
rs17520763	**rs17052671**											
C	C						0.489/0.494	0.801	1	0.462/0.492	0.174	1
T	**T**						**0.338/0.303**	**0.085**	**1**	**0.344/0.301**	**0.040**	**1**
T	C						0.172/0.203	0.084	1	0.186/0.203	0.337	1
rs11725742	**rs13114933**	**rs13102367**	**rs1834833**	**rs17702475**	**rs1593770**	**rs6857340**						
A	G	T	G	A	C	G	0.371/0.409	0.082	1	0.399/0.409	0.683	1
C	G	A	G	A	C	G	0.156/0.144	0.488	1	0.132/0.144	0.491	1
A	G	T	G	A	T	G	0.130/0.130	0.998	1	0.145/0.130	0.346	1
C	A	T	G	A	C	G	0.099/0.106	0.609	1	0.092/0.106	0.269	1

Table 2 continued

rs2318483	rs4602517	rs7689440	rs17052591	rs897511			ADHD Sample ($n = 624$)			PD Sample ($n = 630$)		
							Case/control frequencies	Nominal p value	Permutation p value	Case/control frequencies	Nominal p value	Permutation p value
C	G	T	G	A	C	G	0.083/0.075	0.509	1	0.095/0.075	0.139	1
A	G	T	G	G	C	G	0.077/0.070	0.559	1	0.071/0.070	0.967	1
A	G	T	G	A	C	A	0.053/0.042	0.232	1	0.042/0.042	0.911	1
C	G	T	A	A	C	G	0.018/0.011	0.137	1	–	–	–
rs11722292	**rs7660050**											
T	C						0.432/0.448	0.463	1	0.456/0.448	0.804	1
C	C						0.347/0.343	0.852	1	0.351/0.343	0.620	1
T	T						0.220/0.209	0.527	1	0.188/0.209	0.313	1
rs13128738	**rs1427635**	**rs13117458**	**rs7698061**	**rs6822214**	**rs7683298**							
G	T	C	G	A	A		0.633/0.659	0.309	1	0.669/0.659	0.626	1
G	T	T	G	A	A		0.157/0.145	0.482	1	0.134/0.145	0.477	1
G	C	C	T	A	A		0.118/0.099	0.165	1	0.103/0.099	0.778	1
A	T	C	T	T	A		0.034/0.043	0.326	1	0.046/0.043	0.768	1
G	T	C	T	T	G		0.026/0.029	0.673	1	0.024/0.029	0.524	1
G	T	C	T	T	A		0.023/0.017	0.415	1	0.017/0.017	0.948	1
rs17053121	**rs1485318**	**rs7694278**										
T	C	C					0.465/0.456	0.675	1	0.478/0.456	0.320	1
T	C	A					0.316/0.317	0.997	1	0.347/0.317	0.152	1
T	**T**	**C**					**0.192/0.198**	**0.661**	**1**	**0.145/0.198**	**0.001**	**0.119**
C	C	C					0.027/0.029	0.824	1	0.030/0.029	0.871	1

Haplotype frequencies of cases and controls, nominal and the Bonferroni-corrected (corrected for 26 tests) p values are shown. Bold indicates $p < 0.05$ in at least one of the samples. All p values were rounded to the third decimal place

Table 3 Functional prediction of rs1485318, rs17052671, rs897511, rs7689440 (bold) and their proxy SNPs in high LD ($r^2 \geq 0.8$ and $D' = 1$). All transcription factors (TFs), shown in table, have a core similarity of 1 and a matrix similarity of 0.85 and more. Changes in SREs, like SE (Srp40, SF2/ASF, SC35, SRp55, 9G8) and SI (hnRNP A1, Sironi's Motiv 1, 2 and 3), such as potential splice sites with a match similarity of at least 65.0 are shown for minor and major alleles

Associated SNP	Proxy SNP	Position (bp)	Alleles (d/D)	SNP Function	Transcription Factor (TF)	
					Minor allele (d)	Major allele (D)
5′-Promotor-region						
rs1485318	rs13116056	168,453,407	C/T	Unchanged		
rs1485318	rs35520897	168,441,139	C/T	Changed		RUNX2
rs1485318	rs13117708	168,427,667	A/C	Changed	ATATA (universal)	NANOG
rs1485318	rs4466069	168,423,396	A/G	Unchanged		
rs1485318	**rs1485318**	**168,411,270**	**C/T**	**Unchanged**		
rs1485318	rs10517929	168,405,445	T/C	Changed		OTX2
rs1485318	rs13144552	168,402,551	T/C	Changed		GFI1, OC2
rs1485318	rs34366440	168,400,405	A/G	Unchanged		
rs1485318	rs17600543	168,398,760	A/G	Unchanged		

Associated SNP	Proxy SNP	Position (bp)	Alleles (d/D)	SNP Function	Splice Sites and Splicing Regulatory Elements (SREs)	
					Minor allele (d)	Major allele (D)
Intronic SNPs						
rs17052671	**rs17052671**	**168,077,859**	**C/T**	**Changed**		**9G8, 3xSF2/ASF, Sironi′s Motif 1 und 3**
rs897511	**rs897511**	**167,932,236**	**A/C**	**Changed**	**9G8**	**SRp55, splice site**
rs897511	rs6552223	167,930,857	G/T	Unchanged		
rs897511	rs6822872	167,930,605	G/C	Changed	2xSironi's Motif 2, hnRNP A1, splice site	SC35
rs897511	rs17598006	167,930,176	C/A	Changed	Sironi's Motif 3	
rs897511	rs17597957	167,929,522	A/G	Unchanged		
rs897511	rs3925714	167,929,128	T/C	Changed	SRp40, 2 × 9G8, Sironi's Motif 1	
rs897511	rs1037630	167,929,049	A/C	Changed		Sironi's Motif 2, hnRNP A1, splice site
rs897511	rs1037631	167,928,772	G/A	Unchanged		
rs897511	rs62355207	167,928,569	T/C	Changed	SC35	SF2/ASF
rs897511	rs2318486	167,920,092	C/G	Changed		2xSRp55
rs897511	rs7658810	167,911,970	G/C	Changed	2x9G8, Sironi's Motif 1	SRp40
rs897511	rs2198734	167,910,947	A/G	Changed	2xSF2/ASF, SRp40	
rs897511	rs11929824	167,900,276	T/C	Changed	Splice site	
rs897511	rs7678726	167,898,159	G/A	Changed		SRp40
rs897511	rs9918043	167,897,117	A/C	Changed	Splice site	
rs7689440	rs17519766	167,932,684	A/C	Changed	Sironi's Motif 2	
rs7689440	rs897512	167,931,987	G/A	Changed		2xSF2/ASF
rs7689440	rs72635168	167,930,578	G/A	Changed	Sironi's Motif 3	SRp40

Table 3 continued

Associated SNP	Proxy SNP	Position (bp)	Alleles (d/D)	SNP Function	Splice Sites and Splicing Regulatory Elements (SREs)	
					Minor allele (d)	Major allele (D)
rs7689440	**rs7689440**	**167,921,788**	**C/T**	**Changed**	**SRp40, hnRNP A1, splice site**	**2xSF2/ASF**
rs7689440	rs2318487	167,920,309	G/T	Changed	Sironi's Motif 3, hnRNP A1	SRp40
rs7689440	rs74197863	167,919,727	A/G	Changed	SF2/ASF, 9G8	Sironi's Motif 1
rs7689440	rs6827226	167,913,069	C/T	Changed		2xSF2/ASF
rs7689440	rs79862732	167,901,539	G/A	Changed	2xSRp55, 2xSironi's Motif 2, splice site	3xSF2/ASF
Synonymous-SNP						
rs7689440	rs1057377	167,892,648	C/T	Changed		SC35, Sironi's Motif 2

We furthermore observed differences in counts of predicted SRE between major and minor alleles of associated intronic SNPs and their proxies. Specifically, the PD-specific risk (minor) allele of rs17052671 deletes binding sites for four splicing enhancers (SE) and two splicing inhibitors (SI). Finally, rs897511 and rs7689440 are both located on haploblock 1 with their minor alleles concordantly increasing the predisposition toward ADHD and PD; together with their 21 intronic proxies, the risk alleles overlap with 15 SE and 13 SI binding sites, whereas the protective (major) allele sequence revealed 19 SE and 4 SI sites (see Table 3). Thus, the risk alleles lead to a significant enrichment of SI binding sites (OR = 4.12; $\chi^2 = 4.79$; $p = 0.029$), which may repress alternative splicing of *SPOCK3* transcripts.

Neither the four associated SNPs nor their proxies displayed allele-specific binding of miRNAs.

Discussion

The neurobiological framework of aADHD and comorbid personality disorders is known to overlap [27]. Likewise, the results from a GWAS on ADHD [39] and a pooled GWAS on PD [27, 58] together argue for a high a priori evidence of *SPOCK3* polymorphisms modifying the predisposition toward a comorbid affection. To allow a more detailed characterization, we determined the genetic risk brought about by 48 tag SNPs capturing the common allelic variation in samples of aADHD ($n = 624$), PD ($n = 630$) and healthy controls ($n = 536$). This setting provides a power of 51 % for the aADHD and PD samples to find nominal associations; accordingly, the power to survive Bonferroni correction is lower (aADHD 13 %; PD 13 %). Likewise, our associated SNPs reach only nominal significance in the respective phenotypes. Moreover, the strategy to use of a common control sample has advantages, but also imposes limitations: since the odds ratios of SNPs for each disorder refer to the same apparently healthy set of individuals, genetic effects are well comparable between disorders. The downside of this approach is, however, that improper sampling of the normal population (e.g., by limited sample size) may lead to impaired allele frequencies, thus affecting genetic effect estimation in both examined disorders. Although therefore the possibility of false-positives cannot be ruled out, our overall result of *SPOCK3* being a risk-modifying gene for ADHD and PD is in line with previous findings [39, 58]. However, the present study did not examine the *SPOCK3* candidate SNPs found in previous GWAS [39, 58], and lack of LD hinders the estimation of overlapping SNP effects of these studies. Moreover, a GWAS on aADHD did not reveal association signals of SPOCK3 in the 1,000 top-ranked SNPs [31].

Therefore, an independent replication is required to confirm the cross-disorder impact of *SPOCK3* SNPs.

The *SPOCK3* upstream/promoter region contains risk polymorphisms that together with untyped high LD proxies result in two main functional states, a highly affine state for binding a set of neurorelevant transcription factors (RUNX2, NANOG, OTX2, GFI1 and OC2) and a low-affinity state with binding predicted only for the universal response element ATATA. RUNX2 (also known as AML3), which is primarily known for its influence on differentiation of osteoplasts [59], was also found to be involved in the regulation of glutamic acid dehydrogenase 1 (GAD1) in GABAergic interneurons of the adult hippocampus in bipolar patients [7, 54]. The transcription regulator NANOG supports embryonic stem cell proliferation and therefore conceivably affects neurogenesis in general, and early-stage serotonergic neurons in particular [8]. OTX2 is required for differentiation of thalamic, glutamatergic, mesencephalic and dicephalic dopaminergic progenitors, all of which were associated with psychiatric disorders [44, 53]. The nuclear repressor protein GFI1, which is suspected to regulate dendritic cell development [46] and neurite outgrowth [41], is expressed in a risk locus for multiple sclerosis, the most common central nervous system disease in young adults [1]. Finally, the transcriptional activator OC2, also known as ONECUT2, is supposed to be involved in the development of spinal motor neurons [19]. Due to the association pattern of upstream risk polymorphisms, the *SPOCK3* promoter region seems to be particularly relevant to Cluster C PD. Our failure to confirm the regulatory influence of the promoter polymorphism rs1485318 on *SPOCK3* expression in the HapMap dataset does not necessarily weaken this hypothesis, because we propose an effect on gene expression in brain, while in the GSE6536 dataset expression profiles were derived from lymphoblastoid cells, which likely have a differently composed transcription factor repertoire. Furthermore, the unrelated CEU dataset comprises only 30 individuals, which may not provide enough power to detect effects of the *SPOCK3* promoter SNP on gene expression.

The associated variants in haploblock 1 have several intronic high LD proxies that putatively target splicing regulatory sites; particularly, the risk haplotype 5′-CATTC-3′ contains a significantly increased proportion of SI sites compared to the protective 5′-CGCTA-3′ haplotype. We therefore hypothesize that the molecular mechanism underlying the genetic risk conveyed by *SPOCK3* is due to repressed alternative splicing. The *SPOCK3* gene is expressed in 29 different transcripts, 19 of which result in a translated protein (Ensembl database, release 70). Repression of alternative splicing may therefore lead to paucity of SPOCK3 isoforms and associated functional consequences. Literature about SPOCK3 isoforms is sparse; however, splicing variants have a characteristic subcellular localization: the full-length protein comprising 436 amino acids (AA) is deposited in the ECM, which is not observed in the 316 AA truncated isoform, which lacks two putative glycosaminoglycan attachment sites [38]. *SPOCK3* as well as the structurally similar family members *SPOCK1* and *SPOCK2* were shown to be strongly expressed in brain and the central nervous system [34, 38, 51]. Their gene products were furthermore shown to inhibit neurite growth [35, 51]. Due to the high similarity of SPOCK proteins and the inhibitory effect of SPOCK3 on matrix metalloprotease function [38], an influence on brain development and function is also conceivable for SPOCK3. Given our association results, a fine-tuning of isoform expression and its effect on brain function is particularly relevant for triggering the risk for both aADHD and Cluster B PD.

While we hypothesize that joint risk variants in SPOCK3 contribute to the comorbidity between ADHD and personality disorders, we consider it interesting that the ADHD risk SNPs were specifically found to be associated with dramatic, emotional and erratic Cluster B PD. Both aADHD and Cluster B PD display by emotional dysregulation with increased impulsivity, so that these variants affecting SPOCK3 splicing might contribute to the regulation of impulsive behaviors. On the other hand, the promotor SNP rs1485318 that affect expression levels and is mainly associated with Cluster C PD might go along with a changed predisposition toward anxiety, probably due to interactions with the GABAergic system, as we have preliminary evidenced that it is also associated with panic disorder (not shown, manuscript in preparation).

Taken together, *SPOCK3* variants modulating gene expression preferably influence Cluster C PD risk, and polymorphisms targeting splicing regulatory sites convey both aADHD and Cluster B PD risk. These are only speculations; apart from the strong expression of *SPOCK3* in neurons during embryonic development of mice, it is known that mRNA expression in adults is restricted to the brain, with high protein levels in the bulbus olfactorius, thalamus, hippocampus, cortex und striatum [10, 24, 34, 48]. Especially changes in function and/or formation of the latter four brain regions are associated with many psychiatric disorders, like schizophrenia, bipolar disorder, depression and ADHD [17, 22, 23, 30, 33, 42]. Knowledge about spatial gene expression in brain regions or cell types could provide an indication of gene function. The four brain areas are known for their involvement in movement (prefrontal cortex), social adaption (prefrontal cortex), executive control (prefrontal cortex and striatum), working and short-term memory (prefrontal cortex, striatum, thalamus, hippocampus), attention (striatum and thalamus) and recognition and learning (hippocampus), all characteristics which are impaired in ADHD, underlying the potential

pathophysiological role of *SPOCK3*, but the exact physiological pathway remained to be unclear. Findings of [55] showed that mRNA expression of *SPOCK3* is going along with *GAD1* mRNA levels in GABAergic interneurons in the visual cortex of monkeys. GAD1, the key enzyme for synthesis of the inhibitory and anxiolytic neurotransmitter GABA, is supposed to influence various mental disorders [57], since cortex, striatum, thalamus and hippocampus are all interconnected by GABAergic neurons, arguing for a role of *SPOCK3* in regulatory mechanisms of the GABAergic system. However, the only sparse information about *SPOCK3* impede more detailed conclusions and thus further analyses will be necessary to clarify the contribution of *SPOCK3* to neurobiological processes and behavior.

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Conflict of interest The authors declare that they have no conflict of interest.

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