

Epistatic and Gene Wide Effects in *YWHA* and Aromatic Amino Hydroxylase Genes Across ADHD and Other Common Neuropsychiatric Disorders: Association With *YWHA*E

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Monoamines critically modulate neurophysiological functions affected in several neuropsychiatric disorders. We therefore examined genes encoding key enzymes of catecholamine and serotonin biosynthesis (tyrosine and tryptophan hydroxylases—TH and TPH1/2) as well as their regulatory 14-3-3 proteins (encoded by *YWHA*-genes). Previous studies have focused mainly on the individual genes, but no analysis spanning this regulatory network has been reported. We explored interactions between these genes in Norwegian patients with adult attention deficit hyperactivity disorder (aADHD), followed by gene-complex association tests in four major neuropsychiatric conditions; childhood ADHD (cADHD), bipolar disorder, schizophrenia, and major depressive disorder. For interaction analyses, we evaluated 55 SNPs across these genes in a sample of 583 aADHD patients and 637 controls. For the gene-complex tests, we utilized the data from large-scale studies of The Psychiatric Genomics Consortium (PGC). The four major neuropsychiatric disorders were examined for association with each of the genes individually as well as in three complexes as follows: (1) *TPH1* and *YWHA*-genes; (2) *TH*, *TPH2* and *YWHA*-genes; and (3) all genes together. The results show suggestive epistasis between *YWHA*E and two other 14-3-3-genes - *YWHA*Z, *YWHA*Q - in aADHD (nominal *P*-value of 0.0005 and 0.0008, respectively). In PGC data, association between *YWHA*E and schizophrenia was noted ($P=1.00E-05$), whereas the combination of *TPH1* and *YWHA*-genes revealed signs of association in cADHD, schizophrenia, and bipolar disorder. In conclusion, polymorphisms in the *YWHA*-genes and their targets may exert a cumulative effect in ADHD and related neuropsychiatric conditions, warranting the need for further investigation of these gene-complexes. © 2015 The Authors. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* Published by Wiley Periodicals, Inc.

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INTRODUCTION

Psychiatric disorders are a major cause of loss of quality of life, residing among the top twenty origins of disability world wide [Vos et al., 2012]. Clinical, epidemiological, and genetic studies have

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demonstrated high levels of co-occurrence of psychiatric symptoms and disorders, and indicated some shared environmental and genetic risk factors across multiple conditions [Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Lee et al., 2013]. Such observations have spurred an increased interest in studying genetic risk factors across formal diagnostic boundaries.

Attention-deficit/hyperactivity disorder (ADHD) is the most common childhood onset neurodevelopmental disorder, often persisting into adulthood [Franke et al., 2011]. The disorder is characterized by high levels of inattention, hyperactivity, and impulsivity that cause functional impairments [Banaschewski et al., 2010]. Individuals with ADHD less often complete their university education, are more likely to be unemployed, and have an increased risk of developing anxiety, depression, or substance abuse [Halmøy et al., 2009]. Adult ADHD (aADHD) has a prevalence of 2.5–4.9%, making it a common condition also among adults [Franke et al., 2011]. Combined, cADHD, and aADHD have a high socioeconomic impact, though the number and size of genetic studies on ADHD are small compared to other neuropsychiatric disorders such as schizophrenia (SCZ) and bipolar disorder (BPD). Despite high heritability (56–84%), the underlying genetic architecture of ADHD is poorly understood [Franke et al., 2011; Larsson et al., 2013].

Pharmacological treatment of psychiatric disorders is mainly based on modulation of catecholamine and serotonin signalling. This is also the case for ADHD, where the discovery of amphetamine and its derivatives as effective therapeutic substances early implicated altered catecholamine signalling [Robertson et al., 2009]. Studies linking these neurotransmitters to brain functions where ADHD patients show impairment, such as sustained attention and response inhibition, further support this theory [Barnes et al., 2011]. Although genetic variants, particularly in dopamine receptors and transporter, have been scrutinized in many ADHD samples, their association with the disorder is still being debated, and other genes and mechanisms need to be studied [Franke et al., 2011, 2009b].

Tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) are the rate-limiting enzymes of the catecholamine and serotonin synthesis, respectively [Lenartowski and Goc, 2011] (Fig. 1A). TPH has two forms encoded by separate genes - *TPH1* and *TPH2*- that possess different regulatory properties and are expressed in peripheral endocrine systems, including the pineal gland (*TPH1*) as well as in the central nervous system (CNS) (*TPH2*) [Walther et al., 2003; McKinney et al., 2005]. TH is encoded by one gene (*TH*), and is expressed both in the periphery neuroendocrine system and in the CNS [Haavik et al., 2008].

The activity, subcellular localization and turnover of TH, TPH1, and TPH2 enzymes are regulated by the members of 14-3-3 protein family through direct phosphorylation-mediated protein–protein interactions (Fig. 1A) [Banik et al., 1997; Itagaki et al., 1999; Toska et al., 2002; Winge et al., 2008; Kleppe et al., 2014]. There are seven different 14-3-3 protein forms (β , γ , ϵ , ζ , η , θ/τ , and σ), all known by their gene names *YWHA*B/G/E/Z/H/Q/S, respectively, though *YWHAS* is also recognized as *SFN* (*stratifin*). For convenience, we will use the term “*YWHA*-genes” to denominate all 14-3-3-protein coding genes. Different 14-3-3 monomers can combine into various dimer types of 14-3-3 proteins, the main functional

form of 14-3-3s (Fig. 1B). We have recently reported differences between the 14-3-3 forms in their interaction with TPH2 [Winge et al., 2008]. We have also found distinctions between 14-3-3 proteins in their potency to activate phosphorylated bovine TH (Fig. 1C, Ghorbani et al. unpublished). The 14-3-3 proteins could, therefore, differentially regulate monoamine levels in the nervous system, making them promising candidate genes for ADHD and related neuropsychiatric conditions.

Following the observation of physical interaction between 14-3-3 proteins with tyrosine and tryptophan hydroxylases (Fig. 1, (Toska et al., 2002; McKinney et al., 2005; Yang et al., 2006; Wang et al., 2009; Ramshaw et al., 2013; Kleppe et al., 2014)), we postulated that such interaction might also manifest itself as epistasis between regulatory *YWHA*-genes and the genes of their target enzymes, *TH*, *TPH1* and *TPH2*.

While there have been numerous small studies on polymorphisms within the *YWHA*-genes, *TH* and *TPH1/2*, findings are inconsistent and no single SNP has provided strong, replicable association signal with neuropsychiatric disorders such as ADHD, BPD, SCZ, depression, and autism spectrum disorders. One study investigated gene–gene interaction in several ADHD candidate genes including TH, TPH1, and TPH2, but none of these three genes showed significant interactions [Segurado et al., 2011]. Furthermore, the gene network including the regulatory *YWHA*-genes has not been analyzed together.

Given that no SNP in *YWHAs* nor *TH*, *TPH1/2* genes achieved significance below threshold corrected for multiple testing in genome-wide meta-analysis of either SCZ, BPD, major depressive disorder (MDD), or ADHD, it is unlikely that any single, common variant in these genes would have a strong impact on the development of major neuropsychiatric disorders [Lasky-Su et al., 2008; Neale et al., 2010; Cichon et al., 2011; Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium et al., 2013; Ripke et al., 2013]. However, small effects of singular SNPs may result in a stronger, cumulative signal.

In this study, we performed genetic exploration of the genes involved in the synthesis of monoamines with respect to ADHD and related major neuropsychiatric disorders, such as SCZ, BPD, and MDD. The analyses were structured to examine the complexity of possible contribution of the aforementioned genes to the risk of these disorders in the following manner: (1) single SNP interaction tests (epistasis); (2) gene-wide association tests; and (3) regulatory gene-complex evaluation.

MATERIALS AND METHODS

Subjects in the Norwegian Sample

Single SNP-based interaction analyses were performed in our Norwegian sample. DNA samples of all participants were obtained from the Norwegian adult ADHD biobank. Patients were recruited through the national ADHD registry and outpatient clinics. Participants were diagnosed with ADHD in accordance with either ICD-10 or DSM-IV criteria as previously described [Johansson et al., 2008]. Controls were recruited through the Medical Birth Registry of Norway [Johansson et al., 2008; Halmøy et al., 2009; Jacobsen et al., 2013]. In total, 583 patients and 637 controls were included in the analyses. All participants gave written, informed

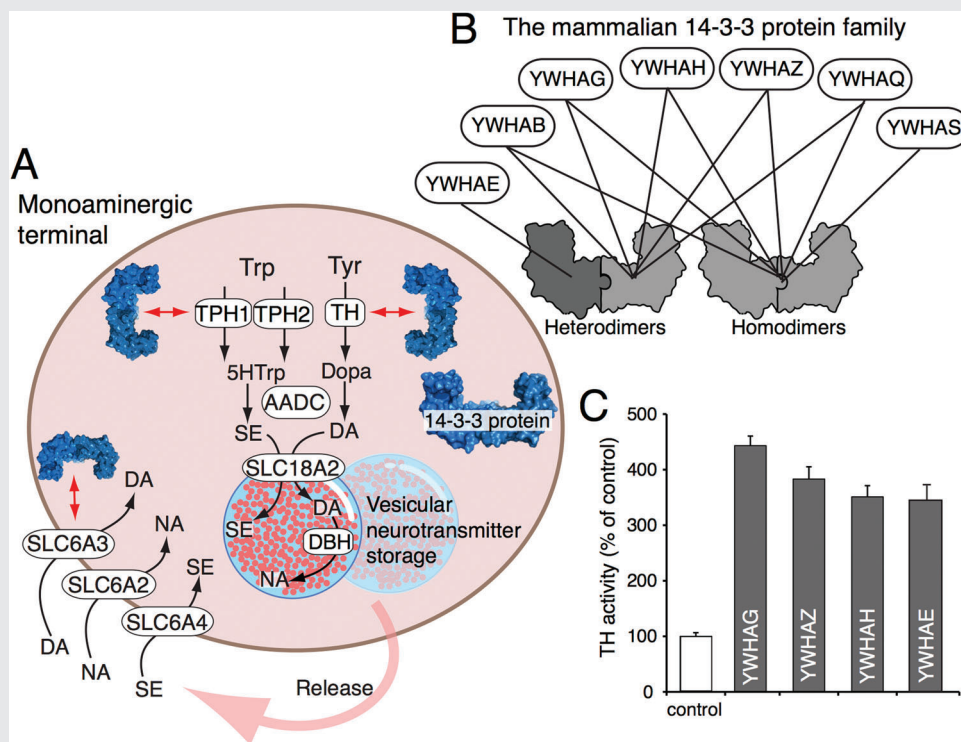


FIG. 1. 14-3-3 proteins in the regulation of monoamine biosynthesis. **(A)** shows a literature based illustration of the presynaptic biosynthesis of dopamine (DA) and noradrenaline (NA) from tyrosine (Tyr) via dihydroxyphenylalanine (L-Dopa), involving the enzymes tyrosine hydroxylase (TH), aromatic amino acid decarboxylase (AADC/DDC), dopamine β -hydroxylase (DBH), and the vesicular monoamine transporter (VMAT2/SLC18A2). Black arrows illustrate chemical transformations or transport processes, whereas red double arrows illustrate protein–protein interactions. Biosynthesis of serotonin (SE) from tryptophan (Trp) via 5-hydroxytryptophan (5HTp) by tryptophan hydroxylase 1 or 2 (TPH1 or TPH2) and AADC with subsequent transport into storage vesicles is also shown. Reuptake of released DA, NA, or SE by dopamine transporter (DAT1/SLC6A3), noradrenaline transporter (NET/SLC6A2), or serotonin transporter (SERT/SLC6A4) is also shown. Phosphorylated TH, TPH1, and TPH2 may interact with members of the mammalian 14-3-3 protein family (YWHA proteins), which may modulate the enzymatic activities, stability, or cellular localization [McKinney et al., 2005; Kleppe et al., 2014]. A reported interaction between SLC6A3 and YWHAZ is also depicted [Ramshaw et al., 2013]. **(B)** illustrates the formation of possible dimers within the mammalian 14-3-3 protein family. The reported interactions are shown between different monomers by formation of heterodimers [14-3-3 ϵ /YWHAE with 14-3-3 β /YWHAB, 14-3-3 γ /YWHAH, 14-3-3 η /YWHAB, 14-3-3 ζ /YWHAZ, and 14-3-3 τ /YWHAQ] and by formation of homodimers [Yang et al., 2006]. 14-3-3 σ /YWHAS is found to preferentially form homodimers, whereas YWHAE is found as heterodimers in cells. **(C)** shows the activation of bovine TH, phosphorylated on Ser19 by p38-regulated/activated protein kinase, in the presence of different 14-3-3 isoforms reported in midbrain [Wang et al., 2009]. The experiments were performed essentially as described [Toska et al., 2002, Ghorbani et al. in preparation]. Different activation potency between the YWHAs suggests a variable risk for association with monoamine related disorders between the YWHA-genes.

consent and were above 18 years of age at the time of recruitment, with no known intellectual disability. The study was approved by the Norwegian Regional Medical Research Ethics Committee West (IRB #3 FWA00009490, IRB00001872).

Selection of SNPs and Genotyping in the Norwegian Sample

The markers in *TPH1* and *TPH2* were chosen from previous candidate gene studies performed by our group as well as others [Johansson et al., 2010; Gao et al., 2012]. All *YWHA*-genes and *TH* were tagged using the aggressive tagging algorithm with default settings of Haploview, based on CEU data from the HapMap release 28 [International HapMap Consortium, 2003; Barrett

et al., 2005]. Each gene was tagged with approximately 5,000 basepair flanking sequence included.

Genotyping was done in two batches using MassArray iPLEX system (Sequenom, San Diego, CA) at CIGENE center for genotyping (University of Life Sciences, Ås, Norway). The genotyping of all selected SNPs was attempted in the first batch. Genes containing failed or low quality SNPs in the first batch were re-tagged using the “force exclude” and “force include” functions of Haploview and the new tagging SNPs were genotyped in the second batch. There was no SNP-overlap between the two batches. To assess the genotyping concordance within each of the two batches, two internal controls were used with a total number of 83 duplicates of the two samples.

Genotyping quality control (QC) was done in PLINK for each batch separately, and then again after combining the two batches

[Purcell et al., 2007]. The inclusion criteria was defined as individual and SNP genotyping rate above 95%, minor allele frequency (MAF) above 5% in controls and a Hardy–Weinberg disequilibrium threshold of $P > 0.01$ in controls.

Data From the Psychiatric Genomics Consortium

For gene-based and gene-complex analyses we utilized the data from the large-scale association meta-analysis of childhood ADHD (cADHD) performed by The Psychiatric Genetics Consortium (PGC, <http://www.med.unc.edu/pgc/downloads>) as it would have a larger power and provide more accurate estimates than our own discovery sample. Since there is a phenotypic overlap between ADHD and several other neuropsychiatric disorders, we also examined possible contribution of our proposed regulatory gene-complexes to SCZ, BPD and MDD using large-scale genome-wide association data reported by the PGC [Neale et al., 2010; Psychiatric GWAS Consortium Bipolar Disorder Working Group et al., 2011; The Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium et al., 2011; Foote and Zhou, 2012; Gao et al., 2012; Mandelli et al., 2012; Watanabe et al., 2012; Yang et al., 2012; Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium et al., 2013; Fromer et al., 2014; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014]. To ensure high quality data in SCZ, BPD, and MDD PGC datasets, we utilized only those SNPs whose imputation INFO measure was reported to be above or equal to 0.6. This measure was not available for the cADHD dataset.

Statistical Analyses

Epistasis in the Norwegian sample. Single SNP-based interaction analyses were performed in the form of likelihood ratio tests implemented in SNPAssoc package [González et al., 2007]. SNPAssoc was carried out in R-software, using gender as a covariate, testing for association with aADHD status (www.r-project.org). Only interaction between genes was evaluated, meaning that each SNP was tested for interaction with all SNPs in other genes, but not with SNPs in the same gene. Thus, in total, 495 interaction SNP pairs were tested in this study.

In order to further explore the strongest epistasis observed, we performed logistic regressions with the two-way interaction term between the pair of SNPs revealing the signal. The regressions were performed in R software.

Bonferroni correction for 495 SNP interaction tests was used to adjust for multiple testing, resulting in the corrected significance threshold of $1.01E-04$.

To evaluate the power of our study to detect a true association, basic power calculations was performed using the Genetic Power Calculator, applying the sample size of our data to a single SNP with minor allele frequency of 0.1 and 0.2 and a genotype relative risk (GRR) of 1.5, given ADHD disease prevalence of 3% [Purcell et al., 2003].

Gene-wide association in four PGC datasets. Gene-wide evaluation of *TH*, *TPH1/2*, and the seven *YWHA*s was done using JAG

software [Lips et al., 2012; Hammerschlag et al., 2014]. For each gene, the test statistic was defined as the sum of the $-\log_{10}$ association *P*-values of individual SNPs annotated to each of the 10 genes of our interest (*TH*, *TPH1*, *TPH2*, and the seven *YWHA*-genes). Gene annotation of the variants from PGC data included 5,000 basepair region around each gene.

To define the significance threshold, 100,000 permutations were carried out. The statistics of each gene were computed for each permutation and the final gene-based *P*-value was calculated as the proportion of test statistics in the permuted data that was higher than the original test statistic.

For permutations and to account for LD effects between examined SNPs, we utilized the genotype data of the European ancestry samples from the 1000 Genomes project [1000 Genomes Project Consortium et al., 2012]. Thus, only SNPs that were available in both the PGC data and the 1000 Genomes genotype data were used to calculate the test statistics.

Regulatory gene-complex analyses in four PGC datasets.

Similarly to gene-based tests, regulatory gene-complexes were evaluated using JAG software. The genes were tested as gene-complex combinations based on their possible regulatory role in the synthesis of monoamines. We defined the following gene-complexes to test: (1) seven *YWHA*-genes and *TPH1* gene to test the effect of genes involved in peripheral monoamine synthesis; (2) seven *YWHA*-genes, *TPH2*, and *TH* to test the effect of genes involved in monoamine synthesis inclusive of central nervous system; and (3) all 10 genes to test their overall effect.

The analyses were performed in two steps: first self-contained association was tested for each gene-complex, followed by a competitive test. A self-contained assessment examines whether a gene-complex is associated with a trait, while the competitive test reveals whether the observed self-contained association is stronger than expected by chance for a random gene-complex with the same number of SNPs.

The self-contained tests were performed in the same manner as the gene-based analyses, where instead of a gene we tested a gene-complex. For the competitive tests, we generated random size-matching gene-complexes and performed self-contained tests for each of these random gene-complexes. The competitive tests' *P*-value was calculated as the proportion of random gene-complexes with self-contained *P*-values lower than the self-contained *P*-value for the gene-complex itself. A significant competitive test would indicate that the evaluated gene-complex is more strongly associated with a trait than any other size-matched set of randomly drawn genic SNPs.

Correction for multiple testing in PGC data was achieved by Bonferroni method, with corrected significance threshold of 0.0031 (to correct for 10 gene-based, three self-contained, and three competitive gene-complex tests).

RESULTS

Subjects in the Norwegian Sample

After QC, a total of 583 adult ADHD cases and 637 controls were available for analyses. The percentage of males was 48% in cases and 40% in controls. Average age at recruitment was 34 years in cases and 29.4 years in controls.

Selection of SNPs and Genotyping in the Norwegian Sample

In total, 71 SNPs were selected: 10 in *TPH1* and *TPH2* from previous studies, one SNP in *YWHAE* from Ikeda et al., and one SNP in *YWHAG* from an exome sequencing database in our genetics laboratory [Ikeda et al., 2008]. In order to tag *TH*, two tagging SNPs were included and for *YWHA*s 57 tagging SNPs were submitted for multiplexes. Sixteen SNPs were removed due to incompatible multiplex design or low genotyping quality, 11 of these in the first round of genotyping. These SNPs were re-tagged and genotyped in the second batch. Overall, 55 SNPs across 10 genes were available for the analyses (Supplementary Table S1). Concordance rate in duplicate samples was >99% within each of the two batches. Supplementary Figure S1 shows an overview of the LD structure for each gene, with tested markers indicated.

Data From the Psychiatric Genomics Consortium

After selecting SNPs with imputation INFO measure above or equal to 0.6 and annotating the variants to the genes of our interest, the PGC's SCZ, BPD, and MDD datasets, contained, 825 SNPs in SCZ, 354 SNPs in BPD, and 204 SNPs in MDD available for the analyses (Table II). The PGC's cADHD dataset comprised a total of 210 SNPs in aforementioned genes. The SNPs were distributed among all 10 genes, though the BPD dataset did not contain any SNPs in *TH*.

Epistasis in the Norwegian Sample

A total of 495 SNP pairs were tested for interaction using the likelihood ratio test in SNPAssoc. The strongest signals were observed between *YWHAE* and two other 14-3-3 genes - *YWHAZ*, *YWHAQ* - in adult ADHD, with P-values of 0.0005 and 0.0008 respectively (Table I, Fig. 2). None of these passed the strict

Bonferroni-corrected significance threshold ($P < 0.0001$). Supplementary Table S2 details the results of all interaction pairs.

The nature of epistasis between the two pairs of SNPs with the strongest signal was further explored in logistic regression. In the *YWHAE/YWHAZ* interaction pair, having the rare alleles of both SNPs appeared to decrease the risk of ADHD, while the combination of rare alleles of interacting SNPs in *YWHAE* and *YWHAQ* revealed the opposite effect. (Table I, Supplementary Table S3).

Power calculations revealed 89% power for detecting a single SNP association with minor allele frequency of 20% and a genotype relative risk of 1.5 in our sample, and 80% for a SNP with 10% minor allele frequency.

Gene-Wide Association in Four PGC Datasets

We performed gene-based association tests for 10 genes: *TH*, *TPH1/2*, and the seven *YWHA*s. The most significant signal, surviving correction for multiple testing ($P < 0.0031$), was observed for *YWHAE* in SCZ ($P = 1.00E-05$). In cADHD, nominal associations were noted for *YWHAQ* and *TPH1*. The summary of these analyses is presented in Table II, while LocusZoom plots of the nominally significant genes is presented in Supplementary Figure S2.

Regulatory Gene-Complex Analyses in Four PGC Datasets

We used the PGC data on SCZ, cADHD, BPD, and MDD to investigate the association between the four disorders and three sets of gene-complexes containing *TH*, *TPH*s, and *YWHA*s. For self-contained tests, examining the association between a gene-complex and a phenotype, the gene-complex including *TPH1* and the seven regulatory *YWHA*-genes revealed the most consistent signal across the examined four disorders, with signals of association for cADHD, SCZ, and BPD. However, competitive tests, indicating the probability of observed association being due to the function of the examined gene-set and not a random chance, resulted in significant

TABLE I. Details of the Strongest Signals of Epistasis in Our Norwegian Sample. Supplementary Table S2 Shows the Details of All Interaction Pairs

(A) P-values of log likelihood tests Bonferroni-corrected significance threshold $P = 1.01E-04$.			
Interaction pair	Gene	SNP	P-value
1	YWHAQ	rs4145375	0.0008
	YWHAE	rs17625475	
2	YWHAE	rs28365859	0.0005
	YWHAZ	rs17365305	
(B) Results of exploring logistic regressions			
		OR	95% confidence interval
YWHAE × YWHAZ interaction			
	rs28365859	1.11	0.92–1.34
	rs17365305	1.87	1.13–3.11
	rs28365859 × rs17365305	0.33	0.18–0.61
YWHAE × YWHAQ interaction			
	rs17625475	1.03	0.80–1.34
	rs4145375	0.75	0.51–1.10
	rs17625475 × rs4145375	3.72	1.60–8.67

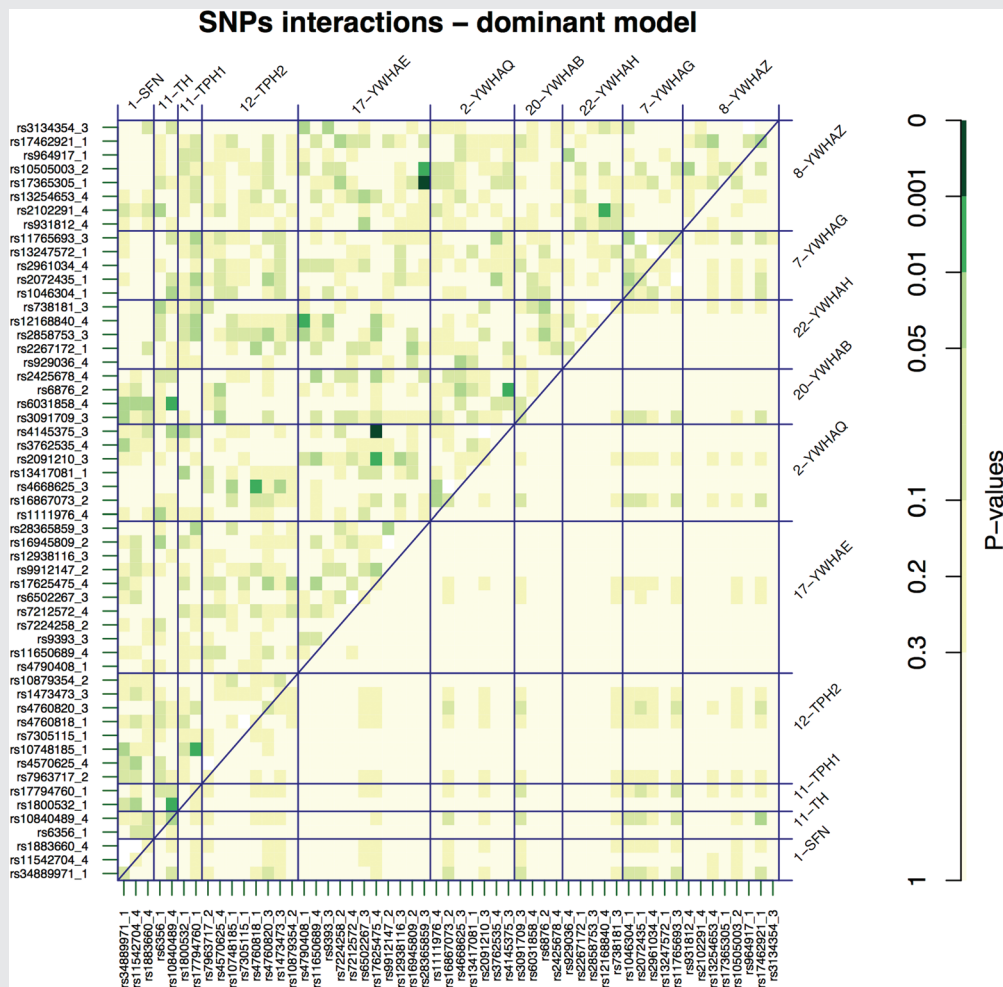


FIG. 2. Likelihood ratio tests of all interactions examined in this study. Each small square represents the *P*-value of a single test. As indicated by the gradient color of green, the darker shades represent smaller, more significant *P*-values. SNPs are grouped along the axes by genes, listed alphabetically and separated by grid lines. Along the diagonal, as indicated by a line, are the *P*-values of the single SNP tests. The lower right triangle represents additive tests, which were not the focus of this study. The upper left triangle represents interaction tests between a pair of SNPs. Two dark green squares correspond to the SNP interactions represented in Table I. The numerical values of each test are presented in Supplementary Table S2.

outcome for SCZ only. While *TPH1* and *YWHA*s complex as well as all gene altogether revealed SCZ association signals passing the correction for multiple testing, none of the competitive test results passed the Bonferroni adjusted significance threshold. Table III shows the details of these analyses.

DISCUSSION

This is the first systematic investigation of genetic variants spanning the whole *YWHA* gene-family and their regulatory targets in monoamine synthesis in association with ADHD and related neuropsychiatric disorders (SCZ, MDD, and BPD). Given results from previous studies, we hypothesized that these genes might influence neuropsychiatric traits through their cumulative effects.

As several of the 14-3-3 proteins are known to physically interact with each other as well as with TH/TPH to regulate their functions

(Fig. 1), genetic interaction analyses could reveal coupled risk variants. We explored epistasis between these genes utilizing our adult ADHD sample of 1,220 individuals. Two nominally significant SNP interactions between the *YWHA*-family members were observed. Interestingly, the strongest evidence for epistasis was detected for the *YWHA E* gene, whose product, 14-3-3 ϵ , is known to mainly form heterodimers with many other 14-3-3 proteins. *YWHA E* revealed possible interaction with *YWHA Z* and *YWHA Q* in association with adult ADHD (Table I). Detailed exploration of these SNP pairs indicate that the epistasis between *YWHA E* and *YWHA Z* may be of protective nature as opposed to that between *YWHA E* and *YWHA Q*, where accumulation of rare alleles of both SNPs was observed in cases compared to controls (Supplementary Table S3). Given that each SNP pair contains transcription factor binding sites—rs28365859 in the 5'UTR region of *YWHA E* interacting with *YWHA Z* and rs4145375 in the vicinity of *YWHA Q*

TABLE II. Gene-Based Association Results Using PGC Data. The Reported *P*-Values are Unadjusted for Multiple Testing and are Based on 10,000 Permutations. Bonferroni Corrected Significance Threshold *P*-value is 0.0031. *P*-Values Below 0.1 are Highlighted in Italics. *P*-Values Surviving Bonferroni Correction are Highlighted in Bold and Italics

Gene symbol	Gene-based association <i>P</i> -value (number of SNPs tested)			
	cADHD	SCZ	MDD	BPD
TH	0.176 [8]	0.683 [51]	0.967 [5]	no data
TPH1	<i>0.081 [15]</i>	<i>5.40E-03 [54]</i>	0.516 [15]	0.219 [25]
TPH2	0.164 [78]	0.247 [285]	0.757 [77]	0.784 [152]
SFN	0.737 [1]	<i>0.052 [4]</i>	0.664 [1]	0.223 [1]
YWHAQ	<i>0.024 [18]</i>	0.294 [82]	<i>0.023 [17]</i>	0.993 [39]
YWHAG	0.412 [14]	<i>0.189 [58]</i>	0.235 [13]	<i>0.031 [24]</i>
YWHAZ	0.344 [20]	0.028 [66]	0.364 [20]	0.337 [25]
YWHAE	0.174 [25]	<i>1.00E-05 [132]</i>	0.467 [25]	<i>0.034 [48]</i>
YWHAB	0.504 [14]	<i>3.96E-03 [52]</i>	0.069 [14]	0.786 [17]
YWHAH	0.424 [17]	0.125 [41]	0.949 [17]	0.212 [23]

interacting with *YWHAE*—multiple scenarios for dynamic heterodimer composition can be postulated. Consequently, various heterodimer types might have different targets with opposing effects. Similarly, in PGC cADHD data, nominal significance was observed for *YWHAQ*, the gene that is nominally associated with adult ADHD through epistasis with *YWHAE* in our independent Norwegian sample. Although none of these interactions reached the Bonferroni corrected significance level, they represent interesting findings that should be taken forward for further analysis.

Curiously, apart from the interaction effect in adult ADHD examined in our Norwegian sample, *YWHAE* also showed the strongest signal in gene-based association tests in PGC datasets (Table II). Specifically, *YWHAE* association with SCZ passed the Bonferroni-corrected significance threshold and showed nominal association with BPD. For PGC's cADHD sample, nominal significance was observed for *YWHAQ* and *TPH1*. The latter also showed nominal association with SCZ in PGC data (Table II).

14-3-3 ϵ , the protein encoded by *YWHAE*, is crucial for neuronal development [Toyo-oka et al., 2003; Bradshaw and Porteous, 2012]. Mice deficient in 14-3-3 ϵ have severe defects in neuronal migration, resulting in growth restriction, craniofacial dysmorphisms, structural abnormalities of brain, and cognitive impairment [Toyo-oka et al., 2003; Nagamani et al., 2009]. The latter often co-occurs with ADHD [Armstrong et al., 2001]. In addition, dysregulation of brain development has been shown to play an important role in the etiology of ADHD [Lesch et al., 2008; Franke et al., 2009a; Poelmans et al., 2011; Yang et al., 2013]. Furthermore, *YWHAE* has been implicated in ADHD and autism through CNVs or de novo mutations [Neale et al., 2012; Ramos-Quiroga et al., 2014]. Several previous studies have also found associations of *YWHAE* with intellectual disability, SCZ, and BPD, albeit some studies have also been negative [Ikeda et al., 2008; Liu et al., 2010, 2012; Takahashi and Nakamura, 2014; Schubert et al., 2015]. Thus, taken together with previous reports, our findings lend further support to the central role of *YWHAE* in schizophrenia and several other neuropsychiatric conditions.

The regulatory gene-complex analysis performed in this study showed nominal evidence of cumulative effect of *TPH1* and the

seven *YWHA*s in SCZ as well cADHD (PGC data, Table III). *TPH1* catalyzes the rate-limiting step of serotonin production in non-neuronal cells, such as enterochromaffin cells of the gut, mast cells, and the pineal gland [Suidan et al., 2013]. So far, the role of peripheral serotonin in nervous system function is poorly understood. However, serotonin has been shown to be of developmental importance, modulating the construction and plasticity of brain circuits [Gaspar et al., 2003; Neckameyer et al., 2007]. Immunohistochemical analyses revealed that in the first 2 hr of *Drosophila* embryonic stage there is ubiquitous presence of *TPH1*-equivalent enzyme, which is the result of maternal deposition rather than zygotic expression of the protein [Neckameyer et al., 2007]. In line with this observation, it has been reported that maternal *Tph1* genotype has large impact on embryonic development and *Tph1* knockout mice display altered gait dynamics, suggesting that *Tph1* may have an impact on the development of nervous system [Côté et al., 2007]. Furthermore, neonatal exposure to serotonin reuptake inhibitors has lasting effects on behavior [Moses-Kolko et al., 2005; Maciag et al., 2006]. Similarly, our group has shown that *TPH1* mutations giving impaired maternal serotonin production may have long-term consequences for brain development and increase the risk of ADHD-related symptoms in offspring [Halmøy et al., 2010].

While our results are intriguing, this study should be viewed in the light of its limitations. The comparatively small size of our aADHD sample limits our power to detect variants with small effect sizes, especially when performing more complex analyses than single marker association. Another possible bias is the LD-structure of the genes, especially for *TPH1/2*, which were not tagged. Thus, we only performed epistasis evaluation in our sample of adult ADHD, while for gene-wide and gene-complex based analyses we utilized the published large-scale genome-wide analyses of the PGC. Given that we did not have access to raw genotypes of all PGC datasets, we were unable to explore epistasis in that data. We found the most compelling evidence for association in SCZ, which is by far the best powered sample in PGC, and it is possible that the signal for cADHD is weaker due to the lack of power. Furthermore, the PGC's SCZ dataset provides information on over 8 million

TABLE III. Gene-Complex Based Association Results Using PGC Data. The Reported P-Values are the Crude Ones. Bonferroni Adjusted Significance Threshold $P = 0.0031$. Self-Contained Tests Examine the Association Between a Gene Set and the Respective Phenotypes, While the Competitive Test Reflects the Likelihood of the Self-Contained Test Being Significant by Chance

Gene-complex	Gene-complex association P-value and the number of SNPs examined											
	CADHD			SCZ			MDD			BPD		
	# of genes (SNPs)	self-contained test	competitive test	# of genes (SNPs)	self-contained test	competitive test	# of genes (SNPs)	self-contained test	competitive test	# of genes (SNPs)	self-contained test	competitive test
TPH1 + YWHAS	8 (124)	0.078	0.476	8 (492)	5.91E-10	0.019	8 (122)	0.151	0.224	8 (202)	0.069	0.195
TH + TPH2 + YWHAS	9 (195)	0.091	0.789	9 (777)	4.7E-04	0.154	9 (189)	0.450	0.552	8 (329)	0.428	0.635
All genes	10 (210)	0.086	0.088	10 (831)	1.8E-04	0.166	10 (204)	0.470	0.566	9 (354)	0.395	0.625

SNPs as opposed to 1.2 million in cADHD. This discrepancy is echoed in the number of available variants in the examined genes in various PGC datasets (Table II), suggesting that some signals may have been detected in SCZ only. Lastly, phenotypic heterogeneity is a major challenge for neuropsychiatric studies, creating a potential loss of power.

In our gene-based and gene-complex evaluation, we have examined childhood ADHD only. However, slightly different genetic risk factors may be associated with the persistent and remitting forms of this disorder [Franke et al., 2011]. Similarly, epistasis has been tested in our Norwegian sample of adult ADHD only and its further exploration in childhood ADHD (as well as other neuropsychiatric disorders) is warranted.

In conclusion, this study is the first to thoroughly investigate *YWHA*-gene involvement in the etiology of ADHD. It strengthens the role of these genes in neuropsychiatric disorders, especially schizophrenia. Our results provide new targets for further exploration in genetics of ADHD and highlights the role of regulatory genes in neuropsychiatric disorders.

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