Supplemental Data

Human CRY1 variants associate with attention deficit/hyperactivity disorder

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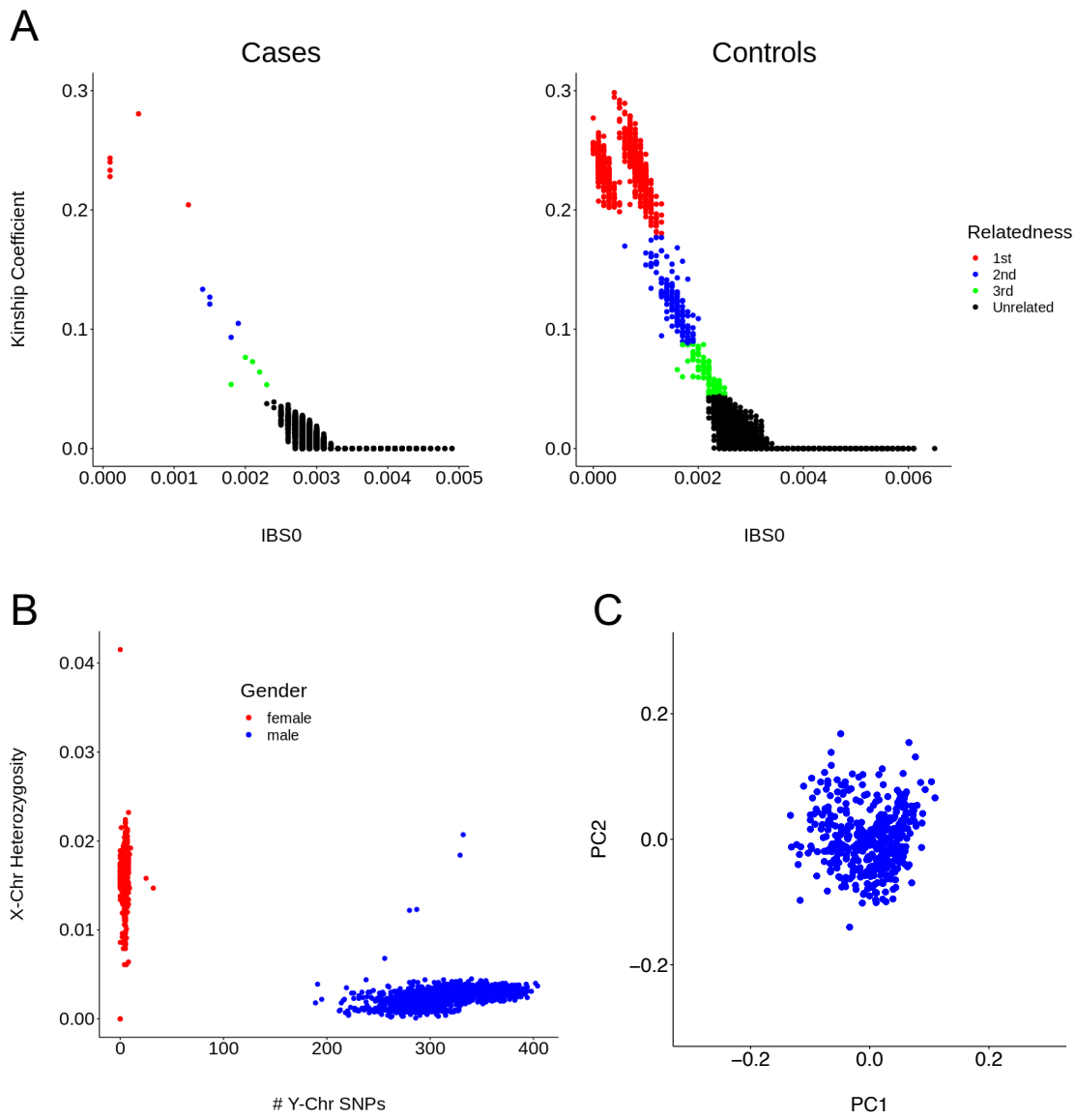
This PDF file includes:

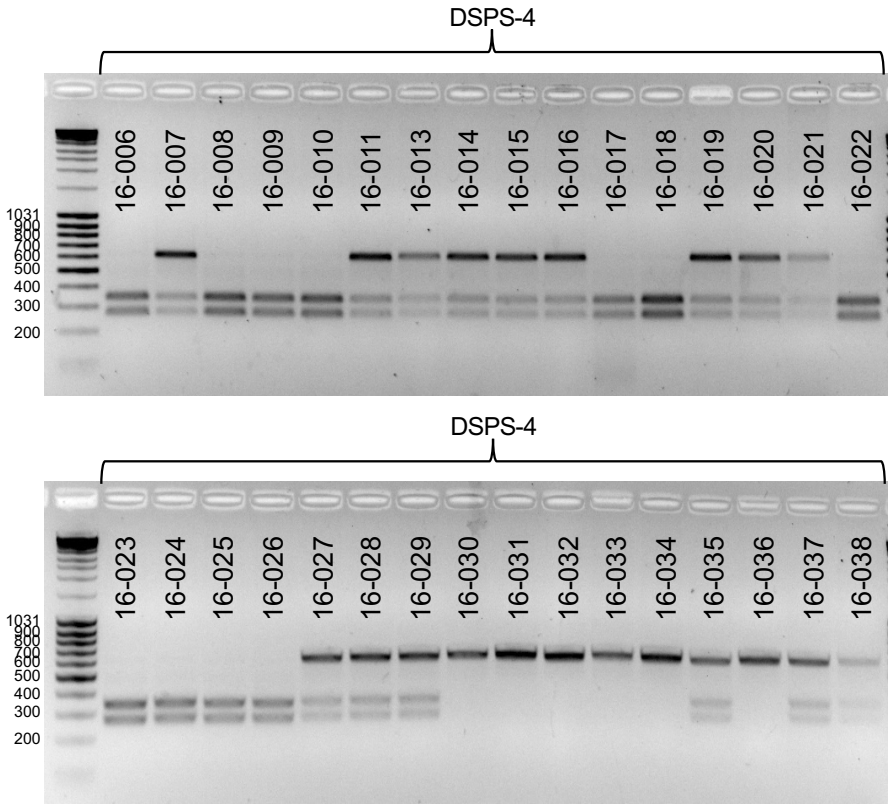
- Supplemental Figures 1 & 2
- Captions for Supplemental Tables 1 to 14
- Supplemental Tables 15 to 24

Other Supplementary Materials for this manuscript include the following:

- Supplemental Tables 1 to 14 (single Excel file)



Supplemental Figure 1. Kinship, sex-determination and principal component analysis of the 447-individual validation cohort. Reported relationships and gender are illustrated by different colors. (**A**) Proportion of SNPs with identical-by-state zero (IBS0) against estimated kinship coefficient from the SNP data using KING software, (**B**) number of Y-Chr SNPs against X-Chr heterozygosity are plotted. (**C**) Principal component analysis projected along the PC1 and PC2 axes.



Supplemental Figure 2. *CRY1* c.1657+3A>C genotyping by PCR and Hpy188I restriction enzyme digestion. Heterozygotes for the c.1657+3A>C mutation yielded 3 fragments of 623 bp + 347 bp + 276 bp. In homozygous individuals the 623 bp product was completely digested. Positive and negative controls were included in each amplification and digestion reaction.

SUPPLEMENTAL TABLES (please see xlsx document for Supplemental Tables 1-14)

Supplemental Table 1. DSM-5 ADHD, ASRS scores and demographics of the 14-family discovery cohort (genotype denotes CRY1Δ11 status).

Supplemental Table 2. DSM-5 ADHD & ASRS symptoms of the 14-family discovery cohort.

Supplemental Table 3. Sleep behavior of 12 families from the 14-family discovery cohort. Families DSPD-1, -4, -6, -9 have been partially reported and families -7, -14 fully reported elsewhere (ref. 1); genotype denotes CRY1Δ11 status.

Supplemental Table 4. Comorbidities in the 14-family discovery cohort (genotype denotes CRY1Δ11 status).

Supplemental Table 5. ADHD severity and sunlight exposure times in the 14-family discovery cohort (genotype denotes CRY1Δ11 status).

Supplemental Table 6. DSM5 ADHD & ASRS scores, and sleep behavior of the 447-individual whole exome sequenced validation cohort (genotype denotes CRY1Δ11 status).

Supplemental Table 7. Gene-based prioritization using SKAT-O test of the 447-individual whole exome sequenced validation cohort.

Supplemental Table 8. CRY1Δ11 (rs184039278) allele frequencies in different populations.

Supplemental Table 9. Association of CRY1Δ11 with the BioMe™ BioBank phenotypes after filtering.

Supplemental Table 10. BioMe™ BioBank filtered phenotypes.

Supplemental Table 11. BioMe™ BioBank phenotypes of the CRY1Δ11 carriers.

Supplemental Table 12. Coding, rare and deleterious variations of core clock (CRY1, CRY2, PER1, PER2, PER3, ARNTL, CLOCK and CSNK1D) and additional candidate clock genes

(CSNK1E, ARNTL2, FBXL3, FBXL21, BHLHE40, BHLHE41, NR1D1 and RORA) that were identified by whole exome sequencing in the 447-individual validation cohort.

Supplemental Table 13. DSM5 ADHD & ASRS scores, and sleep behavior of family DSPD-36 with CRY1Δ6.

Supplemental Table 14. HADDOCK results.

Supplemental Table 15. ADHD and DSPD phenotypes of the 447-individual validation cohort.

	DSPD+	DSPD-	Total
ADHD+	62	16	78
ADHD-	123	246	369
Total	185	262	447

Supplemental Table 16. Filtering criteria for variant discovery.

	Dominant mutations	Recessive mutations
MQ (mapping quality)	< 20	< 20
VQSR (variant quality score recalibration)	Except "PASS" and "."	Except "PASS" and "."
Q (Phred quality score)	< 30	< 30
FS (Fisher's strand)	> 60	none
SOR (strand odds ratio)	> 3.0	none
MQRankSum	< -12.5	none
ReadPosRankSum	< -8.0	none
QD (qual by depth)	< 2.0	none
MAF big databases: GnomAD (n138,632), ExAC (n60,706), KaviAR (n77,781)	≤ 0.005	≤ 0.05
MAF additional databases: 1000g (n2,504), EVS (n6,503)	≤ 0.005	≤ 0.05
MAF Turkish cohorts: In-house: exome _seq (n2,671)/genome_seq (n703)	≤ 0.01	none
Functional annotation (selected for further analysis)	**LoF** (splice-site, frameshift indel, stop-gain, stop- & start-loss) **Missense** with MetaSVM "D" and CADDphred≥ 25.0 **Start-gain** and **inframe indels** with CADDphred ≥ 20.0 **Non-essential splice-site** (±3 bp) with dbscSNV-ADA & dbscSNV-RF < 0.6, Spidex < 5.0	
Low complexity region variants	excluded	

Supplemental Table 17. Summary of core and candidate clock gene variants.

Gene	Annotation	HGVS.c	HGVS.p	Affected Hom/Het	Unaffected Hom/Het	MAF-TR
Core clock genes						
CRY1	splice_donor	c.825+1G>A	-	0/1	0/0	0
	splice_donor	c.1657+3A>C	-	0/9	0/0	8.06E-03
PER1	splice_donor	c.3072+3G>A	-	0/0	0/2	3.10E-04
	splice_acceptor	c.104-4_-3dup	-	0/1	0/0	0
PER2	inframe_deletion	c.2910_2936del	p.971_979del	0/0	0/1	0
CRY2	no variant	-	-	-	-	-
PER3	no variant	-	-	-	-	-
ARNTL	no variant	-	-	-	-	-
CLOCK	no variant	-	-	-	-	-
CSNK1D	no variant	-	-	-	-	-
Candidate clock genes						
CSNK1E	structural_interaction	c.108C>A	-	0/0	0/1	6.20E-04
	missense	c.736A>G	p.Met246Val	0/0	0/3	1.55E-03
FBXL3	structural_interaction	c.978T>C	-	0/1	0/0	0
BHLHE40	missense	c.673C>T	p.Arg225Trp	0/1	0/0	0
BHLHE41	inframe_insertion	c.1212_1214dup	p.Ala405dup	0/1	0/0	0
	inframe_deletion	c.1197_1205del	p.400_402del	0/0	0/1	1.24E-03
	inframe_deletion	c.900_902del	p.Ala301del	0/0	0/2	0
	frameshift	c.877_902del	p.Pro293fs	0/0	0/1	0
	inframe_deletion	c.1215_1229del	p.406_410del	0/1	0/0	0
	inframe_deletion	c.1230_1232del	p.Ala411del	0/4	0/5	3.10E-03
	inframe_deletion	c.1215_1232del	p.406_411del	0/1	0/0	6.20E-04
	inframe_insertion	c.1212_1214dup	p.Ala405dup	0/1	0/0	0
	inframe_insertion	c.1227_1232dup	p.410_411dup	0/0	0/1	0
NR1D1	structural_interaction	c.1318T>G	-	0/0	0/1	0
	missense	c.1576A>C	p.Lys526Gln	0/0	0/1	0
	inframe_insertion	c.252_260dup	p.85_87dup	0/0	0/1	0
	missense	c.1075C>T	p.Arg359Cys	0/0	0/2	6.20E-04
	protein contact	c.1332G>A	-	0/0	0/0	3.10E-04
	inframe_insertion	c.324_326dup	p.Glu108dup	0/1	0/3	3.10E-04
RORA	missense	c.109C>T	p.Arg37Cys	0/1	0/0	3.10E-04
ARNTL2	no variant	-	-	-	-	-

*Affected: DSPD and/or ADHD; Unaffected: No DSPD or ADHD; MAF-TR: Minor allele frequency of the variant in in-house cohort.

64 **Supplemental Table 18.** Oligonucleotide primers used in the cloning of *CRY1*.

pMU2	XbaI_hCRY1_F	5'gcattctagaatgggggtgaacgccgtgc3'
	NotI_hCRY1_R	5'gcatgcggccgcttaattagtgctctgtctctggactttaggacc3'
pcDNA4/ myc-HisA	EcoRV_hCRY1_F	5'gcgatatcatgggggtgaacgccgtgc3'
	NotI_hCRY1_R	5'gcatgcggccgcgcattagtgctctgtctctggactttaggacc3'

65 **Supplemental Table 19.** Oligonucleotide primers used in the mutagenesis of *CRY1*.

Exon 6 Deletion	hCRY1_del-exon6_F	ggaaagaaaagtaaagaagaacagttcccctcccc
	hCRY1_del-exon6_R	tcttctttacttttctttccaaatgcctttccaaacgagtaag
Exon 11 Deletion	hCRY1_del-exon11_F	agcagtggaagaagaagctccatgggcactggtc
	hCRY1_del-exon11_R	gagcttcttcttccactgctgctacaacctggg

66

67 **Supplemental Table 20.** Reaction conditions for Phusion polymerase amplification.

	Volume	Final Concentration
5X Phusion HF Buffer	10 µl	1X
10 mM dNTP Mix	1.0 µl	200µM
10µM Forward Primer	2.5 µl	0.5µM
10µM Reverse Primer	2.5 µl	0.5µM
100% DMSO	1.5 µl	3%
cDNA Template	2.0 µl	
Phusion Polymerase (2 U/µl)	0.5 µl	1 U
ddH_2O	30 µl	
Total	50 µl	

68 **Supplemental Table 21.** Cycling conditions for touchdown PCR.

	Temperature	Time	Cycles
Initial Denaturation	98 °C	2 min	
Denaturation	98 °C	20 sec	12 cycles
Annealing	72 °C – 1 °C/cycle	30 sec	
Elongation	72 °C	45 sec	
Denaturation	98 °C	20 sec	23 cycles
Annealing	60 °C	30 sec	
Elongation	72 °C	45 sec	
Final Elongation	72 °C	5 min	

69 **Supplemental Table 22.** Reaction conditions for Phusion polymerase amplification.

	Volume	Final Concentration
5X Phusion HF Buffer	10µl	1X
10 mM dNTP Mix	1.0µl	200µM
10µM Forward Primer	1.0µl	0.2µM
10µM Reverse Primer	1.0µl	0.2µM
100% DMSO	1.5µl	3%
Plasmid Template (50 ng/µl)	0.5µl	25 ng
Phusion Polymerase (2 U/µl)	0.5µl	1 U
ddH_2O	34.5µl	
Total	50µl	

70 **Supplemental Table 23.** Cycling conditions for touchdown PCR.

	Temperature	Time	Cycles
Initial Denaturation	98 °C	2 min	
Denaturation	98 °C	20 sec	20 cycles
Annealing	64 °C	30 sec	
Elongation	72 °C	3 min 30 sec	
Final Elongation	72 °C	5 min	

71

Supplemental Table 24. Statistics

	Carrier	Non-carrier	*P*	OR	95% CI
14-families from Turkey and Italy					
ADHD phenotype					
Affected	51	4	1.99 x 10-21	280.5	49-1,606
Not-affected	2	44			
Depression					
Affected	34	5	1.65 x 10-8	15.4	5.21-45.4
Not-affected	19	43			
447-individual validation cohort					
ADHD phenotype					
Affected	9	69	8.64 x 10-7	48.0	5.98-384
Not-affected	1	368			
DSPD phenotype					
Affected	10	175	1.2 x 10-4	31.4	1.83-539
Not-affected	0	262			