Genome-wide copy number variation study associates metabotropic glutamate receptor gene networks with attention deficit hyperactivity disorder

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Attention deficit hyperactivity disorder (ADHD) is a common, heritable neuropsychiatric disorder of unknown etiology. We performed a whole-genome copy number variation (CNV) study on 1,013 cases with ADHD and 4,105 healthy children of European ancestry using 550,000 SNPs. We evaluated statistically significant findings in multiple independent cohorts, with a total of 2,493 cases with ADHD and 9,222 controls of European ancestry, using matched platforms. CNVs affecting metabotropic glutamate receptor genes were enriched across all cohorts (P = 2.1 × 10–9). We saw *GRM5* (encoding glutamate receptor, metabotropic 5) deletions in ten cases and one control (P = 1.36 × 10–6). We saw *GRM7* deletions in six cases, and we saw *GRM8* deletions in eight cases and no controls. *GRM1* was duplicated in eight cases. We experimentally validated the observed variants using quantitative RT-PCR. A gene network analysis showed that genes interacting with the genes in the *GRM* family are enriched for CNVs in ~10% of the cases (P = 4.38 × 10–10) after correction for occurrence in the controls. We identified rare recurrent CNVs affecting glutamatergic neurotransmission genes that were overrepresented in multiple ADHD cohorts.

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ADHD is a common neuropsychiatric disorder with heritability estimates as high as 90%[1-3]. Most neurodevelopmental disorders have been difficult to study by GWAS, apart from some progress that has been made in autism[4–6]. A GWAS was performed on 958 ADHD trios through the International Multicentre ADHD Genetics (IMAGE) study, but no locus reaching genome-wide significance was found[7,8]. However, a PBAT (see URLs) analysis of the quantitative measures showed nominal significance of SNPs tagging *CDH13* (rs6565113) and *GFOD1* (rs552655)[9]. A SNP in linkage disequilibrium affecting *CDH13* has previously been reported in two independent ADHD GWAS[10]. Duplications of 16p13.11 have also been shown to be associated with ADHD[11]. We previously reported CNV loci that we observed in the first 335 cases with ADHD that we recruited[12]. Although in that previous study, no CNV loci met the criteria for significance (P < 5 × 10–4), one family in the study had a *GRM5* deletion that affected all three children with ADHD and was inherited from the mother, who also had ADHD.

Our discovery cohort included a total of 1,013 cases of European descent with ADHD recruited and genotyped at The Children's

Hospital of Philadelphia (CHOP) and was comprised of 664 cases without available parental information and 349 cases from trios with complete information (**Supplementary Tables 1 & 2**). We established a minimum inclusion intelligence quotient threshold of 70 to exclude individuals with intellectual disability[13]. The control group included 4,105 healthy children of European ancestry that were 6–18 years old, 32% of which were female and 68% of which were male. We screened medical records and parental- or self-reported questionnaires for individuals with developmental delays or special educational needs. For the replication, we used 624 samples from the IMAGE cohort that met quality control criteria: the PUWMa consortium contributed 864 cases with ADHD and 1,258 parents, and the IMAGE II consortium contributed 787 cases with ADHD and 898 unrelated controls. We used an additional 128 cases from the US National Institute of Mental Health (NIMH) and 90 cases from the University of Utah for the replication. We genotyped the DNA samples on different platforms. To manage differences in CNV detection between the arrays, we used controls genotyped on platforms that matched the platforms used for the cases (**Supplementary Table 3**).

We used PennCNV (see URLs) to produce CNV calls for the cases and controls[14]. Ninety-three percent of the subjects had 8–45 CNV calls after quality control filtering (**Supplementary Fig. 1**). We called four different copy number states: 3,172 homozygous deletions (copy number of 0), 27,810 hemizygous deletions (copy number of 1), 14,806 hemizygous duplications (copy number of 3) and 581 homozygous duplications (copy number of 4). The raw data and the resulting CNV calls are shown in **Supplementary Figure 2**. CNV calls spanned between 3 and 598 SNPs, with an average of 14 SNPs per CNV call and an average CNV size of 62 kb.

Ninety-three percent of controls also had 8–45 CNV calls (**Supplementary Fig. 1**). Among these CNV calls, we identified 4,471 homozygous deletions, 49,726 hemizygous deletions, 27,032 hemizygous duplications and 1,480 homozygous duplications. These CNV calls spanned between 3 and 708 SNPs, with an average of 12.8 SNPs per CNV call and an average CNV size of 53.6 kb. We also ran QuantiSNP15 to evaluate CNV calls that had a minimum of three SNPs on autosomes. We observed the same range of CNV calls (10–50 calls) and a 58% average direct overlap concordance with the PennCNV calls.

We did not detect any genome-wide significant associations in our genotype GWAS analysis (**Supplementary Note** & **Supplementary Tables 4**–**6**). However, we replicated SNPs in *GFOD1* in the families from the CHOP study using a transmission disequilibrium test (TDT) (the *P* values we found ranged from $P = 8 \times 10–4$ to $P = 1 \times 10–2$). The significance values we observed for previously reported ADHD SNPs[10, 16] are listed in **Supplementary Table 7**.

To identify CNVs associated with ADHD, we applied a segment based approach for consecutive SNPs with more CNVs in cases than in controls[4, 14]. The genomic span for consecutive SNPs delineates the shared CNV regions (CNVRs). In the CHOP cohort, we identified ten CNVRs that were present in multiple cases but not in controls and two CNVRs that had a higher frequency in cases than in controls (**Table 1**). We observed the previously reported duplication of 16p13.11 (ref. 11) in three cases and no controls ($P = 0.013$). To ensure CNV reliability, we experimentally validated all CNVRs using quantitative RT-PCR (qRT-PCR) (**Supplementary Fig. 3**). To rule out false negatives in the controls, we performed qRT-PCR on 908 controls across the *GRM5* and *GRM7* loci, which confirmed that no CNVs were present (**Supplementary Fig. 4**). In addition, significantly associated loci were called by both QuantiSNP and PennCNV and were absent in controls.

Replication of the significant findings, including the ten case specific CNVs from the discovery cohort, showed that three CNVs were exclusive to cases from the IMAGE, PUWMa, IMAGE II, NIMH and University of Utah studies, notably *GRM7* ($P = 8.14 \times 10–5$), *GRM8* ($P = 3.52 \times 10−6$) and *NEGR1* ($P = 3.91 \times 10–4$) (**Table 1**). We observed a deletion in *GRM5* in ten cases with ADHD (10/3,506) and one control (1/13,327) ($P = 1.36 \times 10–6$)(**Table 1**). We observed a duplication in *GRM1* in eight cases and two controls ($P = 1.05 \times 10–4$). The odds ratios for *GRM5* and *GRM1* were 38.12 (95% confidence interval, 5–298) and 15.24 (95% confidence interval, 3–72), respectively (**Table 1**).

Table 1 New CNVRs overrepresented in individuals with ADHD

CNVR (hg18)	CHOP cases[a]	CHOP controls[b]	Replication cases[c]	Replication controls[d]	Inherit	Combined P	OR (95% CI)	Type	Gene	Exon distance
chr11: 88,269,449–88,351,661	**4**	**0**	**6**	**1**	**4:1:3 (62.5%)**	**1.36×10^{-6}**	**38.12 (5–298)**	**Del**	***GRM5***	**5,858**
chr7: 126,525,124–126,536,202	**3**	**0**	**5**	**0**	**0:1:0 (100%)**	**3.52×10^{-6}**	**Infinity**	**Del**	***GRM8***	**0**
chr3: 7,183,953–7,197,236	**4**	**0**	**2**	**0**	**0:2:0 (100%)**	**8.14×10^{-5}**	**Infinity**	**Del**	***GRM7***	**20,598**
chr6: 146,657,076–146,694,047	**5**	**2**	**3**	**0**	**2:0:0 (100%)**	**1.05×10^{-4}**	**15.24 (3–72)**	**Dup**	***GRM***	**0**
chr1: 72,317,292–72,328,395	4	0	1	0	0:3:0 (100%)	3.91×10^{-4}	Infinity	Dup	*NEGR1*	10,621
chr7: 153,495,598–153,564,827	5	0	3	2	1:2:0 (100%)	4.08×10^{-4}	15.24 (3–72)	Dup	*DPP6*	68,453
chr5: 65,027,976–65,046,520	4	0	2	1	2:0:2 (50%)	4.68×10^{-4}	22.85 (3–190)	Del	*SGTB/NLN*	0
chr1: 56,053,497–56,064,495	2	0	4	2	1:0:3 (25%)	1.54×10^{-3}	11.42 (2–57)	Del	*USP24*[e]	80,234
chr19: 38,427,720–38,444,834	5	2	2	3	2:2:1 (80%)	4.95×10^{-3}	5.33 (2–17)	Del	*SLC7A10*[e]	19,172
chr3: 1,844,168–1,859,889	4	0	3	6	2:4:0 (100%)	8.81×10^{-3}	4.44 (1–13)	Del	*CNTN4*e	255,661
chr2: 81,419,297–81,446,082	2	0	2	3	1:0:1 (50%)	3.83×10^{-2}	5.07 (1–23)	Dup	*CTNNA2*[e]	152,417
chr4: 113,772,340–113,788,584	2	0	2	3	0:0:0 (NA)	3.83×10^{-2}	5.07 (1–23)	Dup	*LARP7*	0

Loci significantly replicating are shown in bold. The 'inherit' column lists the inheritance pattern of each CNV from parents to cases in the format 'inherited from mother: inherited from father: *de novo*', with the percent of inheritance listed in parentheses. Note that information about the parents was not available for all subjects. Rare variants that were recurrent and observed to be enriched among cases with ADHD relative to controls and that were detected in multiple independent cohorts are reported. All GRM genes were directly affected by the CNVR. Regions listed represent the optimal overlap of cases and significance with respect to controls, as described in the Online Methods and Supplementary Figure 10. The closest gene is listed for each CNVR locus, as this is the gene most likely to be affected. For detailed counts from each contributing project, see Supplementary Table 14. [a]n = 1,013. [b]n = 4,105. [c]n = 2,493. [d]n = 9,222. [e]No gene was directly affected, so the closest proximal gene is listed. Individual CNV boundaries are provided in Supplementary Table 15. OR, odds ratio; CI, confidence interval; del, deletion; dup, duplication. 'Replication' includes the combined IMAGE, PUWMa, IMAGE II, NIMH and Utah data.

Table 2 Discovery, replication and combined significance of CNV regions

CNVR (hg18)	Discovery *P*	Replication *P*	Combined *P*	Permuted discovery *P*	Permuted replication *P*	Permuted combined *P*	type	gene
chr11: 88,269,449–88,351,661	**1.53 × 10−3**	**5.29 × 10−4**	**1.36 × 10−6**	**0.025**	**0.001**	**0.002**	**Del**	***GRM5***
chr7: 126,441,593–126,621,501	**7.74 × 10−3**	**4.35 × 10−4**	**3.52 × 10−6**	**0.013**	**<0.001**	**<0.001**	**Del**	***GRM8***
chr3: 7,183,953–7,197,236	**1.53 × 10−3**	**4.53 × 10−2**	**8.14 × 10−5**	**0.011**	**0.039**	**<0.001**	**Del**	***GRM7***
chr6: 146,657,076–146,694,047	**4.42 × 10−3**	**9.63 × 10−3**	**1.05 × 10−4**	**0.006**	**<0.001**	**<0.001**	**Dup**	***GRM1***
chr1: 72,317,292–72,328,395	1.53 × 10−3	2.13 × 10−1	3.91 × 10−4	0.036	0.213	0.011	Dup	*NEGR1*
chr7: 153,495,598–153,564,827	1.53 × 10−3	6.82 × 10−2	4.08 × 10−4	<0.001	0.058	<0.001	Dup	*DPP6*
chr5: 65,027,976–65,046,520	1.53 × 10−3	1.17 × 10−1	4.68 × 10−4	0.003	0.108	0.001	Del	*SGTB/NLN*
chr1: 56,053,497–56,064,495	3.91 × 10−2	2.12 × 10−2	1.54 × 10−3	0.035	0.024	<0.001	Del	*USP24*
chr19: 38,427,720–38,444,834	4.42 × 10−3	2.89 × 10−1	4.95 × 10−3	0.002	0.262	0.007	Del	*SLC7A10*
chr3: 1,844,168–1,859,889	1.53 × 10−3	4.12 × 10−1	8.81 × 10−3	0.008	0.416	0.015	Del	*CNTN4*
chr2: 81,419,297–81,446,082	3.91 × 10−2	2.89 × 10−1	3.83 × 10−2	0.046	0.294	0.032	Dup	*CTNNA2*
chr4: 113,772,340–113,788,584	3.91 × 10−2	2.89 × 10−1	3.83 × 10−2	0.033	0.288	0.042	Dup	*LARP7*

The top four most significant loci are shown in bold. Del, deletion; dup, duplication.

Figure 1

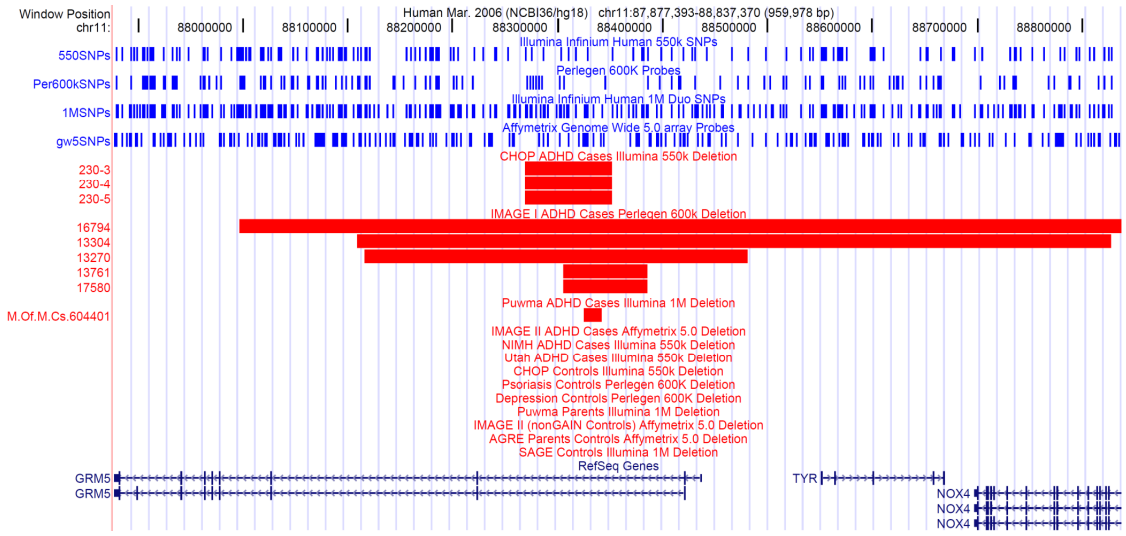

Figure 1: A deletion directly affecting *GRM5* that is exclusive to cases with ADHD and that was replicated in the IMAGE and PUWMa studies. Four hemizygous deletions in *GRM5* in cases with ADHD from the CHOP study that were replicated by two deletions and three larger deletions found in the IMAGE study and one deletion found in the PUWMa study. The SNP coverage of the Illumina 550K, Perlegen 600K, Illumina 1M and Affymetrix 5.0 arrays is shown by vertical blue lines. M.Of.M.Cs. Massachusetts General Hospital offspring male case; W.Fa.M.Cn. Washington University father male control.

Thus, we identified four genes in multiple independent cohorts that belong to the metabotropic glutamate receptor gene family and are directly affected by CNVRs (combined P = 2.1 × 10–9) (**Table 1 & Table 2**). Several other CNV loci were enriched in cases with nominal significance (**Table 1** & **Table 2**). In **Figure 1**, we show representative CNV deletions at the *GRM5* locus (found in ten cases and one control) identified using the UCSC Genome Browser17. Experimental validation of the CNVs present in individuals from the IMAGE, PUWMa, IMAGE II, NIMH and University of Utah studies identified using qRT-PCR and raw B allele frequency (BAF) and log R ratio (LRR) plots is shown in **Supplementary Figures 5–7**. We also detected *GRM2* and *GRM6* deletions in single cases with ADHD from the CHOP and IMAGE II studies, respectively, that were not present in controls. Referencing CNV-associated loci to their genotype, a TDT analysis revealed the strongest support for association of *GRM7* to ADHD (P = 8.35 × 10–5) (**Supplementary Table 8**). We evaluated family based CNV statistics of transmission disequilibrium and *de novo* events in a subset of 311 families from the CHOP study and 422 families from the IMAGE study (**Table 3** and **Supplementary Tables 9** & **10**). We first verified trios by genotype inheritance. The Illumina CHOP data had a combined deletion and duplication *de novo* rate of 4.81%, with 16 deletion and 8 duplication CNVRs, and the Perlegen IMAGE data had a *de novo* rate of 7.43%, with 2 deletion and 5 duplication CNVRs. *GRM5* deletions were *de novo* in three cases (**Table 1**). We evaluated the association of homozygous deletion CNVs separately and found six loci with nominal significance, including a locus in *AKNAD1* (**Supplementary Table 11**).

We hypothesized that genes interacting with *GRM* genes would collectively have more CNVs enriched in cases compared to controls. We identified 228 genes within two degrees of relation to *GRM* genes in the merged human interactome using Cytoscape Software18. We evaluated these genes in the CHOP cohort for enrichment in cases with ADHD ($P < 0.05$). We detected 67 genes interacting with *GRM* genes (not including the *GRM* genes themselves) enriched for CNVs in cases compared to 16 such genes in controls, showing a threefold enrichment of the *GRM* network CNVs in individuals with ADHD (P = 4.38 × 10–10; **Fig. 2a**). We excluded large CNVs spanning

multiple genes in the network to ensure that the network enrichment was not skewed. A GWAS analysis of *GRM* network genes did not result in a significant association (overall P = 0.142), confirming that the GWAS analysis did not capture association to CNVs. We then clustered the second degree *GRM* network to define interconnected modules of genes and scored the enrichment of gene ontology annotations (**Fig. 2b**).

GRMs are G-protein–coupled receptors involved in the modulation of excitatory synaptic transmission[19]. There are three receptor groups that are based on sequence homology, putative signal transduction mechanisms and pharmacologic properties20. *GRM5* and *GRM1* are members of group 1 and are expressed in the basal ganglia and cerebellum[21].

Table 3 Genome-wide analysis of *de novo* CNVs in the CHOP discovery cohort

CNVR (hg18)	Inherited	*De novo*	Parent only	Type	Gene	Distance[a]
chr19: 15,992,679–15,997,923	15	6	15	Del	*LOC126536*	0
chr22: 38,384,374–38,403,731	4	4	13	Del	*CACNA1I*	0
chr17: 71,112,486–71,120,734	12	3	16	Del	*KIAA1783*	0
chr12: 55,902,280–55,923,860	9	3	19	Del	*NDUFA4L2*, *NXPH4*, *SHMT2*, *STAC3*	0
chr19: 59,423,491–59,428,132	74	3	38	Dup	*LILRB3*, *LIR-3*	0
chr16: 87,694,595–87,778,383	32	2	21	Del	*AX748415*, *CDH15*, *LOC197322*	0
chr18: 65,358,832–65,367,619	33	2	21	Del	*DOK6*	0

[a] The respective CNVs directly affect the gene(s) at these loci. CNVs are visually validated as being either inherited or *de novo*. Confidence in the *de novo* calls was derived from multiple observations of the AA genotype in a specific parent and the BB genotype in child (or BB in the parent and AA in the child), suggesting a consistent parental origin. Del, deletion; dup, duplication.

These receptors activate phospholipase C and may have a role in addiction, anxiety and behavioural disorders[22]. *GRM7* and *GRM8* are members of group 3 and inhibit the cyclic AMP cascade. *GRM7* has been linked to anxiety[23] and is the most highly conserved *GRM* member across multiple species[24]. Evidence for glutamatergic involvement in ADHD is emerging from diverse fields. Although association studies investigating *GRM* genes and transporters have reported mixed results[25–28], a GWAS examining the methylphenidate response in children with ADHD found an association with a SNP in *GRM7* (rs3792452)[29]. *GRIN2A* was found to be associated with ADHD in a linkage study[25], and *GRIN2B* was also found to be associated with ADHD using a TDT30. Magnetic reson-ance spectroscopy showed an increased glutamate-ergic tone in the frontal and striatal brain of subjects with ADHD[31–33] that normal-ized with stimulants and atomoxetine[34]. Mice in the ADHD *Slc6a3* knockout model remain responsive to methylphenidate and lack the dopamine transporter[35], and the hyper-activity that is increased by *N*-methyl-d-aspartic acid (NMDA) receptor blockers is suppressed by drugs that increase gluta-matergic transmission[36]. Increased *Slc6a3* and *Drd4* expression in the midbrain were reported in rats that had an increase of glutamate transporter in the striatum[37], suggesting that decreases in dopamine alter glutamate signalling. *GRIN2A* disruption increased *N*-methyl d-aspartate and serotonin metabolism in the frontal cortex and striatum of mice and increased locomotor activity that had been reversed by dopamine or serotonin receptor antagonists[38]. Dysregulated expression of glutamatergic pathway genes has been observed in spontaneously hyper-tensive rat models [39–42] and in ADHD rat models with exposure to polychlorinated biphenyls[41]. Increased concentrations of glutamate were also reported in the neuro-metabolism of ADHD brains, which is consistent with altered glutamate trans-mission in ADHD[33].

Apart from genes in the *GRM* family, we detected association at eight other loci, four of which directly affect genes (**Table 1**). *DPP6* has been associated with amyotrophic lateral sclerosis in previous GWAS[43, 44], and CNVs have been associated with autism[45]. *DPP6* and *CTNNA2* were also implicated in a previous GWAS[10]. *NLN* is responsible for the metabolic

Figure 2

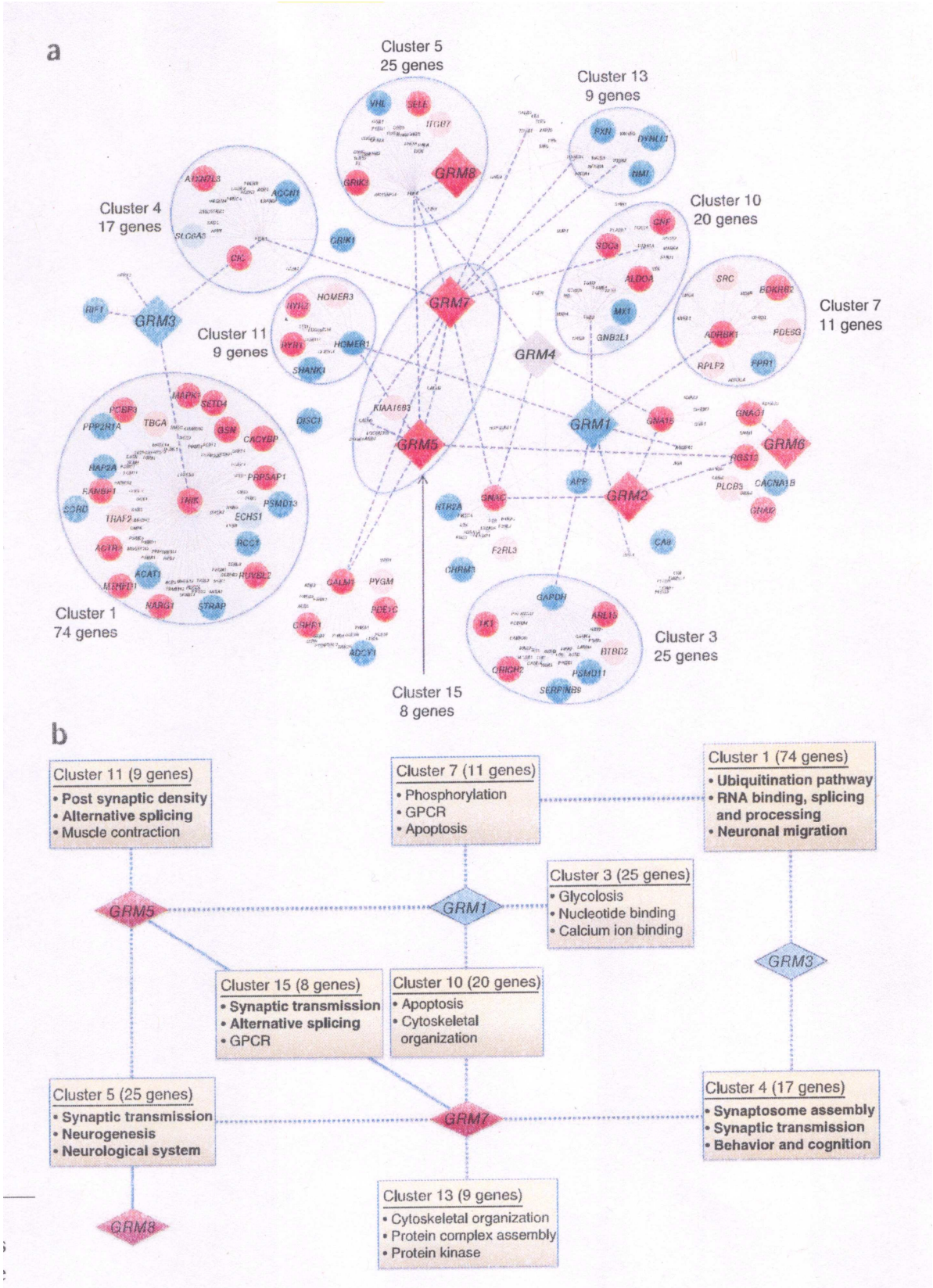
a
Cluster 5
25 genes
Cluster 13
9 genes
Cluster 4
17 genes
Cluster 10
20 genes
Cluster 11
9 genes
Cluster 7
11 genes
Cluster 1
74 genes
Cluster 3
25 genes
Cluster 15
8 genes
GRM1
GRM2
GRM3
GRM4
GRM5
GRM6
GRM7
GRM8
b
Cluster 11 (9 genes)
• Post synaptic density
• Alternative splicing
• Muscle contraction
Cluster 7 (11 genes)
• Phosphorylation
• GPCR
• Apoptosis
Cluster 1 (74 genes)
• Ubiquitination pathway
• RNA binding, splicing and processing
• Neuronal migration
Cluster 3 (25 genes)
• Glycolosis
• Nucleotide binding
• Calcium ion binding
Cluster 15 (8 genes)
• Synaptic transmission
• Alternative splicing
• GPCR
Cluster 10 (20 genes)
• Apoptosis
• Cytoskeletal organization
Cluster 5 (25 genes)
• Synaptic transmission
• Neurogenesis
• Neurological system
Cluster 4 (17 genes)
• Synaptosome assembly
• Synaptic transmission
• Behavior and cognition
Cluster 13 (9 genes)
• Cytoskeletal organization
• Protein complex assembly
• Protein kinase

Figure 2 *GRM receptor gene interaction networks affected in ADHD*. **(a)** *GRM* receptor genes are shown as large diamond-shaped nodes, and other genes within two degrees of interaction with *GRM* genes are shown as smaller circular nodes. Nodes are coloured to represent the enrichment of the CNVs: dark red represents deletions enriched in cases, light red represents deletions enriched in controls, dark turquoise represents duplications enriched in cases, light turquoise represents duplications enriched in controls, and gray represents diploids that are devoid of CNVs. Thick blue dashed lines highlight edges that are connected to at least one *GRM* gene, and thin gray lines represent all other gene interactions. Highly connected modules enriched for significant functional annotations are highlighted by blue shaded ellipses. Details on the gene-based CNV observations are included in Supplementary Table 16, and the respective gene functional clusters are listed in Supplementary Table 17. **(b)** *A schematic overview showing the interaction of GRM receptors affected in ADHD with modules of genes enriched for functional significance*. *GRM* receptor genes are shown as diamonds coloured either turquoise or red to represent duplications and deletions, respectively that were enriched in cases. Boxes highlight the functional modules defined by the network of interacting genes (a) that are significantly enriched for Gene Ontology annotations. The functional modules describe significant functional annotations and are labelled with the cluster name and the number of component genes in parenthesis. Functional annotations that may be particularly pertinent to the underlying pathophysiology of ADHD are shown in bold. The edges of the network connect *GRM* receptor genes to functional modules: solid lines indicate membership of the *GRM*-interacting gene in the functional module, and dotted lines indicate a first degree relationship between *GRM* receptor genes and at least one component gene of a functional module.

inactivation of neural peptides, including neuropeptide Y, which has been implicated in ADHD [46, 47]. *SLC7A10* modulates glutamatergic synapse transmission. *LARP7* is important for small nuclear ribonucleoprotein integrity. *NEGR1* encodes the neural cell adhesion and growth molecule, which is associated with obesity[48].

ADHD is phenotypically complex. In addition to ADHD, one of the three siblings with a deletion in *GRM5* that we studied also had symptoms of social avoidance, one sibling had coexisting obsessive compulsive symptoms, and one sibling was free of comorbidity. Assessment of the mother of these three siblings using the adult ADHD self-report scale[49] indicated a likelihood of ADHD. In subjects with *GRM7* CNVs, one had comorbid anxiety and another had coexisting oppositional defiant disorder. In subjects with *GRM1* CNVs, one had comorbid minor depression that was considered secondary to the ADHD symptoms, two had oppositional defiant disorder and a third had obsessive compulsive symptoms. Subjects with *GRM* CNVs showed a truncated normal distribution of intelligence quotient and all had intelligence quotients above 75 (**Supplementary Fig. 8**). In the IMAGE cohort, two subjects with *GRM* CNVs had a comorbidity of conduct disorder and four had oppositional defiant disorder.

Evaluation of the genes interacting with *GRM* genes for CNV frequency in cases and controls allowed for the inclusion of marginally significant loci, given the prior knowledge of robust association of the *GRM* receptor gene family. Whereas most reported disease-associated CNVs are rare (<1%), they have strong correlation to disease. For example, the *GRM* CNVs associated with ADHD confer large effect sizes (see the odds ratios in **Table 1**). Based on loci that are significant individually, 3.66% of the cases with ADHD have the newly discovered CNVs, and this number increases to 9.94% when genes interacting with *GRM* genes are included. Major hubs of the network include *TNIK*[50], *GNAQ*[51] and *CALM1* (ref. 52) (**Fig. 2a**). The network gene *GRIK1* was previously associated with the hyperactive and impulsive symptoms of ADHD[9].

Taken together, our CNV analysis shows that the *GRM* gene family and genes interacting with it are enriched for CNVs in individuals with ADHD. Several of these genes are crucial in the process of synaptic transmission, in neurogenesis and in neuronal processes that may be defective in ADHD. The observed *GRM* gene modules regulate RNA binding, processing and alternative splicing, which are processes known to influence brain-specific synaptic activity[53, 54]. Abnormal brain connectivity has also been observed in developmental brain disorders with cognitive dysfunction, including ADHD [55, 56].

In conclusion, using two-stage genome-wide association for high resolution CNV detection, we identified 12 loci showing enrichment of CNVs in cases with ADHD

compared to controls and successfully replicated four genes using independent datasets of cases with ADHD and healthy controls genotyped on matched case-control platforms. The four replicating genes belong to the metabotropic glutamate receptor gene family. Extended studies identified over 200 genes interacting with glutamate receptors that were collectively affected by CNVs, suggesting that up to 10% of individuals with ADHD may be enriched for *GRM* network variants. This *GRM*-interacting gene network defines a set of functional modules that, when affected by CNVs, may contribute to the pathogenesis of ADHD. Therefore, enrichment of CNVs in genes within this molecular system that are associated with ADHD has suggested new susceptibility mechanisms and is likely to spur assessment of additional variations, including single-base changes, and expression and functional assays to evaluate the biological effects of these CNVs. Future work will determine whether clinical studies using selective *GRM* agonists as a potential treatment for ADHD are warranted in individuals with ADHD and variants in *GRM* genes.

URLs. PBAT,http://www.biostat.harvard.edu/~clange/default.htm; PennCNV, http://www.open bioinformatics.org/penncnv/; ParseCNV, http://parsecnv.sourceforge.net/; transformation into LRR and BAF values using PennCNV, http://www.openbioinformatics.org/penncnv/penncnv_tutorial_affy_gw6.html.

Methods

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

***Note**: Supplementary information is available on the Nature Genetics website.*

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AUTHOR CONTRIBUTIONS

H.H. and J.E. designed the CHOP study and supervised the data analyses and interpretation. S.V.F., M.G., P.A. and J. Buitelaar designed the IMAGE and IMAGE II studies. S.V.F. designed the PUWMa study and coordinated the analyses for the IMAGE, IMAGE II and PUWMa studies. J.T.G. and K.W. conducted the statistical analyses. C.E.K. and E.C.F. directed the stage 1 genotyping. J.D.B. coordinated the validation analyses. N.T. performed the qRT-PCR validation of the CNVs. J.T.G. and H.H. drafted the manuscript. J.E. collected the CHOP samples. C.R., P.S. and J.L.R. collected the NIMH samples. C.M.F., H.-C.S., A.A.T., A. Reif, A. Rothenberger, B.F., E.O.M., H.R., J. Buitelaar, K.-P.L., L.K., T.B., R.P.E., F.M., R.D.O., J.S., E.S.-B., T.J.R., M.R., J.R., A.W., S.W., J.M., H.P., C.S., S.K.L., S.L.S., J. Biederman, L.K., P.A. and R.J.L.A. collected data for the IMAGE, IMAGE II and PUWMa projects. J. Biederman, E.O.M., S.V.F., S.K.L., S.L.S. and A.A.T. collected samples for the PUWMa study. F.A.M. genotyped the IMAGE II data. H.H. directed and D.H. and J.T.G. performed the gene interaction network and functional enrichment analyses. All authors contributed to the manuscript preparation. S.F.A.G. accessed the public domain data, assisted with the interpretation of the data and edited the manuscript. All other authors contributed samples and/or were involved with data mining and processing.

COMPETING financial INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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ONLINE METHODS

Illumina Infinium assay for CNV discovery. We performed high-throughput genome-wide SNP genotyping using the InfiniumII HumanHap550 BeadChip (Illumina) at the Centre for Applied Genomics at the CHOP. The genotype data content together with the intensity data from the genotyping array provided high confidence for the CNV calls. A simultaneous analysis of intensity data and genotype data in the same experimental setting established a highly accurate definition for normal diploid states and any deviation thereof. To call CNVs, we used PennCNV and QuantiSNP. PennCNV combines multiple sources of information, including LRR and BAF, SNP spacing, trained Hidden Markov Models and the population frequency of the B allele to generate CNV calls. The replication case and control cohorts performed genome-wide SNP genotyping using the Perlegen 600K, Illumina 1M and Affymetrix 5.0 arrays. Raw X and Y values were log10 normalized and clustered to establish the BAF and LRR values using the PennCNV-Affy protocol (Online Methods and **Supplementary Table 12**).

CNV quality control. We calculated quality control measures on our HumanHap550 GWAS data based on statistical distributions to exclude DNA samples of poor quality and false-positive CNVs. The first quality control threshold used was the percentage of attempted SNPs that were successfully genotyped. Only samples with call rate >98% were included. The genome-wide intensity signal should have as little noise as possible. Only samples within the s.d. of the normalized intensity (LRR < 0.35) were included. All samples were required to be of European descent based on principle components analysis (**Supplementary Fig. 9**). Furthermore, case and control matching was insured by calculating a genomic inflation factor (which was 1.024) between groups. Wave artifacts roughly correlating with GC content resulting from hybridization bias of low full-length DNA quantity are known to interfere with the accurate inference of copy number variations[57]. Only samples where |GC base pair wave factor (GCWF)| < 0.05 were accepted. If the count of CNV calls made by PennCNV exceeds 70 (**Supplementary Fig. 1**), the DNA quality is usually poor. Therefore, only samples with CNV call count <70 were included. One sample from any duplicate samples (such as those from monozygotic

twins) was excluded. **Supplementary Table 13** lists the number of samples excluded using each quality control measure.

Statistical analysis of CNVs. The CNV frequency between cases and controls was evaluated at each SNP using a Fisher's exact test. We only considered loci that were nominally significant between cases and controls ($P < 0.05$) when cases in the CHOP discovery cohort had the same variation, which was replicated in the IMAGE, PUWMa or IMAGE II studies, or loci that were not observed in any of the control subjects and were validated using an independent method. We reported statistical local minimums to narrow the association in reference to a region of nominal significance, including SNPs residing within 1 Mb of each other (**Supplementary Fig. 10**). The resulting nominally significant CNVRs were excluded if they met any of the following criteria: (i) residing on telomere- or centromere-proximal cytobands; (ii) located in a 'peninsula' of common CNVs arising from variation in boundary truncation of CNV calling (**Supplementary Fig. 11**); (iii) genomic regions with extremes of GC content, which produces hybridization bias; or (iv) samples contributing to multiple CNVRs. See ParseCNV for details (see URLs). We statistically adjusted for the relatedness of cases using permutation (1,000×). Three lines of evidence were considered to establish statistical significance: independent replication with $P < 0.05$, permutation significance of the observations and no control-enriched significance. We used DAVID (Database for Annotation, Visualization and Integrated Discovery) [58] to assess the significance of the functional annotation clustering of independently associated CNV results into InterPro categories.

Network analysis. We used Cytoscape software18 to identify 228 genes within two degrees of relation to eight *GRM* genes based on the merged human interactome. We clustered this network of genes into 17 distinct modular clusters based solely on network topology using the ClusterViz plug-in for the software using the FAG-EC algorithm with default parameters. Component genes in each of the 17 modules were submitted to DAVID[58] to assess the significance of functional enrichment using *Homo sapiens* Gene Ontology annotations.

CNV validation by qRT-PCR. Universal Probe Library (Roche) probes were selected using the ProbeFinder v2.41 software (Roche). QRT-PCR was performed on an ABI 7500 Real Time PCR Instrument or on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Each sample was analyzed in quadruplicate either in 25 µl of reaction mixture (250 nM probe, 900 nM of each primer, the Fast Start TaqMan Probe Master from Roche and 10 ng of genomic DNA) or in 10 µl of reaction mixture (100 nM probe, 200 nM of each primer, 1× Platinum Quantitative PCR SuperMix-Uracil-DNAGlycosylase with ROX from Invitrogen and 25 ng of genomic DNA). The results were evaluated using Sequence Detection Software v2.2.1 (Applied Biosystems). Further data analysis was performed using the ΔΔ*C*T method. Reference genes, chosen from *COBL*, *GUSB* and *SNCA*, were included based on the minimal coefficient of variation, and data was then normalized by setting a normal control to a value of 1.

PennCNV-Affy workflow adapted to Perlegen 600K data. CNV calling on Perlegen used a similar algorithm to the Illumina arrays but had additional pre-processing. To perform data normalization and signal extraction from the raw final report files generated in the genotyping experiments, we first reformatted data from dbGaP into the format produced by the following Affymetrix Power Tools: birdseed.calls.txt, birdseed.confidences.txt & quant-norm.pm-only.med-polish.expr.
summary.txt (**Supplementary Table 11**). We calculated the log10 of the X and Y values provided in the sample-based report files. For each SNP marker, the allele-specific intensity for the AA, AB and BB genotypes on all genotyped samples was used to construct three canonical genotype clusters in the polar coordinates θ and R. Once canonical genotype clusters were constructed, signal intensity values for each SNP were transformed into LRR and BAF values. For details, see URLs.

To optimize the Hidden Markov Model, we used the reference file hh550. hmm and ran "-

train" in PennCNV in one batch of thirty samples with the lowest s.d. of the LRR value followed by two batches that included random representative samples. We also created a population B allele frequency definition file specifically adapted to the Perlegen data. This allowed for CNV calls to be made in 1,887 (642 cases and 1,245 parents) out of 2,789 Perlegen 600K samples available. Although the global s.d. of the LRR value was below 0.2 for the majority (84%) of the samples, the intensity data was noisy in regions of called CNVs and showed subpopulations of SNPs that were unable to differentiate the deletion signal, perhaps as a result of PCR saturation during lab processing. Nevertheless, deletion and duplication features were detected with confirmation of homozygote and AAB and ABB genotypes (**Supplementary Figs. 6** & **7**). Lastly, Perlegen CNV calls were screened for overlap with the 12 loci associated with ADHD based on the CHOP Illumina data. To ensure that each detected CNV was a true DNA feature, we validated each CNV using qRT-PCR (**Supplementary Fig. 5**).

Permutation to adjust significance for relatedness. For the initial Fisher's exact test, related individuals were not controlled for, as our primary objective was to detect CNVs in multiple samples regardless of relatedness. CNVRs passing this initial screen were scored for statistical significance based on a permuted *P* value, which permutes case and control labels randomly for all samples, with the condition that related individuals have same label. Based on the number of samples with the CNVR being calculated in randomly assigned 'cases' and 'controls', a Fisher's exact test *P* value was assigned. The number of hypothetical scenarios with significance equal or greater to this *P* value provides the permuted *P* value, which corrects for relatedness. The Fisher's exact test *P* value and the counts of cases and controls with each CNVR are provided in **Table 1** for transparency.

Study criteria for inclusion in IMAGE. The probands were required to have combined subtype ADHD, were children aged 6–17 years (inclusive), had one or more sibling(s) in same age range, had both parents available or one parent and two or more siblings available to provide a DNA sample, an intelligence quotient above 70, were free of single-gene disorders associated with ADHD, were free of neurological disease and damage, were living at home with at least one biological parent and one full sibling and did not meet the criteria for autism or Asperger's syndrome.

Study criteria for inclusion in IMAGE II. The probands were required to have ADHD according to the standards of the current diagnostic and statistical manual of mental health (DSM-IV-TR) and had a semi-structured diagnostic interview based on KSADS-PL or Kinder-DIPS, the Child Behaviour Checklist, the Conners parent and teacher Scales or the German Teachers Report on children aged 6–18 years. Probands also had an intelligence quotient above 70, a birth weight >2,000 g, no major medical events during their mother's pregnancy and no drug abuse in their mother during pregnancy. They were also free of single-gene disorders known to be associated with ADHD and free of neurological disease and damage, and they did not meet the criteria for autism, Asperger's syndrome, schizophrenia, bipolar disorder, primary major depressive episode, anxiety disorder or Tourette's Syndrome.

Controls for IMAGE II. The control subjects used were drawn from Affymetrix 6.0 genotyped subjects from the NIMH genetics repository collected through the United States nationally representative survey panel. Participants were screened for psychosis and bipolar disorder. Control participants were not screened for ADHD. Control participants gave written consent for their biological materials to be used for medical research at the discretion of NIMH. Controls were genotyped using the Affymetrix 6.0 array at the Broad Institute National Centre for Genotyping and Analysis. Genotype calls were made with the BIRDSEED program, a module of the BIRDSUITE package.

Ethics Statement. This research was approved by the institutional review board of the Children's Hospital of Philadelphia. The appropriate informed consent was obtained for all sample donors.

Supplementary Information
Genome Wide Copy Number Variation Study Associates Metabotropic Glutamate Receptor Gene Networks With Attention Deficit Hyperactivity Disorder

Josephine Elia, Joseph T. Glessner, Kai Wang, Nagahide Takahashi, Corina J. Shtir, Dexter Hadley, Patrick M.A. Sleiman, Haitao Zhang, Cecilia E. Kim, Reid Robison, Gholson J. Lyon, James H. Flory, Jonathan P. Bradfield, Marcin Imielinski, Cuiping Hou, Edward C. Frackelton, Rosetta M. Chiavacci, Takeshi Sakurai, Cara Rabin, Frank A. Middleton, Kelly A. Thomas, Maria Garris, Frank Mentch, Christine M. Freitag, Hans-Christoph Steinhausen, Alexandre A. Todorov, Andreas Reif, Aribert Rothenberger, Barbara Franke, Eric O. Mick, Herbert Roeyers, Jan Buitelaar, Klaus-Peter Lesch, Tobias Banaschewski, Richard P. Ebstein, Fernando Mulas, Robert D. Oades, Joseph Sergeant, Edmund Sonuga-Barke, Tobias J. Renner, Marcel Romanos, Jasmin Romanos, Andreas Warnke, Susanne Walitza, Jobst Meyer, Haukur Pálmason, Christiane Seitz, Sandra K. Loo, Susan L. Smalley, Joseph Biederman, Lindsey Kent, Philip Asherson, Richard J.L. Anney, J. William Gaynor, Philip Shaw, Marcella Devoto, Peter S. White, Struan F.A. Grant, Joseph D. Buxbaum, Judith L. Rapoport, Nigel M. Williams, Stanley F. Nelson, Stephen V. Faraone, Hakon Hakonarson

Supplementary Notes
CNV Calls and Review of Significant Loci

No additional "CNV burden" was observed in cases vs. controls, rather the distribution of calls made was highly comparable (Supplementary Fig. 1). We established CNV call reliability in Illumina and Perlegen data by observing Mendelian patterns of inheritance. Trios were first verified by genotype inheritance and analyzed to establish the quality of CNV calls from both Illumina and Perlegen platforms based on observed inheritance. Based on all CNV calls called in trios from the Illumina CHOP data, 8,647 CNVs observed in offspring were inherited from a parent while 437 CNVs were putatively *de novo* which is a *de novo* rate of 4.811%. Based on all CNV calls called in trios from the Perlegen IMAGE data, 1,862 CNVs observed in offspring were inherited from a parent while 505 CNVs were putatively *de novo* which is a *de novo* rate of 21.335%. 51 IMAGE cases, 22 deletion loci, and 5 duplication loci had multiple *de novo* events due to low data quality and were excluded as outliers; once excluded, 785 CNVs were inherited and 63 were *de novo* which lowered the observed *de novo* rate to an acceptable level of 7.429%. Based on CNVs observed in parents from Illumina CHOP data, 9,305 CNVs were passed to the child while 7,432 CNVs were not inherited resulting in a 55.595% inheritance rate. Based on all CNVs observed in parents from Perlegen IMAGE data, 2,114 CNVs were passed to the child while 3,789 CNVs were not inherited resulting in a 35.812% inheritance rate. We excluded 65 parent samples that were outliers with 20 or greater CNVs not inherited to offspring and filtering these samples out resulted in 1,204 CNVs were passed to the child while 1,221 were not inherited resulting in a 49.650% inheritance rate which established confidence in this CNV call set. It is intractable to review all PennCNV calls and wasteful to exclude CNVs smaller than a size threshold. Instead, we statistically score the loci based on all CNVs detected and review these nominally associated CNVR loci for appropriate overlap, signal quality, and Mendelian inheritance. As in Table 1, all reported loci show at least one case with the CNV inherited from a parent, in cases where both parents were available.

In total, there are 3,506 cases and 13,327 controls, representing greater than a three-fold abundance of control samples to robustly define CNVs to be absent or at a lower frequency than case samples. Although the number of CNVs detected per sample was as high as 70,

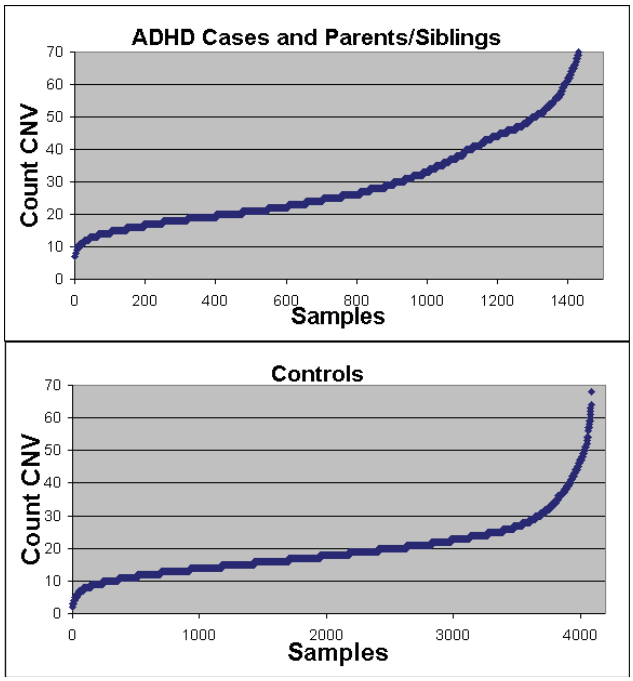
there are actually inferred normal diploid (CN=2) calls which make every sample equivalent. These CNVs are very rare and thus the number of observed CNV calls will vary between samples.

Analysis of Genotype Call Genome-Wide Association

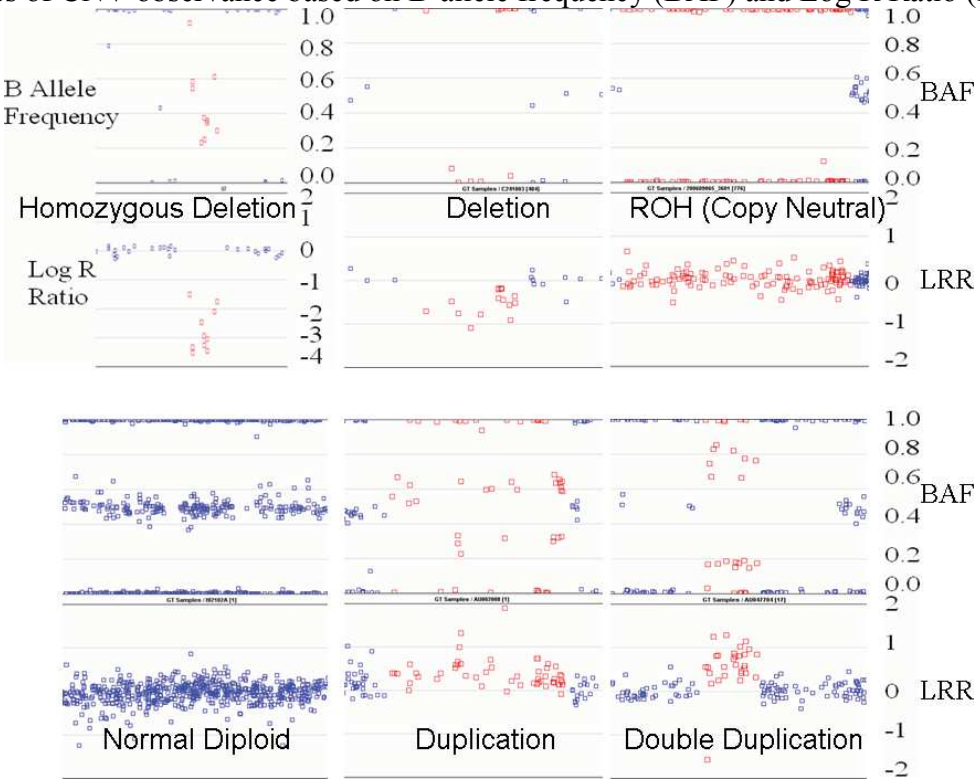
Full scale genotype genome-wide association was performed and the genomic inflation factor (GIF) was at an acceptable level (GIF=1.02409). We also checked pair-wise population concordance to check for and filter out cryptic relatedness which could give rise to rare CNVs specific to ultra-stratified subpopulations of Europe. We performed Transmission Disequilibrium Test (TDT) statistic using Plink on 397 ADHD cases with both parents on the CHOP Illumina HumanHap550 genotype data (Supplementary Table 3). The top result with more than one significant SNP in a region was chr4p12 P(rs1018199)=$2.71x10^{-5}$ and P(rs11724347)=$6.19x10^{-5}$ which impacts *TEC*. We also performed a case:control genotype genome-wide association on 735 cases and 2,298 controls using the same Illumina data set (Supplementary Table 4). The strongest signal was chr19p12 P(rs2081051)=$4.60x10^{-6}$ and P(rs399686)=$4.72x10^{-6}$ residing between *ZNF66* and *ZNF85*. Lastly, 623 ADHD cases with both parents on the IMAGE Perlegen 600K data were analyzed with TDT statistic (Supplementary Table 5). The most significant signal was chr5q23.1 P(rs17144308)= $9.70x10^{-6}$ and P(rs2043053)=$3.36x10^{-5}$ which is 237 kb from the closest proximal gene *DTWD2*. Taken together, SNPs residing around exon 4 of contactin 3 (*CNTN3*) appear to replicate most consistently between Illumina and Perlegen ADHD TDT statistics. SNP rs12488030 is common to both platforms P=$2.51x10^{-3}$ Illumina and P=$4.97x10^{-3}$ Perlegen. There are two supporting SNPs in close proximity also showing significance Illumina: P(rs4073942)= $2.78x10^{-3}$ and P(rs9869828)=$8.61x10^{-3}$ in addition Perlegen: P(rs11915713) =$1.86x10^{-5}$ and P(rs7372975) =$7.59x10^{-5}$

Supplementary Figures:

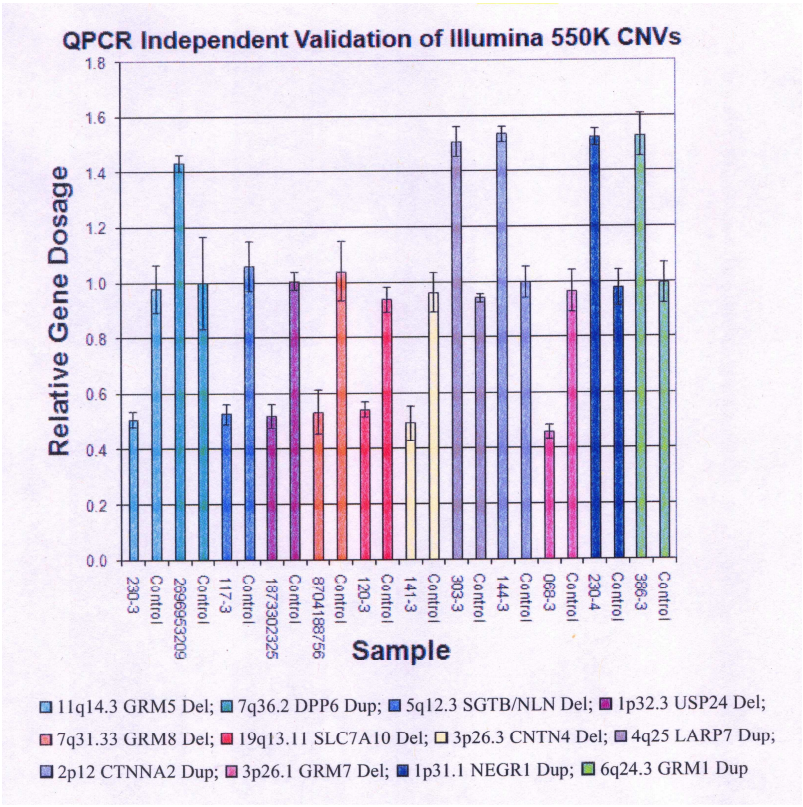
Supplementary Figure 1.
Distribution of CNV calls per individual cases (top panel) *vs*. controls (bottom panel).



Supplementary Figure 2.
Examples of CNV observance based on B-allele frequency (BAF) and Log R Ratio (LRR).

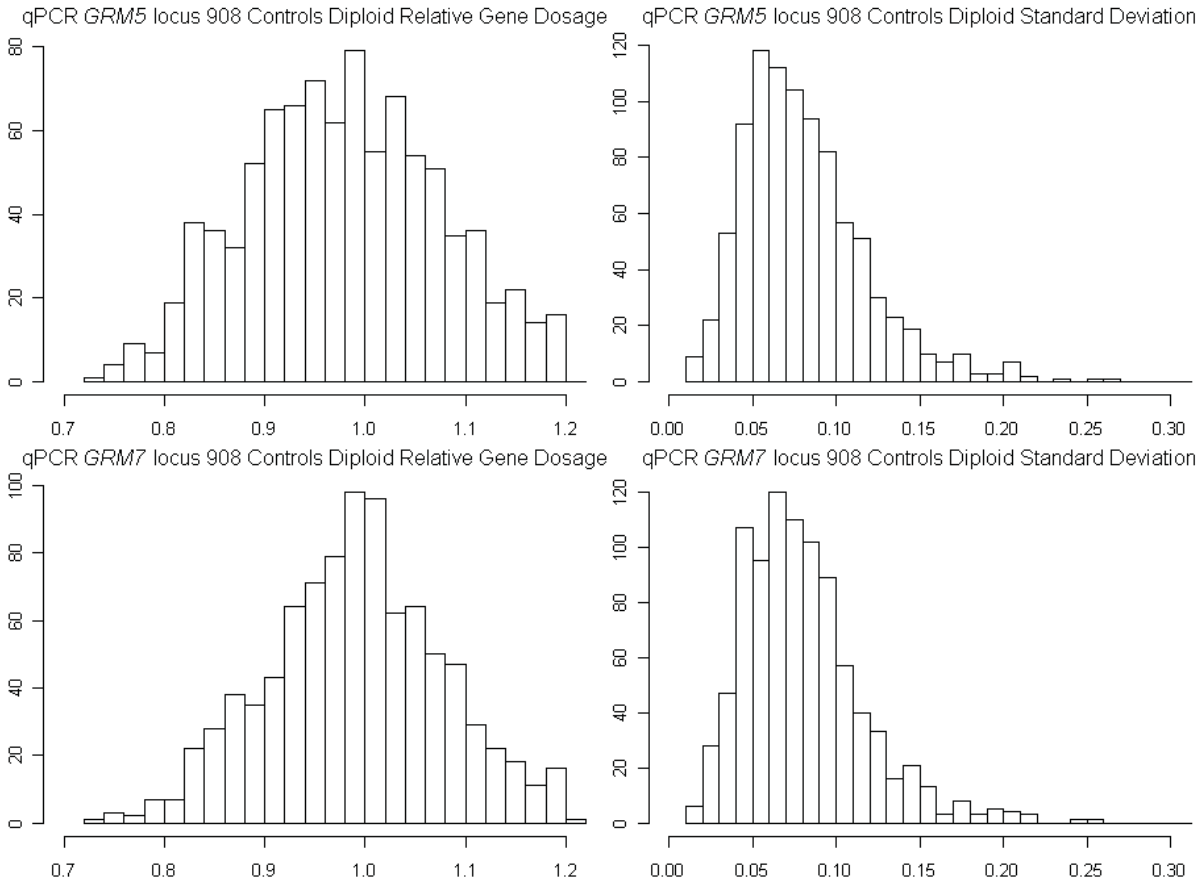


Supplementary Figure 3. Independent qPCR Validation of CHOP ADHD Case Discovery Cohort (Illumina Human Hap550).



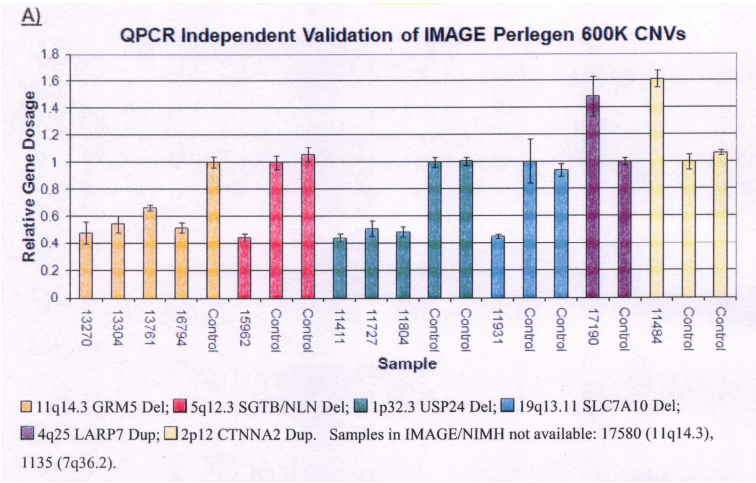
Fluorescent probe-based qPCR assays using Roche Universal probe were designed to validate every candidate CNV with a completely independent test (representative series shown for each locus in case and control pairs). Error bars denote the standard deviation of quadruplicate runs. Del, deletion; Dup, duplication.

Supplementary Figure 4.
Independent qPCR Validation of Controls Negative for CNVs



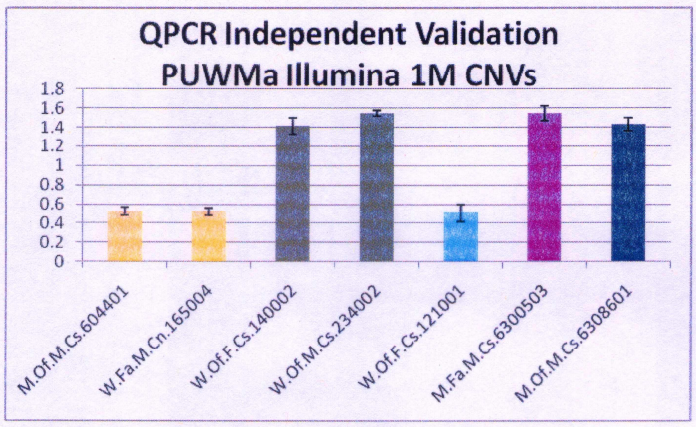
908 controls called for absence of CNV (diploid) by PennCNV in associated regions were screened by qPCR to limit the possibility of false negative calls. Controls were all confirmed to be diploid tightly distributed around one with low level of standard deviation.

Supplementary Figure 5.
Independent qPCR Validation of Replication Cohorts ADHD Cases A) IMAGE (Perlegen 600k) B) PUWMa (Illumina 1M) C) IMAGE II (Affymetrix 5.0)



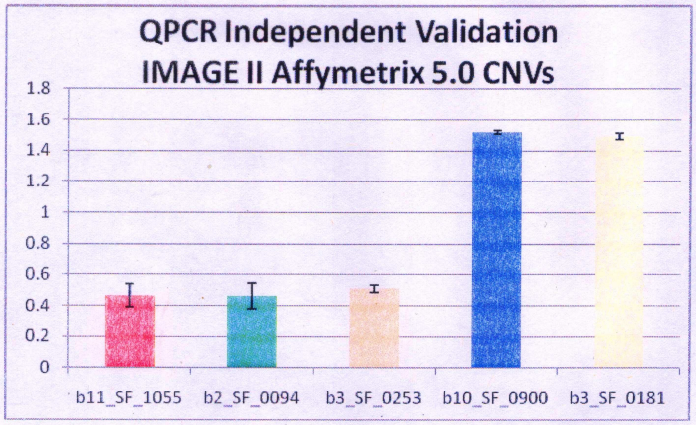
Fluorescent probe-based qPCR assays using Roche Universal probe were designed to validate every candidate CNV with a completely independent test (11 of the 14 IMAGE samples with replicating CNV calls for the loci reported were available for validation and all validated in comparison with control pairs; the other 3 loci were visually validated – see Supplementary Figures 6 and 7). Error bars denote the standard deviation of quadruplicate runs. Del: deletion; Dup: duplication.

B)



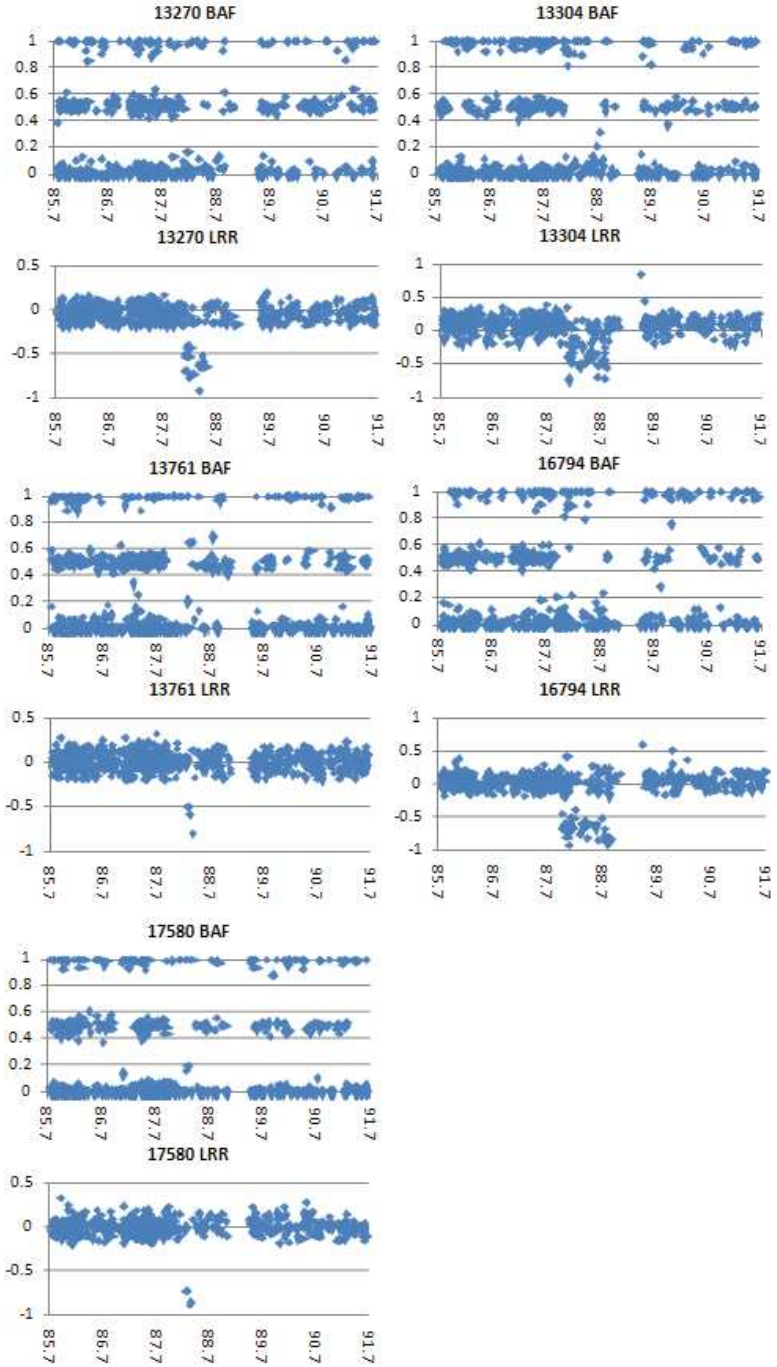
11q14.3 GRM5 Del; 7q36.2 DPP6 Dup; 19q13.11 SLC7A10 Del; 4q25 LARP7 Dup; 1p31.1 NEGR1 Dup;

C)

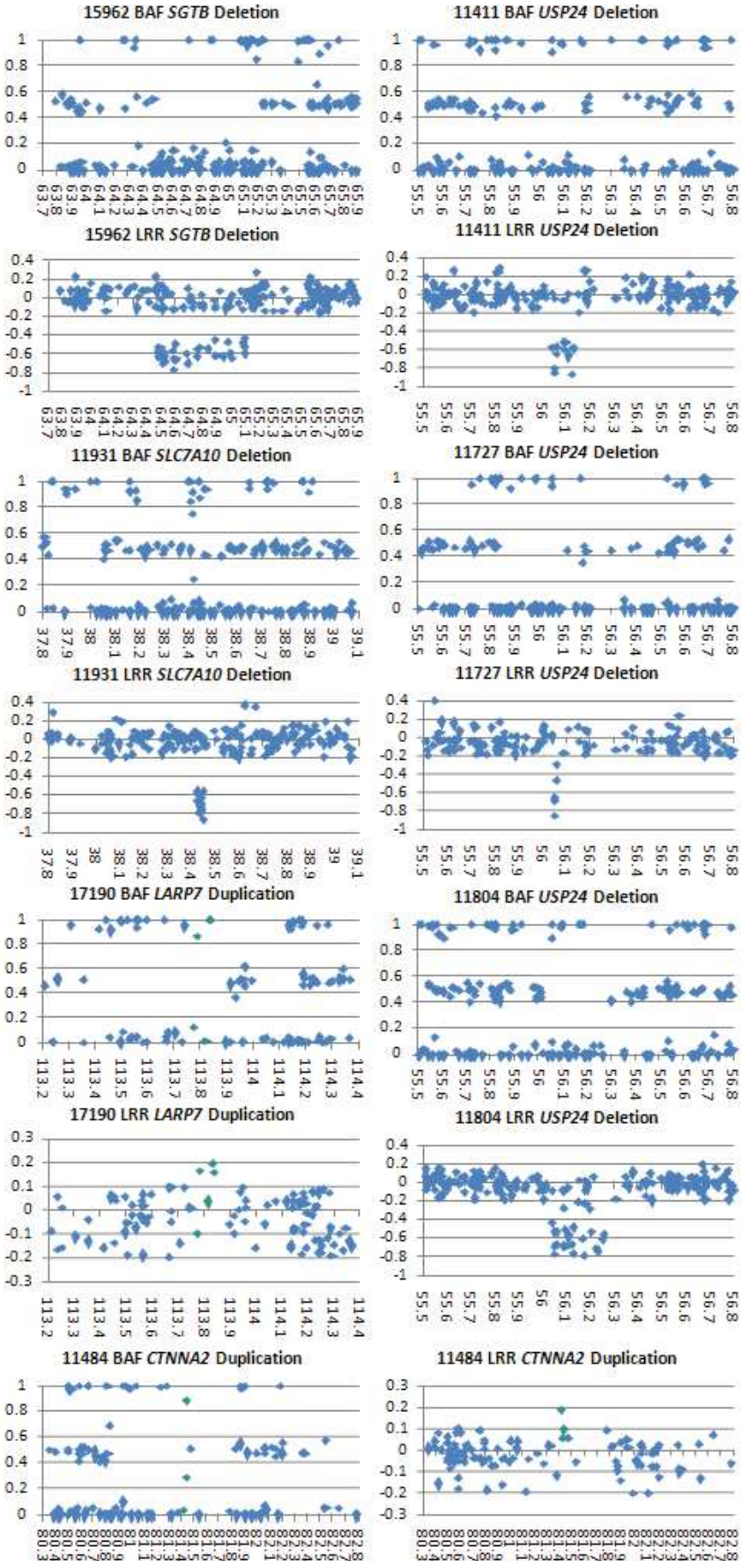


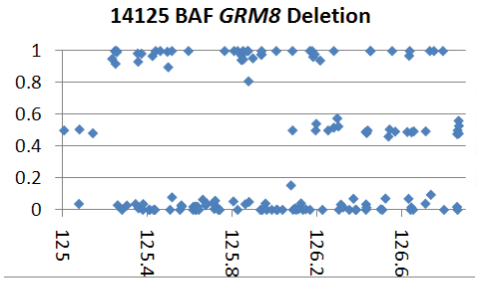
5q12.3 SGTB/NLN Del; 1p32.3 USP24 Del; 3p26.3 CNTN4 Del; 2p12 CTNNA2 Dup; 6q24.3 GRM1 Dup

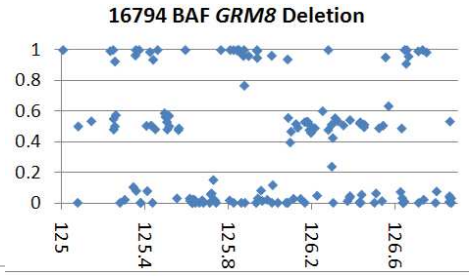
Supplementary Figure 6.
Normalized SNP Level Perlegen 600K Data. The X axis shows base pair position in Mega-bases on chromosome 11. Raw SNP Level Data Showing GRM5 Deletion in five samples from IMAGE Perlegen 600K Data Normalized by Adapted PennCNV-Affy Protocol. Geno-type data termed B-allele frequency (BAF) and intensity data termed Log R Ratio (LRR) plotted.

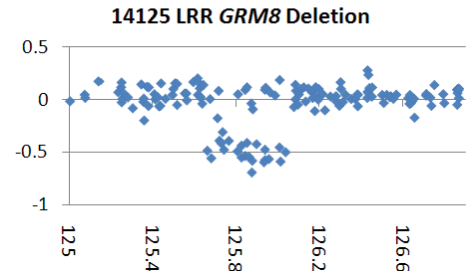


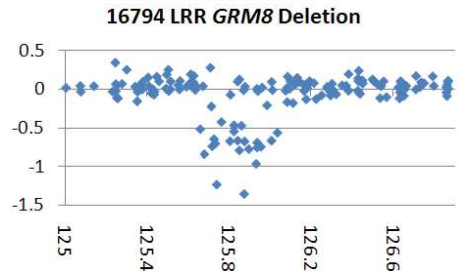
Supplementary Figure 7A
Full SNP-Level Normalized Perlegen 600K Data.




14125 BAF GRM8 Deletion
1
0.8
0.6
0.4
0.2
0
125
125.4
125.8
126.2
126.6


16794 BAF GRM8 Deletion
1
0.8
0.6
0.4
0.2
0
125
125.4
125.8
126.2
126.6


14125 LRR GRM8 Deletion
0.5
0
-0.5
-1
125
125.4
125.8
126.2
126.6


16794 LRR GRM8 Deletion
0.5
0
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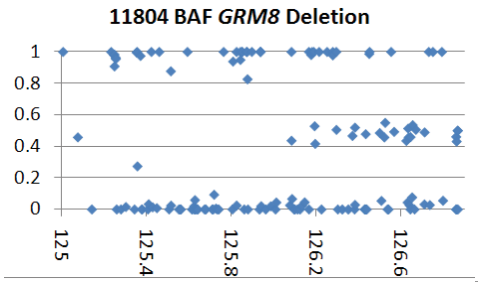
-1
-1.5
125
125.4
125.8
126.2
126.6


11804 BAF GRM8 Deletion
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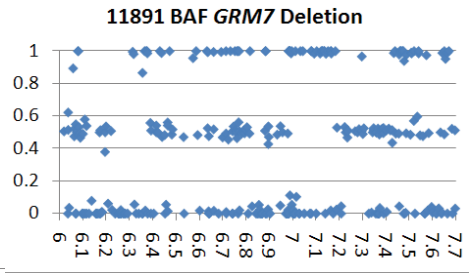
0.8
0.6
0.4
0.2
0
125
125.4
125.8
126.2
126.6


11891 BAF GRM7 Deletion
1
0.8
0.6
0.4
0.2
0
6
6.1
6.2
6.3
6.4
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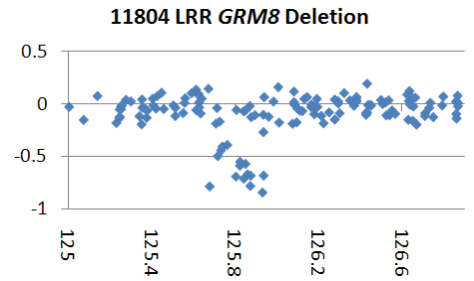
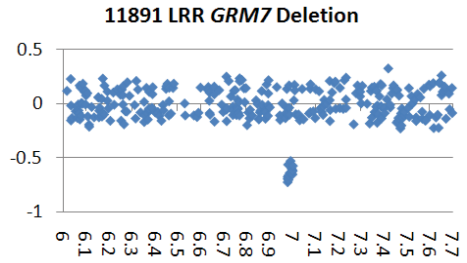
7.2
7.3
7.4
7.5
7.6
7.7


11804 LRR GRM8 Deletion
0.5
0
-0.5
-1
125
125.4
125.8
126.2
126.6


11891 LRR GRM7 Deletion
0.5
0
-0.5
-1
6
6.1
6.2
6.3
6.4
6.5
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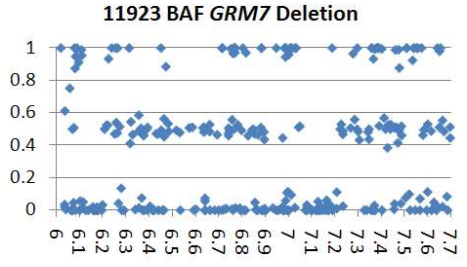
6.8
6.9
7
7.1
7.2
7.3
7.4
7.5
7.6
7.7


11923 BAF GRM7 Deletion
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0.8
0.6
0.4
0.2
0
6
6.1
6.2
6.3
6.4
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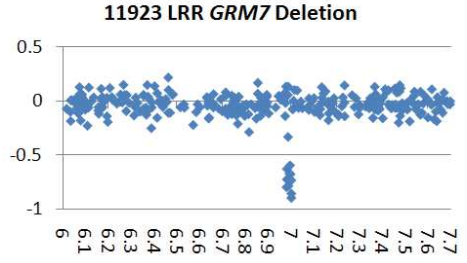
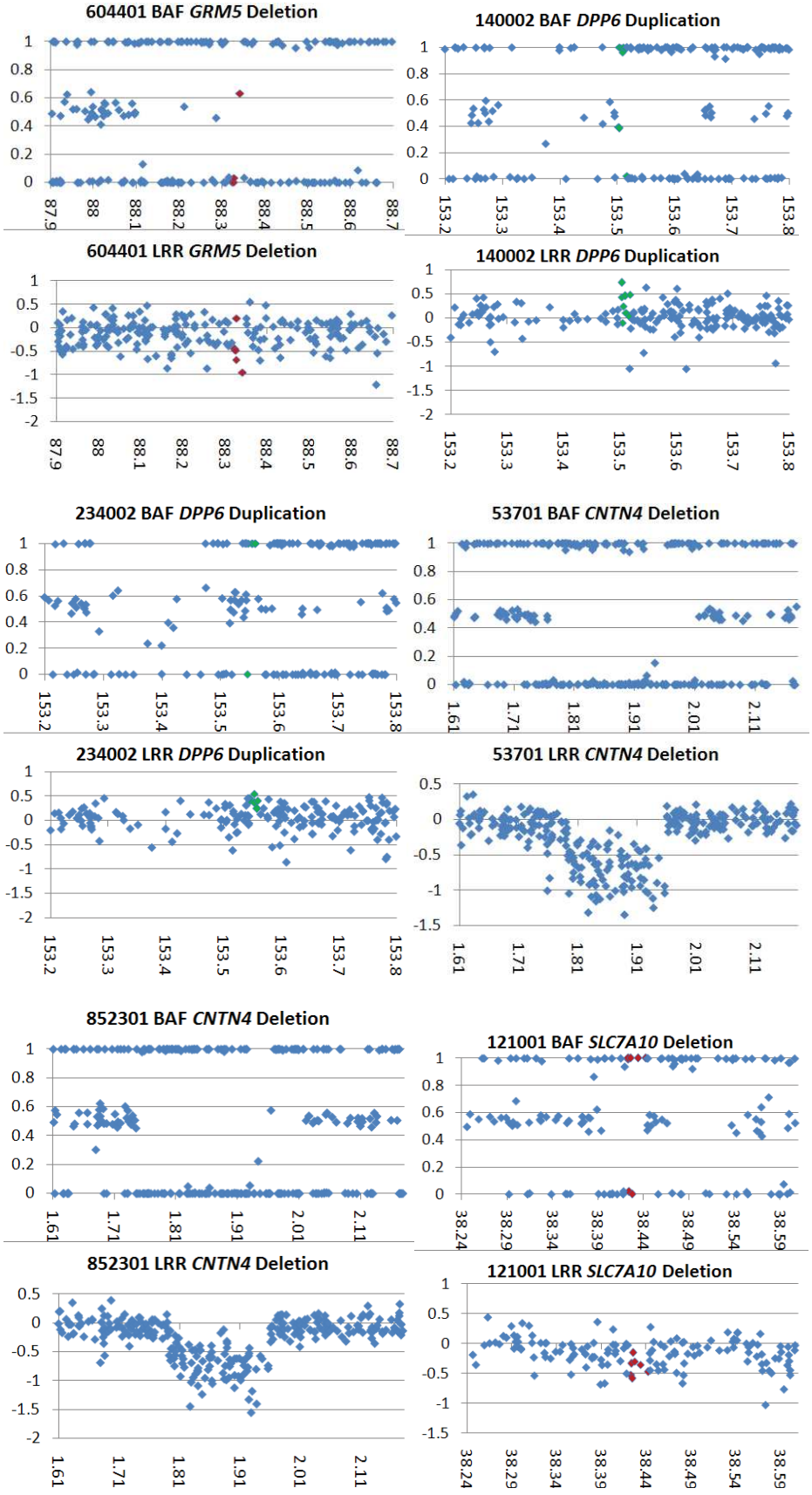
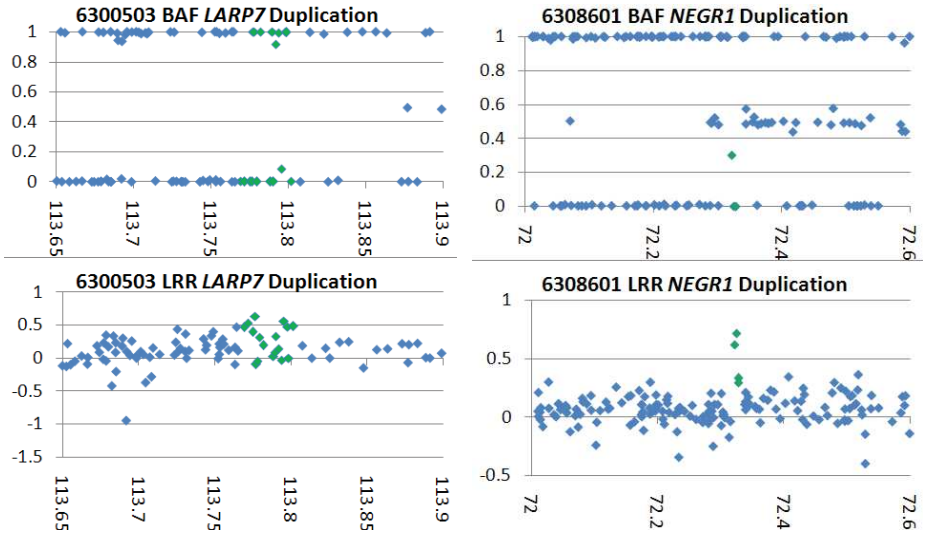
6.8
6.9
7
7.1
7.2
7.3
7.4
7.5
7.6
7.7


11923 LRR GRM7 Deletion
0.5
0
-0.5
-1
6
6.1
6.2
6.3
6.4
6.5
6.6
6.7
6.8
6.9
7
7.1
7.2
7.3
7.4
7.5
7.6
7.7

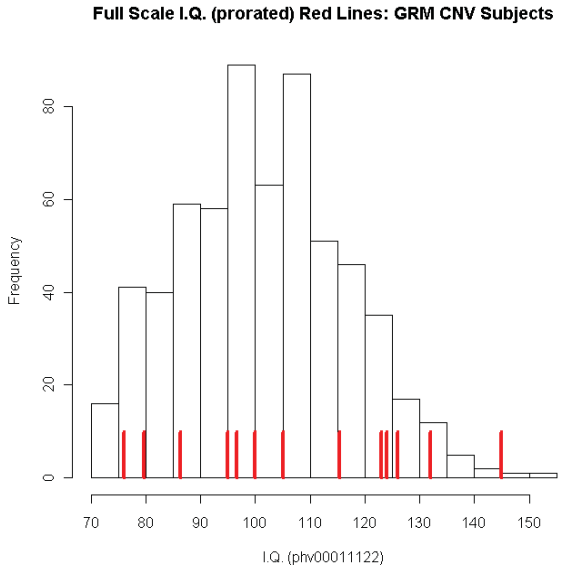
Supplementary Figure 7B.
Full SNP-Level Normalized Illumina 1M PUWMa Data.



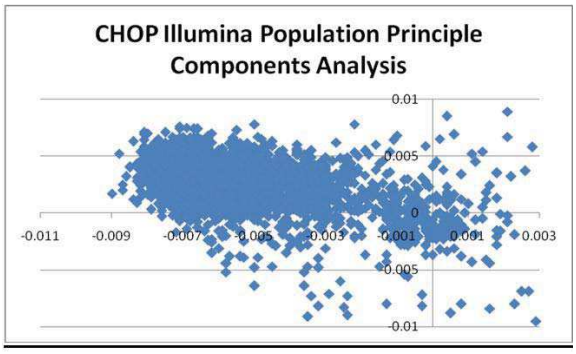

6300503 BAF *LARP7* Duplication
1
0.8
0.6
0.4
0.2
0
113.65
113.7
113.75
113.8
113.85
113.9
6308601 BAF *NEGR1* Duplication
1
0.8
0.6
0.4
0.2
0
72
72.2
72.4
72.6
6300503 LRR *LARP7* Duplication
1
0.5
0
-0.5
-1
-1.5
113.65
113.7
113.75
113.8
113.85
113.9
6308601 LRR *NEGR1* Duplication
1
0.5
0
-0.5
72
72.2
72.4
72.6

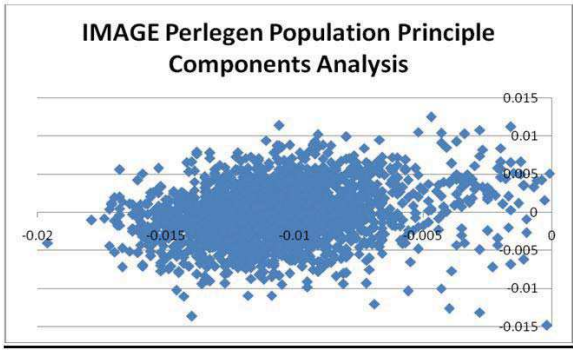
Supplementary Figure 7C.
Full SNP-Level Normalized Affymetrix 5.0 IMAGE II Data.

Supplementary Figure 8. Intelligence Quotient (IQ) ADHD Population Distribution. IQ measures in the ADHD subjects ranged from 70-155, with mean IQ of 103). ADHD subjects with GRM CNV (*GRM5*, *GRM7* and *GRM8* deletions *GRM1* duplication) are inserted in red color showing normal distribution across the ADHD cohort.



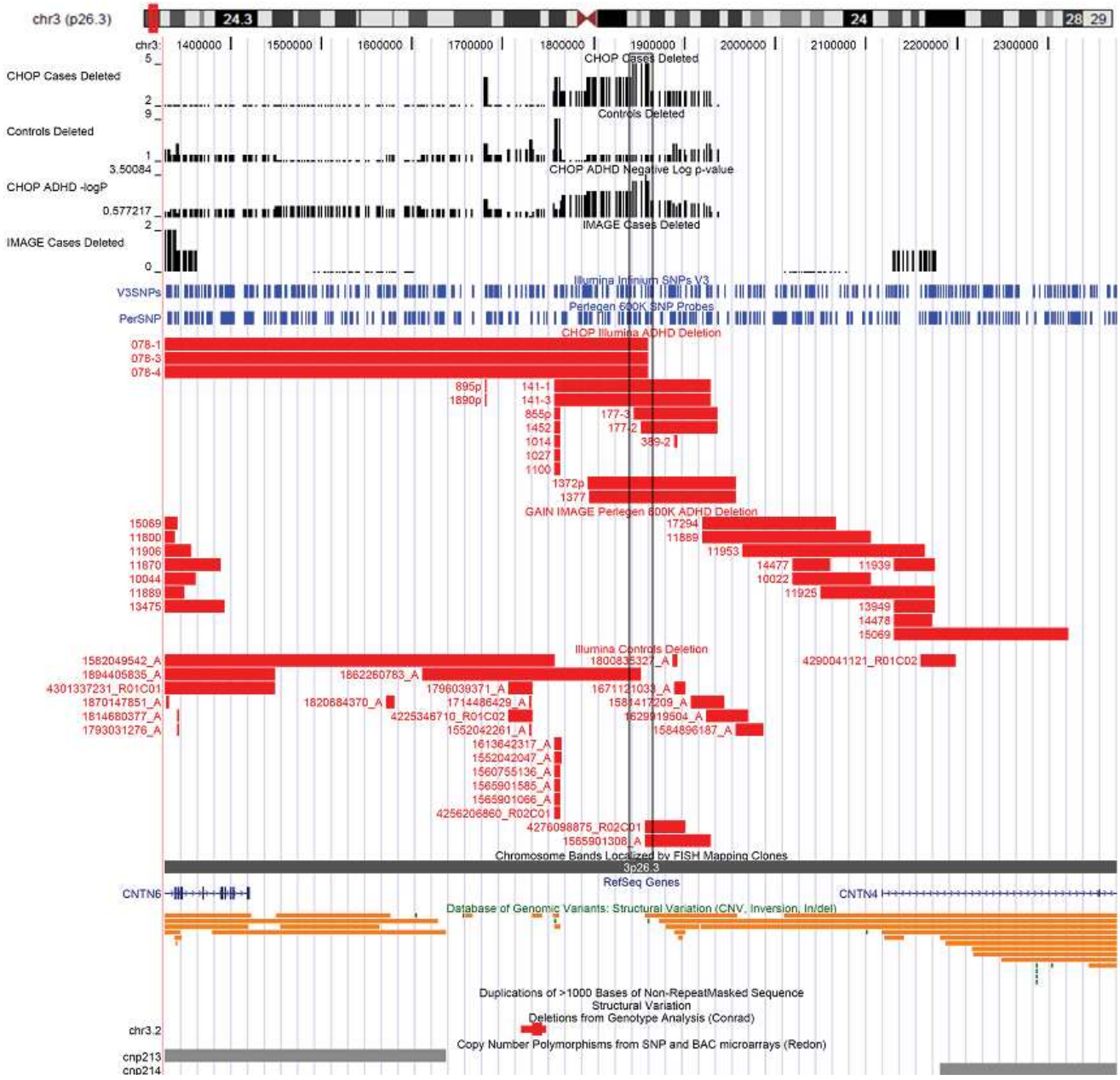
Supplementary Figure 9.
Eigenstrat Principle Components Analysis. Cases and Controls were simultaneously analysed to minimize population substructure in case control CNV association. Samples deviating from the Caucasian cluster shown were removed. The genomic inflation factor (GIF) within Plink was at an acceptable level (GIF=1.02409). We also checked pair-wise population concordance to check for and filter out cryptic relatedness which could give rise to rare CNVs specific to ultra-stratified subpopulations of Europe.



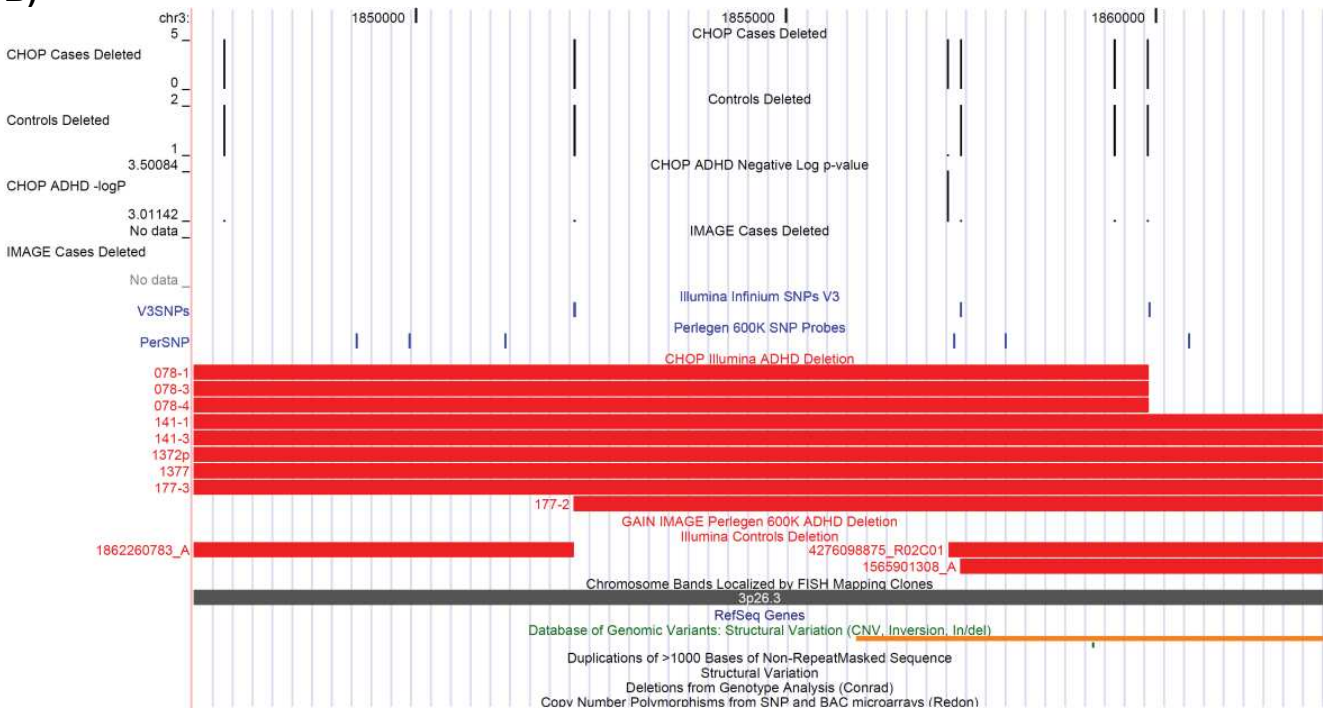


Supplementary Figure 10.
Example of the SNP-based statistics applied and the resulting highest significance region Called**.** Examples from chr 3 are shown; **A)** 1,327,963-2,376,095 and **B)** 1,847,000-1,862,261. Complex CNV overlap is simplified by producing SNP-based statistics. As seen in plots for cases deleted and controls deleted, each SNP has a specific number of CNVs. The cases and controls are compared with a Fisher's exact test and the negative log p value is shown in the third plot. Regions of significance ranging within a power of ten are reported and the region of highest significance (local minimum p-value) within 1MB is reported. The IMAGE cases deleted plot shows only one case sample #11939 since the remaining red regions 3' are parents.

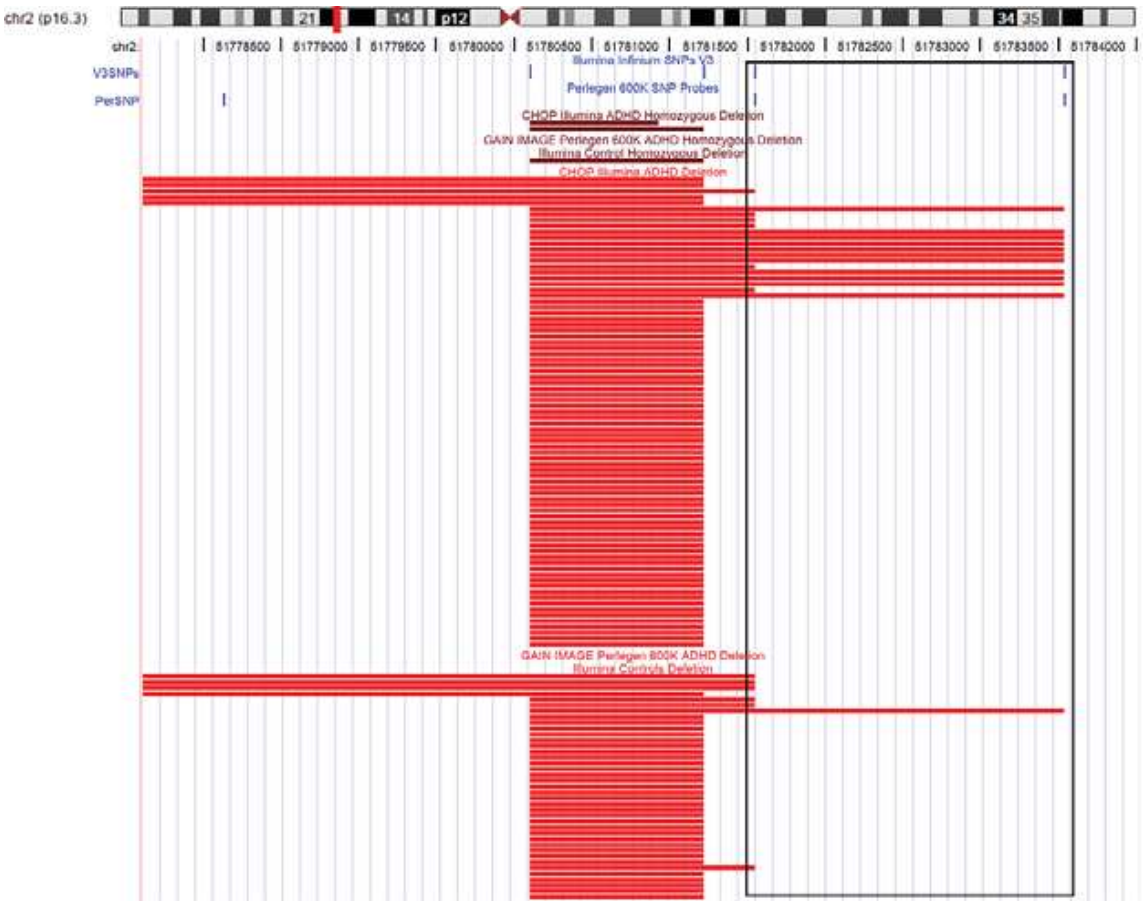
A)



B)



Supplementary Figure 11.
CNV peninsula false positive association example. An example from chr 2 is shown (location 51,777,616-51,784,033**).** All significant CNVRs are reviewed for CNV peninsulas indicating uncertainty in boundary truncation.



Supplementary Tables

Supplementary Table 1.
Clinical Demographics of Study Participants.

ADHD Cohort	N	ADHD subjects age range	Ancestry	ADHD ascertainment
CHOP ADHD trios	349	6-18	European	K-SADS-IVR
CHOP ADHD cases	664	6-18	European	Clinical ADHD diagnosis & treatment with ADHD meds; K-SADS-IVR on majority
NIMH ADHD trios	128	6-12	European	DICA; Conners Scales
UTAH cases	90	19-60	European	WRAADDS, WURS, PRS, strict DSM-IV criteria, including age of onset before 7
IMAGE ADHD trios	642	6-17	European	PACS, Conners, SDQ, WISC
IMAGE II ADHD trios	787	5-14	European	K-SADS German version, Kinder-DIPS, Conners parent & teacher scales, WISC, KABC
PUWMa trios	864	6-18	European	K-SADS

PACS: Parental Account of Child Symptoms; Conners: Behavioural rating scales; SDQ: Strength and Difficulties Questionnaire; WISC: Wechsler Intelligence Scale for Children (WISC-IV); KSADS-IVR: Schedule for affective Disorders and Schizophrenia for School-Age Children-IVR; DICA: Diagnostic Interview for Children and Adolescents; Kinder-DIPS: Diagnostic Interview for Psychiatric Disorders in Children, K-ABC: Kaufman-ABC intelligence scale. WRAADDS=Wender-Reimherr Adult Attention Deficit Disorder Scale; WURS=Wender Utah Rating Scale; PRS=Parent Rating Scale.

Supplementary Table 2.
K-SADS ADHD Severity of CHOP Study Participants in Inattentive, Impulsive, and Hyperactive Domains.

Diagnostic Criteria	Score 1	Score 2	Score 3	Score 4
Often Careless	7	40	372	81
Loses Things	18	126	277	79
Difficulty Finishing	16	90	311	83
Listening	10	22	320	148
Concentration*	2	25	337	135
Distracted	1	10	307	182
Organizing	19	79	304	98
Avoiding	19	55	278	148
Forgetful	19	75	290	116
Interrupts	28	73	305	94
Acts before Thinking	28	112	283	77
Shifts Activities	72	134	247	47

Blurts†	135	82	232	48
Difficulty Waiting Turn	80	172	200	48
Hyperactive	53	127	227	93
Fidgeting	15	47	301	137
Difficulty Staying Seated	45	80	287	88
On the Go	49	89	255	107
Talks Excess	37	77	255	131
Difficulty Playing Quietly	98	120	233	49

*Concentration 1 record missing †Blurts 3 records missing. Scores 1 and 2 means that symptoms are within the normal range while scores 3 and 4 are excessive.

Supplementary Table 3.
Sample Cohorts (Cases and Controls) Used in the Study and Source of Sample DNA

Sample Source	Array Platform	Number	Case/Control	DNA Source
CHOP	Illumina 550k	1,013	Case	Blood/Cell line
NIMH	Illumina 550k	128	Case	Blood
Utah	Illumina 550k	90	Case	Blood
CHOP	Illumina 550k	4,105	Control	Blood
IMAGE	Perlegen 600k	624	Case	Blood /Cell line
Psoriasis	Perlegen 600K	1,600	Control	Blood /Cell line
Depression Control	Perlegen 600K	1,697	Control	Blood
PUWMa	Illumina 1M	864	Case	Blood
SAGE	Illumina 1M	2,211	Control	Blood
PUWMa Parents	Illumina 1M	1,258	Control	Blood
IMAGEII	Affymetrix 5.0	787	Case	Blood/Saliva
IMAGEII (nonGAIN)	Affymetrix 6.0	898	Control	Blood
AGRE Parents	Affymetrix 5.0	1,558	Control	Cell line

Supplementary Table 4.
TDT Analysis of 397 ADHD Cases and Parents from CHOP genotyped on the Illumina HH550 chip.

CHR	SNP	BP	A1	A2	T	U	OR	CHISQ	P
18	rs8095193	58834095	1	2	167	92	1.815	21.72	3.16E-06
17	rs4357980	13498634	1	2	99	174	0.569	20.6	5.65E-06
18	rs8091710	72897492	1	2	29	73	0.3973	18.98	1.32E-05
14	rs899116	97495185	1	2	101	172	0.5872	18.47	1.73E-05
13	rs9595945	48099556	1	2	245	160	1.531	17.84	2.40E-05
4	rs1018199	47927632	1	2	35	80	0.4375	17.61	2.71E-05
1	rs3795324	157456184	2	1	91	157	0.5796	17.56	2.78E-05
3	rs6444186	188156541	1	2	81	36	2.25	17.31	3.18E-05
9	rs11144627	75654927	2	1	46	14	3.286	17.07	3.61E-05
8	rs1462011	108104653	1	2	199	125	1.592	16.9	3.94E-05
X	rs5991935	100480088	1	2	22	59	0.3729	16.9	3.94E-05
7	rs1013572	78350227	1	2	63	118	0.5339	16.71	4.35E-05
11	rs952619	20316347	1	2	108	177	0.6102	16.71	4.37E-05
4	rs7689018	85116479	1	2	41	87	0.4713	16.53	4.79E-05
18	rs1943825	69128567	2	1	97	162	0.5988	16.31	5.37E-05
4	rs4696821	8473961 1	2	2	10	135	1.556	16.3	5.39E-05
18	rs1943823	69131624	2	1	157	237	0.6624	16.24	5.57E-05
4	rs11724347	47923023	1	2	26	64	0.4062	16.04	6.19E-05
1	rs7530899	76950752	2	1	89	151	0.5894	16.02	6.28E-05
18	rs4890560	41457783	1	2	93	156	0.5962	15.94	6.54E-05
6	rs2677099	45527900	1	2	220	144	1.528	15.87	6.79E-05
12	rs11067228	113556980	2	1	231	153	1.51	15.84	6.88E-05
6	rs2790102	45540192	1	2	222	146	1.521	15.7	7.44E-05
1	rs4926757	48961624	1	2	192	122	1.574	15.61	7.80E-05
11	rs17147479	84055504	1	2	137	79	1.734	15.57	7.93E-05
17	rs9913261	12026365	2	1	89	150	0.5933	15.57	7.96E-05
9	rs7041883	135352660	1	2	17	49	0.3469	15.52	8.19E-05
12	rs7309946	103478293	2	1	119	188	0.633	15.51	8.22E-05
7	rs10226468	42907176	2	1	144	219	0.6575	15.5	8.27E-05
5	rs438418	2902436	2	1	78	36	2.167	15.47	8.37E-05
8	rs12682232	108078371	2	1	199	128	1.555	15.42	8.63E-05
X	rs5956634	123092612	2	1	59	110	0.5364	15.39	8.74E-05
7	rs7786719	42850356	1	2	133	205	0.6488	15.34	8.99E-05
6	rs910586	45518290	1	2	221	146	1.514	15.33	9.04E-05
6	rs9395010	44453984	1	2	152	91	1.67	15.31	9.11E-05
14	rs11844273	97489409	1	2	100	163	0.6135	15.09	1.02E-04
2	rs11904235	36288350	1	2	64	27	2.37	15.04	1.05E-04
11	rs487518	131283728	1	2	150	225	0.6667	15	1.08E-04
6	rs6920606	33105652	2	1	164	242	0.6777	14.99	1.08E-04
14	rs2014525	97491178	1	2	109	174	0.6264	14.93	1.12E-04
11	rs7948111	23403649	1	2	65	117	0.5556	14.86	1.16E-04
16	rs12598067	60940038	2	1	65	117	0.5556	14.86	1.16E-04
6	rs9472494	45559814	1	2	223	149	1.497	14.72	1.25E-04
7	rs533486	99085345	2	1	163	240	0.6792	14.71	1.25E-04
8	rs7835921	96345468	1	2	157	96	1.635	14.71	1.26E-04
4	rs827019	8460842	2	1	69	122	0.5656	14.71	1.26E-0

CHR: Chromosome number, SNP:SNP identifier, A1:Minor allele code, A2:Major allele code, T: Transmitted minor allele count, U: Untransmitted allele count, OR:TDT odds ratio, CHISQ:TDT chi-square statistic, P:TDT asymptotic p-value

Supplementary Table 5. Case:Control Analysis of 735 ADHD Cases and 2,298 Unrelated Controls from CHOP genotyped on the Illumina HH550 chip.

CHR	SNP	BP	A1	A2	F_A	F_U	OR	CHISQ	P
18	rs16943400	23086102	1	2	0.02778	0.08875	0.2934	57.53	3.33E-14
3	rs7649108	166136126	1	2	0.3156	0.2497	1.386	24.88	6.11E-07
6	rs9390261	145283744	1	2	0.02585	0.009072	2.899	24.54	7.29E-07
X	rs4609327	37790223	2	1	0.1441	0.08032	1.928	24.48	7.50E-07
X	rs5917547	37803525	2	1	0.1578	0.09074	1.878	24.22	8.59E-07
16	rs2278656	54885245	1	2	0.01443	0.04091	0.3432	22.04	2.67E-06
8	rs17834541	2674349	2	1	0.1083	0.1565	0.6545	21.01	4.56E-06
19	rs2081051	20866811	1	2	0.1382	0.1911	0.6786	21	4.60E-06
19	rs399686	20772798	1	2	0.143	0.1962	0.6833	20.95	4.72E-06
X	rs5917937	39750534	2	1	0.1195	0.06572	1.929	20.93	4.76E-06
19	rs10419820	20943636	2	1	0.1789	0.2357	0.7067	20.9	4.84E-06
X	rs10522011	32517409	1	2	0.05924	0.02509	2.447	19.48	1.02E-05
8	rs11203872	17531028	2	1	0.4342	0.37	1.306	19.34	1.09E-05
X	rs9633179	3535471	2	1	0.1089	0.05969	1.925	19.24	1.15E-05
4	rs10519629	143040375	2	1	0.1864	0.1398	1.409	18.81	1.44E-05
19	rs7253306	20951939	2	1	0.219	0.2759	0.736	18.77	1.48E-05
13	rs9569383	55299477	1	2	0.1415	0.1909	0.6984	18.64	1.58E-05
12	rs12229174	62532933	1	2	0.06054	0.03502	1.776	18.56	1.64E-05
19	rs6511169	20893589	1	2	0.1461	0.1961	0.7014	18.51	1.69E-05
11	rs10833476	21190445	1	2	0.1224	0.08502	1.502	18.48	1.72E-05
2	rs1821659	212064488	2	1	0.3109	0.2527	1.334	18.15	2.05E-05
X	rs2480443	53212284	2	1	0.06525	0.02994	2.262	18.1	2.09E-05
7	rs1486173	45965025	2	1	0.1131	0.07764	1.515	17.91	2.32E-05
15	rs4381545	93039961	2	1	0.2296	0.18	1.358	17.8	2.45E-05
7	rs10265665	96175055	1	2	0.0619	0.0365	1.742	17.79	2.46E-05
10	rs11593585	44391199	1	2	0.1286	0.09093	1.475	17.69	2.60E-05
X	rs4134188	17474194	1	2	0.1016	0.05571	1.917	17.62	2.69E-05
4	rs11131363	63013616	2	1	0.2643	0.212	1.335	17.6	2.72E-05
19	rs1469402	20738115	2	1	0.145	0.1934	0.7075	17.52	2.85E-05
11	rs12279152	133861485	1	2	0.02653	0.01139	2.365	17.43	2.98E-05
X	rs5957334	119125665	2	1	0.06667	0.03136	2.206	17.13	3.49E-05
X	rs6632558	36075450	2	1	0.0812	0.04176	2.028	16.94	3.85E-05
1	rs2057594	117348535	1	2	0.2483	0.1983	1.335	16.89	3.96E-05
8	rs17834523	2672777	1	2	0.09592	0.1367	0.6699	16.84	4.06E-05
7	rs10485959	78702412	2	1	0.3007	0.3595	0.7659	16.83	4.09E-05
X	rs5945330	152438289	2	1	0.08807	0.04698	1.959	16.63	4.55E-05
3	rs16854851	145238402	1	2	0.02381	0.009916	2.435	16.62	4.56E-05
8	rs2237826	17519195	2	1	0.4355	0.376	1.28	16.59	4.65E-05
X	rs16987407	35968032	2	1	0.1041	0.05857	1.868	16.5	4.87E-05
X	rs4089885	22878045	2	1	0.1193	0.07027	1.792	16.47	4.94E-05
1	rs2024766	181385290	2	1	0.5027	0.4424	1.274	16.45	4.99E-05
4	rs9312518	173526549	1	2	0.4639	0.4042	1.276	16.45	5.00E-05
4	rs9997484	173517324	2	1	0.4639	0.4042	1.276	16.45	5.00E-05
17	rs4338847	7870502	1	2	0.3102	0.3679	0.7725	16.35	5.28E-05
12	rs17497206	113000660	2	1	0.1537	0.2011	0.7219	16.33	5.32E-05

CHR:Chromosome, SNP:SNP ID, BP:Physical position (base-pair), A1:Minor allele name (based on whole sample), F_A:Frequency of this allele in cases, F_U:Frequency of this allele in controls, A2:Major allele name, OR:Estimated odds ratio (for A1, i.e. A2 is reference), CHISQ:Basic allelic test chi-square (1df), P:Asymptotic p-value for this test.

Supplementary Table 6. TDT Analysis of 623 ADHD Cases and Parents from IMAGE genotyped on the Perlegen platform.

CHR	SNP	BP	A1	A2	T	U	OR	CHISQ	P
12	rs3782309	26750663	1	2	172	99	1.737	19.66	9.23E-06
5	rs17144308	117965870	2	1	244	352	0.6932	19.57	9.70E-06
2	rs7609261	80530821	2	1	199	297	0.67	19.36	1.08E-05
3	rs1344870	21282405	2	1	16	52	0.3077	19.06	1.27E-05
18	rs7244637	17876224	1	2	134	215	0.6233	18.8	1.45E-05
1	rs3850879	48004718	1	2	226	143	1.58	18.67	1.56E-05
14	rs2295426	58446208	2	1	209	307	0.6808	18.61	1.60E-05
16	rs7204253	5576184	2	1	114	189	0.6032	18.56	1.64E-05
4	rs1378945	25382295	2	1	212	310	0.6839	18.4	1.79E-05
3	rs11915713	74568983	1	2	176	266	0.6617	18.33	1.86E-05
12	rs11830382	41718893	2	1	198	122	1.623	18.05	2.15E-05
12	rs4761641	93525817	2	1	137	215	0.6372	17.28	3.22E-05
5	rs2043053	117958083	2	1	126	201	0.6269	17.2	3.36E-05
18	rs12965880	22313077	1	2	235	333	0.7057	16.91	3.92E-05
9	rs17306197	97862011	1	2	162	96	1.688	16.88	3.97E-05
8	rs17668689	96254526	1	2	216	310	0.6968	16.8	4.16E-05
2	rs4852567	80556890	2	1	206	298	0.6913	16.79	4.17E-05
13	rs1002468	93085569	2	1	287	197	1.457	16.74	4.30E-05
1	rs10873925	77234323	2	1	305	212	1.439	16.73	4.31E-05
16	rs12596741	17345435	1	2	228	324	0.7037	16.7	4.39E-05
9	rs2991298	3284851	2	1	81	142	0.5704	16.69	4.41E-05
14	rs1427324	58434446	1	2	206	297	0.6936	16.46	4.96E-05
10	rs11258682	13951273	1	2	204	130	1.569	16.4	5.14E-05
4	rs10520276	175420068	2	1	216	140	1.543	16.22	5.63E-05
1	rs17375519	179499648	1	2	75	133	0.5639	16.17	5.78E-05
1	rs10800069	163296159	1	2	232	327	0.7095	16.14	5.87E-05
7	rs13340504	75277632	1	2	142	82	1.732	16.07	6.10E-05
2	rs6543239	104056246	2	1	251	349	0.7192	16.01	6.31E-05
2	rs4664452	162762970	1	2	30	6	5	16	6.33E-05
4	rs16889099	13341184	2	1	48	96	0.5	16	6.33E-05
5	rs12520147	2000122	1	2	158	237	0.6667	15.8	7.04E-05
11	rs10400283	23523711	1	2	222	314	0.707	15.79	7.07E-05
4	rs1378946	25382548	1	2	197	284	0.6937	15.74	7.28E-05
3	rs7372975	74602140	2	1	169	250	0.676	15.66	7.59E-05
17	rs11654470	74388926	2	1	82	141	0.5816	15.61	7.79E-05
3	rs9878591	121464488	1	2	107	173	0.6185	15.56	8.01E-05
12	rs1553953	28724544	1	2	76	133	0.5714	15.55	8.06E-05
11	rs7121790	45021541	1	2	171	252	0.6786	15.51	8.20E-05
12	rs1452231	83750252	2	1	223	314	0.7102	15.42	8.60E-05
7	rs194847	103560404	1	2	347	251	1.382	15.41	8.65E-05
2	rs11902138	80565100	1	2	173	254	0.6811	15.37	8.86E-05
16	rs12932714	80320240	1	2	150	226	0.6637	15.36	8.88E-05
1	rs1015144	200004976	2	1	204	291	0.701	15.29	9.22E-05
22	rs6009441	47873456	1	2	107	172	0.6221	15.14	9.97E-05
8	rs4734069	104169047	1	2	275	191	1.44	15.14	9.97E-05
20	rs2024946	61678306	2	1	112	61	1.836	15.03	1.06E-04

CHR:Chromosome number, SNP:SNP identifier, A1:Minor allele code, A2:Major allele code, T:Transmitted minor allele count, U:Untransmitted allele count, OR:TDT odds ratio, CHISQ:TDT chi-square statistic, P:TDT asymptotic p-value

Supplementary Table 7. SNP GWAS Significance of Top Ranked ADHD Associated SNPs Reported by Lesch and Zhou. A) ADHD TDT CHOP Illumina 550k data; B) ADHD Case:Control CHOP Illumina 550k data; C) ADHD IMAGE Perlegen 600k data.

A)

CHR	SNP	BP	A1	A2	T	U	OR	CHISQ	P
2	rs2241685	1896290	1	2	72	62	1.161	0.7463	0.3877
2	rs13395022	79793915	2	1	136	136	1	0	1
2	rs2587695	120038047	1	2	183	197	0.9289	0.5158	0.4726
2	rs2242073	208819551	2	1	108	106	1.019	0.01869	0.8913
2	rs1110998	217169458	1	2	175	159	1.101	0.7665	0.3813
3	rs10510238	2876647	2	1	84	93	0.9032	0.4576	0.4987
3	rs9879164	54040611	2	1	185	198	0.9343	0.4413	0.5065
3	rs2084358	57457928	2	1	182	198	0.9192	0.6737	0.4118
3	rs10490808	59939739	2	1	175	204	0.8578	2.219	0.1363
3	rs10510850	60542142	1	2	90	83	1.084	0.2832	0.5946
4	rs755403	6507714	2	1	195	180	1.083	0.6	0.4386
4	rs10516182	7143981	2	1	155	169	0.9172	0.6049	0.4367
4	rs7697323	7801488	1	2	180	222	0.8108	4.388	0.03619
5	rs173754	65102081	1	2	218	202	1.079	0.6095	0.435
5	rs258082	66166352	1	2	199	205	0.9707	0.08911	0.7653
6	rs160666	2719051	2	1	179	181	0.989	0.01111	0.9161
6	rs2842643	41758714	2	1	180	149	1.208	2.921	0.08744
6	rs3799977	44945334	2	1	209	183	1.142	1.724	0.1891
6	rs8180608	89064414	2	1	178	218	0.8165	4.04	0.04442
6	rs1358601	91532294	1	2	180	181	0.9945	0.00277	0.958
6	rs6921403	154156020	2	1	86	90	0.9556	0.09091	0.763
7	rs2237349	28536203	2	1	176	191	0.9215	0.6131	0.4336
7	rs2002865	154132035	2	1	134	157	0.8535	1.818	0.1776
8	rs6991017	5508780	2	1	127	126	1.008	0.003953	0.9499
8	rs2248529	14657354	1	2	188	190	0.9895	0.01058	0.9181
8	rs4961315	142110882	2	1	186	152	1.224	3.42	0.06441
9	rs2418326	114759028	1	2	141	142	0.993	0.003534	0.9526
9	rs2502731	128056111	2	1	170	178	0.9551	0.1839	0.668
14	rs10483393	31530235	1	2	146	137	1.066	0.2862	0.5927
15	rs2556560	42609135	2	1	169	171	0.9883	0.01176	0.9136
16	rs8060494	78808972	2	1	190	174	1.092	0.7033	0.4017
17	rs4790372	2701606	2	1	163	169	0.9645	0.1084	0.7419
17	rs12453316	69027654	1	2	177	179	0.9888	0.01124	0.9156
19	rs997669	34996323	2	1	201	183	1.098	0.8438	0.3583
20	rs1555322	33312595	1	2	94	79	1.19	1.301	0.2541

B)

CHR	SNP	BP	A1	F_A	F_U	A2	OR	CHISQ	P
2	rs2241685	1896290	1	0.09116	0.09283	2	0.9802	0.03733	0.8468
2	rs13395022	79793915	2	0.2088	0.2095	1	0.9961	0.002865	0.9573
2	rs2587695	120038047	1	0.4973	0.4922	2	1.021	0.1161	0.7333
2	rs2242073	208819551	2	0.1605	0.1568	1	1.029	0.1216	0.7273
2	rs1110998	217169458	1	0.3116	0.2928	2	1.093	1.886	0.1697
3	rs10510238	2876647	2	0.1293	0.1376	1	0.9304	0.6621	0.4158
3	rs9879164	54040611	2	0.4218	0.4359	1	0.9441	0.9084	0.3406
3	rs2084358	57457928	1	0.5184	0.4722	2	1.203	9.597	0.001949
3	rs10490808	59939739	2	0.4068	0.4266	1	0.9218	1.8	0.1797
3	rs10510850	60542142	1	0.1211	0.1116	2	1.097	1.001	0.3172
4	rs755403	6507714	2	0.3985	0.3973	1	1.005	0.007242	0.9322
4	rs10516182	7143981	2	0.2801	0.2954	1	0.9279	1.274	0.259
4	rs7697323	7801488	1	0.3782	0.38	2	0.9927	0.0142	0.9051
5	rs173754	65102081	1	0.4925	0.4915	2	1.004	0.004285	0.9478
5	rs258082	66166352	1	0.4619	0.4521	2	1.04	0.4342	0.5099
6	rs160666	2719051	2	0.2857	0.3025	1	0.9222	1.515	0.2183
6	rs2842643	41758714	2	0.2932	0.2909	1	1.011	0.02797	0.8672
6	rs3799977	44945334	2	0.4306	0.4076	1	1.099	2.452	0.1174
6	rs8180608	89064414	2	0.4101	0.4441	1	0.8703	5.265	0.02176
6	rs1358601	91532294	1	0.3852	0.3846	2	1.003	0.002076	0.9637
6	rs6921403	154156020	2	0.1373	0.1405	1	0.9736	0.09408	0.7591
7	rs2237349	28536203	2	0.4109	0.4082	1	1.011	0.03276	0.8564
7	rs2002865	154132035	2	0.2075	0.217	1	0.9445	0.6065	0.4361
8	rs6991017	5508780	2	0.1891	0.1873	1	1.012	0.02315	0.8791
8	rs2248529	14657354	1	0.3604	0.363	2	0.9888	0.03305	0.8557
8	rs4961315	142110882	2	0.2959	0.2995	1	0.983	0.06846	0.7936
9	rs2418326	114759028	1	0.2534	0.252	2	1.007	0.01179	0.9135
9	rs2502731	128056111	2	0.3626	0.3508	1	1.053	0.6767	0.4107
14	rs10483393	31530235	1	0.2272	0.2203	2	1.041	0.3146	0.5749
15	rs2556560	42609135	2	0.419	0.4215	1	0.9899	0.02811	0.8668
16	rs8060494	78808972	2	0.3215	0.3228	1	0.9943	0.008131	0.9282
17	rs4790372	2701606	2	0.3014	0.3112	1	0.9546	0.5122	0.4742
17	rs12453316	69027654	1	0.3612	0.3662	2	0.9788	0.1193	0.7298
19	rs997669	34996323	2	0.4023	0.3876	1	1.064	1.025	0.3114
20	rs1555322	33312595	1	0.1279	0.1277	2	1.002	0.0004034	0.984

C)

CHR	SNP	BP	A1	A2	T	U	OR	CHISQ	P
1	rs2281597	34132445	0	2	0	0	NA	NA	NA
1	rs642969	197590139	0	2	0	0	NA	NA	NA
2	rs2587695	120038287	1	2	320	294	1.088	1.101	0.2941
2	rs2242073	208702290	2	1	185	182	1.016	0.02452	0.8756
3	rs10510850	60542142	1	2	109	115	0.9478	0.1607	0.6885
3	rs17233461	125807474	2	1	305	322	0.9472	0.4609	0.4972
4	rs755403	6440543	2	1	296	278	1.065	0.5645	0.4525
4	rs3857174	7089831	2	1	202	217	0.9309	0.537	0.4637
4	rs7697323	7734317	1	2	269	278	0.9676	0.1481	0.7004
5	rs1457720	110998762	2	1	247	260	0.95	0.3333	0.5637
6	rs160666	2719051	2	1	248	262	0.9466	0.3843	0.5353
6	rs3799977	44945334	2	1	302	282	1.071	0.6849	0.4079
6	rs6921403	154105599	2	1	149	150	0.9933	0.003344	0.9539
8	rs6991017	5508780	2	1	193	191	1.01	0.01042	0.9187
9	rs2418326	116719295	1	2	236	210	1.124	1.516	0.2183
9	rs2416606	119862757	2	1	264	262	1.008	0.007605	0.9305
10	rs16928529	72652991	2	1	277	312	0.8878	2.08	0.1493
10	rs11594082	72969259	1	2	126	138	0.913	0.5455	0.4602
10	rs10786284	98125495	0	1	0	0	NA	NA	NA
10	rs515910	105956394	2	1	300	272	1.103	1.371	0.2417
11	rs3893215	17721406	0	2	0	0	NA	NA	NA
11	rs10830468	87604834	0	2	0	0	NA	NA	NA
12	rs4964805	102716954	0	2	0	0	NA	NA	NA
13	rs7995215	93206507	1	2	279	317	0.8801	2.423	0.1196
14	rs2239627	22705999	0	2	0	0	NA	NA	NA
14	rs10483286	24273582	0	2	0	0	NA	NA	NA
16	rs10514604	83003885	0	2	0	0	NA	NA	NA
17	rs2440129	6847295	0	2	0	0	NA	NA	NA

Supplementary Table 8. ADHD Genotype GWAS of Glutamatergic Genes. The most significant SNP genotype association in each of the eight GRM gene regions. A) ADHD TDT CHOP Illumina 550k B) ADHD Case:Control CHOP Illumina 550k C) ADHD IMAGE Perlegen 600k.

A)

CHR	SNP	BP	A1	A2	T	U	OR	CHISQ	P	Gene
11	rs4237549	88407924	2	1	31	61	0.5082	9.783	0.001762	GRM5
7	rs17864159	126444172	1	2	22	46	0.4783	8.471	0.003609	GRM8
6	rs3887555	34177040	1	2	208	161	1.292	5.986	0.01442	GRM4
7	rs6943762	86047914	2	1	69	99	0.697	5.357	0.02064	GRM3
3	rs7623055	7485891	1	2	151	193	0.7824	5.128	0.02354	GRM7
6	rs362839	146721428	2	1	125	161	0.7764	4.531	0.03328	GRM1
3	rs4687770	51730105	2	1	114	94	1.213	1.923	0.1655	GRM2
5	rs2078183	178357150	2	1	190	210	0.9048	1	0.3173	GRM6

B)

CHR	SNP	BP	A1	F_A	F_U	A2	OR	CHISQ	P	Gene
3	rs7623055	7485891	1	0.3582	0.4129	2	0.7936	15.48	8.35E-05	GRM7
11	rs1354411	88016449	2	0.03643	0.0566	1	0.6302	10.21	0.001396	GRM5
7	rs2283100	126643293	2	0.2281	0.193	1	1.235	9.527	0.002024	GRM8
6	rs1873250	34130718	2	0.2134	0.2455	1	0.8338	7.062	0.007873	GRM4
7	rs10952890	86193151	1	0.02753	0.03917	2	0.6945	4.782	0.02877	GRM3
5	rs2078183	178357150	2	0.4593	0.4897	1	0.8852	4.605	0.03189	GRM6
6	rs1983635	146707365	2	0.316	0.2917	1	1.122	3.515	0.06081	GRM1
3	rs4687592	51630896	1	0.03442	0.04041	2	0.8464	1.191	0.2752	GRM2

C)

CHR	SNP	BP	A1	A2	T	U	OR	CHISQ	P	Gene
6	rs12206652	34173960	2	1	265	216	1.227	4.992	0.02547	GRM4
11	rs160195	87932621	2	1	302	253	1.194	4.326	0.03753	GRM5
7	rs11563486	126621501	1	2	130	162	0.8025	3.507	0.06112	GRM8
3	rs11717471	7599469	2	1	238	280	0.85	3.405	0.06498	GRM7
6	rs2300620	146745874	2	1	160	133	1.203	2.488	0.1147	GRM1
7	rs1468413	86271589	1	2	190	162	1.173	2.227	0.1356	GRM3
5	rs7725272	178338994	2	1	289	261	1.107	1.425	0.2325	GRM6
3	rs6445959	51747387	2	1	169	153	1.105	0.795	0.3726	GRM2

Supplementary Table 9. ADHD CNV Family Based Transmission Disequilibrium and *de novo* Statistical Tests.

A) Illumina CHOP Deletions Enriched for Inheritance

CNVR	Count SNPs	P TDT Del	**Inh Del**	*de novo* Del	Par Del Not Inh	Gene	Distance
chr18:74258734-74260996	3	0.001953	**9**	0	0	*SALL3*	580267
chr7:120092385-120099982	3	0.001953	**9**	0	0	*KCND2*	0
chr4:92499956-92502794	8	0.001953	**9**	0	0	*KIAA1680*	0
chr11:69755529-69759313	12	0.007813	**7**	0	0	*FADD*	24395
chr4:42400885-42403451	15	0.007813	**7**	0	0	*ATP8A1*	47238
chr5:104463047-104518786	17	0.007813	**7**	0	0	*NR_000039*	0
chr13:69637654-69666685	18	0.015625	**6**	0	0	*NR_002717*	25969
chr3:195971510-195982215	5	0.03125	**5**	1	0	*FAM43A*	80455
chr19:44369918-44376749	3	0.03125	**5**	1	0	*LOC342897*	2695
chr1:2349841-2356176	4	0.03125	**5**	1	0	*PEX10*	15971
chr21:45777720-45782727	3	0.03125	**5**	0	0	*SLC19A1*	0
chr10:67748487-67785209	30	0.03125	**5**	0	0	*CTNNA3*	0

B) Illumina CHOP Duplications Enriched for Inheritance

CNVR	Count SNPs	P TDT Dup	**Inh Dup**	*de novo* Dup	Par Dup Not Inh	Gene	Distance
chr20:59015708-59022667	4	0.007813	**7**	0	0	*CDH4*	238287
chr12:72808323-72832667	5	0.015625	**6**	0	0	*BC061638*	0
chr6:73021641-73023171	3	0.03125	**5**	0	0	*RIMS1*	0
chr17:74089903-74106726	9	0.03125	**5**	0	0	*DNAHL1*	10904
chr1:9243828-9310031	22	0.03125	**5**	0	0	*H6PD,SPSB1*	0

C) Illumina CHOP Deletions Enriched for *de novo*

CNVR	Count	Inh	***de***	Par Del	Gene	Distance

	SNPs	Del	***novo* Del**	Not Inh		
chr19:15992679-15997923	2	15	**6**	15	*LOC126536*	0
chr22:38384374-38403731	8	4	**4**	13	*CACNA1I*	0
chr17:71112486-71120734	4	12	**3**	16	*KIAA1783*	0
chr12:55902280-55923860	3	9	**3**	19	*NDUFA4L2,NXPH4,SHMT2,STAC3*	0
chr16:87694595-87778383	16	32	**2**	21	*AX748415,CDH15,LOC197322*	0
chr18:65358832-65367619	18	33	**2**	21	*DOK6*	0
chr17:74544687-74596676	20	5	**1**	8	*C1QTNF1,CTRP1,DKFZp434P174,FLJ21865*	0
chr20:35485009-35485260	2	14	**1**	9	*SRC*	17774
chr17:76554452-76570569	5	7	**1**	9	*AK127919,KIAA1303*	0
chr19:40353627-40354649	6	20	**1**	9	*FXYD5*	1002
chr1:6071709-6289806	34	2	**1**	9	*ACOT7,CHD5,GPR153,HES3,ICMT,KCNAB2,RNF207,RPL22*	0
chr21:44535630-44541978	5	10	**1**	10	*AIRE*	0
chr9:137731051-137745745	6	10	**1**	10	*KCNT1,SOHLH1*	0
chr19:14102205-14159442	7	14	**1**	11	*ASF1B,BX537706,LPHN1*	0
chr14:104256711-104750100	30	8	**1**	11	*ADSSL1,AHNAK2,BRF1,CDCA4,GPR132,JAG2,KIAA0284,NUDT14,PLD4,SIVA1*	0
chr11:68514489-68538799	8	13	**1**	12	*BC039516,MRGPRF*	0

D) Illumina CHOP Duplications Enriched for *de novo*

CNVR	Count SNPs	Inh Dup	***de novo* Dup**	Par Dup Not Inh	Gene	Distance
chr19:59423491-59428132	12	74	**3**	38	LILRB3,LIR-3	0
chr5:180108611-180122934	13	17	**1**	11	OR2Y1	8947
chr9:138606913-138647195	17	10	**1**	10	AF161442	15688

chr16:87399730-87430019	22	7	**1**	7	APRT,CDT1,FLJ00319,GALNS	0
chr22:23994408-24235668	63	18	**1**	7	BC004918,BC037884,BC040576,BC047380,LOC91353,LRP5L	0
chr14:104225150-104339273	35	7	**1**	11	ADSS,ADSSL1,AKT1,AX721091,C14orf151,C14orf173,SIVA1	0
chr12:31248369-31298174	30	27	**1**	17	OVOS2	0
chr20:61642713-61668792	11	4	**1**	7	C20orf195,PRIC285,SRMS	0

E) Perlegen IMAGE Deletions Enriched for Inheritance

CNVR	Count SNPs	P TDT Del	**Inh Del**	*de novo* Del	Par Del Not Inh	Gene	Distance
chr7:19828746-19840916	7	0.041656	**4**	0	11	*MGC42090*	49005
chr2:180271795-180274556	5	0.003204	**2**	1	13	*ZNF533*	0
chr14:79919894-79924934	5	0.03125	**1**	0	7	*BC039670*	0

F) Perlegen IMAGE Duplications Enriched for Inheritance

CNVR	Count SNPs	P TDT Dup	**Inh Dup**	*de novo* Dup	Par Dup Not Inh	Gene	Distance
chr22:17361563-17369020	3	0.015625	**6**	0	0	*CR623368, KIAA1647*	0
chr15:30088094-30090949	3	0.03125	**5**	1	0	*CHRNA7*	19069
chr7:71664963-71712086	5	0.03125	**5**	0	0	*MGC87315*	0

G) Perlegen IMAGE Deletions Enriched for *de novo*

CNVR	Count SNPs	Inh Del	***de novo* Del**	Par Del Not Inh	Gene	Distance
chr2:180271795-180274923	6	2	**1**	13	*ZNF533*	0
chr10:85445139-85446804	7	5	**1**	7	*GHITM*	442361

H) Perlegen IMAGE Duplications Enriched for *de novo*

CNVR	Count SNPs	Inh Dup	***de novo* Dup**	Par Dup Not Inh	Gene	Distance
chr6:168234697-168295618	13	5	**2**	8	*FLJ00181*	9639

chr12:31276361-31285014	9	15	**1**	17	*OVOS2*	26006
chr10:47089854-47154881	31	11	**1**	17	*AK057316*	0
chr7:140018-162903	13	10	**1**	10	*AL137655*	23529
chr8:2437197-2492653	23	4	**1**	7	*BC045738*	0

Supplementary Table 10. ADHD CNV Family Based Transmission Disequilibrium and *de novo* Statistical Tests.

CNVR (hg18/B36/ Mar2006)	Type	P TDT Del	P TDT Dup	Inh Del	*de novo* Del	Par Del Not Inh	Inh Dup	*de novo* Dup	Par Dup Not Inh
chr7:126441593-126621501	Del	1	1	0	0	0	0	0	0
chr11:88269449-88351661	Del	0.125	1	3	0	0	0	0	0
chr3:7183953-7197236	Del	0.25	1	2	0	0	0	0	0
chr6:146657076-146694047	Dup	1	1	0	0	0	0	0	0
chr7:153495598-153564827	Dup	0.205	1	4	0	6	0	0	0
chr5:65027976-65046520	Del	1	0.5	0	0	0	1	0	0
chr1:56053497-56064495	Del	1	1	0	0	0	0	0	0
chr1:72317292-72328395	Dup	1	1	0	0	0	0	0	0
chr19:38427720-38444834	Del	0.183	1	6	0	8	0	0	0
chr3:1844168-1859889	Del	0.063	1	4	0	0	0	0	0
chr2:81419297-81446082	Dup	1	0.5	0	0	0	1	0	0
chr4:113772340-113788584	Dup	0.375	1	2	0	1	0	0	0

Supplementary Table 11. Homozygous Deletion Analysis for CNVRs.

CNVR	P-value	OR	Cases Deletion	Control Deletion	Gene	Exon Distance
chr4:64398956-64398956	0.010184	8.066572	5	2	*SRD5A2L2*	427060
chr4:87198171-87198951	0.012506	3.695184	8	7	*MAPK10*	5492
chr1:109169467-109173397	0.013403	0	3	0	*AKNAD1*	0
chr22:21254995-21262241	0.013403	0	3	0	*DKFZp667J0810,abParts*	905
chr4:10007525-10009254	0.036934	1.320968	91	228	*KIAA1729*	41348
chr12:69160993-69162296*	0.041022	0.638139	26	128	*BC031864*	12619

* significant control enrichment. Based on discovery cohort, subjects with a homozygous deletion detected included Cases: 711 and Controls: 2280.

Supplementary Table 12. Perlegen Data Reformatted File Samples to match Affymetrix Power Tools output format.

A) Genotype Calls File (0=AA,1=AB,2=BB,-1=NoCall).

probeset_id	10009	10010	10021	10022
SNP_rs10000023	1	1	2	1
SNP_rs10000030	1	0	0	1
SNP_rs10000037	0	0	1	1
SNP_rs10000068	2	2	2	2

B) Genotype Calls Confidence Scores (All set to 1).

probeset_id	10009	10010	10021	10022
SNP_rs10000023	1	1	1	1
SNP_rs10000030	1	1	1	1
SNP_rs10000037	1	1	1	1
SNP_rs10000068	1	1	1	1

C) Intensity Summary (-A=log10(X), -B=log10(Y) (X and Y value from dbGaP Single Sample Final Report files).

probeset_id	10009	10010	10021	10022
SNP_rs10000023-A	2.85	2.78	2.07	2.89
SNP_rs10000023-B	2.86	2.84	2.98	2.96
SNP_rs10000030-A	2.9	2.99	2.95	3.02
SNP_rs10000030-B	2.91	2.4	2.38	3.05

Supplementary Table 13.
Sample exclusion based on quality control measures.

Exclusion Criteria	CHOP	Control
Call Rate < 98%	170	271
SD LRR > 0.35	73	124
Ethnicity non-Caucasian	71	48
|GCWF|>0.05	251	1040
Count CNVs > 70	197	237
Monozygotic Twin	31	38

Samples excluded based on Quality Control (QC) measures on our HumanHap550 GWAS data based on statistical distributions to exclude poor quality DNA samples and false positive CNVs.

Supplementary Table 14. Sample Source Contributions to Impacting CNV Loci.

CNVR	CHOP Cases	CHOP Controls	NIMH cases	Utah cases	IMAGE cases	Per Psoriasis Control	Per Depression Control	PUW Ma Cases	PUW Ma Parents	IMAGE II Cases	IMAGE II Controls	SAGE Illumina 1M Controls	AGRE Affy 5.0 Parents Controls	Type	Gene
chr11:88269449-88351661	4	0	0	0	5	0	0	1	1	0	0	0	0	Del	*GRM5*
chr7:126441593-126621501	3	0	0	0	3	0	0	2	0	0	0	0	0	Del	*GRM8*
chr3:7183953-7197236	4	0	0	0	2	0	0	0	0	0	0	0	0	Del	*GRM7*
chr6:146657076-146694047	5	2	1	1	0	0	0	0	0	1	0	0	0	Dup	*GRM1*
chr1:72317292-72328395	4	0	0	0	0	0	0	1	0	0	0	0	0	Dup	*NEGR1*
chr7:153495598-153564827	5	0	1	0	0	0	0	2	0	0	1	0	1	Dup	*DPP6*
chr5:65027976-65046520	4	0	0	0	1	0	0	0	0	1	1	0	0	Del	*SGTB/NLN*
chr1:56053497-56064495	2	0	0	0	3	0	0	0	0	1	0	0	2	Del	*USP24*
chr19:38427720-38444834	5	2	0	0	1	0	0	1	3	0	0	0	0	Del	*SLC7A10*
chr3:1844168-1859889	4	0	0	0	0	0	0	2	2 (inh)	1	1	4	1	Del	*CNTN4*
chr2:81419297-81446082	2	0	0	0	1	0	3	0	0	1	0	0	0	Dup	*CTNNA2*
chr4:113772340-113788584	2	0	0	0	1	0	0	1	1	0	0	1	1	Dup	*LARP7*

Supplementary Table 15. Boundaries of Individual CNVs in Table 1A and 1B.

CNVR	Gene	Type	Sample ID	Region Called in Sample	Exon Distance*	Sample Validation Run
chr11:88269449-88351661	*GRM5*	Del	230-3	chr11:88269449-88351661	5,858	Y
chr11:88269449-88351661	*GRM5*	Del	230-4	chr11:88269449-88351661	5,858	Y
chr11:88269449-88351661	*GRM5*	Del	230-5	chr11:88269449-88351661	5,858	Y
chr11:88269449-88351661	*GRM5*	Del	497	chr11:83876556-91038751	0	Y
chr11:88269449-88351661	*GRM5*	Del	16794	chr11:87996654-88837360	0	Y
chr11:88269449-88351661	*GRM5*	Del	13304	chr11:88109331-88827923	0	Y
chr11:88269449-88351661	*GRM5*	Del	13270	chr11:88115425-88481107	0	Y
chr11:88269449-88351661	*GRM5*	Del	13761	chr11:88305340-88385387	0	Y
chr11:88269449-88351661	*GRM5*	Del	17580	chr11:88305340-88385387	0	N^
chr11:88269449-88351661	*GRM5*	Del	M.Of.M.Cs.604401	chr11:88324615-88342595	14,924	Y
chr7:126441593-126621501	*GRM8*	Del	1953313026_A	chr7:126532786-126536202	0	Y
chr7:126441593-126621501	*GRM8*	Del	1965040688_A	chr7:126463602-126478050	54,536	Y
chr7:126441593-126621501	*GRM8*	Del	4011452014_A	chr7:126532786-126536202	0	Y
chr7:126441593-126621501	*GRM8*	Del	14125	chr7:125660695-126036276	0	N^
chr7:126441593-126621501	*GRM8*	Del	16794	chr7:125660695-126036276	0	N^
chr7:126441593-126621501	*GRM8*	Del	11804	chr7:125679479-125937528	0	N^
chr7:126441593-126621501	*GRM8*	Del	987314	chr7:126503602-126563602	0	Y
chr7:126441593-126621501	*GRM8*	Del	987124	chr7:126463602-126603602	0	Y
chr3:7183953-7197236	*GRM7*	Del	2023340146	chr3:7053179-7144453	18,686	Y
chr3:7183953-7197236	*GRM7*	Del	068-3	chr3:7183954-7197236	20,599	Y
chr3:7183953-7197236	*GRM7*	Del	068-4	chr3:7183954-7197236	20,599	Y
chr3:7183953-7197236	*GRM7*	Del	4079019863_A	chr3:7183954-7197236	20,599	Y
chr3:7183953-7197236	*GRM7*	Del	11891	chr3:6979874-7003319	101,280	Y
chr3:7183953-7197236	*GRM7*	Del	11923	chr3:6980446-7001696	101,852	Y
chr6:146657076-146694047	*GRM1*	Dup	388-3	chr6:146657077-146675511	0	Y
chr6:146657076-146694047	*GRM1*	Dup	387-3	chr6:146657077-146675511	0	Y
chr6:146657076-146694047	*GRM1*	Dup	386-3	chr6:146657077-146675511	0	Y
chr6:146657076-146694047	*GRM1*	Dup	4301337678_R02C01	chr6:146657077-146675511	0	Y
chr6:146657076-146694047	*GRM1*	Dup	4305910011_R01C02	chr6:146657077-146675511	0	Y
chr6:146657076-146694047	*GRM1*	Dup	1181	chr6:146657077-146694047	0	Y
chr6:146657076-146694047	*GRM1*	Dup	83158	chr6:146657077-146694047	0	Y
chr6:146657076-146694047	*GRM1*	Dup	b3_SF_0181	chr6:146685878-146701196	13,883	Y
chr1:72317292-72328395	*NEGR1*	Dup	230-3	chr1:72317292-72328395	10,621	Y
chr1:72317292-72328395	*NEGR1*	Dup	230-4	chr1:72317292-72328395	10,621	Y
chr1:72317292-72328395	*NEGR1*	Dup	230-5	chr1:72317292-72328395	10,621	Y
chr1:72317292-72328395	*NEGR1*	Dup	TD207.1	chr1:71648994-73025013	0	Y
chr1:72317292-72328395	*NEGR1*	Dup	M.Of.M.Cs.6308601	chr1:72322424-72328395	10,621	Y
chr7:153495598-153564827	*DPP6*	Dup	332-3	chr7:153495598-153578582	54,698	Y
chr7:153495598-153564827	*DPP6*	Dup	4079019863_A	chr7:153495598-153564827	68,453	Y

chr7:153495598-153564827	*DPP6*	Dup	4193372403_B	chr7:153495598-153554210	79,070	Y
chr7:153495598-153564827	*DPP6*	Dup	4243114113_R01C02	chr7:153495598-153577484	55,796	Y
chr7:153495598-153564827	*DPP6*	Dup	1135	chr7:153495598-153576455	56,825	N^
chr7:153495598-153564827	*DPP6*	Dup	8201671744	chr7:153118878-153338318	0	Y
chr7:153495598-153564827	*DPP6*	Dup	W.Of.F.Cs.140002	chr7:153502896-153517548	115,317	Y
chr7:153495598-153564827	*DPP6*	Dup	W.Of.M.Cs.234002	chr7:153545279-153559377	73,903	Y
chr5:65027976-65046520	*SGTB/NLN*	Del	067-3	chr5:65027976-65046520	0	Y
chr5:65027976-65046520	*SGTB/NLN*	Del	117-3	chr5:65027976-65046520	0	Y
chr5:65027976-65046520	*SGTB/NLN*	Del	152-3	chr5:65027976-65046520	0	Y
chr5:65027976-65046520	*SGTB/NLN*	Del	1670639198_A	chr5:65027976-65046520	0	Y
chr5:65027976-65046520	*SGTB/NLN*	Del	15962	chr5:64483534-65101307	0	Y
chr5:65027976-65046520	*SGTB/NLN*	Del	b11_SF_1055	chr5:65020291-65030503	3,236	Y
chr1:56053497-56064495	*USP24*	Del	4147907208_B	chr1:56053497-56064495	80,234	Y
chr1:56053497-56064495	*USP24*	Del	393-3	chr1:56053497-56064495	80,234	Y
chr1:56053497-56064495	*USP24*	Del	11411	chr1:56040939-56132401	67,676	Y
chr1:56053497-56064495	*USP24*	Del	11804	chr1:56040939-56263366	67,676	Y
chr1:56053497-56064495	*USP24*	Del	11727	chr1:56053497-56064840	80,234	Y
chr1:56053497-56064495	*USP24*	Del	b2_SF_0094	chr1:56051215-56057576	77,952	Y
chr19:38427720-38444834	*SLC7A10*	Del	120-3	chr19:38415546-38444834	6,998	Y
chr19:38427720-38444834	*SLC7A10*	Del	224-3	chr19:38415546-38444834	6,998	Y
chr19:38427720-38444834	*SLC7A10*	Del	305-3	chr19:38415545-38434210	6,997	Y
chr19:38427720-38444834	*SLC7A10*	Del	134-4	chr19:38418216-38444834	9,668	Y
chr19:38427720-38444834	*SLC7A10*	Del	168-3	chr19:38423641-38444834	15,093	Y
chr19:38427720-38444834	*SLC7A10*	Del	11931	chr19:38427721-38455315	19,173	Y
chr19:38427720-38444834	*SLC7A10*	Del	W.Of.F.Cs.121001	chr19:38423391-38442154	14,843	Y
chr3:1844168-1859889	*CNTN4*	Del	078-3	chr3:1273990-1859889	0	Y
chr3:1844168-1859889	*CNTN4*	Del	078-4	chr3:1273990-1859889	0	Y
chr3:1844168-1859889	*CNTN4*	Del	141-3	chr3:1756625-1928413	187,137	Y
chr3:1844168-1859889	*CNTN4*	Del	177-3	chr3:1844168-1936623	178,927	Y
chr3:1844168-1859889	*CNTN4*	Del	M.Of.F.Cs.53701	chr3:1793056-1956567	158,983	Y
chr3:1844168-1859889	*CNTN4*	Del	U.Of.F.Cs.852301	chr3:1835561-1852134	263,416	Y
chr3:1844168-1859889	*CNTN4*	Del	b3_SF_0253	chr3:1797102-1930071	185,479	Y
chr2:81419297-81446082	*CTNNA2*	Dup	134-4	chr2:81035643-81654296	0	Y
chr2:81419297-81446082	*CTNNA2*	Dup	144-3	chr2:81035643-81654296	0	Y
chr2:81419297-81446082	*CTNNA2*	Dup	11484	chr2:81419297-81446082	152,417	Y
chr2:81419297-81446082	*CTNNA2*	Dup	b10_SF_0900	chr2:81352586-81386102	85,706	Y
chr4:113772340-113788584	*LARP7*	Dup	303-3	chr4:113744172-113798058	0	Y
chr4:113772340-113788584	*LARP7*	Dup	314-3	chr4:113744172-113798058	0	Y
chr4:113772340-113788584	*LARP7*	Dup	17190	chr4:113772340-113788584	0	Y
chr4:113772340-113788584	*LARP7*	Dup	M.Fa.M.Cs.6300503	chr4:113769438-113801755	0	Y

*exon distance of '0' indicates that exon is impacted by the CNV
^ sample not available for qPCR validation (sample visually validated in Bead Studio).

Supplementary Table 16. Frequency of CNVs in GRM Receptor Interacting Genes in ADHD Cases and Controls.

Gene	Del Counts (cases:controls)	Dup Counts (cases:controls)	ADHD Enrichment
ACAT1	0:0	1:0	Yes
ACCN1	0:0	3:1	Yes
ACTR2	1:0	0:1	Yes
ADCY1	0:0	1:1	Yes
ADRBK1	1:0	0:0	Yes
ALDOA	3:8	2:6	Yes
APP	0:0	8:2	Yes
ARL15	1:1	2:0	Yes
ATXN7L3	1:1	0:0	Yes
BDKRB2	1:1	0:0	Yes
CA8	0:0	1:0	Yes
CACNA1B	0:0	2:2	Yes
CACYBP	1:0	0:0	Yes
CALM1	1:2	0:0	Yes
CHRM3	0:0	2:1	Yes
CIC	1:1	0:0	Yes
CNP	1:2	0:0	Yes
CRHR1	1:0	0:0	Yes
DISC1	0:0	4:7	Yes
DYNLL1	0:0	1:0	Yes
FPR1	0:0	1:1	Yes
GAPDH	0:2	1:1	Yes
GNA15	1:1	1:0	Yes
GNAI2	2:4	0:0	Yes
GNAO1	0:0	1:1	Yes
GNAQ	1:0	0:0	Yes
GRIK1	0:0	8:2	Yes
GRIK3	1:0	0:0	Yes
GRM1	0:0	7:2	Yes
GRM2	1:0	1:0	Yes
GRM3	0:0	1:0	Yes
GRM5	4:0	3:2	Yes
GRM6	1:0	0:4	Yes
GRM7	4:0	0:0	Yes
GRM8	3:0	1:1	Yes
GSN	1:0	1:0	Yes
HOMER1	0:0	1:0	Yes
HTR2A	0:0	1:0	Yes
MAPK1	1:0	0:0	Yes
MTHFD1	1:1	0:0	Yes
MX1	0:0	7:2	Yes
NARG1	1:0	0:0	Yes
NMI	0:0	1:0	Yes
PCBP3	3:2	6:3	Yes
PDE1C	1:0	1:1	Yes

PPP2R1A	0:0	1:0	Yes
PRPSAP1	1:0	1:1	Yes
PSMD11	2:24	1:0	Yes
PSMD13	0:4	1:2	Yes
PXN	0:0	1:0	Yes
QRICH2	1:1	0:1	Yes
RANBP1	2:3	0:9	Yes
RAP2A	0:0	1:1	Yes
RCC1	0:0	1:0	Yes
RGS12	2:0	0:0	Yes
RIF1	0:0	1:0	Yes
RUVBL2	1:0	0:3	Yes
RYR1	1:2	1:1	Yes
RYR2	1:0	1:0	Yes
SDC3	1:0	0:1	Yes
SELE	1:0	0:0	Yes
SERPINB9	0:0	1:0	Yes
SETD4	2:0	8:3	Yes
SHANK1	0:0	1:0	Yes
SORD	0:0	1:0	Yes
STRAP	0:0	1:1	Yes
TK1	2:0	0:2	Yes
TNIK	1:0	0:0	Yes
VHL	0:0	1:0	Yes
BTBD2	0:7	1:15	No
ECHS1	0:1	1:22	No
F2RL3	1:16	0:0	No
GNB2L1	0:0	0:4	No
HOMER3	1:12	1:9	No
ITGB7	0:5	1:12	No
KIAA1683	3:18	0:3	No
PDE6G	3:26	1:12	No
PLCB3	0:5	0:2	No
PYGM	3:29	0:4	No
RPLP2	8:92	1:6	No
SLC6A3	0:0	0:11	No
SRC	1:19	0:2	No
TBCA	1:10	0:0	No
TRAF2	5:24	1:11	No
40425	0:0	0:0	NoSNPsOnGene
ADRA2A	0:0	0:0	NoSNPsOnGene
ADRA2C	1:1	0:1	NoSNPsOnGene
C17orf44	0:0	0:0	NoSNPsOnGene
C7orf25	0:0	0:0	NoSNPsOnGene
F2RL2	0:3	0:0	NoSNPsOnGene
FKBP3	0:0	0:0	NoSNPsOnGene
FSCN1	0:0	0:1	NoSNPsOnGene
GRB7	0:0	0:0	NoSNPsOnGene
HSP90AB1	1:0	0:0	NoSNPsOnGene
IMPDH2	0:0	0:0	NoSNPsOnGene
LOC642393	0:0	1:4	NoSNPsOnGene
LOC653098	0:0	0:0	NoSNPsOnGene
MC4R	0:0	0:0	NoSNPsOnGene
MGC11082	0:0	0:0	NoSNPsOnGene
MRPS16	0:0	0:0	NoSNPsOnGene
NPY2R	1:0	0:0	NoSNPsOnGene
PAFAH1B3	1:1	0:0	NoSNPsOnGene

PCBP1	0:0	0:0	NoSNPsOnGene
PCMT1	0:0	0:0	NoSNPsOnGene
PHKG2	0:0	0:0	NoSNPsOnGene
PRLHR	0:0	0:0	NoSNPsOnGene
PSME1	0:0	0:0	NoSNPsOnGene
RAB2	2:2	0:1	NoSNPsOnGene
RGS2	0:0	0:0	NoSNPsOnGene
S100A6	0:0	0:0	NoSNPsOnGene
SET	0:0	0:0	NoSNPsOnGene
SF3B14	0:0	0:0	NoSNPsOnGene
TBXA2R	10:44	0:10	NoSNPsOnGene
TMEM4	0:0	0:0	NoSNPsOnGene
TPI1	0:0	1:1	NoSNPsOnGene
TRMT112	0:1	0:2	NoSNPsOnGene
TUBA1	0:0	0:0	NoSNPsOnGene
TUBA1A	0:0	0:0	NoSNPsOnGene
TUBA2	0:1	0:0	NoSNPsOnGene
TUBB	0:0	0:0	NoSNPsOnGene
TUBG1	0:1	0:0	NoSNPsOnGene
ACAT2	0:0	0:0	
ACCN2	0:2	0:0	
ACP1	0:0	0:3	
ACTB	0:0	0:0	
ADA	0:0	0:0	
ADD1	0:0	0:0	
ADD2	0:0	0:0	
ADORA1	0:0	0:1	
ADRA1B	0:0	0:0	
ADRB2	0:0	0:0	
ANXA2	0:0	0:0	
APTX	0:0	0:0	
AQP1	0:0	0:1	
ARHGAP24	0:0	0:0	
ARRB1	0:0	0:0	
ARRB2	0:0	0:1	
BDKRB1	0:0	0:0	
BTG2	0:0	0:1	
C1orf116	0:0	0:1	
CALB2	0:0	0:0	
CALM2	0:0	0:0	
CALM3	0:0	0:0	
CAMK1	0:0	0:0	
CAMK2B	0:0	0:0	
CAMK4	0:0	0:0	
CCNB1	0:0	0:0	
CDC42	0:0	0:0	
CENTG1	0:1	0:0	
CHGB	0:0	0:0	
CHP	0:0	0:0	
CHRM2	0:0	0:0	
CMPK	0:0	0:0	
CNR1	0:0	3:8	
COPB2	0:0	0:0	
CYCS	0:0	0:0	
DCN	0:0	0:0	
DHCR7	0:0	0:1	
DLST	0:0	0:0	
DRD2	0:0	0:0	
DRD3	0:0	0:0	
DSTN	0:0	0:0	
EGFR	0:0	0:0	
EIF3S3	0:0	0:1	
ERBB2	0:0	0:0	

F2R	0:0	0:0	
F3	0:0	0:0	
FURIN	0:0	0:0	
FYN	0:0	0:0	
GLP1R	0:0	0:0	
GLP2R	0:0	0:0	
GNAI1	0:0	0:0	
GNAI3	0:0	0:0	
GOT1	0:0	0:0	
GP1BA	0:0	0:0	
GPR26	0:0	0:0	
GRB2	0:0	0:0	
GRIA1	0:0	0:0	
GRM4	0:0	0:0	
HBXIP	0:0	0:0	
HD	0:0	0:0	
HNRPA3	0:0	0:0	
IL8RB	0:0	0:0	
IQGAP2	0:0	0:0	
ITGB1	0:0	0:0	
ITPR1	0:0	0:0	
KIAA0090	0:1	0:0	
LAMA4	0:0	0:0	
LRP2BP	0:3	0:0	
LRRC59	0:0	0:0	
LTA	0:0	0:0	
LYAR	0:0	0:0	
LYN	1:3	0:0	
MAP4	0:0	0:0	
MAPT	0:0	0:0	
MARK4	0:0	0:0	
MRPL14	0:0	0:0	
MTNR1A	0:3	0:0	
MTNR1B	0:0	0:0	
MYC	0:1	0:0	
MYO6	0:0	0:0	
NANS	0:0	0:0	
NCK1	0:0	0:0	
NFKBIA	0:0	0:0	
NUDC	0:0	0:1	
OPRD1	3:13	0:0	
PCDHA4	0:0	0:0	
PCID1	0:0	0:0	
PDCD5	0:0	0:0	
PDE1B	0:0	0:0	
PGM1	0:0	0:0	
PHKB	0:0	0:0	
PICK1	0:3	0:1	
PIK3CA	0:0	0:0	
PIK3R1	0:0	0:0	
PLA2G7	0:0	0:0	
PLCB1	0:0	0:0	
PLCG2	0:0	0:0	
PPIH	0:0	0:0	
PRDX1	0:0	0:0	
PRKCA	0:0	0:0	
PRMT1	0:0	0:1	
PSAT1	0:0	0:0	
PSEN1	0:0	0:0	
PSMA1	0:0	0:1	
PSMC1	0:0	0:0	
PSMD1	0:0	0:0	
PSMD6	0:0	0:0	

PTHR2	0:0	0:0	
PYGL	0:0	0:0	
RALA	0:0	0:0	
RCC2	0:0	0:0	
RHOA	0:0	0:0	
RPA2	0:0	0:0	
RPN2	0:0	0:0	
RPS14	0:0	0:0	
RRM1	0:0	0:0	
SACS	0:0	0:1	
SARS	0:0	0:0	
SCTR	0:0	0:0	
SHBG	0:0	0:0	
SIAH1	0:0	0:0	
SLC2A1	0:0	0:0	
SNCA	0:0	0:0	
SNRPB2	0:0	0:0	
SOCS6	0:0	0:0	
SOCS7	0:0	0:0	
STAU1	0:0	0:0	
STX12	0:0	0:0	
SYK	0:0	0:0	
TCP1	0:0	0:0	
TEAD3	0:0	0:0	
TFAM	0:0	0:0	
TGM2	0:0	0:3	
TJP1	0:0	0:2	
TLR10	0:0	0:0	
TUBA1B	0:0	0:0	
TXN	0:0	0:0	
TXNDC4	0:2	0:1	
TXNL2	0:0	0:1	
TYMS	0:0	0:2	
UBQLN4	0:0	0:0	
UCHL1	0:0	0:0	
VIPR1	0:0	0:0	
YWHAQ	0:0	0:0	
ZAP70	0:0	0:0	

Supplementary Table 17. Gene clusters based on the network of interacting genes.

Cluster #	Genes
1	*SET, HNRPA3, RRM1, SORD, PSMC1, MTHFD1, CACYBP, PCBP1, TXNL2, 40425, SARS, PCID1, GSN, PSMD6, TBCA, MRPS16, RCC2, COPB2, RANBP1, PRMT1, ANXA2, FSCN1, RCC1, ACAT1, NUDC, EIF3S3, UCHL1, FKBP3, PDCD5, ACTR2, PSAT1, LYAR, PCBP3, SF3B14, LRRC59, ACP1, ACAT2, RUVBL2, GPR26, MAPK1, CYCS, MGC11082, STRAP, RAP2A, IMPDH2, ACTR2, PSMD1, SETD4, TRMT112, CMPK, MRPL14, SNRPB2, TEAD3, TMEM4, TFAM, DSTN, PRPSAP1, KIAA0090, PPIH, PSMA1, RPS14, DHCR7, PSMD13, TRAF2, TNIK, RPN2, TYMS, NCK1, NANS, NARG1, PPP2R1A, ECHS1, GOT1, PCMT1*
2	*GRB7, PYGL, CRHR1, PDE1C, CALM1, GLP1R, PYGM, PHKG2, PTHR2, PDE1B, GLP2R, ADD2, ADCY1, SCTR, PHKB, VIPR1, ADD1, PGM1, PGM1, IQGAP2*
3	*HBXIP, S100A6, TXN, SLC2A1, CAMK1, RAB2, PCDHA4, QRICH2, GAPDH, BTBD2, PAFAH1B3, SERPINB9, PSMD11, PRDX1, RPA2, CAMK2B, LAMA4, ARL15, TPI1, CAMK4, TK1, FYN, PGM1, ACTB, CHP*
4	*SLC6A3, UBQLN4, PRLHR, PICK1, CIC, APTX, ERBB2, ATXN7L3, ACCN2, AQP1, GRIA1, ACCN1, ECHS1, SACS, BTG2, LRP2BP, PRKCA*
5	*RALA, CDC42, DRD3, ITGB1, ITGB7, TLR10, HSP90AB1, TJP1, FURIN, VHL, MTNR1B, PSEN1, SHBG, DCN, F3, GRIK3, GP1BA, RHOA, SELE, DRD2, ARHGAP24, MTNR1A, FKBP3, ARRB2, GRM8*
6	*NPY2R, RGS12, GNAI3, ADRA2C, GNAI2, GNAO1, CACNA1B, GNAI1, GRM6, IL8RB, PLCB3*
7	*ADRA2A, PDE6G, SRC, MC4R, ARRB1, SNCA, RPLP2, FPR1, BDKRB2, ADRBK1, OPRD1*
8	*PLCB1, TXNDC4, ITPR1, CCNB1, LYN, CA8, PLCG2*
9	*F2RL3, HTR2A, ADRA1B, F2R, RGS2, HTR2A, GNAQ, F2RL2, CHRM3, PIK3CA, BDKRB2, TBXA2R, BDKRB1*
10	*GNB2L1, CNP, STAU1, CHGB, PSME1, SOCS7, DLST, ALDOA, SYK, SDC3, TUBB, TGM2, HD, MARK4, MAP4, MX1, TUBA1A, SOCS6, C7orf25, PLA2G7*
11	*HOMER1, STX12, CENTG1, RYR2, LOC653098, HOMER3, C1orf116, SHANK1, RYR1*
12	*CNR1, GNA15, CHRM2, ADRB2*
13	*DYNLL1, PIK3R1, NMI, TUBA2, PXN, TUBG1, NFKBIA, TUBA1B, YWHAQ*
14	*HRPT2, RIF1, GRM3*
15	*CALM3, GRM5, MYO6, KIAA1683, GRM7, LOC642393, C17orf44, CALM2*
16	*CALB2, TCP1, LTA, TUBA1, ZAP70*
17	*ADA, ADORA1*