Genome-Wide Association Study of Intelligence: Additive Effects of Novel Brain Expressed Genes

Sandra K. Loo, Ph.D., Corina Shtir, Ph.D., Alysa E. Doyle, Ph.D., Eric Mick, Sc.D., James J. McGough, M.D., James McCracken, M.D., Joseph Biederman, M.D., Susan L. Smalley, Ph.D., Rita M. Cantor, Ph.D., Stephen V. Faraone, Ph.D., Stanley F. Nelson, M.D.

Objective: The purpose of the present study was to identify common genetic variants that are associated with human intelligence or general cognitive ability. **Method:** We performed a genome-wide association analysis with a dense set of 1 million single-nucleotide polymorphisms (SNPs) and quantitative intelligence scores within an ancestrally homogeneous family sample of 656 individuals with at least one child affected by attention-deficit/hyperactivity disorder (ADHD). **Results:** Haplotype trend regression analysis with sliding four-SNP windows identified haplotypes of genome-wide significance in genes involved in synaptic signaling (*KIF16B*; p = 1.27E-08) and neurodevelopment (*PAX5*; p = 3.58E-08), and highlight findings from a recent genetic study of cognitive ability (*RXRA*; p = 7.7E-08; *GYPC*; p = 2.5E-07). Further interrogation of SNPs within top haplotypes reveals that the minor alleles are associated with higher intelligence, whereas others are associated with relatively lower (but still average range) intelligence. Effects of the eight genes are additive, as a greater number of the associated genotypes in a given individual predict higher intelligence (p = 5.36E-08) and account for 8% of variance in intelligence. **Conclusions:** Analyses that examine additive genetic effects may be useful in identifying regions where the additive effects of SNPs have a significant effect on phenotype. These results describe novel variants and additive effects of genes involved in brain development on variability in intelligence within an ADHD sample. The precise mechanisms of these loci in relation to determining individual differences in general cognitive ability require further investigation. J. Am. Acad. Child Adolesc. Psychiatry, 2012;51(4):432–440. **Key Words:** cognitive ability, genetics, SNPs, ADHD, haplotype

Individual differences in human intelligence (IQ) or general cognitive ability have been widely studied, as they are thought to represent an inborn potential that correlates with future academic success, occupational status, and health.[1] Intelligence is a quantitative trait with substantial heritability estimates that increase with age. Heritability increases from about 30% in very young children to approximately 80% in adulthood.[2,3] Despite evidence of substantial genetic effects on intelligence, previous genome-wide studies have been largely unsuccessful in identifying regions and single nucleotide polymorphisms (SNPs) that reach genome-wide significance. For example, in the first large-scale genome-wide association scan for general intelligence using 500K SNPs with 3,000 children, only six SNPs were significant and one (rs249613) survived after a using a false discovery rate of 0.05.[4] Genes identified thus far generally account for less than 1% of the phenotype variance,[4] and there is not a single gene or genetic association for intelligence that has received unequivocal support. This suggests that genetic influences on intelligence are likely to involve many genes of small effect[5] and that single SNPs may not carry enough information to describe the underlying genetic variation without very large sample sizes.

Recently, there has been increased interest and support for genes involved in brain develop-


Supplemental material cited in this article is available online.

ment, integrity, and efficiency as playing a role in intelligence[6] and psychiatric disorders that involve reduced intellectual functioning such as autism,[7] attention-deficit hyperactivity disorder (ADHD)[8] and schizophrenia.[9] For example, Ruano et al.[6] recently reported a functional gene group analysis of synaptic heterotrimeric guanine nucleotide binding proteins (G proteins) that revealed a significant association with cognitive ability (p = 1.9E-04). Although most of the 25 genes that make up this functional group were not individually associated with intelligence, the combination of small genetic effects was significant in a sample of children ascertained for ADHD and was replicated in a larger population sample. These data suggest that the ability to account for the additive effects of SNPs may lead to increased power to identify genetic variants influencing intelligence.

Cognitive phenotypes (or endophenotypes) have been proposed for ADHD genetic studies,[10] and because ADHD is associated with lower IQ, intelligence has often been studied in ADHD. Recent studies have found a modest and significant phenotypic correlation,[11-13] but low genetic correlation between IQ and ADHD symptoms.[11,13] With this in mind, we look for genes contributing to IQ, knowing that we may detect genes that are largely independent of ADHD, but that of these there may be a subset that contributes in small part to the genetic variability underlying ADHD. The Ruano et al. findings support the idea that the genetic association of G-proteins is not specific to cognitive ability within ADHD, but with cognitive ability in general. Similar results for general and patient populations have been found in linkage and candidate gene studies. For example, the 6p25-p22 region has been linked to intelligence in several family studies, one of which was collected on the basis of alcoholism,[14-16] and the cholinergic muscarinic 2 receptor gene (CHRM2) has been associated with intelligence in several population studies and one ADHD sample.[17-20] Similarly, brain-derived neurotrophic factor (BDNF) has been significantly associated to cognitive abilities in samples containing individuals who are typically developing and those with schizophrenia and ADHD.[20-22] Within several ADHD samples, Rizzi et al.[20] replicated genetic associations resulting from population studies; however, the two strongest candidate gene associations with intelligence, ATXN1 and TRIM31 were only in ADHD groups and not found in general population samples. This raises the question of whether different genes play a role in intelligence for the general population and those ascertained on the basis of a psychiatric disorder. This question is complicated by the lack of consistent results among general population studies; however, the data suggest that similar genes are associated with intelligence across non-patient and psychiatric disorder samples. Further research on genetic associations with intelligence across different samples can help to elucidate the specificity of such genes to samples with psychiatric disorder or not.

Because the ability to account for additive SNP effects may lead to increased power to identify genetic variants influencing intelligence, we sought to do that in the current study. It is premature, however, to focus on specific genes or gene networks, given the lack of consensus. Thus, examining additive genetic influences in a genome-wide fashion is a logical next step. With this in mind, we conducted a genome-wide association (GWA) study with a dense, 1-million (1M) SNP platform that accounted for additive genetic influences by analyzing haplotypes with sliding windows across the genome. In addition, we wanted to capitalize on parental IQ data that were collected on a significant subset of the sample (N = 526 adults). Omitting a large portion of data would result in reduced power to detect association. Therefore, we used haplotype trend regression (HTR) analysis, which analyzes parent and child phenotype (for the largest possible sample) in sliding haplotype windows across the genome (to account for additive genetic effects). This analysis was used with permutation testing to account for family substructure (i.e., related individuals are included in the same analysis). Finally, to avoid the strong confounding effects of race on IQ[23] and potential false associations based on population stratification, we performed the GWA analysis on an ancestrally homogeneous subgroup of the total sample.

METHOD

The original sample consists of 498 trios (N = 1494 individuals) with children diagnosed with attention-deficit/hyperactivity disorder (ADHD) who were ascertained from the University of California, Los Angeles (UCLA) and Massachusetts General Hospital (MGH). The trios consisted of two biological parents

and their offspring who were 6 to 17 years of age and met diagnostic criteria for ADHD[24] at initial assessment. IQ phenotype data were available on a subset of the sample (N = 440 children and N = 526 adults). All study procedures were reviewed and approved by the subcommittee for human subjects of each respective institution. All subjects' parents or guardians signed written informed consent forms, and children signed written assent forms.

Assessment Procedures

DSM-IV-TR criteria for ADHD require at least six of nine symptoms of inattention and/or hyperactivity impulsivity to be endorsed. Symptoms must be present by age 7 years and associated with clinically significant impairment in social, academic, or occupational functioning. Subjects meeting full diagnostic criteria for any *DSM-IV-TR* ADHD subtype were enrolled in the study. Subjects were evaluated using a semi-structured diagnostic interview, the Schedule for Affective Disorders and Schizophrenia for School-Age Children (K-SADS).[25] Subjects were excluded from participation if they were positive for any of the following: neurological disorder, genetic anomaly, or lifetime diagnoses of schizophrenia or autism. (A complete description of diagnostic methods is provided by Mick et al.[26]).

Intelligence (IQ) was estimated using the Vocabulary and Block Design subtests from the age appropriate form of the Wechsler Intelligence Scales: Wechsler Intelligence Scale for Children (WISC)[27] and Wechsler Adult Intelligence Scale (WAIS)[28] for parents. The two-subtest estimate of IQ is has a 0.9 correlation with the full-scale IQ generated from the whole battery of tests. The mean IQ score for the WISC/WAIS is 100 and standard deviation is 15. All participants with ADHD were assessed off of psycho-stimulant medications.

Genotyping Procedures

All samples were genotyped using the Illumina 1M (MGH) or 1M Duo Bead (UCLA) Array at Genizon BioSciences Inc. with funding from Pfizer Inc. Genotyping calls were generated after clustering all available data within platform at Genizon and then merged into a single file of 1,172,613 SNPs. To generate a data set of markers common to both sites, we removed SNPs that were either not included on both arrays (N = 128,718 SNPs) or failed preliminary quality-control (QC) procedures conducted at Genizon (99% call rate for all samples and for all SNPs, gender check, Mendelian errors) on both the 1M and 1M-Duo arrays (N = 9,500 SNPs), the 1M array only (N = 39,753 SNPs) or the 1M-Duo array only (N = 11,201 SNPs). Once the data from the two sites and different Illumina arrays were merged, there were 983,441 SNPs genotyped across the complete sample of 1,494 individuals.

Before HTR analysis, we performed additional QC procedures. SNPs were excluded under the following conditions: missing in more than 5% of markers or samples; minor allele frequency is less than 1%; out of Hardy–Weinberg equilibrium (parents only; $p < 1.0E-06$); gender discrepancies; Mendelian errors. This resulted in a final dataset of 795,637 SNPs for association analyses.

Definition of Homogeneous Ancestral Group

Population structure has a strong confounding effect on genetic association studies. Previous studies identified ancestry informative marker polymorphisms that exhibit large differences in allele frequencies across populations of European (EU), Asian, and African descent, and therefore confer increased power for detecting levels of population stratification. We studied the genomic ancestry characterizing the cohort by analyzing 3,000 ancestry informative markers identified by Tian et al.[29] as being informative for detecting allele divergence frequency among European, African, and Asian ancestries. We also incorporated European, African, and Asian samples from the Study of asthma genes and environment (SAGE) cohort, to increase the power in STRUCTURE 2.1 for divergence frequency within the cohort ancestries. Based on a three-cluster model (K = 3), the most homogeneous group of individuals (n = 656) within the cohort were selected, for, which an average of 98% EU ancestry, was identified. These individuals were further used for the subsequent IQ genome-wide analysis.

Statistical Analyses

Haplotype Analysis. Genome-wide association analysis of the IQ phenotype was conducted using haplotype trend regression (HTR) with a four-SNP sliding window in HelixTree version 7 (Golden Helix Inc., Bozeman, MT). This analysis was used to identify regions that harbor sets of SNPs whose additive contributions have a significant effect on phenotype, thus increasing power to detect signals where single marker effects may not carry enough information to describe the underlying genetic variation. The HTR analysis fits a unified model of additive effects of haplotypes and tests association of haplotype frequencies with either a discrete or continuous phenotype. Haplotype probabilities for each observation were computed using the expectation-maximization (EM) algorithm, formalized by Dempster et al.,[30] and a linear regression of phenotype was formed by using haplotype probabilities as the regression matrix. The EM algorithm is a commonly used iterative method[31] for obtaining maximum likelihood estimates of sample haplotype frequencies. The F statistic was used for testing haplotype association with a continuous phenotype, such as IQ.

Permutation Testing. Permutation tests were per-

formed within Golden Helix using the Helix module to determine the likelihood of the obtained p values while controlling for multiple comparisons and non-independent data (i.e., related individuals). After identifying the haplotype (e.g., H1) of interest, the permutation analysis was performed as follows. First, IQ is shuffled across the whole sample. This can be done because the IQ has the same standard score across adults and children (mean = 100, SD = 15). Then the HTR analysis is re-run across the whole genome. A new p value is derived for the H1 haplotype (with the shuffled IQ scores). From this we will get a distribution of where the p value falls each time the HTR analysis is run (which was 100,000,000 times). From this we can determine where the obtained p value of H1 is in the distribution of possible p values for H1s. The permuted p value is the fraction of permutations for which the most significant result over all haplotypes was as significant as or more significant than the obtained p value for H1. This global p value controls for non-independence of people and non-independence of the haplotypes. For all analyses, we adopted the conservative threshold value of $p < 5.0E\text{-}08$ to be considered genome-wide significant[32,33] and $p < .05$ for replication of previously identified significant loci.

Single SNP Analysis. We then examined single markers within the significant haplotype blocks to characterize the contribution of single SNPs to the overall haplotype association. The Golden Helix regression module for SNP association tests was used to determine the most informative markers; permutation testing was used to control for family structure. Analysis of additive SNP effects was run in SPSS version 19 (SPSS Inc., Chicago, IL) using a mixed-model analysis of variance for all subjects, including parents and children. We tested for association between the number of homozygous minor SNP genotypes and IQ using socioeconomic status (SES) as a covariate.

RESULTS

Phenotype

IQ scores were available for 966 individuals (n = 440 children, n = 526 adults), of which 656 individuals of European ancestry (n = 283 children, n = 373 adults) were identified. The mean IQs for the child (mean = 109, SD = 16, range 54-154) and adult (mean = 111, SD = 13, range 75-143) samples were similar across the UCLA and MGH sites ($p > .05$). IQ differed by age group, with children having a slightly lower IQ overall compared with adults ($F_{1,\ 654} = 3.9$, $p = .05$); however a difference of 2 IQ points is clinically insignificant. Skewness and kurtosis for child (skewness = 0.07, kurtosis = 0.36) and adult (skewness = −0.08, kurtosis = −0.23) samples indicate a normal distribution of scores. All children had a diagnosis of ADHD: 64% had combined type, 30% had predominantly inattentive type, and 6% had predominantly hyperactive-impulsive type. Of parents affected with ADHD, 32% had combined type, 55% had predominantly inattentive type, and 13% had predominantly hyperactive-impulsive type. The mean IQ for affected parents (n = 84, mean = 109, SD = 14) was slightly lower than for unaffected parents (n = 286, mean = 112, SD = 12) ($F_{1,368} = 4.27$, $p = .04$). There were no significant differences in IQ by ADHD subtype in either the child or parent groups ($F < 1$, both analyses). SES significantly predicted IQ ($F_{4,336} = 12.9$, $p < .01$) and accounted for 8% of phenotypic variance.

Haplotype Trend Regression Analyses

Haplotype trend regression (HTR) analyses revealed several loci that reached genome-wide significance as seen in Table 1. QQ and Manhattan plots are presented in Figures S1 and S2, available online. The majority of haplotypes identified by the HTR analyses are in genes expressed in the brain, many of which have been previously implicated in neurodevelopment and neural functioning, or previously associated with cognitive ability. For example, haplotypes within genes involved in synaptic signaling (*KIF16B)* and neurodevelopment (PAX5) reached genome-wide significance, and those previously associated with general cognitive ability (*RXRA)* and verbal memory *(LUZP2*) were of marginal genome-wide significance. Haplotypes within three genes (*KIF16B, PAX5, ELSBP1*) remained significant and all haplotypes remained strongly associated ($p \leq 5.00E\text{-}07$) after permutation testing. Detailed information on the specific haplotypes is presented in Table 2.

Within the haplotypes that were significant at 5E-07, regression analyses were run for each SNP with the intelligence phenotype and the results for SNPs that demonstrate a strong individual effect ($p \leq 10^{-5}$) on intelligence are summarized in Table 3. None of the single markers reached genome-wide significance. Although a minor allele frequency of less than 1% was an exclusion criterion, examination of the genotype frequencies (D = minor allele, d = major allele) revealed that the minor allele frequency of the alleles most strongly associated with high intelligence was 14% and ranged from 7% to 18%. The relatively lower frequency of the alleles is likely the reason

TABLE 1 Haplotype Trend Regression Analysis for Intelligence Phenotype

Haplotype	*p* Value	Perm *p* Value	CHR	Gene	Brain Exp	Assoc Phenotype/Brain Function
rs6043979-rs932541-rs6044001-rs6044003	**1.27E-08**	**2.00E-08**	**20p12.1**	**KIF16B**	**Yes**	**Synaptic signaling/transport**
rs3758171-rs1329573-rs3824344-rs7020413	**3.58E-08**	**3.00E-08**	**9p13**	**PAX5**	**Yes**	**Neurodevelopment**
rs3815908-rs2303690-rs3936340-rs2560966	**3.19E-08**	**4.00E-08**	**19q13**	**ELSPBP1**	**Yes**	
Rs2540051-rs7563911-rs7589014-rs2348114	5.94E-08	1.70E-07	2q33	AOX1	Yes	Amyotrophic Lateral Sclerosis
rs11703808-rs761746-rs12627933-rs9621305	6.78E-08	9.00E-08	22q12.3	PISD	Yes	
rs34312136-rs35079168-rs4501664-rs11102986	7.70E-08	1.00E-07	9q34.3	RXRA	Yes	Schizophrenia, Cognitive ability
rs16835742-rs7533254-rs528059-rs544991	7.50E-08	2.00E-07	1p34	CSMD2	Yes	Brain structure
rs10834449-rs2716458-rs12798374-rs1021261	7.86E-08	1.90E-07	11p14.3	LUZP2	Yes	Prader-Willi syndrome; verbal memory
rs10262915-rs6465411-rs13221576-rs4729127	8.50E-08	8.00E-08	7q21	COL1A2	Yes	
rs1987511-rs7475343-rs9423406-rs7896729	9.20E-08	1.40E-07	10p15	UCN3	Yes	Depression; med response/addiction
rs1908039-rs11099040-rs13113376-rs1908038	9.39E-08	1.10E-07	4q28	CYCSP14		
rs6781149-rs17584516-rs4629318-rs11713158	1.15E-07	2.00E-07	3p26	CNTN4	Yes	Cell adhesion; neurodevelopment

Note: Haplotypes in boldface type meet criteria (p < 5E-08) for genome-wide significance. Brain Exp = brain expressed; CHR = chromosome; Perm p Value = permuted p value.

that each sample individually was underpowered to observe association. Across these markers, subjects homozygous for the associated minor alleles (DD) had significantly higher IQ scores (average IQ = 120) when compared with those with Dd (average IQ = 114) and with the dd alleles (average IQ = 109).

The additive effect of the genes associated with intelligence was examined among subjects who were homozygous for the minor alleles (DD) presented in Table 3. To do this, the number of homozygous minor genotypes for each subject was counted. Results of a mixed-model analysis of covariance, using SES as a covariate, between the number of minor gentoypes and intelligence was significant ($F_{3,509.58} = 12.67$, $p = 5.36E\text{-}08$), indicating that having more of the minor (DD) genotypes was associated with higher IQ. For example, having one or more minor genotypes was associated with high average range intelligence, as defined by the Wechsler scales (FSIQ = 110-119) and two or more minor genotypes were associated with an IQ in the superior range (FSIQ = 120-129). This suggests an additive effect of less common variants on IQ (Figure 1). The distribution of minor genotypes did not differ significantly according to age group (i.e., parent or child; $\chi^2 = 1.8$, $p = .61$), sex ($\chi^2 = 4.7$, $p = .20$), or ADHD status ($\chi^2 = 1.3$, $p = .73$), suggesting that the effect of these genes is not specific to any of these subgroups.

DISCUSSION

The goal of the current study was to conduct a genome-wide association study of intelligence in families with an affected child with ADHD using a dense SNP set and an analysis that accounted for the additive genetic influences. The analyses yielded genome-wide significant signals for genetic variants involved in neurodevelopment and synaptic signaling as being associated with human intelligence, and suggest three conclusions. First, these findings indicate that intelligence is a complex trait that is polygenic in nature and associated with many genes of small to moderate effect. Second, our data indicate that there is an additive effect of relatively less common gene variants that are significantly correlated with higher IQ scores. Finally, these results suggest biologically plausible mechanisms for intelligence distribution, such as variations in brain development, integrity, and efficiency.

TABLE 2 Haplotypes Associated With Intelligence

Gene	Haplotype	Frequency	β	SE	t Value	p Value
KIF16B	B,A,B,B	0.03	−18.08	4.71	−3.84	9.79E-05
	A,A,B,B	0.05	15.09	4.02	3.76	0.0005
	B,A,A,B	0.12	8.31	2.47	3.37	0.0005
PAX5	B,A,A,A	0.10	16.64	2.85	5.85	6.70E-07
ELSPBP1	B,B,B,A	0.07	12.81	3.31	3.87	4.51E-06
	B,A,A,A	0.06	−11.40	3.63	−3.14	0.0028
	A,A,A,A	0.32	−4.66	1.80	−2.59	0.0016
AOX1	B,A,B,B	0.05	19.30	4.11	4.70	3.39E-05
	B,B,A,B	0.09	11.98	2.97	4.04	0.0007
PISD	A,B,A,B	0.06	−17.02	3.68	−4.63	8.58E-07
	A,A,B,A	0.18	6.25	2.10	2.98	0.0005
RXRA	{RareHaps}	0.003	−76.70	14.11	−5.44	7.70E-08
CSMD2	{RareHaps}	0.01	−36.36	8.45	−4.30	7.16E-06
	A,B,B,A	0.05	−15.39	4.30	−3.58	0.0001
LUZP2	{RareHaps}	0.004	−76.29	14.04	−5.43	7.86E-08
COL1A2	A,A,B,A	0.69	−11.70	2.07	−5.65	3.57E-07
UCN3	{RareHaps}	0.01	30.77	7.80	3.94	1.98E-05
	A,B,A,B	0.13	9.47	2.50	3.79	3.65E-05
CYCSP14	{RareHaps}	0.002	−95.63	19.69	−4.86	2.06E-06
	B,A,A,A	0.23	−5.86	1.86	−3.15	0.0024
CNTN4	A,A,B,B	0.48	−9.56	1.74	−5.50	3.29E-06

Note: β = β coefficient, RareHaps = rare haplotypes that occur at a frequency of <0.01 in the sample; SE =standard error.

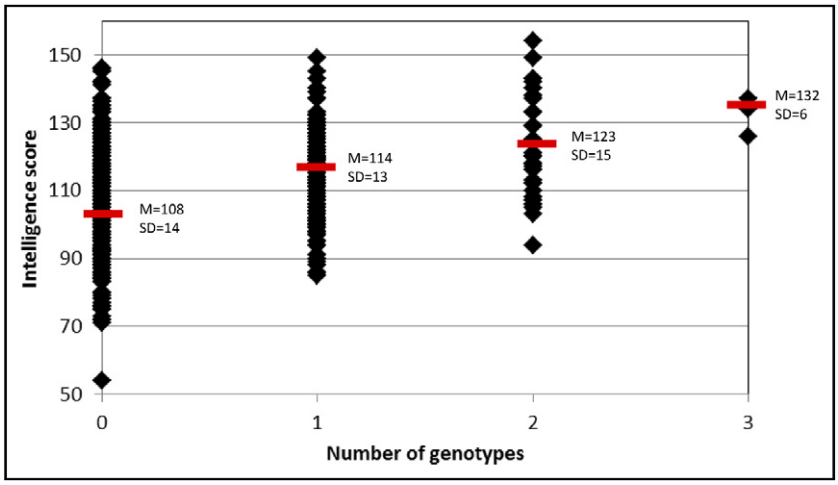
It is widely accepted that hundreds, and possibly thousands, of genes are associated with intelligence. In this context, it is not surprising that our most significant findings did not replicate many of the loci previously associated with intelligence, such as *CHRM2*,[18] *ALDH5A1*,[34] *ATXN1* or *TRIM31*,[20] or heterotrimeric G-proteins.[6] In addition, several large population GWA stud-

TABLE 3 Single Nucleotide Polymorphisms (SNPs) Strongly Associated With Intelligence

				Genotype Count		IQ Average for Genotype						
Marker	CHR	Gene	DD	Dd	dd	DD	Dd	dd	β	SE	p	Perm p
High intelligence												
rs10995170	10	ZNF365	61	260	335	118	112	108	4.42	0.84	2.50E-07	3.00E-07
rs7792596	7	COL1A2	73	264	319	116	112	108	4.13	0.82	6.45E-07	8.00E-07
rs11713158	3	CNTN4	23	190	443	115	115	109	5.04	1.01	8.72E-07	2.00E-06
rs1550404	2	GYPC	5	93	558	125	116	109	7.05	1.44	1.39E-06	2.00E-06
rs3936340	19	ELSPBP1	8	88	559	126	116	109	6.80	1.40	1.56E-06	2.00E-06
rs1865721	18	C18orf62	63	279	314	115	112	108	3.90	0.84	5.46E-06	3.00E-06
rs12125971	1	PRMT6	3	89	561	128	116	110	6.93	1.50	7.20E-06	4.00E-06
rs9423406	10	UCN3	10	145	501	117	115	109	5.17	1.18	1.47E-05	1.90E-05
Average intelligence												
rs5994434	22	DEPDC5	9	126	521	102	105	112	−6.29	1.23	4.78E-07	6.00E-07
rs240657	8	TUSC3	19	145	492	99	108	112	−5.22	1.08	1.84E-06	3.00E-06
rs3758171	9	PAX5	37	230	388	105	108	112	−3.89	0.92	2.78E-05	2.70E-05

Note: SNPs for high intelligence are included here if average IQ for the homozygous minor allele genotype is 1 or more standard deviations (SD = 15) above the mean IQ (100). SNPs for average intelligence are included here if average IQ for the minor allele is within 1 SD from the mean IQ. CHR = chromosome; D = minor allele; d = major allele; Perm p = permuted p value.

FIGURE 1 Additive effects of genes on intelligence. Note: The number of minor genotypes (i.e., homozygous for minor allele) that a person had and their intelligence were significantly associated ($F_{3,651} = 15.4$, $p = 1.24E\text{-}09$), indicating that having an increased number of minor genotypes is positively associated with higher IQ. This suggests an additive effect of less common variants on intelligence. M = mean; SD=standard deviation.



ies of cognitive abilities[4,35,36] were unable to identify SNP associations that reached genome-wide significance. It is therefore more notable that our findings highlight the same SNPs from a large, three-stage genome-wide association study of cognitive ability.[37] We obtained near–genome-wide significance for the top finding in the Davis et al. study,[37] the retinoid x receptor, alpha (*RXRA*); however, the SNP implicated in the Davis et al. study was intergenic between *RXRA* and *WDR5*, whereas the haplotype we identified is in an intronic region of *RXRA*. Nonetheless, *RXRA* has been associated with schizophrenia in numerous reports,[38-40] potentially through an interaction with *Nurr1*.[41] In addition, we also found a strong association of a SNP in glycoprotein, *GYPC*, which was also in the final SNP set implicated from the first two waves of GWAS in the Davis et al. study. In addition, SNPs in the *BDNF* (rs6265) and *COMT* (rs4680) genes were significantly associated with intelligence ($p = .0004$ and $p = .002$, data not shown) in the current sample. Although these results replicate previous findings, they do not meet the corrected threshold for genomewide significance. The current data suggest that replication (or non-replication) occurred regardless of whether the samples were ascertained on the basis of ADHD or other psychiatric disorder (as in Rizzi et al.,[20]) or were part of a larger general population sample, as in (Davis et al.[37]). As well-replicated genetic variants associated with intelligence (or ADHD, for that matter) emerge, further research on whether different genes are related to cognitive abilities within samples based on psychiatric disorder can move forward.

We identified novel variants that are nearly all brain expressed and the majority (75%) have been previously associated with neural functioning (*KIF16B*) and neurodevelopment (*PAX5*, *CNTN4*). *KIF16B* belongs to Kinesin superfamily proteins, which have been reported to be molecular motors that transport components of synaptic vesicles along the axon making these molecules critical for synaptic transmission.[42] *PAX5*, or paired homeobox genes are transcription factors involved in neurogenesis and cell differentiation. This gene is expressed in the hippocampus and is involved in the regulatory network implicated in the development of GABAergic cells as well as the ventral midbrain, where it is involved in development of adult dopaminergic neurons.[43] Cell-adhesion genes are thought to be involved in synapse formation and maintenance. *CNTN4* is a member of the neurexin family and may play a role in neurodevelopment of axons into distinct functional subdomains. This gene has been previously associated with mental retardation,[44] suggesting that it may play a role in both high and low IQ. Finally, *CSMD2* was recently reported to be the most strongly associated gene with brain structure in a voxel-wise GWAS.[45] It is highly expressed in the brain and has been previously associated with ADHD,[46]

suggesting potentially pleiotropic effects for ADHD and intelligence. Although greater characterization of the functional gene effects is needed, this gene appears to play a substantial role in cortical development and organization related to intelligence. Taken together, our data suggest the possibility that several key pathways exert important effects on neural development and functioning and are also associated with intelligence.

Given the low rate of gene discovery and replication for previous findings of genetic linkage and association with intelligence, the current findings should be considered preliminary until independent replication of results occurs. The sample that was used here is small relative to other GWAS, and that may have led to spurious results. Because the heritability estimates for human intelligence change over development, the possibility exists that the parents and children contribute differentially to the genetic results presented here. In a recent study by Haworth et al.,[47] heritability was estimated to be 55% by age 12 years and 66% in a young adult sample. This suggests relatively similar heritability estimates in the parent and child samples; nonetheless, we are more likely to identify genes that are associated with IQ across the lifetime. The mean IQ in this sample is significantly higher than what was expected for a general population sample (mean FSIQ = 100), and may reflect the relatively higher SES of the sample or the Flynn[48] effect, where scores rise steadily over time (~5 IQ points per decade). Although the mean is shifted slightly to the right, there is a normal distribution and the full range of IQ scores are included in the sample. This further highlights the need for replication in samples with IQ across the spectrum (e.g., low, normal, and high IQ). In addition, we used an unconventional analytic strategy to account for additive effects of SNPs, and increased power by using the largest sample possible to detect SNPs of smaller effect. Although it allowed us to double the sample size, the use of related individuals could have resulted in inflation of significant findings. However, to deal with the family structure present in the data, we used permutation testing to account for the effect of data non-independence. This HTR method is likely to highlight only haplotypes in which all SNPs are associated with IQ in the same direction (either high or low, but not both within the same haplotype). That significant haplotypes were identified despite non-significant single SNP results suggests that this could be a potential strategy for other GWAS that wish to account for additive SNP effects.

In conclusion, these GWAS results suggest that there is a strong and additive effect of genes that influence brain development, integrity, and efficiency on intelligence. This study provides partial support for previously identified genetic influences, and presents several potentially new biological mechanisms that are associated with intelligence and may be targets in future studies of both single genes and functional gene groups. &

Accepted January 18, 2012.

This article was reviewed under and accepted by ad hoc editor Robert R. Althoff, M.D.

Drs. Faraone and Nelson contributed equally to this work.

Drs. Loo, McGough, McCracken, Smalley, Cantor, and Nelson are with the University of California, Los Angeles. Dr. Shtir is with the Cambridge Institute for Medical Research, University of Cambridge. Drs. Doyle and Biederman are with the Center for Human Genetic Research, Massachusetts General Hospital. Dr. Mick is with the University of Massachusetts Medical School. Dr. Faraone is with SUNY Upstate Medical University.

This work was supported by National Institute of Health grants MH059126, MH085518, MH01966, MH071852, MH058277, and NS54124. We acknowledge the support and advice regarding study analyses provided by the UCLA Informatics Center for Neurogenetics and Neurogenomics funded by grant NS062691.

The authors thank the families that participated in this study, and the medical team and staff members from the Massachusetts General Hospital and University of California, Los Angeles (UCLA) centers for their hard work in the recruitment, assessment, and diagnosis process.

Disclosure: Dr. Faraone has served as a consultant to, served on the advisory boards for, or participated in continuing medical education (CME) programs sponsored by Shire, McNeil, Janssen, Novartis, Pfizer, and Eli Lilly and Co. He has received research support from Shire and the National Institutes of Health (NIH). He receives royalties from Guilford Press. Dr. McGough has served as a consultant for Eli Lilly and Co., NextWave, Noven, and Shire. Dr. McCracken has received research support from Seaside Therapeutics and Bristol-Myers Squibb. He has served as a consultant for Shionogi, BioMarin, Novartis, PharmaNet, and Noven. Dr. Biederman has received research support from, served as a consultant to, or served on the speakers' bureau for Abbott, Alza, AstraZeneca, Bristol-Myers Squibb, Celltech, Cephalon, Eli Lilly and Co., Elminda, Esai, Forest, GlaxoSmithKline, Gliatech, Janssen, McNeil, Merck, the National Alliance for Research on Schizophrenia and Depression, the National Institute on Drug Abuse, New River, NextWave, the National Institute of Child Health and Human Development, the National Institute of Mental Health, Novartis, Noven, Neurosearch, Organon, Otsuka, Pfizer, Pharmacia, the Prechter Foundation, Shire, the Stanley Foundation, UCB Pharma, and Wyeth. Drs. Loo, Shtir, Doyle, Smalley, Cantor, and Nelson report no biomedical financial intersts or potential conflicts of interest.

Correspondence to Sandra Loo, Ph.D., 760 Westwood Plaza Ste# 47- 406, Los Angeles, CA 90024; e-mail: SLoo@mednet.ucla.edu

0890-8567/$36.00/©2012 American Academy of Child and Adolescent Psychiatry

DOI: 10.1016/j.jaac.2012.01.006

REFERENCES

1. Batty GD, Kivimaki M, Deary IJ. Intelligence, education, and mortality. BMJ. 2010;340:c563.
2. Edmonds CJ, Isaacs EB, Visscher PM, *et al.* Inspection time and cognitive abilities in twins aged 7 to 17 years: age-related changes, heritability, and genetic covariance. Intelligence. 2008;36:210-225.
3. Jacobs N, van Os J, Derom C, Thiery E. Heritability of intelligence. Twin Res Hum Genet. 2007;10:11-14.
4. Butcher LM, Davis OS, Craig IW, Plomin R. Genome-wide quantitative trait locus association scan of general cognitive ability using pooled DNA and 500K single nucleotide polymorphism microarrays. Genes Brain Behav. 2008;7:435-446.
5. Deary IJ, Penke L, Johnson W. The neuroscience of human intelligence differences. Nat Rev Neurosci. 2010;11:201-211.
6. Ruano D, Abecasis GR, Glaser B, *et al.* Functional gene group analysis reveals a role of synaptic heterotrimeric G proteins in cognitive ability. Am J Hum Genet. 2010;86:113-125.
7. Pinto D, Pagnamenta AT, Klei L, *et al.* Functional impact of global rare copy number variation in autism spectrum disorders. Nature. 2010;466:368-372.
8. Elia J, Gai X, Xie HM, *et al.* Rare structural variants found in attention-deficit hyperactivity disorder are preferentially associated with neurodevelopmental genes. Mol Psychiatry. 2010;15:637-646.
9. Walters JT, Corvin A, Owen MJ, *et al.* Psychosis susceptibility gene ZNF804A and cognitive performance in schizophrenia. Arch Gen Psychiatry. 2010;67:692-700.
10. Castellanos FX, Tannock R. Neuroscience of attention-deficit/hyperactivity disorder: the search for endophenotypes. Nat Rev Neurosci. 2002;3:617-628.
11. Paloyelis Y, Rijsdijk F, Wood AC, Asherson P, Kuntsi J. The genetic association between ADHD symptoms and reading difficulties: the role of inattentiveness and IQ. J Abnorm Child Psychol. 2010;38:1083-1095.
12. Rommelse NN, Altink ME, Oosterlaan J, Buschgens CJ, Buitelaar J, Sergeant JA. Support for an independent familial segregation of executive and intelligence endophenotypes in ADHD families. Psychol Med. 2008;38:1595-1606.
13. Wood AC, Rijsdijk F, Johnson KA, *et al.* The relationship between ADHD and key cognitive phenotypes is not mediated by shared familial effects with IQ. Psychol Med. 2011;41:861-871.
14. Posthuma D, Luciano M, Geus EJ, *et al.* A genomewide scan for intelligence identifies quantitative trait loci on 2q and 6p. Am J Hum Genet. 2005;77:318-326.
15. Dick DM, Aliev F, Bierut L, *et al.* Linkage analyses of IQ in the collaborative study on the genetics of alcoholism (COGA) sample. Behav Genet. 2006;36:77-86.
16. Luciano M, Wright MJ, Duffy DL, *et al.* Genome-wide scan of IQ finds significant linkage to a quantitative trait locus on 2q. Behav Genet. 2006;36:45-55.
17. Comings DE, Wu S, Rostamkhani M, *et al.* Role of the cholinergic muscarinic 2 receptor (CHRM2) gene in cognition. Mol Psychiatry. 2003;8:10-11.
18. Dick DM, Aliev F, Kramer J, *et al.* Association of CHRM2 with IQ: converging evidence for a gene influencing intelligence. Behav Genet. 2007;37:265-272.
19. Gosso MF, van Belzen M, de Geus EJ, *et al.* Association between the CHRM2 gene and intelligence in a sample of 304 Dutch families. Genes Brain Behav. 2006;5:577-584.
20. Rizzi TS, Arias-Vasquez A, Rommelse N, *et al.* The ATXN1 and TRIM31 genes are related to intelligence in an ADHD background: evidence from a large collaborative study totaling 4,963 subjects. Am J Med Genet B Neuropsychiatr Genet. 2011;156:145-157.
21. Egan MF, Goldberg TE. Intermediate cognitive phenotypes associated with schizophrenia. Methods Mol Med. 2003;77:163-197.
22. Harris SE, Fox H, Wright AF, *et al.* The brain-derived neurotrophic factor Val66Met polymorphism is associated with age-related change in reasoning skills. Mol Psychiatry. 2006;11:505-513.
23. Payton A. The impact of genetic research on our understanding of normal cognitive ageing: 1995 to 2009. Neuropsychol Rev. 2009;19:451-477.
24. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders—TR. 4th ed*. Washington, DC: American Psychiatric Association; 2000.
25. Kaufman J, Birmaher B, Brent D, *et al.* Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry. 1997;36:980-988.
26. Mick E, Todorov A, Smalley S, *et al.* Family-based genome-wide association scan of attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry. 2010;49:898-905.
27. Wechsler D. *Examiner's Manual: Wechsler Intelligence Scale for Children, 3rd Edition*. New York: Psychological Corporation; 1991.
28. Wechsler D. *WIAS-III: Administration and Scoring Manual.* San Antonio, TX: Psychological Corporation; 1997.
29. Tian C, Gregersen PK, Seldin MF. Accounting for ancestry: population substructure and genome-wide association studies. Hum Mol Genet. 2008;17:R143-R150.
30. Dempster AP, Laird NM, Rubin D. Maximum likelihood from incomplete dta via the EM algorithm. J R Stat Soc B. 1977;39:1-38.
31. Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evolut. 1995;12:921-927.
32. Dudbridge F, Gusnanto A. Estimation of significance thresholds for genomewide association scans. Genet Epidemiol. 2008;32:227-234.
33. Pe'er I, Yelensky R, Altshuler D, Daly MJ. Estimation of the multiple testing burden for genomewide association studies of nearly all common variants. Genet Epidemiol. 2008;32:381-385.
34. Plomin R, Turic DM, Hill L, *et al.* A functional polymorphism in the succinate-semialdehyde dehydrogenase (aldehyde dehydrogenase 5 family, member A1) gene is associated with cognitive ability. Mol Psychiatry. 2004;9:582-586.
35. Davies G, Tenesa A, Payton A, *et al.* Genome-wide association studies establish that human intelligence is highly heritable and polygenic. Mol Psychiatry. 2011;16:996-1005.
36. Need AC, Attix DK, McEvoy JM, *et al.* A genome-wide study of common SNPs and CNVs in cognitive performance in the CANTAB. Hum Mol Genet. 2009;18:4650-4661.
37. Davis OS, Butcher LM, Docherty SJ, *et al.* A three-stage genome-wide association study of general cognitive ability: hunting the small effects. Behav Genet. 2010;40:759-767.
38. Goodman AB. Three independent lines of evidence suggest retinoids as causal to schizophrenia. Proc Natl Acad Sci U S A. 1998;95:7240-7244.
39. Lee CH, Liu CM, Wen CC, Chang SM, Hwu HG. Genetic copy number variants in sib pairs both affected with schizophrenia. J Biomed Sci. 2010;17:2.
40. Krezel W, Ghyselinck N, Samad TA, *et al.* Impaired locomotion and dopamine signaling in retinoid receptor mutant mice. Science. 1998;279:863-867.
41. Wallen-Mackenzie A, Mata de Urquiza A, Petersson S, *et al.* Nurr1-RXR heterodimers mediate RXR ligand-induced signaling in neuronal cells. Genes Dev. 2003;17:3036-3047.
42. Hirokawa N, Niwa S, Tanaka Y. Molecular motors in neurons: transport mechanisms and roles in brain function, development, and disease. Neuron. 2010;68:610-638.
43. Simon HH, Bhatt L, Gherbassi D, Sgado P, Alberi L. Midbrain dopaminergic neurons: determination of their developmental fate by transcription factors. Ann N Y Acad Sci. 2003;991:36-47.
44. Dijkhuizen T, van Essen T, van der Vlies P, *et al.* FISH and array-CGH analysis of a complex chromosome 3 aberration suggests that loss of CNTN4 and CRBN contributes to mental retardation in 3pter deletions. Am J Med Genet A. 2006;140:2482-2487.
45. Stein JL, Hua X, Lee S, *et al.* Voxelwise genome-wide association study (vGWAS). Neuroimage. 2010;53:1160-1174.
46. Lesch KP, Timmesfeld N, Renner TJ, *et al.* Molecular genetics of adult ADHD: converging evidence from genome-wide association and extended pedigree linkage studies. J Neural Transm. 2008;115:1573-1585.
47. Haworth CM, Wright MJ, Luciano M, *et al.* The heritability of general cognitive ability increases linearly from childhood to young adulthood. Mol Psychiatry. 2010;15:1112-1120.
48. Flynn J. *What Is Intelligence?* Cambridge, UK: Cambridge University Press; 2007.

FIGURE S1 Quantile-Quantile (QQ) plot for haplotype trend regression (HTR) analysis. Note: The observed negative log base 10 *p* values (–log10 *p*) from the HTR analysis are plotted against expected –log10 *p* from the null distribution. The plot shows the deviation from the null distribution suggesting that single nucleotide polymorphisms (SNPs) are enriched for associations most likely because of relatedness of family members in the sample ($\lambda = 1.3$). Permutation analysis was used to control for family structure.

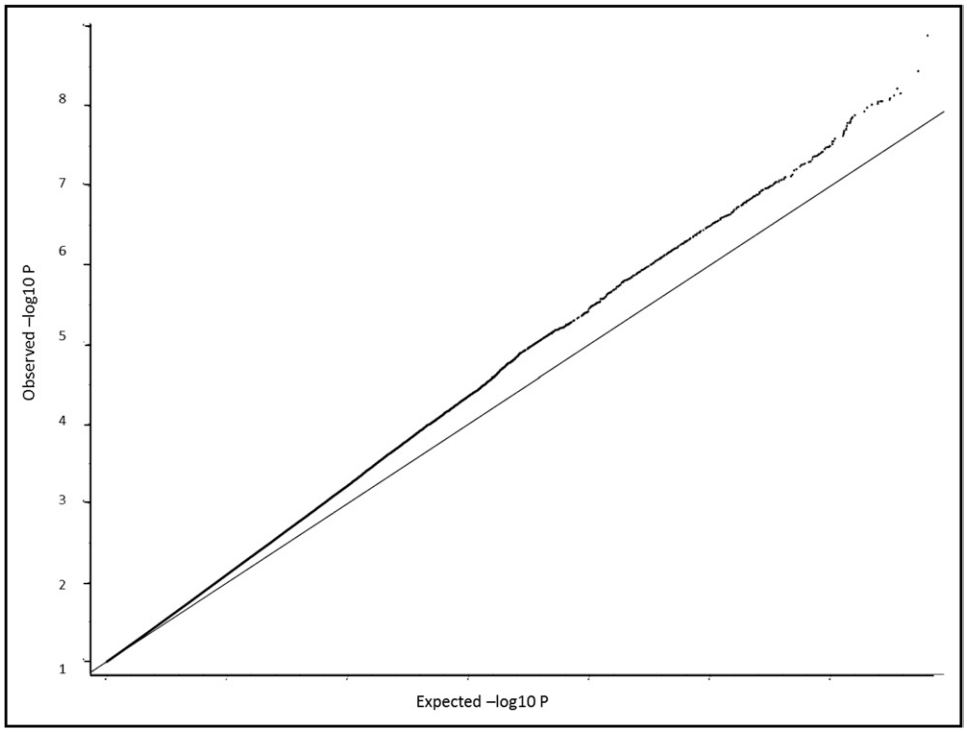


FIGURE S2 Manhattan plot of haplotype trend regression analysis. Note: Negative log base10 *p* (-log10p) values for each haplotype across the genome are plotted by chromosome.