Research Paper

Parent-of-origin effects of *FAS* and *PDLIM1* in attention-deficit/hyperactivity disorder

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Background: Previous studies have suggested that there may be a parent-of-origin effect for attention-deficit/hyperactivity disorder (ADHD) candidate genes. The objective of the present study was to investigate parent-of-origin effects using a genome-wide association analysis of the International Multicentre ADHD Genetics (IMAGE) study sample. **Methods:** Family-based association analysis for ADHD using 846 ADHD probands and their parents was performed using the PLINK program, and parent-of-origin effects were studied using a *Z* score for the difference in paternal versus maternal odds ratios. **Results:** We identified 44 single nucleotide polymorphisms (SNPs) showing parent-of-origin effects at a significance level of $p < 0.001$. The most significant SNP, rs7614907, is at position 3q13.33 in the *CDGAP* gene ($p = 0.000064$ for parent-of-origin effect). Furthermore, 2 genes (*FAS* and *PDLIM1*) showed moderate parent-of-origin effects ($p = 0.00086$ for rs9658691 and $p = 0.00077$ for rs11188249) and strong maternal transmission ($p = 0.000059$ for rs9658691 and $p = 0.0000068$ for rs11188249). In addition, *ZNF775* showed a moderate parent-of-origin effect ($p = 0.00036$ for rs7790549) and strong paternal transmission ($p = 0.000041$ for rs7790549). **Limitations:** We only had 1 sample available for analysis. **Conclusion:** These results suggest several genes or regions with moderate parent-of-origin effects, and these findings will serve as a resource for replication in other populations to elucidate the potential role of these genetic variants in ADHD.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a common, highly heritable childhood-onset psychiatric disorder affecting 2%–6% of children worldwide and is characterized by developmentally inappropriate levels of inattention, hyperactivity and impulsivity. A previous review of genetic epidemiologic studies, including more than 14 published twin studies and 5 adoption studies, indicated that most of the variation was attributable to genetic factors, consistently demonstrating high heritability in the range of 75%–91%.[1] Recent reviews further showed that twin studies of ADHD had been consistent with an average heritability rate of 76%.[2–5]

Previous studies have suggested that there may be a parent-of-origin effect for ADHD candidate genes. For example, a generalized parent-of-origin effect was observed in an Irish ADHD study.[6] Furthermore, gene-specific parent-of-origin effects have been observed for *BDNF*,[7] *DAT1* (*SLC6A3*),[8] *DDC*,[9,10] *GNAL*,[11] *HTR1B*,[12] *SLC6A2*,[10] *SLC6A4*,[6,13] *SNAP25*,[14,15] *TPH2*, *DRD4*, *DRD5* and *SLC6A3*,[6] *FADS2* and *ADRBK2*.[10] Several studies have failed to confirm an overall parent-of-origin effect;[10,15,16] however, gene-specific parent-of-origin effects cannot be excluded.[10]

The conventional genome-wide association (GWA) study approach is a hypothesis-free, systematic search of tagging single nucleotide polymorphisms (SNPs) across the genome to identify novel associations with common diseases. It has emerged as a powerful tool to identify disease-related genes for many common human disorders and other phenotypes.[17] Recently, several GWA studies of ADHD and related phenotypes were reported, and 4 of them were based on a sample set of the International Multicentre ADHD Genetics (IMAGE) study and genotyped with funds from the Genetic Association Information Network (GAIN).[18] For example, the first GWA scan of ADHD was completed on a sample of 909 complete proband–parent trios with a child with the combined subtype of ADHD from the IMAGE project.[19] To

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our knowledge, an examination of parent-of-origin effects in a GWA study of ADHD has not been undertaken. Therefore, we conducted a GWA analysis of ADHD to search for genetic variants showing the difference in transmission frequency between paternal and maternal alleles.

Methods

Study sample

Families were collected for the IMAGE project. A total of 924 affected proband–parent trios were initially selected for the GWA scan. Family members were primarily of Western European origin, hailing from 8 countries: Belgium, Germany, Ireland, Israel, the Netherlands, Spain, Switzerland and the United Kingdom. To reduce the genetic heterogeneity, we chose 846 probands initially ascertained to have DSM-IV[20] combined subtype ADHD. Demographic and clinical characteristics of these participants have been described elsewhere.[19] Genotyping data using the Perlegen (600K) genome-wide association platform (599 164 SNPs) were available for these 846 probands and their parents.

Assessment of Hardy–Weinberg equilibrium

We tested departure from the Hardy–Weinberg equilibrium for unaffected founders using PLINK version 1.07,[21] and we also estimated the genotype call rate and minor allele frequency (MAF).

Family-based association analyses

Family-based association studies, such as the transmission disequilibrium test (TDT), are preferable to case–control studies of allelic association when there is population admixture.[22] In this study, family-based association analysis (i.e., TDT) for ADHD was performed using PLINK. We studied the parent-of-origin effect using the *Z* score to assess the difference in paternal versus maternal odds ratios in PLINK.

Multiple testing

For statistical significance of parent-of-origin effects, we used a conservative per test significance level of $\alpha = 0.0000005$.[17] At the same time, we also used a less stringent criterion of "suggestive association" with a cut-off of $\alpha = 0.001$. In addition to obtaining nominal *p* values, empirical *p* values were generated by 100 000 permutation tests using a Max (T) permutation procedure implemented in PLINK. In this procedure, 2 sets of empirical significance values were calculated: pointwise estimates of an individual SNP's significance (empirical pointwise *p* values) and corrected values for multiple testing (corrected empirical *p* values).

Haplotype block and fine-mapping

We assessed pairwise linkage disequilibrium statistics (D') for unrelated founders using Haploview.[23] We identified haplotype blocks, within which SNPs have strong linkage disequilibrium (D' > 0.8), for interesting candidate genes or regions. Then we chose several SNPs within blocks, including the associated SNPs in the IMAGE data. Haplotype analysis based on a sliding window of fixed haplotype size was performed using the TDTPHASE program in UNPHASED version 2.404.[24] We performed a Fisher exact test in SAS version 9.2 based on a 2×2 contingency test (χ^2 test) to evaluate the difference in transmission frequency between paternal and maternal haplotypes (parent-of-origin effect).

Results

Genotype quality control

We removed SNPs with the Hardy–Weinberg equilibrium of $p < 0.0001$, with call rates less than 95% or with an MAF less than 5%. There were 491 705 SNPs left for further analysis.

Genome-wide association analysis

In total, we identified 44 SNPs showing parent-of-origin effects with $p < 0.001$, and 22 of them were located within 19 genes (Table 1). A more comprehensive list of SNPs (all 44 showing parent-of-origin effects) with $p < 0.001$ is presented in Appendix 1 (Table S1), available at www.cma.ca/jpn. The most significant SNP, rs7614907, was at position 3q13.33 in *CDGAP* ($p = 0.000064$). Two SNPs in *SLC4A4* (rs1452898 and rs7673301), 2 in *NXN* (rs2644700 and rs6042229) and 2 in *STARD13* (rs10492402 and rs7322586) showed parent-of-origin effects. In addition, SNPs in *AK5*, *HLA-DOA*, *TAAR9*, *TAAR6*, *NKAINS*, *FAS* and *PDLIM1* showed parent-of-origin effects.

Table 1 showed that only 3 of 22 SNPs (rs7790549 in *ZNF775*, rs9658691 in *FAS* and rs11188249 in *PDLIM1*) were associated with ADHD in the whole sample ($p < 0.05$). Furthermore, 4 SNPs (rs1452898 in *SLC4A4*, rs13255144 in *SAMD12*, rs9658691 in *FAS* and rs11188249 in *PDLIM1*) showed associations with ADHD from maternal transmission ($p < 0.001$). Four SNPs showed paternal transmission $p < 0.001$ (rs7614907 in *CDGAP*, rs7790549 in *ZNF775*, and rs2644700 and rs604229 in *NXN*).

The Q-Q plot of parent-of-origin effects is presented in Appendix 1 (Fig. S1). As shown in Figure S1, the observed *p* values gradually depart from expected *p* values when $-\log_{10}(p) > 3$. The pattern suggests evidence for parent-of-origin effects.

Permutation results

Table 1 also shows that the empirical pointwise *p* values ranged from $p = 0.00001$ to $p = 0.00043$. Applying a permutation procedure for multiple test correction also yielded significant *p* values (corrected empirical *p* values), which ranged from $p = 0.00053$ to $p = 0.013$.

Fine-mapping

We examined all the SNPs within 19 genes in the IMAGE sample (2258 SNPs) and found an additional 33 SNPs within

Table 1: Twenty-two single nucleotide polymorphisms within genes showing parent-of-origin effects with $p < 0.001$

						All transmissions			Maternal			Paternal			Parent-of-origin effect				
Chr.	SNP database	Base pair position*	Known gene	GT	AF	T/NT	χ^2†	*p*	T/NT	χ^2	*p*	T/NT	χ^2	*p*	χ^2‡	*Z*§	*p*¶	Point. *p***	Corr. *p*††
1	rs17098286	77640412	*AK5*	G/C	0.08	120/103	1.30	0.26	45/62	2.73	0.099	76/42	9.88	0.0017	11.28	3.34	0.00083	0.00028	0.011
1	rs542494	232490912	*SLC35F3*	C/T	0.15	206/203	0.02	0.88	120/80	8.00	0.0047	86/123	6.55	0.01	14.53	3.79	0.00015	0.00002	0.0014
3	rs7614907	120559179	*CDGAP*	T/C	0.05	83/68	1.49	0.22	26/43	4.70	0.030	58/25	13.3	0.00027	15.80	4.00	0.000064	0.00004	0.00053
4	rs1452898	72359433	*SLC4A4*	C/A	0.14	180/200	1.05	0.30	73/119	11.0	0.00090	107/81	3.60	0.058	13.61	3.67	0.00025	0.00006	0.0025
4	rs7673301	72406891	*SLC4A4*	T/C	0.12	157/173	0.78	0.38	61/101	9.94	0.0016	97/73	3.41	0.065	12.52	3.53	0.00042	0.00008	0.0051
4	rs17484427	115089316	*ARSJ*	G/C	0.15	216/211	0.06	0.809	90/123	5.10	0.023	126/88	6.75	0.0094	11.80	3.42	0.00063	0.00014	0.0081
6	rs2582	33082529	*HLA-DOA*	T/G	0.13	207/190	0.73	0.39	90/117	3.52	0.061	117/73	10.2	0.0014	13.01	3.59	0.00034	0.00002	0.0037
6	rs9389004	132901953	*TAAR9*	A/G	0.05	79/66	1.17	0.28	34/47	2.11	0.14	46/20	10.4	0.0013	11.27	3.33	0.00087	0.00043	0.012
6	rs8192624	132933946	*TAAR6*	A/G	0.08	128/125	0.04	0.85	51/76	4.96	0.026	78/50	6.17	0.013	11.01	3.31	0.00094	0.00042	0.013
7	rs7790549	149720965	*ZNF775*	A/G	0.08	143/109	4.59	0.032	67/76	0.57	0.45	77/34	16.8	0.000041	12.90	3.57	0.00036	0.00005	0.004
8	rs12114607	63416967	*NKAIN3*	A/G	0.13	180/173	0.14	0.71	109/73	7.12	0.0076	71/100	4.92	0.027	11.90	3.43	0.0006	0.00006	0.0077
8	rs13255144	119516196	*SAMD12*	T/C	0.35	373/409	1.66	0.20	167/233	11.2	0.00081	206/175	2.52	0.11	11.87	3.47	0.00052	0.00002	0.0064
10	rs9658691	90746143	*FAS*	C/T	0.10	128/169	5.66	0.017	53/103	16.1	0.00059	76/67	0.57	0.45	11.18	3.33	0.00086	0.00011	0.012
10	rs11188249	97000369	*PDLIM1*	G/A	0.10	122/174	9.14	0.0025	45/99	20.3	0.0000068	77/75	0.03	0.87	11.50	3.37	0.00077	0.0002	0.010
13	rs10492402	32606789	*STARD13*	T/C	0.07	108/111	0.04	0.83	44/72	6.75	0.0093	64/39	6.07	0.014	12.79	3.54	0.00041	0.00025	0.0049
13	rs7322586	32610785	*STARD13*	A/G	0.07	110/110	0.01	1.00	44/70	5.93	0.015	66/40	6.37	0.012	12.31	3.47	0.00051	0.00024	0.0063
15	rs4777414	69675927	*THSD4*	C/T	0.10	159/140	1.21	0.27	92/53	10.6	0.0012	68/88	2.58	0.10	11.90	3.44	0.00059	0.0002	0.0074
16	rs4412964	82113739	*CDH13*	T/C	0.37	359/376	0.39	0.53	197/157	4.53	0.033	163/219	8.51	0.0035	12.39	3.55	0.00039	0.00001	0.0046
17	rs2644700	729799	*NXN*	T/C	0.09	116/140	2.25	0.13	70/54	2.07	0.15	46/86	12.1	0.00050	12.04	3.44	0.00058	0.0002	0.0073
17	rs604229	733578	*NXN*	T/C	0.08	101/123	2.17	0.14	65/49	2.27	0.13	37/75	13.0	0.00031	13.12	3.60	0.00032	0.00013	0.0034
18	rs1452643	29741426	*NOL4*	T/A	0.19	236/244	0.13	0.72	91/131	7.21	0.0073	145/113	3.97	0.046	11.05	3.31	0.00093	0.00012	0.013
19	rs17325700	38923667	*CHST8*	T/A	0.10	149/145	0.05	0.82	60/88	5.33	0.021	90/58	6.97	0.0083	12.16	3.48	0.00051	0.00011	0.0063

AF = minor allele frequency of the SNP in founders; Chr. = chromosome; GT = genotype for the SNP; SNP = single nucleotide polymorphism; T/NT = transmitted/nontransmitted allele.
*Physical position is based on NCBI Genome Build 36.3.
†χ^2 transmission disequilibrium test statistic.
‡χ^2 based on a 2×2 contingency test to evaluate the difference in transmission frequency between paternal and maternal haplotypes.
§*Z* score for difference in paternal versus maternal odds ratios.
¶Nominal *p* value, asymptotic for parent-of-origin test.
**Empirical pointwise *p* value, computed by 100 000 permutation tests using a Max (T) permutation procedure implemented in PLINK.
††Empirical *p* value corrected for multiple testing, generated by 100 000 permutation tests using a Max (T) permutation procedure implemented in PLINK.

9 genes that had parent-of-origin effects with nominal $p < 0.05$ (Table 2).

Two genes, *FAS* and *PDLIM1*, which have been previously associated with psychiatric disorders, showed moderate parent-of-origin effects ($p = 0.00086$ for rs9658691 and $p = 0.00077$ for rs11188249) and strong maternal transmission ($p = 0.000059$ for rs9658691 and $p = 0.0000068$ for rs11188249). Therefore, we chose SNPs within *FAS* and *PDLIM1* genes from the IMAGE sample for fine mapping. Part of the haplotype analysis results is presented in Table 3. The haplotype C-A based on rs9658691 and rs1926194 (D′ = 0.91) demonstrated a significant parent-of-origin effect with $p = 0.000157$, whereas for *PDLIM1*, the haplotype T-G based on rs17525659 and rs11188249 (D′ = 1.00) revealed a parent-of-origin effect with $p = 0.000759$. The associations of 8 SNPs within *FAS* and *PDLIM1* are presented in Appendix 1 (Table S2).

Discussion

We tested parent-of-origin effects using a genome-wide design of IMAGE data with 600K SNPs. Forty-four SNPs, of which 22 were within 19 genes, were identified to have suggestive parent-of-origin effects at a nominal allelic $p < 0.001$. In particular, haplotype analyses for 2 genes, *FAS* and *PDLIM1*, further supported the parent-of-origin effects in those genes.

Interestingly, *HLA-DOA* at position 6p21.3 showed parent-of-origin effects. The *HLA-DOA* gene belongs to the HLA class II α chain paralogues. It has been reported that HLA showed strong paternal transmission in celiac disease[25] and autism.[26] Furthermore, *HLA-DQB1*, *DQA1* and *DRB1* are 230kb, 252kb and 306kb, respectively, away from *HLA-DOA*. Owing to the very strong linkage disequilibrium within the

Table 2: Thirty-three single nucleotide polymorphisms showing parent-of-origin effects with $p < 0.05$

Chr.	SNP database	Base pair position*	Known gene	GT	AF	All transmissions			Maternal			Paternal			Parent-of-origin effect		
						T/NT	χ^2†	*p*	T/NT	χ^2	*p*	T/NT	χ^2	*p*	χ^2‡	*Z*§	*p*¶
1	rs2799561	77542078	*AK5*	C/T	0.19	246/294	4.27	0.039	142/142	0.00	1.00	105/153	8.97	0.0028	4.72	2.17	0.030
1	rs2815324	77564277	*AK5*	C/T	0.20	251/301	4.53	0.033	144/146	0.01	0.91	108/156	8.76	0.0031	4.26	2.07	0.039
1	rs2815326	77571533	*AK5*	T/C	0.20	255/300	3.65	0.056	149/144	0.09	0.77	107/157	9.51	0.0020	5.96	2.44	0.015
1	rs2054017	77583700	*AK5*	G/A	0.06	101/84	1.56	0.21	39/49	1.14	0.29	62/35	7.52	0.0061	7.15	2.67	0.0079
1	rs9633478	77657870	*AK5*	A/G	0.08	109/118	0.37	0.55	43/64	4.16	0.041	67/55	1.19	0.28	4.96	2.23	0.026
1	rs1463502	232337970	*SLC35F3*	C/T	0.14	176/208	2.67	0.10	101/96	0.13	0.72	75/112	7.32	0.0068	4.81	2.19	0.029
1	rs12118979	232524008	*SLC35F3*	C/T	0.09	156/125	3.42	0.06	69/73	0.11	0.74	88/53	8.75	0.0031	5.47	2.34	0.019
2	rs12478741	154950445	*GALNT13*	T/A	0.07	92/106	0.99	0.32	52/43	0.85	0.36	40/63	5.14	0.023	5.02	2.23	0.025
2	rs1366750	155047405	*GALNT13*	A/G	0.15	192/218	1.65	0.20	107/97	0.49	0.49	86/122	6.26	0.012	5.10	2.05	0.04
2	rs1830018	155089021	*GALNT13*	G/T	0.19	259/278	0.67	0.41	139/122	0.98	0.32	121/155	4.44	0.035	4.76	2.26	0.024
2	rs11895478	155279369	*GALNT13*	T/C	0.30	353/326	1.07	0.30	168/182	0.64	0.42	186/143	5.36	0.02	4.95	2.18	0.029
2	rs3106653	155283806	*GALNT13*	G/T	0.30	363/329	1.67	0.20	174/183	0.28	0.60	190/145	5.78	0.016	4.41	2.10	0.036
4	rs2602049	72365934	*SLC4A4*	A/C	0.18	222/225	0.02	0.89	93/125	4.70	0.030	129/100	3.67	0.055	8.35	2.88	0.004
4	rs6854303	72501617	*SLC4A4*	G/A	0.24	289/311	0.81	0.37	134/174	5.20	0.023	155/137	1.11	0.29	5.51	2.34	0.019
4	rs4626166	72513226	*SLC4A4*	A/C	0.16	206/241	2.74	0.10	91/134	8.25	0.0041	116/108	0.29	0.59	5.81	2.41	0.016
4	rs4130912	72541637	*SLC4A4*	T/C	0.15	201/215	0.47	0.49	84/119	6.03	0.014	117/96	2.07	0.15	7.64	2.76	0.0059
4	rs4484264	72564200	*SLC4A4*	A/G	0.15	186/219	2.69	0.10	78/121	9.29	0.0023	108/98	0.49	0.49	7.13	2.66	0.0077
4	rs9997927	72576964	*SLC4A4*	C/A	0.29	321/358	2.02	0.16	149/196	6.42	0.011	173/163	0.30	0.58	4.70	2.17	0.030
4	rs10009080	72578718	*SLC4A4*	T/C	0.19	226/288	7.48	0.0062	100/165	16.0	0.000063	127/124	0.04	0.85	8.65	2.94	0.0033
4	rs4469035	72579342	*SLC4A4*	T/C	0.19	224/264	3.28	0.07	100/150	10.0	0.0015	125/115	0.42	0.52	7.20	2.68	0.0073
4	rs17484427	115089316	*SLC4A4*	G/C	0.15	216/211	0.06	0.81	90/123	5.11	0.024	126/88	6.75	0.0094	11.8	3.42	0.00063
6	rs149392	32997949	*HLA-DOA*	C/T	0.07	112/121	0.35	0.56	41/62	4.28	0.039	71/59	1.11	0.29	5.05	2.34	0.025
6	rs1044429	33080620	*HLA-DOA*	T/C	0.15	223/195	1.88	0.17	103/115	0.66	0.42	121/81	7.96	0.0047	6.74	2.60	0.0094
8	rs16928749	63436497	*NKAIN3*	T/C	0.09	130/131	0.01	0.96	76/53	4.13	0.042	55/79	4.33	0.037	8.40	2.89	0.0038
8	rs2351667	63440763	*NKAIN3*	G/A	0.14	191/178	0.46	0.95	112/76	6.93	0.0085	80/103	2.91	0.088	9.34	3.05	0.0023
8	rs16928789	63449820	*NKAIN3*	G/A	0.12	165/166	0.01	0.50	97/71	4.05	0.044	69/96	4.45	0.035	8.44	2.90	0.0037
8	rs1993126	63558534	*NKAIN3*	A/G	0.13	182/167	0.64	0.87	108/78	4.87	0.027	75/90	1.37	0.24	5.57	2.36	0.018
10	rs9658786	90766329	*FAS*	T/C	0.14	189/205	0.65	0.42	83/118	6.10	0.014	106/87	1.87	0.17	7.33	2.70	0.007
10	rs11188256	97027568	*PDLIM1*	C/T	0.22	306/238	8.50	0.0036	165/104	13.9	0.00019	142/135	0.18	0.67	5.63	2.37	0.018
15	rs12913412	69549033	*THSD4*	C/G	0.19	243/260	0.57	0.45	136/120	1.00	0.32	107/140	4.41	0.036	4.84	2.20	0.028
15	rs11635579	69577402	*THSD4*	A/C	0.47	400/443	2.19	0.14	184/236	6.69	0.0097	217/206	0.24	0.63	4.74	2.18	0.030
15	rs12912888	69643738	*THSD4*	G/A	0.14	220/188	2.51	0.11	122/83	7.46	0.0063	99/106	0.24	0.62	5.19	2.28	0.023
15	rs8026019	69644931	*THSD4*	T/A	0.11	191/145	6.30	0.012	99/59	10.2	0.0014	93/87	0.20	0.65	4.14	2.04	0.042

AF = minor allele frequency of the SNP in founders; Chr. = chromosome; GT = genotype for the SNP; SNP = single nucleotide polymorphism; T/NT = transmitted/nontransmitted allele.
*Physical position is based on NCBI Genome Build 36.3.
†χ^2 transmission disequilibrium test statistic.
‡χ^2 based on a 2×2 contingency test to evaluate the difference in transmission frequency between paternal and maternal haplotypes.
§*Z* score for difference in paternal versus maternal odds ratios.
¶Nominal *p* value, asymptotic for parent-of-origin test.

HLA region,[27] the parent-of-origin effects in *HLA-DOA* may result from the linkage disequilibrium with flanking genes.

More interestingly, several genes, including *FAS* and *PDLIM1*, have been reported to be related to psychiatric disorders. For example, the *FAS* antigen (*CD95*) is a cell surface receptor that mediates cell apoptosis signalling, and recent investigations have shown that *FAS*-regulated apoptosis is linked to neurodegenerative lesions in the brains of patients with Alzheimer disease. One polymorphism of the *FAS* promoter region was associated with the risk of Alzheimer disease developing.[28] The *FAS* gene, which plays a role in apoptosis, may be associated with Alzheimer disease by modulating the apoptosis and neuronal loss secondary to Alzheimer disease neuropathology.[29] However, no significant differences in allelic and genotypic distributions were found between cases and controls, or patients with late- and early-onset Alzheimer disease, thus suggesting that these polymorphisms did not represent a risk factor for Alzheimer disease in the Italian population.[30] Furthermore, *PDLIM1* at position 10q22 might play a role in Alzheimer disease,[31,32] serving as a scaffold to form a multiprotein complex that regulates actin cytoskeleton dynamics and playing a role in controlling neurite outgrowth.[33] It has been reported that *TAAR6* was associated with both schizophrenia and bipolar disorder in a Korean study[34] and with schizophrenia in an Irish study,[35] although the results need to be confirmed.

Several genes have been reported to be associated with other diseases. For example, *CDGAP* properties are well conserved between human and mouse species, and *CDGAP* may play an unexpected role in apoptosis and has suggestive association with coronary artery disease.[36,37] In the present study, *SLC4A4* at position 4q21 showed maternal transmissions, and this gene encodes a sodium bicarbonate cotransporter involved in the regulation of bicarbonate secretion and absorption and intracellular pH. Mutations in this gene are associated with cystic fibrosis.[38] The SNP rs7790549 within *ZNF775* at position 7q36.1 showed strong paternal transmission; however, the function of this gene is still unclear. In addition, other genes, such as *AK5* and *KAIN3* at position 8q12.3, have not been associated with any disease. The roles of these genes in ADHD need further study.

To compare our findings with those from previous studies of parent-of-origin effects, we examined the SNPs for the following 12 genes in the IMAGE sample: *BDNF*, *DAT1* (*SLC6A3*), *DDC*, *GNAL*, *HTR1B*, *SLC6A4*, *SNAP25*, *TPH2*, *DRD4*, *DRD5*, *FADS2* and *ADRBK2*. These genes have been reported to have paternal or maternal transmission or parent-of-origin effects in ADHD in previous studies.[6–15] In the cleaned SNP data, we did not find any SNPs in the *DRD4* gene. Furthermore, we could not confirm the paternal or maternal transmission or parent-of-origin effects ($p < 0.01$) for *HTR1B*, *SLC6A4*, *DRD4*, *DRD5* and *FADS2*. However, we confirmed 14 SNPs within 7 genes (*BDNF*, *DAT/DAT1/SLC6A3*, *DDC*, *GNAL*, *SNAP25*, *TPH2* and *ADRBK2*) showing paternal or maternal transmission or parent-of-origin effects with $p < 0.01$ (Table S3 in Appendix 1). Of these genes, our findings for *TPH2* and *ADRBK2* are consistent with those of Anney and colleagues[10] using the IMAGE data. Consistent with the results of Mill and colleagues,[14] we found nominal significance for paternal transmissions for the SNPs rs3787303 and rs3787283 in *SNAP25*. We also found similar maternal transmission in *GNAL* ($p = 0.0081$ for rs1477941, $p = 0.002$ for

Table 3: Haplotype analyses for *FAS* and *PDLIM1* genes

Gene	Haplotype		All transmissions			Maternal			Paternal			Parent-of-origin effect	
			T/NT	χ^{2}*	*p*†	T/NT	χ^{2}	*p*	T/NT	χ^{2}	*p*	χ^{2}‡	*p*¶
FAS	rs9658691	rs1926194		7.31	0.063		16.07	0.0011		8.61	0.035		
	C	A	100/125	2.54	0.11	39/81	15.00	0.00011	61/44	2.77	0.096	14.86	0.000157
	T	A	316/312	0.02	0.88	176/150	2.08	0.15	140/162	1.60	0.21	3.65	0.066
	T	G	300/270	1.18	0.28	158/139	1.22	0.27	142/131	0.44	0.51	0.08	0.80
PDLIM1	rs17525659	rs11188249		9.00	0.029		23.64	0.000029		0.42	0.94		
	G	A	155/129	2.17	0.14	80/55	4.66	0.031	75/74	0.007	0.93	2.28	0.15
	T	A	261/239	0.88	0.35	130/103	3.14	0.077	131/136	0.094	0.76	2.26	0.15
	T	G	110/158	7.63	0.0057	39/90	20.7	0.0000053	71/68	0.065	0.80	12.00	0.000759
	rs11188249	rs2296961		13.11	0.0044		22.41	0.000054		3.63	0.30		
	A	C	246/288	2.46	0.12	127/143	0.95	0.33	119/145	2.57	0.11	0.21	0.66
	A	T	368/282	9.17	0.0025	186/126	11.60	0.00066	182/156	2.00	0.16	2.20	0.15
	G	T	99/133	4.53	0.033	37/74	12.57	0.00039	62/59	0.07	0.79	7.59	0.0078
	rs17453855	rs11188256		12.47	0.0059		20.37	0.00014		1.45	0.69		
	A	T	99/96	0.043	0.84	48/52	0.16	0.69	51/44	0.52	0.47	0.63	0.47
	G	C	260/184	10.85	0.00099	142/77	19.59	0.0000096	118/107	0.54	0.46	7.03	0.0093
	G	T	252/328	8.63	0.0033	116/175	12.05	0.00052	136/153	1.00	0.32	3.06	0.094

AF = minor allele frequency of the SNP in founders; Chr. = chromosome; GT = genotype for the SNP; SNP = single nucleotide polymorphism; T/NT = transmitted/nontransmitted allele.

*χ^{2} transmission disequilibrium test statistic.

†The globe *p* value for 2-SNP haplotype analysis based on a χ^{2}_{3} test or the *p* value for a single haplotype based on a χ^{2}_{1} test using UNPHASED.

‡χ^{2} based on a 2×2 contingency test to evaluate the difference in transmission frequency between paternal and maternal haplotypes.

¶A 2-sided Fisher exact *p* value in SAS version 9.2 based on a 2×2 contingency (χ_{0}) test to evaluate the difference in transmission frequency between paternal and maternal haplotypes.

rs10468679 and $p = 0.0003$ for rs8087897) to those reported by Laurin and colleagues.[11] Furthermore, Table S3 in Appendix 1 confirms the paternal transmission ($p = 0.0019$ for rs12288512) in *BDNF* found by Kent and colleagues.[7] In addition, we confirmed the paternal transmission ($p = 0.022$ for rs3863145) for *DAT1* reported by Hawi and colleagues.[6,8]

Based on QUANTO software,[39] we had greater than 80% power at $\alpha = 5\%$ to detect maternal and paternal transmission for a sample size of 846 (trios), relative risk of 1.3, population risk of 0.1 and allele frequency of 20%.

The mechanism of parent-of-origin effects is still unclear. One potential mechanism is "genomic imprinting" owing to epigenetic modification of the genome. For example, it has been shown that there is evidence to suggest that nearly 80 human genes show monoallelic expression consistent with imprinting,[40] whereas the mechanism underlying the reading of the imprint can involve many aspects of gene expression, and the silencing can be stable throughout the individual's life.[41] Previous linkage and expression data showed that there are maternal-expressed imprinted genes at position 10q22, where *PDLIM1* is located.[42] However, imprinting is only one mechanism, and the utero maternal environment may influence parent-of-origin effect.[43] It has been reported that the *DDC* gene may be imprinted in ADHD.[9,10] However, the results need to be further confirmed.

This study has several strengths. First, we performed GWA analyses to identify genetic variants with parent-of-origin effects in ADHD. Based on our results, genes with strong parent-of-origin effects may not have large main effects in the whole sample. Second, we used a large sample with 846 trios from the IMAGE project. Furthermore, we used a Max (T) permutation procedure implemented using PLINK to correct multiple testing. The corrected p values ranged from $p = 0.00053$ to $p = 0.013$ (Table 1). Finally, we performed haplotype analyses for 2 genes (*FAS* and *PDLIM1*), and the haplotype analysis results further supported parent-of-origin effects in both genes.

Limitations

One limitation is that we had only 1 sample available for analysis. Furthermore, instead of reaching significant genome-wide association significance ($p < 0.0000005$), our study only reached suggestive associations with parent-of-origin effect ($p < 0.001$). Therefore, the findings in this study need to be further confirmed using other samples.

Conclusion

We have identified several interesting genes or regions with parent-of-origin effects using GWA analysis of a large sample from the IMAGE project. These findings will serve as a resource for replication in other populations to elucidate the potential role of these genetic variants in ADHD. Further work to identify additional variants and the disease-causing polymorphisms in the loci, and to examine the functions of these polymorphisms, will help us to better understand the pathogenesis of ADHD.

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