Genome-wide association study of motor coordination problems in ADHD identifies genes for brain and muscle function

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Abstract

Objectives. Motor coordination problems are frequent in children with attention deficit/hyperactivity disorder (ADHD). We performed a genome-wide association study to identify genes contributing to motor coordination problems, hypothesizing that the presence of such problems in children with ADHD may identify a sample of reduced genetic heterogeneity. ***Methods***. Children with ADHD from the International Multicentre ADHD Genetic (IMAGE) study were evaluated with the Parental Account of Children's Symptoms. Genetic association testing was performed in PLINK on 890 probands with genome-wide genotyping data. Bioinformatics enrichment-analysis was performed on highly ranked findings. Further characterization of the findings was conducted in 313 Dutch IMAGE children using the Developmental Coordination Disorder Questionnaire (DCD-Q). ***Results***. Although none of the findings reached genome-wide significance, bioinformatics analysis of the top-ranked findings revealed enrichment of genes for motor neuropathy and amyotrophic lateral sclerosis. Genes involved in neurite outgrowth and muscle functions were also enriched. Among the highest ranked genes were *MAP2K5,* involved in restless legs syndrome, and *CHD6,* causing motor coordination problems in mice. Further characterization of these findings using DCD-Q subscales found nominal association for 15 SNPs. ***Conclusions***. Our findings provide clues about the aetiology of motor coordination problems, but replication studies in independent samples are necessary.

Key words: *Motor coordination problems, ADHD, genome-wide association study (GWAS), neurite outgrowth, and skeletal muscle function*

Introduction

With a prevalence of 5% at school age, motor coordination problems are common in children and are usually referred to as developmental coordination disorder (DCD) (American Psychiatric Association 2000; Kirby and Sugden 2007; Missiuna et al. 2008; Lingam et al. 2009). DCD is a heterogeneous condition. Motor milestones such as crawling and walking may be delayed, while some children show hypotonia and/or clumsiness (Green et al. 2008; Wilson and Larkin 2008). The motor problems lead to difficulties in everyday living and often have an effect on academic performance, sports, play and self-esteem (Cummins et al. 2005; Polatajko and Cantin 2005; Miyahara and Piek 2006; Piek et al. 2008). Delay of brain maturation as well as functional deviations in basal ganglia, parietal lobe and cerebellum have been suggested as the dominant source of neuropathology in motor coordination problems (Zwicker et al. 2009). DCD is considered a multifactorial disorder in which genetic factors and environmental factors such as perinatal adversity play a role (Pearsall-Jones et al. 2009). The one study formally examining the heritability of DCD in a population-based twin study (Martin et al. 2006) estimated it to be 0.69. In our study of sib pairs, we found a familial component (comprising genetic and environmental effects) of 0.47 (Fliers et al. 2009). The genetic component appears polygenic, with many genes of small effect thought to causing the disorder in unfavourable environmental circumstances.

Children with motor coordination problems usually have problems in other areas of development as well, including dyslexia, autistic spectrum disorders and attention deficit/hyperactivity disorder (ADHD). The other way around, we and others have found that of children with ADHD, 30 – 50% also suffer from motor coordination problems (Gillberg et al. 2004; Fliers et al. 2008). The combination of ADHD and motor coordination

problems has previously been named deficits of attention and motor perception, DAMP (Kadesjo and Gillberg 1998; Gillberg et al. 2004). At present, we can only speculate about the underlying neurobiological mechanisms for this comorbidity, but a dopamine-induced imbalance of basal ganglia neurocircuits may play a role (Arnsten 2006).

Previous work on the familiality of these two disorders identified a possible shared etiological background. In the Dutch sample of the International Multicenter ADHD Genetics (IMAGE) study, we found that ADHD and motor coordination problems have a common basis that may be due to genetic factors and/or shared environmental factors. The familial correlation between motor performance measures and ADHD was found to be 0.38 (Fliers et al. 2009). These results are in line with a twin study of the shared background of ADHD and DCD, in which a shared heritability of between 29 and 51% was observed (Martin et al. 2006).

Despite the considerable familial component involved in motor coordination problems (Fliers et al. 2009), little is known about the specific genetic factors involved. Since such knowledge may help to better understand the aetiology of motor coordination problems, we set out to perform a hypothesis generating genome-wide association study (GWAS) to search for DNA variation contributing to the condition. Genome-wide association studies are a powerful tool to identify genetic factors of limited effect size (McCarthy et al. 2008). In GWAS, hundreds of thousands of single nucleotide polymorphisms (SNPs) across the genome are tested independently for their association with a trait/disorder. This method has revolutionized the search for genetic influences on complex traits such as ADHD, in which both genetic and environmental factors work together (Franke et al. 2009). The SNPs analyzed in GWAS are selected to "tag" or capture the majority of genetic variation in their vicinity, which is possible because the human DNA is organized in blocks of genetic material that is transmitted together across generations. This means that if we genotype one given SNP, we will be able to flag or predict the variation of several other SNPs including those contributing to the trait/disease.

We hypothesized that studying motor coordination problems in a sample of ADHD-affected children might reduce the phenotypic and genetic heterogeneity of motor problems. In the current study, 890 children from the IMAGE study were included. We performed bioinformatics analysis on the highest ranked findings to test for enrichment of gene functional groups. Findings were further characterized in more detail using a second phenotyping instrument in the Dutch IMAGE subsample.

Methods

Participants:

Children with ADHD and their siblings were recruited for the IMAGE study that aims at identifying genes that increase the risk for ADHD using QTL linkage and association strategies (Brookes et al. 2006; Kuntsi et al. 2006). Families were identified through ADHD probands aged 5 – 17 years attending outpatient clinics at the data collection sites in Europe (Belgium, Germany, Ireland, The Netherlands, Spain, Switzerland, and the United Kingdom) and Israel. Families of European Caucasian ancestry were recruited based on having one child with ICD-10 or DSM-IV ADHD and at least one other child who would provide DNA and quantitative trait data. In addition, both parents had to be available for DNA-sampling. The ADHD diagnosis was based on DSM-IV criteria using both parent and teacher questionnaires and standardized

interviewing. Diagnostic instruments used were the Parental Account of Childhood Symptoms (PACS) interview (see below), Conners' Parents and Teachers long versions, and the SDQ (Strengths and Difficulties Questionnaire).

Exclusion criteria included an IQ < 70, known genetic syndromes (Down, Turner, Fragile X), autism, seizures (current or in the past), brain disorders (such as periventricular haemorrhage, cerebral palsy and epilepsy), and disorders mimicking ADHD symptoms. When children were using medication to treat their ADHD symptoms, the parents were asked to report on their children's behaviour off medication. For additional details about the clinical characteristics and the diagnostic process, see Brookes et al. 2006; Kuntsi et al. 2006; Chen et al. 2008; Christiansen et al. 2008; Zhou et al. 2008; Mulligan et al. 2009.

The following disorders co-occur with ADHD in the IMAGE sample: mood disorder (15%), anxiety disorder (44%), oppositional defiant disorder (64%) and conduct disorder (24%) (Muller et al. 2011). Both the mean and median IQs were 98. Children with and without motor problems did not differ according to age, gender and also severity of ADHD symptoms (Fliers et al. 2008).

Motor measures:

Parental Account of Children's Symptoms (PACS) interview.

The PACS, a semi-structured, standardized, investigator-based interview (Taylor et al. 1986), was administered to all parents. To ensure cross-site consistency in measurement and coding, all interviewers attended a 5-day PACS training course in the UK. The sites' chief investigators additionally attended annual inter-rater reliability exercises. The mean kappa coefficient across all sites was high (0.88). The PACS also includes questions regarding motor development of which we analysed the question "does your child have motor coordination problems" , with three possible answers: "no" , "maybe", or "yes definitely" as the primary phenotype for genetic analysis.

Developmental Coordination Disorder Questionnaire (DCD-Q).

In the Dutch participants of IMAGE, we collected additional data on motor performance with the DCD-Q, completed by parents (Fliers et al. 2008). The DCD-Q identifies children with motor coordination problems in daily life and is widely used in international studies (Wilson et al. 2000, 2009; Loh et al. 2009) The Dutch DCD-Q has been validated (Schoemaker et al. 2006). The internal consistency of the questionnaire is high ($\alpha = 0.88$). The DCD-Q contains 17 items that are rated on a five-point scale (1 = not at all like this child; 5 = extremely like this child) and 4 subscales: motor control in motion, fine motor control/handwriting, gross motor control/planning and general coordination. In this study DCD-Q scores (on a continuum) were tested as secondary phenotypes in the genetic analysis of candidate SNPs. We tested 5 traits: the total score on the DCD-Q (range from 17 to 85), and the 4 subscale scores.

Genetic analysis

The IMAGE consortium is a part of the Genetic Association Information Network (GAIN), a public private partnership of FNIH (Foundation for the National Institutes of Health, Inc.) (http:// www.fnih.org). A total of 958 affected proband – parent trios from IMAGE were initially selected for genome-wide genotyping conducted at Perlegen Sciences using their genotyping platform of approximately 600,000 tagging SNPs designed to be in high linkage disequilibrium with untyped SNPs for the HapMap populations. Quality control of the genotype data was performed by NCBI (The National Center for Biotechnology

Information) using the GAIN QA/QC Software Package (version 0.7.4). Details of the genotyping and data cleaning process for the ADHD GAIN study (Study Accession, phs000016.v1.p1) have been reported elsewhere (Neale et al. 2008). Briefly, we selected only SNPs with minor allele frequency (MAF) ≥ 5% and Hardy – Weinberg equilibrium (HWE) *P* ≥ 1.00E-06. Genotypes causing Mendelian inconsistencies were identified by PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) and removed (Purcell et al. 2007).

We additionally removed SNPs that failed the quality control metrics for the other two GAIN Perlegen studies (major depression disorder (dbGAP Study Accession phs000020.v1.p1) and psoriasis (dbGAP Study Accession phs000019.v1.p1)). With this filtering, 384,401 autosomal SNPs were retained in the final dataset. To increase coverage of the genome, we used the imputation approach implemented in PLINK (v1.04), which imputes genotypes of SNPs that are not directly genotyped in the dataset, but that are present on a reference panel. The reference panel used consisted of 2,543,285 polymorphic autosomal SNPs genotyped on the 60 HapMap Caucasian (CEU) founders which are publicly available for download from the HapMap website (HapMap r23 build, http://www.hapmap.org). A threshold of 0.95 confidence level was set for a hard genotype call to be included in association testing. Most likely genotypes for imputed SNPs were then used in association analyses.

Statistical analysis

For statistical analysis, the PACS motor answers "no motor problems" and "possible motor problems" were combined into an " unaffected " category creating a binary outcome variable. We chose this rather strict way of analysis because standard deviations of motor scores were overlapping for the groups "no motor problems" and "possible motor problems" whilst the definitely affected category formed a truly different group (not shown). An ANOVA was performed with the binary PACS trait as independent and DCD-Q total scores as dependent variables to validate the motor question in a sample of 313 Dutch IMAGE participants for whom scores from PACS and DCD-Q were available. Of these, 296 also had complete data for covariates required for the genetic analysis. Association analysis of 890 ADHD probands with motor data was conducted using the logistic procedure implemented in PLINK with the motor variable from PACS as a binary outcome. The analysis was adjusted for age, gender, Conners' hyperactive/impulsive score, Conners' inattentive score and the country in which the motor variable had been measured.

SNPs showing association *P* values < 10.00E-05 in the GWAS were tested for their association with the 4 subscales (fine and gross motor scores, general coordination and control during movement) of the DCD-Q. This association analysis was conducted in 296 Dutch ADHD probands using the linear procedure implemented in PLINK. Each DCD-Q variable was a continuous outcome and the models were adjusted for age, gender, Conners' hyperactive/impulsive score and Conners' inattentive score. In order to control for multiple testing, an extra permutation step was added to the linear test by applying the max(T) permutation approach implemented in PLINK. A total of 10,000 permutations were done for the subset of SNPs passing the *P* value threshold to determine empirical (EMP) *P* values for association.

Bioinformatics analysis

To detect significantly enriched gene functional groups in 97 genes from the GWAS containing at least one SNP

Table 1.
Descriptives of the study population measured with the PACS (n=890) & the DCD-Q (n=313)*

Sample of children with ADHD and PACS (n)	890
Age (years, mean, SD)	10.8 (2.8)
Gender (% male)	85.3
Conners score (mean, SD) hyperactivity/impulsivity	78.8 (10.3)
Conners score (mean, SD) inattentiveness	71.3 (9.0)
Sample of children with DCD-Q scores (n)	313
DCD-Q total score (SD)	53.7 (9.5)
DCD-Q control during movement (SD)	19.9 (5.4)
DCD-Q fine motor (SD)	11.2 (3.2)
DCD-Q gross motor (SD)	13.1 (2.9)
DCD-Q general coordination (SD)	9.6 (2.8)

Table 2.
Comparison PACS and DCD motor affection in 296 children participating in the Dutch part of IMAGE

N children	DCD-Q unaffected	DCD-Q affected
PACS motor-affected	121	83
PACS motor-unaffected	26	66

showing association with the PACS motor variable at $P < 10.00E-04$, we performed functional analyses using Ingenuity Pathway Analysis (http://www.ingenuity.com). In the presentation of the results of these analyses, only gene categories with significant enrichment (i.e. false discovery rate corrected $P < 0.05$) and containing more than one gene were taken into account. The Ingenuity software package uses information from the published literature as well as many other sources, including gene expression and GO (gene ontology) terms data-bases, to assign genes to different groups and categories of functionally related genes. "Ingenuity genes" are assigned to one or more of three groups of gene functional categories, i.e. "diseases and disorders", "canonical pathways" and "physiological systems development and function". Each main category can be further divided into many subcategories (http://www.ingenuity.com).

In this study, we specifically looked at the five top-ranked "diseases and disorders" gene functional categories and subsequently at the five top-ranked subcategories within the "neurological disease" gene functional category. In addition, we looked at the top five "canonical pathways" and "physiological systems development and function" gene functional categories.

The NCBI databases (http://www.ncbi.nlm.nih.gov/sites/entrez/), the UCSC Genome Browser (http://genome.ucsc.edu), the HapMap project website (http://www.hapmap.org) and the website of the Sullivan Lab Evidence Project (http://slep.unc.edu) were used to retrieve information on gene function and prior association of the genes of interest with psychiatric disorders.

Results

A sample of 890 children with ADHD combined subtype had complete data. The mean age of the sample was 10.8 years (SD 2.8, age range 5 – 17 years) and 85.3% was male (see Table I). Of these, 199 children (22.4%) were reported by their parents to have definite motor problems, and 225 (25.3%) were noted with possible motor problems. Scores for the DCD-Q were available for 313 Dutch IMAGE individuals (Table I). Groups based on PACS motor scores showed a significant difference in DCD-Q motor scores, both in total score ($F = 36.89$, $P < 0.001$) and subscale-scores (motor control in motion $F = 16.45$, $P < 0.001$; fine motor control/handwriting $F = 13.93$, $P < 0.001$; gross motor control/planning $F = 14.27$, $P < 0.001$; general coordination $F = 8.40$, $P = 0.004$). Of those children showing definite motor problems in PACS ($n = 92$), 66 children (72%) also scored clinically on the DCD-Q total score (in the lowest 15th percentile of the normal population) (see

Table 3. Top single SNPs with $P < 1.00E-04$ from the GWAS for motor coordination problems in children with ADHD and DCD-Q results. The 24 SNPs showing a significant *P*-value for one of the DCD-Q results are indicated in bold.

chr	SNP	Position (base pair)	*P*-values	position ~ gene	gene	*P*-values DCD-Q control	*P*-values DCD-Q fine motor	*P*-values DCD-Q gross motor	*P*-values DCD-Q general coord
1	**rs6687919**	111198699	9.29E-05	< 20 kb upstream	*CD53*	7.24E-01	9.47E-01	**3.29E-02**	6.02E-01
1	**rs6687898**	111198839	9.29E-05	< 20 kb upstream	*CD53*	7.24E-01	9.47E-01	**3.29E-02**	6.02E-01
1	**rs6690536**	111198974	9.29E-05	< 20 kb upstream	*CD53*	7.24E-01	9.47E-01	**3.30E-02**	6.02E-01
2	**rs17762507**	85247495	1.98E-05	intron	*TCF7L1*	1.09E-01	5.72E-02	4.66E-01	4.44E-01
2	rs6733332	231346384	8.99E-05	intron	*CAB39*	9.42E-01	5.41E-01	9.56E-01	5.40E-01
3	rs6550788	23734941	3.43E-05	< 100 kb upstream	*UBE2E1*	3.78E-01	2.52E-01	2.22E-01	2.29E-01
4	rs12643829	16989235	5.26E-05	< 100 kb upstream	*CLRN2*	3.81E-01	3.21E-01	5.92E-01	4.20E-01
4	rs7442317	29512150	3.62E-06	intergenic	-	3.83E-01	7.65E-01	7.69E-01	6.94E-01
4	rs16882428	29512172	3.62E-06	intergenic	-	3.83E-01	7.65E-01	7.69E-01	6.94E-01
4	rs7690092	29516307	3.62E-06	intergenic	-	3.83E-01	7.65E-01	7.69E-01	6.94E-01
4	rs953797	29523996	3.62E-06	intergenic	-	3.83E-01	7.65E-01	7.69E-01	6.94E-01
4	rs10023178	29526536	3.62E-06	intergenic	-	3.83E-01	7.65E-01	7.69E-01	6.94E-01
4	rs1503966	29538600	1.93E-05	intergenic	-	3.81E-01	1.84E-01	7.49E-01	7.42E-01
4	rs6837917	29558689	7.87E-05	intergenic	-	8.80E-01	1.40E-01	1.07E-01	9.44E-01
4	rs12511112	85895123	9.16E-05	intron	*WDFY3*	2.28E-01	1.09E-01	7.59E-02	8.23E-01
4	rs3098928	85898827	9.16E-05	intron	*WDFY3*	2.28E-01	1.09E-01	7.59E-02	8.23E-01
4	rs6858666	85948960	9.16E-05	intron	*WDFY3*	2.28E-01	1.09E-01	7.59E-02	8.23E-01
4	rs6531775	85949938	9.16E-05	intron	*WDFY3*	2.28E-01	1.09E-01	7.59E-02	8.23E-01
4	rs6835046	85973968	9.16E-05	intron	*WDFY3*	2.28E-01	1.09E-01	7.59E-02	8.23E-01
4	rs2046402	85981409	9.16E-05	intron	*WDFY3*	2.28E-01	1.09E-01	7.60E-02	8.23E-01
4	rs2869216	85984565	9.16E-05	intron	*WDFY3*	2.28E-01	1.09E-01	7.60E-02	8.23E-01
4	**rs11097028**	86088807	5.61E-05	intron	*WDFY3*	8.57E-01	3.08E-01	**4.72E-03**	9.10E-01
4	**rs6820517**	86089649	5.61E-05	intron	*WDFY3*	8.57E-01	3.08E-01	**4.72E-03**	9.10E-01
4	**rs12502559**	86094664	5.61E-05	intron	*WDFY3*	8.57E-01	3.08E-01	**4.72E-03**	9.10E-01
4	rs10012888	182392020	7.21E-05	intergenic	-	5.09E-02	2.98E-01	3.74E-01	4.94E-01
5	**rs10462643**	7720153	8.40E-05	intron	*ADCY2*	4.90E-01	1.16E-01	3.45E-01	**1.92E-02**
5	**rs747243**	7736784	8.40E-05	intron	*ADCY2*	4.90E-01	1.16E-01	3.45E-01	**1.92E-02**
5	**rs1366414**	7743296	8.40E-05	intron	*ADCY2*	4.90E-01	1.16E-01	3.45E-01	**1.92E-02**
5	**rs6895553**	114849566	8.63E-05	<30 kb downstream	*FEM1C*	9.30E-01	**2.27E-02**	2.21E-01	9.16E-01
6	**rs4413658**	2313641	3.37E-05	100 kb upstream	*GMDS*	**3.19E-02**	2.79E-01	4.69E-01	2.58E-01
6	**rs7449538**	2314638	3.37E-05	100 kb upstream	*GMDS*	**3.20E-02**	2.79E-01	4.69E-01	2.58E-01
6	**rs9503158**	2315074	3.37E-05	100 kb upstream	*GMDS*	**3.20E-02**	2.79E-01	4.69E-01	2.58E-01
6	**rs1883587**	2319820	3.37E-05	100 kb upstream	*GMDS*	**3.20E-02**	2.79E-01	4.69E-01	2.58E-01
6	**rs1883588**	2319887	3.37E-05	100 kb upstream	*GMDS*	**3.19E-02**	2.79E-01	4.69E-01	2.58E-01
6	rs4507577	19564453	3.38E-05	intergenic	-	2.76E-01	5.78E-01	3.46E-01	4.19E-01
7	**rs2075000**	150764725	4.99E-05	intron	*CRYGN*	**2.90E-02**	1.55E-01	3.32E-01	5.02E-01
7	**rs12534366**	150769315	5.27E-05	intron	*CRYGN*	**3.43E-02**	1.95E-01	2.53E-01	5.62E-01
7	**rs11766792**	152862485	1.20E-05	intergenic	-	9.08E-01	2.16E-01	9.24E-02	**4.33E-03**
8	rs7819754	16125110	6.75E-05	< 50 kb upstream	*MSR1*	3.06E-01	3.96E-01	3.12E-01	6.64E-01
8	rs10090333	16131941	6.37E-05	< 50 kb upstream	*MSR1*	1.07E-01	3.21E-01	3.94E-01	2.39E-01
8	rs2248010	17460770	1.90E-06	intron	*SLC7A2*	7.00E-02	6.55E-01	2.71E-01	4.29E-01
9	rs13283363	34832242	2.66E-05	< 10 kb upstream	*C9ORF144*	7.86E-01	1.29E-01	7.67E-01	2.48E-01
9	rs12726	35394840	9.45E-05	exon	*UNC13B*	2.78E-01	1.42E-01	2.37E-01	8.92E-01
10	**rs11002745**	80370924	1.98E-05	intergenic	-	6.53E-01	4.89E-01	**4.49E-03**	1.86E-01
10	**rs6480913**	80379260	7.26E-05	intergenic	-	5.13E-01	4.87E-01	**4.93E-02**	3.14E-01
10	rs7092666	125267555	1.01E-05	intergenic	-	1.16E-01	4.38E-01	2.49E-01	7.95E-01
11	rs1393878	13869322	7.27E-05	<100 kb upstream	*SPON1*	2.03E-01	3.23E-01	8.02E-01	8.25E-01
15	rs16951001	65641295	6.78E-05	intron	*MAP2K5*	9.61E-02	9.53E-01	9.42E-01	3.01E-01
15	rs11638507	65661099	6.72E-05	intron	*MAP2K5*	1.42E-01	9.96E-01	7.65E-01	3.24E-01
15	rs17241403	65662816	6.72E-05	intron	*MAP2K5*	1.42E-01	9.96E-01	7.65E-01	3.24E-01
15	rs1878699	65687937	6.72E-05	intron	*MAP2K5*	1.42E-01	9.96E-01	7.65E-01	3.24E-01

15	rs17811219	85564053	2.35E-05	intergenic		6.14E-01	2.01E-01	3.40E-01	3.06E-01
17	rs14003	17045439	5.37E-05	exon	*PLD6*	5.08E-02	2.59E-01	7.66E-01	6.93E-01
17	**rs9894565**	17047909	6.74E-05	exon	*PLD6*	**3.13E-02**	1.43E-01	4.20E-01	9.84E-01
17	**rs1736217**	17068881	6.74E-05	intron	*FLCN*	**3.13E-02**	1.43E-01	4.20E-01	9.84E-01
18	rs4800802	23179814	6.49E-05	intergenic	-	8.48E-01	7.81E-01	4.72E-01	6.69E-01
20	rs4812506	39487624	1.80E-05	intron	*CHD6*	3.25E-01	2.06E-01	9.95E-01	1.51E-01
20	rs761024	39490051	1.98E-05	intron	*CHD6*	3.26E-01	2.30E-01	8.60E-01	2.02E-01
21	**rs2839083**	46268084	8.87E-05	< 20 kb downstream	*COL6A1*	**2.39E-03**	**4.79E-04**	3.00E-01	5.64E-01

Table 4.
Top 5 'diseases and disorders' gene functional categories that are significantly enriched in the top 97 ADHD candidate genes from the GWAS for motor coordination problems in children with ADHD (see Supplementary Table 1) using Ingenuity pathway analysis. The 6 genes containing at least one SNP that yielded a *P*- value < 1.00E-04 (see Table 3) are indicated in bold.

Category	Genes	Significance [a]	Adjusted significance [b]
Cardiovascular disease (35/97 genes)	*ACPP, AKAP6, BMPER, BRUNOL4, C3ORF31, CDH13, CNTN3, CNTNAP2, DAB1, ENPP1, EPB41L4A, FAM130A2, GMDS, MAML2,* ***MAP2K5****, MEF2B, MICAL2, NR3C1, PKD1L2, PKP2, PNPLA7, RBMS3, RELN, RYR2, RYR3, SASH1, SCAPER,* ***SLC7A2****, SORCS3, SOX5, SPAG16, THRB, TMEM132D, TRIO, UNC13B*	5.96E-09	2.68E-06
Neurological disease (45/97 genes)	*ACPP,* ***ADCY2****, ANXA6, ATP6V0A4, BRUNOL4, CAB39, CDH13, CNTNAP2, DAB1, GAD2, GMDS, GPR88, GRM4, MAML2, MICAL2, MLLT3, NF1, NGFB, NR3C1, PIP4K2A, PKD1L2, PLA2G4A, PTPRG, RAG1, RBMS2, RBMS3, RELN, RYR2, RYR3, SCN11A, SLC1A3, SLC35C1, SLC6A1,* ***SLC7A2****, SNX27, SORCS3, SOX5, SPAG16,* ***TCF7L1****, THRB, TMEM132D, TRIO, TRIP12, TUFT1,* ***WDFY3***	3.84E-08	6.57E-06
Endocrine system disorders (31/97 genes)	***ADCY2****, AKAP6, CDH13, CNTN3, CNTNAP2, DAB1, ENPP1, EPB41L4A, FARP2,* ***FLCN****, GMDS, MAML2, ME3, MICAL2, NR3C1, PIP4K2A, PTPRG, RBMS3, RYR2, RYR3, SASH1, SCN11A, SLC6A1, SORCS3, SOX5, SPAG16,* ***TCF7L1****, THRB, TMEM132D, TRIO,* ***WDFY3***	5.36E-06	2.19E-04
Gastrointestinal disease (21/97 genes)	*ACPP, AKAP6, CDH13, CNTNAP2, DAB1, EPB41L4A, GMDS, MAML2,* ***MAP2K5****, MICAL2, NR3C1, PKD1L2, PTPRG, RBMS3, RYR2, SLC6A1, SORCS3, SOX5, TMEM132D, TUFT1,* ***WDFY3***	1.74E-05	5.60E-04
Inflammatory disease (32/97 genes)	*ACPP,* ***ADCY2****, AKAP6, BRUNOL4, CDH13, CNTNAP2, DAB1, ELMOD2, ENPP1, EPB41L4A, FARP2, GAD2, GMDS, MAML2,* ***MAP2K5****, MICAL2, MLLT3, NGFB, NR3C1, PKD1L2, PTPRG, RBMS3, RYR2, RYR3, SCN11A, SLC1A3, SLC6A1, SORCS3, SOX5, SPAG16, TMEM132D,* ***WDFY3***	1.74E-05	5.60E-04

Abbreviations: GWAS, genome-wide association study, ADHD, attention-deficit hyperactivity disorder, SNP, single nucleotide polymorphism, [a] Single test *P*-values [b] Multiple test-corrected *P*-values using the Benjamini-Hochberg correction

Table II). The Spearman correlation between the scores on the motor coordination item of the PACS and the DCD-Q total score was – 0.340 (*P* < 0.001).

A total of 580 SNPs showed association with PACS motor score at *P* values < 0.00E-04. The most significant associ-ation was observed for an intronic SNP in *SLC7A2* (*P*

value = 1.90E-06), 58 additional SNPs showed association *P* values < 10.00E-05 (Table III). Of the 580 PACS-associated SNPs, 174 were located in 97 genes (Supplementary Table 1). Bioinformatics analysis using the Ingenuity program revealed that 45 of the 97 genes from the GWAS fell into the "neurological disease" gene category (P = 6.57E-06; Table IV). These 45 genes were most significantly enriched in 5 subcategories of the "neurological disease" category: "neurodegenerative disorder" (22/97 genes; *P* = 6.57E-06), "progressive motor neuropathy" (23/97 genes; *P* = 2.10E-05), "amyotrophic lateral sclerosis" (15/97 genes; *P* = 5.42E-05) and two psychiatric disorders, "bipolar affective disorder" (19/97 genes; *P* = 7.40E-04) and "schizophrenia" (10/97 genes; *P* =1.01E-02) (Table V).

Other gene functional subcategories found significantly enriched in the 97 top candidate genes were "synaptic long term depression" (6/97 genes; *P* = 1.54E-02) and "nervous system development and function" (6/97 genes; *P* = 4.00E-02) (Table VI).

Further characterization of the 59 SNPs showing *P* values = 10.00E-05 for association with the PACS motor score using a more elaborate measure of motor coordination, the DCD-Q, revealed 15 SNPs associated with different subscales with *P* values < 0.05 (Table III). Permutation testing showed that two SNPs had significant empirical *P* values: rs11002745 for the gross motor scale (EMP *P* = 0.045) and rs2839083 for the fine motor scale (EMP *P* = 0.014). While most DCD-Q subscale associated SNPs influenced only one of the subscales, one SNP near the *COL6A1* gene influenced control during movement and fine motor control (Table III).

Of the 59 SNPs (Table III), 17 were located within exonic, intronic or untranslated regions of nine different genes (see Supplementary Table 1 for information regarding gene function and published association with psychiatric disorders). A comprehensive search of the literature and databases indicated that eight of the nine encoded proteins function in a signalling network that operates in functional processes linked to neurite outgrowth, as recently also implicated in ADHD aetiology (Poelmans et al. 2011).

Interestingly, the same eight proteins are expressed in skeletal muscle, where they play important roles in basic muscle function (see Figure 1 and Supplementary File 1).

Discussion:

This report describes the first GWAS of motor coordination problems. Although none of the associations reached genome-wide significance, i.e. a *P* value ≤ 7.20E-08 (Dudbridge and Gusnanto 2008), the findings are intriguing and can give input to further hypothesis-driven follow-up studies.

The finding that eight of the nine proteins encoded by the top-ranked findings from our GWAS (with *P* values < 10.00E-05) function in a signalling network operating in neurite outgrowth is in line with another recent study of our group finding that 45 of the 85 top-ranked ADHD candidate genes from the five reported GWAS for ADHD are involved in neurite outgrowth (Poelmans et al. 2011). The finding that the same eight genes/proteins are also involved in muscle function is in line with the view that motor coordination problems should not be viewed merely as a neuronal problem. They are related to the whole range of functional processes located in the cerebrum, cerebellum, motor neurons, neuromuscular junctions, muscle sensors and muscle cells. Motor skills are also the result of many different processes such as perceptual, feedback and learning processes, motor preparation

Table 5.
Top 5 gene functional subcategories of the 'neurological disease' category that are significantly enriched in the top 97 candidate genes from the GWAS for motor coordination problems in children with ADHD using Ingenuity pathway analysis. The 4 genes containing at least one SNP that yielded a *P* value ≤ 10.00E-05 are indicated in bold.

Subcategory	Genes	Significance [a]	Adjusted significance [b]
Neurodegenerative disorder (22/97 genes)	***ADCY2***, *ATP6V0A4, CDH13, CNTNAP2, DAB1, GAD2, GMDS, GRM4, MICAL2, NR3C1, PLA2G4A, RELN, RYR2, RYR3, SCN11A, SLC1A3, SLC6A1,* ***SLC7A2***, *SORCS3, TMEM132D, TRIO, TUFT1*	3.84E-08	6.57E-06
Progressive motor neuropathy (23/97 genes)	***ADCY2***, *BRUNOL4, CDH13, DAB1, GAD2, GMDS, MAML2, MLLT3, NF1, NR3C1, PKD1L2, RBMS2, SCN11A, SLC1A3, SLC35C1, SLC6A1, SOX5, SPAG16, THRB, TMEM132D, TRIP12, TUFT1,* ***WDFY3***	3.73E-07	2.10E-05
Amyotrophic lateral sclerosis (15/97 genes)	***ADCY2***, *BRUNOL4, CDH13, DAB1, GAD2, GMDS, RBMS2, SCN11A, SLC1A3, SLC35C1, SLC6A1, SPAG16, TMEM132D, TUFT1,* ***WDFY3***	1.09E-06	5.42E-05
Bipolar affective disorder (19/97 genes)	*ACPP, CDH13, CNTNAP2, DAB1, GAD2, GMDS, GRM4, NR3C1, PIP4K2A, PTPRG, RBMS3, RELN, SCN11A, SLC1A3, SNX27, SOX5,* ***TCF7L1***, *THRB, TMEM132D*	2.64E-05	7.40E-04
Schizophrenia (10/97 genes)	*CNTNAP2, DAB1, GAD2, GRM4, NR3C1, PIP4K2A, PLA2G4A, RELN, SLC6A1, SNX27*	5.78E-04	1.01E-02

Abbreviations : GWAS, genome-wide association study, ADHD, attention-deficit hyperactivity disorder, SNP, single nucleotide polymorphism [a] Single test *P*-values: [b] Multiple test-corrected *P*-values using the Benjamini-Hochberg correction

Table 6.
Top 5 'canonical pathways' (1) and 'physiological system development and function' (2) gene functional categories that are significantly enriched in the top 97 candidate genes from the GWAS for motor coordination problems in children with ADHD using Ingenuity pathway analysis. The ADCY2 gene is indicated in bold because it contains 3 SNPs that yielded a *P*-value ≤ 10.00E-05.

Category	Genes	Significance [a]	Adjusted significance [b]
Synaptic long term depression (1) (6/97 genes)	***ADCY2***, *ADCY6, GRM4, PLA2G4A, RYR2, RYR3*	1.29E-04	1.54E-02
Behaviour (2) (2/97 genes)	*GAD2, NGFB*	5.79E-03	4.00E-02
Embryonic development (2) (3/97 genes)	*EZR, FARP2, SCN11A*	5.79E-03	4.00E-02
Haematological system development & function (2) (2/97 genes)	*GAD2, NGFB*	5.79E-03	4.00E-02
Nervous system development & function (2) (6/97 genes)	*FARP2, GAD2, GRM4, NGFB, SLC1A3, SLC6A1*	5.79E-03	4.00E-02

Abbreviations : GWAS, genome-wide association study, ADHD, attention-deficit hyperactivity disorder, DCD, developmental coordination disorder, SNP, single nucleotide polymorphism. [a] Single test *P*-values. [b] Multiple test-corrected *P*-values using the Benjamini-Hochberg

and movement execution processes. These processes rely on the visual system, memory, attention, the balance system, the kinaesthetic system ("feeling one's body") and the motor effector system (Raynor 2001; Schoemaker et al. 2001; Visser 2003; Geuze 2005; Smits-Engelsman et al. 2008). Any defect in one of these processes or systems may lead to motor coordination problems. Thus, our findings of motor coordination associated genes that are expressed in both nerve tissue and muscle may provide a rationale for further studies of basic muscle function in DCD.

Forty-five of the 97 genes identified by the GWAS (P < 10.00E-04) fell into the "neurological disease" functional gene category. Among the most significantly enriched subcategories were "progressive motor neuropathy" and "amyotrophic lateral sclerosis". Indeed, a relationship between ADHD and amyotrophic lateral sclerosis (ALS), an adult onset, polygenic disease of motor neuron degeneration (Ravits and La Spada 2009; Valdmanis et al. 2009; Van der Graaff et al. 2009), has been hypothesized (Lule et al. 2008).

Many patients developing ALS seem to have fulfilled clinical characteristics of ADHD earlier in their lives. At the neurobiological level, there is evidence for hyperactivity of the glutamatergic system and a dopaminergic hypoactivity in both ADHD and ALS (Lule et al. 2008). Therefore, our finding provides further input to the hypothesis that ADHD may be a risk factor for the development of ALS. However, whether children with ADHD and motor coordination problems might be at a particularly high risk for developing ALS in later life needs to be explored in further studies.

The Ingenuity analysis further showed that the categories "synaptic long term depression" and "nervous system development and function" were significantly enriched in the 97 top-ranked genes. Long-term depression of neurotransmission leads to physical changes in neuronal circuits (Johnston 2009) and this neuronal plasticity allows reorganization of neuronal networks and learning. Given that motor learning disturbances such as difficulties in mastering new motor skills like swimming and riding a bicycle are a hallmark of motor coordination problems in children (Sugden 2007), our results are particularly interesting.

Apart from the enrichment of motor neuropathy and ALS genes in the top-ranked findings from the GWAS, more evidence of genes involved in motor dysfunction is present in our data: *COL6A1* codes for a collagen found in most connective tissues and important in organizing extracellular matrix components. Mutations in this gene are known to cause motor problems in Bethlem myopathy and Ullrich scleroatonic muscular dystrophy (Lampe and Bushby 2005; Baker et al. 2007; Nadeau et al. 2009). Several patients with autosomal recessive myosclerosis also have mutations in this gene (Merlini et al. 2008). The *MAP2K5* gene, a member of the mitogen-activated protein kinase family, has been consistently associated with restless legs syndrome (RLS) – a neurological disorder characterized by uncomfortable and unpleasant sensations in the legs that occur at rest and induce an irresistible desire to move the legs – in GWAS (Winkelmann 2008; Kemlink et al. 2009; Trenkwalder et al. 2009). A large population-based study has reported a prevalence of RLS of 2% in children and adolescents without ADHD (Picchietti and Picchietti 2008), whereas up to 44% of children with ADHD have symptoms of RLS (Cortese et al. 2005). Several authors have already suggested that RLS and ADHD

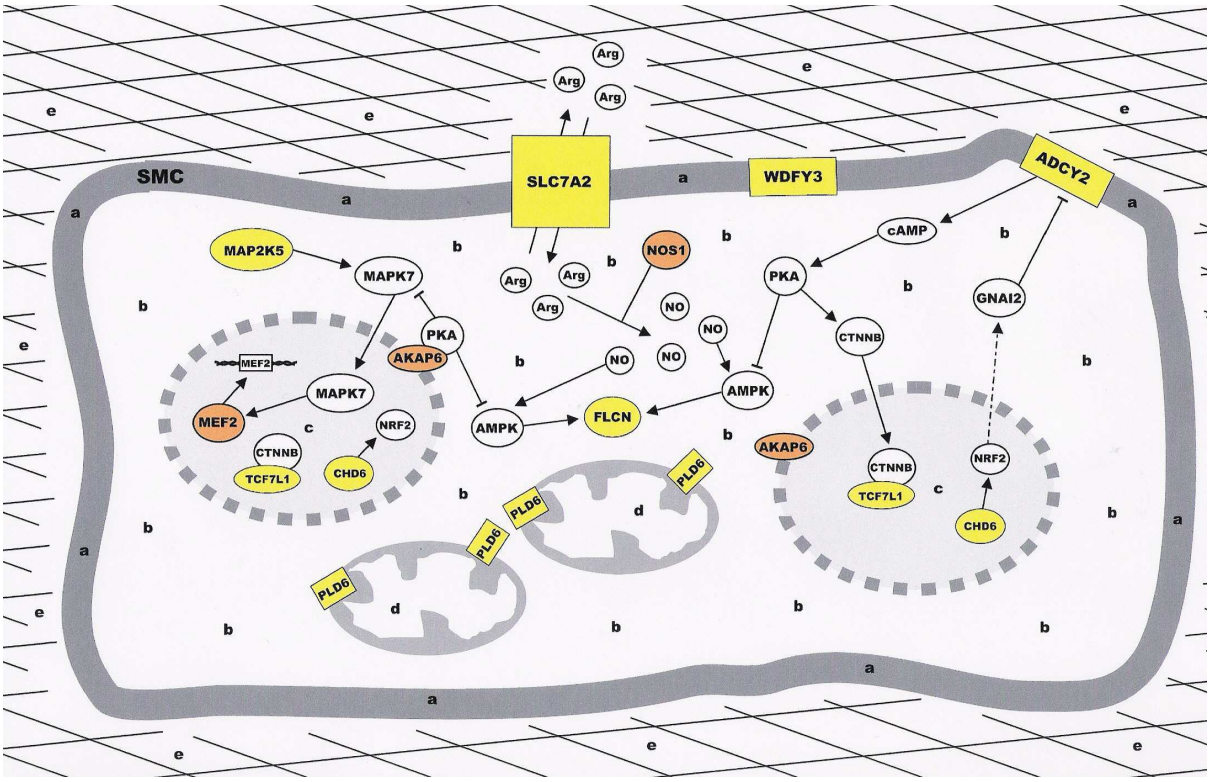

Figure 1: Schematic representation of the function of a gene/protein network potentially contributing to motor coordination problems in children with ADHD by influencing skeletal muscle cell (SMC) function. The eight proteins encoded by genes containing at least one SNP yielding a *P* value < 1.00E-04 in the GWAS for motor coordination problems in children with ADHD are indicated in yellow. The proteins that are encoded by *AKAP6*, *MEF2B* - two genes that contain at least one SNP associated at *P* < 1.00E-03 (Supplementary Table 1) - and *NOS1* - a gene found associated with ADHD in the GWAS by Lasky-Su et al. - are indicated in orange. A more elaborate description of the network can be found in Supplementary File 1.

a : cell membrane ; **b :** cytoplasm ; **c :** nucleus ; **d :** mitochondrion ; **e :** extracellular matrix/compartment

share common risk genes (Schimmelmann et al. 2009; Reif 2010). Lastly, *CHD6* , one of our other main findings, is linked to motor behaviour, as a deletion of exon 12 of this gene leads to motor coordination problems in a mouse model (Lathrop et al. 2010).

The association analysis of the candidate genes with the DCD-Q subscales provided insight into the sources of motor impairment at an additional level, in that it allowed us to characterize the movement "domain" that was influenced by the genetic variants identified. For 15 out of 59 tested SNPs, we found DCD-Q associations with *P* values < 0.05. The intergenic SNP rs11002745, located on chromosome 10, and SNP rs2839083, located 18.7 kb downstream of the *COL6A1* gene on chromosome 21, survived multiple testing correction. The former SNP showed association with gross motor problems, the latter SNP was associated with fine motor problems and control during movement. As children with motor coordination problems show a heterogeneous phenotype, with some of them being mainly disturbed in fine and others in gross motor performance (Polatajko and Cantin 2005; Green et al. 2008), it is not surprising that we find these different associations.

This is the first GWAS of motor coordination problems and should be viewed as only a first step in identifying genetic factors contributing to these

problems. Our study was underpowered, even though we collected a large sample of children with motor coordination problems in which we tried to maximize genetic homogeneity of the motor coordination problems by just focusing on children with ADHD. Another potential limitation of our study is the sparseness of the motor assessment in the international IMAGE sample, with only one question pertaining to motor problems in the PACS. Recognizing this, we chose a conservative approach in pooling the unaffected and possibly affected individuals together as non-affected, which has probably reduced the power of our study. Still, the affected group might show different types of motor problems, as is also suggested by the fact that 28% of people scoring positive for motor problems on PACS scored negative on the more extensive DCD-Q.

The overall correlation of the PACS item with the total DCD-Q score was thus modest, which supports the validity of the PACS item but also indicates that this item and the DCD-Q measure somewhat different movement problems. In addition, it would have been preferable to use objective motor tests in our study. However, these tests are time-consuming, expensive and less compatible with testing large samples of children, as was done in our study. Nevertheless, the substantial evidence of the involvement of the genes from the top-ranks of this GWAS in other movement disorders strongly validates our approach.

Replication studies in independent samples are necessary to confirm or refute the presented results. In addition, replication of these findings is needed in samples of children with DCD who were not primarily selected as suffering from ADHD. However, despite extensive efforts from our side to find such samples, at the current time, they do not seem to be available in the international research community.

Taken together, our findings raise the intriguing possibility that motor coordination problems are associated with genes expressed in both nerve tissue and skeletal muscle.

Statement of Interest

Authors Fliers, Arias Vasquez, Poelmans, Rommelse, Altink, Buschgens, Ebstein, Gill, Miranda, Mulas, Oades and Franke declare no conflicts of interest.

Philip Asherson has been a consultant to/member of advisory board of/and/or speaker for Janssen Cilag, Eli Lilly, Shire and Flynn Pharma in the last 3 years. He has a research grant funded by Shire and an educational grant from Janssen-Cilag. He is not an employee of any of these companies. He is not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents, and royalties.

Tobias Banaschewski served as an advisor or consultant for Desitin, Lilly, Medice, Novartis, Pfizer, Shire, UCB, and Viforpharma. He received conference attendance support and conference support or received speaker's fee by Lilly, Janssen McNeil, Medice, Novartis, Shire, and UCB. He is or was involved in clinical trials conducted by Lilly, Shire and a study on ADHD care management conducted by Novartis. He is not an employee of any of these companies. He is not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents, and royalties. The present study is unrelated to the above grants and relationships, and there are no conflicts of interest of any type concerning this article.

Herbert Roeyers has served as an advisor to Shire and received research support from Shire and Lilly and conference attendance support from Lilly. The present study is unrelated to these relationships.

Aribert Rothenberger has been a consultant to/member of Advisory Board and/ or Speaker for Lilly, Shire, Medice, Novartis, and UCB. He got Research Support from Shire, German Research Society, Schwaabe and Travel Support as well as an Educational Grant from Shire. He is not an employee of any of these companies. He is not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents, and royalties.

Joseph A Sergeant has been on the advisory board of Lilly and Shire, has received research grants from Lilly and speaker's fees from Shire, Lilly, Janssen Cilag and Novartis.

Edmund J S Sonuga-Barke has served on the speakers' bureau and as a consultant for Shire and UCB. He has received research support from Janssen Cilag, Shire, Flynn and Qbtech. He has served on the advisory board for Shire, Flynn, UCB, and Astra Zeneca. He has received conference support from Shire.

Hans-Christoph Steinhausen has worked as an advisor and speaker for the following pharmaceutical companies: Janssen-Cilag, Eli Lilly, Novartis, Medice, Shire, and UCB. He has received unrestricted grants for postgraduate training courses or conferences and research by Janssen-Cilag, Eli Lilly, Novartis, Medice, and Swedish Orphan International.

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Chief Investigators at each site are Rafaela Marco, Nanda Rommelse, Wai Chen, Henrik Uebel, Hanna Christiansen, Ueli Mueller, Cathelijne Buschgens, Marieke Altink, Barbara Franke, Lamprini Psychogiou. We thank all the families who kindly participated in this research. The genetic dataset used for the analyses described in this manuscript was obtained from the dbGaP Database through dbGaP accession number phs000016.v1.p1. Statistical analyses were carried out on the Genetic Cluster Computer http//www.geneticcluster.org), which is financially supported by the Netherlands Scientific Organization (NWO 480-05-003).

Supplementary material available online (and below):

Supplementary Table 1. List of 97 genes harbouring at least one SNP located in an exonic, intronic or untranslated region of the gene and with association at $P < 10.00E-04$ (after correction for multiple testing).

Supplementary Table 2. Additional information about the nine genes harbouring SNPs with $P < 10.00$ E-05 from the GWAS for motor coordination problems in children with ADHD.

Supplementary File 1. Description of the identified gene/protein network potentially contributing to motor coordination problems in children with ADHD

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Supplementary file 1

Signalling through the proposed network can be initiated at the skeletal muscle cell membrane (**Fig.1a**) by ADCY2 (adenylate cyclase 2), a cell membrane protein (Uniprot Consortium 2010) that is also found in the cytoplasm and that is expressed in skeletal muscle and brain (Sunahara and Taussig 2002). ADCY2 is activated by the binding of hormones such as dopamine and prostaglandin to cell surface receptors which interact with intracellular G proteins (Sunahara and Taussig 2002) (not shown in Fig. 1).

ADCY2 produces cAMP (Jones and Kuhar 2006), which subsequently activates PKA (protein kinase A) (**Fig.1b**) and can be negatively regulated by the inhibitory G protein subunit GNAI2 (Grishina and Berlot 1997). In skeletal muscle, PKA activates the transcription factor function of catenin beta (CTNNB) (Hino et al. 2005) and inhibits AMPK (Djouder et al. 2010) and MAPK7 (Pearson et al. 2006) (see below) (**Fig.1b**). PKA is targeted to the proteins it activates or inhibits by the PKA anchoring protein AKAP6 (**Fig.1c**), expressed in the nucleus membrane of skeletal muscle and brain cells, and encoded by *AKAP6* (Dodge-Kafka and Kapiloff 2006; Uniprot Consortium 2010), one of the 97 candidate genes containing at least one GWAS SNP with $P < 10.00E-04$ (see Supplementary Table 1). CTNNB can also be bound and trans-activated by TCF7L1, another transcription factor (Uniprot Consortium 2010) (**Fig.1c**). In the nucleus of skeletal muscle cells, CTNNB functions as a transcription factor (**Fig.1c**) that promotes the self-renewal of these cells (Perez-Ruiz et al. 2008). The kinase MAPK7 (also known as: ERK5), which is highly expressed in brain and skeletal muscle (**Fig.1b**), is activated by MAP2K5 (Uniprot Consortium 2010). MAP2K5 is itself a cytoplasmic kinase (**Fig.1b**) that is expressed in many tissues, including skeletal muscle - where it is particularly abundant - and brain (Uniprot Consortium 2010), and that is activated in a signalling cascade downstream of IGF-2, a growth factor that initiates important signals in myogenesis (Carter et al. 2009). Upon activation, MAPK7 translocates to the nucleus where it activates/phosphorylates transcription factors of the MEF2 (myocyte enhancer factor 2) protein family (Uniprot Consortium 2010) (**Fig.1c**).

The MEF2 proteins, in turn, up-regulate the transcription and expression of numerous muscle specific genes by specifi cally binding to the MEF element/domain in these genes (Uniprot Consortium 2010) (**Fig.1c**). In this way, the MEF2 proteins are e.g. involved in skeletal muscle glucose uptake by up-regulating the expression of the GLUT4 glucose transporter (Zorzano et al. 2005; Lira et al. 2007; Wright 2007).

Another ubiquitously expressed transcription factor in the network is CHD6 (Uniprot Consortium 2010). CHD6 activates NRF2 (Nioi et al. 2005), another transcription factor that is also widely expressed, with highest expression in (adult and foetal) muscle (Uniprot Consortium 2010) (**Fig.1c**). NRF2 up-regulates the expression of *GNAI2*, the negative regulator of ADCY2 (see above), by trans-activating the *GNAI2* promoter (Arinze and Kawai 2005).

Also contributing to the network is SLC7A2, a membrane transporter (**Fig.1a**) for the cationic amino acids (arginine, lysine and ornithine) (Uniprot Consortium 2010) that is (highly) expressed in skeletal muscle (Uniprot Consortium 2010) and brain (Colton et al. 2006). One of the major products of intracellular arginine is nitric oxide (NO) (**Fig.1b and Fig.1e**), which is synthesized by the NOS1 enzyme in skeletal muscle (Grozdanovic 2001; Harris et al. 2008) (**Fig.1b**). NO stimulates the expression of the *GLUT4* glucose transporter in skeletal muscle through AMPK and MEF2 proteins (Zorzano et al. 2005; Lira et al. 2007; Wright 2007) (**Fig.1b and Fig.1c**). *FLCN* encodes the cytoplasmic folliculin (**Fig.1b**), which is expressed in many tissues including skeletal muscle and brain (Uniprot Consortium 2010) and is directly activated by the AMPK kinase (Wang et al. 2010). FLCN is also directly involved in mTOR kinase signalling pathways (not shown in Fig.1), which are important for skeletal muscle protein synthesis and hence skeletal muscle mass (Fujita et al. 2007; Uniprot Consortium 2010).

In addition to the network involved in muscle maintenance and function as described above, two additional genes from the top findings play a role in muscle function: PLD6 is a protein that is located in the (outer) membrane of mitochondria (**Fig.1b** and **Fig.1d)**.

It induces mitochondrial fusion through the formation of a dimer with a PLD6 protein on the outer membrane of a nearby mitochondrion (Choi et al. 2006).

WDFY3 is highly expressed in skeletal muscle and brain and encodes WD repeat and FYVE domain containing protein 3, a membrane protein that targets cytosolic protein aggregates for autophagic degradation (Simonsen et al. 2004). Both mitochondrial fusion (Zorzano 2009; Ding et al. 2010; Zorzano et al. 2010) and autophagic degradation (Raben et al. 2009; Schoser 2009) play important roles in (ab) normal skeletal muscle function.

Importantly, most of the genes and signalling cascades described above, and most notably the GNAI2-ADCY2-PKA-CTNNB (Grishina and Berlot 1997; Sunahara and Taussig 2002; Hino et al. 2005; Votin et al. 2005; Jones and Kuhar 2006) and MAP2K5-MAPK7-MEF2 (Li et al. 2001; Liu et al. 2003; Lam and Chawla 2007) cascades and NRF2 (Kosaka et al. 2010) also function in neurite outgrowth (Poelmans et al. 2011).

Supplementary Table 1. List of 97 genes harboring at least one SNP located in an exonic, intronic or untranslated region of the gene and with association at P ≤ 10.00E-04 (after correction for multiple testing)

Gene	Full name
A2BP1	ataxin-2-binding protein 1
ACACA	acetyl-coenzyme A carboxylase alpha
ACPP	acid phosphatase, prostate
ADCY2	adenylate cyclase 2
ADCY6	adenylate cyclase 6
AKAP6	A kinase anchor protein 6
ANXA6	annexin A6
ARMC3	Armadillo repeat containing 3
ATP6V0A4	ATPase, H+ transporting, lysosomal V0 subunit a4
BFSP1	beaded filament structural protein 1, filensin
BMPER	BMP binding endothelial regulator
BRUNOL4	bruno-like 4, RNA binding protein (Drosophila)
C3orf31	chromosome 3 open reading frame 31
C8A	complement component 8, alpha polypeptide
CAB39	calcium binding protein 39
CAPN9	calpain 9
CDH13	cadherin 13, H-cadherin
CHD6	chromodomain helicase DNA binding protein 6
CNTN3	contactin 3
CNTNAP2	contactin associated protein-like 2
CRYGN	crystallin, gamma N
DAB1	disabled homolog 1 (Drosophila)
DCPS	decapping enzyme, scavenger
DIP	encodes mitochondrial protein DIP
ELMOD2	ELMO/CED-12 domain containing 2
ENPP1	ectonucleotide pyrophosphatase/phosphodiesterase 1
EPB41L4A	erythrocyte membrane protein band 4.1 like 4A
EZR	ezrin
FAM130A2	cysteine-serine-rich nuclear protein 3
FAM155A	family with sequence similarity 155, member A
FARP2	FERM, RhoGEF and pleckstrin domain protein 2
FLCN	folliculin
FLJ45455	-
FLJ45994	-
GAD2	glutamate decarboxylase 2 (pancreatic islets and brain, 65kDa)
GMDS	GDP-mannose 4,6-dehydratase
GORASP1	golgi reassembly stacking protein 1, 65kDa
GPR88	G protein-coupled receptor 88
GRM4	glutamate receptor, metabotropic 4
LCMT2	leucine carboxyl methyltransferase 2
LIPA	lipase A, lysosomal acid, cholesterol esterase
LRRC50	leucine rich repeat containing 50
MACROD2	MACRO domain containing 2
MAML2	mastermind-like 2 (Drosophila)
MAP2K5	mitogen-activated protein kinase kinase 5
ME3	malic enzyme 3, NADP(+)-dependent, mitochondrial
MEF2B	myocyte enhancer factor 2B
MICAL2	microtubule associated monoxygenase, calponin and LIM domain containing 2
MLLT3	myeloid/lymphoid or mixed-lineage leukaemia; translocates to, 3
MYO1B	myosin IB
NCKAP1L	NCK-associated protein 1-like

NF1	neurofibromin 1
NGFB	nerve growth factor (beta polypeptide)
NIP30	-
NR3C1	nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
PIP4K2A	phosphatidylinositol-5-phosphate 4-kinase, type II, alpha
PKD1L2	polycystic kidney disease 1-like 2
PKP2	plakophilin 2
PLA2G4A	phospholipase A2, group IVA (cytosolic, calcium-dependent)
PLD6	phospholipase D6
PNPLA7	patatin-like phospholipase domain containing 7
PTPRG	protein tyrosine phosphatase, receptor type, G
PTPRQ	protein tyrosine phosphatase, receptor type, Q
RAG1	recombination activating gene 1
RBMS2	RNA binding motif, single stranded interacting protein 2
RBMS3	RNA binding motif, single stranded interacting protein
RELN	reelin
RNF20	ring finger protein 20
RYR2	ryanodine receptor 2
RYR3	ryanodine receptor 3
SASH1	SAM and SH3 domain containing 1
SCAPER	S-phase cyclin A-associated protein in the ER
SCN11A	sodium channel, voltage-gated, type XI, alpha subunit
SH3D19	SH3 domain containing 19
SLC1A3	solute carrier family 1 (glial high affinity glutamate transporter), member 3
SLC35C1	solute carrier family 35, member C1
SLC6A1	solute carrier family 6 (neurotransmitter transporter, GABA), member 1
SLC7A2	solute carrier family 7 (cationic amino acid transporter, y+ system), member 2
SNX27	sorting nexin family member 27
SORCS3	sortilin-related VPS10 domain containing receptor 3
SOX5	SRY (sex determining region Y)-box 5
SPAG16	sperm associated antigen 16
ST8SIA4	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 4
SVOPL	SVOP-like
TBCA	tubulin folding cofactor A
TCF7L1	transcription factor 7-like 1
THRB	thyroid hormone receptor, beta
TMEM132D	Trans-membrane protein 132D
TMPRSS5	Trans-membrane protease, serine 5
TNRC4	trinucleotide repeat containing 4
TRIO	triple functional domain (PTPRF interacting)
TRIP12	thyroid hormone receptor interactor 12
TUFT1	tuftelin 1
TYW1B	tRNA-yW synthesizing protein 1 homolog B (S. cerevisiae)
UCP1	uncoupling protein 1 (mitochondrial, proton carrier)
UNC13B	unc-13 homolog B (C. elegans)
WDFY3	WD repeat and FYVE domain containing 3

Supplementary Table 2. Additional information about the nine genes harbouring SNPs with $P \leq 10.00E-05$ from the GWAS for motor coordination problems in children with ADHD.

Gene	Full name	Locus	Gene description	Involvement in relevant disorders *
SLC7A2	solute carrier family 7 member 2	8p22	encodes a membrane transporter for the cationic amino acids (arginine, lysine and ornithine); highly expressed in brain and skeletal muscle	-
CHD6	chromodomain helicase DNA binding protein 6	20q12	encodes an ubiquitously expressed transcription factor	lies within linkage region for autism (NPL 5.56, *P*=2.9E-7) (Allen-Brady et al., 2008); was found in GWAS for SCZ (*P* =0.0004771) (Sullivan et al., 2008); deletion of exon 12 causes motor coordination problems in the mouse (Lathrop et al., 2010)
TCF7L1	transcription factor 7-like 1	2p11.2	encodes transcription factor that is involved in the self-renewal of skeletal muscle cells	lies within linkage region for eating disorder (LOD 2.22; *P* =0.0007) (Devlin et al. 2002)
CRYGN	crystallin, gamma N	7q36.1	encodes an eye lens protein, is very probably a pseudogene	lies within linkage meta-analysis region for autism (HEGESMA 3.9, *P* =0.0027) (Trikalinos et al., 2005); was found in GWAS for depression (Muglia et al., 2009)
PLD6	phospholipase D6	17p11.2	encodes a protein that is located in the mitochondrial membrane and that is involved in mitochondrial fusion	-
WDFY3	WD repeat and FYVE domain containing 3	4q21.23	encodes a protein with WD repeats and a FYVE domain; highly expressed in brain and skeletal muscle	lies within linkage region for SCZ (NPL 2.00) (Faraone et al., 2006); bipolar disorder (LOD 2.00) (McAuley et al., 2008)
MAP2K5	MAP kinase kinase 5	15q23	encodes the dual specificity mitogen-activated protein kinase kinase 5; expressed in brain and skeletal muscle	related to Restless Legs Syndrome (Schimmelmann et al., 2009)
FLCN	folliculin	17p11.2	encodes a protein that is expressed in many tissues including brain and skeletal muscle	-
ADCY2	adenylate cyclase 2	5p15.31	encodes a cell membrane protein that is expressed in brain and skeletal muscle	-

* from SLEP (Sullivan Lab Evidence Project) website and literature

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