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

Short title: Genome-wide association study of motor coordination problems

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Abstract

Objectives:

Motor coordination problems are frequent in children with attention deficit/hyperactivity disorder (ADHD). We performed the first 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 study (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 involved in motor neuropathy and Amyotrophic Lateral Sclerosis (ALS). Genes involved in neurite outgrowth and basic muscle function 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 the top-ranked findings using DCD-Q subscales found nominal association for 15 SNPs.

Conclusions:

Our findings provide clues about the etiology of motor coordination problems, but replication studies in independent samples are necessary.

Key words: motor coordination problems, ADHD, genome-wide association study (GWAS), bioinformatics analysis, neurite outgrowth, 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 marked 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 maturation in the brain 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). Only one study has formally examined the heritability of DCD in a population-based twin study (Martin et al. 2006) and 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, all of small effect, thought to cause the disorder together or in interaction with unfavorable 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 found that of children with ADHD, 30 to 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 a considerable familial component involved in motor coordination problems of 0.47 as measured by the Developmental Coordination Disorder Questionnaire (DCD-Q) in sib pairs (Fliers et al. 2009), little is known about the specific genetic factors involved. Since more knowledge about genetic factors involved in motor coordination problems may help to better understand their etiology, we set out to perform a hypothesis-generating genome-wide association study (GWAS) to search for DNA variation contributing to the condition. GWA 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) are tested for association with a disease. This method has revolutionized the search for genetic influence on complex traits such as ADHD, in which both genetic and environmental factors work together. GWAS build on the mapping of SNPs, that are transmitted in blocks over the generations. This way one particular SNP is able to capture the majority of SNP variation in a block. Recent technology now allows reliable genotyping of up to 1 million SNPs in a single person. 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, phenotypic information on motor problems and genome-wide genotyping data were available for 890 children from the IMAGE study. 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.

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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. ADHD Diagnosis was based on DSM-IV criteria using both parent and teachers questionnaires and standardized interviewing. Instruments used were the SDQ, Conners P and T long version, and Parental Account of Children's Symptoms (PACS) interview

Exclusion criteria applying to all children included an IQ <70, known genetic syndromes (Down, Turner, Fragile X), brain disorders, for example periventricular hemorrhage, cerebral palsy and epilepsy, autism, seizures , current or in the past, as well as all disorders with symptoms potentially mimicking ADHD. Additional details about the clinical characteristics and the diagnostic process of this sample have been described earlier (Brookes et al. 2006; Kuntsi et al. 2006; Chen et al. 2008; Christiansen et al. 2008; Zhou et al. 2008; Mulligan et al. 2009). Briefly, co-occurring disorders were the following: Mood disorder: 23.5% (n 69), Anxiety disorder: 52.7% (n 155), ODD: 56.5% (n 166), Conduct disorder : 18.7% (n 55). Mean IQ was 98. Children with and without motor problems did not differ according to age, gender and also severity of ADHD symptoms (Fliers, 2008). In case of the use of medication parents were asked to report on their children's behavior without medication.

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Verwijderd: Mood disorder : 19.5%, Anxiety disorder: 53.6%, ODD: 54.5%, Conduct disorder : 19.6%

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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. In order to ensure cross-site consistency in measurement and coding of the PACS all interviewers from each site attended a 5 day PACS training course in the UK. The chief investigator at each site attended an annual inter-rater reliability exercise. A mean Kappa coefficient across all sites was 0.88 indicating a substantial level of interrater agreement. The PACS covers DSM-IV symptoms of ADHD, conduct disorder, oppositional defiant disorder, anxiety, mood, and other internalizing disorders. Moreover, questions regarding motor development are included. For this specific study, we analysed the question "does your child have motor coordination problems", with 3 possible answers: "no", "maybe", or "yes definitely" as the primary phenotype for genetic analysis.

Verwijderd: Interviewers were all trained in the United Kingdom and inter-rater reliability tests were performed regularly during the period of data collection in all participating countries.

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Developmental Coordination Disorder Questionnaire (DCD-Q)

In the Dutch participants of IMAGE, we collected additional data on motor performance by means of 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 5-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 were tested as secondary phenotypes in the genetic analysis of candidate SNPs. The scores were used on a continuum. We tested five traits: the total score on the DCD-Q (range from 17 to 85), and the four 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.) that currently involves NIH, Pfizer, Affymetrix, Perlegen Sciences, Abbott, and the Eli and Edythe Broad Institute (of MIT and Harvard University) (http://www.fnih.org). A total of 958 affected proband-parent trios from IMAGE were initially selected for a GWAS. Genotyping was conducted at Perlegen Sciences using their genotyping platform, which comprises approximately 600,000 tagging single –nucleotide polymorphisms (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) developed by Gonçalo Abecasis and Shyam Gopalakrishnan at the University of Michigan. 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) $P \geq$ 1.00E-06. Genotypes causing Mendelian inconsistencies were identified by PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) and removed (Purcell et al. 2007). PLINK is the name of a tool that offers a powerful, user-friendly performance of many common analyses with whole-genome data.

We additionally removed SNPs that failed the quality control metrics for the other two GAIN Perlegen studies (for 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 in the targeted genomic areas, 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 PLINK algorithm is an extension of multimarker tagging. The reference panel used consisted of 2,543,285 polymorphic autosomal SNPs genotyped on the 60 HapMap CEU founders which are publicly available for download from the HapMap website (Caucasian sample included in the HapMap r23 build, http://www.hapmap.org). A threshold of 0.95

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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 both PACS and DCD-Q were available. *A total of 296 children out of these 313 had complete data for all covariates and were included in the 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 was measured.

Verwijderd: Due to missing covariate data we analyzed 296 of these 313 children.

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SNPs showing association *P*-values < 10.00E-05 in the GWAS were tested for their association with the four subscales (fine and gross motor scores, general coordination and control during movement) of the DCD-Q. This association analysis was conducted in 313 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 10000 permutations were done for the subset of SNPs passing the *P*-value threshold to determine empirical (EMP) *P*-values for association.

Bioinformatics analysis

In order to detect significantly enriched gene functional groups in 97 genes from the GWAS containing at least one SNP showing association with the PACS motor variable at $P < 10.00E-04$, we performed functional analyses using the Ingenuity Pathway Analysis software package (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 databases, to assign genes to different groups and categories of functionally related genes. Broadly speaking, '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 of these categories can be further divided into many subcategories (http://www.ingenuity.com). In this study, we specifically looked at the 5 top-ranked 'diseases and disorders' gene functional categories and subsequently at the 5 top-ranked subcategories within the 'neurological disease' gene functional category. In addition, we looked at the top 5 '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 find information on gene function and prior association of the genes of interest with psychiatric disorders.

Results

A sample of 890 children with ADHD combined type had complete data for the PACS interview including information on motor development and had valid genotyping data. The mean age of the sample

was 10.8 years (SD 2.8, age range 5 to 17 years) and 85.3% was male (see Table 1). A total of 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 1). 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 in scores of the subscales (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 15^{th} percentile of the normal population), see Table 2. 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 the PACS motor scores at *P*-values < 10.00E-04. The most significant association was observed for a SNP in an intron of *SLC7A2* (*P*-value = 1.90E-06), 58 additional SNPs showed association *P*-values < 10.00E-05 (Table 3). Of the 580 PACS-associated SNPs, 174 were located in 97 genes (Supplementary Table 1). Bioinformatics analysis using the Ingenuity pathway program revealed that 45 of the 97 primary genes from the GWAS fell into the *'neurological disease'* gene category ($P = 6.57E-06$; Table 4). These 45 genes were most significantly enriched in five 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 5).

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 6).

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 with *P*-values < 0.05 that were associated with different subscales (Table 3). 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 3).

Of the 59 SNPs (Table 3), 17 were located within exonic, intronic or untranslated regions of nine different genes (see Supplementary Table 2 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 etiology (Poelmans et al., submitted). 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 44 of the 85 top-ranked ADHD candidate genes from the five reported GWAS for ADHD are involved in neurite outgrowth (Poelmans et al., submitted).

The finding that the same eight genes/proteins are also involved in muscle function is particularly intriguing. 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 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.

The bioinformatics analysis revealed that 45 of the 97 primary genes from 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'*. Interestingly, 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 recently been hypothesized (Lule et al. 2008). The authors argue that many patients developing ALS fulfilled clinical characteristics of ADHD in earlier years of 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, Lule et al. hypothesized that clinical features of ADHD may be a risk factor for the development of ALS, and our finding from the Ingenuity pathway analysis may provide further input to this hypothesis.

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 functional categories '*synaptic long term depression*' and *'nervous system development and function'* were significantly enriched in the 97 top-ranked genes. It has

been shown that long-term depression of neurotransmission leads to physical changes in neuronal circuits (Johnston 2009). Moreover, it is this neuronal plasticity that 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.

In addition to 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 have also shown mutations in this gene (Merlini et al. 2008). Another interesting finding was the association of motor coordination problems with the *MAP2K5* gene, a member of the mitogen-activated protein kinase family. Previously, this gene has been consistently associated with Restless Legs Syndrome (RLS) in GWAS (Winkelmann 2008; Kemlink et al. 2009; Trenkwalder et al. 2009). RLS is a neurologic disorder characterized by uncomfortable and unpleasant sensations in the legs that occur at rest, usually at night, and induce an irresistible desire to move the legs. A large population-based study has recently 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 suggested that RLS and ADHD share common risk genes (Schimmelmann et al. 2009; Reif 2010). In this light, our finding of the *MAP2K5* gene being associated with motor coordination problems in children with ADHD is interesting.

A recent finding also links the *CHD6* gene, one of our other main findings, 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 (i.e. fine and gross motor scores, general coordination and control during movement) provided insight into the sources of motor

impairment at an additional level. In that way, we were able 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 as well as 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.

Since this is the first GWAS of motor coordination problems, it is only a first step in identifying genetic factors contributing to these problems. Our study was also underpowered, even though we collected a large sample of children with motor coordination problems in which we tried to increase genetic homogeneity of the motor coordination problem by focusing on children with ADHD only.

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 on the one hand supports the validity of the PACS item but on the other hand 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.

Taken together, our findings raise the intriguing possibility that motor coordination problems are associated with genes expressed in both nerve tissue and skeletal muscle. Replication studies in independent samples are necessary to confirm or refute the presented results. 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.

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Conflicts of interest

As for conflicts of interest and financial disclosures, authors Fliers, Arias Vasquez, Poelmans, Rommelse, Altink, Buschgens, Gill, Miranda, Oades and Franke declare none.

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Table 1. Descriptives of the study population measured with the PACS (n=890) and 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-Q motor affection in 296 children participating in the Dutch part of IMAGE

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

Verwijderd: un

Table 3. Top single SNPs with *P* < 10.00E-05 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 < 10.00E-05 (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 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
Hematological system development and function (2) (2/97 genes)	*GAD2, NGFB*	5.79E-03	4.00E-02
Nervous system development and 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 correction

Figure legends

Figure 1: Schematic representation 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 < 10.00E-05 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* < 10.00E-04 (Supplementary Table 1) - and *NOS1* - a gene found associated with ADHD in the GWAS by Lasky-Su et al. (Lasky-Su et al. 2008) - 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