

# Association of Carboxylesterase 1 Gene (CES1) Polymorphism with Weight loss in Children with Attention Deficit Hyperactivity Disorder during Methylphenidate Treatment

Oxenbøll Maria<sup>1,2</sup>, Kaalund-Jørgensen Kristine<sup>1</sup>, Rasmussen Simone<sup>1</sup>, Bjerre Ditte<sup>3</sup>, Jürgens Gesche<sup>4</sup>, Hansen Ebba Holme<sup>2</sup>, Plessen Kerstin Jessica<sup>1</sup>, Rasmussen Henrik Berg<sup>3</sup>, Anne Katrine Pagsberg<sup>1\*</sup> and The INDICES Consortium<sup>5</sup>

<sup>1</sup>Child and Adolescent Mental Health Center, Mental Health Services Capital Region of Denmark and Department of Clinical Medicine, University of Copenhagen, Denmark

<sup>2</sup>Department of Pharmacy, Section for Clinical and Social Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

<sup>3</sup>Copenhagen University Hospital, Capital Region of Denmark, Mental Health Centre Sct. Hans, Institute of Biological Psychiatry, Institute of Biological Psychiatry, Copenhagen University Hospital, Denmark

<sup>4</sup>Roskilde University Hospital, Unit of Clinical Pharmacology, Denmark

<sup>5</sup>List of all partners in INDICES Consortium: Henrik Berg Rasmussen, Institute of Biological Psychiatry, Mental Health Centre Sct. Hans, Copenhagen University Hospital, Roskilde, Denmark; Ditte Bjerre, Institute of Biological Psychiatry, Mental Health Centre Sct. Hans, Copenhagen University Hospital, Roskilde, Denmark; Majbritt Busk Madsen, Institute of Biological Psychiatry, Mental Health Centre Sct. Hans, Copenhagen University Hospital, Roskilde, Denmark; Laura Ferrero, Institute of Biological Psychiatry, Mental Health Centre Sct. Hans, Copenhagen University Hospital, Roskilde, Denmark; Kristian Linnet, Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health Sciences, University of Copenhagen, Denmark; Ragnar Thomsen, Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health Sciences, University of Copenhagen, Denmark; Gesche Jürgens, Roskilde University Hospital, Unit of Clinical Pharmacology, Denmark; Kim Dalhoff, Department of Clinical Pharmacology, Bispebjerg University Hospital, Copenhagen, Denmark; Claus Stage, Department of Clinical Pharmacology, Bispebjerg University Hospital, Copenhagen, Denmark; Hreinn Stefansson, CNS Division, deCODE Genetics, Reykjavik, Iceland; Thomas Hankemeier, The Leiden/Amsterdam Center for Drug Research LACDR, Leiden University, Gorlaeus laboratories, Leiden, The Netherlands; Rima Kaddurah-Daouk, Department of Psychiatry and Behavioural Sciences, Duke University, Durham, NC, USA; Søren Brunak, Center for Biological Sequence Analysis, Technical University of Denmark, Kgs. Lyngby, Denmark; Olivier Taboureau, Center for Biological Sequence Analysis, Technical University of Denmark, Kgs. Lyngby, Denmark; Grace Shema Nzabonimpa, Center for Biological Sequence Analysis, Technical University of Denmark, Kgs. Lyngby, Denmark; Tine Houmann, Centre for Child and Adolescent Mental Health, Mental Health Services in the Capital Region of Denmark; Pia Jeppesen, Centre for Child and Adolescent Mental Health, Mental Health Services in the Capital Region of Denmark; Kristine Kaalund-Jørgensen, Centre for Child and Adolescent Mental Health, Mental Health Services in the Capital Region of Denmark; Peter Riis Hansen, Department of Cardiology, Copenhagen University Hospital, Hellerup, Denmark; Karl Emil Kristensen, Department of Cardiology, Copenhagen University Hospital, Hellerup, Denmark; Anne Katrine Pagsberg, Centre for Child and Adolescent Mental Health, Mental Health Services in the Capital Region of Denmark; Kerstin J Plessen, Centre for Child and Adolescent Mental Health, Mental Health Services in the Capital Region of Denmark; Poul-Erik Hansen, Department of Science, Systems and Models, Roskilde University, Roskilde, Denmark; Thomas Werge, Institute of Biological Psychiatry, Mental Health Centre Sct. Hans, Copenhagen University Hospital, Roskilde, Denmark; Jørgen Dyrborg, Centre for Child and Adolescent Mental Health, Mental Health Services in the Capital Region of Denmark; Maj-Britt Lauritzen, Centre for Child and Adolescent Mental Health, Mental Health Services in the Capital Region of Denmark

## Article Information

Received date: Feb 17, 2017

Accepted date: Mar 17, 2017

Published date: Mar 21, 2017

## \*Corresponding author

Anne Katrine Pagsberg, Research Unit, Child and Adolescent Mental Health Center, Mental Health Services Capital Region of Denmark & Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark;  
Tel. +4538641180; Fax +4538644487;  
Email: anne.katrine.pagsberg@regionh.dk

**Distributed under** Creative Commons CC-BY 4.0

**Keywords** CES1 polymorphism; ADHD; Methylphenidate; Adverse effects

## Abstract

Children with Attention Deficit Hyperactivity Disorder (ADHD) show large variations in response to methylphenidate (MPH) treatment, which may result from genetic factors associated with MPH metabolism. We aimed at investigating a possible link between the -75 T>G polymorphism in the 5' untranslated region of the gene coding for carboxylesterase 1 (CES1) and a common adverse effect, weight loss, during the first three months of MPH treatment.

We analyzed the association between a *CES1* polymorphism and longitudinal clinical data based on retrospective analysis of medical records, from first to last recorded visit at the clinic. By use of poly chain reaction and the Sanger method we genotyped the -75 T>G gene polymorphism and examined the association to clinical response, which was based on retrospective analysis of longitudinal clinical data from medical records. The primary clinical outcome measure was weight loss during the first 3 months of MPH treatment.

Data from 74 MPH treated children with ADHD, mean age 8.6 years, 57% males, were analysed. There were n=26 G-carriers (heterozygote TG and homozygote GG) and n=48 TT-homozygotes. The proportion of weight loss and mean weight change differed significantly in G-carriers (88% / -0.279 kg) compared with TT-homozygotes (31% / +0.157 kg). This study shows an association between the -75 T>G polymorphism in *CES1* and MPH treatment response, demonstrated by a significantly higher frequency and extent of weight loss in G-carriers compared to TT-homozygotes.

## Introduction

Attention Deficit Hyperactivity Disorder (ADHD) has a prevalence of 4-7 % in children [1-3]. Methylphenidate (MPH) is the first-line pharmacological treatment for the core symptoms of inattention, hyperactivity and impulsivity, and the use in children has increased rapidly in recent years [4-7]. Response rate to MPH is about 70-77 % [8,9] but with a marked individual variation in the observed effect and tolerability [8,10]. This response variation may relate to individual differences in the MPH metabolism. Varying drug metabolism can lead to differential beneficial effects and side effect profiles, resulting in diverse prescription patterns. Hence, there is a need for tools to assist the evaluation of individual treatment strategies.

Pharmacogenetic approaches can assist in the prediction of MPH drug response as already used in other areas of medicine [11]. Investigations of genetic factors that affect drug metabolism may help determine relevant factors for individual tailoring of medications [12]. MPH is primarily metabolized by isozyme carboxylesterase 1 (CES1) that converts MPH to the inactive metabolite, ritalinic acid, in the liver [13]. The gene encoding CES1 is located on chromosome 16 and harbor variations that influence the MPH response [14-16]. Several variations in the *CES1* gene with a potential impact on drug metabolism or drug response have been reported including several Single Nucleotide Polymorphisms (SNPs) [17-21]. Bruxel et al. found a possible association between the *CES1* variation -75 T>G (rs3815583), a polymorphism in the 5' untranslated region of this gene, and severity of appetite reduction in MPH treated children with ADHD. The G allele carriers had a trend towards a higher risk of appetite reduction, and a significant elevated risk for worsening of reduced appetite over time, compared to T allele homozygotes [22]. This indicates that the gene variation -75 T>G in *CES1* affects the functionality of CES1 to a degree that influences the clinical response.

The adverse drug reactions associated with MPH treatment are usually mild to moderate and occur mainly within the first months of treatment and most often in the form of appetite reduction, weight loss, headache, gastrointestinal pain, insomnia and irritability [23-26]. The severity of adverse reactions might reflect the dosing, since some adverse reactions, such as decreased appetite, are more common at higher dose levels [8,27]. Thus, the measure of appetite reduction may serve as an early indicator of reduced metabolism. However, this parameter is primarily evaluated subjectively by the child (and/or

parents) whereas an associated parameter, namely weight loss, is easy to quantify and may be a more objective indicator.

The aim of this study was to investigate the association between the -75 T>G polymorphism in *CES1* and the clinical response in children and adolescents with ADHD who are treated with MPH. Primarily, we aimed at testing whether the association between the -75 T>G polymorphism and appetite reduction could be reproduced when using weight loss as a proxy for appetite reduction. We hypothesized that the *CES1* polymorphism would be associated with differential clinical response to MPH; specifically that the *CES1* polymorphism G-carriers, due to a reduced MPH metabolism, would exhibit a greater weight loss compared to TT-homozygotes during the first 3 months of MPH treatment. This study was part of the INDICES programme (INDividualized drug therapy based on pharmacogenomics: focus on CES1) [28], which aims at developing strategies for individualized treatment with MPH and Angiotensin-Converting-Enzyme (ACE) inhibitors.

## Materials and Methods

### Design

This was a retrospective longitudinal study of ADHD patients from the time of MPH treatment onset to end of treatment. Herein, we report data from the first 3 months of MPH treatment in a naturalistic clinical setting. We analyzed the association between a *CES1* polymorphism and longitudinal clinical data based on retrospective analysis of medical records, from first to last recorded visit at the clinic. The primary clinical outcome variable was weight change during the first 3 months of MPH treatment. This time-period was chosen because weight change usually is seen in the first months of treatment [25,26]. The secondary outcomes were the proportions of weight loss and appetite reduction, adherence, and efficacy quantified by change in Clinical Global Impression (CGI) scores [29].

The study was approved by the local committees of bioethics protocol H-B-2009-026. Parents or legal caretakers provided written informed consent.

### Subjects

Participants eligible for this study, were children and adolescents diagnosed with ADHD according to ICD-10 [30] and treated with MPH. The participants were outpatients, recruited at the Copenhagen University Hospital, Child and Adolescent Mental Health Center,

**Table 1:** Demographic and clinical baseline characteristics of the sample according to genotype.

	All	G-carriers	TT-homozygotes	P <sup>a</sup>
	N=74	N=26	N=48	
Male, n (%)	57 (77.0)	19 (73.1)	38 (79.2)	0.55
Female, n (%)	17 (23.0)	7 (26.9)	10 (20.8)	
Age, years, mean (SD) (range)	8.6 (3.1) (3.8-15.8)	8.3 (3.3) (3.8-15.4)	8.8 (3.1) (4.3-15.8)	0.54
<b>Origin</b>				
Origin, Danish, n (%)	56 (75.7)	16 (61.5)	40 (83.3)	0.04*
Origin, other <sup>b</sup> , n (%)	18 (24.3)	10 (38.5)	8 (16.7)	
<b>ADHD type (ICD-10)</b>				
Disturbance of activity and attention (F90.0), n (%)	61 (82.4)	24 (92.3)	37 (77.1)	0.53
Hyperkinetic conduct disorder (F90.1), n (%)	4 (5.4)	1 (3.8)	3 (6.3)	
Other hyperkinetic disorders (F90.8), n (%)	2 (2.7)	0 (0.0)	2 (4.2)	
Other specified or unspecified behavioral and emotional disorders (F98.8/F98.9), n (%)	7 (9.5)	1 (3.8)	6 (12.5)	
<b>Comorbidity</b>				
No comorbid condition, n (%)	22 (29.7)	7 (26.9)	15 (31.3)	0.43
+1 comorbid condition, n (%)	27 (36.5)	12 (46.2)	15 (31.3)	
+2 comorbid conditions, n (%)	20 (27.0)	7 (26.9)	13 (27.1)	
+3 comorbid conditions, n (%)	4 (5.4)	0 (0)	4 (8.3)	
+4 comorbid conditions, n (%)	1 (1.4)	0 (0)	1 (2.1)	
<b>Comorbid Conditions (ICD-10)</b>				
Behavioral and emotional disorders with onset usually occurring in childhood and adolescence (F90-F98), n (%)	11 (14.9)	1 (3.8)	10 (20.8)	0.08
Disorders of psychological development (F80-F89), n (%)	38 (51.4)	15 (57.7)	23 (47.9)	0.48
Mental retardation (F70-F79), n (%)	6 (8.1)	4 (15.4)	2 (4.2)	0.18
Other and unspecified symptoms and signs involving cognitive functions and awareness (R41.8), n (%)	10 (13.5)	2 (7.7)	8 (16.7)	0.48
Neurotic, stress-related and somatoform disorders (F40-F48), n (%)	3 (4.1)	3 (11.5)	0 (0.0)	0.02*

<sup>a</sup>Calculated by *t*-test or Wilcoxon Mann Whitney U-test (quantitative variables).  $\chi^2$  test or Fisher's exact test (categorical variables). \*Significance level set at  $P < 0.05$

<sup>b</sup>Other: Europe (Scotland, UK, Germany, Estonia, Macedonia and Turkey), Asia (Arabia, Iran, Lebanon, India and China), Africa (Somalia, Uganda and South Africa), South America (Argentina, Uruguay and Ecuador)

Department Bispebjerg, Mental Health Services Capital Region of Denmark. The participants were recruited through the health professional staff at the Child and Adolescent Mental Health Center whom through their work had knowledge of eligible subjects.

### Inclusion criteria

1. Children aged 3-18 years attending the ADHD clinic, both sexes, with a confirmed ICD-10 diagnosis of either F90.0 Disturbance of activity and attention, F90.1 Hyperkinetic conduct disorder, F90.8 Other hyperkinetic disorders, F90.9 Hyperkinetic disorder, unspecified or F98.8 Other specified behavioral and emotional disorders (subtype attention deficit disorder without hyperactivity).
2. Initiated MPH treatment and continued for at least 3 months.
3. Naïve of other pharmacologic ADHD treatments.

### Exclusion criteria

1. Lack of informed consent
2. Quality of DNA samples insufficient

### Diagnostic and clinical assessment

The observation period for each participant was the entire treatment period from their first visit to their last recorded visit at the clinic. The data were retrieved retrospectively from the medical records at three time points: 1) at baseline just before MPH treatment was initiated; 2) after 3 months of MPH treatment; and 3) at endpoint, which is end of observation period for the individual patient. The following data were retrieved: age; gender; country of origin; diagnoses; weight; appetite; height; MPH dosages; dates of treatment start/end; additional medications; and changes in medication regime. For each patient, the average daily dose of MPH was calculated (mg/kg/day) at all available time points. Explicit clinical data from every patient in the medical record were reviewed to assess the child's beneficial effects of MPH treatment and the occurrence of adverse drug reactions. In addition, we reviewed all scores on standardized

scales of adverse drug reactions and on Attention Deficit Hyperactivity Disorder Rating Scale (ADHD-RS) schemes [31] when available in the medical records. If schemes were not available, other relevant data (i.e. assessments by physician or nurse) relating to treatment were used.

In order to systematically evaluate psychiatric status and MPH response in every case, a specialist in child- and adolescent psychiatry (AKP), who was blinded to patient genotype, reviewed all patient records. Each patient assessment was based on all available information, i.e. clinical descriptions; Global Assessment of Psychosocial Disability rating (GAPD) [32]; tests of variables of attention scores [33]; ADHD-RS schemes (parent and school versions) when available; and adverse drug reaction scores on a clinical scale adapted from Barkley's Side Effect Rating Scale [34]. The global review of every patient's illness severity was rated on the CGI severity score (CGI-S). The patients were retrospectively rated at baseline and after 3 months of treatment on a scale of 1 (normal, not at all ill) to 7 (extremely ill). At 3 months the CGI improvement score (CGI-I) was used to rate illness improvement during treatment on a scale from 1 (very much improved) to 7 (very much worse).

### Collection of samples and isolation of DNA

**Table 2:** Treatment related effects according to genotype.

	All N=74	G-carriers N=26	TT-homozygotes N=48	P <sup>a</sup>
<b>Weight, kg, mean (SD)</b>				
Baseline	35.3 (15.8)	35.3 (16.0)	35.3 (15.9)	0.99
3 months <sup>b</sup>	35.3 (15.9)	35.0 (16.1)	35.5 (16.0)	0.90
Change extrapolated (n=74) <sup>b</sup>	+0.004 (0.8)	-0.279 (0.8)	+0.157 (0.8)	0.03*
Change non-extrapolated (n=44)	-0.030 (1.0)	-0.423 (1.2)	+0.135 (0.8)	0.10
<b>Height, cm, mean (SD)</b>				
Baseline	136.4 (18.1)	135.4 (18.6)	137.0 (18.0)	0.73
3 months <sup>b</sup>	137.1 (18.0)	137.0 (19.1)	137.8 (18.0)	0.87
<b>Body Mass Index, kg/m<sup>2</sup>, mean (SD)</b>				
Baseline	18.1 (4.0)	18.4 (4.1)	17.9 (3.9)	0.63
3 months <sup>b</sup>	18.0 (4.8)	18.0 (3.7)	17.8 (4.0)	0.51
<b>Clinical Global Impression score, mean (SD)</b>				
CGI-S baseline	5.0 (0.9)	5.0 (1.0)	5.0 (0.8)	0.67
CGI-I (at 3 months)	2.2 (0.6)	2.3 (0.7)	2.2 (0.5)	0.24
CGI-S follow-up (at 3 months)	3.7 (0.9)	3.8 (0.9)	3.6 (0.8)	0.78
CGI-S mean change (Baseline to 3 months)	1.3 (0.7)	1.2 (0.7)	1.4 (0.7)	0.43
<b>MPH treatment</b>				
Mean MPH dose, baseline <sup>c</sup> , mg/kg, mean (SD)	0.34 (0.2)	0.37 (0.3)	0.32 (0.2)	0.41
Mean MPH dose, 3 months <sup>c</sup> , mg/kg, mean (SD)	0.66 (0.2)	0.73 (0.3)	0.63 (0.2)	0.11
MPH Treatment period, years, mean (SD)	3.5 (2.2)	3.5 (2.4)	3.5 (2.2)	0.98
Lack of adherence to treatment, 3 months, n (%)	3 (4.1)	2 (7.7)	1 (2.1)	0.24
Concomitant medication, n (%)	25 (33.8)	7 (26.9)	18 (37.5)	0.36
Appetite reduction, n (%)	21 (28.4)	9 (34.6)	12 (25)	0.43

<sup>a</sup>Statistics by *t*-test or Wilcoxon Mann Whitney U-test (quantitative variables).  $\chi^2$  test or Fisher's exact test (categorical variables). Weight change statistics by ANCOVA

<sup>b</sup>30 extrapolated data points

<sup>c</sup>Patients with adherence problems are not included in this calculation (No. of excluded patients: TT-homozygotes=7, G-carriers=6)

MPH=Methylphenidate. CGI-I=Clinical global impression – improvement; CGI-S=Clinical global impression - severity

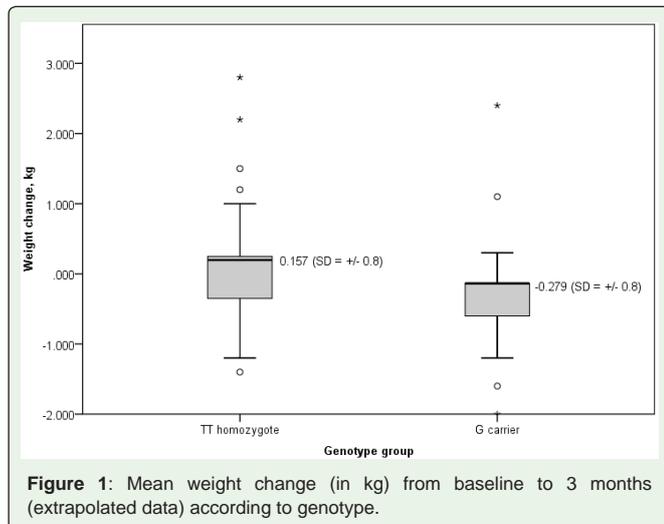
Samples of saliva were collected from the patients using the Oragene DNA (OG-250) or Oragene DNA (OG-500) kits from DNA Genotek Inc., Kanata, Canada. Genomic DNA was isolated from the saliva samples using the guidelines of the manufacturer of these kits.

### Genotyping

A CES1 fragment of 2.6 kb containing the -75 T>G polymorphism was amplified by Polymerase Chain Reaction (PCR) using primers with the sequences ACTATGGGGGACGGAGTTCA (forward) and GACTGTGAGGGTACATACGG (reverse), respectively. The amplified fragment was purified by vacuum filtration through a silica membrane (Qiaquick 96, QIAGEN GmbH, Hilden, Germany) and sequenced by the Sanger method (Bjerre and Rasmussen, unpublished). Determination of the genotype of the -75 T>G polymorphism was done by visual inspection of the electropherograms.

### Statistical analyses

We used SPSS version 18.0 (SPSS, Chicago, IL, USA) software. The three patient genotypes were grouped into TT-homozygotes and G-carriers (TG-heterozygotes and GG-homozygotes). We assessed whether the genotype proportions corresponded to those expected under Hardy-Weinberg conditions using chi-squared test. Student's



t-test and Wilcoxon-Mann Whitney U-test were used for analysis of continuous variables with and without normal distribution, respectively. The chi-squared test or Fisher's exact test was used for categorical variables. To test our hypothesis of more pronounced weight loss in G-carriers, we performed an ANCOVA, with weight change from baseline to 3 months being the dependent variable, whereas genotype (G-carriers or TT-homozygotes) was a fixed factor; and age, weight at baseline and MPH dose at 3 months served as covariates. In addition, we performed a linear regression analysis to investigate the effect of relevant parameters on the primary outcome in the total sample. In case of missing data on height and weight; we extrapolated values based on available data on the average weight and height change of the cohort of G-carriers and TT-homozygotes respectively. A two-tailed p-value <0.05 was considered statistically significant.

## Results

### Sample characteristics

Between January 2011 and March 2012, 76 subjects gave consent to participate. The DNA quality of the saliva samples in two participants was not satisfactory and did not support amplification of the desired fragment of *CES1* thus excluding sequencing. Hence, 74 participants were included in this analysis. The genotype frequencies for the TT, TG and GG genotype amounted to 0.62; 0.32; and 0.06 respectively. This genotype distribution did not deviate from that expected under conditions of Hardy-Weinberg equilibrium ( $P=0.63$ ). The frequency of the G allele amounted to 0.22. There were  $n=26$  (35.1%) G-carriers (GG and TG genotypes) and  $n=48$  (64.9%) TT-homozygotes.

Demographic and clinical characteristics were not different between the G-carriers and TT-homozygotes, with the exception of more G-carriers with a non-Danish ethnic origin compared to the TT-homozygotes ( $P=0.04$ ) and more G-carriers than TT-homozygotes with a co-morbid diagnosis of neurotic, stress-related or somatoform disorder (Table 1). The observation period ranged from 0.24 months to 10.3 years. Some subjects did not have a consultation at the exact 3 months follow-up date therefore the first new record of weight and height around the 3 months follow-up date was used. The observation period had a mean (SD) of 3.5 (2.1) years with no

significant differences between groups. In our further analysis of data, we focused on the initial 3 months of treatment.

### Treatment characteristics and effects

The treating physicians had decided titration and adjustment of MPH dosing over time based on clinical evaluations of benefits and harms of MPH treatment in the individual patient. Mean baseline and follow-up doses of MPH and discontinuation rates did not differ significantly between groups (Table 2). The age group of the included children was broad (3-18 years) and we therefore tested mean dose (mg/kg) at baseline between two age-groups (3-9 years, children) and (10-18 years, adolescents). However, no significant difference in dose between the two age groups was observed ( $p=0.72$ ). Adherence at 3 months in the two age groups was also tested, however no significant difference was found ( $p=0.80$ ). Of 74 patients, 25 received concomitant medication other than MPH at their latest visit: melatonin ( $n=19$ ), bronchodilators ( $n=3$ ) and antipsychotics ( $n=3$ ) with no significant differences between groups. The treatment related effects are shown in Table 2.

As a group, the patients pre-treatment illness severity were on average rated as "markedly ill" on the CGI-S (mean score 5.0) at baseline and "much improved" on the CGI-I (mean score 2.2) after 3 months of treatment, also reflected in a significantly reduced mean CGI-S score at 3 months of 3.7 ( $t=16.26$ ,  $p<0.001$ ), i.e. mildly to moderately ill. There were no significant differences at any time point between groups on these measures.

Twenty-one out of 74 patients indicated reduction of appetite, with an overrepresentation among G-carriers (34.6%) compared to TT-homozygotes (25%), although not statistically significant. At the 3 month time point, 13 (50%) participants in G-carriers and 17 (35%) in TT-homozygotes lacked registrations on height and/or weight in their medical record. These data were extrapolated based on the average registered weight and height change at 3 months in the remaining G-carriers and TT-homozygotes (non-extrapolated values, mean (SD) change weight/height: G-carriers:  $-0.42$  (1.2) kg/+ 1.40 (2.1) cm; TT-homozygotes:  $+0.14$  (1.2) kg / $+0.75$  (1.0) cm). On non-extrapolated data, groups did not differ with respect to the growth parameters weight, height and BMI at baseline and after 3 months. The mean change in height did not differ significantly between the groups ( $p=0.21$ ). As shown in Figure 1, G-carriers had lost more weight than TT-homozygotes after 3 months of treatment (based on extrapolated data,  $n=74$ ). In our ANCOVA, genotype was statistically associated with weight change at 3 months ( $F=5.036$ ,  $p=0.028$ ). None of the other variables included in the model were significantly associated with weight change. When repeating the ANCOVA in the non-extrapolated data ( $n=44$ ) the finding was no longer significant ( $F=1.04$ ,  $p=0.31$ ). The incidence of patients with weight loss registered on extrapolated data was significantly higher (88%) in the G-carriers compared to the TT-homozygotes (31%) (Chi square=22.01,  $p<0.001$ ). When repeated on the non-extrapolated data, the incidence of weight loss was still larger in the G-carriers (77%) than the TT-homozygotes (48%), but not significantly (chi square=3.04,  $p=0.08$ ). We also tested whether mean dose between outcome-groups (weight-loss vs no weight-loss) was significantly different. The mean dose at 3 months (subjects with adherence problems not included) registered on extrapolated data was not significantly different between subjects with and without weight-loss ( $P=0.23$ ). When repeated on non-

extrapolated data, the mean dose was still not significantly different ( $p=0.11$ ). Mean BMI decreased (extrapolated data) significantly more in the G-carriers than in the TT-homozygotes ( $-1.12\text{kg/m}^2$  and  $-0.12\text{kg/m}^2$  respectively) ( $t=2.869$   $p=0.006$ ).

Due to the difference between genotype-groups concerning ethnicity, we performed a sensitivity analysis and repeated the ANCOVA in the group of patients with Danish origin ( $n=56$ ) and in the group of patients of non-Danish origin ( $n=18$ ). In neither of these two subgroup analyses, the effect of genotype on weight change was significant. When performing a t-test comparing Danish and non-Danish participants, there was a significant difference in weight change, with patients of Danish origin gaining a mean (SD) of 0.13 (0.8) kg, while patients of non-Danish origin lost a mean of 0.40 (0.7) kg during the 3 months treatment period ( $t=2.54$ ,  $p=0.013$ ). In the group of Danish origins, mean (SD) weight change was  $-0.09$  kg (0.85) in G-carriers ( $n=16$ ) and  $+0.22$  (0.79) in TT-homozygotes ( $n=40$ ), a non-significant finding ( $t=1.316$ ,  $p=0.194$ ). In the group of non-Danish origins, mean (SD) weight change was  $-0.58$  kg (0.69) in G-carriers ( $n=10$ ) and  $-0.18$  kg (0.59) in TT-homozygotes ( $n=8$ ), a non-significant finding ( $t=1.361$ ,  $p=0.207$ ). When performing a regression analysis of the whole sample ( $n=74$ ) with weight change as the dependent variable, genotype independently had a significant regression coefficient ( $\beta=-259$ ,  $t=-2.280$ ,  $p=0.026$ ), while origin did not ( $\beta=-208$ ,  $t=-1.801$ ,  $p=0.076$ ). When both independent variables were included in the same regression analysis, none of the coefficients were significant ( $p=0.66$ ;  $p=0.210$  respectively).

## Discussion

We examined the association between the *CES1* polymorphism -75 T>G and response to MPH in the first 3 months of treatment in 74 children and adolescents with ADHD aged 4-16 years. We tested the hypothesis that G-carriers would exhibit more severe side effects expressed as a greater weight loss compared to TT-homozygotes. Our main finding was an average weight loss of 0.280 kg in G-carriers, while TT-homozygotes on average gained 0.157 kg, thus confirming our hypothesis of an association of the *CES1* polymorphism and an increased risk of side effects to MPH, which presumably reflects a decreased MPH metabolism by *CES1* [16]. This decreased metabolism could result from a lower level of expression of the enzyme due to an effect of the G allele on the process of translation initiation. Alternatively, this allele may not be causal in it but is in linkage disequilibrium with a variant in *CES1* that influences the level of transcription or translation of the gene.

The clinical characteristics of this initially ADHD medication-naïve sample resembled the typical description of children and adolescents with ADHD [35]. Kaplan et al. found similar CGI-S scores in children with ADHD as in our study, indicating that our sample is representative with respect to illness severity [36]. The mean MPH dose at 3 months of 0.66 mg/kg/day is in line with that of other reports [37]. We identified a similar prevalence of appetite reduction (28%) in our sample, in accordance with several studies [38–40], including a randomized, double-blind, placebo-controlled study where more than 30% of the patients treated with MPH experienced decreased appetite [41]. Moreover, we found the expected distribution of the investigated genotype frequencies with 26 (35%) patients carrying the TG or GG genotype, while 48 (65%) carried the TT genotype at position -75 in the 5' untranslated region of *CES1*. The two groups

were demographically and clinically similar, except for a higher prevalence of non-Danish origin among the G-carriers.

Our study results extend the findings of the study by Bruxel et al. [22] with equivalent frequencies of the G-allele, that demonstrated an association between the -75 T>G polymorphism and appetite reduction. They found an excess of appetite reduction in the G-carriers vs. the TT-homozygotes (34% vs 13%, Odds Ratio (OR) = 3.47, confidence interval (CI)=1.4-8.8) and we reproduced this finding, although not reaching significance (35% vs 25%, OR=1.59, CI=0.6-4.5). However, in our sample, the association was significantly expressed as a failure to gain or sustain average bodyweight in the group of G-carriers. The finding is underlined by our finding of a significantly higher number of patients in G-carriers with weight loss and a larger mean BMI decrease. In addition, although not statistically significant, we found less adherence to treatment in the G-carriers, which might indicate less tolerance to MPH treatment. To our knowledge, no further studies have investigated the association between the -75 T>G polymorphism and MPH drug response. However, others have examined the role of other *CES1* gene variations in MPH treatment. Johnson et al. investigated seven SNPs in the *CES1* gene, and found no associations to clinical beneficial response or dose prescribed, but a significant association between two *CES1* SNP markers and occurrence of sadness [17]. This supports the association of *CES1* variations to another known side effect of MPH. The findings of significant associations between several variations of the *CES1* gene and side effects to MPH may pave the way for an improved understanding of the genetics underlying the clinical response induced by MPH.

## Strengths and Limitations

The retrospective design of this study was a limitation to the quality of our clinical data and did not allow for MPH plasma monitoring, which could have revealed more insight to variations in drug metabolism. However, concrete measurable observations such as MPH doses; height and weight are easily sampled from medical records, and retrospective CGI ratings of medical charts has been used in other studies on child- and adolescent psychiatric samples [39]. We chose to use weight change as a proxy for appetite reduction in order to rely on an objective parameter for our primary outcome, which we expect to have better validity than retrospective reports on subjective measures of adverse events as registered in medical records. Moreover, a single trained specialist, blinded for case genotype, evaluated all medical records with CGI, which is a valid clinical outcome measure across diagnostic groupings [42]. This limits the effects of variations in the clinical evaluation of treatment efficacy that may occur when carried out by different clinicians. The use of CGI enabled comparisons across studies. Although this study design has its limitations, it also represents a unique strength because the naturalistic setting allows us to describe the treatment of unselected subjects meeting research criteria for ADHD over a longer period.

The amount of missing data on our primary outcome measure may limit the validity of our findings. The commonly used method of Last Observation Carried Forward did not seem attractive given only two data points of major interest. We therefore used extrapolations based on average values from the sampled data sets without missing data. When reanalyzing findings from the extrapolated data sets in the non-extrapolated data sets, the direction of the results were

reproduced, although not significantly, probably due to lack of power. We acknowledge that the extrapolation method used has its limitations however it can give a hint of a direction. Another limitation was the small sample size. Although we had sufficient statistical power to identify a significant effect on our primary outcome, we may lack power to identify other relevant possible differences in treatment response, and the small sample size limits the possibility of further exploring the implications of ethnic origin as a possible confounder of the findings. The number of subjects in this present study did not provide enough power to investigate effects of the subgroup of the GG-homozygote genotype, due to the low frequency. Finally, our study design was not adequately powered to explore the association of MPH dose-dependent adverse events that has been shown in other studies [8,27].

## Relevance/Implication

Our results indicate that genotype assisted individualized MPH treatment could support future clinical practice in the treatment of ADHD. However, the results need to be replicated prospectively in a larger scale.

## Funding and Disclosures

The present study is a part of INDICES (INDividualised drug therapy based on pharmacogenomics: focus on carboxylesterase 1, CES1), a project supported by grant 10-092792/DSF (DKK 17,2 million) from the Danish Council for Strategic Research, Programme Commission on Individuals, Disease and Society.

Mental Health Services of the Capital Region of Denmark financial support: DKK 112,000.

Equipment: was not supported by any pharmaceutical company.

Drugs: Methylphenidate treatment was not supported by any pharmaceutical company.

Maria Oxenbøll, Kristine Kaalund-Jørgensen, Ditte Bjerre, Gesche Jürgens, Ebba Holme Hansen, Kerstin J Plessen, Henrik B Rasmussen and Anne Katrine Pagsberg declare no conflicts of interest.

Simone Rasmussen: HB Pharma sponsored her participation in 5<sup>th</sup> World Congress on ADHD.

## References

- Faraone SV, Sergeant J, Gillberg C, Biederman J. The worldwide prevalence of ADHD: is it an American condition? *World Psychiatry Off J World Psychiatr Assoc WPA*. 2003; 2:104–113.
- Polanczyk G, de Lima MS, Horta BL, Biederman J, Rohde LA. The worldwide prevalence of ADHD: a systematic review and meta regression analysis. *Am J Psychiatry*. 2007; 164:942–948.
- Thomas R, Sanders S, Doust J, Beller E, Glasziou P. Prevalence of attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. *Pediatrics*. 2015; 135:e994–1001.
- Bruckner TA, Hodgson A, Mahoney CB, Fulton BD, Levine P, Scheffler RM. Health care supply and county-level variation in attention-deficit hyperactivity disorder prescription medications. *Pharmacoepidemiol Drug Saf*. 2012; 21:442–449.
- Dalsgaard S, Nielsen HS, Simonsen M. Five-fold increase in national prevalence rates of attention-deficit/hyperactivity disorder medications for children and adolescents with autism spectrum disorder, attention-deficit/hyperactivity disorder, and other psychiatric disorders: a Danish register-based study. *J Child Adolesc Psychopharmacol*. 2013; 23:432–439.
- Hsia Y, Maclennan K. Rise in psychotropic drug prescribing in children and adolescents during 1992-2001: a population-based study in the UK. *Eur J Epidemiol*. 2009; 24:211–216.
- Pottegård A, Bjerregaard BK, Glintborg D, Hallas J, Moreno SI. The use of medication against attention deficit hyperactivity disorder in Denmark: a drug use study from a national perspective. *Eur J Clin Pharmacol*. 2012; 68:1443–1450.
- Greenhill LL, Swanson JM, Vitiello B, Davies M, Clevenger W, Wu M, et al. Impairment and deportment responses to different methylphenidate doses in children with ADHD: the MTA titration trial. *J Am Acad Child Adolesc Psychiatry*. 2001; 40:180–187.
- Spencer T, Biederman J, Wilens T, Harding M, O'Donnell D, Griffin S. Pharmacotherapy of attention-deficit hyperactivity disorder across the life cycle. *J Am Acad Child Adolesc Psychiatry*. 1996; 35:409–432.
- Vitiello B, Severe JB, Greenhill LL, Arnold LE, Abikoff HB, Bukstein OG, et al. Methylphenidate dosage for children with ADHD over time under controlled conditions: lessons from the MTA. *J Am Acad Child Adolesc Psychiatry*. 2001; 40:188–196.
- Dezentjé VO, Guchelaar H-J, Nortier JWR, Velde CJH van de, Gelderblom H. Clinical Implications of CYP2D6 Genotyping in Tamoxifen Treatment for Breast Cancer. *Clin Cancer Res*. 2009; 15:15–21.
- Meyer UA, Meyer, U. A. Pharmacogenetics and adverse drug reactions. *Lancet* 356, 1667-1671. *Lancet*. 2000; 356:1667–1671.
- Nemoda Z, Angyal N, Tarnok Z, Gadoros J, Sasvari-Szekely M. Carboxylesterase 1 gene polymorphism and methylphenidate response in ADHD. *Neuropharmacology*. 2009; 57:731–733.
- Hosokawa M. Structure and catalytic properties of carboxylesterase isozymes involved in metabolic activation of prodrugs. *Mol Basel Switz*. 2008; 13:412–431.
- McGough JJ, McCracken JT, Loo SK, Manganiello M, Leung MC, Tietjens JR, et al. A candidate gene analysis of methylphenidate response in attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry*. 2009; 48:1155–1164.
- Rasmussen HB, Bjerre D, Linnet K, Jürgens G, Dalhoff K, Stefansson H, et al. Individualization of treatments with drugs metabolized by CES1: combining genetics and metabolomics. *Pharmacogenomics*. 2015; 16:649–665.
- Johnson KA, Barry E, Lambert D, Fitzgerald M, McNicholas F, Kirley A, et al. Methylphenidate side effect profile is influenced by genetic variation in the attention-deficit/hyperactivity disorder-associated CES1 gene. *J Child Adolesc Psychopharmacol*. 2013; 23:655–664.
- Marsh S, Xiao M, Yu J, Ahluwalia R, Minton M, Freimuth RR, et al. Pharmacogenomic assessment of carboxylesterases 1 and 2. *Genomics*. 2004; 84:661–668.
- Yamada S, Richardson K, Tang M, Halaschek-Wiener J, Cook VJ, Fitzgerald JM, et al. Genetic variation in carboxylesterase genes and susceptibility to isoniazid-induced hepatotoxicity. *Pharmacogenomics J*. 2010; 10:524–536.
- Yoshimura M, Kimura T, Ishii M, Ishii K, Matsuura T, Geshi E, et al. Functional polymorphisms in carboxylesterase1A2 (CES1A2) gene involves specific protein 1 (Sp1) binding sites. *Biochem Biophys Res Commun*. 2008; 369:939–942.
- Zhu H-J, Patrick KS, Yuan H-J, Wang J-S, Donovan JL, DeVane CL, et al. Two CES1 gene mutations lead to dysfunctional carboxylesterase 1 activity in man: clinical significance and molecular basis. *Am J Hum Genet*. 2008; 82:1241–1248.
- Bruxel EM, Salatino-Oliveira A, Genro JP, Zeni CP, Polanczyk GV, Chazan R, et al. Association of a carboxylesterase 1 polymorphism with appetite reduction in children and adolescents with attention-deficit/hyperactivity disorder treated with methylphenidate. *Pharmacogenomics J*. 2013; 13:476–480.
- Aagaard L, Hansen EH. The occurrence of adverse drug reactions reported for attention deficit hyperactivity disorder (ADHD) medications in the pediatric

- population: a qualitative review of empirical studies. *Neuropsychiatr Dis Treat*. 2011; 7:729–744.
24. Charach A, Ickowicz A, Schachar R. Stimulant treatment over five years: adherence, effectiveness, and adverse effects. *J Am Acad Child Adolesc Psychiatry*. 2004; 43:559–567.
25. Graham J, Coghill D. Adverse effects of pharmacotherapies for attention-deficit hyperactivity disorder: epidemiology, prevention and management. *CNS Drugs*. 2008; 22:213–237.
26. Greenhill LL, Pliszka S, Dulcan MK. Practice Parameter for the Use of Stimulant Medications in the Treatment of Children, Adolescents, and Adults. *J Am Acad Child Adolesc Psychiatry*. 2002; 41:26S – 49S.
27. Stein MA, Waldman ID, Charney E, Aryal S, Sable C, Gruber R, et al. Dose effects and comparative effectiveness of extended release dexamethylphenidate and mixed amphetamine salts. *J Child Adolesc Psychopharmacol*. 2011; 21:581–588.
28. INDICES. Individualiseret behandling med fokus på carboxylesterase 1 (CES1). Farmakogenetiske undersøgelser af CES1-geneti relation til behandling med methylphenidat og trandolapril hos personer med henholdsvis ADHD eller kronisk hjertesygdom. - Research - Region Hovedstadens Psykiatri [Internet]. [cited 2015 Sep 9]. Available from: [https://forskning.regionh.dk/psykiatri/en/projects/indices-individualiseret-behandling-med-fokus-paa-carboxylesterase-1-ces1-farmakogenetiske-undersogelser-af-ces1genet-i-relation-til-behandling-med-methylphenidat-og-trandolapril-hos-personer-med-henholdsvis-adhd-eller-kronisk-hjertesygdom\(32708ca8-4748-4632-96a0-0cea903e4c9c\).html](https://forskning.regionh.dk/psykiatri/en/projects/indices-individualiseret-behandling-med-fokus-paa-carboxylesterase-1-ces1-farmakogenetiske-undersogelser-af-ces1genet-i-relation-til-behandling-med-methylphenidat-og-trandolapril-hos-personer-med-henholdsvis-adhd-eller-kronisk-hjertesygdom(32708ca8-4748-4632-96a0-0cea903e4c9c).html)
29. Guy W. CGI Clinical Global Impressions. In: ECDEU assessment manual for psychopharmacology. Rockville, Maryland: U. S. Dept. of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, National Institute of Mental Health, Psychopharmacology Research Branch, Division of Extramural Research Programs. 1976.
30. World Health Organization. International Statistical Classification of Diseases and Related Health Problems 10th Revision - Online Version. World Health Organization; 2010.
31. DuPaul GJ. Parent and Teacher Ratings of ADHD Symptoms: Psychometric Properties in a Community-Based Sample. *J Clin Child Psychol*. 1991; 20:245–253.
32. World Health Organization, Rutter M. Axis Six - Global Assessment of Psychosocial Disability. In: Multiaxial classification of child and adolescent psychiatric disorders : the ICD-10 classification of mental and behavioural disorders in children and adolescents. Cambridge: Cambridge University Press. 1996; 271–272.
33. AbuRuz SM, Bulatova NR, Yousef AM. Validation of a comprehensive classification tool for treatment-related problems. *Pharm World Sci PWS*. 2006; 28:222–232.
34. Barkley RA, McMurray MB, Edelbrock CS, Robbins K. Side effects of methylphenidate in children with attention deficit hyperactivity disorder: a systemic, placebo-controlled evaluation. *Pediatrics*. 1990; 86:184–192.
35. Powell SG, Thomsen PH, Frydenberg M, Rasmussen H. Long-term treatment of ADHD with stimulants: a large observational study of real-life patients. *J Atten Disord*. 2011; 15:439–451.
36. Kaplan S, Heiligenstein J, West S, Busner J, Harder D, Dittmann R, et al. Efficacy and safety of atomoxetine in childhood attention-deficit/hyperactivity disorder with comorbid oppositional defiant disorder. *J Atten Disord*. 2004; 8: 45–52.
37. Vitiello B, Abikoff HB, Chuang SZ, Kollins SH, McCracken JT, Riddle MA, et al. Effectiveness of methylphenidate in the 10-month continuation phase of the Preschoolers with Attention-Deficit/Hyperactivity Disorder Treatment Study (PATS). *J Child Adolesc Psychopharmacol*. 2007; 17:593–604.
38. Lee J, Grizenko N, Bhat V, Sengupta S, Polotskaia A, Joobar R. Relation between therapeutic response and side effects induced by methylphenidate as observed by parents and teachers of children with ADHD. *BMC Psychiatry*. 2011; 11:70.
39. Shon S-H, Joo Y, Lee J-S, Kim H-W. Lamotrigine treatment of adolescents with unipolar and bipolar depression: a retrospective chart review. *J Child Adolesc Psychopharmacol*. 2014; 24:285–287.
40. Sonuga-Barke EJS, Coghill D, Wigal T, DeBacker M, Swanson J. Adverse reactions to methylphenidate treatment for attention-deficit/hyperactivity disorder: structure and associations with clinical characteristics and symptom control. *J Child Adolesc Psychopharmacol*. 2009; 19:683–690.
41. Greenhill LL, Muniz R, Ball RR, Levine A, Pestreich L, Jiang H. Efficacy and safety of dexamethylphenidate extended-release capsules in children with attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry*. 2006; 45:817–823.
42. Berk M, Ng F, Dodd S, Callaly T, Campbell S, Bernardo M, et al. The validity of the CGI severity and improvement scales as measures of clinical effectiveness suitable for routine clinical use. *J Eval Clin Pract*. 2008; 14:979–983.