UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDIACAL SCIENCES

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PhD Thesis

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Predictors of methylphenidate treatment outcome

The outcome and predictors of outcome after 12 weeks treatment with methylphenidate:

A clinical observational study of the relationship between *CES1* genotype and methylphenidate response in children with ADHD

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Paper II

- Association of carboxylesterase 1 gene variants with 12-week outcome of methylphenidate treatment of children with ADHD.

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3 Dansk resumé

ADHD er den hyppigst forekomne neuropsykiatrisk udviklingsfortyrrelse i barn- og ungdommen med en prævalens på 3-4%. Det antages, at ADHD er af multifaktoriel oprindelse hvor både miljømæssige (præ-, peri- og postnatalefaktorer) og hundredvis af gener ligger til grund for sygdommen, der endnu ikke er præcist klarlagt.

Methylphenidat (MPH) er første valgs præparatet til behandling af børn over fem år med moderat til svær ADHD og med betydelig funktionsnedsættelse indenfor flere områder. MPH behandlingsresponset viser stor individuel variation forhold til effekt, optimal dosis og bivirkninger. MPH metaboliseres næsten udelukkende af carboxylesterase (CES1) i leveren fra de farmakologiske aktive former, *d* og *l*-MPH til de inaktive enantiomerer *d*- og *l*-ritalinsyre. CES1 anses for at være et vigtigt og måske essentielt enzym, der også metaboliserer mange andre lægemidler samt endogene komponenter som f.eks. lipider. Farmakogenetiske studier har vist, at *CES1* varianter var associeret til en ændring i behandlingsresponset af CES1 metaboliserede medikamenter. Det er lavet få kandidatgen associationsstudier mellem *CES1* varianter fra DNA fra ADHD patienter og MPH behandlingsresponset.

Det overordnede formål med det observationelle farmakogenetiske studie var at bidrage til evidensgrundlaget for behandlingsresponset og prædiktorerne for behandlingsresponset af en grundigt monitoreret og individuelt tilpasset MPH behandling af børn, der netop var diagnosticeret med ADHD i klinikken. De specifikke formål var at identificere og karakterisere de kliniske karakteristika (paper I) og *CES1* varianterne (paper II), der var var associeret til MPH behandlingsresponset og at undersøge potentialet for personlig medicin til behandling af ADHD.

Studiet var et prospektivt longitudinelt observationelt farmakogenetisk 12 ugers studie, der inkluderede en klinisk repræsentativ population af MPH naïve børn i alderen 7 til 12 år (i gennemsnit 9.6 år), der netop var diagnosticeret med ADHD i Børne- og Ungdomspsykiatrien i Region H. Børnene blev individuelt titreret med MPH indtil et klinisk signifikant respons var opnået (defineret ved en normalisering eller borderline normalisering af kliniker vurderet ADHD symptomer), eller bivirkninger hindrede dosis øgning, eller den maximale dosis var opnået. Desuden var behandlingsresponset også målt på ADHD symptomer vurderet af forældre og lærere, den samlede sygdomssværhedsgrad, det daglige og sociale funktionsniveau, samt barnets vedvarende opmærksomhed. Studiet undersøgte den prædiktiv værdi af; køn, alder, komorbiditet, vægt, adfærdsforstyrrelse, ADHD subtype, intelligensniveau og vægt, samt varianter af *CES1* i forhold til behandlingsresponset ud fra ADHD symptomer, bivirkninger og MPH slut dosis.

4 English summary

Attention deficit/hyperactivity activity disorder (ADHD) is the most common neuropsychiatric developmental disorder in children and adolescents with a prevalence of 3-4%. It is believed that ADHD has a multifactorial origin where both environmental (pre-, peri- and postnatal factors) and hundreds of genes underlie the disease, which has not yet been precisely elucidated.

Methylphenidate (MPH) is the first-choice drug for the treatment of children over five years of age with moderate to severe ADHD and functional impairments in one or several domains of the child's life. The MPH treatment response shows large individual variation in terms of beneficial effects, optimal dose and adverse reactions. MPH is primarily metabolized by carboxylesterase (CES1) in the liver from the pharmacologically active forms, *d* and *l*-MPH to the inactive enantiomers *d*- and *l*-ritalic acid. CES1 is an important and maybe an essential enzyme that also metabolizes many other drugs as well as endogenous components such as lipids. Pharmacogenetic studies have shown that variants of *CES1* were associated with a change in the response of CES1 metabolized drugs. Few candidate gene association studies have explored the association between *CES1* variants identified in DNA from ADHD patients and their MPH response.

The overall aim of this observational pharmacogenetic study was to contribute to the evidence base on the outcome and the predictors of outcome of a carefully monitored and individually adapted first-time treatment with MPH for children recently diagnosed with ADHD in a routine clinical setting. The specific objectives were to identify and characterize the clinical characteristics (paper I) and the variants of *CES1* (paper II) that were associated with the MPH treatment response and to explore the potential for personalized medicine in the treatment of ADHD.

The study was a prospective longitudinal observational pharmacogenetic 12-week study that included a clinical representative sample of 207 MPH naïve children aged 7 to 12 years (mean 9.6 years), who had recently been diagnosed with ADHD at the Child and Adolescent Mental Health Centre, Mental Health Services, Capital Region Denmark. The children were individually titrated with MPH until a clinically significant response (defined as a normalization or a borderline normalization on clinician rated ADHD symptoms) was achieved, or until adverse reactions prohibited further up-titrations, or the maximum dose was reached. Furthermore, the treatment response of MPH was measured on parent and teacher rated ADHD symptoms, global impairments, daily and social functioning, and indices of sustained attention. The study explored the predictive value of the following baseline characteristics; age, sex, comorbidity, intelligence level, conduct disorder, global impairments, ADHD

subtype, and weight, as well as the variants of *CES1* with regard to the outcomes and course of outcomes of MPH treatment, which included ADHD symptoms, adverse reactions, and end-dose of MPH.

In the observational study (paper I), 168 out of 207 children were normalized or borderline normalized on the clinician rated ADHD symptoms (Inattention and/or Hyperactivity-Impulsivity) at study-end. The non-responders comprised 19 children not normalized or borderline normalized on the clinician rated ADHD symptoms, and 12 children who dropped out due to adverse reactions. Eight children dropped out because of other reasons. There were few adverse reactions, and the standardized total scores of adverse reactions decreased during the follow-up; the only exception was an increase in the number of children with reduced appetite and weight loss. The individual titrated treatment response showed a favourable balance between ADHD symptom reductions across informants, improved daily and social functioning and indices of sustained attention improvements, and few adverse reactions in this representative population of children with ADHD. A high level of Hyperactivity-Impulsivity symptoms before initiation of MPH were associated with nonresponse. Even though global impairments improved during treatment, the presence of more severe global impairments and psychiatric comorbidity before initiation of MPH were associated with nonresponse, higher end-dose, and/or more reporting of adverse reactions. The reduced sum score of adverse reactions that was recorded during the treatment period questions the validity of the currently available standard instruments designed to measure adverse reactions of MPH.

The pharmacogenetic study (paper II) systematically sequenced and identified seven representative variants of *CES1*, and a duplication of this gene in the clinical population of children with ADHD. In the study lower end-dose of MPH was associated with rs3815583 (GT/GG) and rs2307240 (GA), and higher response rates (normalization or borderline normalization of Inattention and/or Hyperactivity-Impulsivity) were associated with rs2302722 (GT/TT), rs111604615 (CC), and re2307240 (GA) compared to the wild types. However, the results were of small effect-size, and the genetic variants were identified in a small patient population and the results might be of limited clinical relevance. Children with variants of rs2307240 (GA) or rs3815583 (GG/GT) might be at risk of being *slow metabolizers* of MPH. There will be a need to replicate these findings in a larger population before conclusions can be made and translated into clinical guidelines for personalized medication of children with ADHD. The results have contributed to the understanding of the pharmacogenetic behind the MPH treatment response, which is probably influenced by hundreds of genetic variants. In the future, it may be possible to calculate a pharmacogenetic risk score containing many pharmacokinetic and pharmacodynamic gene variants before initiating MPH treatment.

This study contributed to the knowledge of MPH treatment response in a clinical representative sample of children with ADHD. Variants of *CES1* might in the future contribute to personalized MPH treatment for children with ADHD, but for now stepwise careful titration of MPH seemed to be an important first step in the implementation of personalized MPH treatment in the routine care. This knowledge will be worth to implement in the clinical guidelines.

5 Introduction

5.1 General introduction

Attention deficit/hyperactivity activity disorder (ADHD), consisting of maladaptive levels of inattention, hyperactivity, and impulsivity, is the most common neurodevelopmental disorder of childhood. ADHD often co-occurs with other conditions including autism spectrum disorder, anxiety, and oppositional or conduct problems and can profoundly affect academic achievement, general well-being, and personal relationships of children. ADHD risk consists of multiple genetic and environmental factors and is mediated by a range of brain networks.

Treatment of children with ADHD consists of psychological and pharmacological interventions and are applied according to national guidelines which may vary by country. Severity of the problems, comorbidity, intellectual ability and age of the child are factors that may influence the type of treatment chosen. Despite these different treatment options, medication plays an important role in most cases.

Central nervous system stimulants are usually the first choice of medication, and methylphenidate (MPH) is usually the first choice of stimulant. There is a solid evidence base of good quality randomized controlled trials (RCT) and systematic reviews that indicates that MPH has a large effect in reducing ADHD symptoms. MPH is usually prescribed with a dosage at the low end of the range and is gradually built up to reach the best possible balance between beneficial and adverse effects.

Although this sounds quite simple, there is large variation across individual children in the quantity and timing of the required dosage of MPH, and since there is no alternative for careful monitoring during the titration phase, dosage adjustment requires adequate professional time as well as cooperation of the child, the parents, and the teachers. Therefore, treatment with MPH might benefit from approaches that take individual variations in beneficial effects and adverse reactions (ARs) into account. Factors that are associated with individual variations in effects of MPH include clinical (e.g. age, gender, comorbidity, intelligence quotient (IQ), etc.) and pharmacogenetic factors. Knowledge about factors that explain variation in treatment outcomes may help adjust treatments to a more individual level. This is part

of what is called personalized medicine, which is defined as the tailoring of therapy with the best response and highest safety margin to ensure better patient care.

This thesis seeks to answer the potential for a more personalized approach to the treatment with MPH of children with ADHD based on clinical and genetic information. To this end, we conducted an observational prospective longitudinal 12-week study of immediate release MPH (IR-MPH) treatment of stimulant naïve children with ADHD individually titrated to an optimal end-dose.

The specific sub-targets were to:

a. examine the associations of clinical characteristics with outcome, ARs, and end-dose in children with ADHD treated with IR-MPH;
b. examine the association of one genotype (*CES1* gene) associated with the rate of IR-MPH metabolism with outcome, ARs, and end-dose of children with ADHD treated with IR-MPH.

5.2 ADHD

5.2.1 Definition

ADHD is a neurodevelopmental disorder characterized by pervasive and impairing symptoms of inattention and/or hyperactivity and impulsivity. ADHD persists into adulthood in about two third of the cases[1–3], and the Diagnostic and Statistical Manual of Mental Disorders (DSM-5)[4] states that symptoms of ADHD may change during the lifespan[5,6]. The diagnostic criteria of ADHD require that symptoms persist for more than six months, occur across two or more settings, and interfere with social and academic performance according to the DSM-5 and the International Classifications of Diseases and Related Health Problems (ICD-10)[7]. DSM-5 requires an onset before age 12, whereas ICD-10 requires an onset before age 7. In Denmark, the term hyperkinetic disorder is used as the term for ADHD (ICD-10 F90-F90.9)[8].

5.2.2 Prevalence

ADHD is the most common neurodevelopmental disorder in children and adolescents with a mean prevalence of 3.4%[9]. The prevalence of ADHD during the school years is about 5%[10], and is more frequent in boys than in girls [8,11,12]. Boys are diagnosed at a younger age than girls[8]. Prevalence estimates show large variations due to methodological differences in sample characteristics, assessments procedures, and diagnostic criteria across studies[9–11,13–15].

5.2.3 Comorbidity

Comorbidity is common in ADHD. A register-based study of children and adolescent aged 4-17 years from inpatient and outpatient clinics in Denmark showed that 52% with a first-time diagnosis of hyperkinetic disorder

had one or more and 26% had two or more comorbid psychiatric diagnoses[8]. Learning disability and mental retardation represented 2.8% and 7.9% respectively of the children and adolescents with comorbidity in the study[8], whereas subnormal intelligence (IQ = 70-85, *inferioritas intellectualis*) is not very often reported in studies[16].

The most common comorbid psychiatric diagnoses reported in various epidemiological and clinical studies are: autism spectrum disorders, tic disorder, anxiety, depression, and oppositional/conduct disorder[8,11,17–20].

5.2.4 Risk factors and causes of ADHD

ADHD is believed to be multifactorial and polygenetic in origin with an estimated heritability of 74-88%[21,22], where each factor have small effects on the causal pathway in the majority of cases[23,24] and ADHD might arise from a "pool" of these factors[23]. Furthermore, one meta-analysis of genome-wide association studies (GWAS) did not identify genome-wide significant associations for ADHD[25]. The polygenetic origin of ADHD means that ADHD is a result of the effect of combined and interaction of thousands of genes, where each variant contribute with a low-effect size and together be calculated as a polygenetic risk factor[26–28]. Genes that have been associated with genetic susceptibility to ADHD include: *DR4*, *DR5* (dopamine D4 and D5 receptors, *DAT1* (DAT transporter), *5-HTT* (serotonin transporter), *HTR1B* (serotonin receptor), *SNAP-25* (Synaptosomal-Associated Protein), *DBH* (DA β-hydroxylase), *MAOA* (monoamine oxidase A), *ADRA2A* (alpha 2A adrenergic receptor), *TBH2* (tryptophan hydroxylase)[29–31].

Environmental risk factors are estimated to account between 10 to 40% of the variance associated with ADHD[22]. Environmental risk factors include prenatal (e.g. low birth weight, maternal smoking), perinatal, as well as postnatal factors as early maternal deprivation[22,23,32]. However, the evidence that these factors play a causal role is conflicting[22,23,32].

ADHD is found associated to structural alterations of the brain, which include lower maturation and smaller volumes of the prefrontal cortex, the cerebellum, caudatus nucleus, right globus pallidus, and right putamen[33,34]. The delay of maturation of the cerebral cortex in children with ADHD may alter during development and may be associated with the change or remission of ADHD symptoms later on in life[33].

The pathophysiology of ADHD is an imbalance of dopamine (DA) and norepinephrine (NE) in these areas[35]. ADHD has also been linked to dysfunction of serotonin, acetylcholine, opioid, and glutamate pathways[36–41], which affect brain structures that moderate executive functions, working memory, sustained attention, and reward processing[23,41].

5.3 Treatment

5.3.1 International guidelines

The National Institute for Health and Care Excellence guideline (NICE 2019) of ADHD: diagnosis and management, recommend ensuring children with ADHD to have *a comprehensive, holistic shared treatment plan that addresses psychological, behavioral, and educational needs. Take into account: severity of ADHD symptoms and impairment, and how these affect or may everyday life (including sleep), their goals, their resilience and protective factors, and the relative impact of other neurodevelopmental or mental health conditions*[42].

The Parents of children with ADHD who are under five years should be offered parent-training with a focus on environmental modifications to minimize the impact of the child's ADHD on the everyday life[42,43]. As a starting point pharmacological treatment is not advised[42].

The parents of children with ADHD who are older than five years should be informed of how the ADHD diagnosis could affect the child's life[42]. Instructional, behavioral, environmental, and psychosocial interventions with the involvement of parents, school, and kindergarten should be offered[42–44]. Furthermore, the parents should be offered parent-training if the child have symptoms of oppositional defiant disorder or conduct disorder[42]. If there after these interventions still persists moderate impairments of ADHD symptoms and functional challenges in at least one domain of the child's life then pharmacological treatment is advised[42–44].

International guidelines, including Danish guidelines, recommend MPH as the first choice of pharmacological treatment for children older than five years and adolescents with moderate to severe impairing ADHD [42,43,45–47].These guidelines advice that titration of MPH should take at least four weeks, with three to four evaluations until no further reduction of ADHD core symptoms, or intolerable adverse reactions occur[43]. Despite the key role of pharmacological treatment for ADHD in children, the guidelines recommend psychoeducation and behavioral intervention to treat associated impairment of ADHD and comorbidities before pharmacological treatment is started[42,43,47,48].

Optimization of initial MPH treatment includes careful evaluation of the beneficial effects of increasing steps in dosage (ADHD symptoms, behavior, educational performance and social relationships) with tolerable ARs. International guidelines encourage the use of standard ADHD rating scales in the evaluation of treatment effects[42,43,45–47]. Unfortunately, these guidelines do not provide recommendations for criteria for positive treatment response.

5.3.2 Methylphenidate

5.3.2.1 Clinical optimal dose

The optimal dose of MPH varies among children. A RCT of 7- to 9-year old children with ADHD showed that the optimal dose of MPH varied from a low dose (≤15 mg/day) for one third of the responders, intermediate dose (16-34 mg/day) for another third of the responders, and high dose (>34 mg/day) for the last third of the responders[49]. A Cochrane review of 185 RCTs of children and adolescents with ADHD treated with MPH found a variation of end-dose from 4 mg to 77 mg a day and a mean end-dose of 24.5 mg/day or 0.6 mg/kg/day[50]. The study defined a low dose as ≤20 mg/day or ≤0.6 mg/kg/day and moderate to high dose as >20 mg/day or >0.6 mg/kg/day.

Although one would expect that ARs increase with increasing dose, the evidence of dose-related ARs is inconclusive. Some studies did not find dose-related ARs[51,52], but other studies reported that high doses of MPH were related to parent reported *insomnia*, *nightmares*, and *reduced appetite*[53,54].

Most international guidelines advice a maximum dose of 0.7 to 1.4 mg/kg/day (60 to 80 mg/day) for the treatment of ADHD in children and adolescents[43,55]. However, Danish guidelines as well as the British National Formulary for Children (BNF) guidelines advice a somewhat higher maximum dose of 2.1 mg/kg/day (90 mg/day)[56–58].

5.3.2.2 Pharmacology

Methylphenidate belongs to the group of stimulants. It is most commonly prescribed as a racemix mixture consisting of the *d*- and *l*-form with the former of possessing significantly higher pharmacological activity than the latter[59]. So far, the mechanism of action of MPH is poorly understood. However, it is generally believed that it acts by blocking the DAT and the NE transporters (NET)[60,61], thereby increasing the levels of DA and NE in the synaptic clefts in the brain[60–62]. The block of the DAT1 is associated with a decrease in hyperactive-impulsive symptoms in children with ADHD[63].

Immediate-Release-MPH (IR-MPH) is rapidly and completely absorbed in the intestine after oral administration and the peak blood concentration (T_{max}) occurs between one and three hours[64]. 11-52% (bioavailability) of the active form of MPH enters the system circulation[64]. MPH has a low degree of protein binding and high lipid solubility and therefore enters the central nervous system rapidly[65]. MPH is primarily metabolized by carboxylesterase 1 (CES1) in the liver[66]. This hydrolysis results in the formation of the inactive enantiomers *l*- and *d*-ritalinic acid (*d*-RA)[59], which are eliminated by urinary excretion[67].

5.3.2.3 Carboxylesterase 1

CES1 belongs to the multigene family of human carboxylesterases[68]. CES1 is one of the most important members of this family being responsible for 80-95 % of hydrolytic activity in the liver[68,69 69 68]. The gene encoding

CES1 is located on chromosome 16 and is primarily expressed in the liver (1% of the entire liver proteome)[69–71]. CES1 is responsible for the *inactivation* of a wide range of pharmaceutical agent and in, the *activation* of prodrugs[72–77] (Table 1), besides playing a role in the metabolism of several drugs of abuse[78,79]. Important substrates of CES1 are listed in (Table 1). Besides being implicated in the metabolism of xenobiotics CES1 hydrolyzes cholesterol esters[80], triglycerides[81], and bioactive lipids[82], which suggests that this enzyme has a physiological role[83].

Table 1. Important drugs metabolized by CES1 (modified)[84]	
Substrate	**Product Activity**
Cardiovascular agents	
Clopidogrel	Inactive
Dabigatran etexilate	Active
Prodrugs of ACE inhibitors [1]	Active
Antihyperlipidemic Agents	
Simvastatin	Active
Lovastatin	Active
Clofibrate	Active
Fenofibrate	Active
Antiviral Agents	
oseltamivir	Active
CNS Agents	
Methylphenidate	Inactive
Cocaine	Inactive
Meperidine	Inactive
Flumazenil	Inactive
Rufinamide	Inactive
Immunosuppressive Agents	
Mycophenolate mofetil [2]	Active
Ciclesonide	Active
Oncology Agents	
Capecitabine	Inactive
[1] Almost all Angiotensin Converting Enzyme (ACE) inhibitors are ester prodrugs and potential substrates for CES1. Most commonly prescribed ACE inhibitors are enalapril, ramipril, and trandolapril [2] Hydrolyzed by both CES1 and CES2. CES=Carboxylesterase, CNS=Central Nervous System	

5.3.2.4 Pharmacogenetics in general

Pharmacogenetics is the study of the association of genetic variation with pharmacokinetics or pharmacodynamics and outcome of drug treatments, thus enabling personalization of drug treatments[85–89]. The aim of pharmacogenomics is to optimize the individual medication response through the knowledge of the patients' genotype and thereby ensuring the best drug effect with a minimum of ARs[89,90].

The simplest and most common genetic variation belongs to a substitution of one single nucleotide for another, a single nucleotide polymorphism (SNP)[91,92]. SNPs consist of two alleles. The genotype can be either heterozygotic or homozygotic. Copy number variation (CNV) is repetition of a section of the genome which vary among individuals, which can either be of a deletion or a duplication[93].

This discipline is highly relevant since drug responses and susceptibility to adverse drug reactions vary markedly between different individuals with efficacy rates varying from 25 to 80% depending upon drug[94]. A large part of the variation in drug efficacy and susceptibility to ARs is believed to be genetically determined, most likely, having a polygenic background with contributions from small-effect sizes genetic variant, probably of 1000 to 10.000

variants[89,95]. However, this does not include genetic variants with a moderate or high-effect playing a role in a drug responses or ARs.

Genetic variation in drug metabolizing enzymes have been studied intensively through the past decades, in particular variation in the cytochrome P450 (CYP) enzymes. This has resulted in the identification of variants with moderately high-effects on drug responses and on the susceptibility to drug ARs[96]. More than 180 allelic variants of *CYP2D6* are identified, one of the most important drug metabolizing enzymes (around 160 different drugs)[92,95–97]. Interestingly, several of these *CYP2D6* variants are nonfunctional present at relatively high frequencies leading to poor metabolizer status in 1-6% of the individuals in different populations[97]. Importantly, the *CYP2D6* is also subjected to duplication, which confers increased enzyme activity and increased drug metabolism[92,97]. Being involved in the metabolism of several important and commonly used drugs, the pharmacogenetics of *CES1* would be important to examine hypothesizing that this gene also affects the metabolism and outcome of drug treatments[69,72].

The heterogeneity especially due to the methodology (especially the study design) in pharmacogenetic studies makes such studies difficult to compare[14]. Furthermore, pharmacogenetic association studies often failed to be replicated, because of the small sample sizes (the minor allele frequency (MAF) differs with the ethnicity), which is one of the criticism of pharmacogenomic research[98].

5.3.2.5 Pharmacogenetics of carboxylesterase 1

So far, pharmacogenetic studies of MPH primarily have mostly focused on pharmacodynamics[31,99–107]. It has not been possible to replicate most of the findings in these studies. This study focused on the pharmacogenetics of *CES1*.

A total of 8600 SNPs have been identified in *CES1* and deposited in the NCBI SNP-database[108]. However, many of these are rare[69,72,109] apparently only being detected in a single individual. Interestingly, *CES1* is also subjected to duplication leading to the formation of *CES1A2*, which consists of a copy of *CES1* fused with the promoter region of pseudogene *CES1P1*[110,111].

Polymorphisms in the *CES1* gene (especially loss-of-function variants (LOF)) should theoretically result in decreased or complete loss of enzyme activity (decrease in clearance) with an increase in the exposure of *d*-MPH and thereby increase the beneficial effect but also increase in risk of ARs[72]. No *CES1* gain-in-function polymorphism (duplication of the active promotor may confer increased enzyme activity) have been identified so far, but only a limited number of SNPs has been assessed for their effect on CES1[72].

So far, association of SNPs in *CES1* with beneficial effect of MPH in the treatment of ADHD have not been found but several SNPs of *CES1* have been associated with ARs or end-dose of MPH[112–115] (SI Table 1 in paper

II). Zhu et al associated the heterozygous GA genotype of rs71647871 in *CES1* (LOF variation) with significant reduced enzyme activity of CES1 in healthy adults and suggested this allele to have clinical relevance[116]. However, the frequency of the allele responsible for this was only 0.037 which limits its clinical utility. Nemoda et al which also examined the heterozygous genotype of rs71647871 found that it was associated with a lower end-dose of MPH than the genotype homozygous for the wildtype allele in children with ADHD (0.41mg/kg vs 0.57mg/kg, $p = 0.022$)[114]. Bruxel et al found that the GG and GT genotypes of rs3815583 were associated with *reduced appetite* ($p = 0.01$)[112]. This finding was supported by Oxenbøll et al who found that the same two genotypes were associated with weight loss (-0.28 kg vs +0.16 kg, $p = 0.030$)[115]. Johnson et al associated the AA genotype of rs2244613 and the GG genotype of rs2002577 with sadness ($p = 0.032$)[113].

The structural variant *CES1A2* has been found to be associated with formation of the active metabolite of irinotecan in cancer patients from Japan suggesting that this variant confers increased CES1 activity[117]. By contrast, Stage et al reported *CES1A2* decreased the metabolism of MPH[118]. Since both studies examined a limited number of subjects the importance of *CES1A2* in drug metabolism, if any, remains uncertain.

As CES1 is implicated in the metabolism a wide range of drugs, decreased activity of this enzyme may also affect the treatment outcome of range of disorders (Table 2). For example, rs151291296, rs121912777, rs146456965, rs148947808, rs201065375, rs4784575, and rs143718310 in *CES1* have been associated with decreased *in vitro* metabolism of enalapril, clopidogrel and sacubitril used in the treatment of hypertension and chronic heart failure. Additionally, rs200707504, rs71647871, rs202001817 and rs202121317 in *CES1* have been associated with decreased *in vitro* metabolism of enalapril, clopidogrel, sacubitril, oseltamivir and dabigatran etexilate[72]. However, due to low frequencies of the alleles conferring decreased drug metabolism their clinical utility of applying in pharmacogenetic screening may be limited.

Table 2 Human studies of the effect of carboxylesterase SNPs on drug disposition, and therapeutic and toxic effects (modified from[69,72])					
dbSNP ID	**Location**	**Amino acid change**	**Drugs affected**	**Comment**	**MAF[108]**
rs71647871	*CES1*, Exon 4	p.Gly143Glu	oseltamivir	Decreased formation of the active metabolites of oseltamivir but minimal effects on therapeutics[119].	0.02 (European)
			clopidogrel	Increased clopidogrel active metabolite exposure and antiplatelet effect[120].	
			methylphenidate	Decreased elimination of methylphenidate with increased therapeutic and toxic effects[114,118].	
			enalapril quinapril	Decreased formation of the active metabolites of quinapril and enalapril but minimal effects on therapeutics[121].	
rs200707504	*CES1*, Exon 5	p.Glu220Gly	oseltamivir	Decreased formation of the oseltamivir active metabolite[122].	0.00 (1000Genomes)
rs2307240	*CES1*, Exon 2	p.Ser75Asn	clopidogrel	Decreased incidence of cerebrovascular events and acute myocardial infarction[123].	0.06 (European)
rs3815583	*CES1*, 5'-UTR		methylphenidate	Increased methylphenidate appetite suppression[112].	0.06 (unknown)
rs2244613	*CES1*, intron		dabigatran etexilate	Decreased dabigatran trough concentrations and decreased bleeding risk[124].	0.19 (European)
			capecitabine	Increased toxicity of capecitabine[77].	
rs2244614	*CES1*, intron		capecitabine	Increased toxicity of capecitabine[77].	0.41 (European)
rs8192935	*CES1*, intron		dabigatran	Decreased dabigatran peak plasma concentrations[124].	0.32 (European)
rs8192950	*CES1*, intron		clopidogrel	Decreased recurrence of ischemic events[125].	0.36 (European)
rs3217164	*CES1*, intron		capecitabine	Increased toxicity of capecitabine[77].	0.45 (European)
MAF=minor allele frequency, *CES1*=carboxylesterase 1 gene, SNP=single nucleotide polymorphism					

5.3.2.6 Adverse reactions

A Cochrane review of 260 non-randomized controlled studies of children aged 5-18 years with ADHD treated with MPH found the most frequent ARs to be: insomnia (17.9 %), headache (14.4 %), abdominal pain (10.7 %), and reduced appetite (31.1 %)[126]. The most common reason for discontinuation of treatment with MPH are ARs . The average of study duration was from 4.6 to 7.9 months depending on classification of ARs.

In most studies on ARs of MPH treatment in children, ARs were assessed and reported in a non-systematic way[126,129]. However, if ARs were assessed in is a standardized way, the most frequently used rating scale was the 17-item Barkley Stimulant Side Effect Rating Scale (BSSERS), which is a 17-item clinician reported scale[130]. Principal component analyses revealed six groups of problems for this scale[53]:

- emotionality (*irritable, anxious, sadness, prone to crying*)
- sleep/appetite (*insomnia, nightmare, reduced appetite*)
- disengaged (*talks less, drowsiness, staring more*)
- euphoric/dizzy (*euphoria, dizziness*)
- uninterested (*disinterested in others*)
- aches (st*omachaches*)

The items on *nail biting*, *tics/nervous movements* and *headaches* were not included in the scales because they correlated with more than one group.

Studies that used standardized rating scales for assessing ARs over time showed that a number of ARs tend to decrease during treatment, including *irritable*, *prone to crying*, *anxious*, *nail biting*, *euphoria*, and *sadness*[51,53,54].

International guidelines, including Danish guidelines, stress the importance of monitoring weight, height, blood pressure, pulse, and ARs every third or sixth month[43,45–47,131]. The NICE guideline also advice the monitoring of tics, seizures, and sleep using an ARs checklist or a standard ARs rating scale[42]. Of course, it is important to keep ARs as low as possible.

5.3.3 Treatment effects

Guidelines for pharmacological treatment of children with ADHD are based on studies that are evaluated by the Grading of Recommendations Assessment, Development, and Evaluation (GRADE[132,133]) system[42,47] or evaluated by RCTs[43,45,46]. Studies with the highest rate of evidence (and highest degree in the GRADE system) are almost always RCTs[133–135]. RCTs seek to achieve optimal internal validity with a minimum of bias[136,137]. The randomization balances the baseline characteristics and the methods of analysis can be simple and straightforward (due to the random sampling)[138,139]. Thereby RCTs are well-designed for measuring the effectiveness of a drug over a short study period[133,139]. But the internal validity in RCTs comes at the cost of markedly reduced external validity or generalizability to the daily clinic[136]. RCTs may be biased by selection according to the studies' inclusion and exclusion criteria, the patients' willingness to participate in a randomized experimental situation, and the clinicians' willingness to allocate patients to RCTs. For example, a classical RCT can have such strict inclusion and exclusion criteria that most routinely treated patients will not be eligible[140]. This is seen in a review of RCTs of adolescents with ADHD treated with MPH excluded RCTs with adolescents with common comorbidities such as conduct disorder, autism, and intellectual disability[141]. Another strength of RCTs are the possibility to differentiate between efficacy of MPH because of the comparable groups. This was seen in the Multimodal Treatment Study of Children with ADHD (MTA), where the children were randomized into different treatment; intensive medication management alone, intensive behavioral treatment alone, combination of both, or routine community care (with medication), and the MTA study found that intensive medication management was as good as the combination of treatment and better than the two other treatments[142].

Observational studies (large-scale) can determinate long-term treatment outcomes of beneficial effects and ARs/SARs of the use of a drug because of the longer observation period compared to RCTs[133,136,139]. It is found important with clinical observational studies that measures MPH treatment outcomes in clinical representative samples

of children with ADHD regardless of ADHD severity and comorbidity in routine settings because most evidence of MPH treatment outcomes are based on RCTs[143]. Also, it can be difficult to blind placebo-controlled studies with MPH because the appetite reduction is often very obvious for the child, the parents, and the rater[44].

5.3.3.1 Treatment effects in RCTs

RCTs usually measure the efficacy of MPH between treated and untreated groups as differences over time in mean or SD of the rating scale score rated by parents or teacher[50,144,145].

A meta-analysis of 62 RCTs of IR-MPH treatment in 2897 children with ADHD showed large effect sizes (0.54 to 0.78) for mean teacher- and parent reported ADHD core behavioral scores[145]. The mean follow-up was 3.3 weeks (range 2-196 days). Patients were excluded who required highly specialized school or/and home environment (due to mental retardation, autism spectrum disorder, psychosis). Comorbidity was identified in 59.7% of the trials.

In a Cochrane review of original 185 RCT met 19 RCTs (parallel-group and cross-over trials) the primary outcome of teacher reported ADHD core behavioral scores and an effect size of 0.77 was reported[50]. The 1698 children were treated with MPH (IR-MPH, extended-release (ER-MPH), or modified-release (MR-MPH)) with a mean follow-up of 10.7 weeks (range 1-425 days). The Cochrane inclusion criteria of each RCT was that at least 75% the included patents had an IQ above 70, whereas the exclusion criteria of comorbidity belonged to each RCT.

It was not possible to identify duplets of the included studies in the meta-analysis and the Cochrane review[50,145]. The list of included studies are no longer available in Schachter et al, but studies were only included from 1981 to 1999[145]. The much newer Cochrane review included studies up to 2015[50]. There might few of the same studies in both articles.

Other RCTs on MPH treatment for ADHD in children reported improvements in cognitive functions[146], classroom behavior, and academic performance[147], although in a recent meta-analysis only small positive effects of MPH treatment on school performance was found[148].

There are not many studies of long-term effects of MPH. Therefore, a meta-analysis of long-term effect (>12 weeks) of IR-MPH included both three RCTs and four observational studies of 444 children with ADHD[144]. Two of the RCTs[127,142] were also included in the Cochrane review. The exclusion criteria were IQ < 70 and neurological comorbidity. The follow-up in weeks was 17-60.5 in the RCTs, and 13-52.1 in the observational studies. Effect sizes were large for parent and teacher reported ADHD core behavioral problem scores in both types of studies (0.96-1.25).

5.3.3.2 Treatment effects in clinical observational studies

A systematic literature search was done with the aim to review observational treatment outcome studies of MPH treatment in clinical samples of children with ADHD. The aim of the literature research was to identify

observational longitudinal cohort studies that included a non-selected group of children with ADHD who were MPH free at the time of baseline and measured the response to MPH using a standardized psychometric measurement over a specific treatment period. See below, Appendix A (Table A and B), and Supplementary materials of paper I (S1 Figure and S2 Table) for details.

Inclusion criteria

1) Sample size above 50 patients. No upper limit.
2) Mean or median age between 7 and 12 years old.
3) ADHD/ADD diagnosed as *DSM*-IIIR, *DSM*-IV, *DSM*-5 or ICD-10
4) Prospective longitudinal cohort studies with a psychiatric measurement as an outcome over time.
5) Treatment with MPH as tablets. A group of patients should be treated only with MPH and the MPH outcomes should be reported separately.
6) Consecutively selected patients from child and adolescent, pediatric departments or from a specific geographic area.
7) Patients should be methylphenidate-free at baseline (no MPH treatment at the time of inclusion).

Exclusion criteria

1) Epidemiological studies using register-based data for prescriptions and/or diagnosis.
2) Only subgroups of patients with ADHD and a specific comorbidity.
3) Studies which only measure treatment outcomes after switching of medication.
4) Reviews, case reports, and RCTs.
5) Articles before 1987.

A total of 690 studies were identified and finally 11 studies (14 publications) were included in the review[113,149–161] . The 11 studies of MPH naïve children (subgroup of the included MPH free children) included a total of n = 1537 patients (51-280 per study) with a mean age of 9.3 (range 8.0-10.3) years. The mean end-dose of MPH was 0.80 mg/kg/day (range 0.50-1.06). The mean follow-up time was 9.5 weeks (range 4-24), with several follow-up points in time varying from 1 to 6 weeks. Only nine studies monitored the response to MPH using well-known psychometric instruments and a priori defined a response criterion for symptom reduction measured with validated instruments and the reported response rates varied from 15.1% to 81.5% (see S2 Table in paper I)[149–152,154,156,159–161] . The mean reduction of ADHD core symptoms (in percentage of symptom level at entry) varied from 23.8% to 62.7%[113,150–152,154,158]. Only one study divided ADHD core behavioral problem scores into subscales with mean reductions of 41.4% to 59.3% on the Inattention and 47.3% to 66.0% on the Hyperactivity-Impulsivity subscales, respectively[152]. Seven of the 11 studies apparently monitored ARs[112,113,150,152,153,155], but the reporting was incomplete and thus difficult to summarize.

5.3.3.3 Predictors of outcome

Several RCTs and observational studies looked at predictors that moderate MPH treatment outcome with often conflicting findings. In the Cochrane review[50] of 19 RCTs there were no differences in treatment outcome between age groups, sex, comorbidity (without comorbidity versus with any psychiatric comorbidity), and ADHD subtypes (inattention subtype versus combined subtype)[50]. However, studies not included in the Cochrane review and other meta analyses reported associations between predictors and MPH treatment outcome. Below is an overview of

commonly tested predictors (not all predictors were included in the Cochrane review) of treatment outcome in children with ADHD treated with MPH.

Age. Polanczyk et al found that young age was associated with a positive treatment response in a multivariate regression analysis of data from nine studies[14].

Sex. Most studies reported no sex differences in treatment response between boys and girls[14]. Only one study found that boys had a better treatment response than girls[162].

Intelligence. A systematic review and meta-analysis of data from eight RCTs showed that children with ADHD and borderline mental retardation or mental retardation responded positively to MPH treatment compared to placebo[163]. A review by Gray and Kagan of 59 RCTs of 2950 children showed that children with an IQ <77 had less or the same treatment responses versus children with IQ in the normal range[164], and one RCT found equal MPH response between borderline mental retardation and high IQ (IQ >120)[165]. Furthermore two RCTs found that children with high IQ responded better than children with IQ in the normal range[166,167]. Overall all children regardless to level of IQ might response positively to treatment with MPH.

Comorbidity. Most studies have shown no difference in treatment response in children with or without comorbid anxiety[14,149,168,169], but there are studies that showed that both low and high levels of anxiety[164,166,167] were associated with better treatment response. In the earlier cited Cochrane review the most common comorbidities were oppositional defiant disorder and conduct disorder[50]. Polanczyk et al found that children with ADHD without oppositional defiant disorder had a better treatment response than children with oppositional defiant disorder[14]. Overall, patients with comorbidity might response positively to MPH.

ADHD subtypes and severity. A high level of inattentiveness was associated with positive treatment response in one study[118], and another study showed that a high level of the combined type symptoms was associated with a reduced treatment response[171]. Polanczyk et al did not find any difference in treatment outcome for ADHD subtypes, but a high level of ADHD severity (baseline severity score) was associated with positive treatment response[14], whereas high levels of ADHD severity (baseline severity score) was associated to less positive treatment response in the MTA study[172]. Low level global severity of disorder has also been associated with higher response rates in another study[167]. Overall might patients of attention deficit disorder without hyperactivity subtype response better to MPH treatment than patients with the combined subtype. It is difficult to compare studies of ADHD severity as a predictor of outcome, because it will also depend on how the severity and response are measured[14].

Adverse reactions. Sex and age were not associated with ARs in studies[51,53,113]. Furthermore, a study found no associations between parent-rated BSSERS, height, weight, comorbid anxiety, or comorbid oppositional

defiant disorder in a multiple regression analysis[53]. Another study did not find end-dose and severity of ADHD associated with *reduced appetite*, whereas the study found that comorbidity was associated with *reduced appetite*[51]. The evidence of clinical baseline characteristics as predictors for ARs was little, and comorbidity might be associated to ARs.

End-dose of MPH. Patients with the combined subtype of ADHD showed higher end-dose of MPH than patients with the inattentive subtype in one RCT[173]. Another RCT found higher levels of parent rated ADHD symptoms associated to higher end-dose of MR-MPH, whereas other baseline characteristics as sex, age (13-18 years), weight, and clinician rated ADHD symptoms did not differ in end-dose[52]. High level of ADHD symptoms before in initiation of MPH treatment might predict a higher optimal end-dose of MPH.

In summary, there is limited conformity of evidence for clinical predictors that moderate treatment outcome of MPH. The heterogenous group of children with ADHD will all theoretically respond positively to MPH despite of sex, age, comorbidity, intelligence level, and ADHD diagnosis and severity, and it will not be possible to predict if the beneficial effect, the end-dose, or the level of ARs will deviate from the mean of the heterogenic population of children with ADHD. Therefore guidelines have not included any of the potential predictors of outcome as moderators for treatment outcome[42,43,47].

6 Hypotheses and aims

6.1 Hypotheses

This present research study is a part of a Danish multicenter study named INDICES (INDIvidualized drug therapy based on pharmacogenetics: focus on CES1) which aimed at personalizing the treatment of drugs metabolized by CES1[174] and included several clinical and non-clinical sub-studies.

The overall subject of this prospective longitudinal observational pharmacogenetic ecological valid 12-week intervention study was to characterize the outcome and the course of outcome of individually weekly titrated IR-MPH treatment in a clinical representative sample of stimulant naïve children recently diagnosed with ADHD. We aimed to identify predictors of end-dose, beneficial effects, and ARs of IR-MPH when the primary treatment outcome was determined which was the rate of a normalization (Nor) or borderline normalization (Bnor) of the clinician rated ADHD symptoms (ADHD-Rating-Scale-Clinician (ADHD-RS-C)) in week 12.

In the pharmacogenetic literature few studies have examined the associations between SNPs or *CES1A2* of *CES1* and MPH treatment outcome in a clinical representative population of children with ADHD[112–115]. Other

candidate gene association studies of variants (SNPs or *CES1A2*) of *CES1* and CES1 metabolized drugs have found significant associations between genetic variants of *CES1* and the treatment outcome[69,72].

We hypothesize;

- Patients with variants of *CES1* will have a *slow metabolism* of MPH and will be of higher risk of ARs, will need a lower end-dose of MPH, but may have sufficient or even better beneficial effect[72,114,116].
- Patients with 3 or 4 copies of *CES1* (*CES1A2*) will theoretically have a *fast metabolism* and will need a higher end-dose of IR-MPH, may have insufficient beneficial effect of IR-MPH, and may not be of higher risk of ARs (most studies found no difference in outcome[175–178]).
- A proportion of children with ADHD will differ in MPH treatment outcome; end-dose, beneficial effects, and/or ARs with regard to clinical baseline characteristics (paper I) and variants of *CES1* (paper II) compared to other the children with ADHD.

6.2 Aims

Paper I aim:

1) to describe the first 12 weeks of IR-MPH treatment outcome based on parent and teacher rated ADHD core symptoms and behaviors, the parent rated daily and social functioning, indices of sustained attention, physical examination, nonreponder status, clinician rated global severity of psychiatric disorder, the weekly clinician rated ADHD core symptoms and behaviors and the weekly clinician rated ARs.
2) to provide information of the predictive value of clinical baseline characteristics (sex, age group, global severity of psychiatric disorder, psychiatric comorbidity, weight, and subtype of ADHD diagnoses) for end-dose of IR-MPH, nonresponder status, the weekly clinician rated ADHD total core symptoms and rate of Nor/Bnor, and the weekly clinician rated ARs.

Paper II aims:

1) to sequence DNA from children with ADHD and select variants of *CES1* throughout predefined criteria
2) to provide information of explorative associations between the selected variants of *CES1* and end-dose of IR-MPH, the weekly clinician rated ADHD core symptoms and rate of Nor/Bnor, and the weekly clinician rated ARs when adjusted for the clinical baseline characteristics (models identified in paper I).

7 Material and methods

7.1 Power considerations

The different rare SNPs of *CES1* were estimated to be of a total frequency of 0.05 and *CES1A2* was estimated to be of a frequency of 0.20 in the Danish population. In the simulated models each single variant of *CES1* was assumed to be of an additive effect on a continuedly normally distributed outcome. The power estimations are measured in "Quanto" (version 1.2.4, May 2009, http://hydra.usc.edu/gxe/).

When it was assumed that the outcome was the end-dose of IR-MPH = 1 mg/kg/day (SD 0.5 mg/kg/day) *or* it was assumed that the outcome was the average of IR-MPH titration duration = 6 weeks (SD 2 weeks) *and* the significance level was of 0.05 (one-sided) *and* the power was of 0.80 then a minimum effect size measured as a significant regression-coefficient of $\beta = 0.25$ (mg/kg/day) *or* $\beta = 0.1$ (weeks) would be detectable in

- 94 children (frequency of 0.20 in *CES1A2*)
- 257 children (total frequency of 0.05 of SNPs of *CES1*)

It was considered that inclusion of at least 200 children would be enough because it was assumed that the rare SNPs of *CES1* would have a relatively larger impact on the treatment outcome compared to *CES1A2*. The power estimations were the same for the two outcomes because it was assumed that the variance was of approximately the same size.

There was not made a power calculation for the observational study since this part of the study was based on the pharmacogenetic study.

7.2 Settings and ethics

The study presents data from children from the Child and Adolescent Mental Health Centre, Mental Health Services (Capital Region of Denmark). The study was defined in a protocol (Appendix B). The study followed the protocol with the only deviations that the results of Clinical Global Impression Efficacy (CGI-E)[179], ASK-ME attitude[180], and blood samples measuring the concentration of IR-MPH were not included in the two papers . The study was registered in ClinicalTrials.gov (NCT04366609) and was approved by the Danish Data Protection Agency (P-2019-851). The Local Committee on Health Research Ethics was consulted (J.nr.H-B-2009-026) in accordance with national guidelines and the Declaration of Helsinki. The parents provided written informed consent for DNA samples for research purposes. The IR-MPH treatment was part of the observational study, and did not provide written inform consent, but the parents and the patients were informed (written and oral) about pharmacological treatment of ADHD and IR-MPH as a part of the routine in the clinic. Participation was voluntary and data were kept confidential. The

participants could withdraw their consent at any time without having to give reasons and with no consequences for their further treatment options.

7.3 Participants

7.3.1 Identification of the children

All patients who were referred to Child and Adolescent Mental Health Centre, Mental Health Services, Capital Region Denmark in the period from 1st of May 2012 to 1st of August 2014 and who were suspected of having ADHD were consecutively screened for study eligibility. This is a well-defined catchment area. All patients who met the criteria for inclusion were offered to participate in INDICES and were consecutively included (Figure 2). The study included stimulant naïve boys and girls aged 7-12 years with a recent ICD-10 diagnosis of hyperkinetic disorder (F90.0-90.9) or attention deficit disorder without hyperactivity (F98.8) and clinical indication for treatment with IR-MPH.

Exclusion criteria for **paper I and II** were mental retardation (ICD-F70.X or IQ < 70), previous treatment with drugs metabolized by CES1, severe comorbid psychiatric or somatic disease that resulted in contraindication for treatment with MPH (e.g., schizophrenia or cardiac disease), language barriers, refused treatment with MPH, and refused participation in INDICES.

Exclusion criteria for **paper II** were lack of informed consent for DNA, consanguine patients, non-Caucasian patients, no available data of SNPs or *CES1A2* of *CES1* when sequenced, SNPs or *CES1A2* of *CES1* with a MAF below 0.05, and SNPs or *CES1A2* of *CES1* that did not meet Hardy-Weinberg equilibrium.

7.3.2 Diagnostic evaluation

The diagnostic procedure was performed in accordance with the Danish "National clinical guideline for the assessment of ADHD in children and adolescents"[44,47,57 181] and was based on best practice and routine examination using standardized instruments. All children were assessed with the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children, Present and Lifetime Version (K-SADS-PL)[182], parent rated ADHD-RS (ADHD-RS-P) and teacher rated ADHD-RS-T (ADHD-RS-T)[183–185], parent rated Child Behavior Checklist (CBCL) and teacher rated Teacher's Report Form (TRF)[186–188], Weiss Functional Impairment Rating Scale–Parent version (W-FIRS-P)[189,190], and Test of Variables of Attention (TOVA)[191,192]. A child and adolescent psychiatrist confirmed the ADHD diagnosis and any psychiatric comorbidities disorders.

7.3.3 Assessment procedure and testing reliability

The enrolment in the study was of 12 weeks and the initial assessments were done immediately before initiation of IR-MPH in week 0. The clinical investigator undertook the weekly evaluation of ADHD symptoms on ADHD-RS-C[185,193] and ARs on clinician-rated BSSERS[130] **(**BSSERS-C) (Figure 1). The weekly evaluations were based

on telephone interviews with the parent in week 1, 2, 3, 5, 6, 7, 9, 10, 11, and on more thorough clinical evaluation at baseline (week 0) and in week 4, 8, and 12, including interviews with the parents about the children's daily functioning and symptoms, clinical observation and physical examination of the patients, and parent- and teacher-rated ADHD symptoms and behaviors (ADHD-RS-P and ADHD-RS-T). Consensus ratings of the children's ADHD symptoms on ADHD-RS-C in week 0 and 12 were conducted by the clinical investigator and the children's regular clinicians. The same pair consisting of the clinical investigator and a child's regular clinician followed the same patient over 12 weeks, and the PhD was the clinical investigator and rater in 71% of cases. In weeks 0 and 12, each rater performed an independent rating of the ADHD-RS-C before they agreed on a consensus rating.

The two independent ratings were used for calculation of the interrater reliability for the pair at each time point using the intraclass correlation coefficient (ICC). The mean of the ICCs was calculated for each pair across time points. The mean ICCs ranged from 0.72 to 0.99 (mean of ICC = 0.90). If any disagreement was found, the single items of ADHD-RS-C were discussed until consensus was reached. Consensus ratings of ADHD-RS-C scores in week 0 and 12 were measured to set an individual standard for the clinical investigator ratings in week 1 to 11. Decisions about changes in IR-MPH dosing or discontinuation due to ARs were made in collaboration with the children's' regular clinicians and the families at the study site.

Furthermore, the body weight, height, heart rate and blood pressure were measured every fourth week with the same equipment each time. The study followed the European guidelines[194] and measured blood pressure after 10 minutes of rest with a sphygmomanometer (nonelectrical) three times and the average of the last two measures was used.

7.3.4 Administration of IR-MPH

Based on British and Danish guidelines[42,44,47,57], we developed an enhanced IR-MPH-treatment manual for the present study. This included weekly evaluation of symptom change and presence of ARs to allow for an individually titrated optimal dosing of IR-MPH (Figure 1). The initial dose and weekly dose increment were 2.5 or 5 mg IR-MPH (depending on weight </> 30 kg) and were given two or three times a day. Individual titration continued until a clinically significant response, defined as normalization or borderline normalization (Nor/Bnor) on clinician rated ADHD-RS (ADHD-RS-C) was achieved (see 7.4.1), ARs prohibited further up-titrations of the total daily dose of IR-MPH, or the maximum dose was reached (2.1 mg/kg/day).

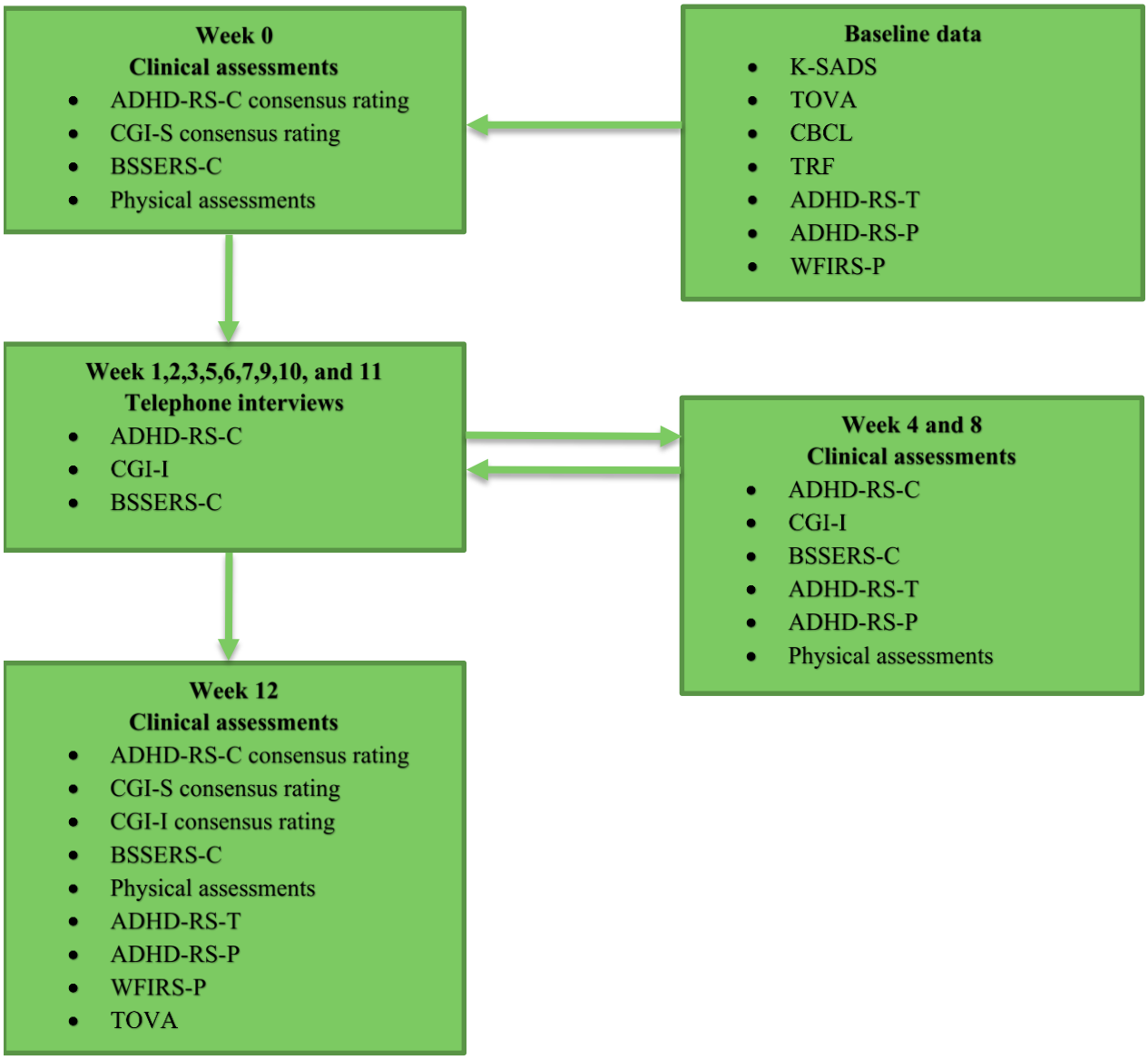

Figure 1 Flow chart of psychometric measurements, questionnaires, and physical assessments in the study.

7.4 Definition of primary and secondary outcome measures

7.4.1 Primary treatment outcome measure of paper I and paper II

The primary treatment outcome was a Nor or Bnor (Nor/Bnor) of sum scores of Inattention and Hyperactivity-Impulsivity on ADHD-RS-C[185,193] for both papers. The 18-item ADHD-RS-C is identical to the ADHD-

RS-P and ADHD-RS-T subscales measuring Inattention and Hyperactivity-Impulsivity, whereas Conduct problems are not scored[193] (see section 7.4.2.1). High correlations between ADHD-RS-C and ADHD-RS-P have been documented[195]. ADHD-RS-P and ADHD-RS-T have previously been validated and normed in Denmark[185], and the standardized scores (*t-scores*) stratified for each sex and age group (7-9 and 10-12 years) were used to delineate the cut-off for Nor (≤ 60 *t-scores*) or Bnor (60-70 *t-scores*). ADHD-RS-C scores were determined using all available information including interviews with the parents, the ADHD-RS-P and the ADHD-RS-T scores, psychiatric assessment, and observation of the children. ADHD-RS-C were used to describe the change in Inattention and Hyperactivity-Impulsivity symptoms (sum scores) and the proportion of patients who meet the primary treatment response defined as Nor/Bnor Inattention and Hyperactivity-Impulsivity symptoms using standardized scores of ADHD-RS-P.

7.4.2 Secondary outcome measures of paper I

7.4.2.1 Parent and teacher rated ADHD symptoms

The ADHD-RS-P and the ADHD-RS-T[183–185] each consist of nine questions of inattention, nine questions of hyperactivity and impulsivity, and six questions of conduct problems evaluated on a four-point Likert scale from 0 (*none = never or rarely*) to 3 (*severe = very often*). ADHD-RS-P and ADHD-RS-T were done in week 0, 4, 8, and 12.

7.4.2.2 Severity of symptoms

The clinician-rated CGI-Severity (CGI-S)[179] is a seven-point Likert scale, rated 1 (*normal, not ill at all*) to 7 (*among the most extremely ill patients*), used to measure the global severity of symptoms and functional impairments of mental health disorder including ADHD and psychiatric comorbidities. Consensus ratings were performed in week 0 and 12. A beneficial symptom reduction was defined by a CGI-S score of 1 or 2 (*normal, not ill at all* or *borderline ill*).

The clinician-rated CGI-Improvement (CGI-I)[179] is also a seven-point Likert scale rated from 1 (*very much improved*) to 7 (*very much worse*). Evaluation on CGI-I was done in week 4 and 8, and consensus ratings were performed in week 12. A beneficial symptom reduction was defined by a CGI-I score of 1 or 2 (*very much improved* or *much improved*).

7.4.2.3 Daily and social functioning

The child's daily and social functioning was measured with the parent reported W-FIRS-P[128,189,190] which consists of: family (10 items), learning and school (10 items), activities of daily living (10 items), self-concept (3 items), social activities (7 items), and risky activities (10 items). The W-FIRS-P is scored on a four-point Likert scale

from 0 (*never or not at all*) to 3 (*very often or very much*). The W-FIRS-P was administered W-FIRS-P in week 0 and 12.

7.4.2.4 Sustained attention

TOVA[191,192] (version 7.3 and 8.0[196,197]) is a computerized, continuous performance test and is validated to evaluate the objective response to the treatment of ADHD as the persistent attention over time and was administrated in week 0 and 12. Number of commission errors (response to non-target), number of omission errors (non-response to target), the response time in microseconds, and the variability of response time in correct responses to target were measured over the 21.6-minute-long test used as total raw scores. Consistently, IR-MPH was dosed at 1 p.m. and TOVA was performed at 2 p.m.[198] to ensure an equal and sufficient exposure of IR-MPH at the week 12-assessment (maximal plasma concentration is achieved 1-2 hours after administration of IR-MPH[67]). In week 0 and 12, total raw scores were converted to *z*-scores based on mean and standard deviation of week 0 raw scores due to the lack of standard of TOVA for Danish children.

7.4.3 Secondary outcome measures of paper I and paper II

7.4.3.1 Adverse reactions

ARs were measured on the clinician rated BSSERS-C[130] based on information from patients, parents, and clinical observations scored before initiation of IR-MPH-treatment (week 0) and weekly during treatment. A manual for interviewing and rating using BSSERS-C was elaborated for the study, anchoring the 10-point problem scores from 0 (*problem absent*) to 9 (*problem evokes serious impairment*) of the 17 effect items (e.g., specifying the time to fall asleep when scoring insomnia). Severities of ARs were calculated as the sum of problem scores [range 0-153]. *Reduced appetite* [range 0-9] was the only single item of significant increase and moderately to severely *reduced appetite* was defined as a score of ≥ 4 when dichotomized.

7.4.3.2 Dosing of IR-MPH

The end-dose in week 12 was measured as IR-MPH mg/kg/day. Low end-dose of IR-MPH was defined as < 0.80 mg/kg/day.

7.4.3.3 Nonresponder status

The nonresponder status was defined as;

1) discontinued treatment due to ARs or serious ARs (SARs) *or*

2) not Nor/Bnor on any of the subscales of ADHD-RS-C in week 12.

Based on the clinical experience that these two types of poor outcome are interrelated.

7.4.4 Secondary outcome measure of paper II

7.4.4.1 Identification and selection of variants of *CES1*

DNA was extracted from saliva collected in the Oragene OG-250 DNA kit (DNA Genotek Inc., ON, Canada) as described by the manufacturer. Using the Illumina Infinium OmniExpressExome-8v1-3_A array the samples of DNA had been genotyped in another study (Madsen et al, unpublished), which is a genetical principal component analysis to detect and exclude DNA of consanguine and Non-Caucasian patients (available on request). Determination of *CES1* copy number by real-time PCR served to detect presence of *CES1A2.* This was followed by PCR-based amplification of fragments of *CES1* and Sanger sequencing of these fragments[111,174]. The amplification reaction for production of a fragment of *CES1* containing exon 6-14 also amplifies *CES1A2*. Variants with a MAF above 0.05 and with genotype proportions that did not deviate from those expected under conditions of Hardy-Weinberg equilibrium after correction for multiple testing were selected for subsequent association analyses. To avoid testing of intercorrelated SNPs and avoid redundant analyses, the selected SNPs also had to be independent defined as lack of strong linkage disequilibrium, namely, D' < 0.80.

The homozygotic variant was combined with the heterozygotic variant if $n \leq 5$ of homozygotic variant. If n = 6-10 of homozygotic variant, the variant was analyzed twice i.e. first kept separately and secondly combined with the heterozygotic variant. The wild type was the reference to the homozygotic and/or heterozygotic variant in all analysis.

7.4.5 Definitions of predictors

Paper I explored the predictive value of following baseline characteristics: age (7-9 or 10-12 years), sex, comorbidity (two/more or none/one comorbid psychiatric diagnosis), intelligence level (IQ = 70-85 (*inferioritas intellectualis*) or IQ > 85), conduct disorder (ICD10 DF90.1/DF91.X/DF92.X or no conduct disorder), CGI-S in week 0 as a continuous variable [range 1-7] or as a dichotomized variable ([range 1-5] or [range 6-7]), and weight in week 0 (≤ 30kg or > 30kg) with regard to the outcomes and course of outcomes of IR-MPH treatment. Conduct disorder and *inferioritas intellectualis* were tested as individually covariable, but these variables were also included the as a part of the comorbidity variable.

Paper II explored the associations between the identified and selected variants (SNPs and *CES1A2*) of *CES1* (see results for variants of *CES1* in 8.2, Table 6) and treatment outcomes course of outcomes of IR-MPH with adjustment for clinical baseline characteristics.

7.4.6 The role of the PhD student

The clinical investigator was primarily the PhD student, who did most of the weekly follow-ups including IR-MPH treatment evaluation by psychiatric and physical examination in collaboration with the children's' regular clinicians. A nurse, a medical student, and a senior MD in child and adolescent psychiatry also helped with including data collection.

7.5 Statistical analyses

SPSS version 22 was used for the statistical analyses in both paper I and paper II[199]. For overview of the statistic models see Table 3.

Comparisons of baseline characteristics of included and excluded patients were carried out with the independent *t*-test for continuous variables and X^2 (chi-squared) test for categorical variables (n = 418). Changes from week 0 to week 12 in treatment outcomes were analyzed with paired *t*-test for continuous variables and X^2 test for categorical variables (n = 187).

Univariate associations of the variants of *CES1* (SNPs or *CES1A2*) and categorical and continuous outcomes were analyzed with X^2-test, independent t-test, and one-way analysis of variance, respectively (n = 172).

Linear mixed effect models for repeated measures were used on the responses of the sum scores of Inattention and Hyperactivity-Impulsivity (ADHD-RS-C) to explore the predictive value of clinical baseline characteristics and were used on the responses of the sum score of ARs (BSSERS-C) to explore the predictive value of clinical baseline characteristics and the variants of *CES1*. Time was measured as a continuous variable coded 0, 1, 2…12 weeks, and all other explanatory variables were measured as categorical variables. It was problematic to estimate the full mixed models including all explanatory variables simultaneously.

Repeated measures of reduced appetite (single item of BSSERS-C) were not normally distributed and was dichotomized into mildly reduced or no reduced appetite (BSSERS-C single score ≤ 3) and moderately to severely reduced appetite (BSSERS-C single score ≥ 4). To identify potential predictors (the clinical baseline characteristics and the variants of *CES1*) of *reduced appetite* over 12 weeks, generalized estimation equations (GEEs) were used[200].

To determine independent predictors for the chance of normalization or borderline normalization (Nor/Bnor) on ADHD-RS-C Inattention and Hyperactivity-Impulsivity, Cox regression analysis with backward elimination (clinical baseline characteristics) and without backward elimination (clinical baseline characteristics and variants of *CES1*) were used. Patients were registered as an event if Nor/Bnor during the study and the first week of Nor/Bnor was the event time. Patients for whom no Nor/Bnor was registered, were censured and the censure time was the last week of a registered no Nor/Bnor.

The association between the end-dose of IR-MPH per day at week 12 and clinical baseline characteristics was explored using linear regression with backward eliminations.

The association between variants at *CES1* and the end-dose of IR-MPH-dose in week 12 was explored using linear regression estimated using generalized least squares (GLS) with adjustments for clinical baseline characteristics without backward elimination.

Table 3 Overview of statistic models used in paper I and paper II

Statistic method	Outcome	Variables	Significant model
Paper I			
Cox regression Backward elimination	**Inattention** Nor/Bnor on ADHD-RS-C	Age, sex, intelligence level, conduct disorder, and comorbidity	Sex, intelligence level
Cox regression Backward elimination	**Hyperactivity-Impulsivity** Nor/Bnor on ADHD-RS-C	Age, sex, intelligence level, conduct disorder, and comorbidity	Age, intelligence level, comorbidity
Linear mixed effect model Pair wise testing	**Inattention** Sum score on ADHD-RS-C	Week, *week, sex, *sex, age, *age, intelligence level, conduct disorder, and comorbidity	Time, age, sex, age*sex.
Linear mixed effect model Pair wise testing	**Hyperactivity-Impulsivity** Sum score on ADHD-RS-C	Week, *week, sex, *sex, age, *age, intelligence level, conduct disorder, and comorbidity	Time, age, time*age
Linear mixed effect model Pair wise testing	**Adverse reactions** Sum score on BSSERS-C	Week, *week, sex, *sex, age, *age, intelligence level, conduct disorder, comorbidity, ADHD diagnosis, and CGI-S	Week, CGI-S
Generalized estimation equations Pair wise testing	**Reduced appetite** Single score on BSSERS-C	Week, *week, sex, *sex, age, *age, intelligence level, conduct disorder, comorbidity, ADHD diagnosis, and CGI-S	Week, CGI-S
Linear regression Backward elimination	**End-dose of IR-MPH**	CGI-S, ADHD diagnosis, sex, age, and weight	Age, CGI-S
Paper II			
			Only significant variants of *CES1* of each model
Cox regression Without backward elimination	**Inattention** Nor/Bnor on ADHD-RS-C	Variants of *CES1,* age, sex, intelligence level, conduct disorder, and comorbidity	rs56278207 rs2302722
Cox regression Without backward elimination	**Hyperactivity-Impulsivity** Nor/Bnor on ADHD-RS-C	Variants of *CES1,* age, sex, intelligence level, conduct disorder, and comorbidity	rs2302722 rs2307240 rs111604615
Linear mixed effect model Pair wise testing	**Adverse reactions** Sum score on BSSERS-C	Variants of *CES1,* week, *week, CGI-S, sex, *sex, age, and *age	rs2244614*week
Generalized estimation equations Pair wise testing	**Reduced appetite** Single score on BSSERS-C	Variants of *CES,* week, *week, CGI-S, sex, *sex, age, and *age	None
Generalized least squares Without backward elimination	**End-dose of IR-MPH**	Variants of *CES1,* ADHD diagnosis, CGI-S, sex, *sex, age, *age, and weight	rs3815583 rs2307240

CES1 = Carboxylesterase 1, ADHD-RS-C = ADHD-Rating Scale (DuPaul) clinician rated, BSSERS-C = Barkley Stimulant Side Effects Rating Scale, clinician rated, CGI-S = Clinical Global Impression Severity.
Week = time, *week, *age, and *sex were tested in interactions with other covariates.
All covariates were tested pair wise with *week in linear mixed effect model and generalized estimation equations in paper I.
All variants of *CES1* were tested pair wise with *week in linear mixed effect model and generalized estimation equations in paper II

8 Results of paper I and paper II

8.1. Study population

Paper I included 207 children out of 418 children who were screened for eligibility (Figure 2).

Paper II included 189 patients of the 207 patients who were included in paper I. The reasons for exclusion were: lacking DNA because of the impossibility to sequence the variants of *CES1* from the sputum or did not what to participate with DNA (four patients), non-Caucasian origin (nine patients), and consanguinity (five patients). There by we included 7 children more in the observational study and 11 children less in the pharmacogenetic study than what was estimated through power calculation.

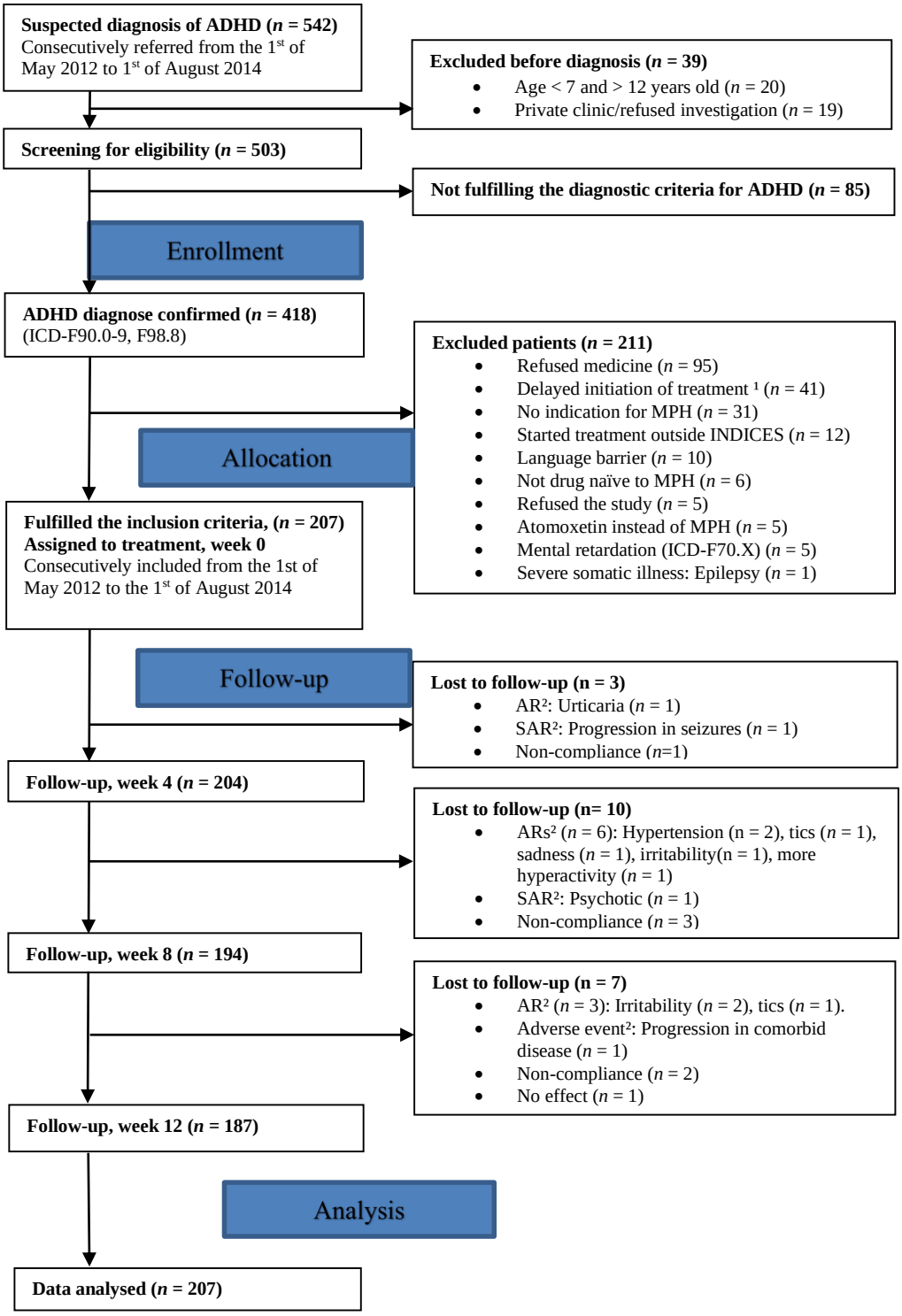

Figure 2 TREND, flow diagram of inclusion
ICD-10 = International Classification of Diseases and Related Health Problems, ADHD = Attention-deficit/hyperactivity disorder, MPH = Methylphenidate, INDICES = INDIvidualised drug therapy based on pharmacogenetics: focus on carboxylesterase 1, AR = Adverse reaction, SAR = Serious adverse reaction.
¹ = Treatment initiated after the study inclusion period was terminated.
² = Clinician decision of patient's discontinuation of treatment with MPH due to adverse events, ARs, and SARs.

8.1.1 Characteristics of the included and excluded children

The main reasons for non-participating were that the parents did not consent to MPH treatment of their children or a delayed decision to initiate MPH treatment of the eligible group of children (Figure 2). Only five children were excluded because of parents' refusal to participate in INDICES. There were relatively more males among the excluded patients than included in the study ($p = 0.030$) (Table 4).

	Included (*n* = 207)	**Excluded (*n* = 211)**	**Refused medicine[1] (*n* = 95)**
Boys, *n* (%)	156 (75.4)	177 (83.9)	75 (78.9)
Age, years, mean (SD)	9.6 (1.5)	9,88 (1.5)	9.64 (1.6)
Age, 7-9 years, *n* (%)	133 (64.3)	119 (56.4)	56 (58.9)
Age, 10-12 years, *n* (%)	74 (35.7)	92 (43.6)	39 (41.1)
ADHD diagnoses (ICD-10)			
Disturbance of activity and attention, hyperkinetic disorder other, hyperkinetic disorder unspecified. (F 90.0, F 90.8, F 90.9), *n* (%)	172 (83.1)	163 (77.3)	74 (77.9)
Hyperkinetic conduct disorder. (F 90.1), *n* (%)	12 (5.8)	14 (6.6)	5 (5.3)
Attention deficit disorder without hyperactivity. (F 98.8,), *n* (%)	23 (11.1)	34 (16.1)	16 (16.8)
Comorbidity (ICD-10)			
Tic disorders. (F95.X), *n* (%)	17 (8.2)	14 (6.6)	4 (4.2)
Externalizing disorders. Conduct disorders, mixed disorders of conduct and emotions. (F 91.X, F 92.X), *n* (%)	14 (6.8)	9 (4.3)	4 (4.2)
Specific developmental disorders. (F81.X-83.X, F88.X), *n* (%)	46 (22.2)	46 (21.8)	26 (27.4)
Cognitive deficits. Inferioritas intellectualis, mental retardation. (R41.8[2], F70-79), *n* (%)	56 (27.1)	62 (29.4)	27 (28.4)
Encopresis and/or enuresis. (F98.0-98.1), *n* (%)	24 (11.6)	18 (8.5)	8 (8.4)
Autism spectrum disorders. (F84.X), *n* (%)	26 (12.6)	40 (19.0)	15 (15.9)
Attachment disorders. (F94.X), *n* (%)	3 (1.4)	7 (3.3)	1 (1.1)
Emotional disorders. Depressive episode, persistent mood-, obsessive-compulsive-, reaction to severe stress, and adjustment disorders, emotional disorders with onset specific to childhood. (F 32.X, F 34.X, F 42.X, F 43.X, F 93.X), *n* (%)	27 (13.0)	23 (10.9)	10 (10.5)
Number of comorbid psychiatric diagnoses			
No comorbidity, *n* (%)	72 (34.8)	64 (30.3)	29 (30.5)
1 comorbid diagnosis, *n* (%)	78 (37.7)	85 (40.3)	39 (41.1)
2 comorbid diagnoses, *n* (%)	39 (18.8)	52 (24.6)	25 (26.3)
3 comorbid diagnoses, *n* (%)	15 (7.2)	10 (4.7)	2 (2.1)
4 comorbid diagnoses, *n* (%)	3 (1.4)	0 (0.0)	0 (0.0)

ICD-10 = International Classification of Diseases and Related Health Problems.
[1] Patients, who refused medicine, were a subgroup of excluded patients.
[2] R41.8 referred to diagnose from the diagnostic conference and/or from the intelligence test (WISC) (IQ 70-85) depending on data access.

Table 4 Characteristics of included and not included patients at study entry

The comparison of included versus excluded patients furthermore showed that the included patients had significantly higher ADHD-RS-P scores, and significantly lower ADHD-RS-T scores compared with those excluded (Table 5). The group of patients who were excluded solely because of their parents' refusals ($n = 95$) were characterized by lower ADHD-RS-P scores compared to patients excluded due to other reasons ($n = 116$), 10.9 (SD 5.3), and 13.9 (SD 6.3), respectively, ($p < 0.001$). More baseline characteristics are listed in S4 Table in paper I am including CBCL, TRF, and W-FIRS-P.

Table 5 Comparison of ADHD symptoms severity between included and excluded patient

ADHD symptoms before inclusion	Included (*n* = 207) M (SD)	Excluded (*n* = 211) M (SD)	Included versus excluded			Refused medicine (*n* = 95) M (SD)	Included versus refused medicine		
			M dif. (SD dif.)	95% CI	*p*-value		M dif. (SD dif.)	95% CI	*p*-value
Parent									
Inattention	17.4[1] (5.05)	15.1[7] (5.6)	2.3 (0.6)	(1.3, 3.4)	< 0.001	14.6[12] (5.3)	2.8 (0.7)	(1.5, 4.1)	< 0.001
Hyperactivity-Impulsivity	15.3[2] (6.1)	12.5[7] (6.0)	2.9 (0.6)	(1.6, 4.1)	< 0.001	10.9[13] (5.3)	4.5 (0.8)	(3.0, 6.0)	< 0.001
Conduct problems	10.5[3] (5.9)	8.1[8] (5.5)	2.4 (0.6)	(1.3, 3.6)	< 0.001	7.5[14] (5.1)	3.0 (0.7)	(1.6, 4.5)	< 0.001
Teacher									
Inattention	17.1[4] (4.9)	18.5[9] (5.1)	-1.4 (0.5)	(-2.4, -0.3)	< 0.010	18.2[15] (4.6)	-1.1 (0.6)	(-2.4, 0.1)	0.081
Hyperactivity-Impulsivity	9.0[5] (5.7)	15.1[10] (7.2)	-6.0 (0.7)	(-7.4, -4.7)	< 0.001	14.3[16] (7.3)	5.2 (0.8)	(3.6, 6.8)	< 0.001
Conduct problems	4.5[6] (4.6)	9.0[11] (6.5)	-4.4 (0.6)	(-5.6, -3.3)	< 0.001	8.2[17] (6.4)	-3.6 (0.7)	(-5.0, 2.3)	< 0.001

Independent t-test: Comparison between included vs. excluded patients and comparison between included and patients excluded due to parent's decision of refusing medicine (a subgroup of excluded patients)
M = Mean. M dif. = Mean difference. SD dif. = Standard deviation difference.
ADHD-Rating Scale (DuPaul). Inattention subscale: 9 items [range 0-27]. Hyperactivity-Impulsivity subscale: 9 items [range 0-27]. Conduct problems subscale: 8 items [range 0-24]. Rated by parent (ADHD-RS-P) or teacher (ADHD-RS-T).
[1] *n* = 200, [2] *n* = 197, [3] *n* = 204, [4] *n* = 187, [5] *n* = 191, [6] *n* = 193, [7] *n* = 174, [8] *n* = 181, [9] *n* = 170, [10] *n* = 173, [11] *n* = 177, [12] *n* = 78, [13] *n* = 80, [14] *n* = 84, [15] *n* = 78, [16] *n* = 77, [17] *n* = 79.

The 189 patients who also were included in the pharmacogenetic part of the study in paper II did not differ in baseline characteristics from the whole sample: 144 boys (76.2 %) with a mean age of 9.6 (SD 1.5), 156 (82.5 %) with hyperkinetic disorder, 12 (6.3 %) with hyperkinetic conduct disorder, and 21 (11.1 %) with attention deficit disorder without hyperactivity (Table 1, paper II).

8.2 Selection of variants of *CES1*

Accordingly, we did not perform amplifications of this *CES1* fragment from individuals harboring, *CES1A2*. Since deletion of *CES1* is extremely rare, the detection of three and four copies by the real-time PCR analysis was interpreted as the presence of one and two copies of *CES1A2*, respectively. 44 SNPs of *CES1* were identified besides the variation creating *CES1A2*. Twenty of the SNPs had MAFs below 0.05 and were thus considered rare. In total, six SNPs in *CES1* (rs3815583, rs111604615, rs56278207, rs2302722, rs2307240 and rs2244614) and the, *CES1A*2, fulfilled our predetermined criteria for subsequent association analyses (Table 6). rs71647871 (p.Gly143Glu) was included, because of the possible effect on the metabolism of MPH[116]. The remaining SNPs selected for the association analyses were all located in intervening and therefore noncoding sequences. In the power estimation the study should include at least 200 individuals of each single genetic variant of *CES1*, which the study after sequencing and selection were not able to. More characteristics of the variants of *CES1* are listed in SI Table 2 in paper II.

Table 6 Characteristics of variants of *CES1*.

CES1 marker	Location in gene	Genotype frequency, *n* (%)	MAF	% genotyped
Copy number *n* = 182	NA	3,4: *n* = 54 (29.7)	0.154	96.3
		2: *n* = 128 (70.3)		
rs3815583 *n* = 176	5'UTR	GT/GG: *n* = 67 (38.1)	0.210	93.1
		TT: *n* = 109 (61.9)		
rs111604615 *n* = 175	X1	CC: *n* = 9 (5.1)	0.174	92.6
		GC: *n* = 43 (24.6)		
		GG: *n* = 123 (70.3)		
rs56278207 *n* = 167	IVS1	del/del: *n* = 18 (10.8)	0.269	88.4
		del/ins: *n* = 66 (39.5)		
		ins/ins: *n* = 83 (49.7)		
rs2307240 *n* = 122	X2	GA: *n* = 12 (9.8)	0.049	64.6
		GG: *n* = 110 (90.2)		
rs2302722 *n* = 84	IVS9	GT/TT: *n* = 18 (21.4)	0.131	44.4
		GG: *n* = 66 (78.6)		
rs2244614 *n* = 110	IVS10	CC: *n* = 8 (7.3)	0.286	58.2
		CT: *n* = 47 (42.7)		
		TT: *n* = 55 (50.0)		
rs71647871 *n* = 184	X4	GA: *n* = 8 (4.3)	0.022	97.3
		GG: *n* = 176 (95.7)		

CES1 = Carboxylesterase 1 gene, MAF = Minor Allele Frequency, NA = not applicable, 5' UTR = 5' untranslated region, X = exon, IVS = intervening sequence. Baseline sample (*n* = 189).

8.3 Treatment outcomes

In the study, 187 patients (90.3% mean age of 9.6 (SD 1.5), 140 boys (74.9%)) (paper I) and 172 (91.0%) patients (paper II) completed the 12 weeks treatment period. In this section only treatment outcomes of ADHD symptoms and behavior, end-dose, ADHD related symptoms and impairments, and ARs from paper I are mentioned.

8.3.1 ADHD symptoms and end-dose

The primary treatment outcome was determined as the rate of Nor or Bnor (Nor/Bnor) of ADHD-RS-C in week 12. In week 12, 137 (73.2%) of the patients obtained Nor/Bnor on the Inattention subscale, 157 (84.0%) of the patients obtained Nor/Bnor on the Hyperactivity-Impulsivity subscale, 19 (10.1%) of the patients were not Nor/Bnor on any of the subscales, 126 (67.4%) of the patients were Nor/Bnor on both subscales, and 42 (22.5%) of the patients were Nor/Bnor on either the Inattention or the Hyperactivity-Impulsivity subscale of ADHD-RS-C (Table 7, divided into Nor and Bnor). The mean end-dose of IR-MPH was 1.0 mg/kg/day (SD 0.3).

Table 7 Distribution of number of patients with normalization and borderline normalization of clinician rated ADHD symptoms score in week 0 and 12 (*n* = 187)

Inattention subscale					
		Week 0	**Week 12 Normalization** *n (%)*	**Week 12 Borderline normalization** *n (%)*	**Week 12 No normalization or borderline normalization** *n (%)*
Normalization	*n (%)*	**1** (0.5%)	**1**	**0**	**0**
Borderline normalization	*n (%)*	**3** (1.6%)	**2**	**1**	**0**
No normalization or borderline normalization	*n (%)*	**183** (97.9%)	**67**	**66**	**50**
Total week 12	*n (%)*	**187** (100%)	**70** (37.4%)	**67** (35.8%)	**50** (26.7%)
IR-MPH, mg/kg/day, week 12	M(SD)		**1.0** (0.3)	**1.0** [1] (0.3)	**1.1**[2] (0.3)
Hyperactivity-Impulsivity subscale					
		Week 0	**Week 12 Normalization** *n (%)*	**Week 12 Borderline normalization** *n (%)*	**Week 12 No normalization or borderline normalization** *n (%)*
Normalization	*n (%)*	**10** (5.3%)	**10**	**0**	**0**
Borderline normalization	*n (%)*	**17** (9.1%)	**17**	**0**	**0**
No normalization or borderline normalization	*n (%)*	**160** (85.6%)	**68**	**62**	**30**
Total week 12	*n (%)*	**187** (100%)	**95** (50.8%)	**62** (33.2%)	**30** (16.0%)
IR-MPH, mg/kg/day week 12	M(SD)		**1.0** (0.3)	**1.0**[3] (0.3)	**1.1** (0.3)

N = number, M = mean. SD = Standard deviation. Missing data: [1] *n* = 66, [2] *n* = 48, [3] *n* = 59.
ADHD-Rating Scale, clinician rated (ADHD-RS-C, DuPaul). Inattention subscale: 9 items [range 0-27]. Hyperactivity-impulsivity subscale: 9 items [range 0-27]. Normalization (*t-score* ≤ 60), borderline normalization (*t-score* ≤ 70), no normalization or borderline normalization of ADHD symptoms (ADHD-RS) due to Danish norms of sex and age.
In the article normalization (Nor) and borderline normalization (Bnor) on ADHD-RS-C are multiplied to Nor/Bnor = *n* (%). Number of patients who were Nor/Bnor on the Inattention subscale = 137 (73.2%) and number of patients who were Nor/Bnor on the Hyperactivity-Impulsivity subscale = 157 (84.0%).

Changes in mean sum scores from week 0 to week 12 showed a significant reduction of severity of inattention symptoms and hyperactivity-impulsivity symptoms rated by clinicians, parents, and teachers (ADHD-RS-C, ADHD-RS-P, and ADHD-RS-T; Table 8, SI Table 3, paper II). Reduction of conduct problems was statistically significant in parents' ratings ($p < 0.001$) but not in teachers' ratings ($p = 0.293$).

8.3.2 ADHD related symptoms and impairments

The patients' daily and social functioning were improved on W-FIRS-P with a significant reduction of problems of all six subscales. The mean subscale index scores of W-FIRS-P at week 0 and 12 were ≤ 1, therefore the overall level of functioning was mildly affected in each domain (range of index score 0-3). The largest mean reductions on the W-FIRS-P were on the school subscale (0.4, SD 0.4) and the social life (0.4, SD 0.5, S7 Table in paper I). The four mean TOVA outcomes showed significant improvements in both raw scores and z-scores in response time, variability of response time, omission errors, and commission errors ($n = 115$) from week 0 to week 12 (S7 Table in paper I). The mean time from midday IR-MPH dose to TOVA test was 108 minutes (range 9 to 193), and the mean midday dose was 0.42 mg IR-MPH per kg (SD 0.13) ($n = 106$) at week 12.

Table 8 Clinical follow-up from week 0 to week 12 (*n* = 187)

ADHD rating scales	Week 0 M (SD), *n* (%)	Week 12 M (SD), *n* (%)	Week 0 versus week 12			
			M dif. (SD)	95% CI	t (df)	*p*-value
Parent						
Inattention	17.7[1] (5.0)	9.2[1] (4.2)	8.2 (5.1)	(7.5, 9.0)	21.3(173)	< 0.001
Hyperactivity-Impulsivity	15.6[2] (6.0)	8.6[2] (4.7)	7.0 (5.7)	(6.2, 7.9)	16.2(170)	< 0.001
Inattention and Hyperactivity-Impulsivity	33.2[3] (9.4)	17.7[3] (7.9)	15.5 (9.4)	(14.1, 17.0)	21.1(162)	< 0.001
Conduct problems	10.7[4] (5.9)	5.3[4] (4.1)	5.4 (5.1)	(4.7, 6.2)	14.2(178)	< 0.001
Teacher						
Inattention	16.9[5] (4.7)	11.2[5] (5.1)	5.6 (5.1)	(4.7, 6.5)	12.4(126)	0.001
Hyperactivity-Impulsivity	9.1[6] (5.7)	7.7[6] (4.9)	1.4 (5.2)	(0.5, 2.3)	3.0(124)	0.004
Inattention and Hyperactivity-Impulsivity	25.9[7] (9.0)	18.6[7] (8.4)	7.2 (8.9)	(5.6, 8.9)	8.8 (117)	< 0.001
Conduct problems	4.8[8] (4.7)	4.4[8] (4.1)	0.4 (4.4)	(-0.4, 1.2)	1.1(127)	0.293
Clinician						
Inattention	19.9 (3.7)	9.6 (3.7)	10.3 (4.0)	(9.7, 10.9)	35.2(186)	< 0.001
Hyperactivity-Impulsivity	18.0 (5.8)	7.9 (4.0)	10.1 (5.1)	(9.4, 10.8)	27.3(186)	< 0.001
Inattention and Hyperactivity-Impulsivity	37.9 (7.8)	17.5 (6.8)	20.4 (7.7)	(19.3, 21.5)	36.5(186)	<0.001
CGI-S	5.3 (1.0)	3.0 (1.1)	2.3 (1.0)	(2.2, 2.5)	31.5(186)	< 0.001
CGI-S ≤ 2	0 (0.0%)	*n* = 67 (35.8%)	0.4 (0.5)	(0.3, 0.4)	10.2(186)	< 0.001
CGI-I	-	1.8 (0.6)	-	-	-	-
CGI-I ≤ 2	-	*n* = 171 (91.5%)	-	-	-	-
Weight, kg	36.3[9] (11.0)	35.4[9] (11.1)	0.9 (1.5)	(0.7, 1.15)	8.3(183)	< 0.001
Height, cm	140.1[9] (10.8)	141.3[9] (10.7)	-1.2 (1.1)	(-1.4, -1.0)	-14.8(183)	< 0.001
Diastolic blood pressure, mmHg	67.1[10] (7.1)	66.4[10] (7.4)	0.7 (9.4)	(-0.7, 2.0)	0.9(182)	0.344
Systolic blood pressure, mmHg	102.2[10] (8.9)	101.8[10] (9.7)	0.3 (9.9)	(-1.2, 1.7)	0.4(182)	0.693
Heart rate, bp/m	79.2[11] (10.9)	78.2[11] (11.9)	1.0 (15.1)	(-1.2, 3.3)	0.9 (180)	0.341
BSSERS-C	17.3[9] (10.5)	14.5[9] (9.0)	2.8 (8.6)	(1.5, -4.0)	4.4(183)	< 0.001
Reduced appetite	0.7[12] (1.3)	1.9[12] (1.5)	-1.2 (1.8)	(-1.5, 1.0)	-9.2(185)	< 0.001
Methylphenidate mg/kg/day	0.3 (0.1) (initial dose)	1.0 (0.3)	-0.7 (0.3)	(-0.7, -0.7)	-34.2(186)	< 0.001

Paired t-test between outcomes of week 0 and week 12. M = mean. M dif. = Mean difference. SD dif. = Standard deviation difference.
Missing data: [1] *n* = 174, [2] *n* = 171, [3] *n* = 163, [4] *n* = 179, [5] *n* = 127, [6] *n* = 125, [7] *n* = 118, [8] *n* = 128, [9] *n* = 184, [10] *n* = 183, *n* [11] = 181, [12] *n* = 186.
ADHD-Rating Scale (ADHD-RS, DuPaul). Inattention subscale: 9 items, [range 0-27]. Hyperactivity-Impulsivity subscale: 9 items [range 0-27]. Conduct problems subscale: 8 items, [range 0-24]. Rated by clinician, parent, or teacher.
Clinical Global Impression Severity (CGI-S): 1 item, [range 1-7]. CGI-S, 1 = *Not ill at all*. CGI-S, 2 = *Borderline ill*.
Clinical Global Impression Improvement (CGI-I): 1 item, [range 1-7]. CGI-I, 1 = *Very much improved*. CGI-I, 2 = *Much improved*.
Barkley's Stimulant Side Effects Rating Scale, clinician rated (BSSERS-C). Whole scale: 17 items, [range 0-153]. Reduced appetite: single item, [range 0-9].

Patients significantly improved on the CGI-S scale from week 0 to week 12, where a total of 67 (35.8%) patients were rated with a score of 1 or 2 (*normal* to *mildly ill*) on CGI-S (Figure 3). Furthermore, 171 patients (91.5%) had a score of 1 or 2 (*very much* or *much improved*) on CGI-I after 12 weeks of treatment (Table 8).

Figure 3

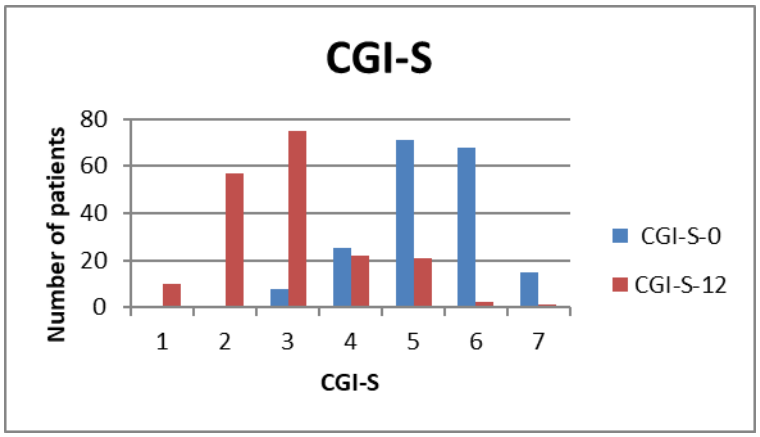

Figure 3 Clinical Global Impression Severity (CGI-S) in week 0 and in week 12 [range 1-7]

8.3.3 Adverse reactions

12 (5.8%) patients discontinued medication with IR-MPH due to ARs (*n* = 10, 4.8%) or SARs (*n* = 2, 1.0%), and only six these patients had ARs or SARs that were detected by the weekly monitoring with BSSERS-C, whereas the remaining ARs were reported spontaneously or measured during the physical examination. These SARs were: onset of psychotic symptoms (*n* = 1, 0.5%) and progression in seizures (*n* = 1, 0.5%), and these ARs were hypertension (*n* = 2, 1.0%) (increase in a systolic blood pressure and/or diastolic blood pressure to a level corresponding to the 95-99 percentile for age and gender), hyperactivity (*n* = 0.5%) , and urticarial reaction (*n* = 0.5%). The ARs measured on BSSERS were: irritability (*n* = 3, 1.4%), tics (*n* = 2, 1.0%), and sadness (*n* = 1, 0.5%). Overall, the mean sum problem score of the BSSERS-C rating scale significantly declined over 12 weeks of treatment (pre-post mean difference 2.8 (SD 8.6), $p < 0.001$) (Table 8, Figure 4, S8 Table in paper I). Reduced appetite was the only BSSERS-C-rated AR problem score that increased significantly over time, whereas the other BSSERS-C-rated AR problem scores were either stable or decreased over time (e.g., the largest decrease on any single AR problem score was found for euphoria with a mean decrease of 0.7 (SD 1.4) $p < 0.001$, CI 95% 0.5 to 0.9). There was no significant increase in mean blood pressure, no change in the blood pressure percentiles (data not shown), and no change in heart rate. There was a significant increase in children's height with a mean of 1.2 cm (SD 1.1) despite a mean loss of weight of 0.9 kg (SD 1.5).

Figure 4

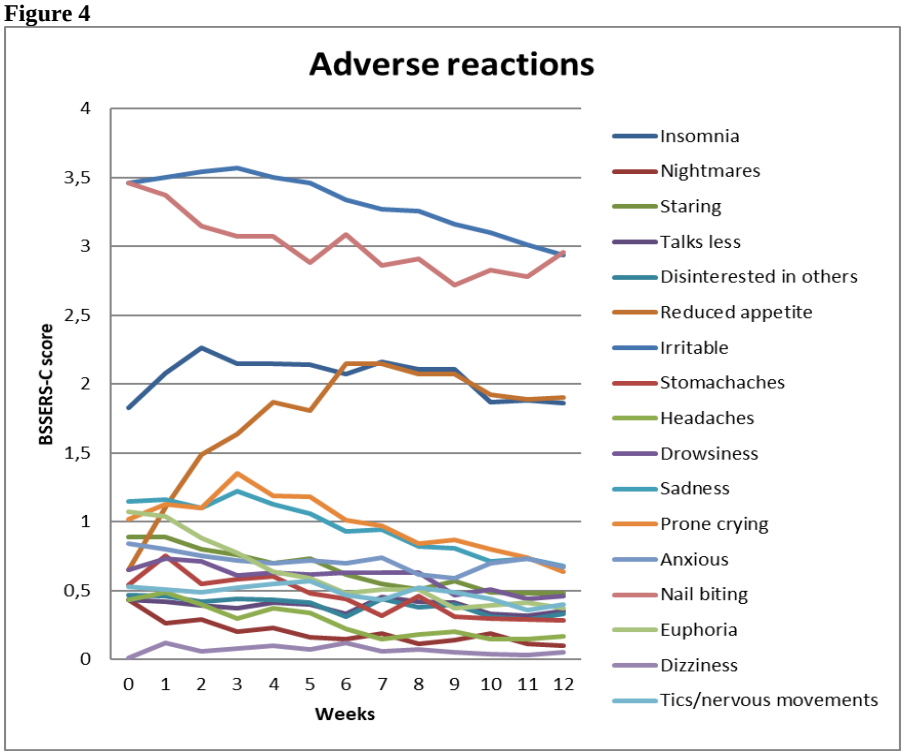

Figure 4 Single items of adverse reactions on Barkley's Stimulant Side Effect Rating Scale, clinician rated (BSSERS-C) from week 0 to week 12 [range 0-9].

8.4 Predictors of end-dose of IR-MPH

Paper I CGI-S in week 0, ADHD diagnosis, sex, age, and weight were tested as predictors for end-dose in a linear regression model. Table 9 shows that higher CGI-S scores at week 0 predicted a higher end-dose of IR-MPH. CGI-S at week 0 and age explained together 12.7% ($p < 0.001$) of the variance of the end-dose IR-MPH. The estimated effect of being "*among the most extremely ill patients*" in week 0 (CGI-S 7) corresponded to an end-dose of IR-MPH 1.19 mg/kg/day, whereas being rated "*mildly ill*" in week 0 (CGI-S 3) corresponded to an end-dose of IR-MPH 0.85 mg/kg/day. Young age predicted a lower end-dose of IR-MPH. Patients aged 7 to 9 years were estimated to receive a 0.13 mg/kg/day lower end-dose of IR-MPH than patients aged 10-12 years.

Table 9 Baseline characteristics as predictors for end-dose of IR-MPH (*n* = 187)

Predictors for end-dose of IR-MPH dose/mg/kg/day						
	Estimate	**SD Error**	**Beta**	**t**	***p*-value**	**CI95 % of estimate**
Constant	0.56	0.12		4.84	< 0.001	(0.33, 0.79)
CGI-S, week 0	0.09	0.02	0.29	4.32	< 0.001	(0.05, 0.13)
Age (7-9 years)	-0.13	0.04	-0.16	-3.05	0.003	(-0.21, -0.05)

Linear regression for end-dose of Immediate Release Methylphenidate (IR-MPH).
Explaining variables are measured in week 0. Clinical Global Impression Severity (CGI-S) in week 0, [range 1-7].

Paper II. Only genotypes of variants of *CES1* which were significant different associated to end-dose are present in the text. Each variant of *CES1* were both tested as predictors for end-dose in univariate and multivariate

analyzes. An overview of significant associations is presented in Table 10 (overview of univariate association analyzes are seen in SI Table 4 in paper II). Variants of *CES1*, ADHD diagnosis, CGI-S in week 0, sex, age, and weight (sex and age were also tested in interactions with the mentioned covariates) were tested as predictors for end-dose in the GLS model.

Patients with copy numbers of 3 or 4 (*CES1A2*) more often received the low end-dose (17 out of 51, 33.3%) than patients with 2 copies (21 out of 115, 18.3%), $p = 0.003$.

More patients with GT/GG at rs3815583 (22 out of 59, 37.3%) received a low end-dose compared with TT (14 out of 101, 13.9%) at this polymorphic site), $p = 0.001$. This was confirmed with a significantly lower mean end-dose of IR-MPH for GT/GG carriers (0.90 mg/kg/day, SD 0.33) than for TT carriers (1.01 mg/kg/day, SD 0.24) with a mean difference of -0.12 (95% CI -0.21 to -0.03), $p = 0.001$. Table 11 shows that patients with GT/GG at rs3815583 were also estimated to have a significantly lower end-dose (0.86 mg/kg/day) than the common homozygotic genotype (0.96 mg/kg/day) in GLS.

More patients with GA at rs2307240 (6 out of 11, 54.5%) compared with GG at this polymorphic site (23 out of 99, 23.2%) received a low end-dose, $p = 0.025$. This was also seen with a lower mean end-dose of IR-MPH for GA carriers (0.74 mg/kg/day, SD 0.22) than for GG carriers (0.96 mg/kg/day, SD 0.29) with a mean difference of -0.22 (95% CI -0.40 to -0.04), $p = 0.017$. Table 11 shows that patients with GA at rs2307240 were estimated to have a borderline significant lower end-dose (0.75 mg/kg/day) than those with the common homozygotic GG genotype (0.89 mg/kg/day), $p = 0.088$ in GLS.

Only in one univariate analysis of dichotomized end-dose where patients with 3 or 4 (*CES1A2*) associated to lower end-dose compared to patients with 2 copies. This was not confirmed in the other univariate and multivariate analysis of end-dose. Thereby we could not confirm our hypotheses of patients with copy numbers of 3 or 4 (*CES1A2*) needed a higher end-dose of IR-MPH and patients with GA of rs71647871 needed a lower end-dose of IR-MPH.

Table 10 Summary of significant associations between variants of *CES1* and outcomes

***CES1* marker**	**Univariate analysis *P*-value (outcome)**	**Multivariate analysis *P*-value (outcome)**	**Summary of findings**
Copy number *n* = 182	*P* = 0.033 (end-dose)[1] *P* = 0.021 (reduced appetite)	NS	3 or 4 copies were associated to • low end-dose[1] (< 0.80 mg/kg/day) • reduced appetite
rs3815583 *n* = 176	*P* = 0.012 (end-dose)[1] *P* < 0.001 (end-dose)[2]	*P* = 0.018 (end-dose)	GT/GG were consistently associated to • low end-dose
rs111604615 *n* = 175	NS	*P* = 0.032 (Hyperactivity-Impulsivity)	CC (vs GG and vs CG) was associated to • higher response rate
rs56278207 *n* = 167	*P* = 0.021 (adverse reactions)	*P* = 0.057 (Inattention)	del/del was associated to • increase of adverse reactions ins/ins (vs del/ins) was associated to • higher response rate
rs2307240 *n* = 122	*P* = 0.017 (end-dose)[1] *P* = 0.025 (end-dose)[2]	*P* = 0.029 (Hyperactivity-Impulsivity) *P* = 0.088 (End-dose)	GA was consistently associated to • low end-dose • higher response rate
rs2302722 *n* = 84	NS	*P* = 0.002 (Inattention) *P* = 0.005 (Hyperactivity-Impulsivity)	GT/TT were associated to • higher response rates
rs2244614 *n* = 110	NS	*P* = 0.035 (adverse reactions)	The adverse reactions did not differ in rs2244614 polymorphisms after 12 weeks of treatment.
rs71647871 *n* = 184	NS	NS	-

NS = Not significant. See methods and results in the thesis for statistics. The genotypes were tested against a total of 12 drug response phenotypes. [1] = Low IR-MPH end-dose (< 0.80 mg/kg/day), [2] = mean IR-MPH end-dose.
CES1 = Carboxylesterase 1 gene
The wildtype genotype of *CES1* is the reference in the multivariate analyzes.
Responder = normalization or borderline normalization of Inattention and/or Hyperactivity-Impulsivity (ADHD-RS) in week 12

Table 11 Variants of *CES1* associated with end-dose of IR-MPH

Variant	Estimate	t	95% CI	*P*-value
Copy number 3-4 copies	-0.07	-1.53	-0.15 to 0.02	0.128
rs3815583 GT/GG	-0.10	-2.40	0.02 to 0.04	0.018
rs111604615	-0.06	-1.19	-0.15 to 0.04	0.235
CG	0.10	1.05	-0.09 to 0.28	0.294
CC				
rs111604615 CG/CC	0.03	-0.67	-0.12 to 0.06	0.503
rs56278207	-0.06	-1.34	-0.15 to 0.03	0.182
del/ins	-0.10	-1.42	-0.23 to 0.04	0.158
del/del				
rs2307240 GA	-0.15	-1.72	-0.31 to 0.02	0.088
rs2302722 GT/TT	-0.02	-0.29	-0.16 to 0.12	0.773
rs2244614	-0.09	-1.51	-0.20 to 0.03	0.135
CT	0.09	0.26	-0.18 to 0.24	0.795
CC				
rs2244614 CT/CC	-0.07	-1.26	-0.18 to 0.04	0.212
rs71647871 GA	-0.04	-0.43	-0.25 to 0.16	0.671

End-dose of immediate release methylphenidate (IR-MPH); Linear regression estimated using generalized least squares (GLS).
CES1=Carboxylesterase 1 gene. The reference is the wild type genotype. The estimation methods composed of; SNPs or CNVs of *CES1*, ADHD ICD10 diagnoses, CGI in week 0, weight in week 0, sex, and age.
SNPs=Single Nucleotide Polymorphisms, CNV=Copy number variation
n = *n* in week 12, see S Table 4 and 5

8.5 Predictors of beneficial effects

8.5.1 Normalization of ADHD symptoms

Paper I Age, sex, intelligence level, conduct disorder, and comorbidity were tested as predictors for the chance of Nor/Bnor on the Inattention or Hyperactivity-Impulsivity subscales (ADHD-RS-C) in the cox regression model. Table 12 shows that female sex and a diagnosis of *inferioritas intellectualis* (patients with IQ = 70-85) were significant predictors of a lower chance of Nor/Bnor on the Inattention subscale. Young age (7-9 years) and *inferioritas intellectualis* (patients with IQ = 70-85) predicted lower chance of Nor/Bnor on the Hyperactivity-Impulsivity subscale. Comorbidity significantly improved the chance of Nor/Bnor on the Hyperactivity-Impulsivity subscale. Post hoc

analysis did not show any significant differences in sum scores of Inattention or Hyperactivity-Impulsivity sub scale (ADHD-RS-C) in week 0 between patients with none or one psychiatric comorbid diagnosis and those with two or more psychiatric comorbid diagnoses.

Table 12 Baseline characteristics as predictors for the chance of normalization or borderline normalization of the clinician rated ADHD symptoms score (*n* = 207)

Inattention	**HR**	**CI 95%**	***p*-value**
Sex (girls)	0.64	(0.54, 0.75)	< 0.001
Inferioritas intellectualis	0.74	(0.63, 0.86)	< 0.001
Hyperactivity-Impulsivity	**HR**	**CI 95%**	***p*-value**
Age (7-9 years)	0.88	(0.71, 0.96)	0.031
Inferioritas intellectualis	0.82	(0.78, 0.99)	0.011
Comorbidity	1.17	(1.02, 1.35)	0.030
Cox regressions of time to first normalisation (zero standard deviation) or borderline normalisation (one standard deviation) of ADHD core symptoms due to Danish norms of sex and age on ADHD-Rating Scale, clinician rated (ADHD-RS-C, DuPaul). Inattention subscale: 9 items, range 0-27. Hyperactivity-Impulsivity subscale: 9 items, range 0-27. Only last model of cox regression with backward elimination are listed in the table. HR = Hazard ratio. Independent predictors: Sex, age, comorbidity (≤ 2 comorbid diagnosis), and intelligence level (R41.8) from the diagnostic conference and/or from the intelligence test (WISC) (IQ 70-85) depending on data access)			

Paper II Variants of *CES1*, age, sex, intelligence level, conduct disorder, and comorbidity were tested as predictors of the chance of Nor/Bnor on the Inattention or Hyperactivity-Impulsivity subscale in the cox regression model. Patients with the del/del variant had a significantly lower chance of Nor/Bnor on Inattention than the del/ins variant with pairwise tests (HR 0.73, 95% CI 0.56 to 0.94, $p = 0.016$) during the treatment period (the pairwise test is not shown in Table 13). Table 13 shows that patients with GT/TT at rs2302722 had a significantly higher chance of Nor/Bnor on Inattention during the treatment period. Also, the genotypes CC at rs111604615, GA at rs2307240, and GT/TT at rs2302722 significantly increased the chance of Nor/Bnor on Hyperactivity-Impulsivity.

Table 13 Variants of *CES1* associated with the chance of Nor/Bnor of ADHD symptoms

Variants		Time to Nor/Bnor of Inattention		Time to Nor/Bnor Hyperactivity-Impulsivity	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Copy number	3-4 copies	0.96 (0.83 to 1.11)	0.580	1.03 (0.90 to 1.18)	0.645
rs3815583	GT/GG	1.02 (0.89 to 1.17)	0.786	1.07 (0.94 to 1.21)	0.289
rs111604615	Overall	-	0.618	-	0.028
	GC	1.01 (0.86 to 1.19)	0.862	0.10 (0.86 to 1.06)	0.997
	CC	0.86 (0.62 to 1.19)	0.347	1.40 (1.10 to 1.78)	0.008
rs111604615	GC/CC	0.98 (0.85 to 1.15)	0.833	1.07 (0.94 to 1.22)	0.320
rs56278207	Overall	-	0.026	-	0.510
	del/ins	1.13 (0.98 to 1.31)	0.092	1.06 (0.93 to 1.21)	0.385
	del/del	0.82 (0.63 to 1.05)	0.110	0.95 (0.77 to 1.17)	0.614
rs2307240	GA	1.12 (0.86 to 1.46)	0.384	1.27 (1.02 to 1.58)	0.034
rs2302722	GT/TT	1.45 (1.15 to 1.83)	0.002	1.33 (1.07 to 1.65)	0.010
rs2244614	Overall	-	0.062	-	0.121
	CT	1.13 (0.95 to 1.35)	0.157	1.16 (0.99 to 1.37)	0.075
	CC	0.74 (0.51 to 1.08)	0.119	0.91 (0.66 to 1.25)	0.555
rs2244614	CT/ CC	1.07 (0.90 to 1.27)	0.434	1.12 (0.96 to 1.31)	0.163
rs71647871	GA	1.09 (0.81 to 1.46)	0.578	0.76 (0.55 to 1.05)	0.095

Cox regression used to determine if any genetic variants was associated with time to normalization (Nor) (*t-score* ≤ 60) or borderline normalization (Bnor) (*t-score* ≤ 70) of ADHD core symptoms, Inattention and Hyperactivity-Impulsivity due to Danish norms on ADHD-Rating Scale (ADHD-RS-C, DuPaul), clinician rated.
Inattention subscale: 9 items, [range 0-27]. Hyperactivity-Impulsivity subscale: 9 items, [range 0-27].
CES1 = Carboxylesterase 1 gene, HR = Hazard ratio.
The wild type genotype is the reference. For SNPs with three genotypes and significant *P*-values, see the text.
The model composed of: single nucleotide polymorphisms (SNPs) or copy number variations (CNV), sex, age (7-9 versus 10-12 years), comorbidity (0-1 versus ≤ 2 comorbid diagnoses), conduct disorder, and intelligence level (*inferioritas intellectuals* IQ 70-85, DR41.8 versus normal intelligence level).
n = *n* in week 0, see S Table 4 and 5.

8.5.2 Sum score of ADHD symptoms

Paper I We tested week, sex, age, intelligence level, conduct disorder, and comorbidity (week, sex, and age were also tested in interactions with the mentioned covariates) in the linear mixed effect models for repeated measures of the sum scores of Inattention and Hyperactivity-Impulsivity (ADHD-RS-C). Figure 5 shows time (number of weeks in treatment) and sex in interaction with age were significantly associated with the course of inattention symptoms, scored on the Inattention subscale (for estimates of Figure 5, see Table 3 in paper I) in the mixed effect model. The estimated effects of time corresponded to a mean reduction of 0.8 of the Inattention sum score per week in subgroups of sex and age throughout the treatment period. Girls aged 7 to 9 years had a lower estimated mean score of Inattention symptoms in week 0 (score 17.5) than the group of girls aged 10 to 12 years (score 18.6) in week 0. Boys aged 7 to 9 years had a higher estimated mean score of Inattention in week 0 (score 19.4) than boys aged 10 to 12 years (score 18.2) in week 0. None of the variables (age, sex, intelligence level, conduct disorder, and comorbidity) had a significant interaction with time on Inattention, and the differences in estimated mean scores in week 12 between sex and age were the same as in week 0.

Figure 5

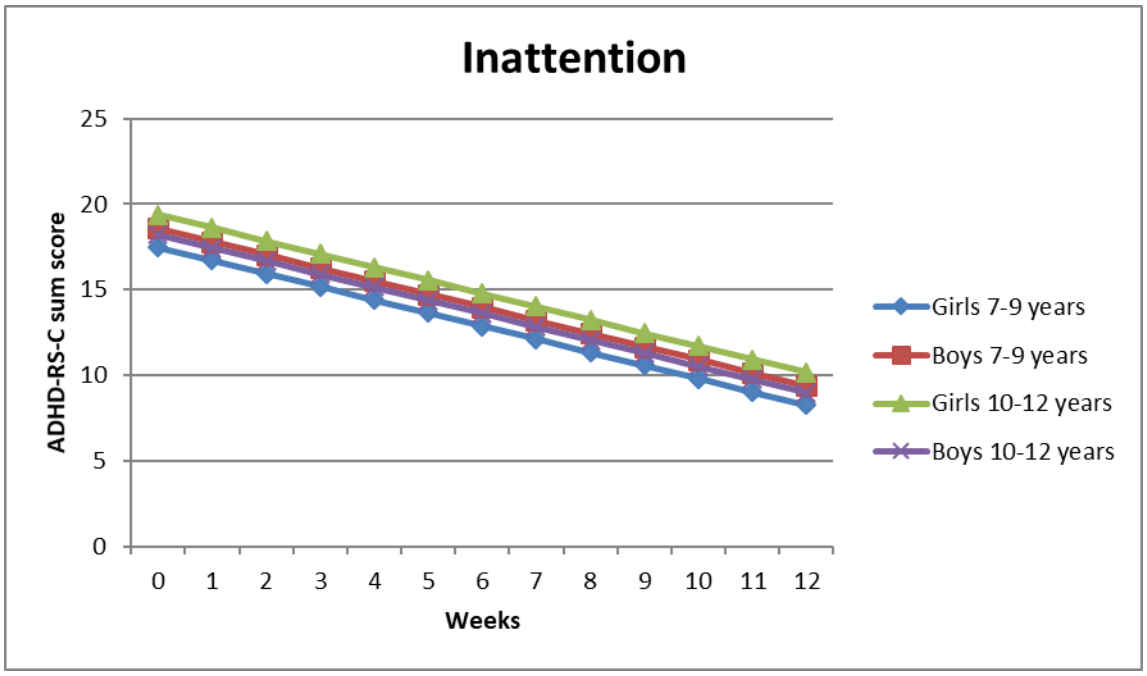

Figure 5 Examples of graphs for estimations sum scores of Inattention throughout the 12 weeks as estimated by the linear mixed models, *n* = 207
Clinician rated ADHD-Rating-Scale (ADHD-RS-C); Inattention subscale, 9 items [range 0-27] and Hyperactivity-Impulsivity subscale, 9 items [range 0-27].
Explanatory variables: Sex and age (7 to 9 years and 10 to 12 years) For estimates see Table 3 in paper I.
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Figure 6 shows time (number of weeks in treatment) and age were significantly associated with the course of hyperactivity-impulsivity symptoms, rated on the Hyperactivity-Impulsivity subscale (for estimates of Figure 6, see Table 3 in paper I). Patients aged 7 to 9 years had an estimated baseline score of 16.0 and an estimated decrease in symptoms of 0.8 per week. Patients aged 10 to 12 years had an estimated baseline score of 13.3 and estimated decrease in symptoms of 0.6 per week. In week 12, patients aged 7 to 9 years had a score of 6.8 and patients aged 10 to 12 years had an estimated score of 6.3 (i.e., the scores from the two age groups of patients ended up at about the same level).

Figure 6

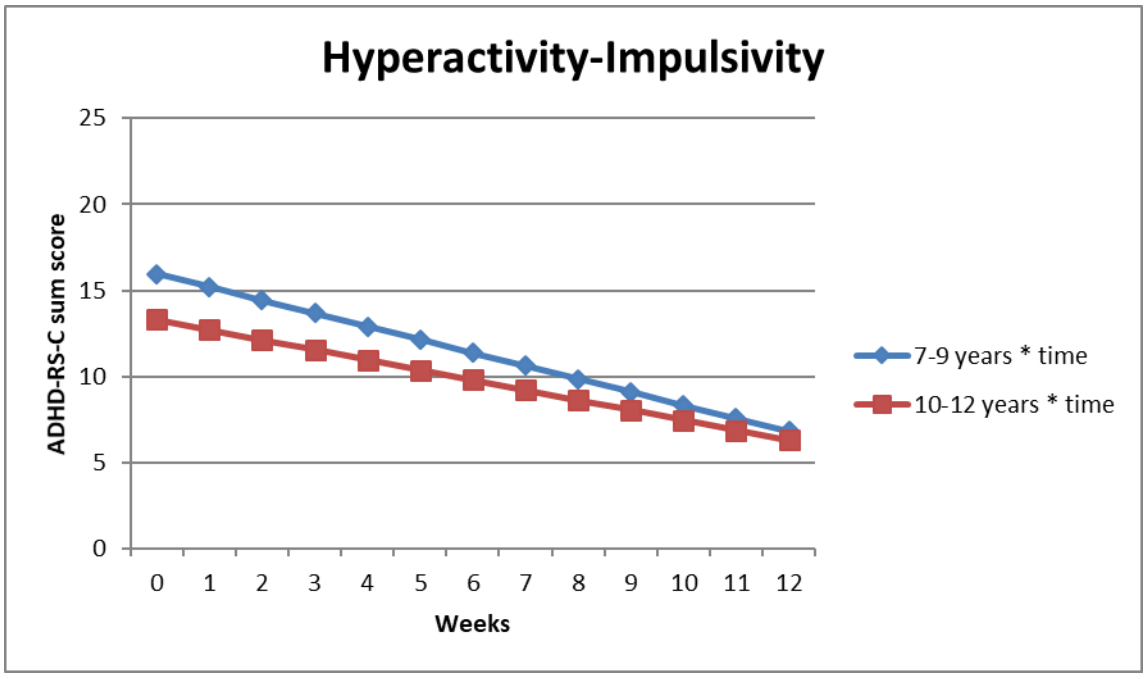

Figure 6 Examples of graphs for estimations sum scores of Hyperactivity-Impulsivity throughout the 12 weeks as estimated by the linear mixed models, *n* = 207
Clinician rated ADHD-Rating-Scale (ADHD-RS-C); Inattention subscale, 9 items [range 0-27] and Hyperactivity-Impulsivity subscale, 9 items [range 0-27].
Explanatory variables: Age (7 to 9 years and 10 to 12 years) and week. For estimates see Table 3 in paper I.

On Inattention none of the tested potential clinical predictors had a significant interaction with time (number of weeks in treatment) and on Hyperactivity-Impulsivity the differences were minimized over 12 weeks period of treatment. There was a limited difference on the scores between girls 7-9 years and girls 10-12 years on Inattention score (1.1 score) and a limited difference on the scores between children 7-9 years and children 10-12 years on Hyperactivity-Impulsivity (0.5 score) in week 12.

8.6 Predictors of adverse reactions

8.6.1 Sum score of adverse reactions

Paper I The 12-week outcome of each 17-items of BSSERS-C are present above in Figure 4 and shows differences in outcome for each item. Here the predictors of the sum score of all 17 items BSSERS-C is present because the ARs symptoms significantly overall decline during the treatment period. We tested week, sex, age, intelligence level, conduct disorder, comorbidity, ADHD diagnosis, and CGI-S (week, sex, and age were also tested in interactions with the mentioned covariates) in the linear mixed effect models for repeated measures of the sum scores of BSSERS-C. Figure 7 shows that CGI-S measured in week 0 and time (number of weeks in treatment) were significantly different associated with the course of ARs when divided into four categories in the linear mixed effect model. There was no significant interaction between CGI-S and time and the mean reduction in the BSSERS-C problem score was 0.3 per week. Patients rated *severely ill* and *most extremely ill* (CGI-S 6-7) in week 0 had a more severe BSSERS-C problem

score (mean score 21.7) before initiation of treatment than patients with a lower-rated CGI-S score in week 0. Patients who were rated *moderately ill* (CGI-S 5) at baseline had an estimated mean BSSERS-C problem score of 17.2, patients rated *markedly ill* on CGI-S (CGI-S 4) had an estimated mean BSSERS-C problem score of 17.4, and patients rated *not ill at all, borderline ill, or mildly ill* (CGI-S 1-3) had an estimated mean BSSERS-C problem score of 13.4 before initiation of treatment. For estimates of Figure 7 see Table 3 in paper I.

Figure 7

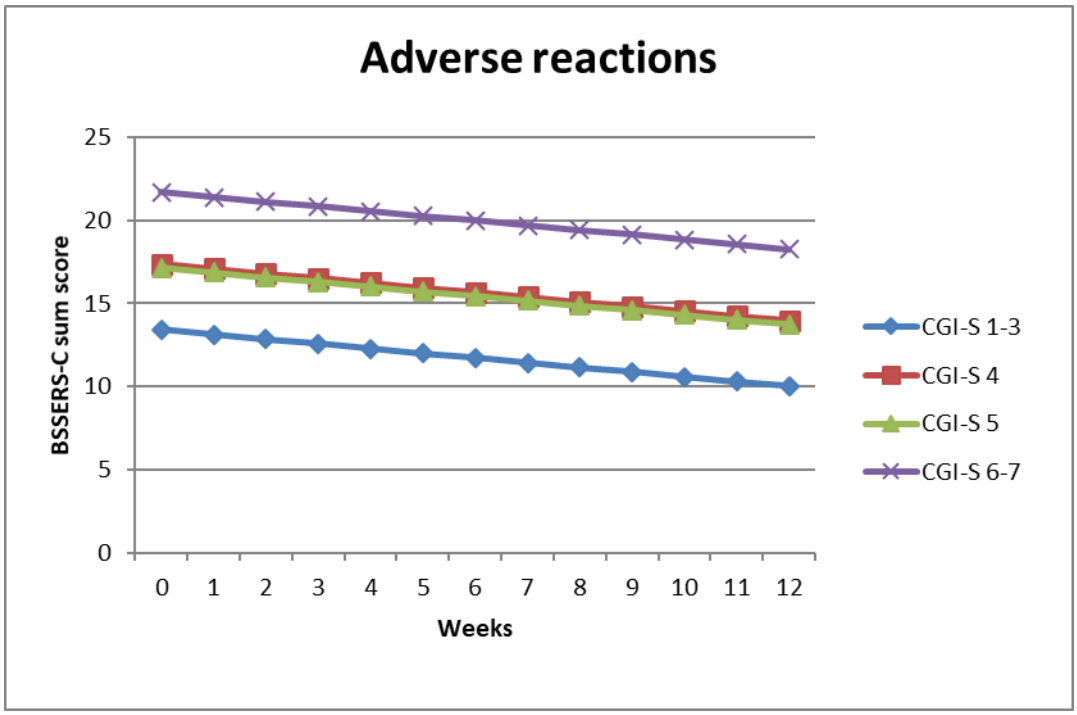

Figure 7 Examples of graphs for estimations sum scores of adverse reactions throughout the 12 weeks as estimated by the linear mixed models, *n* = 207
Clinician rated Barkley's Stimulant Side Effect Rating Scale (BSSERS-C), 17 items, [range 0-153].
Explanatory variables: functional impairments in week 0: Clinical Global Impression Severity (CGI-S). CGI-S devided into: 1-3 = *Not ill, borderline ill, mildly ill*. CGI-S 4 = *Moderately ill*, CGI-S 5 = *Markedly ill*. CGI-S 6-7 = *Severly ill, among the most extreme ill patients*. For estimates see Table 3 in paper I .

Paper II Variants of *CES1*, week, sex, and age (week, sex, and age were also tested in interactions with the mentioned covariates) were tested in the linear mixed effect models for repeated measures of the sum scores of BSSERS-C. Only the variants of *CES1* which had a significant different association to the course of the sum scores of BSSERS-C are present here. Figure 8 shows the homozygotic variant del/del at rs56278207 had a mean increment of ARs (mean = 3.0, SD 8.7) from week 0 to week 12 which was significantly different compared to the other genotypes with mean decreases of ARs (del/ins, mean = -3.2, SD 8.1 and ins/ins, mean = -2.4, SD 7.9), $p = 0.021$ (Table 10). In week 0 (before medication) the ARs sum scores differed significantly between patients with different genotypes at rs2244614 (23.1 (CC), 15.3 (CT), 19.4 (TT)). The sum scores of BSSERS-C changed significantly different over the 12-week period for the genotypes of rs2244614 and after 12 weeks of treatment they were nearly the same (15.8 (CC).

13.7 (CT) and 14.8 (TT)). For *p*-values of the genetic variants associated to ARs see SI Table 5 in paper II in mixed effect model.

Figure 8

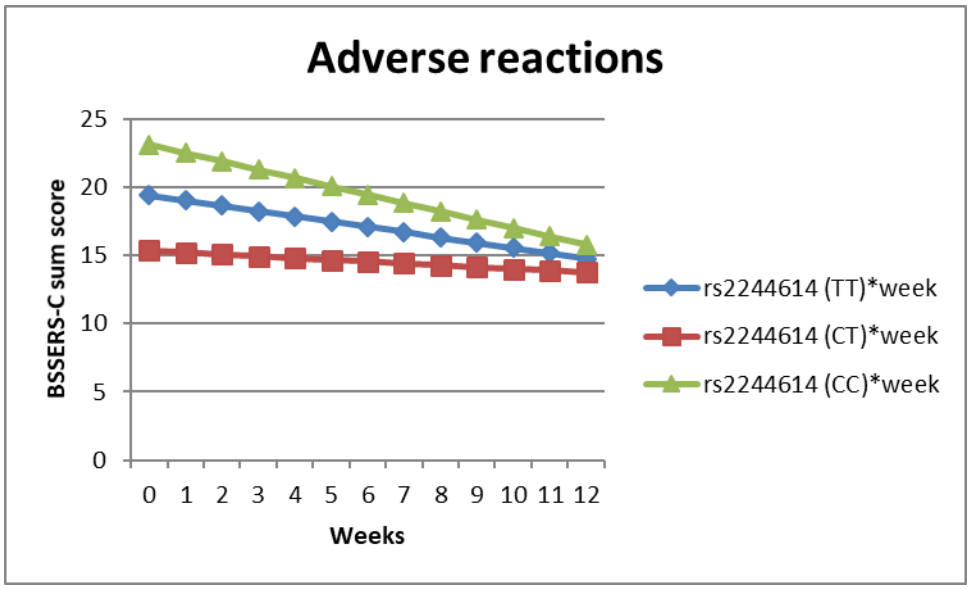

Figure 8 Association of rs2244614 with problem sum scores of adverse reactions through 12 weeks of methylphenidate treatment (*n* = 110).
Linear mixed model for adverse reactions of rs2244614, week, rs2244614 * week.
Barkley's Stimulant Side Effects Rating Scale (BSSERS-C), clinician rated, [range 0-153].
For estimates see SI Table 5 in paper II.

8.6.2 Reduced appetite

Paper I Week, sex, age, intelligence level, conduct disorder, comorbidity, ADHD diagnosis, and CGI-S (week, sex, and age were also tested in interactions with the mentioned covariates) were tested as predictors in the model of GEE for repeated measures in of the single score of reduced appetite on BSSERS-C (Table 3 in paper I). The potential predictors for reduced appetite were tested because it was the only single item of BSSERS-C, which significantly increased during the treatment period. CGI-S was significantly associated with the course of reduced appetite in the model of GEE for repeated measures. *Severely ill* and *most extremely ill* rated patients (CGI-S 6-7) had a significantly higher risk of reduced appetite compared with patients rated not ill to moderately ill (CGI-S 1-5) in week 0 and throughout the whole treatment period (OR = 2.41, CI 95% 1.42 to 4.08). The probability of reduced appetite in week 0 was 13.0% for patients rated severely ill and most extremely ill and 5.8% for patients not ill to *moderately ill*. The proportion of patients with reduced appetite did not change significantly over time (weeks, $p = 0.069$).

Paper II There was a weak correlation (r = 0.27, $P < 0.001$) between weight reduction and *appetite reduction* that explained about 7% of the variation in weight (data not shown). There were relatively more patients with copy numbers of 3 or 4 (*CES1A2*) with reduced appetite in week 12 (10 out of 50, 20.0%) compared with patients with 2 copies (8 out of 115, 7.0%), $p = 0.014$. Variants of *CES1*, week, sex, and age (week, sex, and age were also tested in

interactions with the mentioned covariates) were tested as predictors in the model of GEE for repeated measures in of the single score of reduced appetite on BSSERS-C. There were no associations between any of the genetic variations of *CES1* and reduced appetite over the treatment period (data not shown) in the model of GEE for repeated measures.

8.7 Nonresponders

Paper I Thirty-one patients (15.0%) were nonresponders; of these, 12 (5.8%) patients discontinued IR-MPH treatment because of ARs/SARs, and 19 patients (9.1%) were not Nor/Bnor on any of the ADHD-RS-C subscales. Table 14 shows the responders (n = 168) and nonresponders did not differ with respect to age, sex, and comorbidity. Nonresponders were characterized by having higher mean sum scores of Hyperactivity-Impulsivity ($p \leq 0.001$), higher mean CGI-S scores ($p \leq 0.001$), and a higher mean score of problems on BSSERS-C ($p = 0.034$) at week 0.

Table 14 Baseline characteristics and symptoms in week 0 of responder and nonresponder.

	Responder n = 168	**Nonresponder n = 31**	**Responder versus nonresponder**			
			X^2 (df)		**p-value**	
Boys	125 (74.4)	25 (80.6)	0.5(1)		0.459	
Age (10-12 years)	59 (35.1)	9 (29.0)	0.4(1)		0.511	
Comorbidity ≥ 2 diagnoses	45 (26.8)	9 (29.0)	0.1(1)		0.796	
Cognitive deficits	44 (26.2)	9 (29.0)	0.1(1)		0.742	
Conduct disorder	17 (10.1)	5 (16.1)	1.0(1)		0.327	
Clinician rated symptoms in week 0	**M (SD)**	**M (SD)**	**M dif. (SD error)**	**95% CI**	**t(df)**	**p-value**
Inattention	19.7 (3.7)	20.8 (3.3)	-1.1 (0.7)	(2.6, 0.3)	-1.5(197)	0.127
Hyperactivity-Impulsivity	17.5 (5.7)	21.5 (4.4)	-4.0 (1.1)	(-6.1, -1.8)	-3.7(197)	< 0.001
CGI-S	5.2 (0.9)	5.9 (0.8)	-0.65 (0.2)	(-1.0, -0.3)	-3.6(197)	< 0.001
BSSERS-C	17.0 (10.4)	21.3 (11.3)	-4.3 (2.1)	(-8.4, -0.2)	-2.1(197)	0.038
Reduced appetite	0.6 (1.2)	1.0 (1.8)	-0.4 (0.2)	(-0.9, 0.1)	-1.6(197)	0.114

Paired t-test and X^2 (chi-squared) between responder and nonresponder. M = mean. M dif. = Mean difference. SD dif. = Standard deviation difference.
ADHD-Rating Scale, clinician rated (ADHD-RS-C, DuPaul). Inattention subscale: 9 items [range 0-27]. Hyperactivity-Impulsivity subscale: 9 items [range 0-27]. Normalization (zero standard deviation), borderline normalization (one standard deviation), no normalization or borderline normalization (two standard deviations) of ADHD cores symptoms (ADHD-RS) due to Danish norms of sex and age.
Nonresponder defined by no normalization or borderline normalization of any subscales of ADHD-RS-C in week 12 or by discontinuation of treatment due to adverse reactions.
Age between 7 and 9 years old or between 10 and 12 years old. Cognitive deficits: (DR 41.8) from the diagnostic conference and/or from the intelligence test (WISC) (IQ 70-85) depending on data access or no cognitive deficits (IQ ≤ 86). Conduct disorder: Hyperkinetic conduct disorder, (DF 90.1, DF 91.X, DF 92.X) or no conduct disorder.
Clinical Global Impression Severity (CGI-S) week 0: 1 item [range 1-7]. Barkley's Stimulant Side Effects Rating Scale, clinician rated (BSSERS-C). Whole scale (17 items) [range 0-153]. Reduced appetite (single item) [range 0-9].

Paper II There were no associations between any of the variations of *CES1* and nonresponders.

9 Discussion

9.1 Rationale of the study and main findings

The study investigated the outcome and the course of outcome of the first 12-week treatment with IR-MPH treatment with regard to beneficial effects, ARs and end-doses of MPH, and the clinical and genetic predictors of outcome in a representative clinical population of children (n=207) recently diagnosed with ADHD in the child and adolescent mental health services. The overall purpose of the study was to add clinical and genetical information to improve the personalized medicine of IR-MPH in children with ADHD. The specific objective was to identify variants of *CES1* that could predict the outcome of MPH treatment.

The observational study showed that beneficial effects of IR-MPH with improvements of ADHD symptoms, related symptoms, and impairments across informants, improvements of daily and social functioning, and at the same time few ARs. The only ARs were reduced appetite and weight loss. Greater global impairment before initiation of treatment was associated with nonresponse, and/or high end-dose of IR-MPH, and/or ARs before and during treatment. Furthermore, high level of Hyperactivity-Impulsivity symptoms was associated to nonresponse. Mental and physical complaints, measured as potential ARs on the BSSERS-C before initiation of treatment, decreased during the treatment period. This has raised the question of the validity of the ARs rating scale, and the study advises that a new rating scale for ARs is needed.

The pharmacogenetic study showed that variants of rs3815583 (GT/GG) and rs2307240 (GA) of *CES1* were associated with lower end-dose of IR-MPH, and variants of rs111604615 (CC), rs2307240 (GA), and rs2302722 (GT/TT) of *CES1* where associated to higher treatment response rate than the wildtypes. The *CES1A2* was weakly associated to reduced appetite and low end-dose of IR-MPH. Patients with the variants of rs2307240 (GA) or rs3815583 (GG/GT) may be at risk of being slow metabolizers of MPH. These significant associations were of small effect-sizes and the clinical significance of the variants suggested a minor role - at best - in personalized treatment of ADHD with MPH in the clinic. Still, these findings add to the understanding of the pharmacogenetics of MPH and suggest that the response of MPH is determined by a number of genetics variant with small-effect sizes, which include genetic variants of *CES1*.

9.2 Research findings in context of paper I

9.2.1 Description of the first 12 weeks of IR-MPH treatment outcome

In the present study the children with ADHD were treated individually with carefully titrated doses of IR-MPH based on the weekly monitoring of beneficial effects and ARs. This resulted in a mean end-dose of 1.0 (0.3

SD) mg/kg/day which was within the range of the clinical recommendations[55–57] and in the higher end of the mean doses reported in other observational studies (S2 Table in paper I)

The study population was comparable to the total age-matched population of children with a registered diagnosis of ADHD in Denmark with regard to the distribution of gender and comorbid mental health disorders[201]. Furthermore, the distribution of comorbid mental health disorders in the present study were comparable to an Italian register based study[202].

In the present study, the mean change in total ADHD symptom scores over time were comparable to the mean pre-post changes reported in the systematic reviews of RCTs[141,203]. Furthermore, the mean level of ADHD symptoms at study end were on the level categorized as *a very good or optimal response* (total score < 18 and total subscale score < 9) in the routine care[204].

The present study did also find significant improvements of the sustained attention on all TOVA parameters, which also are seen with in observational studies[154,152] and RCTs[205,206]. The largest improvement was the variability of response time, which might indicate higher stability of attention during the whole test period. This more objective of improvements of inattention and impulsivity symptoms support the findings of ADHD symptoms improvements evaluated by clinicians, parents, and teachers.

Three observational studies (S2 Table in paper I) found lower CGI-I response rates (CGI-I ≤ 2); 79.1%[159], 81.5-78.5%[160,161], and 49.9%[154] than the present study's rate of 91.5% The present study included a clinical representative sample of children that were severely affected according to the CGI-S scores (5.3, SD1.0) however only 35.8% reach the threshold (CGI-S ≤ 2) which is lower than one study 46.1% measured[151].

The extent to which the individually monitored long-term MPH treatment can improve the prognosis is sparsely elucidated. The efficacy pharmacological treatment of ADHD in RCTs are often using fixed doses and short follow up time periods (<six month), whereas the long-term treatment effects are limited studied[50,126]. But new long-term cohort studies have found pharmacological treatment associated to lower risk of accidents[207], criminality[208], and better academical performance[147,148,209]. The limited knowledge of the short- and long-term-outcome of MPH treatment regarding the daily and social functioning, academic performance is problematic since the primary indication for pharmacological treatment[42,47] arguably is the functional impairments of ADHD symptoms in at least one domain of the child's life. The present study demonstrated improvements of daily and social functioning measured by W-FIRS-P in the same range as found in the few other studies that used W-FIRS-P, including one new RCT[201], one observational study[210] (Table C in Appendix A), and the W-FIRS validation study[128]. This study also found that changes in some of the W-FIRS-P domains were associated to the decrease of symptoms measured on ADHD-RS and CGI-S[128].

Furthermore, one study[190] has validated the use of W-FIRS-P on ADHD patients before initiation of treatment and found significant impairments of daily and social life compared to children without ADHD. W-FIRS-P is also implemented in the Canadian guidelines as a baseline assessment before initiation of treatment, but not as an evaluation assessment during treatment. Our results suggested that it is possible to detect clinically meaningful changes in daily and social functioning by repeated use of W-FIRS-P in the routine care of children with ADHD.

As far as we know, this is the first observational study that have reported ARs based on a weekly and systematically monitoring during the first 12 weeks of treatment with MPH. Scores rated by the clinicians as potential ARs were rated highest before initiation of treatment and decreased during the treatment period with the only exception that we saw a significant increase in loss of *appetite*.

The same decreases in ARs are found in few other studies (*proneness to cry*, *irritability*, *nail biting*, *anxiety*, *sadness*, and *euphoria*)[51,53,54]. Many of the AR items rated on BSSERS-C may not be true ARs associated to IR-MPH but may reflect symptoms of ADHD or psychiatric comorbidity[53]. This may challenge the use of BSSERS as a valid instrument for assessment of ARs associated with stimulant treatment[50,112,113,152,153]. This study suggests that ADHD related symptoms and impairments measured on BSSERS-C (S8 Tables in paper I) could be *irritability*, *euphoria*, *sadness, staring, prone crying,* and *nail biting. Euphoria* could be a part of the hyperactivity or impulsivity symptoms. *Staring* could be a part of the inattention symptoms. *Sadness* and *prone crying* could be a part of comorbidity or a part of the challenges of having ADHD. *Nail biting* could be a part of controlling the hyperactivity or impulsivity symptoms. Moreover, BSSERS-C detected only half of the ARs/SARs that were recorded in relation to drop out and possibly caused the drop out. A total of 12 out of 207 (5.8%) children discontinued treatment due to ARs/SARs: six patients reported ARs/SARs detected on BSSERS-C (*irritability* n=3, *tics* n=2, *sadness* n=1,) and another six patients reported ARs/SARs spontaneously or the ARs/SARs were measured as a part of the physical examination (hypertension n=2, hyperactivity n=1, urticarial reaction n=1, progression in seizures n=1, onset of psychotic symptoms n=1).

The discontinuation rate due to ARs/SARs in the present study was of 5.8% which is similar to the documented rates in other observational studies (4.5-8.0%, ARs[150,152]) and in the Cochrane review of ARs in non-randomized studies (1.2%, SARs and 6.2%, ARs)[126]. RCTs seemed to underestimate SARs[129]. In the present study the mean blood pressure was not increased at study-end, however, two patients discontinued treatment due to hypertension. The stable blood pressure was comparable to the reports from other observational studies[126,155], and a RCT[211].

Overall, the patients who completed the 12 weeks of treatment in the present study seemed to report fewer clinically significant ARs than patients in other observational studies[113,126,152,160], but the comparison of ARs

across studies is hampered by the use of different assessment instruments and the lack of consensus on how the ARs should be reported [50,126].

9.2.1 Predictive value of clinical baseline characteristics

9.2.1.1 End-dose

This study showed that severe global impairments (CGI-S) before initiation of treatment and young age (7-9 years) predicted a higher end-dose of IR-MPH compared to patients with less severe global impairments and older age (10-12 years), respectively. The end-dose difference (0.34 mg/kg/day) between "*among the most extremely ill patients*" in week 0 (CGI-S 7) and "*mildly ill*" in week 0 (CGI-S 3) might be of clinical relevance whereas the difference between the age-groups (0.13 mg/kg/day) might be neglectable. ADHD subtype, sex, and weight did not predict any difference in end-dose. No studies or meta-analysis were direct comparable to this study. The Cochrane review also advised future studies to report treatment outcomes by groups of children with different baseline characteristics. This way studies could provide data for metanalytic studies aiming to identify predictors of outcome[50]. We identified two RCTs that studied moderators of end doses of MPH. The two studies found that a high level of ADHD symptoms before initiation of treatment and the ADHD combined type, respectively, was associated to higher end-dose[52,173].

9.2.1.2 Beneficial effect

The same clinical characteristics were tested as predictors for beneficial effects in both Cox regression analyses and mixed effect models. In the Cox regression analysis, the chance of normalization of Hyperactive-Impulsivity scores was increased for children with two or more psychiatric comorbid diagnoses and reduced for children with *inferioritas intellectualis* regarding both Inattention and Hyperactivity-Impulsivity. But the more powerful mixed effect models analysis of the 12-week course of mean sum scores reductions (Inattention and Hyperactivity-Impulsivity) did not confirm these differences by intellectual level or psychiatric comorbidity. Taken together, the results might indicate that the magnitude of improvements are similar across groups, even though the chance of normalization of ADHD symptoms is reduced when IQ is in the lower end. The higher chance of normalization of Hyperactive-Impulsivity scores for children with psychiatric comorbidity is somewhat counter-intuitive. Perhaps the presence of comorbid psychiatric disorders and impairments contributed to the decision to initiate drug treatment, suggesting that the children with comorbidity had milder ADHD symptoms.

This is in line with the literature, which found that patients with lower IQ responded positively to MPH resulting in benefits at the same level of improvement compared to patients with IQ in the normal range[163–165].

Most studies did not find any difference in treatment outcome between patients with and without comorbidity[14,50,149,168,169], whereas few studies found low level of anxiety[164,167] and absence of oppositional defiant[14] associated to better treatment outcome than those patients with comorbidity, even though one study find the opposite[212].

Overall, there were no clinically significant differences by or by age and sex in outcomes of treatment with MPH measured as mean change in ADHD sum score and in rate of normalization on Inattention or Hyperactivity-Impulsivity. However, there were mixed findings of minor differences by age-and-sex, which might be an artefact related to the age and gender specific cut-offs for normalization based on a study conducted nearly 10 years ago[185]. As an example, older girls (10-12 years) had a higher mean sum score on Inattention trough out the treatment period than younger girls (7-9 years) and both age groups of boys, but girls had a lower chance of normalization compared to boys. There might be a need for new *age and gender* specific norms for ADHD symptom scores.

A multivariate regression analysis of nine studies showed that young age was associated to good clinical response[14], whereas the Cochrane review did not find this association[50].

In summary, there were no clear indications of differential response to the individually titrated IR-MPH-treatment across age, gender, IQ, and psychiatric comorbidity. Our results regarding patients with comorbidity improved on ADHD symptoms as much as patients without comorbidity or even better, are important information because of the common appearance of comorbidity in the routine clinic[8].

9.2.1.3 Adverse reactions and nonresponse

In the present study high sum score of ARs (BSSERS-C) throughout the whole treatment period (including week 0) was predicted by greater global impairments (CGI-S 6-7) before initiation of treatment. High risk of complaining about *reduced appetite* with no change during the whole treatment period was also predicted by greater global baseline impairments before initiation of treatment.

A study found comorbidity associated to *reduced appetite*[51]. This present study did not test the comorbidity as a predictor for the *reduced appetite* outcome, but comorbidity was not associated to the ARs outcome. As other studies[53,113], the present study did not find any association between ARs and age or sex.

Overall, the present study showed that patients with greater global impairments before initiation of treatment reported high level of problem scores on BSSERS-C before treatment and during the treatment period, and some of these patients dropped out due to ARs (nonresponders) or did not respond to treatment (nonresponders due to no Nor/Bnor) even though the group received a high end-dose. High level of hyperactivity-impulsivity symptoms before initiation of treatment was also associated with being nonresponder.

9.3 Research findings in context of paper II

9.3.1 Sequencing and selection of variants of *CES1*

The present study systematically sequenced SNPs and determined the presence or absence of *CES1A2*, the duplicated variant of *CES1* which counted 44 SNPs of *CES1* were identified besides the variation creating *CES1A2* and selected the seven including the *CES1A2* variants through predefined criteria in a clinical representative sample of children with ADHD. The advantage of our approach was that we selected collection of variants of *CES1* for out study based on predefined criteria. Furthermore, we excluded DNA data of non-Caucasian patients and DNA of consanguine patients due to the genetic principal component analyses, which avoided redundant data.

We added rs71647871 because of the finding in the literature[114,116], but found no associations between rs71647871 and the treatment outcomes. A decrease in the CES1 activity is showed associated to the change of the amino acid from glycine (Gly) to glutamic (Glu) in rs71647871, as a LOF variation described by Zhu et al[116]. This LOF might be the reason for why Nemoda et al without any predefined selection criteria chose the rs71647871 and found the heterozygotic variant of rs71647871 (GA) associated to low end-dose[114]. Furthermore, Johnson et al selected seven SNPs of *CES1* in her study without any explanation for this selection[113].

9.3.2 Predictive value of selected variants of *CES1*

9.3.2.1 End-dose

In the present study associations between single variants of *CES1* and end-dose were only tested without backward elimination of covariates in the GLS. This was a way of showing the *real effect* of each gene on the end-dose.

3 or 4 copies (*CES1A2*) were weakly associated to lower end-dose compared to 2 copy numbers, meaning only a significant difference in the categorial univariate analysis and not significant, when stratified for baseline characteristics in the GLS analysis, and will not be of clinical relevance.

3 or 4 copies (*CES1A2*) would theoretically lead to a *fast metabolism* and patients with these variants will need a higher end-dose of IR-MPH and might have insufficient beneficial effect, which only was confirmed in one study[117]. But this was not confirmed in the present study and our the results is in line with other studies[175–178,213], where *CES1A2* not was associated to difference in end-dose.

In the present study, a low end-dose was consistently associated with the SNPs, rs3815583 (GT/GG) and rs2307240 (GA), but rs2307240 (GA) was only borderline significant ($p = 0.088$) when adjusted for baseline characteristics maybe because of the low number of GA ($n = 12$) in rs2307240. The differences in end-doses between the variants and the wildtypes where about 0.1 mg/kg/day, which might not be of clinical relevance. One study found

rs2307240 (GA) was associated to a significant lower *d*-RA/*d*-MPH ratio after three hours of treatment compared to the wild type, but no difference between the genotypes of rs3815583[214]. Other studies did not find any differences between variants of rs2307240 and CES1 activity *in vitro*[215] and no associations between variants of rs3815583 and treatment outcome[177,216].

This present study did not find the same associations the LOF variant of rs71647871 (GA) as Nemoda et al[114], other pharmacokinetic *in vivo* studies[118,214], in *in vitro* an study[176], and in reviews[69,72]. The lack of significant difference was maybe because of low frequency of GA in our patients (*n* = 8, 4.3%), but this frequency is comparable to other studies: 3.7%[116] (Caucasian), 1.6%[118] (Danish), and 4.1-5.8%[114] (Hungarian) or because of other definitions of responder status or higher end-dose.

9.3.2.2 Beneficial effect

Also, the present study examined the single variants of *CES1* in the Cox regression without backward elimination of covariates to get the *true effect* of each genetic variant on the ADHD symptoms.

rs2302722 (TT/GT) and rs111604615 (CC) were associated to the chance of normalization of ADHD symptoms compared to the wildtypes, which are novel findings compared to other studies[176,177]. The chance of Nor/Bnor on Inattention was also associated to rs56278207 (ins/ins) but only compared to the heterozygotic variant del/ins (*n* = 66) and no significant difference between the wild type and the homozygotic variant (*n* = 18). Therefore, this finding might not be of clinical relevance. Furthermore, the chance of Nor/Bnor on Hyperactivity-Impulsivity was associated to rs2307240 (GA), which is in contrast to Johnson et al[217] and an *in vitro* study[215]. No studies have so far found any associations between variants of *CES1* and beneficial effect.

9.3.2.3 Adverse reactions

The variations of genotypes of rs2244614 were associated with differences in AR scores before initiation of treatment in the present study. The differences disappeared during treatment, which might reflect the regression towards the mean.

This present study confirmed Johnson et al findings of no association between variants of rs2307240 and ARs[113]. The earlier mentioned observational studies of two different patient samples found the variants of rs3815583 were associated to weight loss by Oxenbøll et al[115], nor the variants of rs3815583 were associated to reduced appetite by Bruxel et al, which this present study did not confirm[112].

9.3.2.4 Interpretations

The present study showed that rs2307240 (GA) and rs3815583 (GG/GT) were associated to low end-dose, and patients with those variants may be *slow metabolizers* of MPH. But these results might also be because of low

individual titrated optimal end-dose in combination with an average or even increased rate of Nor/Bnor of ADHD symptoms. This might have prevented some ARs, like *reduced appetite*, and substituted these outcomes with low end-dose as effect of variants of *CES1*. But personalized medicine is both considering individual titration with MPH and the knowledge of genetic variants associated to MPH treatment outcome. But the difference in end-dose was limited and might not be of clinical relevance.

Furthermore, variants of rs111604615 (CC), rs2307240 (GA), and rs2302722 (GT/TT) of *CES1* where associated to higher treatment response rate than the wildtypes. It is interestingly that rs2307240 (GA) both was associated to low end-dose and higher treatment response and not associated to increased risk of ARs, which could increase the likelihood for rs2307240 (GA) to be associated to *slow metabolism* of MPH.

The present study did not find the same frequency and effect-size as *CYP2D6* variants with LOF, which enables prediction of the pharmacokinetics and treatment outcomes of several *CYP2D6* metabolized drugs[92,97,218]. By contrast, the occurrence of large-effect size variants in *CES1* appears to be rare[116] and therefore not clinically relevant. The low rate of high-effect sizes may related to CES1 encoded by *CES1* also is implicated in important physiological functions (e.g. lipid metabolism[80–82,219]). CES1 has been identified as a backup for other lipases[220]. Therefore the detrimental variants might have been subjected to negative selection during the evolution[220]. This in line with the findings in the present study where variants of *CES1* where were not or only weakly correlated with the MPH treatment outcomes. The pharmacokinetics of MPH might not be sufficiently explained by a single variant of *CES1*[221]. The rate of MPH metabolism and the response to this drug might be of polygenic origin where specific combinations of small-effect size variants in *CES1* and other genes collectively contribute to the activity of CES1 and moderates MPH treatment outcome[69,72,103,160]. But the potential polygene origin complicates identification of these variants. Furthermore, the effect size of a single genetic variant can be difficult to describe with statistical analyses.

Overall the study found rs111604615 (CC), rs2307240 (GA), rs2302722 (GT/TT), and rs3815583 (GG/GT) statistically significant difference between treatment outcomes of beneficial effect and/or end-dose and the wildtypes. But the findings might be of minor clinical relevance because of the small impact on the treatment outcome. But the results were hypothesis-generating and future studies will need to replicate the results in with larger sample sizes.

9.4 Methodological considerations

9.4.1 Paper I

The main strength of this ecological valid observational study was the inclusion of a representative clinical sample of children[201] with recently diagnosed ADHD; low attrition rate, treatment guided by evidence-based

clinical guidelines, and beneficial effects and ARs were closely monitored with standardized instruments. The study population was comparable to other clinical samples of children characterized by high occurrence of comorbid mental disorders, and with moderate impairment[202]. The weekly assessments and monitoring of beneficial effects and ARs were also a main strength compared to other studies[50,106,126].The repeated measurements of the ADHD symptoms and global impairments were performed by the same clinical investigator throughout the 12-week study period and with consensus ratings in week 0 and 12 with the child's child and adolescent psychiatrist. The intensive monitoring of symptom reductions, ARs, and the careful titration of IR-MPH probably contributed to the high maintenance of the participants in the study resulting in an overall low dropout rate. Short-term effects of MPH on the ADHD symptoms are well-documented, whereas the medium- and longer-term effects are less well documented[50]. This study contributed with evidence of the short- and medium-term effect of MPH for the treatment of childhood ADHD in a naturalistic setting using an individually adapted dosing strategy[144].

The lack of an untreated control group is a limitation of this observational study because the natural course of ADHD cannot be differentiated from effects due to IR-MPH treatment, and changes can also be explained by a regression toward the mean and/or unspecific effects of the frequent contact with the clinical investigator. Other limitations include the possibility that information was lost when continuous variables (ADHD sum scores) were dichotomized into the categories of Nor or Bnor of ADHD symptoms[222]. Existing Danish norms and cut-off values for ADHD-RS-P were used to determine Nor and Bnor based on Inattention and Hyperactivity-Impulsivity scores of ADHD-RS-C, because ADHD-RS-C is not validated in Denmark[185,195]. Also, the age and gender specific norms and cut-off value of ADHD-RS-P and ADHD-RS-T might need revision. As examples; girls (< 9 years) need to have less ADHD symptoms (sum score < 6) compared to boys (< 9 years) (sum score < 8) to obtain normalization on the subscale Hyperactivity-Impulsivity of ADHD-RS-P, and especially girls (< 9 years) need to have nearly no symptoms (sum score < 3) on subscale Hyperactivity-Impulsivity of ADHD-RS-T to obtain normalization compared to boys (sum score < 10). TOVA is not validated in Denmark. Therefore z-scores based on mean and standard deviations of week 0 total raw scores were calculated. W-FIRS-P is not validated in Denmark, but is a very common instrument used in the studies[128,189,190]. There is no validation of BSSERS-C in Denmark too. However, the instrument is the most common standardized instrument to measure ARs[126]. Unfortunately there were no instructions available for how to rate each item from 0 (*problem absent*) to 9 (*problem evokes serious impairment*)[130]. Therefore, we developed a manual for ratings of the 17 items of BSSERS-C. The discriminative validity of BSSERS with regard to various mental and physical health problems and ARs are not known. The decrease of systematically measured ARs suggests that the complaints reflected general health and mental health problems which were related to the ADHD illness rather than ARs to IR-MPH

treatment[53]. Even healthy children might complain of some of the effect items measured on BSSERS of during a 12-week period which is a limitation of validation of BSSERS[130]. Like most other studies of ADHD in childhood, the study did not include patients with mental retardation. The group with mental retardation and ADHD is difficult to include because most psychometric instruments are not validated for this group of patents, and the ADHD diagnosis might be inaccurate because of their global mental delay.

9.4.2 Paper II

The way the pharmacogenetic study systematically sequenced variants of *CES1* and selected genetic variants based on predefined criteria was a strength. The study followed the methodological recommendations for pharmacogenetic studies with assessments of multiple continuously and categorically outcomes rated by different raters[104], measuring of baseline data separately[101], and blinding of the patients genotype during the titration phase. There was no lack of baseline data in the present pharmacogenetic study, which has been of one the criticism of pharmacogenetic studies[101]. The study did not grouping genotypes, which also can led to different results if one genotype is more dominant than another[104].

A limitation of the present pharmacogenetic study was the use of flexible dosing of IR-MPH which might lead the associations between the variants of *CES1* and the treatment outcomes to become less transparent[104].

The hypotheses of the variants of *CES1* associated to different treatment outcomes were defined without the knowledge (which variants and sample size) about the variants of *CES1* there were supposed to be included in the study. Thereby the hypothesis testing was of an explorative character and the exact sample size for each variant was not known at study-end. The limitation was also that the pharmacogenetic sample size (n = 189, n for genotyped sites of *CES1* varied among the participants, and n for the baseline characteristics between the polymorphic sites of *CES1* varied too). Even though the present study had a larger sample size than most other pharmacogenetic studies[106]. The sample size might be too small to exclude random significant effects (Bonferroni correction), likewise too small to test the combined effect of several independent variants of *CES1*, and too small to identify the potential influence of *CES1* (type II error). As an example, when the low frequencies of the homozygotic variants at rs111604615 and rs2244614 (n = 9 and n = 8) where merged to heterozygotic variants there were no differences compared to the wild types in the treatment response rates and ARs, respectively. A total number of 12 explorative association analyses of treatment outcomes tested eight different variables of *CES1* in with no adjustment of the level of significance due to multiple testing[223]. But the choice of tests was predetermined due to the protocol and the explorations of associations where not just made by "fishing"[223]. The pharmacogenetic results can be findings by chance. Also, the influence of the variants of *CES1* on the treatment outcomes might be better described by the simple

univariate analyses, as the multivariate analyses may be over-adjusted for genetically mediated variations. Replication in larger studies with enough power to examine for interactive effects of MPH and multiple variants of *CES1* is needed. Furthermore, pharmacogenetic studies have a tendency to underestimate environmental contributions and overestimate genetic effects[224].

9.5 Directions for future research

Future observational studies should

- Replicate the findings of individually and closely titrated MPH with improvements of ADHD symptoms, related symptoms and impairments across informants and few ARs over a longer treatment period than 12 weeks.
- Evaluate of the importance of frequent contact with a clinician.
- Use standard rating scales for measuring ADHD symptoms and ARs.
- Unify a definition of the clinical response to MPH.
- Develop and validate a new rating scale for ARs which includes a definition and way of evaluation of each ARs. The new rating scale for ARs will be of use in studies and in the clinic.
- Divide treatment outcomes in relation to baseline characteristics.
- Re-validate and re-norm the Danish ADHD-RS rated by parents and teachers.
- Evaluate the effect of MPH on daily and social functioning over a longer treatment period than 12 weeks.

These future findings will be important knowledge to improve the standard treatment of ADHD in children and adolescent and might be worth to include in future guidelines.

Future pharmacogenetic studies should

- Replicate the results of associations between variants of *CES1* and IR-MPH treatment outcome in a larger sample of patients before a conclusion about their possible role in the metabolism and outcome of treatment with MPH can be drawn.
- Ideally be conducted on a genome-wide basis to enable identification of gene variants determining the pharmacokinetics and pharmacodynamics of MPH. Preferentially, such studies should be powered to detect gene-gene interactions and gene-environment interactions in addition to the effects of single gene variants[224].
- Enable the construction of a polygenic score for treatment outcome of MPH and inclusion of environmental effects in this score system that collectively may enable accurate prediction of the response to this drug on the individual level[225].

9.6 Potential implications to the clinic

We hoped that the results of the present observational pharmacogenetic 12-week study could provide useful knowledge to improve the use of personalized medicine in the clinic to insure a better MPH response with the highest safety margin for each child with ADHD. But the present study did not show any predictive relationship between baseline characteristics and ADHD symptom reductions or ARs.

The observational study showed that an individually titrated dosing of MPH based on weekly assessments of beneficial and harmful effects produced a favorable balance between symptom reductions and ARs, and a high rate of responders. These results are of relevance for clinicians and guideline developers and add to the current debate about the effectiveness of MPH in the real world setting as compared to the efficacy of MPH in RCTS[226,227]. The positive experience of working together with the child, the parents, and the teachers during the titration of IR-MPH also stresses the clinical importance of a *comprehensive, holistic and shared treatment plan*, as advised in the guidelines[42].

The results of this study suggest that clinical guidelines should advice

- the clinician to obtain information about severity of potential ARs before initiation of treatment and continue to monitor ARs during titration of MPH by use of a standardized rating scale. This means e.g. initial information about sleeping problems and appetite problems, a common understanding of euphoria and sadness. The BSSERS may not differentiate between ADHD related symptoms and ARs.
- the clinician to systematically collect information of the child's ADHD symptoms and behavior by use of standardized rating scales in different settings, e.g. ADHD-RS from home, school, and during leisure activities both before initiation of treatment, during titration phase, and during the continuing treatment with MPH.
- implementation of W-FIRS-P in the routine care as an instrument used before initiation of treatment and as an evaluation assessment during treatment.
- the clinician to integrate and use all this information to individually titrate MPH and thereby ensure a better treatment response with few ARs.
- the clinician to collaborate with the parents and the teachers aiming for a shared understanding and treatment plan that considers the personal preferences of the child and parents and integrates the different viewpoints. In order to clarify the indication for drug treatment, it can be important to explore any differences between the parent and the teacher in ratings of the child's levels of ADHD symptoms.
- the clinician to be particularly aware of children with greater global levels of symptoms and functional impairments as they tend to report severe symptoms mimicking ARs before initiation of treatment and during

the treatment period. These children are also in risk of treatment failure due to discontinuation, nonresponse and/or a high end-dose.

The pharmacogenetic study showed statistically significant associations between variants of *CES1* and IR-MPH treatment outcome, but these results might be of limited clinical relevance because of the small impact on the treatment outcome. The identification and analyses of generic variants as predictors of the MPH treatment response will need to be replicated and expanded before the results will be of clinical relevance for the personalized medicine of IR-MPH in children with ADHD. Furthermore, the MPH response are determined by many other things (e.g. ethnicity, dietary habits, age, weight) that also can influence on the pharmacokinetics of MPH[89].

Pharmacogenetics studies of ADHD are in the rise, and it is one of the largest pharmacogenetics research areas in child and adolescent mental health[102]. In the future it might be possible to shift from trial-and-error approach or the stepwise careful-titration approach for ADHD medication to making use of pharmacogenetic moderators[101,102], which could accelerate the treatment processes[98].

10 Conclusion

This observational study demonstrated beneficial effects on both ADHD symptoms and behaviors, ADHD related symptoms, and the level of functioning in daily and social life over a 12-week treatment period in a clinical representative sample of children with ADHD. Compared with the efficacy of MPH in RCTs, the results suggested effectiveness of MPH in a naturalistic clinical setting with a carefully monitored and individually titrated IR-MPH treatment. The only increase of ARs were reduced appetite and weight loss. Children with greater global impairment before initiation of MPH complained more of ADHD associated symptoms measured as ARs and received more often higher dose of IR-MPH. The commonly used ARs rating scale showed decreases of most symptoms and a new validated ARs rating scale with well-defined symptoms ratings is needed.

The present pharmacogenetic study examined seven representative variants of *CES1* and a duplication of this gene in a population of children with ADHD and found that two of these variants were associated to lower end-dose of IR-MPH, three variants where associated to higher treatment response rate, and one of these variants was associated to both lower end-dose of IR-MPH and higher treatment response rate than the wildtypes. These results add to the understanding of the pharmacogenetics of MPH and expands the knowledge by demonstrating that the MPH response is determined by a large number of genetic variants, each contributing with a small-effect size, which include variants in *CES1*. The increased insights in the pharmacogenomics of MPH may assists in developing strategies for personalized treatment with MPH for children and adolescent with ADHD in the future.

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Paper I

1) **Manuscript**
2) **Supplementary tables and figures**

Short title

Treatment outcomes of a 12-week naturalistic study of children with ADHD.

Full title

Outcomes of a 12-week ecologically valid observational study of first treatment with methylphenidate in a representative clinical sample of drug naïve children with ADHD.

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Abstract

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Randomized placebo-controlled trials have reported efficacy of methylphenidate (MPH) for Attention-deficit/hyperactivity disorder (ADHD); however, selection biases due to strict entry criteria may limit the generalizability of the findings. Few ecologically valid studies have investigated effectiveness of MPH in representative clinical populations of children.

This independently funded study aims to describe treatment responses and their predictors during the first 12 weeks of MPH treatment using repeated measurements of symptoms and adverse reactions (ARs) to treatment in 207 children recently diagnosed with ADHD. The children were consecutively included from the Child and Adolescent Mental Health Centre, Mental Health Services, Capital Region of Denmark.

The children (mean age, 9.6 years [range 7-12], 75.4% males) were titrated with MPH, based on weekly assessments of symptoms (18-item ADHD-rating scale scores, ADHD-RS-C) and ARs. At study-end 187 (90.8%) children reached a mean end-dose of 1.0 mg/kg/day. A normalisation/borderline normalisation on ADHD-RS-C was achieved for 168 (81.2%) children on the Inattention and/or the Hyperactivity-Impulsivity subscale in week 12, and 31 (15.0%) children were nonresponders, which was defined as absence of normalisation/borderline normalisation (n = 19) or discontinuation due to ARs (n = 12), and eight (3.8%) children dropped out from follow-up. Nonresponders were characterised by more severe symptoms of Hyperactivity-Impulsivity and global impairment before the treatment. ARs were few; the most prominent were appetite reduction and weight loss. A decrease in AR-like symptoms during the treatment period questions the validity of currently available standard instruments designed to measure ARs of MPH.

This ecologically valid observational study supports prior randomized placebo-controlled trials; 81.2% of the children responded favourably in multiple domains with few harmful effects to carefully titrated MPH.

Keywords

ADHD; children, drug naïve; ecologically valid 12-week observational study; methylphenidate; treatment response.

Introduction

Attention deficit/hyperactivity disorder (ADHD) is a heterogeneous neurodevelopmental disorder characterised by pervasive and impairing symptoms of inattention and/or hyperactivity and impulsivity with onset of symptoms before age 7 years (International Classification of Diseases and Related Health Problems, ICD-10)[1] or age 12 years (Diagnostic and Statistical Manual of Mental Disorders, *DSM-5*)[2] and high rates of persistence into adulthood[3,4]. The mean estimated worldwide prevalence of ADHD is 3.4% (CI 95% 2.6 to 4.5) in the general population of children and adolescent, making it the most common neurodevelopmental disorder in youth[5]. ADHD has a polygenic and multifactorial aetiology that is only partly understood[6].

The National Institute for Health and Care Excellence (NICE; United Kingdom) recommends methylphenidate (MPH) as a first-line pharmacological treatment of ADHD in children[7]. MPH is a central nervous system stimulant that has been used for treatment of ADHD in youth since the 1960s[8]. Although its mechanism of action is not completely understood, MPH inhibits the dopamine and the norepinephrine transporters[9], primarily resulting in increased extracellular levels of dopamine in the brain[10,11].

Several meta-analyses of the short-term efficacy of immediate release MPH (IR-MPH) in randomized placebo-controlled trials have reported large effect sizes (range from 0.54 to 1.83) on ADHD core symptoms, when effects are measured as differences in endpoint or change in scores of parent-rated and teacher-rated ADHD symptoms and behaviours . A review of treatments for ADHD in adolescents aged 12-18 years found large effects for stimulants (extended-release MPH and amphetamine) describing a mean change in absolute symptoms score ranging from 10 to 18 points on the clinician-rated, 18-item-ADHD-rating-scale (ADHD-RS-C, range 0-54[16,17]).

Beneficial effects of IR-MPH have been demonstrated in several domains including improvements in ADHD symptoms and behaviours at home and in school[12,13], cognitive functions[18], classroom behaviour, and academic performance[19], although a recent meta-analysis found only small effects on school performance [20]. The primary measure of MPH efficacy in randomized controlled trials (RCTs) is usually the endpoint or change in scores of total ADHD symptoms, and clinically meaningful response is generally considered to be a within-group symptom reduction of 25%[21] or 30%[22] on ADHD-RS (corresponding to 10-15 absolute points).

However, there is no generally accepted definition of the clinically significant response to treatment for ADHD, and the response rates obviously vary with the psychometric instrument, the informant, and the defined response criteria[21,23]. In one RCT, the response was defined as a 40% or more decrease in sum scores in ADHD-RS-C after 6 weeks of treatment[24], resulting in a response rate of 64% in youth patients treated with MPH for the first time.

Generally, adverse reactions (ARs) to MPH treatment are poorly described in the literature, partly due to huge variations in the definitions and the instruments for measurement of ARs[25]. A review of ARs associated with MPH in short-term studies showed rates of loss of appetite of 3 to 56%, poor sleep of 9 to 64%, headache of 2 to 33%, and abdominal pain of 4 to 19%[25]. Another review of ARs induced by MPH in long-term studies with a duration of one to two years reported discontinuation rates due to ARs of 8 to 15% and incidences of any ARs of 85 to 89%, loss of appetite 14 to 19%, poor sleep 15 to 19%, headache 25 to 30%, and abdominal pain 8 to 11%[26]. Although the efficacy and safety of MPH is well documented in placebo-controlled RCTs and meta-analyses of RCTs, less is known about the "real world" effectiveness of MPH for treatment of heterogeneous samples of children with ADHD in service of child and adolescent mental health. The RCTs of MPH may be biased by selection according to the studies' inclusion and exclusion criteria (typically excluding comorbidity), the patients' willingness to participate, and the clinicians' willingness to allocate patients to randomised intervention studies. The limited generalizability of the evidence from the RCTs, including the risk of selection bias, calls for more naturalistic observational studies of the effectiveness of MPH in representative clinical samples of children with ADHD, treated in routine settings, including all MPH-treated children regardless of ADHD severity, ADHD subtype and comorbidity[27].

In preparation for the analysis of the present study, we systematically searched PUBMED for naturalistic observational clinical studies of MPH treatment of children with ADHD including studies from the start of the PUBMED database from 1987 to January 6, 2020 (S1 Fig, PRISMA flow chart; S1 Table, criteria for literature search). A total of 43 publications covering 30 naturalistic observational MPH studies were identified of MPH-free (no MPH treatment at the time of inclusion) children aged 7-12 years and of these were 21 publications covering 17 studies of MPH naïve children aged 6-18 years, and of these were 11 studies with 14 publications with subgroup analyses (e.g. outcomes of specific genotypes) or specific neurocognitive outcome[28–41] (S2 Table, included studies). The 11 studies of MPH naïve children included a total of n = 1537 patients (51-280 per study) with a mean age of 9.3 [mean range 8.0-10.3] years. The mean end-dose of MPH was 0.80 mg/kg/day [range 0.50-1.06]. The mean follow-up time was 9.5 weeks [range 4-24], with a number of follow-up points in time varying from 1 to 6 weeks.

Only nine studies monitored the response to MPH using well-known psychometric instruments: DuPaul's ADHD Rating Scale rated by parents (ADHD-RS-P, [range 0-54])[16] and rated by clinicians (ADHD-RS-C [range 0-54])[17], the revised Conner's Parent Rating Scale (CPRS-R, [range 0-144])[42], Swanson Nolan Pelham version IV scale (SNAP-IV, [range 0-60][43], Test of Variables of Attention (TOVA)[44], Clinical Global Impression Improvement (CGI-I, [range 1-7]), and/or CGI Severity (CGI-S, [range 1-7])[45]. Nine studies (11 publications) a priori defined a response criterion for symptom reduction measured with validated instruments and the reported response rates varied from 15.1% to 81.5%[28–31,33,35,39–41]. The mean reduction of ADHD core symptoms (in percentage of symptom level at entry) varied from 23.8% to 62.7%[29–31,33,37,38]. Only one study divided ADHD-RS-P and ADHD-RS-C into subscales of Inattention [range 0-27] and Hyperactivity-Impulsivity [range 0-27], and the mean reductions were 41.4% to 59.3% on the Inattention and 47.3% to 66.0% on the Hyperactivity-Impulsivity subscales, respectively[31]. Seven of the 11 studies apparently monitored ARs[29,31,32,34,37,46], but the reporting was incomplete and thus difficult to summarise. Reduced appetite was present in roughly 25-66% of patients[31,32,37,40] using Barkley's Stimulant Side Effect Rating Scale[47] (BSSERS) and severe appetite reduction in 21.3% of patients[46]. Weight loss was reported in 34% of patients[37]. Intolerable ARs (defined by each study) were found in 8% of patients[29]. The vast majority of patients (75-82%) had one or more ARs during the treatment period[31,32]. One study measured 82.8% of patients having more than one AR during the treatment period while only 4.5% of patients withdrew from the study due to ARs[31].

In summary, there is no consensus on the definition of a clinically significant response to MPH treatment, and the reported response rates, and rates of ARs apparently vary with patient sample, study design, and the applied psychometric instruments and informants.

The overall objective of this ecologically valid prospective observational intervention study was to characterise the beneficial effects and ARs of IR-MPH treatment in a clinical sample of MPH naïve children recently diagnosed with ADHD and offered an individually titrated dosing of IR-MPH. The specific aims were to (a) describe the treatment response during the first 12 weeks after initiation of IR-MPH treatment based on weekly clinician-rated ADHD core symptoms and behaviours, the rate of normalisation/borderline normalisation of ADHD core symptoms, ARs, daily and social functioning, and indices of sustained attention; (b) provide information about the predictive value of clinical characteristics at entry (sex, age group, global severity of psychiatric disorder, psychiatric comorbidity, and subtype of ADHD diagnoses) for these outcomes; and (c) determine the end-dose of IR-MPH.

Materials and methods

Participants

The study included MPH naïve boys and girls aged 7-12 years with a recent ICD-10 diagnosis of hyperkinetic disorder (F90.0-90.9) or attention deficit disorder without hyperactivity (F98.8) and clinical indication for treatment with IR-MPH. Patients referred to the Child and Adolescent Mental Health Centre, Mental Health Services (Capital Region of Denmark) in the period from 1st of May 2012 to 1st August 2014, and who were suspected of having ADHD were consecutively screened for study eligibility.

The exclusion criteria were mental retardation (ICD-F70.X or IQ < 70), previous treatment with drugs metabolised by carboxylesterase 1 (CES1) (see below); e.g., MPH, severe comorbid psychiatric or somatic disease that resulted in contraindication for treatment with MPH (e.g., schizophrenia or cardiac disease), language barriers, and lack of informed consent.

Study design

The study was a prospective, noncontrolled intervention study. It is a part of the Danish INDICES study as work package six (INDIvidualised drug therapy based on pharmacogenetics: focus on CES1) aimed at personalising the treatment of drugs metabolised by CES1[48]. There was made a protocol (S11 and S12). The study followed the protocol and the only deviations from the protocol was that we did not include CGI-Efficacy[45] and ASK-ME attitude[49].

Diagnostic evaluation

The diagnostic procedure was performed in accordance with the Danish "National clinical guideline for the assessment of ADHD in children and adolescents"[50]. A diagnosis of ADHD was posed after a thorough assessment including clinical observations and examinations of the children using the following instruments: Schedule for Affective Disorders and Schizophrenia for School-Age Children, Present and Lifetime Version[51], ADHD-RS-P and ADHD-RS-T[16,52,53], parent rated Child Behaviour Checklist and teacher rated Teacher Report Form[54–56], Weiss Functional Impairment Rating Scale – Parent version (WFIRS-P)[57,58], and TOVA[44,59]. Experienced medical doctors and psychologists, all trained in the instruments used in this study, conducted all assessments.

As a part of the routine clinical care, a senior consultant in child and adolescent psychiatry confirmed the ADHD diagnosis, any psychiatric comorbid disorders, and the indication for treatment with IR-MPH. Patients were consecutively included in the study after their parents had given informed consent to participate in the study.

Administration of IR-MPH treatment

According to the Danish guidelines for treatment of ADHD, MPH is the first-line drug when pharmacological treatment is indicated. The Danish guidelines are similar to The NICE guidelines: use of an initial low oral dose of MPH and an up-titration period of at least 4 weeks, until no further effect is measured on a standard ADHD rating scale, or the appearance of intolerable ARs, or a maximum dose of 2.1 mg/kg/day[7,50]. Based on these guidelines, we developed an enhanced IR-MPH-treatment manual for the present study with weekly telephone-based ratings and monthly clinical assessments of symptom change and the presence of ARs to allow for an individually titrated optimal dosing of IR-MPH. Because the study was an observational study based on "treatment as usual", the study was not registered in a registry for clinical trials (see the ethics section). The study used IR-MPH in the form of Medikinet® 5, 10 and 20 mg because of the option to split the tablet. It was the children's regular clinicians who decided to treat each patient with IR-MPH as a part of the routine care. The initial dose was 2.5 or 5 mg IR-MPH depending upon a patient's weight (</> 30 kg). Tablets were given two or three times a day as determined by each patient's needs and was administered of parents and teachers. The weekly dose increment of 2.5 or 5 mg IR-MPH per dose continued until a clinically significant response, defined as normalisation/borderline normalisation on ADHD-RS-C was achieved, ARs prohibited further up-titrations of the total daily dose of IR-MPH, or the maximum dose was reached. Some circumstances allowed patients to change to other MPH formulations (Motiron® in the event of lactose intolerance or extended-release-MPH if patients were non-compliant or had ARs due to IR-MPH). Patients were excluded from further study assessments if their medication changed to a non-MPH preparation (e.g., atomoxetine) or if they were noncompliant with the treatment.

The assessment programme

The patients were enrolled in the study for a 12-week study period from 1st of May 2012 to 1st August 2014. After enrolment, the initial assessments were done immediately before initiation of IR-MPH in week 0. The clinical investigator undertook the weekly evaluation of ADHD core symptoms and ARs using the ADHD-RS-C and the clinician-rated BSSERS-C. The weekly evaluations were based on telephone interviews with the parent in week 1, 2, 3, 5, 6, 7, 9, 10, 11, and on more thorough clinical evaluation at baseline (week 0) and in week 4, 8, and 12, including interviews with the parents about the children's daily functions and symptoms, clinical observation and physical examination of the patients, and parent- and teacher-rated ADHD core symptoms (ADHD-RS-P and ADHD-RS-T). The clinical investigator and the children's regular clinicians who all used the ADHD-RS-C in week 0 and 12 conducted consensus ratings of the children's ADHD core symptoms. Decisions about

changes in IR-MPH dosing or discontinuation due to ARs were made in collaboration with the children's' regular clinicians and the families at the study site.

The same pair consisting of the clinical investigator and a child's regular clinician followed the same patient over 12 weeks, and the first author was the clinical investigator and rater in 71% of cases. In weeks 0 and 12, each rater performed an independent rating of the ADHD-RS-C before they agreed on a consensus rating. The two independent ratings were used for calculation of the interrater reliability for the pair at each time point using the intraclass correlation coefficient (ICC). The mean of the ICCs was calculated for each pair across time points. The mean ICCs ranged from 0.72 to 0.99 (mean of ICC = 0.90). If any disagreement was found, the single items of ADHD-RS-C were discussed until consensus was reached. Consensus ratings of ADHD-RS-C scores in week 0 and 12 were measured to set an individual standard for the clinical investigator ratings in week 1 to 11. None of the clinicians were blinded to evaluation of the treatment and changes of the treatment with MPH.

Assessment instruments

ADHD-RS-scales.

The primary outcome of the 12-week IR-MPH-treatment was measured with the ADHD-RS scales[16,17,52,53]. The ADHD-RS-P and the ADHD-RS-T each consist of nine questions of inattention, nine questions of hyperactivity and impulsivity, and six questions of conduct problems evaluated on a four-point Likert scale from 0 (*none = never or rarely*) to 3 (*severe = very often*). The 18-item, clinician-rated ADHD-RS (ADHD-RS-C) is identical to the ADHD-RS-P and ADHD-RS-T subscales measuring Inattention and Hyperactivity-Impulsivity, whereas Conduct problems are not scored[17]. ADHD-RS-P and ADHD-RS-T have previously been validated in a Danish general population-based sample (n = 865 children)[53], and the standardised scores (*t-scores*) stratified for each sex and age group (7-9 and 10-12 years) were used to delineate the cut-off for normalisation (≤ 60 *t-scores*) or borderline normalisation (60-70 *t-scores*) on the clinician-rated ADHD-RS-C scores of Inattention and of Hyperactivity-Impulsivity in the present study. High correlations between ADHD-RS-C and ADHD-RS-P have been documented[60]. In the present study, ADHD-RS-C scores were determined using all available information including interviews with the parents, the parent- and teacher-rated ADHD-RS scores, psychiatric assessment, and observation of the children. The ADHD-RS-C scores were used to describe change in symptoms (sum scores) and proportions of children normalised (Nor) or borderline normalised (Bnor); any treatment response was defined as Nor or Bnor (Nor/Bnor).

Clinical Global Impression Severity scale (CGI-S).

The clinician-rated CGI-S[45] is a seven-point Likert scale, rated 1 (*normal, not ill at all*) to 7 (am*ong the most extremely ill patients*), used to measure the global severity of symptoms and functional impairments of mental health disorder including ADHD and psychiatric comorbidities. Consensus ratings were performed in week 0 and 12. A beneficial symptom reduction was defined by a CGI-S score of 1 or 2 (*normal, not ill at all* or *borderline ill*).

Clinical Global Impression Improvement (CGI-I).

The clinician-rated CGI-I is also a seven-point Likert scale[45] rated from 1 (*very much improved*) to 7 (*very much worse*). Consensus ratings were performed in week 12. A beneficial symptom reduction was defined by a CGI-I score of 1 or 2 (*very much improved* or *much improved*).

Test of Variables of Attention (TOVA).

TOVA is a computerized, continuous performance test and is validated to evaluate the objective response to the treatment of ADHD[44,59,61,62]. TOVA was administrated in week 0 and 12, and number of commission errors (response to non-target), number of omission errors (non-response to target), the response time in microseconds, and the variability of response time in correct responses to target were measured over the 21.6-minute-long test used as total raw scores. The study used version 7.3 and 8.0 of TOVA[63,64]. Consistently, IR-MPH was dosed at 1 p.m. and TOVA was performed at 2 p.m.[65] to ensure an equal and sufficient exposure of IR-MPH at the week 12-assessment (maximal plasma concentration is achieved 1-2 hours after administration of IR-MPH[66]). In week 0 and 12, total raw scores were converted to *z*-scores based on mean and standard deviation of week 0 raw scores due to the lack of standard of TOVA for Danish children.

Weiss Functional Impairment Rating Scale - Parent version (WFIRS-P).

WFIRS-P is a parent-rated questionnaire[57,58,67], with 50 questions about a child's daily and social functioning in six different domains of a child's life: family (10 items), learning and school (10 items), activities of daily living (10 items), self-concept (3 items), social activities (7 items), and risky activities (10 items). It is evaluated on a four-point Likert scale from 0 (*never or not at all*) to 3 (*very often or very much*). Index scores of WFIRS-P were measured in week 0 and 12[68]. Single item scores = 2-3 signal impairment (> 2 SD)[57,58,67].

Barkley's Stimulant Side Effect Rating Scale (BSSERS-C).

ARs were measured by the clinical investigator using the BSSERS-C[47]. It consists of 17 effect items rated by the clinician, based on information from patients, parents, and clinical observations: insomnia, nightmares, staring, talks less, disinterested in

others, reduced appetite, irritable, stomachaches, headaches, drowsiness, sadness, prone to crying, anxious, nail biting, euphoria, dizziness, and tics/nervous movements. BSSERS-C is a 10-point Likert scale rated from 0 (*problem absent*) to 9 (*problem evokes serious impairment*). A manual for interviewing and rating using BSSERS-C was elaborated for the study, anchoring the 10-point problem scores (e.g., specifying the time to fall asleep when scoring insomnia). The BSSERS-C was scored before initiation of IR-MPH-treatment (week 0) and weekly during treatment. Severities of ARs were calculated as the sum of problem scores on the 17-item BSSERS-C. Significant changes in single items were also explored (e.g., reduced appetite; 1 item, range 0-9).

Dosing of IR-MPH.

The IR-MPH doses were individually titrated, based on the weekly evaluations of symptom reductions and ARs (and the body weight of a child), which were carried out by the clinical investigator. The dose of IR-MPH (mg/kg/day) at the end of the study was registered.

Physical assessments.

The body weight, height, heart rate and blood pressure were measured every fourth week with the same equipment each time. The study followed the European guidelines[69] and measured blood pressure after 10 minutes of rest with a sphygmomanometer (nonelectrical) three times and the average of the last two measures was used.

Nonresponder.

A combined measure of nonresponder status was defined as patients who discontinued IR-MPH treatment due to ARs or serious adverse reactions (SARs) or patients who did not attain Nor/Bnor status, based on the clinical experience that these two types of poor outcome are interrelated.

Predictors

We explored whether the following clinical baseline characteristics were predictors for outcomes:
Age (7-9 or 10-12 years), sex, comorbidity (two/more or none/one comorbid psychiatric diagnosis), intelligence level (IQ 70-85 (*inferioritas intellectualis*) or IQ > 85), conduct disorder (ICD10 DF90.1/DF91.X/DF92.X or no conduct disorder), CGI-S in week 0 as a continuous variable [range 1-7] or dichotomized to *normal not ill* to *markedly severity* of psychiatric disease (CGI-S 1-5) and *severe* to *extreme among the most ill patients* (CGI-S 6-7), ADHD diagnosis (ICD10 DF90.0/ DF90.8/DF90.9 or DF90.1 or DF98.8), and weight in week 0 (≤ 30kg or > 30kg).

The primary outcomes measures were: (a) the number of patients (percentage) who obtained Nor (*t-score* ≤ 60) or Bnor (≤ 60 *t-score* ≤ 70) of the ADHD-RS-C scores of Inattention and of Hyperactivity-Impulsivity at week 12; (b) the course of the weekly ADHD-RS-C scores of Inattention and of Hyperactivity-Impulsivity during week 0-12; (c) the course of the weekly BSSERS-C scores of ARs during week 0-12; and (d) the end-dose IR-MPH.

Statistical analyses

Comparisons of baseline characteristics of included and excluded patients were carried out with the independent *t*-test for continuous variables and X^2 (chi-squared) test for categorical variables.

Changes from week 0 to week 12 in scores on ADHD-RS-C subscales, ADHD-RS-P subscales, ADHD-RS-T subscales, WFIRS-P subscales, the BSSERS-C sum score, height, weight and blood pressure were analysed with paired *t*-test for continuous variables and X^2 test for categorical variables. Missing data on any items were not allowed on ADHD-RS-C, ADHD-RS-P, ADHD-RS-T and BSSERS-C. Ten percent missing data on items on WFIRS-P subscales were allowed and missing data on items were set as 0 (*never or not at all*). Changes in *z*-scores and raw scores of response time, variability of response time, omission errors, and commission errors of TOVA were tested with paired *t*-test.

To explore the predictive value of clinical baseline characteristics on the responses of 12 weeks of treatment with IR-MPH, linear mixed effect models for repeated measures were used. Unstructured covariance type fitted the data best as judged by the Akaike information criterion (compound symmetry covariance was used in case a model with unstructured covariance was impossible to fit). The outcome variables of the mixed models were the sum scores of Inattention and Hyperactivity-Impulsivity (ADHD-RS-C) and the sum score of ARs (BSSERS-C). The mixed models of Inattention and Hyperactivity-Impulsivity (ADHD-RS-C) tested the potential impact of explanatory variables of age, sex, intelligence level, conduct disorder, comorbidity, and time. A mixed model of ARs (BSSERS-C) tested the impact of age, sex, intelligence level, conduct disorder, comorbidity, time, ADHD diagnosis, and CGI-S in week 0. Time was measured as a continuous variable coded 0, 1, 2…12 weeks, and all other explanatory variables were measured as categorical variables. It was problematic to estimate the full mixed models including all explanatory variables simultaneously. Therefore, the explanatory variables were first tested pairwise (as main factors and interactions) and only significant main factors and interactions were included in the final multiple mixed model analyses.

Repeated measures of reduced appetite (single item of BSSERS-C) were not normally distributed. Reduced appetite was dichotomised into mildly reduced or no reduced appetite (BSSERS-C single score ≤ 3) and moderately to severely reduced

appetite (BSSERS-C single score ≥ 4). To identify potential predictors of reduced appetite over 12 weeks, generalized estimation equations (GEEs) were used. The correlation structure for the model was unstructured or M-independent. It was problematic to estimate the full GEEs including all explanatory variables simultaneously. Therefore, as described above, variables were first tested pairwise and only significant main factors and interactions were included in the final multiple GEE analyses. GEEs of reduced appetite tested the impact of age, sex, intelligence level, conduct disorder, comorbidity, time, ADHD diagnosis, and CGI-S in week 0. The ADHD disorders were categorised into ICD-10 hyperkinetic disorder or hyperkinetic conduct disorder (DF90.0, DF90.8, DF90.9, and DF90.1) versus attention deficit disorder without hyperactivity (DF98.8). CGI-S in week 0 was dichotomised to *not ill to markedly severity* of psychiatric disease (CGI-S 1-5) and *severe to extreme among the most ill patients* (CGI-S 6-7).

To determine independent predictors for the chance of normalisation or borderline normalisation on ADHD-RS-C Inattention and Hyperactivity-Impulsivity, Cox regression analysis with backward elimination was used. The explanatory variables in the Cox regression model were age, sex, cognitive deficits, conduct disorder, and comorbidity (categorical variables). Dropouts were censored out when patients exited the study.

The association between the end-dose of IR-MPH per day at week 12 and the severity of the psychiatric disorder (CGI-S week 0) was explored using linear regression with backward eliminations, with adjustment for sex, age, and weight (week 0).

SPSS version 22 was used for the statistical analyses[70].

Ethics

The study was registered in ClinicalTrials.gov (NCT04366609). The study was approved by the Danish Data Protection Agency (P-2019-851). The Local Committee on Health Research Ethics was consulted (J.nr. H-B-2009-026) in accordance with national guidelines and the Declaration of Helsinki and the study was evaluated not be within their jurisdiction due study design as an observational study. Participation was voluntary and data was kept confidential. The participants could withdraw their consent at any time without having to give reasons and with no consequences for their further treatment options.

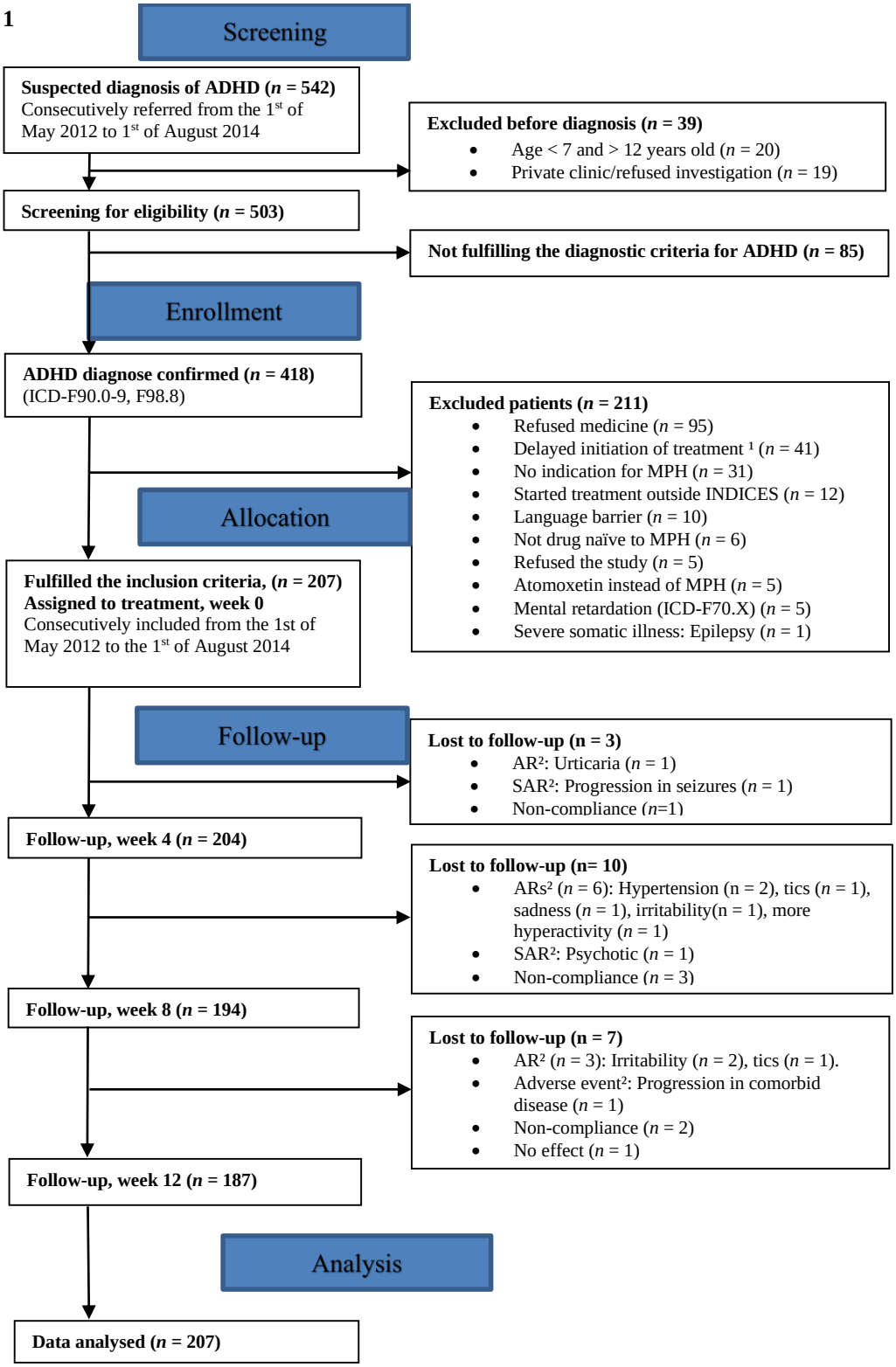
Results

Baseline characteristics of included and excluded patients

Among the 542 patients screened for eligibility (Fig 1), a total of 207 patients (mean age 9.6 SD 1.5), 156 boys (75.4%)) were included in the study (Table 1). Among those with a verified ADHD diagnosis (n = 418), a total of 211 patients were excluded.

The main reason for exclusion were that the parents did not consent to MPH treatment of their children (n = 95, 45.0%), a delayed decision to initiate MPH treatment, or no clinical indication for MPH treatment as judged by the clinician. Of the included patients, 187 (90.3%) patients (mean age 9.6 (SD 1.5), 140 boys (74.9%)) completed the 12 weeks study. There were relatively more males (n = 177, 83.9%) among the excluded patients than included in the study (p = 0.030) (Table 1).
The comparison of included versus excluded patients furthermore showed that the included patients had significantly higher ADHD-RS-P scores, and at the same time, significantly lower ADHD-RS-T scores compared with those excluded (S3 Table). There were no other significant differences in ADHD symptoms between included and excluded patients or between the included patients and the subgroup of patients excluded solely because their parents refused medicine (n = 95). This group of patients who were excluded solely because of their parents' refusals were characterized by lower ADHD-RS-P scores compared to patients excluded due to other reasons (n = 116), 10.9 (SD 5.3), and 13.9 (SD 6.3), respectively, (p < 0.001) (for further baseline characteristics see S4 Table). Only five children were excluded because of parents' refusals to let them participate in the study.

Fig 1



[**Fig 1 TREND, flow diagram, of inclusion**
ICD-10 = International Classification of Diseases and Related Health Problems, ADHD = Attention-deficit/hyperactivity disorder, MPH = Methylphenidate, INDICES = INDIvidualised drug therapy based on pharmacogenetics: focus on carboxylesterase 1, AR = Adverse reaction, SAR = Serious adverse reaction.
¹ = Treatment initiated after the study inclusion period was terminated.
² = Clinician decision of patient's discontinuation of treatment with MPH due to adverse events, ARs, and SARs.]

[Table 1 Characteristics of included and excluded patients at study entry]

	Included (*n* = 207)	**Excluded (*n* = 211)**	**Excluded, refused medicine[1] (*n* = 95)**
Boys, *n* (%)	156 (75.4)	177 (83.9)	75 (78.9)
Age, years, mean (SD)	9.58 (1.51)	9,88 (1.53)	9.64 (1.55)
Age, 7-9 years, *n* (%)	133 (64.3)	119 (56.4)	56 (58.9)
Age, 10-12 years, *n* (%)	74 (35.7)	92 (43.6)	39 (41.1)
ADHD diagnoses (ICD-10)			
Disturbance of activity and attention, hyperkinetic disorder other, hyperkinetic disorder unspecified. (F 90.0, F 90.8, F 90.9) *n* (%)	172 (83.1)	163 (77.3)	74 (77.9)
Hyperkinetic conduct disorder (F 90.1) *n* (%)	12 (5.8)	14 (6.6)	5 (5.3)
Attention deficit disorder without hyperactivity (F 98.8,) *n* (%)	23 (11.1)	34 (16.1)	16 (16.8)
Comorbidity (ICD-10)			
Tic disorders. (F95.X) *n* (%)	17 (8.2)	14 (6.6)	4 (4.2)
Externalizing disorders. Conduct disorders, mixed disorders of conduct and emotions (F 91.X, F 92.X) *n* (%)	14 (6.8)	9 (4.3)	4 (4.2)
Specific developmental disorders. (F81.X-83.X, F88.X) *n* (%)	46 (22.2)	46 (21.8)	26 (27.4)
Cognitive deficits. Inferioritas intellectualis, mental retardation (R41.8[2], F70-79) *n* (%)	56 (27.1)	62 (29.4)	27 (28.4)
Encopresis and/or enuresis. (F98.0-98.1) *n* (%)	24 (11.6)	18 (8.5)	8 (8.4)
Autism spectrum disorders. (F84.X) *n* (%)	26 (12.6)	40 (19.0)	15 (15.9)
Attachment disorders. (F94.X) *n* (%)	3 (1.4)	7 (3.3)	1 (1.1)
Emotional disorders. Depressive episode, persistent mood-, obsessive-compulsive-, reaction to severe stress, and adjustment disorders, emotional disorders with onset specific to childhood. (F 32.X, F 34.X, F 42.X, F 43.X, F 93.X) *n* (%)	27 (13.0)	23 (10.9)	10 (10.5)
Number of comorbid psychiatric diagnoses			
No comorbidity, *n* (%)	72 (34.8)	64 (30.3)	29 (30.5)
1 comorbid diagnosis, *n* (%)	78 (37.7)	85 (40.3)	39 (41.1)
2 comorbid diagnoses, *n* (%)	39 (18.8)	52 (24.6)	25 (26.3)
3 comorbid diagnoses, *n* (%)	15 (7.2)	10 (4.7)	2 (2.1)
4 comorbid diagnoses, *n* (%)	3 (1.4)	0 (0.0)	0 (0.0)

ICD-10 = International Classification of Diseases and Related Health Problems.
[1] Patients, who refused medicine, were a subgroup of excluded patients.
[2] R41.8 referred to diagnose from the diagnostic conference and/or from the intelligence test (WISC) (IQ 70-85) depending on data access.

The treatment response during the first 12 weeks after initiation of IR-MPH treatment

The reasons for discontinuation of IR-MPH treatment.

The clinicians decided when to discontinue medication with IR-MPH due to ARs or SARs (n = 12, 5.8%). Of the patients who discontinued the treatment with IR-MPH, one experienced onset of psychotic symptoms (0.5%) and another patient had progression in seizures (0.5%), both were classified as SARs (1.0%). A total of 10 patients (4.8%) discontinued the treatment because of ARs: three patients (1.4%) because of irritability, two patients (1.0%) because of hypertension (increase in a systolic blood pressure and/or diastolic blood pressure to a level corresponding to the 95-99 percentile for age and gender), two patients (1.0%) because of tics, one patient (0.5%) because of sadness, one patient (0.5%) because of hyperactivity, and one patient (0.5%) because of urticarial reaction. Six of these 12 patients had ARs or SARs that were detected by the weekly monitoring with BSSERS-C, whereas the remaining ARs were reported spontaneously.

One other patient (0.5%) reported psychotic symptoms during treatment, and a thorough clinical evaluation established that intermittent psychotic symptoms had been present for several years before initiation of MPH treatment. The medication was discontinued, and the relapse of psychotic symptoms was classified as an adverse event. Furthermore, six patients (2.9%) were non-compliant/non-adherent and discontinued their treatment and one patient (0.5%) due to no symptom reduction.

ADHD core symptoms, conduct problems, daily and social functioning.

A total of 137 (73.2%) patients obtained Nor/Bnor on the Inattention subscale (ADHD-RS-C) at week 12 (Nor and Bnor separated in S5 Table). Of these patients, 111 (81.0%) were boys and 89 (65.0%) were 7 to 9 years of age. A total of 157 (84.0%) patients obtained Nor/Bnor on the Hyperactivity-Impulsivity subscale (ADHD-RS-C) at week 12. Of these patients, 115 (55.6%) were boys and 103 (65.6%) were 7 to 9 years of age. In summary, 19 (10.1%) patients were not Nor/Bnor on any of the subscales at week 12, 126 (67.4%) patients were Nor/Bnor on both subscales of ADHD-RS-C at week 12, and 42 (22.5%) patients were Nor/Bnor on either the Inattention or the Hyperactivity-Impulsivity subscale of ADHD-RS-C at week 12.

Changes in sum scores from week 0 to week 12 showed a mean significant reduction of severity of inattention symptoms and hyperactivity-impulsivity symptoms rated by clinicians, parents, and teachers (ADHD-RS-C, ADHD-RS-P, and ADHD-RS-T, Table 2). Reduction of conduct problems was statistically significant in parents' ratings ($p < 0.001$) but not in teachers' ratings ($p = 0.293$).

The mean percent reductions of scores on the clinician-rated ADHD-RS-C subscale from week 0 to 12 were 52.0% on Inattention and 56.0% on Hyperactivity-Impulsivity, and the mean percentage reductions on the parent-rated ADHD-RS-P subscales were 48.1% on Inattention, 45.0% on Hyperactivity-Impulsivity, and 50.7% on Conduct problems (S6 Table).

Patients significantly improved on the CGI-S scale from week 0 to week 12. A total of 67 (35.8%) patients were rated with a score of 1 or 2 (*normal to mildly ill*) on CGI-S after 12 weeks of treatment ($p < 0.001$, the mean difference between week 0 and 12 was 0.4 (SD 0.5, CI 95% 0.3 to 0.4). The mean score on CGI-S was 5.3 (SD 1.0) in week 0 and 3.0 (SD 1.1) after 12 weeks of treatment ($p < 0.001$, M difference 2.3, SD 1.0, CI 95% 2.2 to 2.5). A total of 171 patients (91.5%) had a score of 1 or 2 (*very much* or *much improved*) on CGI-I after 12 weeks of treatments. The mean score on CGI-I at week 12 was 1.8 (SD 0.6) (Table 2).

After 12 weeks of treatment, the patients' daily and social functioning were improved on WFIRS-P, which showed a significant reduction of symptoms of all six subscales from week 0 to week 12 (S7 Table). Mean subscale index scores of WFIRS-P at week 0 and 12 were ≤1, meaning that the overall level of functioning was mildly affected in each domain (range of index score 0-3). The largest mean reductions on the WFIRS-P were on the school subscale and the social life subscale with mean reductions of 0.4 (SD 0.4) and 0.4 (SD 0.5), respectively, and the smallest mean reductions on the subscales were on the daily life subscale and the risk behaviour subscale with mean reductions of 0.1 (SD 0.4) and 0.1 (SD 0.2). All reductions were statistically significant.

The four mean TOVA outcomes showed significant improvements in response time, variability of response time, omission errors, and commission errors ($n = 115$) from week 0 to week 12. Compared to week 0, at week 12 there were improvements in standard deviations of 1.0 on response time, 1.4 on variability of response time, 0.9 on omission errors, and 0.7 on commission errors (S7 Table). The mean time from midday IR-MPH dose to TOVA test was 108 minutes (range 9 to 193), and the mean midday dose was 0.42 mg IR-MPH per kg (SD 0.13) ($n = 106$) at week 12.

[Table 2 Clinical follow-up from week 0 to week 12 (*n* = 187)]

ADHD rating scales	Week 0 M (SD), *n* (%)	Week 12 M (SD), *n* (%)	Week 0 versus week 12			
			M dif. (SD)	95% CI	t (df)	*p*-value
Parent						
Inattention	17.7[1] (5.0)	9.2[1] (4.2)	8.2 (5.1)	(7.5, 9.0)	21.3(173)	< 0.001
Hyperactivity-Impulsivity	15.6[2] (6.0)	8.6[2] (4.7)	7.0 (5.7)	(6.2, 7.9)	16.2(170)	< 0.001
Inattention and Hyperactivity-Impulsivity	33.2[3] (9.4)	17.7[3] (7.9)	15.5 (9.4)	(14.1, 17.0)	21.1(162)	< 0.001
Conduct problems	10.7[4] (5.9)	5.3[4] (4.1)	5.4 (5.1)	(4.7, 6.2)	14.2(178)	< 0.001
Teacher						
Inattention	16.9[5] (4.7)	11.2[5] (5.1)	5.6 (5.1)	(4.7, 6.5)	12.4(126)	0.001
Hyperactivity-Impulsivity	9.1[6] (5.7)	7.7[6] (4.9)	1.4 (5.2)	(0.5, 2.3)	3.0(124)	0.004
Inattention and Hyperactivity-Impulsivity	25.9[7] (9.0)	18.6[7] (8.4)	7.2 (8.9)	(5.6, 8.9)	8.8 (117)	< 0.001
Conduct problems	4.8[8] (4.7)	4.4[8] (4.1)	0.4 (4.4)	(-0.4, 1.2)	1.1(127)	0.293
Clinician						
Inattention	19.9 (3.7)	9.6 (3.7)	10.3 (4.0)	(9.7, 10.9)	35.2(186)	< 0.001
Hyperactivity-Impulsivity	18.0 (5.8)	7.9 (4.0)	10.1 (5.1)	(9.4, 10.8)	27.3(186)	< 0.001
Inattention and Hyperactivity-Impulsivity	37.9 (7.8)	17.5 (6.8)	20.4 (7.7)	(19.3, 21.5)	36.5(186)	<0.001
CGI-S	5.3 (1.0)	3.0 (1.1)	2.3 (1.0)	(2.2, 2.5)	31.5(186)	< 0.001
CGI-S ≤ 2	0 (0.0%)	*n* = 67 (35.8%)	0.4 (0.5)	(0.3, 0.4)	10.2(186)	< 0.001
CGI-I	-	1.8 (0.6)	-	-	-	-
CGI-I ≤ 2	-	*n* = 171 (91.5%)	-	-	-	-
Weight, kg	36.3[9] (11.0)	35.4[9] (11.1)	0.9 (1.5)	(0.7, 1.15)	8.3(183)	< 0.001
Height, cm	140.1[9] (10.8)	141.3[9] (10.7)	-1.2 (1.1)	(-1.4, -1.0)	-14.8(183)	< 0.001
Diastolic blood pressure, mmHg	67.1[10] (7.1)	66.4[10] (7.4)	0.7 (9.4)	(-0.7, 2.0)	0.9(182)	0.344
Systolic blood pressure, mmHg	102.2[10] (8.9)	101.8[10] (9.7)	0.3 (9.9)	(-1.2, 1.7)	0.4(182)	0.693
Heart rate, bp/m	79.2[11] (10.9)	78.2[11] (11.9)	1.0 (15.1)	(-1.2, 3.3)	0.9 (180)	0.341
BSSERS-C	17.3[9] (10.5)	14.5[9] (9.0)	2.8 (8.6)	(1.5, -4.0)	4.4(183)	< 0.001
Reduced appetite	0.7[12] (1.3)	1.9[12] (1.5)	-1.2 (1.8)	(-1.5, 1.0)	-9.2(185)	< 0.001
Methylphenidate mg/kg/day	0.3 (0.1) (initial dose)	1.0 (0.3)	-0.7 (0.3)	(-0.7, -0.7)	-34.2(186)	< 0.001

Paired t-test between outcomes of week 0 and week 12. M = mean. M dif. = Mean difference. SD dif. = Standard deviation difference.
Missing data: [1] *n* = 174, [2] *n* = 171, [3] *n* = 163, [4] *n* = 179, [5] *n* = 127, [6] *n* = 125, [7] *n* = 118, [8] *n* = 128, [9] *n* = 184, [10] *n* = 183, *n* [11] = 181, [12] *n* = 186.
ADHD-Rating Scale (ADHD-RS, DuPaul). Inattention subscale: 9 items, [range 0-27]. Hyperactivity-Impulsivity subscale: 9 items [range 0-27]. Conduct problems subscale: 8 items, [range 0-24]. Rated by clinician, parent, or teacher.
Clinical Global Impression Severity (CGI-S): 1 item, [range 1-7]. CGI-S, 1 = *Not ill at all*. CGI-S, 2 = *Borderline ill*.
Clinical Global Impression Improvement (CGI-I): 1 item, [range 1-7]. CGI-I, 1 = *Very much improved*. CGI-I, 2 = *Much improved*.
Barkley's Stimulant Side Effects Rating Scale, clinician rated (BSSERS-C). Whole scale: 17 items, [range 0-153]. Reduced appetite: single item, [range 0-9].

Attrition during the study.

There were no differences between the patients who completed the study (*n* = 187) and those who discontinued IR-MPH treatment due to ARs (*n* = 12) at entry (week 0) in mean sum scores of Inattention and Hyperactivity-Impulsivity (ADHD-RS-C) (19.9 (SD 3.7) and 18.0 (SD 5.8) vs. 20.3 (SD 3.2) and 19.7 (SD 5.2), *p* = 0.742 and *p* = 0.315) and in mean sum score of ARs (BSSERS-C) (17.6 (SD 10.8) vs. 17.9 (SD 7.8), *p* = 0.928)

Adverse reactions.

Overall the mean sum problem score of the BSSERS-C rating scale significantly declined over 12 weeks of treatment (pre-post mean difference 2.8 (SD 8.6), $p < 0.001$) (Table 2, single-item ARs of BSSERS-C in S8 Table). Reduced appetite was the only BSSERS-C-rated AR problem score that increased significantly over time, whereas the other BSSERS-C-rated AR problem scores were either stable or decreased over time (e.g., the largest decrease on any single AR problem score was found for euphoria with a mean decrease of 0.7 (SD 1.4) $p < 0.001$, CI 95% 0.5 to 0.9). There was no significant increase in mean blood pressure, no change in the blood pressure percentiles (data not shown), and no change in heart rate from week 0 to week 12. There was a significant increase in children's height with a mean of 1.2 cm (SD 1.1) despite a mean loss of weight of 0.9 kg (SD 1.5).

Nonresponders.

Thirty-one patients (15.0%) were nonresponders; of these, 12 (5.8%) patients discontinued IR-MPH treatment because of ARs/SARs, and 19 patients (9.1%) were not Nor/Bnor on any of the ADHD-RS-C subscales. Responders ($n = 168$) and nonresponders did not differ with respect to age, sex, and comorbidity (S9 Table). Nonresponders were characterised by having more severe ADHD and global symptoms than responders at week 0 (mean sum scores of Hyperactivity-Impulsivity were 21.5 (SD 4.4) vs. 17.5 (SD 5.7), $p \leq 0.001$, and mean CGI-S scores were 5.9 (SD 0.8) vs. 5.2 (SD 0.9), $p \leq 0.001$), and a higher mean score of problems on BSSERS-C (21.3 (SD 11.3) vs. 17.0 (SD 10.4), $p = 0.034$) at week 0.

Modelling the response and the predictors of response during the first 12 weeks of treatment.

Course of Inattention.

Among the investigated clinical characteristics, sex, age, and time (number of weeks in treatment) were significantly associated with the course of inattention symptoms, rated on the Inattention subscale (ADHD-RS-C, range 0-27), when analysed in the linear mixed effect model (Table 3, graphs of Inattention Fig 2A). The estimated effects of time corresponded to a mean reduction of 0.8 of the Inattention sum score per week in subgroups of sex and age throughout the treatment period. Girls aged 7 to 9 years had a lower estimated mean score of Inattention symptoms in week 0 (score 17.5) than the group of girls aged 10 to 12 years (score 18.6) in week 0. Boys aged 7 to 9 years had a higher estimated mean score of Inattention symptoms in week 0 (score 19.4) than boys aged 10 to 12 years (score 18.2) in week 0. None of the investigated variables (age,

sex, intelligence level, conduct disorder, and comorbidity) had a significant interaction with time, and the differences in estimated mean scores in week 12 between sex and age were the same as in week 0.

Course of Hyperactivity-Impulsivity.

In the linear mixed effect model for repeated measures, only age and time (number of weeks in treatment) were significantly associated with the course of hyperactivity-impulsivity symptoms, rated on the Hyperactivity-Impulsivity subscale (ADHD-RS-C, range 0-27) (Table 3, graphs of Hyperactivity-Impulsivity Fig 2B). Patients aged 7 to 9 years had an estimated baseline score of 16.0 and an estimated decrease in symptoms of 0.8 per week. Patients aged 10 to 12 years had an estimated baseline score of 13.3 and estimated decrease in symptoms of 0.6 per week. In week 12, patients aged 7 to 9 years had a score of 6.8 and patients aged 10 to 12 years had an estimated score of 6.3 (i.e., the scores from the two age groups of patients ended up at about the same level).

[Table 3 Baseline characteristics as predictors for symptom reductions and adverse reactions (*n* = 207)]

Linear mixed model			
Inattention	**Estimate**	**95% CI**	***p*-value**
Intercept	18.21	(17.37, 19.05)	< 0.001
Week	-	-	< 0.001
Week	-0.77	(-0.81, -0.72)	< 0.001
Age	-	-	0.062
10-12 years (ref.)	-	-	-
7-9 years	0.38	(-0.66, 1.30)	0.521
Sex	-	-	0.794
Boys (ref.)	-	-	-
Girls	1.38	(-0.19, 2.94)	0.084
Age*Sex	-	-	0.013
Boys*10-12 years	-	-	-
Boys*7-9 years	-	-	-
Girls*10-12 years	-	-	-
Girls*7-9 years	-2.50	(-4.46, -0.54)	0.013
Hyperactivity-Impulsivity	**Estimate**	**95% CI**	***p*-value**
Intercept	13.30	(12.13, 14.47)	< 0.001
Week	-	-	< 0.001
Week	-0.58	(-0.66, -0.50)	< 0.001
Age	-	-	< 0.001
10-12 years (ref.)	-	-	-
7-9 years	2.65	(1.19, 4.11)	< 0.001
Week*age	-	-	< 0.001
Week*10-12 years (ref.)	-	-	-
Week*7-9 years	-0.18	(-0.28, -0.08)	< 0.001
Adverse reactions	**Estimate**	**95% CI**	***p*-value**
Intercept	21.66	(19.84, 23.49)	< 0.001
Week	-	-	< 0.001
Week	-0.28	(-0.37, -0.19)	< 0.001
CGI-S, week 0	-	-	< 0.001
CGI-S, 6-7 (ref)	-	-	-
CGI-S, 5	-4.53	(-7.00, -2.06)	< 0.001
CGI-S, 4	-4.30	(-7.77, -0.84)	0.015
CGI-S, 1-3	-8.25	(-14.15, -2.35)	0.006
General Estimating Equation			
Reduced appetite	**Estimate**	**95% CI**	***p*-value**
Intercept	1.90	(1.44, 2.37)	< 0.001
Week	-	-	< 0.001
Week	-0.04	(-0.08, 0.00)	0.069
CGI-S, week 0	-	-	< 0.001
CGI-S 6-7 (ref)	-	-	-
CGI-S 1-5	0.88	(0.35, 1.41)	< 0.001

Linear mixed model for repeated measures of sum scores of Inattention, Hyperactivity-Impulsivity, and adverse reactions. ADHD-Rating Scale, clinician rated. (ADHD-RS-C, DuPaul). Inattention: 9 items, [range 0-27]. Hyperactivity-Impulsivity: 9 items, [range 0-27]. Barkley's Stimulant Side Effects Rating Scale, clinician rated (BSSERS-C). Whole scale: 17 items, [range 0-153]. Only significant values ($p < 0.05$) of the explanatory variables as main factors and/or interactions are listed in the table. Explanatory variables; continuous variable: time (defined as 13 weeks from week 0 to 12) and categorical variables: sex, age, Clinical Global Impression Severity (CGI-S) in week 0. CGI-S, [range 1-7], divided into four groups: CGI-S 1-3 = *not ill at all, borderline ill, mildly ill*, CGI-S 4 = *moderately ill*, CGI-S 5 = *markedly ill*, CGI-S 6-7 = *severely ill, among the most extreme ill*.
General Estimating Equation for repeated measures of reduced appetite. BSSERS-C, single item, reduced appetite, [range 0-9]. No or mild reduced appetite 0-3, moderate to server reduced appetite 4-9. Only significant values ($p < 0.05$) of the explanatory variables as main factors and/or interactions are listed in the table. Explanatory variables; continuous variable: time (defined as 13 weeks from week 0 to 12) and categorical variables: CGI-S in week 0 divided into CGI-S 1-5 and CGI-S 6-7.

Fig 2

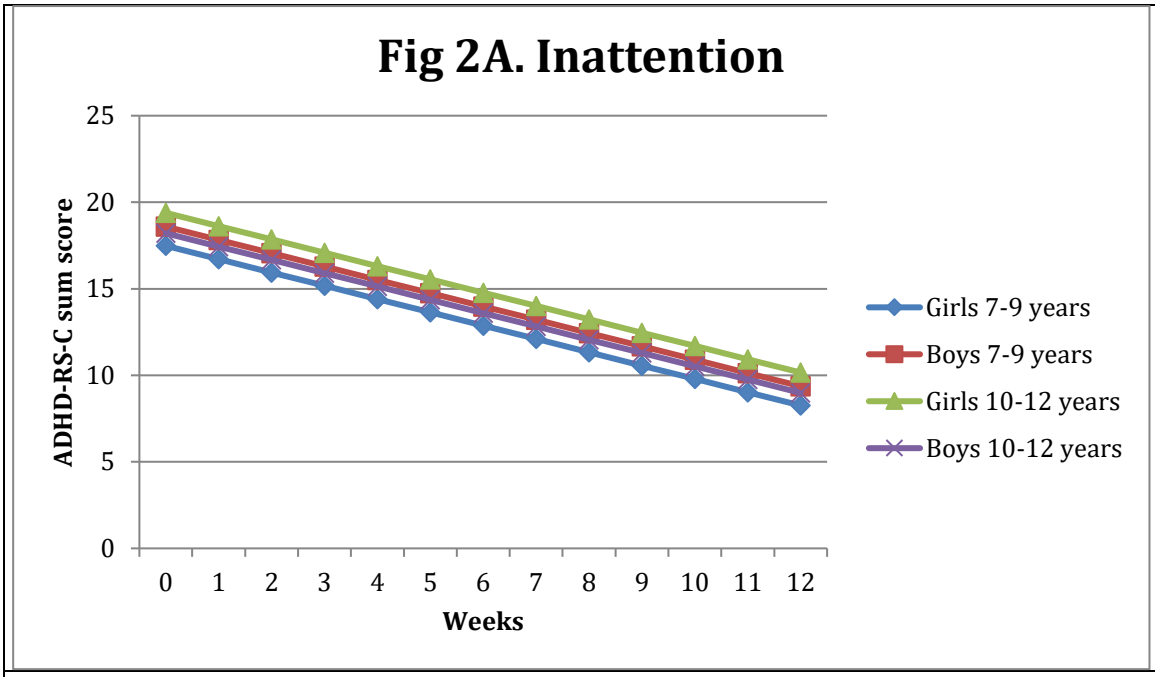


Fig 2A. Inattention
ADHD-RS-C sum score
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Weeks
Girls 7-9 years
Boys 7-9 years
Girls 10-12 years
Boys 10-12 years

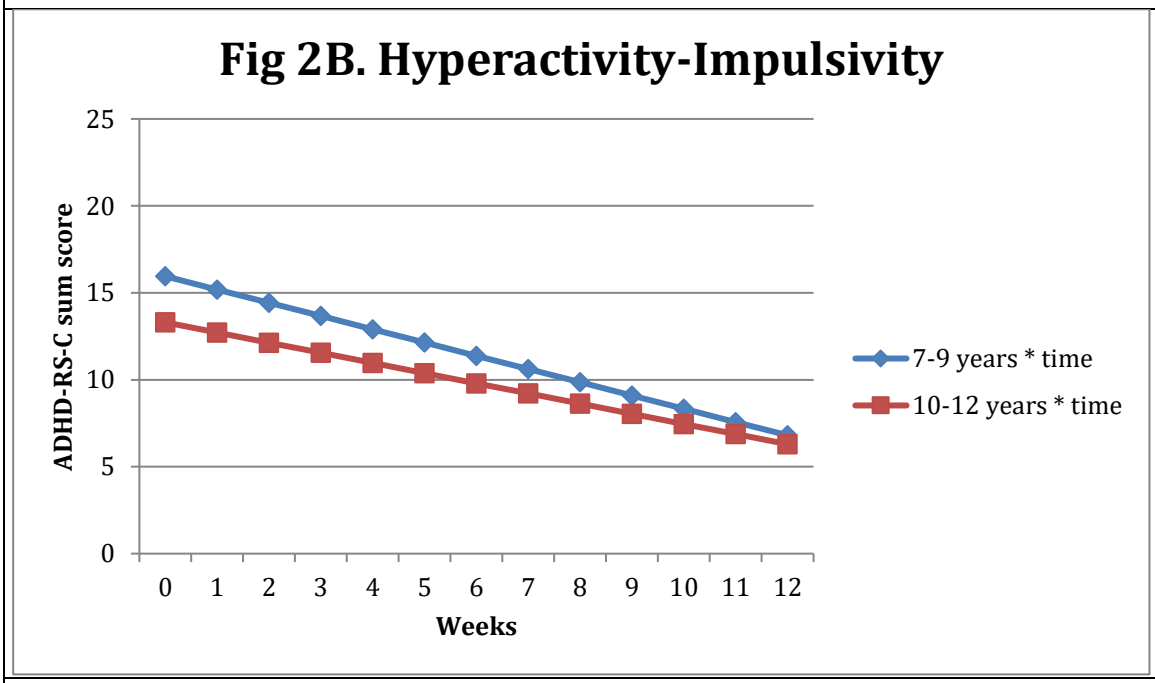


Fig 2B. Hyperactivity-Impulsivity
ADHD-RS-C sum score
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Weeks
7-9 years * time
10-12 years * time

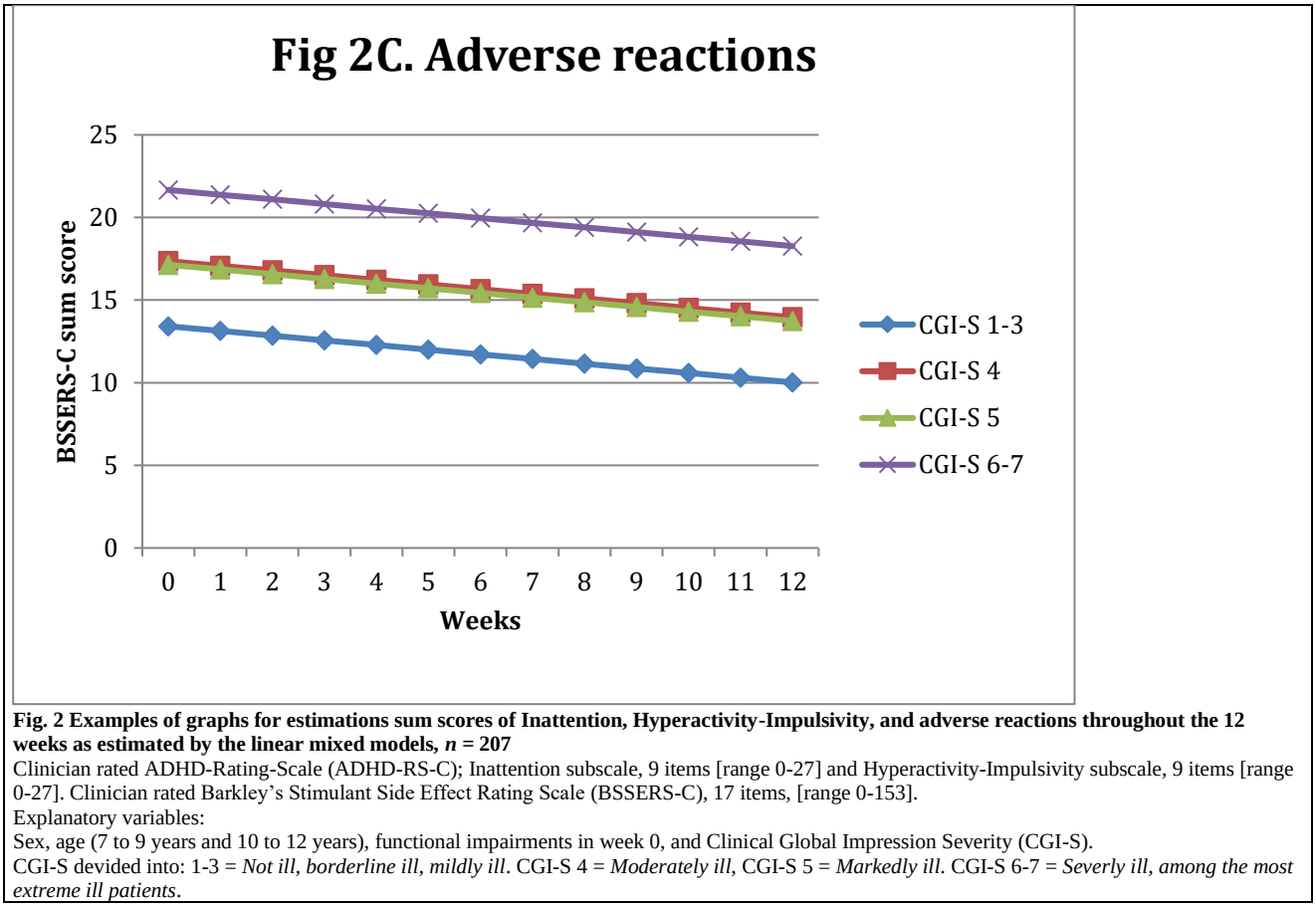


Fig. 2 Examples of graphs for estimations sum scores of Inattention, Hyperactivity-Impulsivity, and adverse reactions throughout the 12 weeks as estimated by the linear mixed models, *n* = 207
Clinician rated ADHD-Rating-Scale (ADHD-RS-C); Inattention subscale, 9 items [range 0-27] and Hyperactivity-Impulsivity subscale, 9 items [range 0-27]. Clinician rated Barkley's Stimulant Side Effect Rating Scale (BSSERS-C), 17 items, [range 0-153].
Explanatory variables:
Sex, age (7 to 9 years and 10 to 12 years), functional impairments in week 0, and Clinical Global Impression Severity (CGI-S).
CGI-S devided into: 1-3 = *Not ill, borderline ill, mildly ill*. CGI-S 4 = *Moderately ill*, CGI-S 5 = *Markedly ill*. CGI-S 6-7 = *Severly ill, among the most extreme ill patients*.

[**Fig. 2 Examples of graphs for estimations sum scores of Inattention, Hyperactivity-Impulsivity, and adverse reactions throughout the 12 weeks as estimated by the linear mixed models, *n* = 207**
Clinician rated ADHD-Rating-Scale (ADHD-RS-C); Inattention subscale, 9 items [range 0-27] and Hyperactivity-Impulsivity subscale, 9 items [range 0-27]. Clinician rated Barkley's Stimulant Side Effect Rating Scale (BSSERS-C), 17 items, [range 0-153].
Explanatory variables:
Sex, age (7 to 9 years and 10 to 12 years), functional impairments in week 0, and Clinical Global Impression Severity (CGI-S).
CGI-S devided into: 1-3 = *Not ill, borderline ill, mildly ill*. CGI-S 4 = *Moderately ill*, CGI-S 5 = *Markedly ill*. CGI-S 6-7 = *Severly ill, among the most extreme ill patients*.]

Normalisation or borderline normalisation of the clinician-rated ADHD core symptoms.

Female sex (HR = 0.64, CI 95% 0.54 to 0.75) and a diagnosis of *inferioritas intellectualis* (HR = 0.74, CI 95% 0.63 to 0.86) were significant predictors of a lower chance of Nor/Bnor on the Inattention subscale (ADHD-RS-C) (Table 4).

Young age (7-9 years) (HR = 0.88, CI 95% 0.71 to 0.96) and *inferioritas intellectualis* (HR = 0.82, CI 95% 0.78 to 0.99) significantly predicted lower chance of Nor/Bnor on the Hyperactivity-Impulsivity subscale (ADHD-RS-C). Comorbidity (HR 1.17, CI 95% 1.02 to 1.35) significantly improved the chance of Nor/Bnor on the Hyperactivity-Impulsivity subscale (ADHD-

RS-C) (Table 10). Post hoc analysis did not show any significant differences in sum scores of Inattention or Hyperactivity-Impulsivity subscale (ADHD-RS-C) in week 0 between patients with none or one psychiatric comorbid diagnosis and those with two or more psychiatric comorbid diagnoses.

[Table 4 Baseline characteristics as predictors for the chance of normalisation or borderline normalisation of the clinician rated ADHD core symptoms score (*n* = 207)]

Inattention	**HR**	**CI 95%**	***p*-value**
Sex (girls)	0.64	(0.54, 0.75)	< 0.001
Inferioritas intellectuals	0.74	(0.63, 0.86)	< 0.001
Hyperactivity-Impulsivity	**HR**	**CI 95%**	***p*-value**
Age (7-9 years)	0.88	(0.71, 0.96)	0.031
Inferioritas intellectuals	0.82	(0.78, 0.99)	0.011
Comorbidity	1.17	(1.02, 1.35)	0.030

Cox regressions of time to first normalisation (zero standard deviation) or borderline normalisation (one standard deviation) of ADHD core symptoms due to Danish norms of sex and age on ADHD-Rating Scale, clinician rated (ADHD-RS-C, DuPaul). Inattention subscale: 9 items, range 0-27. Hyperactivity-Impulsivity subscale: 9 items, range 0-27. Only last model of cox regression with backward elimination are listed in the table. HR = Hazard ratio. Independent predictors: Sex, age, comorbidity (≤ 2 comorbid diagnosis), and intelligence level (*inferioritas intellectuals*, ICD10-R41.8) from the diagnostic conference and/or from the intelligence test (WISC) (IQ 70-85) depending on data access)

Adverse reactions.

The mean reduction in the BSSERS-C problem score was 0.3 per week. Among the investigated clinical characteristics, only CGI-S measured in week 0 and time (number of weeks in treatment) were significantly associated with the course of ARs in the linear mixed effect model for repeated measures (BSSERS-C, range 0-153) (Table 3, graphs of ARs Fig 2C). Patients rated *severely ill* and *most extremely ill* (CGI-S 6-7) in week 0 had a more severe BSSERS-C problem score (mean score 21.7) before initiation of treatment than patients with a lower-rated CGI-S score in week 0. Patients who were rated *moderately ill* (CGI-S 5) at baseline had an estimated mean BSSERS-C problem score of 17.2, patients rated *markedly ill* on CGI-S (CGI-S 4) had an estimated mean BSSERS-C problem score of 17.4, and patients rated *not ill at all, borderline ill, or mildly ill* (CGI-S 1-3) had an estimated mean BSSERS-C problem score of 13.4 before initiation of treatment. None of the investigated variables (age, sex, intelligence level, conduct disorder, comorbidity, time, ADHD diagnosis, and CGI-S in week 0) had a significant interaction with time.

Reduced appetite.

Of the investigated AR items on BSSERS-C, reduced appetite was the only item that significantly increased in problem score over the 12-week treatment period. Reduced appetite was post hoc defined as a problem score of 4-9 on BSSERS-C. Among the investigated clinical characteristics in week 0, only CGI-S was significantly associated with the course of reduced appetite in the model of GEE for repeated measures (BSSERS-C, single item, 0-3 = no reduced appetite, 4-9 = reduced appetite) (Table

3). *Severely ill* and *most extremely ill* rated patients (CGI-S 6-7) had a significantly higher risk of reduced appetite compared with patients rated not ill to moderately ill (CGI-S 1-5) in week 0 and throughout the whole treatment period (OR = 2.41, CI 95% 1.42 to 4.08). The probability of reduced appetite in week 0 was 13.0% for patients rated severely ill and most extremely ill and 5.8% for patients not ill to *moderately ill*. The proportion of patients with reduced appetite did not change significantly over time (weeks, $p = 0.069$).

Baseline characteristics as predictors for end-dose of IR-MPH.

The mean end-dose of IR-MPH was 1.0 mg/kg/day (SD 0.3, Table 2). Higher CGI-S scores at week 0 predicted a higher end-dose of IR-MPH. CGI-S at week 0 and age explained together 12.7% ($p > 0.001$) of the variance of the end-dose IR-MPH. The estimated effect of being "among the most extremely ill patients" in week 0 (CGI-S 7) corresponded to an end-dose of IR-MPH 1.19 mg/kg/day, whereas being rated "mildly ill" in week 0 (CGI-S 3) corresponded to an end-dose of IR-MPH 0.85 mg/kg/day (Table 5). Young age predicted a lower end-dose of IR-MPH. Patients aged 7 to 9 years were estimated to receive a 0.13 mg/kg/day lower end-dose of IR-MPH than patients aged 10-12 years.

[Table 5 Baseline characteristics as predictors for end-dose of IR-MPH ($n = 187$)]

Predictors for end-dose of IR-MPH dose/mg/kg/day						
	Estimate	**SD Error**	**Beta**	**t**	***p*-value**	**CI95 % of estimate**
Constant	0.56	0.12		4.84	< 0.001	(0.33, 0.79)
CGI-S, week 0	0.09	0.02	0.29	4.32	< 0.001	(0.05, 0.13)
Age (7-9 years)	-0.13	0.04	-0.16	-3.05	0.003	(-0.21, -0.05)

Linear regression for end-dose of Immediate Release Methylphenidate (IR-MPH).
Explaining variables are measured in week 0. Clinical Global Impression Severity (CGI-S) in week 0, [range 1-7].

Discussion

This study is a prospective, uncontrolled, ecologically valid study aimed at exploring the course and outcome of a group of patients treated with IR-MPH treatment in a routine child and adolescent clinic in a real-world setting[27]. This heterogeneous group of patients with substantial psychiatric comorbidity, including autism spectrum disorders and *inferioritas intellectualis*, were offered an individually titrated optimal dosing of IR-MPH based on weekly assessments of ADHD core symptoms and ARs. Children with ADHD displayed statistically and clinically significant improvements of these core symptoms across informants, global level of symptoms, and parent-reported functional impairments of daily life functioning. The clinical findings were supported by an objective performance based on the patients' sustained attention by TOVA, which also showed significant improvements after 12 weeks of treatment with IR-MPH. Thirty-one of the children were nonresponders, and compared with the responders, they were characterised by more severe symptoms of hyperactivity-

impulsivity and global impairment before initiation of treatment. Interestingly, the mental and physical complaint, measured as potential ARs, scored highest before initiation of treatment and dropped significantly during the up-titration of IR-MPH, suggesting that the complaints reflected general health and mental health problems which were related to the ADHD illness rather than ARs to IR-MPH treatment. However, two types of well-known ARs to IR-MPH (reduced appetite and weight loss) increased during the period of IR-MPH treatment. To our knowledge this is the first naturalistic observational study that monitored ARs weekly in a 12-week period.

Results were mixed regarding potential predictors of outcome of the individually titrated IR-MPH-treatment, suggesting no need to modify the dosing of IR-MPH according to age, gender, IQ between 70 and 85, or psychiatric comorbidity. Although the chance of Nor/Bnor was reduced for children with *inferioritas intellectualis* and increased for children with psychiatric comorbidity in the Cox regression analyses, this pattern was not confirmed in the more powerful mixed models of the course of symptoms.

The individually titrated doses varied (range 0.3-2.0 mg/kg/day), and higher levels of clinical global impairments before initiation of treatment predicted higher end-doses, whereas being between 7 and 9 years old was associated with slightly lower end-doses. The mean end-dose of 1.0 mg/kg/day was in the higher end of the clinical recommendations.

The pattern of higher parent-rated ADHD core symptoms and, at the same time, lower teacher-rated ADHD core symptoms at entry was seen among included children for whom the parents accepted IR-MPH-treatment, and the opposite pattern was seen among children excluded due to parents' refusals of IR-MPH-treatment. This is a strong reminder that parents' conceptions of child ADHD core symptoms may impact the clinical decision to offer IR-MPH treatment of children.

Outcomes of MPH treatment vary in the literature, which may reflect definitions of response criterions used, the choice of ADHD rating scales, the length of observation period and the type of informants. Compared to a systematic review of efficacy trials (stimulant medication) reporting symptom reductions in the range of 10 to 18 in absolute scores[21], this study found a mean symptom reduction of 20.4 (SD 7.7) on the ADHD-RS-C, suggesting clinical effects within the same range as reported in efficacy trials. Direct comparisons of the present findings and other naturalistic observational studies with MPH naïve patients are only possible by comparing scores on the CGI-S and CGI-I (response rates defined as CGI-I ≤ 2 and CGI-S ≤ 2 at end of study). Kim *et al.*[30] found a higher CGI-S response rate (46.1%) after 12 weeks of treatment compared to the response rate in the present study (35.8%). Whereas two other studies found lower CGI-I response rates; 79.1% after 8 weeks of treatment[39] and 49.9% after 24 weeks of treatment[33] than the response rate of 91.5% in the present study (S2 Table and Table 2). This could indicate that, the present study included very ill patients, but the patients still had high response rates of MPH after 12 weeks of treatments compared to other studies.

The present study found a significant improvement of the level of daily and social functioning (total mean score of WFIRS-P) though the overall level of functioning in daily life rated by parents, was only mildly affected at study entry[58].

In line with findings in RCTs[61,62] and naturalistic observational studies[33,31], we found significant improvements of attention on all TOVA parameters. The variability of response time exhibited the largest improvement, indicating higher stability of attention during the whole test period.

Contrary to expectations, problem scores rated by the clinicians as potential ARs (BSSERS-C) were rated highest before medication and decreased during the treatment period (see S6 Table for single items), with the exception of appetite (BSSERS-C) along with a decrease in weight during treatment. The 17-item rating scale of BSSERS has been validated in a triple-blind placebo-controlled crossover study (n = 83), in which parents and teachers rated ARs of MPH in children with ADHD[47]. BSSERS was found useful to measure reduced appetite, insomnia, headache, and stomachache (rated by parents), and staring, sadness, and anxiety (rated by teachers) during the treatment period, but the same ARs were also reported at lower levels during the placebo periods.

Many of the AR items rated on BSSERS-C may not be true ARs associated to IR-MPH but may reflect symptoms of ADHD or psychiatric comorbidity[71]. This may challenge the frequent use of BSSERS as a valid instrument for assessment of ARs associated with stimulant treatment[23,31,32,37,46]. Furthermore, only half of the patients who dropped out due to ARs experienced ARs detected on BSSERS-C in this study. There are few other validated AR rating scales, such as the Pittsburgh Side-Effects Rating Scale[72] and Subject's Treatment Emergent Symptom Scale[73], which are not very frequently used in research. When studies systematically use the ARs ratings scales, very few ARs increases during treatment with MPH and even decreases of "ARs" are found (*irritability*, *proneness to cry*, *anxiety*, *nail biting*, *euphoria*, and *sadness*)[71,74,75]. But the literature search revealed a general lack of systematic reporting of ARs, and several studies limited AR assessments to spontaneous reports. Reduced appetite and insomnia are the most frequent reported ARs in RCTs[23,76] and in naturalistic observational studies[31,32,37]. The present study had a low discontinuation rate due to ARs (5.8%). Similar rates are found in other naturalistic observational studies (8.0% in[29] and 4.5% in[31]). Our findings are in line with a recent Cochrane review of non-randomized studies of children (5-18 years) with ADHD treated with MPH, which identified a discontinuations rate of 1.2% due to SARs and a discontinuations rate of 6.2% due ARs[77]. The discontinuation rates were low despite of relative high frequencies of reported ARs (insomnia (17.9%), headache (14.4%), abdominal pain (10.7%), and reduced appetite (6.2%)). In the present study, there was no increase in mean blood pressure during IR-MPH-treatment, which is in line with findings from another naturalistic observational study[34]. However, two of our patients discontinued treatment due to

hypertension. Overall, the patients who completed the 12 weeks of treatment in the present study seemed to report fewer clinically significant ARs than patients in other naturalistic observational studies[31,37] (S2 Table).

Children aged 7-9 years had a lower chance of normalisation or borderline normalisation of hyperactivity-impulsivity symptoms (Nor/Bnor of ADHD-RS-C) after 12 weeks of treatment than children aged 10-12 years, even though, children aged 7-9 years had a higher sum score of hyperactivity-impulsivity symptoms (ADHD-RS-C) in week 0 and had a higher weekly reduction of symptoms than children aged 10-12 years. In both cases, the differences were minor and hence of limited clinical significance. Also, the differential reduction of ADHD symptoms was explained by symptom severity differences at entry, whereas the end scores were similar for the two age groups.

The subgroup of girls aged 7-9 years had a lower sum score of inattention symptoms (ADHD-RS-C) than girls of age 10-12 years throughout the treatment period, but the weekly reduction of inattention symptoms was the same regardless of age and gender. The contradicting finding that girls had a lower chance of normalisation or borderline normalisation of inattention symptoms (ADHD-RS-C) compared to boys might be an artefact related to the gender specific cut-offs for normalisation based on a study conducted 10 years ago[53]. This point toward a need for new *age and gender* specific norms for ADHD core symptom scores.

IQ above 85 versus IQ below or equal to 85 but above 70 (*inferioritas intellectualis*) was statistically significantly associated with a better chance of Nor/Bnor of the inattention and hyperactivity-impulsivity symptoms, whereas the weekly reduction of sum scores of inattention and of hyperactivity-impulsivity showed no differences between IQ groups. These seemingly mixed results might indicate equally beneficial effects in both groups even though the chance of normalization is reduced when IQ is reduced.

Having two or more comorbid psychiatric diagnoses predicted a better chance of normalisation or borderline normalisation of hyperactivity-impulsivity symptoms (Nor/Bnor of ADHD-RS-C), but this finding contrasted with the statistical modelling of the course of hyperactivity-impulsivity symptoms, which showed no significant association with comorbidity.

Results from the Multimodal Treatment Attention Deficit Hyperactivity Disorder (MTA) study[78] showed that patients with an intelligence level above the population mean (IQ 100) had a better response to MPH treatment than patients with lower IQ. Other RCTs also found a normal IQ range is a predictor for a good treatment outcome[79–81], which also was found in one naturalistic observational study[82] but not in another[83]. Many different baseline characteristics have been sporadically identified as predictors for good treatment response (and sometimes opposite directed) in RCTs[78,81] and naturalistic observational studies[28,83]. The most consistent result of a predictor for a good treatment outcome might be intelligence within in the normal to upper range.

It is well known that ARs of MPH are related to high doses of MPH as observed in RCTs[74,84] and in a naturalistic observational study[32], but a review of 10 RCTs did not find the same association[76]. Severe global impairment (CGI-S 6-7) in week 0 predicted a higher mean problem score of ARs (BSSERS-C) throughout the whole treatment period including week 0, and severe global impairment (CGI-S 6-7) in week 0 was associated with a high end-dose of IR-MPH. Taken together, these findings indicate that the more severely ill patients with more severe symptoms mimicking ARs before initiation of treatment end up needing higher end-doses of MPH. It is important to notice that the ARs measured by the BSSERS-C may be 'influenced' by the global impairment level (CGI-S) of a patient. In this study, CGI-S proved to be a useful psychometric instrument, which confirmed its wide applicability in the psychiatry for measuring global impairment.

Strength and limitations

The main strength of the study was the inclusion of a consecutive clinical and representative sample of children in their first medical treatment for ADHD in a 'real-life setting'. Moreover, the children were recruited from a well-defined catchment area, and the diagnosis of ADHD was based on best practice and routine examination using standardised instruments[27]. The included children represent a typical population of children with ADHD and one or several comorbid mental disorders, and with moderate to severe global impairment[85].

The clinical investigator performed all the weekly assessments of the same group of patients throughout the 12-week study period and monitored the IR-MPH treatment effects jointly with the clinician. Furthermore, the outcome measures of ADHD core symptoms were based on consensus ratings of all psychometric instruments in week 0 and 12. The intensive monitoring of symptom reductions, ARs, and the careful titration of IR-MPH probably explains the low attrition rate.

The limitation of the study was the lack of an untreated control group. Therefore, the symptom reductions of ADHD core symptoms and ARs due to IR-MPH treatment cannot be differentiated from the natural course of ADHD with a regression toward the mean and determination of the effect of the frequent contact with the clinical investigator. It can also be difficult to define a relevant cut-off for Nor or Bnor of ADHD core symptoms, and information might be lost when continuous variables (the ADHD sum scores) are dichotomised into cut-off values[86].

The study determined Nor and Bnor based on Inattention and Hyperactivity-Impulsivity of ADHD-RS-C using the existing Danish norms and cut-off values for ADHD-RS-P instead of the foreign norms and values for ADHD-RS-C[53,60]. The WFIRS-P and TOVA are widely used but have not been validated in Denmark. The raw scores of TOVA were converted to *z*-scores based on week 0 total raw scores. BSSERS has no instructions for how to rate each item from 0 (*problem absent*) to 9 (*problem evokes serious impairment*)[47]. To overcome this, the authors of this study created a manual for ratings of the 17

items of BSSERS-C. Furthermore, the discriminative validity of BSSERS-C with regard to various mental and physical health problems and ARs are not known, and BSSERS-C has not been validated in Denmark.

Perspectives for the daily clinic

This study showed no causal relationship between baseline characteristics and symptom reductions or ARs of IR-MPH. Most patients continued treatment throughout the 12-weeks. The treatment outcome showed a favourable balance between symptom reductions and ARs. This is assuring compared to the recent debate of the efficacy of MPH[87,88]. Our study also emphasizes that the clinicians should obtain baseline information about severity of ADHD symptoms and potential ARs, and they should continuously monitor these during up-titration of MPH using standardised rating scales. The BSSERS proved ineffective because it may not differentiate between symptoms and ARs, and there is an urgent need for development of a better instrument to monitor ARs of MPH in clinical practice. The parents seemed to be more aware of the severity of the patients' ADHD core symptoms than the teachers. Patients with severe global levels of symptoms and functional impairments appear to report more severe symptoms mimicking ARs before initiation of treatment and during the treatment period.

Research perspectives

Future studies should investigate the importance of thorough individually titrated dosing of MPH, monitored on standard rating scales of ADHD core symptoms and ARs, and the effect of frequent brief contact with a clinician (e.g., a nurse) during the titration period. This will be important knowledge for the standard treatment of ADHD.
This study suggests an alignment of ways to measure and define a clinical response to MPH on a standard ADHD-rating scale and more valid methods to monitor ARs.

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Supporting information of Paper I

S1 Fig.

S1 Table. Criteria for literature search.

S2 Table. Included studies in the review of naturalistic observational clinical prospective studies of MPH treatment of drug naïve children with ADHD.

S3 Table. Comparison of ADHD core symptoms severity between included and excluded patients.

S4 Table. Baseline characteristics of included patients.

S5 Table. Distribution of number of patients with normalisation and borderline normalisation of clinician rated ADHD core symptoms score in week 0 and 12.

S6 Table. Absolute mean reduction score of ADHD core symptoms.

S7 Table. Further clinical follow-up from week 0 to week 12.

S8 Table. Changes of adverse reactions.

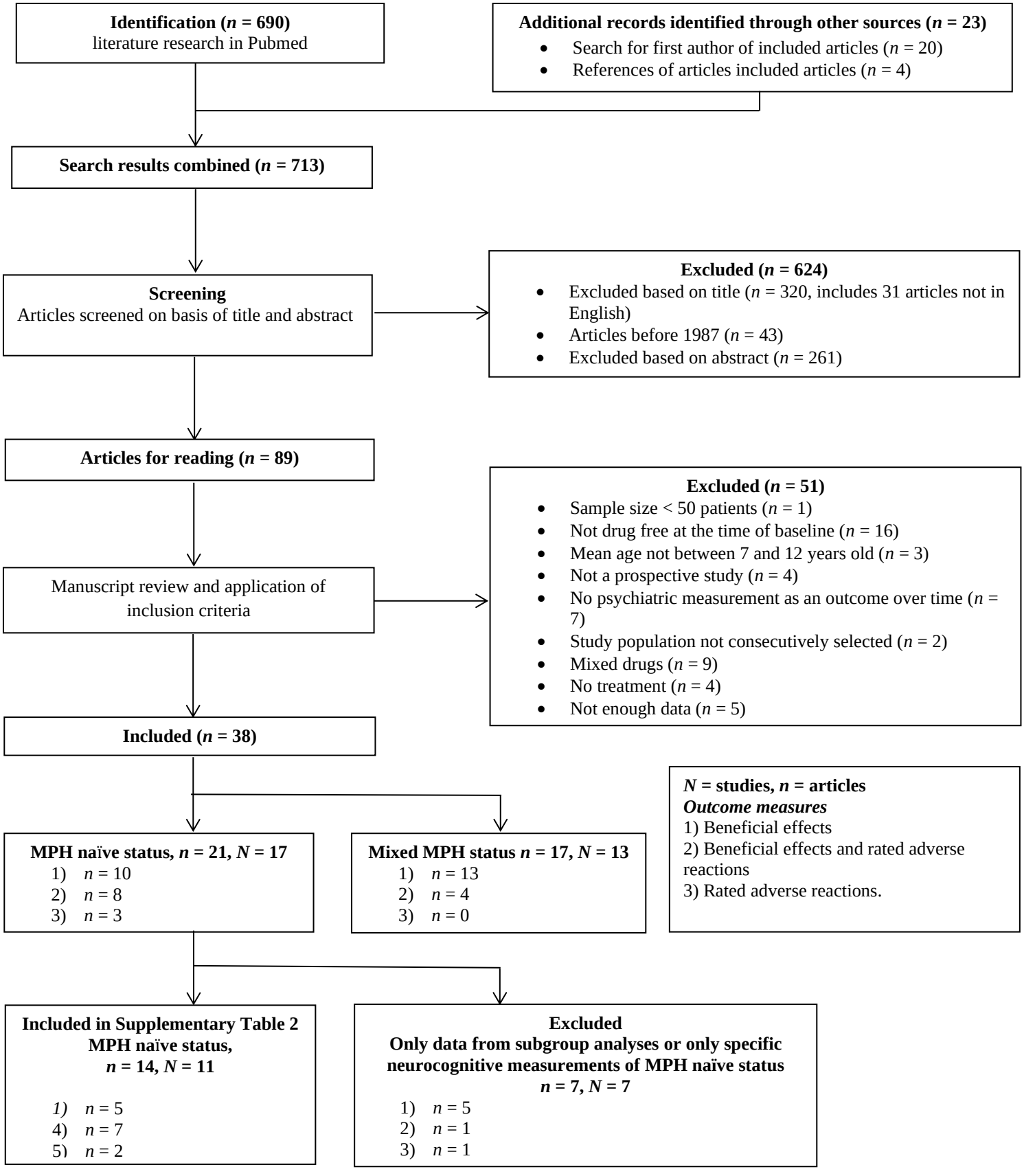
S9 Table. Baseline characteristics and symptoms in week 0 of responder and nonresponder.

S10 Protocol INDICES

S11 Protocol INDICES work package 6.

Supporting information, paper I

S1 Fig. PRISMA flow chart of naturalistic observational clinical prospective studies of methylphenidate treatment of children with ADHD



S1 Table. Criteria for literature search

Inclusion criteria for articles:

1) Sample size above 50 patients. No upper limit.
2) Mean or median age between 7 and 12 years old.
3) ADHD/ADD diagnosed as *DSM*-IIIR, *DSM*-IV, *DSM*-5 or ICD-10
4) Prospective longitudinal cohort studies with a psychiatric measurement as an outcome over time.
5) Treatment with methylphenidate as tablets. A group of patients should be treated only with MPH and the MPH should reported separately.
6) Consecutively selected patients from child and adolescent, pediatric departments or from a specific geographic area.
7) A group of patients should be methylphenidate-free[1] at baseline.

Exclusion criteria for articles:

1) Epidemiological studies using register-based data for prescriptions and/or diagnosis.
2) Only subgroups of patients with ADHD and a specific comorbidity.
3) Studies which only measure treatment outcomes after switching of medication.
4) Reviews, case reports and randomized controlled trials.
5) Articles before 1987.

DSM = Diagnostic and Statistical Manual of Mental Disorders, ICD-10 = International Classification of Diseases and Related Health Problems
[1] = No methylphenidate treatment at the time of inclusion

S2 Table. Included studies in the review of naturalistic observational clinical prospective studies of MPH treatment of drug naïve children with ADHD

Studies	Total included, *N* Boys, *n* (%)	Mean age (SD)	ADHD subtype *DSM*-IV *N* (%)	Follow up after initiation of MPH, weeks	End-dose of MPH mg/kg/day (SD)	Response definitions	Response rates	Calculated absolute mean reduction score of ADHD core symptoms in %
1: *Kim B-L et al.* 2011(1) *	*N* = 102 *n* = 82 (80.4)	8.7 (2.1)	27 ADHD-I (26.4%) 6 ADHD-HI (5.8%) 69 ADHD-C (67.6%)	2, 4 8, 12	0.98 (0.50)	1: Sum score ≤ 18 and single item score ≤ 1 on ADHD-RS-C (1-18), and ≤ 2 on CGI-I 2: ≤ 2 on CGI-S 3: ≥ 50% decrease on ADHD-RS-C (1-18)	1: (*n* = 80) 78.4% 2: (*n* = 47) 46.1% 3: (*n* = 22) 21.6%	ADHD-RS-C (1-18); 62.7%
2: *Lee et al.* 2011(2) *	*N* = 137 Completed, *N* = 112 *n* = 93 (83.0)	*N* = 112 10.2 (2.9)	21 ADHD-I (18.8%) 1 ADHD-HI (0.9%) 90 ADHD-C (80.4%)	1, 2, 4, 8	Responder *n* = 76: 0.85 (0.27) (67.9%) Non-responder *n* = 36: 0.86 (0.30) (32.1%), *n* = 112: 0.85	≥ 50% decrease on ADHD-RS-P (1-18) and ≤ 2 on CGI-I	(*n* = 76) 67.9%	ADHD-RS-P (1-18); 38.5%
3: *Johnson et al.* 2013(3) *	*N* = 77 *n* = 66 (85.7)	8.0 (2.6)	7 ADHD-I (9%), 7 ADHD-HI (9%), 63 ADHD-C (82%).	6	0.57 (0.19) 0.54 (0.20) (IR-MPH)	No definition of response to MPH (CPRS-R:S ADHD index T-score)		CPRS-R:S; 23.8%
4: *Garcia et al.* 2009(4)	*N* = 280 *n* = 211 (75.4)	ADHD+ANX: *N* = 76: 9.9 (3.0) ADHD-ANX: *N* = 204: 10.2 (3.0) ANX=Anxiety *n* = 280: 10.0	No information about ADHD sub-types.	4	ADHD+ANX *n* = 76 (27.1%): 0.45 (0.14). ADHD-ANX, *n* = 204 (72.9%): 0.52 (0.16), *n* = 280: 0.50	≥ 50% decrease on SNAP-IV	(*n* = 88) 31.4%	Not possible
5: *Paton et al.* 2014(5)	*N* = 51 *n* = 43 (84.3)	8.4 (2.4)	4 ADHD-I (8%) 3 ADHD-HI (6%) 44 ADHD-C (86%)	6	0.58 (0.18)	No definition of response to MPH		CPRS-R:S; 24.4%
6: *Song et al.* 2014(6) *	*N* = 139 *n* = 120 (86.3)	10.0 (2.8)	28 ADHD-I (20.1%) 3 ADHD-HI (2.2%) 102 ADHD-C (73.9%) 6 ADHD-NOS (4.3%)	8	31.0 (9.0) mg/day No weight available.	1: ≥ 50% decrease on ADHD-RS-P (1-18) 2: ≤ 2 on CGI-I 3: ≥ 50% decrease on ADHD-RS-P (1-18) and ≤ 2 on CGI-I	1: (*n* = 99) 71.2% 2: (*n* = 110) 79.1% 3: (*n* = 90) 64.7%	Not possible
7: *Kim JI et al.* 2016(7) *	*N* = 75 *n* = 64 (85.3)	8.8 (2.2)	24 ADHD-I (32%) 3 ADHD-HI (4%) 39 ADHD-C (52%) 9 ADHD-NOS (12%)	(2), (4), (6), 8, 16, 24. (limited data of these weeks)	28.1 (12.3) mg/day No weight available	1: ≥ 40% decrease on ADHD-RS-P (1-18) 2: ≤ 2 on CGI-I 3: ≥ 40% decrease on ADHD-RS-P (1-18) and ≤ 2 on CGI-I.	1: (*n* = 38) 50.7% 2: (*n* = 37) 49.3% 3: (*n* = 19) 25.3%	ADHD-RS-P (1-18); 41.8%
8: *Park et al.* 2013(8) *Kim J-W et al* 2013(9)	*N* = 132 *n* = 108 (81.8)	8.8 (1.5)	N = 131 (99.2%) 39 ADHD-I (29.5%) 6 ADHD-HI (4.5%) 78 ADHD-C (59.1%) 8 ADHD-NOS (6.1%)	2, 4, 8, 12	1.00 (0.28)	1: ≥ 50% decrease on ADHD-RS-P (1-18) and no response on TOVA 2: ≤ 15% decrease on mean composite score of TOVA and in 1 3: No response in 1 and 2 4: Response in 1 and 2. 1: ≤ 18 ADHD-RS-C (1-18) and ≤ 2 CGI-I	1: (*n* = 29) 24.4% 2: (*n* = 18) 15.1% 3: (*n* = 43) 36.1% 4: (*n* = 29) 24.4% 1: (*n* = 101) 76.5%	Not possible ADHD-RS-C (1-18): 62.2% ADHD-RS-C (1-9): 59.3% ADHD-RS-C (10-18): 66.0% ADHD-RS-P (1-18): 43.9% ADHD-RS-P (1-9): 41.4% ADHD-RS-P (10-18): 47.3%

9 *Pagerols et al.* 2018(10) * *Pagerols et al.* 2017(11) *	*N* = 173 *n* = 147 (84.9) *N* = 107 *n* = 95 (88.8)	9.6 (2.9) 9.4 (2.8)	37 ADHD-I (21.4%) 5 ADHD-HI (2.9%) 131 ADHD-C (75.7%) 22 ADHD-I (20.6 %) 6 ADHD-HI (5.6 %) 79 ADHD-C (74.0 %)	8	1.06 (0.28) 1.07 (0.30)	1: ≤ 2 on CGI-I 1: ≤ 2 on CGI-I	1: (n =141) (81.5 %) 1: (n = 84) (78.5 %)	No possible
Study						**ARs definition**	**Adverse effect rates**	
1: *Cho et al.* 2012(12)* (*Kim et al.* 2011(1) *)	-	-	-	-	-	No definition	No significant change in blood pressure (BP), heart rate (HR) or electrocardiography. BP changed < 0.1mmHg and HR changed < 4 bpm.	
2: *Lee et al.* 2011(12–15)(2) *	-	-	-	-	-	No definition	Lack of effect; 0.7% (*n* = 1) Intolerable ARs; 8.0% (*n* = 11) Refusal of MPH treatment; 0.7% (*n* = 1) Not attending scheduled clinic visit; 8.8% (*n* = 12)	
3: *Johnson et al.* 2013(3) *	-	-	-	-		BSSERS-C	Reduced appetite 66% (*n* = 50), 0.6 mg/kg/day (0.2) Weight loss 34% (*n* = 26), 0.7 mg/kg/day (0.2) Headache or abdominal pain; 50% (*n* = 38), irritability; 46% (*n* = 35), sadness; 38% (*n* = 29), insomnia (new onset); 33% (*n* = 25), insomnia (exacerbation); 30% (*n* = 23), tics (new onset); 3% (*n* = 2), tics (exacerbation); 7% (*n* = 5)	
8: *Kim J-W et al* 2013(9) (*Park et al*. 2013(8))	-	-	-	-	-	BSSERS-C Not whole scale represented	Any ARs; 75.0% (n = 99) Dropped out due to ARs: 4.5% (*n* = 6): insomnia (*n* = 2), reduced appetite (*n* = 1), tics (*n* = 1), rash (*n* = 1), tremor (*n* = 1). Reduced appetite; 58.3% (*n* = 77), insomnia; 43.9% (*n* = 58), abdominal pain; 28.0% (*n* = 37), Anxiety; 27.3% (*n* = 36), headache; 23.5% (*n* = 31), uninterested; 22.0% (*n* = 29), nail biting; 17.4% (*n* = 23), somnolence; 15.9% (*n* = 21), incommunicative; 15.2% (*n* = 20), daydream; 14.4% (*n* = 19), nausea; 12.1% (*n* = 16), nightmare; 9.8% (*n* = 13), dizziness; 8.3% (*n* = 11) Mean (SD) significant decrease: BMI Z-score of 0.3 (1.1), weight 0.9 kg (1.9) Mean (SD) significant increase: pulse rate 2.8 bpm (10.8) No significant difference: systolic and diastolic blood pressure.	
10: *Bruxel et al.* 2013(13) *	*N* = 213 Completed; *N* = 205 *n* = 151 (73.6)	*N* = 129: 10.4 (2.9) *N* = 76: 10.0 (3.1) *n* = 205: 10.0	N = 205 57 ADHD-I (27.8%) 14 ADHD-HI (6.8%) 117 ADHD-C (57.1%) 17 ADHD-NOS (8.3%)	4, 12		Low score, BSSERS-P = 0-5. High score, BSSERS-P = 6-9.	FU appetite low score BSSERS-P; 79.7% (*n* = 94) FU appetite high score BSSERS-P; 21.3% (*n* = 24)	
11: *Kim et al.* 2013(14) *	*N* = 147 Completed; *N* = 134 *n* = 112 (83.6)	*N* = 134 10.3 (2.9)	23 ADHD-I (17.2%) 2 ADHD-HI (1.5%) 102 ADHD-C (76.1%) 7 ADHD-NOS (5.2%)	1, 2, 4	0.86 (0.30)	BSSERS-P	1 ≥ ARs; 82.8% (*n* = 111) Mean BSSERS-P; 11.3 (SD 14.1) Reduced appetite; 56.7% (*n* = 76) Trouble sleeping; 41.8% (*n* = 56) Talking little with others; 22.4% (*n* = 30) Irritability; 20.9% (*n* = 28)	
9: *Pagerols et al.* 2017(11) *	-	-	-	-	-	BSSERS-C	Any ARs 65.1% (*n* = 69); insomnia 34.3% (*n* = 37), reduced appetite 25.0 % (*n* = 27).	
Total ***Mean*** ***Range***	**1537** **139.7** **(51-280)**	**102.8** **9.3** **(8.0-10.3)**		**104** **9.5** **(4-24)**	**0.80**** **(0.50-1.06)**			

* = Pharmacogenetic studies. ** = Mean dose per kg per day based on 8 studies. ARs = Adverse reactions. FU = Follow-Up.
Only data from *Pagerols et al.* 2018(10) is included in the calculation of included patients and mean of age and end-dose.
ADHD-RS (DuPaul) Single item [range 0-3]. ADHD-RS (1-9) = Inattention subscale [range 0-27], ADHD-RS (10-18) = hyperactivity-impulsivity subscale [range 0-27)], ADHD-RS (1-18) = Inattention and hyperactivity-impulsivity [range 0-54].
ADHD-RS-C = clinician rated, ADHD-RS-P = parents rated, ADHD-RS-T = teacher rated.
CGI-I: Clinical Global Impression Improvement [range 1-7], CGI-S: Clinical Global Impression Severity [range 1-7].
CPRS-R:S: Connors Parent Rating Scale Short version. 27 items, oppositional, cognitive problems/inattention hyperactivity (18 items), ADHD index score (9 items + 3 items from the 18 items) in mixed order. Single item [range 0-3]. Converted to ADHD index T-scores in week 0 and at the time of FU.
SNAP-IV: ADHD Rating scale, Swanson Nolan Pelham. 90 items. 18-20 items of ADHD. SNAP-IV 1-9 = inattention subscale, SNAP-IV 10 = summarize inattention, SNAP-IV 11-19 = hyperactivity-impulsivity subscale, SNAP-IV 20 = summarize hyperactivity-impulsivity. Single item [range 0-3].
BSSERS: Barkley's Stimulant Side Effect Rating Scale. BSSERS-P =parent rated, BSSERS-C = clinician rated.
TOVA: Test of Variables of Attention, Composite score = Mean score of the four domains: Response time, variability of response time, omission errors, and commission errors.
ADHD *DSM-IV*:

- 314.01 Combined subtype = ADHD-comb subtype = ADHD-C subtype
- 314.01 Predominantly hyperactive-impulsive subtype = ADHD-HI subtype
- 314.00 Predominantly inattentive subtype = ADHD-I subtype
- 314.9 Attention-Deficit Hyperactivity Disorder Not Otherwise Specified = ADHD-NOS

Calculated absolute mean reduction score = (sum score of ADHD core symptoms in week 0 - sum score of ADHD core symptoms at time of FU) / sum score of ADHD core symptoms in week 0 in %.

S3 Table. Comparison of ADHD core symptoms severity between included and excluded patients

ADHD symptoms before inclusion	Included (*n* = 207) M (SD)	Excluded (*n* = 211) M (SD)	Included versus excluded			Refused medicine (*n* = 95) M (SD)	Included versus refused medicine		
			M dif. (SD dif.)	95% CI	*p*-value		M dif. (SD dif.)	95% CI	*p*-value
Parent									
Inattention	17.4[1] (5.05)	15.1[7] (5.6)	2.3 (0.6)	(1.3, 3.4)	< 0.001	14.6[12] (5.3)	2.8 (0.7)	(1.5, 4.1)	< 0.001
Hyperactivity-Impulsivity	15.3[2] (6.1)	12.5[7] (6.0)	2.9 (0.6)	(1.6, 4.1)	< 0.001	10.9[13] (5.3)	4.5 (0.8)	(3.0, 6.0)	< 0.001
Conduct problems	10.5[3] (5.9)	8.1[8] (5.5)	2.4 (0.6)	(1.3, 3.6)	< 0.001	7.5[14] (5.1)	3.0 (0.7)	(1.6, 4.5)	< 0.001
Teacher									
Inattention	17.1[4] (4.9)	18.5[9] (5.1)	-1.4 (0.5)	(-2.4, -0.3)	0.010	18.2[15] (4.6)	-1.1 (0.6)	(-2.4, 0.1)	0.081
Hyperactivity-Impulsivity	9.0[5] (5.7)	15.1[10] (7.2)	-6.0 (0.7)	(-7.4, -4.7)	< 0.001	14.3[16] (7.3)	5.2 (0.8)	(3.6, 6.8)	< 0.001
Conduct problems	4.5[6] (4.6)	9.0[11] (6.5)	-4.4 (0.6)	(-5.6, -3.3)	< 0.001	8.2[17] (6.4)	-3.6 (0.7)	(-5.0, 2.3)	< 0.001

Independent t-test: Comparison between included vs. excluded patients and comparison between included and patients excluded due to parent's decision of refusing medicine (a subgroup of excluded patients)
M = Mean. M dif. = Mean difference. SD dif. = Standard deviation difference.
ADHD-Rating Scale (DuPaul). Inattention subscale: 9 items [range 0-27]. Hyperactivity-Impulsivity subscale: 9 items [range 0-27]. Conduct problems subscale: 8 items [range 0-24]. Rated by parent (ADHD-RS-P) or teacher (ADHD-RS-T).
[1] *n* = 200, [2] *n* = 197, [3] *n* = 204, [4] *n* = 187, [5] *n* = 191, [6] *n* = 193, [7] *n* = 174, [8] *n* = 181, [9] *n* = 170, [10] *n* = 173, [11] *n* = 177, [12] *n* = 78, [13] *n* = 80, [14] *n* = 84, [15] *n* = 78, [16] *n* = 77, [17] *n* = 79.

S4 Table. Baseline characteristics of included patients (*n* = 207)

Parents civil status	***n* (%)**
- Parents divorced	96 (46.4)
Maternal education	***n* (%)**
- Primary school (10 years)	24 (11.6)
- Short, higher education (11-14 years)	73 (35.3)
- Medium, higher education (15-14 years)	77 (37.2)
- Long, higher education (17-19 years)	26 (12.6)
- Unknown	7 (3.4)
Paternal education	***n* (%)**
- Primary school (10 years)	35 (16.9)
- Short, higher education (11-14 years)	47 (22.7)
- Medium, higher education (15-14 years)	65 (31.4)
- Long, higher education (17-19 years)	38 (18.4)
- Unknown	22 (10.6)
Ethnicity of parents	***n* (%)**
- Both parents of Nordic origin	190 (91.8)
- Both parents of non-Nordic origin	3 (1.4)
- One parent of Nordic origin, one parent of non-Nordic origin	14 (6.8)
Adopted patients	***n* (%)**
- Adopted	5 (2.4)
Child Behaviour Check List. Parent rated.	**M (SD)**
- CBCL externalizing score	17.9[1] (10.7)
- CBCL internalizing score	11.4[1] (7.6)
- **CBCL total problem score**	**56.0[1] (26.2)**
- **CBCL total T-score**	**79.6[1] (19.1)**
Teacher Report Form. Teacher rated.	**M (SD)**
- TRF externalizing score	23.8[2] (13.9)
- TRF internalizing score	9.6[2] (6.8)
- **TRF total problem score**	**68.7[2] (29.0)**
- **TRF total T-score**	**80.6[2] (18.1)**
WFIRS-P. Parent rated.	**M (SD)**
- WFIRS-P, Family	1.0[3] (0.7)
- WFIRS-P, School	1.0[3] (0.5)
- WFIRS-P, Daily life	0.8[4] (0.5)
- WFIRS-P, Self-perception	1.0[4] (0.8)
- WFIRS-P, Social life	1.0[4] (0.7)
- WFIRS-P, Risk behaviour	0.4[4] (0.3)
- WFIRS-P, total	0.8[4] (0.4)

N = number, M = mean, SD = Standard deviation. Missing data: [1] *n* = 147, [2] *n* = 143, [3] *n* = 195, [4] *n* = 193.
Child Behaviour Check List (CBCL). Parent rated. Externalizing score: 35 items [range 0-70]. Internalizing score: 32 items [range 0-64]. Total problem score: 118 [range 0-236]. Teacher Report Form (TRF). Teacher rated. Externalizing score, 34 items [range 0-68]. Internalizing score: 34 items [range 0-68]. Total problem score: 118 [range 0-236]. Weis Functional Impairment Rating Scale, parent version (W-FIRS-P). Family, school daily life and risk behaviour: 10 items [range 0-30]. Self-perfection: 3 items [range 0-9]. Social life: 7 items [range 0-21]. Each subscale of WFIRS-P is divided by the number of items.

S5 Table. Distribution of number of patients with normalisation and borderline normalisation of clinician rated ADHD core symptoms score in week 0 and 12 (*n* = 187)

Inattention subscale					
		Week 0	**Week 12 Normalisation** *n (%)*	**Week 12 Borderline normalisation** *n (%)*	**Week 12 No normalisation or borderline normalisation** *n (%)*
Normalisation	*n (%)*	**1** (0.5%)	**1**	**0**	**0**
Borderline normalisation	*n (%)*	**3** (1.6%)	**2**	**1**	**0**
No normalisation or borderline normalisation	*n (%)*	**183** (97.9%)	**67**	**66**	**50**
Total week 12	*n (%)*	**187** (100%)	**70** (37.4%)	**67** (35.8%)	**50** (26.7%)
IR-MPH, mg/kg/day, week 12	M (SD)		**1.0** (0.3)	**1.0** [1] (0.3)	**1.1**[2] (0.3)
Hyperactivity-Impulsivity subscale					
		Week 0	**Week 12 Normalisation** *n (%)*	**Week 12 Borderline normalisation** *n (%)*	**Week 12 No normalisation or borderline normalisation** *n (%)*
Normalisation	*n (%)*	**10** (5.3%)	**10**	**0**	**0**
Borderline normalisation	*n (%)*	**17** (9.1%)	**17**	**0**	**0**
No normalisation or borderline normalisation	*n (%)*	**160** (85.6%)	**68**	**62**	**30**
Total week 12	*n (%)*	**187** (100%)	**95** (50.8%)	**62** (33.2%)	**30** (16.0%)
IR-MPH, mg/kg/day week 12	M(SD)		**1.0** (0.3)	**1.0**[3] (0.3)	**1.1** (0.3)

N = number, M = mean. SD = Standard deviation. Missing data: [1] *n* = 66, [2] *n* = 48, [3] *n* = 59.
ADHD-Rating Scale, clinician rated (ADHD-RS-C, DuPaul). Inattention subscale: 9 items [range 0-27]. Hyperactivity-impulsivity subscale: 9 items [range 0-27]. Normalisation (*t-score* ≤ 60), borderline normalisation (*t-score* ≤ 70), no normalisation or borderline normalisation of ADHD cores symptoms (ADHD-RS) due to Danish norms of sex and age.
In the article normalisation (Nor) and borderline normalisation (Bnor) on ADHD-RS-C are multiplied to Nor/Bnor = *n* (%). Number of patients who were Nor/Bnor on the Inattention subscale = 137 (73.2%) and number of patients who were Nor/Bnor on the Hyperactivity-Impulsivity subscale = 157 (84.0%).

S6 Table. Absolute mean reduction score of ADHD core symptoms (*n* = 187)

Mean reduction score of ADHD core symptoms from week 0 to week 12	
Clinician rated	**M in % (SD)**
Inattention	51.7% (17.3)
Hyperactivity-Impulsivity	53.0% (32.0)
Inattention and Hyperactivity-Impulsivity	53.6% (15.7)
Parent rated	
Inattention	45.3%[1] (26.8)
Hyperactivity-Impulsivity	39.9%[2] (51.6)
Inattention and Hyperactivity-Impulsivity	45.2%[3] (24.3)
Conduct problems	43.2%[4] (52.2)
Teacher rated	
Inattention	31.8%[5] (28.1)
Hyperactivity-Impulsivity	[6]
Inattention and Hyperactivity-Impulsivity	[6]
Conduct problems	[6]

Missing data: [1] *n* = 174, [2] *n* = 171, [3] *n* = 163, [4] *n* = 179, [5] *n* = 127, [6] = Too many single items were missing/negative sum scores to calculate. M = mean. SD = Standard deviation.
ADHD-Rating Scale (ADHD-RS, DuPaul). Clinician rated. Inattention subscale: 9 items, range 0-27. Hyperactivity-Impulsivity subscale: 9 items [range 0-27]. Clinician rated (ADHD-RS-C), parent rated (ADHD-RS-P), and teacher rated (ADHD-RS-T).
Absolute mean reduction score = (sum score of ADHD core symptoms in week 0 - sum score of ADHD core symptoms in week 12) / sum score of ADHD core symptoms in week 0 in %.

S7 Table. Further clinical follow-ups from week 0 to week 12 (*n* = 187)

	Week 0 M (SD)	**Week 12** M (SD)	**Week 0 versus week 12**			
			M dif. (SD)	**95% CI**	**t (df)**	***p*-value**
WFIRS-P						
Family	1.0[1] (0.7)	0.7[1] (0.5)	0.3 (0.5)	(0.2, 0.3)	6.6(167)	< 0.000
School	1.0[1] (0.4)	0.6[1] (0.3)	0.4 (0.4)	(0.3, 0.4)	11.5(167)	< 0.000
Daily life	0.8[2] (0.5)	0.7[2] (0.4)	0.1 (0.4)	(0.0, 0.1)	2.1(161)	0.039
Self-perception	1.0[2] (0.8)	0.7[2] (0.7)	0.3 (0.7)	(0.2, 0.4)	5.4(161)	< 0.001
Social life	1.0[2] (0.7)	0.7[2] (0.5)	0.4 (0.5)	(0.3, 0.4)	8.9(161)	< 0.001
Risk behavior	0.4[2] (0.3)	0.2[2] (0.2)	0.1 (0.2)	(0.1, 0.2)	7.4(161)	< 0.001
total	0.8[2] (0.4)	0.6[2] (0.3)	0.2 (0.3)	(0.2, 0.3)	9.5(161)	< 0.001
TOVA						
RT, Raw score	521.3[3](113.1)	423.9[3] (89.6)	97.3 (89.8)	(80.7, 113.9)	11.6(114)	< 0.001
VRT, Raw score	240.2[3] (87.4)	159.2[3] (93.0)	81.0 (76.6)	(66.9, 95.2)	11.3(114)	< 0.001
OE, Raw score	47.8[3] (58.6)	12.3[3] (22.0)	35.6 (55.0)	(25.4, 45.7)	6.9(114)	< 0.001
CE, Raw score	39.4[3] (26.0)	26.1[3] (20.3)	13.3 (24.1)	(8.85, 17.8)	5.9(114)	< 0.001
RT, Z-score	0.0[3] (1.0)	-1.0[3] (1.0)	-1.0 (0.9)	(-1.1, -0.8)	-11.9(114)	< 0.001
VRT, Z-score	0.0[3] (1.0)	-1.4[3] (1.3)	-1.4 (1.0)	(-1.5, -1.2)	-14.3(114)	< 0.001
OE, Z-score	0.0[3] (1.0)	-0.9[3] (0.7)	-0.9 (0.9)	(-1.1, -0.8)	-11.6(114)	< 0.001
CE, Z-score	0.0[3] (1.0)	-0.7[3] (1.03)	-0.7 (1.0)	(0.8, -0.5)	-7.1(114)	< 0.000

Paired t-test between outcomes of week 0 and week 12. M = mean. M dif. = Mean difference. SD dif. = Standard deviation difference.
[1] *n* = 168, [2] *n* = 163, [3] *n* = 115.
Weiss Functional Impairment Rating Scale, parent version (W-FIRS-P). Family, school daily life, and risk behaviour: 10 items, [range 0-30]. Self-perfection: 3 items, [range 0-9]. Social life: 7 items, [range 0-21]. Each subscale of WFIRS-P is divided by the number of items.
Computerized Test of Variables of Attention (TOVA). Four domains: Response time (RT), variability of response time (VRT), omission errors (OE), and commission errors (CE).

S8 Table. Follow-up of adverse reactions (*n* = 187)

BSSERS-C	Week 0 M (SD)	Week 12 M (SD)	Week 0 versus week 12			
			M dif., (SD)	95% CI	t(df)	*p*-value
Insomnia	1.8 (2.2)	1.9 (1.9)	0.0 (2.4)	(-0.4, 0.3)	-0.3(186)	0.782
Nightmares	0.5 (1.1)	0.1 (0.5)	0.4 (1.2)	(0.2, 0.5)	4.2(186)	< 0.001
Staring	0.9 (1.5)	0.5 (1.0)	0.4 (1.3)	(0.3, 0.6)	4.6(186)	< 0.001
Talks less	0.4 (1.2)	0.4 (0.9)	0.0 (1.0)	(-0.1, 0.2)	0.3(186)	0.711
Disinterested in others	0.4 (1.1)	0.3 (0.9)	0.1 (1.0)	(-0.1, 0.2)	1.1(186)	0.257
Reduced appetite	0.7 (1.3)[1]	1.9 (1.5)[1]	-1.2 (1.8)	(-1.5, -1.0)	-9.2(185)	< 0.001
Irritable	3.4 (2.5)	2.9 (2.3)	0.4 (2.2)	(0.1, 0.8)	2.8(186)	0.005
Stomachaches	0.5 (1.1)	0.3 (0.8)	0.3 (1.1)	(0.1, 0.4)	3.2(186)	0.002
Headaches	0.4 (1.1)	0.2 (0.6)	0.3 (1.2)	(0.1, 0.4)	2.9(186)	0.005
Drowsiness	0.6 (1.0)	0.5 (1.0)	0.2 (1.2)	(0.0, 0.3)	1.9(186)	0.053
Sadness	1.1 (1.6)	0.7 (1.2)	0.4 (1.8)	(0.2, 0.7)	3.4(186)	0.001
Prone crying	1.0 (1.7)	0.6 (1.1)	0.4 (1.8)	(0.1, 0.6)	2.9(186)	0.004
Anxious	0.8 (1.5)[1]	0.7 (1.4)[1]	0.1 (1.2)	(-0.1, 0.3)	1.3(185)	0.203
Nail biting	3.5 (4.0)	3.0 (3.7)	0.5 (2.5)	(0.1, 0.9)	2. 8(186)	0.006
Euphoria	1.0 (1.5)	0.4 (0.8)	0.7 (1.4)	(0.5, 0.9)	6.5(186)	< 0.001
Dizziness	0.0 (0.2)	0.1 (0.3)	0.0 (0.3)	(-0.1, 0.0)	-1.3(186)	0.202
Tics/nervous movements	0.5 (1.3)[1]	0.4 (1.2)[1]	0.1 (1.3)	(-0.1, -0.3)	1.1(185)	0.256

Paired t-test between adverse reactions (ARs) of week 0 and week 12. M = mean. M dif. = Mean difference. SD dif. = Standard deviation difference.
Missing data: [1]n = 186.
Barkley's Stimulant Side Effect Rating Scale, clinician rated (BSSERS-C), 17 items, single item [range 0-9].
Irritable symptoms are measured during the day where methylphenidate did not have effect.

S9 Table. Baseline characteristics and symptoms in week 0 of responder and nonresponder (*n* = 199)

	Responder *n* = 168	Nonresponder *n* = 31	Responder versus nonresponder			
			X^2 (df)		*p*-value	
Boys	125 (74.4)	25 (80.6)	0.5(1)		0.459	
Age (10-12 years)	59 (35.1)	9 (29.0)	0.4(1)		0.511	
Comorbidity ≥ 2 diagnoses	45 (26.8)	9 (29.0)	0.1(1)		0.796	
Cognitive deficits	44 (26.2)	9 (29.0)	0.1(1)		0.742	
Conduct disorder	17 (10.1)	5 (16.1)	1.0(1)		0.327	
Clinician rated symptoms in week 0	**M (SD)**	**M (SD)**	**M dif. (SD error)**	**95% CI**	**t(df)**	***p*-value**
Inattention	19.7 (3.7)	20.8 (3.3)	-1.1 (0.7)	(2.6, 0.3)	-1.5(197)	0.127
Hyperactivity-Impulsivity	17.5 (5.7)	21.5 (4.4)	-4.0 (1.1)	(-6.1, -1.8)	-3.7(197)	< 0.001
CGI-S	5.2 (0.9)	5.9 (0.8)	-0.65 (0.2)	(-1.0, -0.3)	-3.6(197)	< 0.001
BSSERS-C	17.0 (10.4)	21.3 (11.3)	-4.3 (2.1)	(-8.4, -0.2)	-2.1(197)	0.038
Reduced appetite	0.6 (1.2)	1.0 (1.8)	-0.4 (0.2)	(-0.9, 0.1)	-1.6(197)	0.114

Paired t-test and X^2 (chi-squared) between responder and nonresponder. M = mean. M dif. = Mean difference. SD dif. = Standard deviation difference.
ADHD-Rating Scale, clinician rated (ADHD-RS-C, DuPaul). Inattention subscale: 9 items [range 0-27]. Hyperactivity-Impulsivity subscale: 9 items [range 0-27]. Normalisation (zero standard deviation), borderline normalisation (one standard deviation), no normalisation or borderline normalisation (two standard deviations) of ADHD cores symptoms (ADHD-RS) due to Danish norms of sex and age.
Nonresponder defined by no normalisation or borderline normalisation of any subscales of ADHD-RS-C in week 12 or by discontinuation of treatment due to adverse reactions.
Age between 7 and 9 years old or between 10 and 12 years old. Cognitive deficits: *Inferioritas intellectualis* (DR 41.8) from the diagnostic conference and/or from the intelligence test (WISC) (IQ 70-85) depending on data access or no cognitive deficits (IQ ≤ 86). Conduct disorder: *Hyperkinetic conduct disorder, conduct disorders, mixed disorders of conduct and emotions* (DF 90.1, DF 91.X, DF 92.X) or no conduct disorder.
Clinical Global Impression Severity (CGI-S) week 0: 1 item [range 1-7]. Barkley's Stimulant Side Effects Rating Scale, clinician rated (BSSERS-C). Whole scale (17 items) [range 0-153]. Reduced appetite (single item) [range 0-9].

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Paper II

1) Manuscript
2) Tables and figures of paper II
3) Supplementary material of paper II

Title:

Association of carboxylesterase 1 gene variants with 12-week outcome of methylphenidate treatment of children with ADHD.

Running title:

Carboxylesterase 1 gene variants associated to methylphenidate treatment outcome.

Abstract

Methylphenidate (MPH) is the first line pharmacological treatment in children with Attention-Deficit/Hyperactivity Disorder (ADHD). Beneficial effects, adverse reactions, and daily dose show huge individual variations of possible genetic origin. Associations between seven single-nucleotide-polymorphisms and one copy-number-variation (CNV) of carboxylesterase 1 (*CES1*) and the 12-week immediate release-MPH (IR-MPH) treatment outcome in 189 MPH naïve children aged 7-12 years were analyzed with univariate and multivariate statistical analyses with adjustments for clinical covariates. Lower end-dose of IR-MPH were associated with rs3815583 (GT/GG) and rs2307240 (GA) compared to the wild types. Higher response rates (normalization of Inattention and/or Hyperactivity-Impulsivity) were associated with rs2302722 (GT/TT), rs111604615 (CC), and re2307240 (GA) compared to the wild types. The CNV *CES1A2* was only weakly associated to lower end-dose of IR-MPH and reduced appetite. Common variants of *CES1* may moderate dosing and response of IR-MPH in children with ADHD. This study supports an individualized treatment informed by genetic variants.

Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder with an estimated worldwide mean prevalence of 3.4% in children of the general population making it the most frequent neurodevelopmental disorder in children and adolescents[1]. In Denmark, the cumulated incidence rates of diagnosed ADHD in childhood is 3% for girls and 6% for boys[2]. Methylphenidate (MPH) is the first-line pharmacological treatment of ADHD in children in many countries[3] and prescription of MPH has increased significantly the last decades[4–6]. The increased use of MPH combined with large individual variation in effect and tolerance suggests a need for studies of the underlying pharmacogenetic mechanisms to pave the way for personalized medicine with MPH[7–13].

The pharmacodynamic effects of MPH are not completely understood. It is generally agreed that MPH enhances the dopamine neurotransmission in the brain by both dopamine transporter dependent and independent mechanisms[14]. Pharmacogenetic studies have focused on associations between the genes encoding the dopamine or the norepinephrine transporters[15] and treatment response to MPH, but findings are inconsistent[12,16–21].

The pharmacokinetics determine the treatment of a drug through the organism and contribute to the treatment outcome. The rate of metabolism plays a major role in determining the pharmacokinetics of a drug. MPH is primarily metabolized by carboxylesterase 1 (CES1), which is responsible for the first-pass metabolism of MPH in the liver[22] and hydrolyses MPH to the inactive metabolite ritalinic acid[23].

CES1 appears to play a role in the response to MPH . Individuals with genetic variations of *CES1* can have a *slow metabolism* of MPH with higher risk of adverse reactions (ARs) and will need a lower dose of MPH[25,26].

Only a limited number of studies have identified associations between variants of *CES1* and response to MPH[24,27–29] (Supplementary Information (SI) Table 1). Nemoda et al. found an association between the heterozygotic variant, GA at rs71647871 (p.Gly143Glu) and low end-dose of MPH[24], and GA at rs71647871 might be involved in reduced CES1 activity and thereby confer slow metabolization[24,26]. Johnson et al. found that homozygosity for both the A allele at rs2244613 and for the G allele at rs2002577 were associated with increased symptoms of sadness[27]. Bruxel et al. found that the two genotypes GG and GT at rs3815583 were associated with reduced appetite[28], and Oxenbøll et al. reported an association between the same genotypes and weight loss[29].

The nonsynonymous variation of rs111604615 is part of the *CES1VAR* haplotype[30] and results in the replacement of arginine with proline[31]. The gene encoding CES1 may be subject to copy number variation (CNV) with the duplicated gene segment, which is found together with a segment of pseudogene *CES1P1* as a hybrid gene, known as *CES1A2*[32,33].

The present study systematically sequenced *CES1* from a clinical representative sample of MPH naïve children with ADHD admitted to a 12-week treatment study with weekly individually titrated dosing of immediate-release MPH (IR-MPH). We investigated the associations between gene variants of *CES1* and treatment responses including dosage of IR-MPH to provide novel insights to aid individualized MPH treatment in children with ADHD.

Materials and methods

Design and subjects

The study is a prospective observational pharmacogenetic study of the initial 12-week of treatment with IR-MPH. It is a part of the Danish INDICES study (INDIvidualised drug therapy

based on pharmacogenetics: focus on *CES1*, a larger investigation of the impact of *CES1* genotypes on pharmacokinetics and clinical outcomes of different drugs including MPH)[34].
We extracted DNA from saliva collected in the Oragene OG-250 DNA kit (DNA Genotek Inc., ON, Canada) as described by the manufacturer. Using the Illumina Infinium OmniExpressExome-8v1-3_A array the samples of DNA had been genotyped in another study (Madsen et al, unpublished). This study included 189 children from the original study population (n = 207[35]), and four excluded children did not obtain a genotype using this platform, nine children due to non-European ethnicity, and five children because of included siblings (both determined by principal components analysis of the genotyping results).
The stimulant naïve children were recruited consecutively from 2012 to 2014 after the first ICD-10 diagnosis[36] (International Classification of Diseases and Related Health Problems) of hyperkinetic disorder (DF 90.0-90.9) or attention deficit disorder without hyperactivity (DF 98.8) from the Child and Adolescent Mental Health Centre, Mental Health Services - Capital Region of Denmark. The exclusion criteria were: mental retardation (IQ < 70), previous treatment with drugs metabolized by CES1, contraindications to treatment with MPH, language barriers, and lack of informed consent.

Diagnostic assessment

All participants were assessed with the Schedule for Affective Disorders and Schizophrenia for School-Age Children – Present and Lifetime Version[37]; parent and teacher rated ADHD rating scales (ADHD-RS)[38–40]; Child Behaviour Checklist; Teacher Report Form[41,42]; Weiss Functional Impairment Rating Scale Parent version[43,44]; and Test of Variables of Attention[45]. The diagnoses of ADHD and psychiatric comorbidities were confirmed by a child- and adolescent psychiatrist.

Pharmacological intervention

Baseline assessments by the ADHD-RS-Clinician rated (ADHD-RS-C)[46] measuring ADHD core symptoms and Barkley's-Stimulant-Side-Effect-RS-Clinician rated (BSSERS-C)[47] measuring ARs were done immediately before initiation of IR-MPH in week 0. The initial dose and the weekly individual dose increase was 2.5 or 5 mg IR-MPH per dose (Medikinet®), according to

the patient's weight (</>30kg) and was given two or three times a day[48]. The increments continued until no further clinically meaningful response was achieved on ADHD-RS-C or until clinically significant ARs measured on BSSERS-C prohibited further up-titrations.
A child and adolescent psychiatrist who was blind to the patients' genotype performed all measurements.

Treatment outcome measures

The primary outcome measure was response to IR-MPH defined as normalization (Nor) (≤ 60 *t-scores*) or borderline normalization (Bnor) (≤ 70 *t-scores*) of the sum scores on any of the two subscales Inattention and Hyperactivity-Impulsivity on ADHD-RS-C (subscale total score of nine items [range 0-27])[40,46,49].

The secondary outcome measures were*:* (1) nonresponder status was defined as discontinued treatment due to ARs or serious ARs (SARs) or not Nor/Bnor on any of the subscales on ADHD-RS-C in week 12, (2) end-dose of IR-MPH mg/kg/day and low end-dose <0.80 mg/kg/day at week 12 (3) ARs measured on BSSERS-C[47] (17 items [range 0-153]), including the single item, *reduced appetite* (≥ 4 BSSERS score).

More details of clinical assessments are described in SI and in[35].

Genotyping

Determination of *CES1* copy number by real-time PCR served to detect presence of *CES1A2.* This was followed by PCR-based amplification of fragments of *CES1* and Sanger sequencing of these fragments[33,34]. The amplification reaction for production of a fragment of *CES1* containing exon 6-14 also amplifies *CES1A2*. Accordingly, we did not perform amplifications of this *CES1* fragment from individuals harboring *CES1A2*. Since deletion of *CES1* is extremely rare, the detection of three and four copies by the real-time PCR analysis was interpreted as the presence of one and two copies of *CES1A2*, respectively. Variants with a minor allele frequency (MAF) above 0.05 and with genotype proportions that did not deviate from those expected under conditions of Hardy-Weinberg equilibrium after correction for multiple testing were selected for subsequent association analyses. To avoid testing of

intercorrelated Single Nucleotide Polymorphisms (SNPs) and avoid redundant analyses, the selected SNPs also had to be independent defined as lack of strong linkage disequilibrium, namely, D' < 0.80. Furthermore, rs71647871 (p.Gly143Glu) was included, because of the possible effect on the metabolism of MPH[26].

Statistical analyses

Univariate associations of the genetic variants of *CES1* (the SNPs and the CNV, *CES1A2*) and categorical and continuous outcomes (Nor/Bnor of Inattention and Hyperactivity-Impulsivity, end-dose of IR-MPH, ARs, and reduced appetite) were analysed with X^2-test, independent t-test, and one-way analysis of variance, respectively.

The multivariate associations of the variants of *CES1* and the same outcome measures of interests were analysed with adjustments for clinically correlates. See Tables 3-5 and SI for co-variables definitions, models, and estimations methods.

Cox regression was used to determine if any genetic variants of *CES1* was associated with our primary measure of response, i.e. time to Nor/Bnor of Inattention and/or of Hyperactivity-Impulsivity (ADHD-RS-C), based on weekly assessments. Patients were registered as an event if Nor/Bnor during the study and the first week of Nor/Bnor was the event time. Patients for whom no Nor/Bnor was registered, were censured and the censure time was the last week of a registered no Nor/Bnor. The cox regressions analysis is a strong statistic method because allows for modelling the time to Nor/Bnor while adjusting for baseline characteristics.

The association between genetic variants at *CES1* and the end-dose of IR-MPH-dose in week 12 was explored using linear regression estimated using generalized least squares (GLS).

Linear mixed effect models for repeated measures were used to detect associations between variants of *CES1* and sum score of ARs (BSSERS-C) during the 12-weeks IR-MPH treatment. Unstructured covariance type fitted the data best as judged by the Akaike information criterion.

To identify the association between variants of *CES1* and reduced appetite (BSSERS-C) over 12 weeks, logistic regression with repeated measures estimated using generalized estimation

equations were used. The correlation structure for the model was unstructured or M-dependent[50].

The homozygotic variant was combined with the heterozygotic variant if $n \leq 5$ of homozygotic variant (Supplementary Figure 1). If n = 6-10 of homozygotic variant, the variant was analysed twice i.e. first kept separately and secondly combined with the heterozygotic variant. The wild type was the reference to the homozygotic and/or heterozygotic variant in all analysis. SPSS version 22 was used for the statistical analyses[51].

Ethics

The study was registered in ClinicalTrial.gov (registration number NCT04366609). The study was approved by The Danish Data Protection Agency (RHP-2012-008, I-suite nr. 01695). The Local Committee on Health Research Ethics was consulted (J.nr. H-B-2009-026) in accordance with national guidelines and the Declaration of Helsinki. Participation was voluntary, and data was kept confidential. Parents provided written informed consent for DNA samples for research purposes.

Results

Baseline assessments

The baseline characteristics of the 189 patients (76.2% males, n = 144) with a mean age of 9.6 (s.d. 1.5) years are listed in Table 1.

Table 1 Baseline characteristics of included patients

[Paste Table 1 here]

We identified 44 SNPs of *CES1* besides the variation creating *CES1A2*. Twenty of the SNPs had MAFs below 0.05 and were thus considered rare. In total, six SNPs in *CES1* (rs3815583, rs111604615, rs56278207, rs2302722, rs2307240 and rs2244614) and the CNV, *CES1A*2, fulfilled our predetermined criteria for subsequent association analyses (Table 2, SI Table 2) and rs71647871 because of variants with los-of-function. The remaining SNPs selected for the association analyses were all located in intervening and therefore noncoding sequences.

Table 2 Characteristics of SNPs and CNVs (*CES1A2*) of *CES1*

[Paste Table 2 here]

Treatment outcomes

The treatment outcomes are shown in SI and SI Table 3. Of the 172 patients (91.0%) who completed the study, Nor/Bnor on ADHD-RS-C was seen in 127 (73.8%) on Inattention, in 144 (83.7%) on Hyperactivity-Impulsivity, and 155 (90.1%) on either subscale at week 12. The 29 nonresponders (15.8%) counted 17 who were not Nor/Bnor in week 12, and 12 who discontinued due to ARs (n = 10) or SARs (n = 2). The mean end-dose of IR-MPH was 0.98 (s.d. 0.28) mg/kg/day, and 38 (22.1%) patients received a low end-dose. The mean sum score of the 17 items of BSSERS-C significantly declined during the 12 weeks and *reduced appetite* was the only single item of BSSERS-C, which significantly increased in the treatment period. There was a weak correlation (r = 0.27, $P < 0.001$) between weight reduction and appetite reduction that explained about 7% of the variation in weight (data not shown).

Univariate associations analyses

Summary of the significant association analyses is listed in Table 3. There were no statistically significant associations in the univariate analyses between any of the variants at *CES1* and Nor/Bnor in week 12 of Inattention or Hyperactivity-Impulsivity, and nonresponder status (SI Table 4). However, several variants were associated with the individually titrated optimal mean end-dose of IR-MPH after categorization into low versus high end-dose. Patients with copy numbers of 3 or 4 (*CES1A2*) more often received the low end-dose (17 out of 51, 33.3%) than patients with 2 copies (21 out of 115, 18.3%), $P = 0.003$. Furthermore, there were relatively more patients with copy numbers of 3 or 4 (*CES1A2*) with reduced appetite in week 12 (10 out of 50, 20.0%) compared with patients with 2 copies (8 out of 115, 7.0%), $P = 0.014$.
More patients with GT/GG at rs3815583 (22 out of 59, 37.3%) received a low end-dose compared with TT (14 out of 101, 13.9%) at this polymorphic site, $P = 0.001$. This was confirmed with a significantly lower mean end-dose of IR-MPH for GT/GG carriers (0.90 mg/kg/day, s.d. 0.33) than for TT carriers (1.01 mg/kg/day, s.d. 0.24) with a mean difference of -0.12 (95% CI -0.21 to -0.03), $P = 0.001$.

More patients with GA at rs2307240 (6 out of 11, 54.5%) compared with GG at this polymorphic site (23 out of 99, 23.2%) received a low end-dose, *P* = 0.025. This was also seen with a significantly lower mean end-dose of IR-MPH for GA carriers (0.74 mg/kg/day, s.d. 0.22) than for GG carriers (0.96 mg/kg/day, s.d. 0.29) with a mean difference of -0.22 (95% CI -0.40 to -0.04), *P* = 0.017. Homozygotes with AA at rs2307240 were not detected.

Patients with del/del at rs56278207 suffered from more ARs in week 12 than the wildtype. To avoid "ARs" before initiation of treatment to interfere ARs in week 12 was measured as the change in BSSERS-C sum score from week 0 to week 12. The homozygotic variant del/del at rs56278207 had an increment of ARs (mean = 3.00, s.d. 8.69) compared to the heterozygotic variant (del/ins) (mean = -3.20, s.d. 8.12) and the wildtype (mean = -2.35, s.d. 7.88), who both did not suffer from ARs in week 12, *P* = 0.021.

Table 3 Summary of significant associations

[Paste Table 3 here]

Multivariate associations analyses

Table 4 shows the chance of Nor/Bnor on Inattention and Hyperactivity-Impulsivity distributed on the SNPs and CVN of *CES1*. With pairwise tests of rs56278207 genotypes patients with the del/del variant had a significantly lower chance of Nor/Bnor on Inattention than the del/ins variant (HR 0.73, 95% CI 0.56 to 0.94, *P* = 0.016) during the treatment period. Patients with GT/TT at rs2302722 had a significantly higher chance of Nor/Bnor on Inattention during the treatment period. Also, the genotypes CC at rs111604615, GA at rs2307240, and GT/TT at rs2302722 significantly increased the chance of Nor/Bnor on Hyperactivity-Impulsivity.

Table 4 Genetic variants of *CES1* associated with time to Nor/Bnor of ADHD core symptoms

[Paste Table 4 here]

Table 5 shows the associations between the variants of *CES1* and the end-dose of IR-MPH. Patients with GT/GG at rs3815583 were estimated to have a significantly lower end-dose than the common homozygotic genotype. The estimated marginal means were 0.86 mg/kg/day (GT/GG) and 0.96 mg/kg/day (TT). Furthermore, patients with GA at rs2307240 were estimated to have a borderline significant lower end-dose than those with the common

homozygotic GG genotype. The estimated marginal means were 0.75 mg/kg/day (GA) and 0.89 mg/kg/day (GG).

Table 5 Genetic variants of *CES1* associated with end-dose of IR-MPH

[Paste Table 5 here]

SI Table 5 shows the associations between the variants of *CES1* and the course of ARs. In week 0 (before medication) the ARs sum scores differed statistically significantly between patients with different genotypes at rs2244614 (23.1 (CC), 15.3 (CT), 19.4 (TT)) (Figure 1). These scores changed significantly different over the 12-week period for the genotypes of rs2244614 and after 12 weeks of treatment they were nearly the same (15.8 (CC). 13.7 (CT) and 14.8 (TT)). Copy number 3 or 4 (*CES1A2*) did not significantly differ from copy number 2 in any of the outcomes in the multivariate analyses.

[Paste Figure 1 here]

We did not find associations between any of the variations of *CES1* and reduced appetite over the treatment period (data not shown).

Discussion

This is the first prospective observational study in the context of a pharmacogenetic study that aimed at detecting *CES1* variants and exploring the association between representative *CES1* variants and the outcome of IR-MPH in stimulant naïve children with ADHD.

The association analyses were explored in six out of the 44 identified SNPs of *CES1* and the CNV, *CES1A*2 which all met the predetermined criteria and in rs71647871 (included too due to the possible influence on the metabolism).

The SNPs, GG/GT at rs3815583 and GA at rs2307240 were consistently associated with lower end-dose of IR-MPH. CC at rs111604615, TT/GT at rs2302722, and GA at rs2307240 were associated with a higher response rate than the wildtypes in Cox regression analysis.

Copy number of 3 and 4 (*CES1A2*) was only weakly associated to end-dose of IR-MPH and reduced appetite. Other pharmacogenetic[52,53] and pharmacokinetic[54] studies have found no associations.

The present study found an association between GT/GG at rs3815583 and low end-dose of IR-MPH, but no association between reduced appetite and weight loss. We could neither confirm the association between rs3815583 and reduced appetite reported by Bruxel et al.[28], nor the association between rs3815583 and weight loss in another patient sample by Oxenbøll et al.[29]. The G carrier frequency (38.1%) at rs3815583 were similar to other studies (37.0%[28], 38.0%[29]).

In contrast to Johnson et al.[27], we found an association between rs2307240 and treatment response, but like Johnson et al., no association between rs2307240 and ARs. GA at rs2307240 was associated with low end-dose in the univariate analyses, but borderline significant (p = 0.088), when adjusted for baseline characteristics in the GLS analysis, which might be because of the low number of GA (n = 12).

GG/GT at rs3815583 and GA at rs2307240 may be slow metabolizers of IR-MPH resulting in low titrated optimal end-dose in combination with an average or even increased rate of normalization.

Zhu et al. found a change of the amino acid from glycine (Gly) to glutamic (Glu) in rs71647871 resulting in decrease CES1 metabolization[26]. The los-of-function might be the reason for Nemoda et al. to choose rs71647871 and found the variant associated to low end-dose[24]. The same association is found in pharmacokinetic studies[54,55] *in vitro* with healthy adults and *in vivo*[56]. A review finds rs71647871 significantly associated to treatment outcomes of seven different CES1 metabolized drugs (depending on drug elimination/activating prodrug)[57]. Maybe because of small number of GA at rs71647871 (n = 8, 4.3%), we did not find the same association, but the number is comparable to other studies: 3.7%[26] (White) , 1.6%[54] (Danish), and 4.1-5.8%[24] (Hungarian).

The variations of rs2244614 was associated with differences in AR scores prior to treatment, but the differences disappeared during treatment, presumably reflecting regression towards the mean.

TT/GT at rs2302722 and ins/ins at rs56278207 were associated with better treatment response, which is a novel finding. Others showed that GT/TT at rs2302722 might not be associated to the pharmacodynamics of CES1 metabolized drugs[58].

The frequencies of the homozygotic variants at rs111604615 and rs2244614 were low (*n* = 9 and *n* = 8) and when the homozygotic- and heterozygotic variants were merged there were no differences compared to the wild types in the treatment response rates and ARs, respectively. The close monitoring of treatment outcome during the individual titration of IR-MPH may have prevented some ARs, like reduced appetite, and substituted these outcomes with low end-dose as effect of variants of *CES1.* In contrast to our study, RCTs have often used a predetermined and fixed titration of MPH[10,18,59], which make the treatment outcomes more comparable[60].

A single variant of *CES1* might not be sufficient to explain the pharmacokinetics[17]. Rather it is possible that specific combinations of variants of *CES1* collectively contribute to the activity of CES1 and rate of metabolism of MPH each with small effects thus contributing to treatment outcomes[57,61]. Overall, a high number of drug responses, probably including the response to MPH, are polygenic determined by contributions from a large number of low-effect size genetic variants in molecules implicated in the pharmacokinetics and the pharmacodynamics with rare appearance[61]. This complicates their identification. But some rare variants of *CES1* might still have high-effect size on a drug mediated treatment outcome in contrast to other pharmacogenetic studies[62–64]. This may reflect that the protein encoded by this gene is implicated in important physiological functions (e.g. lipid metabolism), thus the detrimental variants have been subjected to negative selection during the evolution[31]. This is consistent with the observations from the present study that most variants at *CES1* did not have a marked effect on the metabolism of MPH and hence were not or only weakly correlated with the treatment outcomes.

One limitation of the study is the sample size (n = 189, (n for genotyped sites of *CES1* varied among the 189 participants), even though this study is larger than most other pharmacogenetic studies[18]. We tested eight different variables of *CES1* in a total number of 12 explorative association analyses of treatment outcomes with no adjustment of the level of significance due to multiple testing. The results can be findings by chance and will need replication in larger studies with enough power to examine for interactive effects of MPH and multiple gene variants of *CES1*.

Conclusion

The results of this comprehensive pharmacogenetic study were the detection of eight representative polymorphisms and CNVs of *CES1* indicating clinically significant variations in the metabolism of MPH, for which two variants were associated to lower end-dose of IR-MPH, three variants where associated to higher treatment response rate, and one of these variants was associated to both lower end-dose of IR-MPH and higher treatment response rate than the wildtypes. The results need to be replicated before a conclusion about their possible role in the metabolism and outcome of treatment with MPH can be drawn.

Conflicts of interest

The authors declared no conflict of interest.

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Tables and figures of paper II

Table 1 Baseline characteristics of included patients

	Included (*n* = 189)
Males, *n* (%)	144 (76.2)
Age, years, mean (SD)	9.55 (1.49)
Age, 7-9 years, *n* (%)	125 (66.1)
Age, 10-12 years, *n* (%)	64 (33.9)
ADHD diagnose (ICD10)	
Disturbance of activity and attention, hyperkinetic disorder other, hyperkinetic disorder unspecified. DF 90.0, DF 90.8, DF 90.9, *n* (%)	156 (82.5)
Hyperkinetic conduct disorder. DF 90.1, *n* (%)	12 (6.3)
Attention deficit disorder without hyperactivity. DF 98.8, *n* (%)	21 (11.1)
Comorbidity (ICD10)	
Tic disorders. DF95.X, *n* (%)	17 (9.0)
Externalizing disorders. Conduct disorders, mixed disorders of conduct, and emotions. DF 91.X, DF 92.X, *n* (%)	12 (6.3)
Specific developmental disorder. DF81.X-83.X, DF88.X, *n* (%)	41 (21.7)
Intellectual level. Inferioritas intellectualis. DR41.8, *n* (%)	52 (27.0)
Encopresis and/or enuresis. DF98.0-98.1, *n* (%)	24 (12.7)
Autism spectrum. DF84.X, *n* (%)	22 (11.6)
Attachment disorder. DF94.X, *n* (%)	2 (1.1)
Emotional disorder. Depressive episode, persistent mood-, obsessive-compulsive-, reaction to severe stress, and adjustment disorders, emotional disorders with onset specific to childhood. DF 32.X, DF 34.X, DF 42.X, DF 43.X, DF 93.X, *n* (%)	24 (12.7)
Number of comorbid psychiatric diagnoses (ICD10)	
No comorbidity, *n* (%)	67 (35.4)
1 comorbid diagnosis, *n* (%)	69 (36.5)
2 comorbid diagnoses, *n* (%)	37 (19.6)
3 comorbid diagnoses, *n* (%)	13 (6.9)
4 comorbid diagnoses, *n* (%)	3 (1.6)

Inferioritas intellectualis were based on information from the diagnostic conference and from the intelligence test (WISC) (IQ 70-85) depending on data access. *Inferioritas intellectualis* was included in number of comorbid psychiatric diagnoses.

Table 2 Characteristics of variants of *CES1*.

CES1 marker	Location in gene	Genotype frequency, *n* (%)	MAF	% genotyped
Copy number *n* = 182	NA	3,4: *n* = 54 (29.7)	0.154	96.3
		2: *n* = 128 (70.3)		
rs3815583 *n* = 176	5'UTR	GT/GG: *n* = 67 (38.1)	0.210	93.1
		TT: *n* = 109 (61.9)		
rs111604615 *n* = 175	X1	CC: *n* = 9 (5.1)	0.174	92.6
		GC: *n* = 43 (24.6)		
		GG: *n* = 123 (70.3)		
rs56278207 *n* = 167	IVS1	del/del: *n* = 18 (10.8)	0.269	88.4
		del/ins: *n* = 66 (39.5)		
		ins/ins: *n* = 83 (49.7)		
rs2307240 *n* = 122	X2	GA: *n* = 12 (9.8)	0.049	64.6
		GG: *n* = 110 (90.2)		
rs2302722 *n* = 84	IVS9	GT/TT: *n* = 18 (21.4)	0.131	44.4
		GG: *n* = 66 (78.6)		
rs2244614 *n* = 110	IVS10	CC: *n* = 8 (7.3)	0.286	58.2
		CT: *n* = 47 (42.7)		
		TT: *n* = 55 (50.0)		
rs71647871 *n* = 184	X4	GA: *n* = 8 (4.3)	0.022	97.3
		GG: *n* = 176 (95.7)		

CES1 = Carboxylesterase 1 gene, SNPs = single nucleotide polymorphisms, CNV = copy number variation, MAF = Minor Allele Frequency, NA = not applicable, 5' UTR = 5' untranslated region, X = exon, IVS = intervening sequence. Baseline sample (*n* = 189). See more details in SI Table 2

Table 3 Summary of significant associations

CES1 marker	Univariate analysis *P*-value (outcome)	Multivariate analysis *P*-value (outcome)	Summary of findings
Copy number *n* = 182	*P* = 0.033 (end-dose)[1] *P* = 0.021 (reduced appetite)	NS	3 or 4 copies were associated to • low end-dose[1] (< 0.80 mg/kg/day) • reduced appetite
rs3815583 *n* = 176	*P* = 0.012 (end-dose)[1] *P* < 0.001 (end-dose)[2]	*P* = 0.018 (end-dose)	GT/GG were consistently associated to • low end-dose
rs111604615 *n* = 175	NS	*P* = 0.032 (Hyperactivity-Impulsivity)	CC (vs GG and vs CG) was associated to • higher response rate
rs56278207 *n* = 167	*P* = 0.021 (adverse reactions)	*P* = 0.057 (Inattention)	del/del was associated to • increase of adverse reactions ins/ins (vs del/ins) was associated to • higher response rate
rs2307240 *n* = 122	*P* = 0.017 (end-dose)[1] *P* = 0.025 (end-dose)[2]	*P* = 0.029 (Hyperactivity-Impulsivity) *P* = 0.088 (End-dose)	GA was consistently associated to • low end-dose • higher response rate
rs2302722 *n* = 84	NS	*P* = 0.002 (Inattention) *P* = 0.005 (Hyperactivity-Impulsivity)	GT/TT were associated to • higher response rates
rs2244614 *n* = 110	NS	*P* = 0.035 (adverse reactions)	The adverse reactions did not differ in rs2244614 polymorphisms after 12 weeks of treatment.
rs71647871 *n* = 184	NS	NS	-

NS = Not significant. See methods and results in article for statistics.
The genotypes were tested against a total of 12 drug response phenotypes.
[1] = Low IR-MPH end-dose (< 0.80 mg/kg/day), [2] = mean IR-MPH end-dose
The wildtype genotype of *CES1* is the reference in the multivariate analyzes.
Responder = normalization or borderline normalization of Inattention and/or Hyperactivity-Impulsivity (ADHD-RS) in week 12

Table 4 Variants of *CES1* associated with time to Nor/Bnor of ADHD core symptoms

	Variants	Time to Nor/Bnor of Inattention		Time to Nor/Bnor Hyperactivity-Impulsivity	
		HR (95% CI)	***P*-value**	**HR (95% CI)**	***P*-value**
Copy number	3-4 copies	0.96 (0.83 to 1.11)	0.580	1.03 (0.90 to 1.18)	0.645
rs3815583	GT/GG	1.02 (0.89 to 1.17)	0.786	1.07 (0.94 to 1.21)	0.289
rs111604615	Overall	-	0.618	-	0.028
	GC	1.01 (0.86 to 1.19)	0.862	0.10 (0.86 to 1.06)	0.997
	CC	0.86 (0.62 to 1.19)	0.347	1.40 (1.10 to 1.78)	0.008
rs111604615	GC/CC	0.98 (0.85 to 1.15)	0.833	1.07 (0.94 to 1.22)	0.320
rs56278207	Overall	-	0.026	-	0.510
	del/ins	1.13 (0.98 to 1.31)	0.092	1.06 (0.93 to 1.21)	0.385
	del/del	0.82 (0.63 to 1.05)	0.110	0.95 (0.77 to 1.17)	0.614
rs2307240	GA	1.12 (0.86 to 1.46)	0.384	1.27 (1.02 to 1.58)	0.034
rs2302722	GT/TT	1.45 (1.15 to 1.83)	0.002	1.33 (1.07 to 1.65)	0.010
rs2244614	Overall	-	0.062	-	0.121
	CT	1.13 (0.95 to 1.35)	0.157	1.16 (0.99 to 1.37)	0.075
	CC	0.74 (0.51 to 1.08)	0.119	0.91 (0.66 to 1.25)	0.555
rs2244614	CT/ CC	1.07 (0.90 to 1.27)	0.434	1.12 (0.96 to 1.31)	0.163
rs71647871	GA	1.09 (0.81 to 1.46)	0.578	0.76 (0.55 to 1.05)	0.095

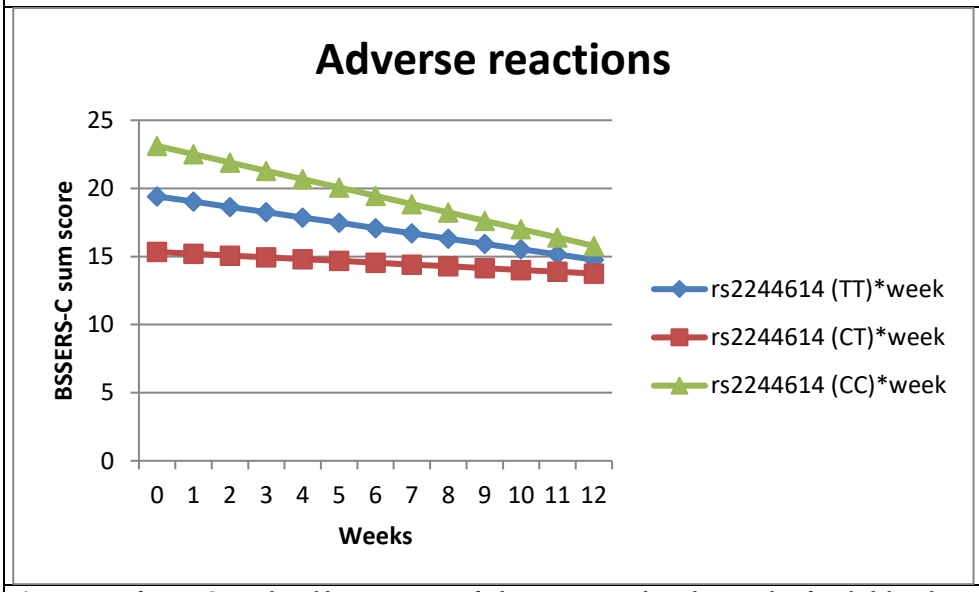
Cox regression used to determine if any genetic variants was associated with time to normalization (Nor) (*t-score* ≤ 60) or borderline normalization (Bnor) (*t-score* ≤ 70) of ADHD core symptoms, Inattention and Hyperactivity-Impulsivity due to Danish norms on ADHD-Rating Scale (ADHD-RS-C, DuPaul), clinician rated.
Inattention subscale: 9 items, [range 0-27]. Hyperactivity-Impulsivity subscale: 9 items, [range 0-27].
CES1 = Carboxylesterase 1 gene, HR = Hazard ratio.
The wild type genotype is the reference. For SNPs with three genotypes and significant *P*-values, see the text.
The model composed of: single nucleotide polymorphisms (SNPs) or copy number variations (CNV), sex, age (7-9 versus 10-12 years), comorbidity (0-1 versus ≤ 2 comorbid diagnoses), conduct disorder, and intelligence level (*inferioritas intellectuals* IQ 70-85, DR41.8 versus normal intelligence level).
n = *n* in week 0, see SI Table 2.

Table 5 Variants of *CES1* associated with end-dose of IR-MPH

	Variant	Estimate	t	95% CI	*P*-value
Copy number	3-4 copies	-0.07	-1.53	-0.15 to 0.02	0.128
rs3815583	GT/GG	-0.10	-2.40	0.02 to 0.04	0.018
rs111604615	CG	-0.06	-1.19	-0.15 to 0.04	0.235
	CC	0.10	1.05	-0.09 to 0.28	0.294
rs111604615	CG/CC	0.03	-0.67	-0.12 to 0.06	0.503
rs56278207	del/ins	-0.06	-1.34	-0.15 to 0.03	0.182
	del/del	-0.10	-1.42	-0.23 to 0.04	0.158
rs2307240	GA	-0.15	-1.72	-0.31 to 0.02	0.088
rs2302722	GT/TT	-0.02	-0.29	-0.16 to 0.12	0.773
rs2244614	CT	-0.09	-1.51	-0.20 to 0.03	0.135
	CC	0.09	0.26	-0.18 to 0.24	0.795
rs2244614	CT/CC	-0.07	-1.26	-0.18 to 0.04	0.212
rs71647871	GA	-0.04	-0.43	-0.25 to 0.16	0.671

End-dose of immediate release methylphenidate (IR-MPH); Linear regression estimated using generalized least squares (GLS).
CES1 = Carboxylesterase 1 gene. The reference is the wild type genotype. The estimation methods composed of; SNPs or CNV *CES1*, ADHD ICD10 diagnoses, CGI in week 0, weight in week 0, sex, and age.
n = *n* in week 12, see SI Table 2.

Figure 1



Association of rs2244614 with problem sum scores of adverse reactions through 12 weeks of methylphenidate treatment (*n* = 110).
Linear mixed model for adverse reactions of rs2244614, week, rs2244614 * week.
Barkley's Stimulant Side Effects Rating Scale (BSSERS-C), clinician rated, [range 0-153].
For estimates see SI Table 5.

Supplementary Information of paper II

Introduction

SI Table 1 Clinical studies of SNPs of *CES1* associated to treatment outcome of methylphenidate

Study	Associations	
	SNPs of *CES1*	**Outcome**
Nemoda et al.[1]	heterozygotic variant of rs71647871 (GA) (p.Gly143Glu) Loss of function	low end-dose of MPH
Johnson et al.[2]*	homozygotic variant of rs2244613 (AA)	sadness
	homozygotic variant of rs2002577 (GG)	sadness
Bruxel et al.[3]	variants of rs3815583 (GG/GT)	reduced appetite
Oxenbøll et al.[4]	variants of rs3815583 (GG/GT)	weight loss

SNP = single nucleotid polymorphisms, *CES1* = Carboxylesterase 1, MPH = methylphenidate
* Tested seven SNPs of *CES1*

Materials and methods

Clinical assessments

The ADHD-RS-Clinician rated (ADHD-RS-C) measured the primary outcome of the 12-week IR-MPH-treatment[6–8,14]. The ADHD-RS-Parents (ADHD-RS-P) rated consist of nine questions of inattention, nine questions of hyperactivity and impulsivity, and six questions of conduct problems evaluated on a four-point Likert scale from 0 (*none = never or rarely*) to 3 (*severe = very often*). The 18-item ADHD-RS-C is identical to the ADHD-RS-P subscales measuring Inattention and Hyperactivity-Impulsivity, whereas Conduct problems are not scored[14]. ADHD-RS-P have previously been validated in a Denmark[8], and the standardized scores (*t-scores*) stratified for each sex and age group (7-9 and 10-12 years) were used to delineate the cut-off for normalization (Nor) (≤ 60 *t-scores*) or borderline normalization (Bnor) (60-70 *t-scores*) on ADHD-RS-C scores of Inattention and of Hyperactivity-Impulsivity in the present study. High correlations between ADHD-RS-C and ADHD-RS-P have been documented[15]. In the present study, ADHD-RS-C scores were determined using all available information including interviews with the parents, the parent- and teacher-rated ADHD-RS scores, psychiatric assessment, and observation of the children.

The clinician rated Clinical Global Impression Severity scale (CGI-S)[16] is a seven-point Likert scale, rated from 1 (*normal, not ill at all*) to 7 (*among the most extremely ill patients*), used to measure the global severity of symptoms and functional impairments of mental health disorder including ADHD and comorbidity. A beneficial symptom reduction was defined by a CGI-S score of 1 or 2 (*normal, not ill at all or borderline ill*).

The Clinical Global Impression Improvement scale (CGI-I)[16] is also a seven-point Likert scale rated from 1 (*very much improved*) to 7 (*very much worse*). A beneficial symptom reduction was defined by a CGI-I score of 1 or 2 (*very much improved or much improved*).

The Barkley's Stimulant-Side-Effect-RS-Clinician rated (BSSERS-C)[17] measured ARs. It consists of 17 effect items rated by the clinician, based on information from patients, parents, and clinical observations: insomnia, nightmares, staring, talks less, disinterested in others, reduced appetite, irritable, stomachaches, headaches, drowsiness, sadness, prone to crying, anxious, nail biting, euphoria, dizziness, and tics/nervous movements. BSSERS-C is a 10-point Likert scale rated from 0 (*problem absent*) to 9 (*problem evokes serious impairment*). A manual for interviewing and rating using BSSERS-C was elaborated for the study, anchoring the 10-point scores (e.g., specifying the time to fall asleep when scoring insomnia). The BSSERS-C was scored before initiation of IR-MPH-treatment (week 0) and weekly during treatment. Severities of ARs were calculated as the sum of scores on the 17-item BSSERS-C [range 0-153]. Significant changes in single items were also explored (e.g., reduced appetite; 1 item, [range 0-9]).

The physical assessments were the body weight, height, heart rate and blood pressure were measured every fourth week with the same equipment each time. The study followed the European guidelines [18] and measured blood pressure after 10 minutes of rest with a sphygmomanometer (nonelectrical) three times and the average of the last two measures was used.

We explored if the following clinical baseline characteristics (Table 1, Supplementary Table 3) and variants of *CES1* (Table 2) were associated to the treatment outcomes

SNPs of *CES1*, CVNs of *CES1*, age (7-9 or 10-12 years), sex, comorbidity (two/more - or one/none comorbid psychiatric diagnosis), intelligence level (IQ 70- 85 or IQ >85), conduct disorder (DF90.1/DF91.X/DF92.X or no conduct disorder), the global severity and functional impairments of mental health in week 0 (CGI-S, [range 1-7]) or dichotomized to *normal not ill* to *markedly ill severity* of psychiatric disease (CGI-S 1-5) and *severe* to *extreme among the most ill patients* (CGI-S 6-7), ADHD diagnose (DF90.0/ DF90.8/DF90.9/DF90.1 or DF98.8), weight in week 0 (≤ 30kg or > 30kg), end-dose of IR-MPH (< 0.80 mg/kg/day or ≥ 0.80 mg/kg/day), appetite (BSSERS-C [range 0-9]) (*no to mild* reduced appetite [0-3] or *moderately to severely* reduced appetite [4-9]).

Statistics

Variants of *CES1* associated to treatment outcome: Models and estimations methods.

Time to Nor/Bnor of Inattention or Hyperactivity-Impulsivity (ADHD-RS-C): Cox regression.

model I; SNPs/CNVs of *CES1*, sex, age, comorbidity, conduct disorder, intellectual level.

End-dose of IR-MPH: Linear regression estimated using generalized least squares (GLS).

Estimations method; SNPs or CNVs of *CES1*, sex, age, weight, ADHD diagnosis, CGI-S in week 0

Adverse reactions (BSSERS-C): Linear mixed effect model.

model I; SNPs/CNVs of *CES1*, SNPs/CNVs of *CES1**time.

model II; SNPs/CNVs of *CES1* has been tested separately with age, sex, *age, *sex, and CGI-S in week 0.

In the mixed effect model time was measured as a continuous variable coded 0, 1, 2…12 week, and all other explanatory variables were measured as categorical variables.

Reduced appetite (BSSERS-C): logistic regression for repeated measures estimated using generalized estimating equation (GEE)

Estimations method I; SNPs/CNVs of *CES1*, SNPs/CNVs of *CES1**time.

Estimations method II; time, CGI-S in week 0, SNPs/CNVs of CES1, SNPs/CNVs of CES1*time.

Estimations method III; SNPs/CNVs of *CES1* has been tested separately with sex, age, *age ,and *sex.

Results

SI Table 2 Characteristics of Variants of *CES1*

CES1 marker	Genotype frequency, *n* (%)	Male, *n* (%)	7-9 years, *n* (%)	Dropouts, *n* (%)
Copy number *n* = 182	3,4: *n* = 54 (29.7)	37 (68.5)	37 (68.5)	3 (5.6)
	2: *n* = 128 (70.3)	101 (78.9)	85 (66.4)	13 (10.2)
rs3815583 *n* = 176	GT/GG: *n* = 67 (38.1)	52 (77.6)	43 (64.2)	8 (11.9)
	TT: *n* = 109 (61.9)	83 (76.1)	74 (67.9)	8 (7.3)
rs111604615 *n* = 175	CC: *n* = 9 (5.1)	8 (88.9)	6 (66.7)	1 (11.1)
	GC: *n* = 43 (24.6)	33 (76.7)	31 (72.1)	7 (16.3)
	GG: *n* = 123 (70.3)	93 (75.6)	80 (65.0)	7 (5.7)
rs56278207 *n* = 167	del/del: *n* = 18 (10.8)	14 (77.8)	9 (50.0)	1 (5.6)
	del/ins: *n* = 66 (39.5)	50 (75.8)	20 (30.3)	7 (10.6)
	ins/ins: *n* = 83 (49.7)	93 (75.9)	29 (34.9)	4 (4.8)
rs2307240 *n* = 122	GA: *n* = 12 (9.8)	7 (58.3)	7 (53.3)	1 (8.3)
	GG: *n* = 110 (90.2)	81 (73.6)	72 (65.5)	11 (10.0)
rs2302722 *n* = 84	GT/TT: *n* = 18 (21.4)	14 (77.8)	11 (61.1)	1 (5.6)
	GG: *n* = 66 (78.6)	51 (77.3)	44 (66.7)	101 (15.2)
rs2244614 *n* = 110	CC: *n* = 8 (7.3)	7 (87.5)	5 (62.5)	1 (12.5)
	CT: *n* = 47 (42.7)	34 (72.3)	32 (68.1)	4 (8.5)
	TT: *n* = 55 (50.0)	45 (81.8)	34 (61.8)	5 (9.1)
rs71647871 *n* = 184	GA: *n* = 8 (4.3)	8 (100.0)	4 (50.0)	1 (12.5)
	GG: *n* = 176 (95.7)	132 (75.0)	116 (65.9)	15 (8.5)

CES1 = Carboxylesterase 1 gene, SNPs = single nucleotide polymorphisms, CNV = copy number variation. Baseline sample (*n* = 189)

Details of clinical treatment outcomes

A total of 17 (9.0%) patients did not complete the study. Three (1.6%) patients dropped out because of non-compliance, one (0.5%) patient due to no beneficial symptom reduction, and one (0.5%) patient had symptoms classified as an adverse event. The clinicians decided when to terminate medications due to adverse reactions (AR) or serious AR (SAR) (n=12, 6.3%). One patient experienced onset of psychotic symptoms (0.5%) and another patient had progression in seizures (0.5%), both where classified as SARs (1.0%). A total of 10 patients (5.3%) dropped out because of ARs: three patients (1.6%) because of irritability, two patients (1.1%) because of hypertension, two patients (1.1%) because of tics, one patient (0.5%) because of sadness, one patient (0.5%) because of more hyperactivity and one patient (0.5%) because of urticarial. Only sex of these 12 patients had ARs or SARs, which were detected by weekly BSSERS-C, while the remaining ARs and SARs were reported spontaneously. Furthermore, one patient (0.5%) had progressed to psychotic comorbid disease, which was classified as an adverse event. There were no significantly changes in mean blood pressure, but two patients dropped out due to hypertension.

A total of 127 (73.8%) patients obtained Nor/Bnor on Inattention (ADHD-RS-C) in week 12, and of these were 84 (66.1%) of young age and 104 (81.9%) were boys. 144 of patients obtained Nor/Bnor on Hyperactivity-Impulsivity (ADHD-RS-C) in week 12, and of these were 96 (66.7%) of young age and 107 (74.3%) were boys. 155 (84.2%) patients obtained Nor/Bnor on Inattention or Hyperactivity-Impulsivity (ADHD-RS-C).

The global severity of symptoms and functional impairments of mental health (CGI-S) was significantly reduced in week 12 (mean difference 2.35 (s.d. 1.03), (CI 95%) 2.19 to 2.50, $P < 0.001$) and 62 patients (32.8%) were rated *not ill at all* or *borderline ill* in week 12 (Supplementary Table 3). The mean global improvement of symptoms and functional impairments of mental health (CGI-I) was 1.75 (s.d. 0.60), and 159 patients (84.1%) were rated *very much improved* or *much improved* in week 12.

SI Table 3 Clinician rated changes from week 0 to week 12

	Week 0 M (SD) *n* (%)	**Week 12** M (SD) *n* (%)	**Week 0 vs. week 12**		
			M dif. (SD)	**95% CI**	***P*-value**
Inattention, sum score	19.83 (3.75)	9.49 (3.70)	10.34 (4.04)	9.73 to 10.95	< 0.001
Hyperactivity-Impulsivity, sum score	18.12 (5.80)	7.88 (4.09)	10.23 (5.09)	9.47 to 11.00	< 0.001
Inattention, Nor/Bnor	4 (2.3%)	127 (73.8%)	0.72 (0.45)	0.65 to 0.78	< 0.001
Hyperactivity-Impulsivity, Nor/Bnor	24 (14.0%)	144 (83.7%)	0.70 (0.46)	0.63 to 0.77	< 0.001
Nonresponder	-	29 (15.8%) [1]	-	-	-
CGI-S	5.29 (0.96)	2.94 (1.07)	2.35 (1.03)	2.19 to 2.50	< 0.001
CGI-S ≤ 2	*n* = 0.0 (0.0%)	*n* = 62 (32.8 %)	0.36 (0.48)	0.29 to 0.43	< 0.001
CGI-I	-	1.75 (0.60)	-	-	-
CGI-I ≤ 2	-	*n* = 159 (84.1%)	-	-	-
IR-MPH, mg/kg/day	0.27 (0.09) (initial dose)	0.98 (0.28)	-0.70 (0.28)	-0.75 to -0.66	< 0.001
IR-MPH end-dose, < 0.80 mg/kg/day	-	38 (22.1%)	-	-	-
BSSERS-C, sum score	17.12 (10.16) [1]	14.52 (8.78) [1]	2.60 (8.47)	1.32 to 3.88	< 0.001
Reduced appetite, single score	0.62 (1.23) [2]	1.94 (1.48) [2]	-1.32 (1.73)	-1.58 to -1.05	< 0.001
Reduced appetite, score difference ≥ - 4	-	14 (7.4%) [2]	-	-	-
Weight, kg	36.64 (11.18)	35.73 (11.24)	0.91 (1.53)	0.68 to 1.14	< 0.001

Paired t-test and X^2-test of clinician rated changes from week 0 to week 12 (*n* = 172).
M = mean. SD = Standard Deviation. M dif. = Mean difference. IR-MPH = immediate release methylphenidate.
Missing data: [1] n = 170, [2] n = 171.
ADHD-Rating Scale, (ADHD-RS-C, DuPaul), clinician rated. Inattention subscale: 9 items, [range 0-27]. Hyperactivity-Impulsivity subscale: 9 items, [range 0-27].
Nor/Bnor = normalization (*t-score* ≤ 60) or borderline normalization (*t-score* ≤ 70) of Inattention and Hyperactivity-Impulsivity on ADHD-RS-C due to Danish norms.
Nonresponder = not Nor/Bnor on ADHD-RS-C on either subscale in week 12 or discontinued treatment due to adverse reactions or serious adverse reactions.
Clinical Global Impression Severity (CGI-S), clinician rated, 1 item [range 1-7]. CGI-S, 1 = *Not ill at all*. CGI-S,2 = *Borderline ill*.
Clinical Global Impression Improvement (CGI-I), clinician rated, 1 item [range 1-7]. CGI-I, 1 = *Very much improved*. CGI-I, 2 = *Much improved.*
Barkley's Stimulant Side Effects Rating Scale (BSSERS-C), clinician rated. Whole scale: 17 items, [range 0-153]. Reduced appetite: single item, [range 0-9]. Reduced appetite defined as an increased single score of 4 or more on BSSERS-C in week 12.

SI Table 4 Significant associations between variants of *CES1* and outcomes after 12 weeks of treatment

Outcome in week 12	**Copy number** *n* = 182	**rs3815583** *n* = 176	**rs111604615** *n* = 175	**rs56278207** *n* = 167	**rs2307240** *n* = 122	**rs2302722** *n* = 84	**rs2244614** *n* = 110	**rs71647871** *n* = 184
Inattention, Nor/Bnor	NS	NS	NS	NS	NS	NS	NS	NS
Hyperactivity-Impulsivity, Nor/Bnor	NS	NS	NS	NS	NS	NS	NS	NS
Nonresponder	NS	NS	NS	NS	NS	NS	NS	NS
IR-MPH end-dose, mg/kg/day	NS	*P* = 0.012	NS	NS	*P* = 0.017	NS	NS	NS
IR-MPH end-dose, < 0.80 mg/kg/day	*P* = 0.033	*P* = 0.001	NS	NS	*P* = 0.025	NS	NS	NS
Adverse reactions, sum score difference	NS	NS	NS	*P* = 0.021	NS	NS	NS	NS
Reduced appetite, score ≥ - 4	*P* = 0.014	NS	NS	NS	NS	NS	NS	NS

Comparison between methylphenidate treatment outcome and genetic variants of carboxylesterase 1 (*CES1*) with X^2-test, oneway analysis of variance, and independent t-test. See the text for details of the association analyses.
IR-MPH = immediate release methylphenidate, NS = not significant ($P > 0.05$).
ADHD-Rating Scale, (ADHD-RS-C, DuPaul), clinician rated. Inattention subscale: 9 items, [range 0-27]. Hyperactivity-Impulsivity subscale: 9 items, [range 0-27].
Nor/Bnor = normalization (*t-score* ≤ 60) or borderline normalization (*t-score* ≤ 70) of Inattention and Hyperactivity-Impulsivity on ADHD-RS-C due to Danish norms.
Nonresponder = not Nor/Bnor on ADHD-RS-C on either subscale in week 12 or discontinued treatment due to adverse reactions or seriously adverse reactions.
Barkley's Stimulant Side Effects Rating Scale (BSSERS-C), clinician rated. Adverse reactions: whole scale consisting of 17 items, [range 0-153] = sum score in week 0 – sum score in week 12. Reduced appetite: single item, [range 0-9]. Reduced appetite defined as a categorical variable of a single score of 4 or more on BSSERS-C in week 12.
n = in week 12, see SI Table 2

SI Table 5 Variants of *CES1* associated to adverse reactions over the 12-week treatment period

Variants of *CES1* * week				
	Variant	**Estimate**	**95% CI**	***P*-value**
Copy number	**3-4 copies**	0.06	-0.15 to 0.28	0.568
rs3815583	**GT/GG**	-0.01	-0.22 to 0.21	0.952
rs111604615	**GC**	-0.04	-0.28 to 0.20	0.734
	CC	0.05	-0.41 to 0.51	0.832
rs111604615	**GC/CC**	-0.02	-0.25 to 0.20	0.839
rs56278207	**ins/del**	0.02	-0.20 to 0.23	0.893
	del/del	0.30	-0.04 to 0.65	0.088
rs2307240	**GA**	0.05	-0.37 to 0.48	0.799
rs2302722	**GT/TT**	-0.16	-0.47 to 0.14	0.306
rs2244614	**CT**	0.26	0.02 to 0.49	0.035
	CC	-0.22	-0.68 to 0.23	0.333
rs2244614	**CT/CC**	0.20	-0.03 to 0.43	0.083
rs71647871	**GA**	0.47	-0.03 to 0.96	0.063

CES1 = Carboxylesterase 1 gene, SNP = single nucleotide polymorphisms, CNV = Copy Number Variation.
Linear mixed model for repeated measures of sum score of adverse reactions.
The model composed of SNPs or CNV (*CES1A2*) of *CES1*, SNPs or CNV, *CES1A2* of *CES1* * week, week.
SNPs or CNV (*CES1A2*) of *CES1* was also tested pairwise with sex and age, which did not make a difference of the estimates and significance level of SNPs or CNV (*CES1A2*) of *CES1*.
Barkley's Stimulant Side Effects Rating Scale (BSSERS-C), clinician rated. Whole scale, 17 items, [range 0-153]. *n* = *n* in week 0, see SI Table 2.

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Appendix A

Systematic literature search of observational studies:

1) S1 Fig. PRISMA flow chart of naturalistic observational clinical prospective studies of methylphenidate treatment of children with ADHD (supplementary tables and figures paper I)
2) S2 Table. Included studies in the review of naturalistic observational clinical prospective studies of MPH treatment of drug naïve children with ADHD (supplementary tables and figures paper I)
3) Table A Search terms
4) Table B Abbreviations of psychometric instruments in S2 Table in paper I and Table C in Appendix A
5) Table C Included studies in the review of naturalistic observational clinical prospective studies of MPH treatment of drug naïve/MPH free children with ADHD.

The inclusion criteria of the systematic literature search are both listed in the introduction of the thesis and in S2 Table in paper I.

Table A Search terms in pubmed.ncbi.nlm.gov

	Population 7-12 years	**Study design**	**Medication**	**Diagnose**
	AND			
OR	Chid	Cohort study Prospective study Follow-up study Longitudinal study Observational study Incidence study	Methylphenidate Brand names	ADHD Attention deficit hyperactivity disorders ADHD primarily inattention disorder Hyperkinetic disorder Hyperkinetic disorder Hyperkinetic disorder, other Hyperkinetic conduct disorder Hyperkinesis Attention Deficit and Disruptive Behavior Disorder Attention Deficit Disorder with Hyperreactivity

Table B Abbreviations of psychometric instruments in S2 Table in paper I and Table C in Appendix A

Instrument	**Full name**	**Measures [Range]**	**Measurements**
IOWA Conners[1]	Inattention/Overactivity (I/O) and oppositional-defiant (O/D) Conners Behavior Rating Scale	Five items of I/O and five items of O/D of each [0-3]	ADHD core symptoms
CGI-I[2]	Clinical Global Impression-Improvement	One item [1-7] from very much improved (1) to very much worse (7).	Global symptoms
Digit Span Test[3]	Subtest of China-Weschler Intelligence Scale	Verbal working memory of both Digit Span Forward and Digit Spam Backward	Cognitive test
Stroop Color-word test[4]		Four tasks: word reading, color reading, word reading of color-words, and color naming of color words.	Cognitive test
Coding Test[5]		Digit Symbol Substitution Test; consisted of digit symbol pairs	Cognitive test
WCST[6]	Wisconsin Card Sorting Test	The ability to display flexibility during schedule changes of 13 stimulus cards (shapes of different color, quantity and designs). Executive functions	Cognitive test
K-SADS-E[7] K-SADS-K[5 6 8] K-SADS-PL[7]	Kiddie Schedule for Affective Disorders and Schizophrenia - Epidemiologic version - Korean version - Present and life time version	Screen interview. Semi-structured interview to measure current and past symptoms of mood, anxiety, psychotic, and disruptive behaviour disorders in children ages 6-18 years old	Screening comorbidity
CHIP-CE*	Child Health and Illness Profile-Child Edition.	5 Domains: Meets the expectations for role performance in school, Satisfaction, Comfort, Resilience, Risk Avoidance	Global symptoms
CGI-ADHD-S[9]	Clinical Global Impressions-ADHD-Severity	Clinician rated ADHD core symptoms and disease associated problems (aggressive behavior, depressive mood, anxiety, tics, and learning difficulties).	ADHD core symptoms

CGI-S[2]	Clinical Global Impressions Severity	7-point Likerts scale of illness; [1], no disease, to [7] *extremely severely ill*. An author[10] expanded this with [0], *cannot be judged.*	Global symptoms
CSI-4*	Child Symptom Inventory-4 Parent Checklist	Assessment of comorbidity	Diagnostics
CBCL[11]	Child Behavior Cheklist	Parent rated	ADHD core symptoms Comorbidity
TRF[11]	Teacher Report Form	Teacher rated	ADHD core symptoms Comorbidity
K-ARS[12] **	Korean ADHD Rating Scale	9 items of inattention [0-27] 9 items of hyperactivity-impulsivity [0-27] Designed by DuPaul	ADHD core symptoms
CDI* BDI[13] **	The Children's Depression Inventory. Beck Depression inventory	Self-rating scale of 27 items Parent rated 21 items of subjective severity of depression.	Comorbidity
The State-Trait Anxiety Inventory*		Subscale of "acute anxiety" Subscale of "chronic anxiety" 20 items, 3-point Likert scale from 1 (almost never) to 2 (often)	Comorbidity
LPS[14]	Life Participation Scale	(ADHD)-Child version parent rated scale that assesses changes in adaptive functioning related to treatment for ADHD. 24 items 4-point scale from scale from *never* to very *often*, divided into self-control subscale (17 items) and happy/social subscale (4 items)	Quality of life
ADS[15] **	Computerized ADHD diagnostic system	Standardized continuous Performance Test of both visual and auditory tasks. Supplement to ADHD diagnosis	Diagnostics
SNAP-IV[16]	Swanson, Nolan, Pelham, Fourth edition	ADHD rating scale [0-60]	ADHD core symptoms
ADHD-RS[17]	ADHD rating scale clinician rated	Nine items of inattention, each item ranged from [0] to [3] Nine items of hyperactivity and impulsivity, each item ranged from [0] to [3].	ADHD core symptoms
BRIEF[18]	Behavior Rating Inventory of Executive Function Scale	86 items of executive behaviors at home and in school rated by parents and teachers. Each item ranges from [1], *never*, to [3] *often*.	Comorbidity
W-FIRS[19]	Weiss Function Impairment Rating Scale	Evaluation of patient's social function. Seven subscales: family, work, school, life skill, self-concept, social, and risk, each item ranges from [0], *never/not at all*, to [3] *very often/very much*.	Daily and social life
CAT[20] **	Computerized Comprehensive Attention Test	Measures the response time variability with; visual and auditory task, flanker interference task, sustained attention task	ADHD core symptoms
CPT[21]	Computerized Continuous Performance test (Conners).	Sustained attention ability (CPT Distraction) Ability to inhibit impulsive responses (CPT Impulsivity)	ADHD core symptoms
FBB-ADHD[22]	Fremdbeurteilungsbogen für Aufmerksamkeitsdefizit-Hyperaktivitätsstörung	A part of the German diagnostic system for mental disorder in children and adolescent. Consists of nine items of inattention, and 11 items of hyperactivity and impulsivity. Each item with a range of [0-3] and rated by parent and teacher.	ADHD core symptoms
DAYAS[23]	Day profile of ADHD symptoms	Day profile of ADHD symptoms and other externalizing symptoms with six items of each range [0-3]. Rated by parent and teacher two to four times a day.	ADHD core symptoms
KINDL[24]	Kinder Lebensqualitätsfragebogen Parent rated of children 6 to 11 years old (KID-KINDL) Parent rated of adolescents 6 to 17 years old (KINDL).	Health related quality of life rated by the patient and parent. 24 items of physical wellbeing, emotional wellbeing, self-esteem, family, friends, and school. Rated from 0 (most negative state) to 100 (most positive state).	Quality of life
Physician-rated adherence and tolerability scale[25]		Clinician rated item from [1] excellent/very good to [6] failed/inadequate made by the authors.	Adverse reactions Global symptoms
CAPA[26]	Parent version of Child and Adolescent Psychiatric Assessment	Clinical assessment of ADHD diagnoses	Diagnostics
CHATTI[27]	The Child Attention-Deficit Hyperactivity Disorder Teacher Telephone Interview.	Clinical assessment of ADHD diagnoses	Diagnostics

CPTRS-R:S [28] or L[29] CPRS-R:S[28] or L[30]	Conner's Teacher Rating Scale Revised Short Version (28 items) or Long version (59 items) Conner's Parent Rating Scale Revised Short Version (27 items) or Long version (80 items)	CPRS-R-L [0-3] (80 items). Oppositional (10), Cognitive/ Inattention (10), Hyperactivity (8), Anxious-shy (8), Perfectionism (7), Social problems (5), Psychosomatic (6). Conners' global index: Restless-impulsive (7), Emotional Lability (3) ADHD index (12) DSM-IV symptoms: DSM-IV Inattentive (9), DSM-IV Hyperactive-Impulsive (9)	ADHD core symptoms
C-GAS[31]	The Children's Global Assessment Scale.	Overall severity of the disorder from [1] needs constant supervision to [100] superior functioning	Global symptoms
ToL [33]	Tower of London	The planning ability. Number of attempts to solve a problem and time of the decision of first attempt, rated from [0] to [3].	Cognitive test
WM	N-Back working memory test	Developed by the authors[34]. A computerized test of the N-back paradigm.	Cognitive test
QoL	Quality of Life	A Likert Scale made by the authors[10] of school, recreation area, family, and peer interaction ranging from [1], very good to [5], very bad at study-entry and rating changes from [1], *much improved* to [5], *much worse.*	Quality of life
CGI-C	Clinical Global Impressions Change	The authors[10] made an expansion of Guy[2] from [0], *cannot be judged*, [1], no disease, to [7] *extremely severely ill.*	Global symptoms
ARs[10]	Adverse reactions	5-point Likert scale made by the authors from [1], very good, to [5], very bad.	Adverse reactions
MINI-kid[35]	Mini-International Neuropsychiatric Interview.	Short standardized diagnostic interview and covers a rather broad range of diagnoses applicable to children and adolescents	Diagnostics
DSMIII	Diagnostic and Statistical Manual Disorders III;	Hyperactivity (5 items), inattention (5 items), impulsivity (6 items), peer interactions (7 items).	Diagnostics
WISC III WISC IV[36]	Weschlers intelligence scale	The WISC takes 45–65 minutes to administer. It generates a Full Scale IQ (formerly known as an intelligence quotient or IQ score) that represents a child's general intellectual ability. It also provides five primary index scores: Verbal Comprehension Index, Visual Spatial Index, Fluid Reasoning Index, Working Memory Index, and Processing Speed Index.	Cognitive test
Tea-Ch[37]	Test of Everyday Attention for Children	The *Test of Everyday Attention for Children* (TEA-Ch) is a standardized and normed clinical battery that measures different attentional capacities in children aged 6–16.	ADHD core symptoms
BSSERS[38]	Barkley Stimulant Side Effect Rating Scale	17 items with each item range [0] absent to [9]. Assessment of severity of changes.	Adverse reactions
ARs questionnaire[39]		The authors made their own clinician rated 18 items questionnaire (two general, four gastrointestinal, three central nervous system, one cardiovascular, one metabolic/nutritional, and seven psychiatric) rated from *mild* to *life-threatening*	Adverse reactions
MFFT[40]	Matching Familiar Figure Test	Assessment of impulse control. The patient where asked to find an identical match for a stimulus from one to six pictures.	ADHD core symptoms
VFT *	Verbal Fluency Test	1) Letter Fluency Test, ability to generate words in response to a letter cue. 2) Category Fluency Test, ability to generate words in response to a category cue (example name as many items in a category in one minute).	Cognitive test
CTT[41]	Color Trails Test	Trail A: 25 numbered circles need to be connected as rapidly as possible Trail B: Circles the pink and yellow colors must be connected in sequences. Scored for frequency and errors.	Cognitive test
CSAICA[42]	Chinese version of Social Adjustment Inventory for Children and Adolescent.	Evaluation of children's functioning in school, in spare time activities, and with peers, siblings, and parents	Social life
Adherence to treatment[42]		Parents satisfactions rate, frequency of ARs (reduced appetite, nausea, somnolence, insomnia, headache, dizziness, abdominal pain, and stomach ache) and mean dose of MPH made by the authors.	Adverse reactions
TOVA[43]	Test Of Variables Attention.	Computerized, continuous performance test comprising a target stimulus and a non-target stimulus.	ADHD core symptoms
APRS[44]	Academic Performance Rating Scale	15 parent rated items of academic success, academic productivity, and academic attention.	Cognitive test
PSI[45]	Parenting Stress Index	Eight child characteristic-related stress items Nine parent-child characteristic-related tress items	Comorbidity

		Three achievement expectation-related items.,	
* = Reference not possible, ** = full text not possible			

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Appendix A **Table C Included studies in the review of naturalistic observational clinical prospective studies of MPH treatment of drug naïve/MPH free children with ADHD.**

Study number **Included studies (x)** **Study name**	**Author**	**Population,** **Recruitment strategy** **N = screened** **n = included**	**Control group** **Age [range]**	**Status of medication at baseline** **A: MPH naïve** **B: MPH free** **C: Naïve or free**	**Inclusion age [range]** **Mean-age (SD)** **Boys (%)**	**Study Design** **Definition by authors**	**Diagnostic instruments. ADHD subtype**	**Baseline assessments**	**Length in weeks** **Follow-up (FU) in weeks**	**Treatments** **Dose (%)** **End-dose** **Mean (SD)**	**Primary outcome measure**	**Secondary outcome measures**	**Findings at study-end and comments** **N (%)** **Mean (SD)**
1	*Zheng et al.* 2015[1]	Centers in China Screened (N = 153) Included (n = 128) Completed (n = 123)	n = 40 [7-13]	B: MPH free period of three days before baseline	[6-9] Median 9.2 (SD 1.5) 108 boys (84.4%)	12-week prospective open-label multicenter self-controlled clinical study	DSMIV No information about ADHD subtypes.	Parent rated IOWA Conners, Clinician rated CGI-I, Digit Span Test, Stroop Color Word Test, WCST Coding Test.	12 FU: 1 ,2 ,3 ,7 ,12	OROS-MPH once-daily 18 mg/day (73.5%) 36 mg/day (26.5%)	(1) IOWA Conners I/O (2) Digit Span Test	(3) IOWA Conners O/D. (4) Remission rate of IOWA Conners total score ≤ 5. (5) CGI-I. (6) Stroop Color-word test. (7) Coding Test. (8) WCST. (9) Academic performance. (10) ARs.	(1) Improvements of IOWA Connors I/O; 3.8 (SD 2.3) ($P < 0.001$). (2) Digit Span Test with higher score ($P < 0.001$). (3) IOWA Conners O/D; 3.3 (SD2.4) ($P < 0.001$). (4) Remission rate of IOWA Conners 103 (81.1%). (5) CGI-I; 36 (32.4%) very much improved, 46 (41.4%) much improved. (6) Stroop Color-word test with decrease (P <0.001 to $P = 0.009$). (7) Coding test; increased ($P < 0.001$). (8) WCST improved ($P < 0.001$). (9) Academic performance improved ($P < 0.001$). (10) ARs; poor sleep (1.6%), tics (1.6%), loss of appetite (12.2%).
2	(1) *Wang et al.* 2011[2] (2) *Wang et al.* 2011[3] (3) *Wang et al.* 2014[4]	Child and Adolescent Psychiatry Outpatients Department of Chang Gung Memorial Hospital in Keelung in Taiwan. (1) Included n = 50. Completed n = 38. (2) Included n = 58. Completed the study n = 40. (3) Included n = 50. Completed the study n = 30.	(1) n = 50 age and gender matched	C: MPH naïve or MPH-free period of six month.	[6 -12] (1) 7.6 (SD 1.6) 40 (80.0%). (2) 7.7 (SD 1.6) 48 (82.8%) (3) 7.8 (1.6) 40 boys and 10 girls	24-week nonrandomized, observational, prospective study.	DSMIV K-SADS-E n = 50: 15 In (30 %), 11 Hyp/im (22 %), 24 Comb (48 %). CBCL subscale N = 28 aggressive type N = 22 non-aggressive type	Clinician rated ADHD-RS, CPT, parent rated SNAP-IV. (1) Dehydroepiandrosterone (DHEA) level (2) DHEA/cortisol-, DHEA, and cortisol level (3) CBCL	24 FU: 4, 12, 24	Formulation (?) (1) 12.8 mg/day (SD 7.6). (2) 12.5 mg/day (SD 7.7). (3) 13.0 mg/day (SD 7.5) and 0.46 mg/kg/day (SD 0.2).	(1) Elucidate relationship between DHEA and ADHD symptoms (2) Elucidate relationship between DHEA/cortisol and ADHD symptoms. (3) To determine the time course of improvements in ADHD (and subtypes) clinical symptoms and neurocognitive function in a realistic setting.	CBCL ARs	(1) Improvement of ADHD-RS hyperactivity and inattention ($P < 0.001$) and CPT impulsivity ($P < 0.001$), but not CPT distraction ($P = 0.592$). DHEA level correlated to CPT but not ADHD-RS and MPH dosage. (2) Increment DHEA level ($P = 0.027$) and DHEA/cortisol level ($P = 0.007$. No change of cortisol ($P = 0.529$). CBCL subscales decreased ($P < 0.001$ to $P = 0.007$). No collation between CBCL subscales and DHEA-, DHEA/cortisol-, or cortisol level ($P < 0.05$). (3) Oppositional score of SNAP-IV decreased ($P < 0.001$) and improvements as (1). Non-aggressive type might improve more on CBT. Discontinuation due to ARs (n = 3)
3	*Kim et al.* 2015[5]	Patients from 7 university hospitals in Soul in Korea. Included n = 143 Completed n = 116	-	B: OROS-MPH free period of three months. 24-hour washout period for all previous ADHD medication.	[6-18] 9.4 (SD 2.4) 100 (86.2%)	12-week prospective multicenter open-label study	DSMIV K-SADS-K n = 116: 39 In (33.6 %), 6 Hyp/Im (5.2 %), 42 Comb (36.2 %), 29 Sub (25.0 %).	Parents rated K-ARS Parent rated IOWA Conners LPS CDI The State-Trait Anxiety Inventory	12 FU: 1, 3, 6, 9, 12.	OROS-MPH; 18 or 27 mg. 34.1 mg/day (SD 13.8) 1.0 mg/kg/day (SD 0,3) Responders: 30.1 mg/day (SD 12.5) 0.9 mg/kg/day (SD 0.3)	Relationship between reduced ADHD symptoms and comorbidity due to treatment with OROS-MPH and adaptive functioning in children. Responder: K-ARS < 18 and CGI-I = 1 or 2.	Parent rated IOWA, CDI, LPS, The State-Trait Anxiety Inventory, ARs.	77 responders (66.4%) Improvements on K-ARDS and LPS ($P < 0.001$) LPS changes were - moderate/strong associated to K-ARS changes. - moderate associated to O/D of IOWA changes. - weak associated to CDI- and Trait Anxiety Inventory changes (chronic anxiety) Discontinuation due to ARs (n = 8)
4	*Kim et al.* 2015[6]	Outpatients from Department of Child and Adolescent Psychiatry at Guro Hospital, Korea University Medical Center. Included n = 86 Completed n = 37	-	C: Mixed patients MPH naïve or MPH-free for a period of two months	[6-12] 75 (87.2%) 8.2 (SD 2.1)	No definition	DSMIV ADS No information but includes inattention or hyperactivity-impulsivity or both.	Heart rate variability (HRV) K-ARS ADS	12 FU: 12.	IR or ER MPH 22.2 mg/day (SD 8.9) 0.7 mg/day/kg (SD 0.2)	Clarify the relationship between the autonomic nervous system and the ADHD measurements. Evaluate the utility of HRV as biomarker for therapeutic change in ADHD. HRV K-ARS	ARs	Significant correlation between K-ARS inattention score and some HRV parameters. Improvements of K-ARS subscale scores ($P < 0.001$) and increase of mean heart rate ($P = 0.007$) Decrease in HRV parameters (low frequency (HF)) and normal-to-normal intervals (RMSSD)) suggests that parasymphatic dominance in

											ADS		ADHD can be altered by methylphenidate treatment.
5 # (1)	(1) *Kim et al.* 2011[7] (2) *Cho et al.* 2012[8] (3) *Kim et al.* 2013[9]	Six Child Psychiatric Clinics at University Hospitals in South Korea. Included n = 101-102	-	A: MPH naïve	[6-12] 81-82 (80.2-80.4%). 8.7 (2.1)	12-week prospective open-label study.	DSMIV K-SADS-P 27 In (26.4 %), 6 Hyp/Im (5.8 %), 69 Com (67.6 %).	Clinician rated K-ARDS, Clinician rated CGI-S, Clinician rated CGI-I, (2) ECG (Electro-cardiographic), (2) Heart rate (HR), (2) Blood pressure (BP) (3) CAT, Blood samples for: (1) Brain-derived neurotrophic factor gene (BDNF), (2) norepinephrine genes, (3) *ADRA2A* and *SLC6A2*	12 FU: 2, 4 8, 12	OROS-MPH. 1.0 mg/kg/day (SD 0.5)	(1) Val/Val genotype associated to remission rates. Response criterions: 1: K-ARDS sum score ≤ 18 and single score 0-1 and ≤2 on CGI-I. 2: ≤ 2 on CGI-S. 3: K-ARS > 50% sum score reduction 4: 1, 2, and 3 are satisfied (2) Norepinephrine genes associated to heart rate or blood pressure. (3) *ADRA2A* or *SLC6A2* associated to response time variability before and after treatment with OROS-MPH.	(1) Associations between *NTF-3*, *SLC62*, and *ADRA2A* and remission rates. ARs	(1) K-ARDS decreased from 33.5 (SD 9.6) to 12.5 (SD 5.2) Val/Val associated to all four response criterions compared to Val/Met and Met/Met. (2) No significant change in BP and ECG but increase of HR (P = 0.001). Increase of diastolic BP was associated to SNPs of *ADRA2A* (P = 0.001). Increase of HR was associated SNPs of *SLC6A2* (P = 0.009). (3) K-ARDS decreased 34.0 (SD 9.6) to 13 (SD 5.2). SNPs of *ADRA2A* were associated to the change of CAT (the flanker interference and sustained attention) (P = 0.001)
6	*Wang et al.* 2013[10]	Child and Adolescent Psychiatry Outpatients Department of Chang Gung Memorial Hospital in Keelung in Taiwan. Included n = 108 Completed n = 79	-	C: Mixed patients. MPH naïve or MPH-free period of six months.	[6-16] n = 79 64 (81.0 %) 9.1 (SD 1.9)	No definition. Treatment in the clinical setting.	DSMIV n = 79 40 In (50.6 %), 5 Hyp/Im (6.3 %), 34 Com (43.0 %).	Clinician rated ADHD-RS, CBCL, TRF, clinician rated CGI-S	1 year. FU: 1 year	IR-MPH 2-3 times/day. No mean end-dose	To determine changes in behavior in ADHD patients (and subtypes) by different informants during treatment in a clinical setting.		No significant improvement of CBCL subscales. Improvements of ADHD-RS (P < 0.001), CGI-S (P < 0.001), and TRF (social problems (P = 0.023), attention problems (P = 0.003), aggressive behavior (P = 0.047), and externalizing behavior (P = 0.017)). ADHD Hyp/In- and ADHD Com subtype were associated to high baseline scores of CBCL (aggressive behavior, p = 0.002, delinquency, p < 0.001, and external problems, p = 0.002)- ADHD Hyp/In- and ADHD Com subtype had a greater improvement (interaction) on TRF (somatic complains (P = 0.027), social problems (p = 0.036), aggressive behavior (p = 0.032), and delinquency (p = 0.047).
7	*Lee et al.* 2009[11]	Seven centers in Korea. Included n = 144 Completed n = 88	-	B: OROS-MPH-free period of three months.	[6-15] Completed: 77 (87.5%) 9.4 (SD 2.2)	Prospective open-label multi-center study.	DSMIV K-SADS-PL ADS No information about ADHD subtype.	K-ARS (clinician rated?), Parent rated IOWA conners, clinician rated CGI-S, clinician rated CGI-I, ADS.	12. FU: 1, 3, 6, 9, 12.	OROS-MPH. 1..0 mg/kg (SD 0.3).	Is great variability in response time in ADS related to poor MPH treatment response? Responder: Sum score < 18 on K-ARS and 1 or 2 on CGI-I.		59 responders (67.0 %), 29 nonresponders (33.0 %). Characteristic of responder and non-responder (IOWA, K-ARS, MPH dose) in the article. Nonresponder greater variability of response time at baseline (p > 0.01). Variability of response time at baseline predicted 94.9 % of responders, 17.2 % of nonresponders, and 69.3 % of overall (p > 0.01) - (great response time – poor response to MPH treatment. Discontinuation due to ARs (n = 8)
8 # (2) **SLC6A2=DAT**	*Lee et al.* 2011[12]	Outpatients from Child and Adolescent department of Hallum University Sacred Heart Hospital in Anyang and CHA University hospital in Seongnam in Korea. Screened N = 192 Included n = 137 Completed n = 112	n = 252 [20-36] randomly selected from DNA bank	A: MPH naïve	[6 -18] Completed: 93 (83.0 %). 10.2 (SD 2.9). Responder n = 76 62 (81.6 %) 10.3 (SD 2.9) Nonresponder n = 36 31 (86.1%) 10.2 (SD 2.7)	Open-label study with case-control	DSMIV K-SADS-PL 21 In (18.8 %), 1 Hyp/Im (0.9 %), 90 Com (80.4 %).	Parent rated K-ARS, clinician rated CGI-S, clinician rated CGI-I, blood samples for DNA.	8 FU: 1, 2, 4, 8.	OROS-MPH. Responder 0.9 mg/kg/day (SD 0.3). Nonresponder 0.9 mg/kg/day (SD 0.3).	Association between *SLC6A2* and methylphenidate response. Response: decrease ≥ 50% in sum score on K-ARS and 1 or 2 on CGI-I	Changes in K-ARS sum score and subscale sum score and CGI-I	76 responders (67.9 %), 36 nonresponders (32.1 %). Responders had lower CGI-S baseline score responder than nonresponders (P = 0.002). No differences in genotype and allele distribution for each SNPS of *SLC6A2* in the patients and the control group (P > 0.05). No differences in change of total score and subscale scores in K-ARDS or CGI-I scores among the genotypes and the allele distribution (P > 0.05). SNPs of *SLC6A2* were not associated to treatment response (P = 0.23 to 92) Discontinuation due to ARs (n = 11)

9 **OBSEER:** Observation of Safety and Effectiveness of Equasym in a Routine care.	(1) *Becker et al.* 2011[13] * (2) *Rothenberger et al.* 2011[14] (3) *Döpfner et al.* 2011[15] (4) *Döpfner et al.* 2011[16] * (5) *Hautmann et al.* 2013[17] * (6) *Hautmann et al.* 2017 [18] * * = results are no represented in the Table (outcomes with mixed baseline drug status)	169 centers in Germany Included n = 852 Completed n = 822-782 Completed MPH naïve n = 208	-	C: Mixed patients. MPH naïve n = 208 (25.3 %) and three other subgroups of prior treatment: 1) IR-MPH once daily 2) IR-MPH more than once daily 3) MR-MPH other than Equasym XL	[6 -17] MPH naïve n = 208 9.8 (SD 2.7)	Open-label prospective multicentre observational post-marketing study.	DSMIV or ICD10 MPH naïve: n = 208 (remains 8): 108 of F90.0 (48.1 %), 69 of F90.1 (33.2 %), 23 of F90.8 (11.1 %).	(2) (3) Parent rated KINDL (6-17 years) & KID-KINDL (6-11 years), (2) Clinician rated adherence and tolerability, (3) Parent and teacher rated FBB-ADHD, (3) Clinician rated CGI-ADHD-S, (3) Parent and teacher rated DAYAS,	6-12. FU: 1-3, 6-12.	Equasym XL 10 to 120 mg All groups: 25.3 mg/day (SD 9.4) Graph in (4) shows approximately 0.7 mg/kg/day	Evaluate the degree and safety of Equasym in routine care. (2) Is quality of life and satisfaction with Equasym XL related to prior MPH status at study-end? (3) Is the degree of symptom control with Equeasym XL related to previous medication		(2) Largest improvement of KINDL in MPH naïve (only figure) compared to other groups (2) 57.1 % was assessed as *very good of* clinician rated adherence and tolerability (only figure). (3) Total mean score (SD) at study-end: CGI-ADHD-S (1.0 (SD 0.6)), FFB-ADHD-P (0.95 (SD 0.6)), FFB-ADHD-T (0.74 (SD 0.5)), DAYAS-P evening (1.1 (SD 0.7)), DAYAS-T morning (0.6 (SD 0.5)), KINDL (72.3 (SD 11.3)), and KID-KIDL (74.7 (SD 10.0)). (3) MANOVA test (comparison of patients stratified by prior treatment, main effect (group) interactions (group-by-time)): MPH naïve patient greatest improvements (as effects size) in CGI-ADHD-S, FFB-ADHD-P & FFB-ADHD-T, no differences in DAYAS, and MPH naïve & IR-MPH once daily greatest improvements in KINDL when compared to the other patients-groups. All groups: Discontinuation due to ARs 26 (3.1 %)
10 # (3)	*Johnson et al.* 2013[19]	Child and adolescent psychiatry clinics in Eastern region of the Republic of Ireland. Screened N = 132 Included n = 77	-	A: MPH naïve	[4-15] 66 boys (85.7%) 8.0 (SD 2.6)	Naturalistic prospective study.	DSMIV CAPA CHATTI 7 IN (9 %), 7 Hyp/Im (9 %), 63 Com (82 %).	CPTRS-R:S, Parent rated BSSERS, ARs as a categorical variable Weight Blood or saliva sample for DNA	6 FU: 6, 24, 1 year.	MPH 0.6 mg/kg/day (SD 0.2) IR-MPH (n = 45) 0.54 mg/kg/day (SD 0.2).	Examine the influence of *CES1* genetic variants (1) end-dose of MPH (2) clinical response (3) ARs		(1) No effect of the SNPs of *CES1* on end-dose of MPH (with and without baseline covariates) (2) Improvements on CPTRS-R:S ($P < 0.001$) and no difference between sex. No genetic variants of *CES1* was associated to the clinical response (3) ARs: 50 reduced appetite (66 %), 26 weight loss (34 %), 38 headache or abdominal pain (50 %), 35 irritability (46 %), 29 sadness (38 %), 25 insomnia, new onset (33 %), 23 insomnia, exacerbation (30 %), 2 tics, new onset (3 %), 5 tics, exacerbation (7 %). High end-dose of MPH was associated to reduced appetite and weight loss, and low age were associated to sadness. rs2244613 (A/A) ($P = 0.0015$) and rs2002577 (G/G) ($P = 0.0003$) were both associated to sadness (in sample of n = 45).
11 #	*Bruxel et al.* 2015[20]	Outpatients from Child and Adolescent psychiatry, Hospital De Clinicas De Porto Alegre in Brazil. Included n = 523 and n = 172	+ n = 132, [6-18] unaffected controls	A: MPH naïve	[4-17] 403 (77.3 %). 10.7 (SD 3.2)	Naturalistic prospective clinical study with a control group.	DSMIV K-SADS-E n = 523 (the study population is a subgroup, at data should be similar). 206 In (39.4%), 26 Hyp/Im (5.0 %), 275 Com (52.6 %), 13 Sub (2.5 %).	Parent rated SNAP-IV, Blood sample for *LPHN3.*	12 FU: 4, 12.	IR- MPH twice or more times a day. 0.6 mg/kg/day (SD 0.2).	Association between *LPHN3* variants and 1) ADHD diagnose (n = 523) and controls (n = 132) 2) MPH response (SNAP-IV) (n = 172). No definition of MPH response.		SNPs from rs6813183, rs1355368 and rs734644 were associated to the ADHD diagnose ($p = 0.02$) Mixed effect models (figures) showed SNPs had a significant faster treatment response (see article).
12 #	*Pasini et al.* 2013[21]	Outpatient from Division of Child Neuropsychiatry at Tor Vergata Hospital in Rome in Italy. Included n = 108 Completed n = 99	- But eight weeks after MPH withdrawal.	A: MPH naïve	[7-15] 108 (100%) 9.9 (2.0)	Longitudinal open label trial	DSMIV K-SADS-PL Clinician rated ADHD-RS 108 Com (100 %)	Clinician rated ADHD-RS, CPTRS-R:S, C-GAS, ToL, CPT, WM, DNA for genotyping *DAT* 3'UTR VNTR.	24. FU: 4, 8, 24, and 8 weeks after MPH withdrawal.	IR-MPH. 0.5 mg/kg/day. No exact mean dose.	Evaluate the effect of MPH on neurocognitive functions	Relationship between *DAT* 3'UTR VNTR genotypes and the neurocognitive response to MPH	No differences at baseline between neurocognitive performance and *DAT* 3'UTR VNTR genotypes (*DAT* 10/10, *DAT* 9/10, *DAT* 9/9). 10/10 *DAT* was associated to improvements of CPT and WM compared to *DAT* 9/10 and *DAT* 9/9 which might indicate an increased expression level of dopamine transporter and mediates the response to MPH. No separate summery of the psychometric measurement over time

13	*Gerwe et al.* 2009[22]	Outpatients from pediatric departments. Included n = 306 Completed n = 263	-	C: Mixed patients. MPH naïve: n = 231 (75.5 %) Switching-group from IR-MPH to OROS-MPH n = 75 (24.5 %)	[6-14] years MPH naïve 241 (78.7 %) 9.7 (SD 2.2)	Prospective open-label single-arm non-interventional study.	ICD10 MPH naïve n = 231. Some patients had more than 1 diagnose: 181 of F90.0 (78.7 %), 71 of F90.1 (30.7 %), 0 of F90.8 (0.0 %).	QoL, CGI-S, CGI-C, Clinical somatic measurements, ARs as quality of sleep and appetite etc.	8. FU: 1, 2, 8.	OROS-MPH MPH naïve 27.8 mg/day (SD 10.8)	Tolerability and effectiveness of ORES-MPH. Change of functioning in four areas of life (school, recreational area, family life and peer interaction) in QoL. Response rate: ≤ 2 CGI-C Remission rate: ≤ 2 CGI-S		Response rate higher in the MPH naïve group than in the switch treatment group (74.7 % vs 64.9 %). No differences in remission rate in the two groups. QoL improvements in 70.6 % (switch treatment group) and 84.0 % (MPH naïve group). Improvements on QoL ($p < 0.0001$); School (-1.3 (SD 1.3)), Recreation (-0.8 (SD 1.3)), family life (-1.1 (SD 1.5), and peer interaction (-0.7 (SD 1.3)). 319 ARs reported by 160 patients (52.3%) 37 discontinued due to ARs (12.1 %), insomnia (10.8%), anorexia (7.8%), ineffectiveness of medication (7.8%), headache (5.6%). SARs in two patients (family stress, loss of consciousness, tremor, and decreased activity) Weight and blood pressure in the articles.
14 # (1) *Kereszturi et al.* 2007[23] * (2) *Kereszturi et al.* 2008[24] (3) *Nemoda et al.* 2009[25] * = Results without MPH outcome		Inpatients from the Vadaskert child and Adolescent psychiatric clinic in Budapest in Hungary. (1) Included n = 173 (2) (3) Included (drug free period) n = 122	+ n = 284 (sex-matched)	B: Drug free period at time of baseline	108 (88.5 %), 9.6 (SD 2.6)	Prospective case-control study.	DSMIV MINI-Kid CBCL ADHD-RS N = 173 22 In (13 %), 26 Hyp/Im (15 %), 125 Com (72 %).	ADHD-RS (unknown rater) CGI-S	24. FU: 4, 8, 12, 16, 20, 24.	MPH. 0.6 mg/kg/day (SD 0.2) Responder: 0.6 mg/kg/day (SD 0.2) Nonresponder 0.5 mg/kg/day (SD 0.1).	(1) Association between ADHD diagnose and DRD4 promotor. Association between responders and genetic markers: (2) SNPs of Catechol-O-Methyltransferase Val158Met, and (3) SNPs of Carboxylesterase 1 (*CES1*).	Responder; ≥ 25 % decrease of sum score in ADHD-RS in six months and ≤ 2 CGI-S in two consecutive months. Non-responder; ≤ 10 % decrease of sum score in ADHD-RS in the first three months.	(2) (3) 90 responders (73.8 %), 32 nonresponders (26.2 %). No differences between the two groups (comorbity, ADHD type, demographic variables). (2) Good association between Val-allele or Val-Val genotype and the responders ($P = 0.009$ and $P = 0.034$). (3) No association between responder/nonresponder and any SNPs of *CES1*. Five responders with 143Glu-variant had a lower end-dose ((0.4 mg/kg/day (SD 0.1)) than re rest of the responders ($P = 0.022$)
15 #	*Zeni et al.* 2007[26]	Outpatients from Child and Adolescent Psychiatric Devision, Hospital de Clinicas de Porte Alegre, Rio Grande do Sul, Brazil. Included n = 111 Completed n = 106	-	A: MPH naïve	[4-17] n = 106 82 (77.4 %) 10.7 (SD 3.1)	Prospective clinical cohort study.	DSMIV No information about ADHD subtypes.	Parent rated SNAP-IV (baseline score > 1) CGAS (baseline score < 70), BSSERS, Blood sample.	4 FU: 4.	IR-MPH. 0.5 mg/kg/day (no SD)	Association between the response to MPH (SNAP-IV) and polymorphism dopaminergic (*DRD4*, *DAT1*) or serotonergic (*HTR1B*, *HTR2A*, *5-HTT*) candidate genes. No definition of response/worse response	CGAS, BSSERS.	No results of CGAS or SNAP-IV sum scores, but improvement of SNAP-IV ($p < 0.001$). No association between response (sum score or subscale sum score of SNAP-IV or CGAS) or ARs or worse response and polymorphisms/genotype.
16 (4)	*Garcia et al.* 2009[27]	Outpatients from Child and Adolescent Psychiatric Devision, Hospital de Clinicas de Porte Alegre, Rio Grande do Sul, Brazil. Screened N = 348 Included n = 280	+ n = 204	A: MPH naïve	[4 -17] ADHD-ANX n = 204; 156 (76.5 %), 10.2 (SD 3.0). ADHD+ANX; n = 76 55 (72.4 %) 9.9 (SD 3.0).	Prospective naturalistic study (quasi-experimental).	DSMIV K-SADS-E No information about ADHD subtypes.	Parent rated SNAP-IV, socioeconomic status (clinical interview), WISC III.	4 FU: 4.	IR-MPH. ADHD-ANX: 0.5 mg/kg/day (SD 0.2). ADHD+ANX: 0.5 mg/kg/day (SD 0.1).	Assess different treatment responses to MPH in children and adolescent with ADHD with and without anxiety (ANX).		SNAP-IV decrease of ≥ 50 %: 27.6 % of ADHD+ANX 32.8 % of ADHD-ANX, no difference between the groups ($P = 0.47$).
17	*Su et al.* 2015[28]	11 centers in China. Screened N = 248 Included n = 239 Completed n = 205	-	C: Mixed patients. MPH naïve or a drug free period of six months.	[6-16]. 203 (84.9 %) 9.2 (SD 2.1).	Prospective single arm open-label study.	DSMIV K-SADS 82 In (34.3 %), 9 Hyp/Im (3.8 %), 146 Com (61.1 %), 2 Sub (0.8 %).	Parent rated SNAP-IV, Clinician rated CGI-I, BREIF, Parent rated W-firs, ARs	24. FU: 1, 2, 3, 8, 12, 16, 20, 24.	OROS-MPH. 8 weeks; Remission: 35.7 mg/day (SD 12.9), Non-remission: 39.8 mg/day (SD 17.2). 24 weeks; Remission: 110.9 mg/day (SD 40.0), Non-remission: 115.3 mg/day (SD 49.5).	Remission rates correlated to executive and social functioning. Remission rates on SNAP-IV: 1: Single scores < 1: - Item 1-9, for In. type, - Item 10-18, for Hyp/im. type, - Item 1-18, < 1 for com. type.	Change of SNAP-IV, CGI-I, Brief, W-firs in week 8 and 24	8 weeks of treatment (tables of ADHD subtypes in the article): Remission 1: 151 in remission (69.3 %), 67 in non-remission (30.7 %) Remission 2: 161 in remission (73.2 %), 59 in non-remission group (26.8 %). 24 weeks of treatment: Remission 1: 158 in remission (72.5 %), 60 in non-remission (27.5 %). Remission 2: 171 in remission (77.7 %), 49 in non-remission (22.3 %).

											2: Sum score for item 1-18 < 18 for all ADHD subtypes.		44-50% of CGI-I had "much improved" after 8 weeks. At week 8 and 24 the remission group had lower scores at CGI-I, BRIEF, and Weiss ($P < 0.001$). 16 discontinued due to ARs (6.7 %)
18 (5)	*Panton et al.* 2014[29]	Child and adolescent psychiatry clinics in Eastern region of the Republic of Ireland. Included n = 51 Completed n = 50	n = 35 sex, age and left-handed matched	A: MPH naïve	6-14 years old. 43 (84.3 %). 8.4 (SD 2.4).	Prospective study with controls.	DSMIV 4 In (8 %), 3 Hyp/Im (6 %), 44 Com (86 %).	Wisc-IV CPRS-R:S TEA-Ch	6. FU: 6.	MPH-based treatment. 0.6 mg/kg/day (SD 0.2)	To investigate the effects of MPH administration on performance on al subtests of TEA-Ch with a group of MPH naïve children with ADHD and a sex and age comparable control group.		Improvement of CPRS-R:S ($P < 0.001$) (index score from 76.8 to 58.1). CPRS-R:S (index score) was higher at baseline and in week 6 in the MPH naïve group than in the control group ($P < 0.001$). Five main results of TEA-Ch in the article. The beneficial effect on only some measures of selective and sustained attention and attention control performance. MPH can aid the performance on certain tasks all though there was no deficit the in the drug naïve status.
19 # (10)	*Bruxel et al.* 2013[30]	Outpatients from Child and Adolescent psychiatry, Hospital De Clinicas De Porto Alegre in Brazil. Included n = 213 Completed n = 205	-	A: MPH naïve	[4-17] T homozygous, n = 129 101 (78.3 %). 10.4 (SD 2.9) G-allele carriers, n = 76 50 (65.8 %). 10.0 (SD 3.1)	Prospective study.	DSMIV K-SADS-E n = 129: 35 In (27.1 %), 11 Hyp/Im (8.5 %), 71 Comb (55.0 %), 12 Sub (9.3 %). n = 76 22 In (28.9 %), 3 Hyp/Im (3.9 %), 46 Com (60.5 %), 5 Sub (6.6 %).	Parent rated BSSERS, Blood samples.	12. FU: 1, 3.	MPH twice daily. approximately 1.0 mg/kg/day.	Evaluate the association between *CES1* (-75 T>G) and MPH induced reduced appetite in children and adolescents with ADHD. Mild reduced appetite: BSSERS = 1-5 Severe reduced appetite: BSSERS = 6-9		T homozygous, n = 129, G-allele carriers, n = 76. Genotype frequency: 0.63 for T/T, 0.35 for G/T, and 0.02 for GG. 10 T homozygous (13.0 %) and 14 (34.1 %) with reduced appetite. Mixed-effect model (full model in supp.) showed G allele carriers present a higher risk of appetite reduction compared with *T allele homozygotes* (odds ratio = 3.49, $P < 0.01$). *CES1* (-75 T>G) might have influence on appetite reduction in children treated with ADHD.
20 # (11)	*Salatine-Oliveira et al.* 2011[31]	Outpatients from Child and Adolescent psychiatry, Hospital De Clinicas De Porto Alegre in Brazil. Screened N = 251 Included n = 134 Completed n = 112	-	A: MPH naïve	[4-17]. 112 (100 %). Val homozygous: n = 35 9.0 (SD 2.9) Met carriers: n = 77 9.9 (SD 2.9)	Prospective study	DSMIV K-SADS-E n = 35: 2 In (5.7 %), 6 Hyp/Im (17.1 %), 25 Com (71.4 %), 2 Sub (5.7 %). n = 77: 11 In (14.5 %), 6 Hyp/Im (7.9 %), 55 Com (71.0 %), 5 Sub (6.6 %).	Oppositional subscale (ODD) on the parent rated SNAP-IV	12 FU: 4, 12.	IR-MPH. 0.7 mg/kg/day (no SD information)	Evaluate any association between the effect of MPH on ODD) and *COMT* Val158Met. No definition of response but baseline ODD score > 1.		Val homozygous, n = 35, Met carriers n = 77. Genotype frequency: 0.32 for Val/Val, 0.50 for Val/Met, and 0.18 for Met/Met. Improvements of ODD ($P = 0.005$, no information of study-end score). Effect of treatment over time for Met carriers ($P = 0.027$) Interaction of treatment over time and baseline ODD score ($P < 0.001$). Mixed effect model (full model in supp.) showed an interaction between Met carriers and improvement of ODD ($P = 0.002$)
21 # (11)	*Kim et al.* 2013[32]	Outpatients from Child and Adolescent department of Hallum University Sacred Heart Hospital in Anyang and CHA University hospital in Seongnam in Korea. Screened N = 154 Included n = 147 Completed n = 134	-	A: MPH naïve	[6-16] n = 134: 112 (83.6 %). 10.3 (SD 2.9) Case-control of mean score on BSSERS: 1) < 1.5 SD, n = 122 2) > 1.5 SD, n = 12	Prospective open label case control study.	DSMIV K-SADS-PL n = 134: 23 In (17.2 %), 2 Hyp/Im (1.5 %), 102 Com (76.1 %), 7 Sub (5.2 %).	Parent rated BSSERS, Parent rated K-ARS, CGI-S.	4. FU: 1, 2, 4.	18 mg OROS-MPH 0.9 mg/kg/day (SD 0.3)	Association between ARs (BSSERS) and *ABCBI* SNPs. Five SNPs were selected.	Association between ARs (BSSERS) and *ABCBI* SNPs as *ABCBI* transport activity by using membrane vesicle assay.	Mean end-score of BSSERS 11.3 (SD 14.1) with no association to end-dose ($P = 0.633$). 82.8 % of all patients experienced at least one ARs. Poor appetite (56.7 %), trouble sleeping (41.8 %), talking little with others (22.4 %), irritability (20.9 %). See supp. for further information. T/T predicted high mean BSSERS score compared to the G-carrier of rs2032582 ($P = 0.008$, odds ratio = 5.72)

22 # (6)	*Song et al.* 2014[33]	3 child and adolescent clinics of university hospitals in South Korea. Included n = 139	-	A: MPH naïve	[6 -18] 120 (86.3 %). 10.0 (SD 2.8)	Prospective study.	DSMIV K-SADS-PL 28 In (20.1 %), 3 Hyp/Im (2.2 %), 102 Com (73.9 %), 6 Sub (4.3 %).	Parent rated K-ARS, CGI-I, Blood sample.	8. FU: 8.	OROS-MPH 31.0 mg/day (SD 9.2)	Evaluate any association between five SNPs of *SNAP-25*, *SLC6A2*, and *LPHN3 SNPs* and OROS-MPH treatment response. Responder: (1) Improvement > 50 % on K-ARS (2) CGI-I score of 1 or 2.		K-ARS: 99 Responders (72.2 %), 40 Non-responders (28.8 %). CGI: 110 Responders (79.1 %), 29 Non-responders (20.9 %). K-ARS and CGI-I: 90 Responders (64.7 %), 49 Non-responders (35.3 %). An association between *SNAP-25* SNPs and responder of K-ARS (P = 0.034), and responder of K-ARS and CGI-I (P = 0.018) but no association to responder of CGI-I. (P = 0.083) An association between *SLC6A2* SNPs and responder of CGI-I (P = 0.009). No significant results when adjusted for multiple comparison.
23	*Huang et al.* 2012[34]	Child and Adolescent Psychiatry Outpatients Department of Chang GungChildren's Hospital in Taiwan (Linkou) Included n = 103 Completed n = 75.	-	C: Mixed patients. MPH naïve or a six months drug-free period.	[6-16]. 82 (79.6 %). 9.1 (SD 1.9)	Prospective study.	DSMIV K-SADS-E. n = 103: 54 In (52.4 %), 5 Hyp/Im (4.9 %), 44 Com (42.7 %).	TOVA	1 year FU: 24, 1 year.	IR-MPH 19.8 mg/day (SD 13.2)	Improvements in TOVA during one year of treatment with MPH. Evaluate difference in gender, age groups and ADHD subtype.		Only commissions of errors improved (P = 0.008). Mixed effect model showed: <9 years performed better in response time (P = 0.020), response time variability (P = 0.013), response sensitivity and ADHD score (P = 0.006) but improved less than older patients in response time (P = 0.007). Boys improved more in omission error (P = 0.022) and response sensitivity (P = 0.017) than girls. ADHD subtypes significantly differed in ADHD scores (P = 0.029). All TOVA indices had no effect of interaction between MPH and ADHD subtypes
24 #	*Park et al.* 2015[35]	Child and adolescent psychiatry clinics of two university hospitals in Korea. Included n = 131 Completed n = 114	-	A: MPH naïve.	[6-15]. 95 (83.3 %) 9.1 (SD 1.9)	Prospective study	DSMIV K-SADS-PL 53 In (46.5 %), 12 Hyp/Im (10.5 %), 49 Com (43.0 %).	Clinician rated CGI-S, Clinician rated CGI-I, Parent rated K-ARS, Blood samples for SLC6A4, Parent rated ARs.	8 FU: 1, 2, 4, 6, 8.	MPH. 29.8 mg/day (SD 7.6).	Relationship between SLC6A4 and response to MPH. Responder: >50% decrease in K-ARS and CGI-I ≤ 2.		SLC6A4 genotype frequency: 70 S/S (60.9 %), 40 S/L (34.8 %), and 4 L/L (3.5 %). CGI-I: 46 S/S Responders (65.7 %), 25 S/L + L/L Responders (56.8 %). K-ARS: 44 S/S Responders (62.9 %), 24 S/L + L/L Responders (54.5 %). No significant difference between SLC6A4 genotypes and responders or end-dose. Difference of new onset/aggravation of tics with 67 in S/S (87.1 %) and 20 in S/L + L/L (45.5 %) (P < 0.001). 15 discontinued due to ARs.
25	*Lee et al.* 2007[36]	12 child and adolescent clinics in South Korea Included n = 119 Completed n = 110	-	C: Mixed patients. MPH naïve or a six months drug-free period.	[6 -13] 107 (90 %) 8.5 (SD 1.6)	Multi-center, open-label, dose adjustment of OROS-MPH.	DSMIV No information about subtype. Included any subtype of AD/HD	Parent rated IOWA Conners, Teacher rated IOWA Conners, SNAP-IV (rater?) (peer interactions rating scale, six items from SNAP-IV) Clinician rated CGI-I, Clinician rated CGI-S, ADS (visual version), MFFT, VFT, CTT, ARs (the study made their own questionnaire), BP, ECG, HR, weight, ARs spontaneously reported.	3. FU: 1, 2, 3.	OROS-MPH 0.9 mg/kg/day (SD 0.3).	Effects of OROS-MPH on behavior and function measured on (1) IOWA Conners, (2) SNAP-IV, (3) CGI-S, (4) CGI-I.	Effects of OROS-MPH on cognitive function measured with (5) CPT (6) MFFT, (7) VFT, (8) CTT. ARs questionnaire.	Improvement of IOWA (I/O and O/D) parent and teacher rated and CGI-S (P < 0.001). CGI-I 15 were *very much* improved (14 %) and 60 were *much improved* (54 %). Improvements of all cognitive test (P < 0.05 to P < 0.001) apart from TMT 45 (37.8 %) of reported at least 1 mild/moderate ARs. 2 discontinued due to ARs. ARs: anorexia (26.1 %), insomnia (21.7 %), headache (14.5 %), vomiting (7.2 %), and abdominal pain (5.8 %), followed by tics (5.8 %), fatigue (4.3 %), nausea (4.3%), dizziness (4.3 %), rash (3.9 %), dyspepsia (1.4 %), and nervousness (1.4 %).
26 # (2)	*Park et al.* 2014[37]	6 child and adolescent clinics in Korea. Included n = 96	-	A: MPH naïve	[6-12] 79 (82.3 %) 8.7 (SD 1.4)	Prospective multicentre clinical study study.	DSMIV-TR K-SADS-PL n = 95 26 In (27.1 %), 5 Hyp/In (5.2 %), 60 Com (62.5 %).	Clinician rated BSSERS. Subtracted baseline-score each visit.	2. FU: 2.	OROS-MPH 18 mg < 30 kg 27 mg > 30 kg	To evaluate the association between SNPs of NTF3 and ARs.		No information of overall sum score or single of BSSERS Genotype frequency of rs6332 was: 34 G/G (35.4 %), 42 G/A (43.8 %), 20 A/A 20.8 %)

								Blood samples for neurotrophin 3 (NTF3) genotype					Genotype frequency of rs1805149 was: 21 G/G (21.9 %), 50 G/A (52.1 %), 25 A/A 26.0 %) A/A at rs6332 was associated to *emotionality*, *over-focus/euphoria*, *nail biting*, and *proneness to crying* (*P* = 0.017 to 0.047)
27 # (7)	*Kim et al.* 2016[38]	Child and adolescent psychiatry clinic, Soul National University Hospital. Screened N = 110 Included n = 86 Completed n = 75	-	A: MPH naïve	[6 -17] n = 86 72 (83.7 %). 8.8 (SD 2.2)	Sample of two prospective studies: An open label 8-week study (n = 43) and a 2-year prospective study (n = 67).	DSMIV K-SADS-PL n = 75: 24 In (32 %), 3 Hyp/Im (4 %), 39 Com (52 %), 9 Sub (12 %).	Parent rated K-ARS, Clinician rated CGI-I, Clinician rated CGI-S, CPT.	24. FU: (2), (4), (6), 8, 16, 24. (only some patients followed these weeks)	MPH 28.1 mg/day (SD 12.3) 0.7-1.5 mg/kg/day (no SD)	Association of the SNPs GRIN2B (rs2284411) or GRIN2A (rs22229193) and good response of MPH. Responder: (1) ≥ 40 % K-ARS (1-9, 10-18, 1-28), (2) CGI ≤ 2, (3) ≥ 40% K-ARS (1-9, 10-18, 1-28) and CGI ≤ 2.	Changes in (4) ADHD-RS score (5) CPT score.	Improvements of K-ARS (*P* < 0.001) Responders: 38 (50.7 %) of (1), 37 (49.3 %) of (2), 19 (25.3 %) of (3). C/C (54 %) at rs2284411 of *GRIN2B* was associated to: (1) (*P* = 0.009-0.028), (2) (*P* = 0.009), (4) *P* = < 0.001-0.007, (5) variability of response time (*P* < 0.001). G/G (92 %) at rs22229193 of *GRIN2A* was associated to: (5) commission errors (*P* < 0.001).
28 (8)	(1) *Park et al.* 2013[39] (2) *Kim J-W et al.* 2013[40]	Soul National University hospital and other hospitals. Included n = 132 Completed n = 119.	-	A: MPH naïve	[6-12] n = 132 108 (81.8 %) 8.8 (SD 1.5)	Multicenter open-label 12-week trial.	DSMIV KADS-PL n = 131: 39 In (29.8 %), 6 Hyp/In (4.6 %), 78 Com (59.5 %), 8 Sub (6.1 %)	(1) (2) Parent rated K-ARS, (1) Clinician rated K-ARS, (1) Parent rated APRS, (1) Parent rated BDI, (1) Parent rated PSI, (1) Clinician rated CGI-S, (1) Clinician rated CGI-I, (1) CAT, (1) Clinician rated BSSERS, (1) Somatic assessments, (2) CPT.	12. FU: 2, 4, 8, 12.	OROS-MPH 18mg < 30kg 27mg > 30kg 1.0 mg/kg/day (SD 0.3)	(1) Parental response to OROS-MPH. (2) Association between baseline characteristics and subjective (K-ARS) and objective (CPT) response to OROS-MPH: (A) ≥ 50% K-ARS, (B) ≤15% in the mean composite score of CPT, (C) no (A) and no (B), (D) both (A) and (B).	(1) Clinician rated response to OROS-MPH. (E) Clinician K-ARS ≤ 18 and CGI-I of 1 or 2 (1) Objective neuropsychological response to OROS-MPH. (1) ARs	(1) Improvements of parent and clinician rated K-ARS and CGI-S (*P* < 0.001). 91 responders (76.5 %) of (E). Improvements of APRS, BDI, CAT, and PSI (*P* < 0.001 to *P* = 0.025). Responders were associated to APRS (academic success subscale (*P* = 0.0018). Changes of PSI scores were positively associated to parent rated K-ARS and negatively associated to changes of APRS. (2) Responders: (A) 29 Responder (24.4%) (B) 18 Responder (15.1 %) (C) 43 Nonresponder (36.1 %) (D) 29 Responder (24.4 %) Nonresponders (C) were older, had higher end-dose of MPH, baseline CPT of omission errors and variability of response time were higher compared with responder (B) (*P* = 0.012-0.049). High inattention baseline K-ARS was associated to higher subjective response in (A). Low inattention baseline K-ARS were associated to higher subjective response in (B). Low baseline omission errors and variability of response time on CPT were associated to (A), (B), and (D). Six patients (4.5 %) discontinued due to ARs (two of insomnia, one of reduced appetite, one of tics, one of rash, and one of tremor).
29 (5)	*Tzang et al.* 2012[41]	Nine centers in Taiwan: Mackay Memorial-; Dr Enherya-; Tri-Service General-; Taiwan Adventist-; Chang Gung Memorial-; National Kaohsiung University-; Taipei Municipal Gan-Dau-; Changhua Christian; Lin Shin Hospital. Included n = 757.	(-) Naturalistic study, medication differed.	C: Mixed patients. MPH-free period of four weeks.	[6-18]. 607 (80.2 %) 10.1 (SD 2.7)	Naturalistic observational prospective study	DSMIV 278 In (36.7 %), 36 Hyp/In (4.8 %), 443 Com (58.5 %).	Parent rated SNAP-IV, Clinician rated SNAP-IV, Clinician rated CGI-ADHD-S, CSAIICA, Parents rated adherence to treatment	48. FU: 4, 12, 24, 36, 48	Naturalistic study: (1) OROS-MPH (n = 265), (2) IR-MPH (n = 293), (3) OROS-MPH +IR-MPH (n = 129), (4) no medication (n = 70). OROS-MPH: 30.2 mg/day (SD 10.8) IR-MPH: 19.4 mg/day (SD 11.4).	To compare the effectiveness of IR-MPH and OROS-MPH. Remission rate: ≤ 1 on each SNAP-IV item or ≤ 2 on CGI-ADHD-S. Recovery rate: A remission rate lasting > 12 weeks.		Remission rate (SNAP-IV): OROS-MPH group > IR-MPH group (*p* = 0.0003). Remission rate (CGI-ADHD-S): OROS-MPH group > IR-MPH group (*p* = 0.0053). Recovery rate (SNAP-IV): OROS-MPH group > IR-MPH group (*p*=0.0016). Recovery rate (CGI-ADHD-S) OROS-MPH group > IR-MPH group (*p*=0.015). CSAIICA: OROS-MPH group > IR-MPH group (*P* < 0.0001). Adherence to treatment: OROS-MPH group > IR-MPH group (*P* < 0.0001). Decrease appetite: 95 in OROS-MPH (35.8 %) group and 63 in the IR-MPH group (21.4 %).

30 # (9)	(1) *Pagerols et al.* 2018 [42] (2) *Pagerols et al.* 2017[43]	Spanish of Caucasian origin (1) n = 173 (2) n = 107	N = 189 adult ADHD patients	A: MPH naïve	[?-18] 147 (84.9 %) 9.6 (SD 2.9)	Genome-wide association study	DSM IV K-SADS-PL n = 173 37 In (21.4 %) 5 Hyp/Im (2.9 %) 131 Com (75.7 %) n = 107 22 In (20.6 %) 6 Hyp/Im (5.6 %) 79 Com (74.0 %)	(1) (2) CGI-I (1) (2) CGI-S (2) Clinician rated BSSERS	8.	1.1 mg/kg/day (SD 0.3) Responder 1.0 mg/kg/day (SD 0.3) Nonresponder 1.1 mg/kg/day (SD 0.3)	(1) GWAS of MPH efficacy to characterize the biological pathways associated with treatment response. Responder: CGI-I of 1 or 2. (2) (A) assesses multiple SNPs covering, in terms of linkage disequilibrium, SNPs of nine dopamine candidate genes; (B) considers gene–gene interactions (C) environmental risk factors interplay with dopaminergic candidate genes in predicting the response and ARs to MPH. Responder: CGI-I of 1 or 2.	(2) Decrease of CGI-S of 2-points (2) BSSERS	(1) 141 (81.5 %) Responder 32 (18.5 %) Nonresponder No differences in age, sex, ADHD subtype, comorbidity, MPH formulation, concomitant medication between responders and nonresponders ($P > 0.05$). CGI-S baseline score was lower in nonresponders than in responders ($P = 0.024$). (2) 84 Responder (78.5 %) of CGI-I Nonresponders (CGI-I and CGI-S) were associated to C/C at rs2070762 (*Tyrosine hydrolase, TH*) ($P = 0.0.55 – 0.0071$). Responders were also associated other SNPs of *TH*, dopamine beta-hydroxylase (*DBH*), and dopamine D2 receptor (*DRD2*). 69 patients (65.1 %) with ARs; insomnia (34.3 %), reduced appetite (25.0 %). High end-dose ($P = 0.005$) and comorbidity ($P = 0.030$) were associated to ARs. Variants of SNPs of *DHB* ($P = 0.0064$) and *DRD2* ($P = 0.0047$) were associated to ARs.

ARs = Adverse reactions, MPH = methylphenidate, IR-MPH = immediate release, MR-MPH = modified release, OROS-MPH = Concerta, SNPs = Single Nucleotide Polymorphism

ADHD subtype DSMIV: In = Inattention, Hyp/im = Hyperactivity/Impulsivity, Comb = combined, Sub = Subthreshold
ADHD ICD10: disturbance of activity and attention (F90.0), hyperkinetic conduct disorder (F90.1), other hyperkinetic disorders (F90.8)

Study populations and outcomes:

(1) Mph naïve; Outcome of beneficial effects
(2) Mph naïve; Outcomes both beneficial effects and ARs
(3) Mph naïve; Outcomes of ARs
(4) Mph free at the time of baseline; Outcome of beneficial effects
(5) Mph free at the time of baseline; Outcomes both beneficial effect and ARs

Articles of MPH naïve patients were included in the final review of included studies (green row, S2 Table in paper I). Some articles were not included in the final review because there were not enough data or only data of subgroups e.g. pharmacogenetic subgroups.
Table B in Appendix A for abbreviations and definitions of psychometric measurements.
= Pharmacogenetic studies.

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Appendix B

1) **INDICES Protocol work package 6**
2) **INDICES Protocol**

The relationship between CES1 genotype and methylphenidate response in children with ADHD – INDICES work package 6
Protocol version 3

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INDICES: (Individualised drug therapy based on pharmacogenomics: Focus on carboxylesterase1)
SUMMARY.
We wish to examine the relationship of Carboxylesterase 1(CES1) genotype with the response to Methylphenidate (MPH) in children with attention deficit hyperactivity disorder (ADHD). This will allow us to develop guidelines for individualized CES1 genotype-based MPH treatment in patients with ADHD.

The INDICES project combines pharmacology, genetics and metabolomics with clinical psychiatry and cardiology. It consists of seven work packages with focus upon carboxylesterase 1 (CES1), an enzyme with a major role in the metabolism of a variety of essential drugs including methylphenidate (MPH) and trandolapril (TA) for treatment of ADHD and cardiovascular disease (CVD), respectively.
The overall aim is to develop and implement guidelines for individualized treatments with MPH and TA and obtain a better drug response and reduce the risk of adverse reactions.

Background (Work package 6):
MPH is first-line treatment for ADHD, which affects 3-5% of children and adolescents. In most cases it persists into adulthood[1]. In Denmark the consumption of MPH has increased by a factor of 10 during the last 10 years[2]
Comorbidity is common, affecting more than 80% of patients with ADHD.
15- 20 % of patients treated with MPH are nonresponders. Adverse reactions are common, but few studies have had adverse reactions as their primary focus[3,4]. Decreased appetite is closely linked to stimulant treatment, and sleep problems occurs in approximately 70% in the initial stages of treatment. Increased blood pressure and heart rate, associated with stimulant treatment, affects a minority of treated individuals (5%). Potentially more serious cardiovascular events, however, seem to appear in stimulant treated children, with a risk, similar to the background population[5].
Discontinuation of treatment because of adverse reactions occurs in 2-10%[4]. Overall, adverse reactions in MPH treatment seem to be linked to individual underlying mechanisms, possibly related to pharmacokinetics and pharmacogenomics. Guidelines for treatment with MPH emphasize the need for individually tailored medication dosage, and further research into pharmacogenetics ,

but the clinical use of these tools is still highly variable[3,6,7] Several studies have found evidence for genetic variations in MPH response, especially variations in the dopamine transporter and dopamine D4 receptor. Variations in candidate genes, predict differences in ADHD treatment response and side effects of immediate release MPH[8-10]

Carboxylesterase 1(CES1) is a key enzyme in the metabolism of MPH, various other drugs, and endogenous lipids. Clinical and preclinical studies indicate that polymorphisms in the CES1 gene are responsible for individual variations in MPH treatment response and drug-drug interactions [11-13]. Recent data from a collaborative study supported by the European Commission (EU-FP7) have revealed duplication of the CES1 gene at frequencies of approximately 0.20 in Europe (Henrik Berg Rasmussen, unpublished). Gene duplication may give rise to increased rate of metabolism of CES1 dependent drugs.

Hypotheses:
Our main hypotheses: Individuals with a gene variant of CES1 resulting in rapid metabolic breakdown of MPH will experience less therapeutic effect and thus need higher dosages.
Individuals with a gene variant of CES 1 resulting in slow metabolizing of MPH will experience a better therapeutic effect and thus need lower dosages.
Our exploratory hypotheses: Dose-related side effects in MPH treatment will be associated with the metabolic rate of MPH breakdown, in line with its therapeutic effect.

Aims:
We aim to examine the influence of the CES1 genotype in children with ADHD on the effectiveness and the pharmacokinetics of MPH. Moreover, we aim to develop guidelines for individualised MPH therapy, based on CES1genotype by combining the results from INDICES Workpackage 2, a pharmacokinetic study of the correlation of CES1 genotype and copy number, with the pharmacokinetics of trandolapril and methylphenidate.
Implementation of guidelines for individualized pharmacotherapy will improve treatment efficacy and safety in this group of children.

Studypopulation:
200 drugnaïve children diagnosed with ADHD according to DSM-IV criteria in the age 7-12 years will be recruited from the three Child- and Adolescent Psychiatric Centers in the Capital Region of Copenhagen. To allow for a naturalistic setting, and because more than 80% of children and adolescents with ADHD have a comorbid psychiatric condition, we will only exclude patients with an IQ < 70, current serious medical or psychiatric illness (e.g. schizophrenia, bipolar disorder, untreated epilepsy, heart failure).

Inclusion criteria:
Age: 7-12 years (both inclusive)
Both sexes
DSM-IVADHD diagnosis (any subtype)
Clinical indication for methylphenidate treatment, based on national guidelines, confirmed by an ADHD-RS score +1.5 SD above mean adjusted for age and sex
Drug naïve to ADHD medication (Methylphenidate, Dexamphetamine, Atomoxetine)
Written informed consent by parents holding custody, and the child, to participate in the study

Exclusion criteria:
Medical conditions that contraindicate treatment with MPH: Cardiovascular or cerebrovascular disease, hyperthyroidism, phaeocromocytoma, glaucoma.
Psychiatric conditions that contraindicate treatment with MPH: Mania, psychosis, former severe depression, bipolar disorder not well controlled, suicidal conduct, anorexia nervosa
Diagnosis of mental retardation (IQ < 70)
Any ADHD medication ever (Methylphenidate, Dexamphetamine, Atomoxetine)
Current treatment with another CES1 related drug (Tamiflu, Trandolapril)
Current treatment with antipsychotics
Treatment with irreversible MAO inhibitors within the last 14 days before treatment start

Methods:
The psychometric instruments used in this study, for diagnostic assessment, are standard procedures for children in the age group, referred with a possible diagnosis of ADHD, in the Capital Region of Copenhagen. The staffs (Child- and adolescent psychiatrists and psychologists) who are conducting the assessments at the three study sites have all been trained in the psychometric instruments used in this study. We will measure interrater-reliability (by using kappa) for the K-SADS interviews.
The INDICES work package 6 study group includes a local child and adolescent psychiatrist, who is the responsible researcher on each of the three study sites.

Diagnostic assessment:
All children referred to the three Centers of Child and Adolescent Psychiatry in the Capital Region of Copenhagen, with a possible diagnosis of ADHD, are assessed by the following procedures:
The CBCL[14] (web-based questionnaires)
K-SADS interview[15]
Clinical assessment and diagnosis of ADHD according to Danish national guidelines, including history of development, symptoms and sociodemographics, clinical observation of symptoms, cognitive test and standard physical examination
ADHD diagnosis confirmed by K-SADS
Comorbidity diagnosed with K-SADS.
Possible cases, based on age and ADHD diagnosis, are evaluated by the local responsible researcher for inclusion in the study, and if relevant, contacted for permission and written informed consent by parent(s) holding custody, and the child, to participate in the study

Baseline ratings and measures on patients meeting inclusion criteria:
ADHD-RS [16] rated by parent
ADHD-RS rated by teacher
Investigator rated ADHD-DSM-IV-RS [17]
CGI- Severity scale [18]
Connors CPT[19] or TOVA[20](to be finally decided)
Adverse reactions-Rating Scale [21]
ASK-ME parent rated questionnaire, attitude scale (7 questions) [22]
Body weight and height
Blood pressure and pulse

DNA samples:
A sample of saliva

Patients included in the study enter a twelve-week study period with dose escalation of methylphenidate, weekly telephone ratings and monthly clinical assessments of effect and adverse reactions to MPH treatment. Weekly telephone ratings are done by the PhD. student, and the following decision on pursuing further dose escalation is made in cooperation with the child's regular psychiatrist at the study site. Monthly clinical assessments and decisions on dose escalation are done by the child's regular psychiatrist.

Weekly assessment and ratings:
(not made in week 4, 8 and 12)
By telephone: ADHD-DSM-IV-RS and Adverse Reaction-Rating Scale .
Weekly decision on pursuing further dose escalation based on the above ratings

Clinical assessment:
Every four weeks:
Measurement of blood pressure, pulse, weight and height
ADHD-DSM-IV-RS
Parent rated ADHD-RS
Adverse reactions-Rating Scale

Supplementary rating and test at week 8:
Teacher rated ADHD-RS
Blood test to measure serum concentration of Ritalin acid, and DNA

Outcome ratings after twelve weeks :
Investigator rated ADHD-DSM-IV-RS (primary outcome measure)
CGI-Severity (secondary outcome measure)
CGI-Improvement (secondary outcome measure)
CGI-Efficacy (secondary outcome measure)
Connors CPT or TOVA(secondary outcome measure)
Parent rated ADHD-RS (secondary outcome measure)
Teacher rated ADHD-RS (secondary outcome measure)
Adverse reactions-Rating Scale (secondary outcome measure)

Outcome measures:
Investigator rated ADHD-DSM-IV-RS score
Parent rated ADHD-RS score
Teacher rated ADHD-RS score
CGI-I
CGI-S
CGI-E
Connors or TOVA
MPH dose required for "Borderline normalisation" on ADHD-DSM-IV-RS: T-score 60-70
MPH dose required for normalisation on ADHD-DSM-IV-RS: T-score < 60
Occurrence (no. of weeks from treatment initiation) and type of adverse reactions
MPH dose at occurrence of adverse reaction
MPH dose at discontinuation, if that is necessary, because of adverse drug reactions or
Treatment failure (no effect of MPH on ADHD core symptoms)

Serum concentration of Ritalin acid at week 8

Body weight, height, blood pressure and pulse

Dose escalation:
MPH is initiated at a low dose: 5mg. MPH-IR three times daily (TID) and escalated weekly with 0 - 2,5 - 5mg MPH-IR TID. Maximum dose is 2mg/kg/day. MPH dose morning, midday and afternoon can be individually escalated, based on effect and adverse reactions. Decisions on dose escalation will be based on a clinical decision manual, elaborated for the study. Individual deviations in dose escalation are noted in the CRF and case records.

Dose escalation is continued on a weekly basis until one of the following:
- Normalisation on ADHD-RS defined as T-score < 60
- Borderline normalisation on ADHD-RS, if normalisation is not obtainable, defined as T-score 60-70
- Cessation of dose escalation because of adverse reactions
- Discontinuation of MPH because of either intolerable adverse reactions, or no effect of MPH on ADHD core symptoms.

On basis of this, we exploratively will categorize patients as
1) Clinically normal reponders
2) Clinically slow responders
3) Clinically rapid responders

Results:
We plan to use multivariate regression models with the change in symptom severity, measured with the ADHD-DSM-IV rating scale score as the dependent measure, whereas CES1genotype, weight adjusted MPH dosage (mg/kg/day), age, gender, occurrence of adverse reactions and ADHD subtype will serve as predictors.
We will repeat the analysis with CGI-I as the dependent measure.
This will allow us to assess whether MPH is less effective in subjects with CES1 gene variants, that are known to have a rapid metabolic breakdown of MPH, whether higher dosages of MPH, necessary to adapt to the genetic differences in these subjects, are associated with more side effects, whether subjects with CES1 gene variants that are known to have a slow metabolic breakdown of MPH, need lower dosages of MPH than expected, to have effect on ADHD core symptoms, and whether lower dosages of MPH, necessary to adapt to the genetic differences in these subjects, are associated with less side effects.
By combining our results with data from the pharmacokinetic work-package in the INDICES study, we will develop guidelines for individualised therapy of ADHD with MPH

Ethics
The clinical work package 6 focused on ADHD is covered by existing permissions from the local committees on scientific ethics (De Videnskabsetiske Komiteer for Region Hovedstaden) and the

Danish Data Protection Agency (Datatilsynet). Those permissions allow the collection and examination of data as well as biological samples from psychiatric patients with ages below 18 years, for research purposes, including genetic research projects. Journal number: H-B-2009-026

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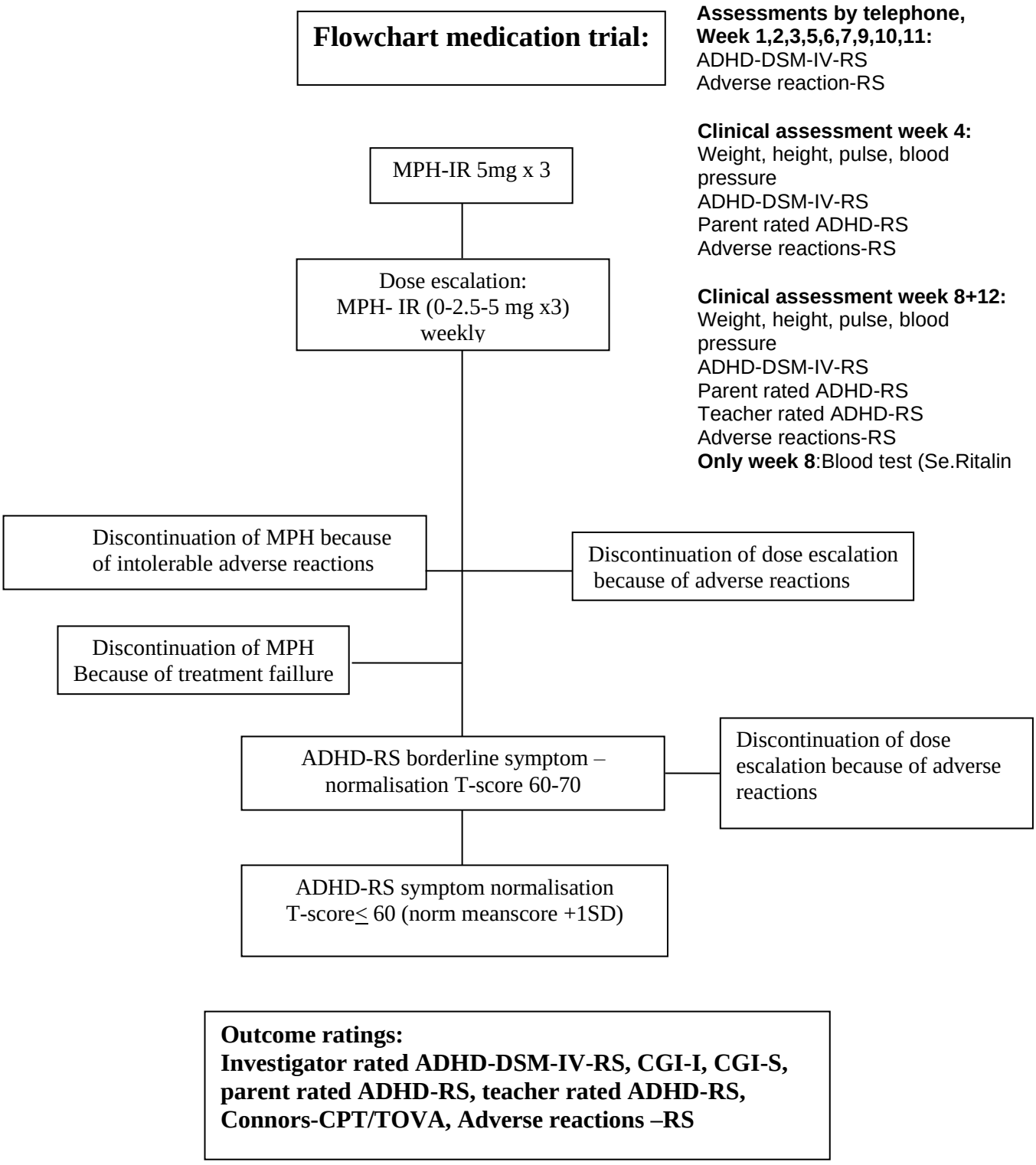
Milestones
2012: Month 1-2: planning and introduction
2012: Month 3-12: recruitment of 100 patients who will complete a 3-month study period of drugescalation
2013: Month 13-24: recruitment of 100 additional patients who will complete a 3-month study period corresponding to a total of 200
2014: Month 25-29: Finalization of entire clinical study. Data analysis. Preparation and submission of two manuscripts.
2014: Month 30-36: study visit at University of Nijmegen
2015: Month 37-39: Statistical treatment of data and elaboration of manuscripts. Advices for improved stimulant therapy developed
2015: Month 40-42: Preparation of PhD thesis. (Month 29-42: preparation of PhD thesis)


Flowchart medication trial:
Assessments by telephone, Week 1,2,3,5,6,7,9,10,11:
ADHD-DSM-IV-RS
Adverse reaction-RS
Clinical assessment week 4:
Weight, height, pulse, blood pressure
ADHD-DSM-IV-RS
Parent rated ADHD-RS
Adverse reactions-RS
Clinical assessment week 8+12:
Weight, height, pulse, blood pressure
ADHD-DSM-IV-RS
Parent rated ADHD-RS
Teacher rated ADHD-RS
Adverse reactions-RS
Only week 8:Blood test (Se.Ritalin
MPH-IR 5mg x 3
Dose escalation: MPH- IR (0-2.5-5 mg x3) weekly
Discontinuation of MPH because of intolerable adverse reactions
Discontinuation of dose escalation because of adverse reactions
Discontinuation of MPH Because of treatment faillure
ADHD-RS borderline symptom – normalisation T-score 60-70
Discontinuation of dose escalation because of adverse reactions
ADHD-RS symptom normalisation T-score≤ 60 (norm meanscore +1SD)
Outcome ratings:
Investigator rated ADHD-DSM-IV-RS, CGI-I, CGI-S, parent rated ADHD-RS, teacher rated ADHD-RS, Connors-CPT/TOVA, Adverse reactions –RS

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INDICES: INDIvidualised drug therapy based on pharmacogenomics: focus on carboxylesterase 1 (CES1)

1. SUMMARY

To a large extent drug treatment is still based upon the one-size-fits-all principle. This is unfortunate as there are large individual variations in the response to many drugs. Often a significant fraction of patients receiving a drug do not experience any benefit from the agent; others develop severe adverse effects. This project proposes the application of a new strategy that combines pharmacological, genetic and metabolomic sciences to improve pharmacotherapy of important mental and somatic disorders. It focuses upon carboxylesterase 1 (CES1), a key enzyme in the metabolism of a variety of essential drugs, including methylphenidate (MPH) and trandolapril (TA) for treatment of attention-deficit hyperactivity disorder (ADHD) and chronic heart failure (CHF), respectively. The aims are to 1) pioneer clinical decision strategies allowing individualisation of treatments with MPH and TA; 2) develop new research strategies that may serve as paradigms for future studies on individualised drug treatment; 3) identify drugs which interact with CES1 and produce toxic effects when administered together with MPH or TA; and 4) develop fast methods with commercial potential for predicting MPH and TA responses. The study is the first to develop guidelines for individualised treatments with MPH and TA. The approach is unique as it combines genomics, metabolomics and systems pharmacology. Implementation of guidelines for individualised pharmacotherapy is expected to improve treatment efficacies and reduce the risk of adverse effects, leading to reductions in health care system costs and improved patient quality of life. Moreover, the project should provide clues to individualisation of therapies with other essential CES1-dependent drugs (e.g. Tamiflu® and antihypertensive agents related to TA) and inspire future studies of the etiology of lipid metabolism disorders.

2. OBJECTIVES

The vision and overall scientific objective of INDICES is to identify causes of individual variation in drug responses to CES1-dependent drugs among patients with selected psychiatric and somatic disorders and translate this knowledge into clinical tools that can improve treatment efficacy and reduce the risk of adverse effects. The specific objectives are to provide psychiatrists and cardiologists with easy-to-use laboratory analyses and clinical guidelines for individualised treatments of ADHD and CHF with MPH and TA, respectively. These clinical objectives are dependent on a series of basic scientific objectives being met: 1) achievement of detailed knowledge of genetic variants of the key hepatic enzyme, CES1, and the impact of these variants on rate of drug metabolism; 2) identification of lipid metabolites and metabolomic signatures acting as proxies for CES1 activity; and 3) the combination of pharmacological, genetic, metabolomic and clinical information to accurately predict individual drug responses. The main objective related to society and the economy in Denmark is to improve the quality of life of patients with ADHD and CHF, reduce health care system expenditures and commercialise developed laboratory analyses into fast and easy-to-use tests.

3. THE MAIN RESULTS OF THE PROJECT

1) **Identification of biomarkers predicting individual drug metabolism**. We expect to develop a catalogue of CES1 gene variants that are phenotypically characterised in terms of pharmacokinetic impact and clinical relevance. This includes CES1 gene duplication and its effect on drug metabolism, i.e. a potential "gene-dosage effect". Furthermore, lipid metabolites

and metabolomics signatures acting as surrogate markers of CES1 activity to predict individual responses to MPH and TA will be identified.

2) **Improvement of drug safety and quality of treatment.** We expect to develop guidelines for individualised treatment with MPH and TA. Also clues to individualisation of other essential drugs metabolised by CES1 will be provided. Furthermore, drugs which interact with CES1 resulting in clinically relevant drug-drug interactions will be identified.
3) **Acquisition of knowledge on the basic regulation of the CES1 enzyme.** We expect to gain new insights into the physiological processes in which CES1 is involved, in particular lipid metabolism, and to formulate hypotheses on the development of disorders such as the metabolic syndrome which may potentially result from deregulation of these processes.
4) **Analytical procedures with commercial potential.** Analytical procedures developed by the study will be made into kits. This includes a procedure for determination of gene copy number as well as CES1 genotyping kits serving as companion diagnostics to treatment with MPH and TA.
5) **Paradigm for future studies.** The use of metabolomics in combination with systems pharmacology and genomics may serve as a conceptual paradigm for future studies of individual variation in drug metabolism.

4. BACKGROUND AND HYPOTHESIS OF THE PROJECT

The prevalence of ADHD in children and CHF is rising in Denmark, where these diseases now affect 6-8% of the population (1,2). This is accompanied by increased use of MPH and ACE inhibitors (ACE-Is). For example, the number of ADHD patients treated with MPH has increased by a factor of 10 over the last 10 years in Denmark (3). The use of this drug is expected to increase further due to increased focus on adult ADHD. In 2009 the consumption of MPH and ACE-Is in Denmark was equivalent to treating a total of 25,000 patients with ADHD and 340,000 with CHF (3).

There are large individual variations in the response to commonly used drugs. Often a large percentage of patients do not experience any benefit from the drug they are given; others develop adverse effects. For MPH the non-response rate is in the range of 15-20% (4). Adverse effects during treatment of ADHD with MPH are exceedingly common and include gastrointestinal disorders, insomnia and nervousness with incidences above 5%. Cardiac symptoms are also common with incidences of 3-5%. Discontinuation of MPH treatment due to severe adverse effects occurs in about 1% of cases.

Antihypertensive drug treatment is largely based on a trial and error approach, reflecting a marked individual variation in the response to different drug classes, including the ACE-Is (5,6). Animal studies have suggested that pharmacokinetic factors are involved in the non-response to ACE-Is, implying that dose adjustments can improve treatment efficacies (7). Non-serious adverse drug reactions are common in treatment with ACE-Is, but some patients develop severe and potentially life-threatening adverse effects such as renal impairment. Together with MPH the ACE-Is are on the top 10 list of drugs causing adverse effects in Denmark (3).

CES1 plays a key role in the metabolism of a variety of ester- and amide-containing xenobiotics and endogenous components, including MPH, TA, oseltamivir (Tamiflu®) for combating flu pandemics, the anticancer drug capecitabine, the analgesic meperidine and cocaine (8). Relatively few single nucleotide variations have been identified in CES1 and overall there is a hiatus in the knowledge of variation at the CES1 locus (9). Recent data from a collaborative study supported by the European Commission (EU-FP7) have revealed duplication of the CES1 gene at frequencies above 0.20 in Europe (Henrik Berg Rasmussen, unpublished observations). Gene duplication may give rise to the formation of increased amounts of the gene product, i.e. a “gene dosage effect”.

Genetic screening of drug metabolising enzymes such as CYP2D6 provides the clinician with the ability to identify patients with abnormal rate of drug metabolism and to individualise the treatment based on this. For example, a CYP2D6 "ultra-rapid metaboliser" may not experience any benefit from treatment with a standard dose of a CYP2D6-dependent drug and should have increased doses (10). Therapeutic drug monitoring is an alternative tool to identify patients with abnormal drug metabolism rate. The main drawback is that it cannot be used until after therapy initiation delaying clinical decision making. Moreover, drug monitoring is of limited value for treatment with drugs, such as MPH, for which there is not a plasma concentration range defining an optimal response. There are large individual variations in the metabolism of MPH, TA and oseltamivir, all CES1-substrates (11,12,13), suggesting a significant potential for individualisation of therapies with these drugs. Except for an association of two single nucleotide polymorphisms (SNPs) in CES1 with poor metabolism of MPH that included a steroselective effect on the metabolism of the l-form of this drug (14), associations between *CES1* genotype and drug metabolism have not been reported. Drug-drug interactions are also a general problem for pharmacotherapy particularly relevant to treatment of ADHD with MPH as this disorder often co-exists with other treatment-requiring mental or somatic disorders. No studies have systematically assessed the risk of toxic effects due to drug-drug interactions during co-medication of MPH with other drugs (15). In this regard systems pharmacology, a new area of pharmacology that uses network analyses to examine drug actions, holds promises for improving understanding of the underlying mechanisms behind a drug's effect, adverse effects and drug-drug interactions (16).
Besides being involved in the metabolism of essential drugs, CES1 is an important player in endogenous lipid metabolism as it hydrolyses cholesteryl esters and triglycerides, suggesting a role for this enzyme in the regulation of intracellular levels of these substances (17). This combined with findings indicating that CES1 activity is regulated by various lipids (18) hints at a relationship between the level of lipid metabolites in the blood and rate of metabolism of CES1-dependent drugs. A powerful tool to identify novel small molecule markers is metabolomics, which in principal targets the entire repertoire of metabolites within a sample, integrating all environmental influences from gene transcription through protein expression to formation of metabolites (19). Pharmacometabolomics, i.e. the use of metabolomics for examination of drug responses, represents a new and promising tool for individualisation of the drug treatments.

We hypothesise that:

1) Several clinically relevant CES1 variants with an effect on enzyme activity remain to be identified.
2) Duplication of CES1 is associated with increased rate of metabolism of MPH and TA through a "gene dosage effect".
3) Guidelines for individualised therapy with MPH and TA can be established.
4) Many commonly used drugs interact with CES1, resulting in metabolism-mediated toxic effects.
5) Lipid metabolites are proxies for CES1 activity and correlated with the rate of metabolism of CES1-dependent drugs.
6) The combination of pharmacology genomics and metabolomics which bridges genotype to phenotype is more effective in predicting individual drug responses to CES1-dependent drugs than each of the two "omics" alone and integration of pharmacology with genomics and metabolomics into "pharmacolomics" is the most promising solution to individualised medicine.

5. INNOVATIVE VALUE, IMPACT AND RELEVANCE OF THE PROJECT

Innovations and novel concepts

1) First application of metabolomic profiling using knowledge on endogenous substrates of a drug-metabolising enzyme to examine the basis of individual differences in pharmacokinetics.

2) A complete catalogue of CES1 variants, including duplication with information about the effect on drug metabolism, is highly desired but currently does not exist.
3) Combination of genomics and metabolomics, which accumulates all environmental influences onto the genetic background with systems pharmacology is unique.
4) First to develop guidelines for individualised treatment with MPH and TA.
5) Identification of hitherto unknown drug-drug interactions may improve therapies with MPH, TA and other drugs.
6) An easy and reliable method for determination of gene copy number.

Impact on future research

1) Redirection of the current line of research by a combination of metabolomics with genomics - a conceptual paradigm for future studies of individual drug metabolism and response.
2) Fuelling studies of genetic variability in the metabolism of an entire range of CES1-dependent drugs, including several ACE-Is, oseltamivir (Tamiflu®) and cocaine.
3) Fuelling studies of individual susceptibility of obesity/lipid metabolism disorders.
4) *In-silico* and *in-vitro* identified drug-drug interactions call for *in-vivo* confirmation.

Relevance to society. Individualised treatment with of ADHD and CHF with MPH and TA, respectively, has the potential to improve treatment efficacy and reduce the risk of adverse effects, resulting in improved patient quality of life and in reduced expenditures to the health care system. We expect to develop an assay with a commercial potential and to materialise new variants of CES1 into a kit for a rapid routine genotyping prior to initiation of treatment with CES1-dependent drugs, a companion diagnostic. This may create new jobs related to kit development and manufacturing.

6. PROJECTS METHODOLOGY AND RESULTS

INDICES consists of seven closely interconnected work packages (WPs).

WP1: "Drug and lipid metabolite study". The purposes are to: 1) develop and validate methods for determination of plasma concentrations of TA, the d- and l forms of MPH, and the major metabolites of these drugs; 2) detect *in-vitro* drug-drug interactions of potential clinical relevance; and 3) identify endogenous lipids modulating the activity of CES1. The methods for quantification of the two drugs and their metabolites will be based on liquid chromatography-tandem mass spectrometry. Plasma extractions of MPH and its major metabolite will be performed by a mixed-mode solid-phase extraction procedure followed by chromatography on a chiral column. The developed methods will be applied for analysis of the samples from WP2 and WP7. We will use *in-silico* data generated by WP5 to select commonly used drugs that potentially interact with CES1. The inhibitory effect of these drugs on CES1will be examined by *in-vitro* systems of pooled human liver microsomes or recombinant CES1. This involves co-incubation of the potential inhibitor with one of the two CES1-selective drugs, MPH or TA, followed by determination of the concentrations of the CES1-selective drug. We will also use the *in-vitro* system to screen lipid libraries for identification of lipids interacting with CES1. Furthermore, lipid metabolites WP4 associates with a pharmacokinetic profile will be examined in the *in-vitro* system to verify an effect on CES1 activity. In the event of problems with the development of the assays for detection of MPH, TA and their metabolites, we will have the samples analysed at an academic institution or analytical companies abroad. Furthermore, an alternative approach based on a "coloured" enzyme substrate for detection of drugs and lipids interacting with CES1 will be developed. Overall, we do not expect delay in excess of two months. Such a delay would not be critical.

WP2: "Pharmacokinetic study". The purposes are to: 1) correlate the CES1 genotype and copy number with the pharmacokinetic profile of TA and MPH and to determine dose adjustment factors for these drugs; 2) provide pharmacokinetically well-characterised DNA samples for the WP 3; and 3) provide data for WP 4, 6 and 7. We will recruit 100 healthy, young adults at the Department of

Science, Systems and Models, Roskilde University, where we have permission to contact the students. After screening the recruited subjects for CES1 gene copy number, we will select 5 CES1-duplication homozygotes, 10 duplication heterozygotes, and 20 subjects' homozygous without duplication. This sample of subjects enriched for the CES1 gene duplication will participate in two pharmacokinetic trials with MPH and TA, respectively, separated by a "wash-out" period. The trials will take place at the experimental pharmacological phase 1 unit, Department of Clinical Pharmacology, Bispebjerg University Hospital. After pre-drug blood sampling and intake of a single dose of one of the two drugs in the morning, there will be blood sampling throughout the rest of the day. We will correlate the pharmacokinetic profile with the CES1 genotype and estimate dose adjustment factors for genotypes conferring decreased or increased metabolism of TA and MPH. Using *in-silico* modelling we will identify other CES1-dependent drugs and evaluate their potential as candidates for individualised therapy. Successful outcome of WP2 depends upon the ability to recruit the desired number of subjects and avoid drop-out of recruited subjects. We will solve these problems by 1) motivating oral presentations, 2) a relatively high economic compensation to each study subject, e.g. DKK 3,000-3,500 (EUR 350 Euros) per day, 3) additional recruitment at the University of Copenhagen, and 4) use of the homepage, www.forsoegsperson.dk, which is dedicated to recruitment of subjects to biomedical studies. A major drop-out of participants after the first pharmacokinetic study may necessitate a new recruitment round. This may cause a two to three-month delay, which is not critical.

WP3: "Genetic study". The purposes are to: 1) identify new variants in the CES1 gene and in its proximity; 2) assess their functional significance; 3) establish analyses for genotyping of clinically relevant CES1 variants; 4) assess a relation between lipid disorders and CES1; and 5) determine the worldwide frequency of CES1 duplication and identify nutritional factors acting in a positive selection. As the first step we will examine the samples from the 100 young adults recruited by WP2 by using paired end-mapping and Solexa sequencing of a 1.5 Mb segment containing CES1. The functional importance of identified variants will be assessed using the pharmacokinetic data from WP2 supplemented with *in-silico* analyses. Based on this we will construct a catalogue of CES1 variants and design genotyping assays for those of potential clinical relevance. SNP genotyping will be based on the 5'exonuclease-based approach. A novel high-throughput method for determination of gene copy number will be developed. The MPH treatment response group will be used as a discovery cohort and IP protected. In collaboration with deCODE, sequence variants associated with MPH treatment response will be imputed into a large ADHD sample (N>1000) and tested for association with ADHD. Likewise, a search for genetic associations of CES1 with lipid metabolism disorders will be conducted in collaboration with deCODE. We will also determine the worldwide frequencies of CES1 duplication and correlate them with the amount of meat and dairy products in the diet as proxies for the levels of triglycerides and cholesterol. In the event of problems with the Solexa-based sequencing, other technical platforms will be applied. The delay resulting from this should not exceed four months, which is not critical.

WP4: "Pharmacometabolomics". The purposes are to: 1) identify lipid metabolites and pathways implicated in variation in MPH and TA metabolism; and 2) improve prediction of individual variation by combination of metabolomic profiles and DNA markers. We will determine metabolomic profiles of plasma samples from all of the participants in the pharmacokinetic study prior to intake of MPH and TA and determine predose-metabolomic profiles. As the participants in the first pharmacokinetic trial (MPH) are identical to those in the second pharmacokinetic trial (TA), we will obtain two independent pre-dose profiles of each participant. Choice of a metabolomic profiling platform will be based upon knowledge on endogenous substrates of CES1 employing cholesterol and triglyceride biochemistry analysis platforms, each targeting about 5,000 different lipid metabolites in an integrative approach with subsequent partial least square modelling

(PLS) of data. The latter is a multivariate statistical approach, which will detect relationships between pharmacokinetic parameters and lipid metabolites, thus allowing us to build a statistical model capable of predicting individual variations in the rate of drug metabolism. Subsequently we will incorporate the data from deep sequencing of the CES1 gene and copy number determinations, into the PLS analyses and remodel to increase the pharmacokinetic prediction ability. To provide insights into the background of individual variability in pharmacokinetics we will construct hypothetical metabolic networks based upon metabolites associated with individual variability in WP2 and their interacting partners using information from the Human Metabolome Database. The information contained in each of these metabolomics networks will be condensed into a single, clinically applicable measure. In the event of problems we will use our extended network which includes the Pharmacometabolomics Center, Duke University to solve the problems.

WP5: "Systems pharmacology and metabolomics". The purposes are to: 1) identify drug-drug interactions of potential importance to the metabolism of CES1 dependent drugs by *in-silico* methods; 2) identify genetic factors associated with adverse effects of MPH and TA; 3) provide a deeper understanding of the basis of individual variation in CES1-mediated drug metabolism; and 4) provide essential bioformatics services to WP1, WP2 and WP3. We will collect information on structural parameters of drugs and drug-like compounds interacting with CES1. Based on this we will construct *in-silico* models for virtual screening of large compound databases (millions of compounds) to detect drugs potentially involved in interactions with CES1-dependent drugs. A systems pharmacology network will be developed by the compilation of drug-target interactions and protein-protein interactions of the targets. Information on adverse effects will be integrated into this network to identify genes and complexes of genes implicated in individual susceptibility to adverse effects of MPH and TA. Using the phenome-interactome platform in combination with the Human Metabolome Database, genes encoding proteins that directly or indirectly interact with CES1 will be identified and mapped to genetic variations obtained from WP3 and the literature. This will allow us to create a platform for understanding individual CES-1 mediated drug metabolism and to develop an *in-silico* model for prediction of individual variation in the metabolism and response of CES1-dependent drugs. The Center for Biological Sequence Analysis is in close contact with other leading bioinformatics clusters. In the event of problems one or more of these will be contacted.

WP6: "Clinical study A - ADHD". The purposes are to: 1) examine the relationship of CES1 genotype with the response to MPH in children with ADHD, and 2) develop guidelines for CES1 genotype-based prescription of MPH for treatment of ADHD in children. We will recruit 200 drug-naïve children aged 7-11 years with ADHD according to ICD-10 criteria. Rating of ADHD symptom severity and adverse effect rating will be conducted at base-line. MPH will be initiated at a low dose and increased until symptom normalisation or treatment failure occurs. CES1 genotypes including copy number will be correlated with the drug response measures: 1) number of weeks required for a predetermined improvement to occur, 2) occurrence of treatment failure, and 3) adverse effects and premature discontinuation of therapy due to unacceptable adverse effects. For example, this will allow us to assess whether the MPH dose should be increased at a faster rate and with larger dose increments than normal in patients with CES1 duplication. We also expect to determine whether patients with defective alleles should be up-titrated using smaller dose increments than normal. By combining the pharmacokinetic data from WP2 we will develop guidelines for individualised therapy of ADHD with MPH. To enhance recruitment participating children will be offered a DKK 50 (EUR 7) gift certificate at a toy shop. In the event of recruitment delay we will also recruit at *Privathospitalet, Hejmdal* with permission from its chief psychiatrist, Dr Torsten Warrer. Professor Hans-Christoph Steinhausen, Aalborg Psychiatric Hospital, will also assist in the recruitment if necessary. Determination of plasma concentrations of MPH has limited therapeutic value and will not be done.

WP7: "Clinical study B – CHF". The purposes are to: 1) examine a relationship of CES1 genotypes with the response to TA in patients with CHF and 2) develop guidelines for genotype-based prescription of TA for treatment of CHF. A similar prospective design as employed in WP6 will be used. A total of 200 patients with CHF, left ventricular ejection fraction <0.45, and clinical indication for TA will be recruited. TA is initiated followed by successive dose-titration to a predetermined target dose with drug discontinuation or dose reduction if adverse effects occur. The investigation duration is 6 months with recruitment of patients at the out-patient clinics at two large cardiology hospital departments in Copenhagen (Gentofte University Hospital and Bispebjerg University Hospital). If necessary, supplementary recruitment will be possible at the Department of Cardiology B, Rigshospitalet and the Department of Cardiology, Hillerød University Hospital. Patients will be clinically examined at t=0, 3 and 6 months and blood and urine samples taken. CES1 genotype will be correlated to clinical and biochemical treatment response measures. In combination with the results from WP2, and drug metabolite measurements in plasma from the CHF patients, guidelines for individualised treatment of CHF patients with TA will be developed. They may include dose adjustments for 'poor metabolisers' and 'ultrarapid metabolisers' or recommendation to use drugs which are not metabolised by CES1 for treatment of patients with abnormal CES1 genotypes. During a PhD student study visit at Newcastle University, the development of the treatment guidelines will take place in collaboration with Simon Thomas, Professor of Clinical Pharmacology and Therapeutics.

Power calculations. Calculations for the pharmacokinetic trial with the ratio between minimum difference in mean area under curve and SD = 3, $\alpha = 0.05$, gives power = 0.94 with 35 participants. For the clinical trials we assume a smaller effect size, namely, 1.5 and for $\alpha = 0.05$, this gives power = 0.90 with 200 participants. Therefore all of the trials are very well powered.

7. PROJECT PLAN

The Gantt diagram only includes scientific personnel. Light brown denotes PhD student and postdoc study visits.

WP1 (Project leader: Kristian Linnet). A PhD associated with WP1 will execute the following activities within the time frame indicated below:

- Month 1-12: development of analyses for quantification of drug and drug metabolites
- Month 12-15: analysis of samples from pharmacokinetic study
- Month 15-21: study visit (Linkobing University)
- Month 21-24: analysis of samples from the CHF study
- Month 24-30: identification of drugs interacting with CES1
- Month 30-36: identification of bioactive lipids interacting with CES1
- Month 36-39: preparation of PhD dissertation

We expect the drug-drug interactions analyses and analyses of bioactive lipids to start after 12 months. This should allow enough time for their completion after 30 months. Kristian Linnet will contribute with 3 months of work for execution of WP1. Olivier Taboureau will contribute with 2 months, Henrik Rasmussen with 2 months, Kim Dalhoff or Gesche Jürgens with 1 month of work. Kristian Linnet will provide 3 months of technical laboratory assistance.

WP2 (Project leader: Kim Dalhoff). A PhD associated with WP2 will execute the following activities within the time frame indicated below:

- Month 1-9: planning and execution of pharmcokinetic trials
- Month 9-15: study visit (Kiel University)

DICES - Gannt diagram

Year	2011				2012				2013				2014				2015				Months							
Projects / quarter	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Sct. Hans**	**UniCph**	**BBH Pharma**	**de CODE**	**Leiden**	**CBS**	**GLO Psyc**	**GEH Card**
Work Package 1																												
Drug metabolite detection																					2	3+1phd	1			2		1
Work Package 2																												
Pharmacokinetic study																					4	1	6+1phd			1		
Work Package 3																												
Genetic study																					6+1postdoc			3		1		
Work Package 4																												
Pharmacometabolomics																					1		1		2+2 years	1		
Work Package 5																												
Systems pharmacology																					2		1			4+1phd		
Work Package 6																												
Clinical study A																					2		1				4+1phd	
Work package 7																												
Clinical study B																					1	1	1					4+1phd

Thomas Hankemeier's staff at the Netherlands Metabolomics Centre will contribute 2 years of work. Thomas Hankemeier will use 2 months on WP4.

- Month 15-21: calculation of pharmacokinetic parameters and analyses of their relationship with CES1 genotype
- Month 21-27: guidelines for genotype-based adjustment of doses of MPH and TA
- Month 27-39: identification of additional candidates for genotype-based adjustment of doses among other CES1 dependent drugs
- Month 39-42: preparation of PhD dissertation

The pharmacokinetic trials are labour-intensive, requiring the study subjects to be split into smaller groups participating on separate days. Therefore a relatively large amount of time has been allocated to the completion of these trials. The study visit is placed in the period where the samples from the pharmacokinetic trials are being analysed, allowing for the best use of the time. Kim Dalhoff and Gesche Jürgens will contribute with 6 months of work and Henrik Rasmussen with 4 months of work for completion of WP2. Olivier Taboureau will use 1 month of work onWP2 and Kristian Linnet, or his PhD student, with 1 month of work. A laboratory technician (self-financed, MHC Sct. Hans) will contribute with 2 months work and be responsible for registering samples, extraction of DNA and CES1 gene copy number determination. In addition it will be necessary to have two nurses contribute 1½ month of work at the experimental pharmacological unit.

WP3 (Project leader: Hreinn Stefansson). A postdoc associated with WP3 will execute the following activities within the time frame indicated below:

- Month 1-3: introduction and planning
- Month 3-9: deep sequencing of the 100 samples (Solexa sequencing)
- Month 9-15: elaboration of a comprehensive catalogue of genetic variants of CES1 and variants in neighbour genes, construction of haplotypes and *in-silico* assessment of the functional importance of the variants
- Month 15-27: development of genotyping assays
- Month 27-33: genotyping of the clinical samples
- Month 33-39: determination of CES1 duplication in different populations of the world and evolutionary analyses
- Month 39-42: preparation of manuscripts and finalisation of the activities

After the introductory period the postdoc will then take residence at deCODE for one year. Hreinn Stefansson is expected to contribute to WP3 with 3 months of work, Henrik Rasmussen with 6 months of work and Olivier Taboureau with 1 month. MHC Sct. Hans will provide 6 months of technical laboratory assistance (self-financed).

WP4 (Project leader: Thomas Hankemeier). The activities in WP4 will take place at the Netherlands Metabolomics Centre and the Metabolomics Center, Duke University. The following activities will be executed within the time frame indicated below:

- Month 1-12: metabolomics analyses of plasma samples
- Month 12-24: building of statistical models for prediction of individual variations in the rate of metabolism of MPH and TA
- Month 24-30: analysis of combined pharmacometabolomics and genetic data
- Month 30-39: construction of hypothetical metabolomic networks based upon metabolites associated with individual variability in pharmacokinetic study.

Thomas Hankemeier will conduct the metabolomics analyses of the plasma samples. Modeling of metabolomics data, pathway analyses and construction of hypothetical metabolomic networks will take place at the Netherlands Metabolomics Centre or the Pharmacometabolomics Center, headed by Rima Kaddurah-Daouk who will collaborate on the project. The PhD associated with WP5 is expected to complete a 3-month study visit at the Netherlands Metabolomics Centre or the Pharmacometabolomics Center. Thomas Hankemeier will contribute to the completion of WP4 with

2 months of work, Olivier Taboureau with 1 month and Kim Dalhoff or Gesche Jürgens with 1 month of work. Henrik Rasmussen will contribute with 1 working month.

WP5 (Project leader: Søren Brunak). A PhD associated with WP5 will execute the following activities within the time frame indicated below:

- Month 0-9: identification of drugs interacting with CES1
- Month 9-15 months: identification of genes involved in adverse effects of MPH and TA
- Month 15-18: study visit at the Netherlands Metabolomics Centre or the Pharmacometabolomics Center, Duke University
- Month 18-21: combining systems pharmacology data and pharmacometabolomic data
- Month 21-33: development of *in-silico* tool for prediction of individual response to CES1-dependent drugs
- Month 33-36: preparation of PhD dissertation

Olivier Taboureau will contribute to the completion of WP5 with 4 months, Henrik Rasmussen with 2 months, and Kim Dalhoff or Gesche Jürgens with 1 month of work.

WP6 (Project leader: Tine Houmann) and WP7 (Project leader: Peter Riis Hansen). A PhD associated with WP6 and another with WP7 will execute the following activities within the time frame indicated below:

- Month 0-3: planning and introduction
- Month 3-15: recruitment of 100 patients who will then complete a 6-month study period
- Month 15-27: recruitment of 100 additional patients who will then complete a 6-month study period corresponding to a total of 200
- Month 27-33: study visit at University of Nijmegen (WP6) and University of Newcastle (WP7)
- Month 33-39: statistical treatment of data and elaboration of manuscripts
- Month 29-42: preparation of PhD thesis

Tine Houmann (WP6) and Peter Riis Hansen (WP7) will both contribute with 4 months of work, Henrik Rasmussen with 2 months on WP6 and 1 month on WP7. Kim Dalhoff and Gesche Jürgens will contribute jointly with 1 month of work for completion of WP 6 and 1 month for completion of WP7. Kristian Linnet will contribute with 1 month of work to WP7. Henrik Rasmussen will provide 3 months of technical laboratory assistance (self-financed).

8. PROJECT'S INTERNATIONAL DIMENSION

Contact and exchange of knowledge with the international science community will be enhanced by:

1) The participation of leading research institutions from abroad in the project
2) PhD student study visits at research institutions abroad for a period of 3-6 months. Agreements have been obtained from the Department of Forensic Genetics and Toxicology in Linkobing, University of Nijmegen, Kiel University, Duke University and Newcastle University
3) Attendance by all of the PhD students and key persons in at least one, preferably two, international high-level scientific congresses annually.
4) Annual work meetings (cf. "11. The Participating parties and project management").
5) Mid-term evaluation (cf. "11. The Participating parties and project management")

The interest in personalised medicine has a high priority globally. The concept of combining metabolomics and genomics to provide insights into the basis for individual variation of drug responses is in line with that of a recent NIH-funded initiative, "National Metabolomics Network for Drug Response Phenotypes". The current application is connected to this network through the Netherlands Metabolomics Centre which is participant of this network. We expect the international activities to have a major impact on the future research of individualised medicine in Denmark by:
1) enhancing contact with leading scientists within the field and facilitating the exchange of ideas

and future collaborations, 2) offering opportunities for the scientific education of young scientists at the highest level, and 3) importing knowledge that Denmark currently does not possess, particularly in regard to pharmacometabolomics. Currently, most research groups in individualized medicine in Denmark focus upon pharmacogenomics. The international activities of INDICES will serve to redirect this research towards a line that combines several "omics". A combination of the two "extremes", namely genomics and metabolomics that accumulate all of the environmental influences onto the genetic background, could become the most rewarding avenue of research.

9. LEGAL AND ETHICAL ASPECTS

The local Committees on Biomedical Research Ethics and the Danish Data Protection Agency have issued permissions for the clinical WP on ADHD that allow for the collection, management and examination of data as well as the taking of biological samples from psychiatric patients below 18 years of age for research purposes. This includes genetic research projects.
Permissions from the local Committees on Biomedical Research Ethics and the Danish Data Protection Agency are also required for completion of WP2, WP3 and WP7. Subjects will be recruited after having given informed consent. They will be adequately informed about potential risks associated with the studies.
We consider the risks to the subjects participating in the pharmacokinetic study as low since the study subjects will all be healthy young adults with no history of heart disease or other diseases that might increase the risk of severe adverse effects. Moreover, the drugs applied in this study, MPH and TA, are each given as a single, relatively low dose. The conduction of the pharmacokinetic study in an experimental pharmacological phase 1 unit with medical doctors and trained nurses being present during the entire trial also serves to increase the safety of the study subjects. We do not anticipate the clinical studies to be associated with safety concerns since dose adjustments are not implemented at this stage of our research.

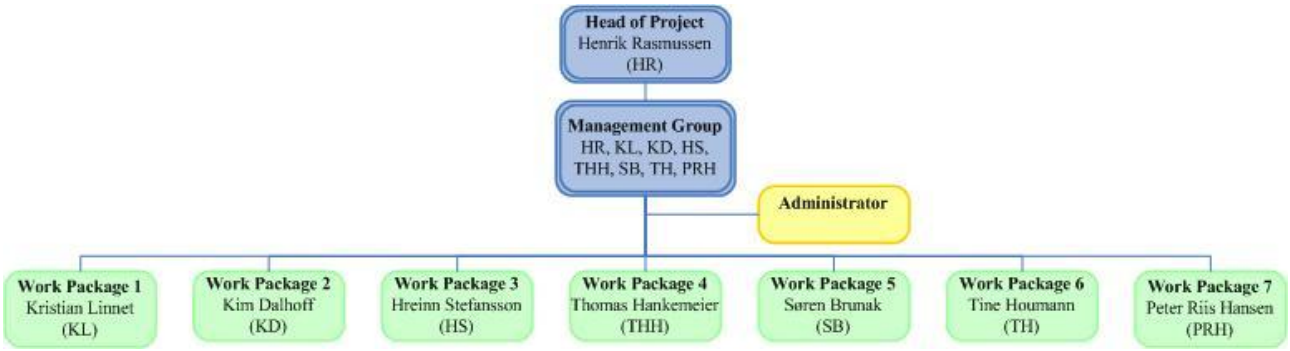
10. PUBLICATION AND PROMOTIONAL STRATEGY AND EXPLOITATION OF RESULTS

We expect the quality of our studies to readily meet the publication standards of high-ranked peer-reviewed scientific journals such as *Nature Genetics, PLOS Genetics, The New England Journal of Medicine, Journal of Pharmacology and Experimental Therapeutics, and The Pharmacogenomics Journal, Bioinformatics, Journal of Child and Adolescent Psychopharmacology, Molecular Psychiatry,* and *Cardiovascular Research.* Information about published studies will be deposited in the Danish National Research Database, a central portal for published Danish research. Here we list the topics of a number of publications that we expect to publish: **WP 1**: 1) quantification of MPH and TA, 2) CES1-mediated drug interactions, 3) lipid interactions with CES1, and 4) review of CES1 interactions. **WP2**: 1) CES1 genotype and drug metabolism, 2) dose-adjustment based upon CES1 genotype, 3) individualised drug therapy with CES1-dependent drugs, 4) CES1 variants in relation to drug metabolism. **WP3**: 1) organisation of the CES1 and CES4 loci, 2) methods for identification of CES1 variants, 3) environment, diet and CES1 gene copy number, 4) CES1 gene and lipid metabolism disorders, and 5) method for gene copy number assessment. **WP4**: 1) metabolomics and individual pharmacokinetic profiles, 2) metabolomics profiling of drug metabolising enzymes, and 3) metabolomic network of CES1 interacting lipids. **WP5**: 1) CES1 metabolism-mediated drug-drug interactions, 2) genetic susceptibility to drug adverse effects, 3) *in-silico* tool for prediction of individual drug responses. **WP6**: 1) CES1 genotype and treatment of ADHD with MPH, 2) metabolomic signatures and ADHD treatment, 3) guidelines for CES1 genotype-based therapy with MPH, and 4) CES1 genotype and MPH treatment responses. **WP7**: 1) CES1 genotype and efficacy

of TA, 2) CES1 genotype and lipid markers in heart failure, 3) guidelines for CES1 genotype-based therapy with TA, and 4) metabolomic signatures and prediction of outcome of treatment with TA. Results from INDICES will also be presented at international congresses, e.g. the World Congress on Psychiatric Genetics, the European Society of Child and Adolescent Psychiatry, the International Society of Metabolomics, and the annual meeting of the European Heart Society. Moreover we will prioritise giving presentations at national scientific meetings, e.g. the annual meeting of the Danish Psychiatric Society and to disseminate knowledge at post-graduate courses for medical doctors and through student courses. We will seek permission from the ADHD Association and the Danish Heart Association to inform about INDICES on their homepages as well as suggest presentations of the project to be held at meetings arranged by these patient associations. Since "tailored medicine" has a broad public appeal we will contact large Danish newspapers and national television channels to present the project's main findings to provide inspiration for thematic articles and broadcasts. The implementation of guidelines for CES1 genotype-based prescription of MPH and TA will start at the departments responsible for the clinical studies (Psychiatric Centre Glostrup and Department of Cardiology, Gentofte University Hospital); the Mental Health Centre Sct. Hans will conduct the genotyping. After implementation at these departments we will identify another two hospital ADHD departments and hospital cardiology departments that are interested in introducing the new treatment principles. We will assist these departments with implementation of the guidelines for individualised treatment. We expect that this combined with other efforts such as presentations at national scientific meetings and lectures at post-graduate courses for medical doctors gradually will lead to the implementation of individualised guidelines at a larger number of hospitals in Denmark. We expect to develop assays that can be transformed into easy-to-use laboratory kits for clinical use. The development and marketing of such kits would be a task for a biotechnological company. The technology transfer unit of the capital region (RegionH), Tectra, will assist with patenting and commercialisation of innovations and hold responsibility for the process of commercialisation. Intellectual property rights (IPRs) and collaboration with project participants not belonging to RegionH will be regulated by collaborative agreements written by Tectra before the project starts. Issues on which several parties might claim IPRs and foreground rights will be discussed and agreed upon with Tectra and partners prior to the initiation of the project.

11. THE PARTICIPATING PARTIES AND PROJECT MANGEMENT

The project will be headed by *HBR*. He will be assisted by a management group consisting of *KL, KD, HS, THH, SB, TH,* and *PRH*. An administrator (0,2 FTE) will be responsible for routine mail, budgets, coordination of meetings and assistance in preparing reports; see organisation chart.



Henrik Berg Rasmussen (HBR), DVM, PhD, is a senior scientist and laboratory manager at the Research Institute of Biological Psychiatry and the Mental Health Centre Sct. Hans. He heads a research group focusing upon pharmacogenetics and individualised medicine. *HBR* is responsible

for the development and implementation of new genotyping techniques and the maintenance of the Danish Psychiatric Biobank. Furthermore, he holds responsibility for the CYP2D6 and CYP2C19 genotyping of clinical samples at the Mental Health Centre Sct. Hans, a service provided to hospitals and general practitioners in Denmark, and for quality control of these analyses through collaboration with international partners. *HBR* is the primary investigator of the ADME (structural variation in genes associated with absorption, distribution, metabolism and excretion of drugs) project, a sub-project of PSYCH GENE (EU-FP7) and of a study on the genetic variability of the dopamine transporter together with four international partners. *HBR* will be heading the consortium. Accordingly, he will be involved in overall coordination of the research efforts of the single WPs, maximising the synergies between the participants. He will also actively take part in the genetic WP (planning and conduction of pharmacokinetic trials, supervision of PhD student, development and design of genetic assays and analyses of samples from the clinical WPs) and the two clinical WPs (development of guidelines for genotype-based treatment with MPH and TA).

Kristian Linnet (KL), MD, DMSc, is a professor of forensic chemistry and the director of the Section of Forensic Chemistry, University of Copenhagen. This unit consists of about 50 employees, including scientific and technical staff. *KL* heads a research group currently including 4 PhD students focusing upon the development of new analytical methods, drug metabolism and pharmacogenetics. *KL* participates in several international collaborations and is advisor of the Clinical and Laboratory Standards Institute and a regional representative of the International Association of Forensic Toxicologists. The Section of Forensic Chemistry, University of Copenhagen, is highly experienced in analysis of psychoactive and cardiovascular drugs in human samples. *KL* will be responsible for the drug and lipid metabolite WP. *KL* will also take part in and interact with the pharmacokinetic WP (provision of drug metabolite data), the pharmacometabolomics WP (exchange of data) and the CHF WP (provision of drug metabolite data).

Kim Dalhoff (KD), MD, DMSc, consultant specialist in Clinical Pharmacology and Hepatology, Bispebjerg University Hospital and associate professor at the Medical School, University of Copenhagen. He is the head of the Danish Poison Control Centre and supervisor of several PhD students investigating the influence of genes on drug metabolism and toxicity. He has been involved in several clinical pharmacokinetic studies, maintains a large international network and was the PI of a large GCP study with a new drug against liver cancer. *KD* will be the primary person responsible for the pharmacokinetic study (WP2). He will also be involved in other WPs, in particular the drug metabolite WP (contributing with expertise on drug-drug interactions), the metabolomics WP (provision of pharmacokinetic data) and the two clinical WPs (collaborating in the development of guidelines for genotype-based treatment with MPH and TA).

Gesche Jürgens (GJ), MD, PhD is a clinical pharmacologist. She is employed as specialist registrar at the Department of Clinical Pharmacology (Bispebjerg University Hospital) and the Danish Poison Control Centre. *GJ* is experienced in the planning and conduction of clinical and pharmacokinetic studies and has worked as principle and co-investigator in research projects dealing with pharmacokinetics, drug metabolism and toxicology. *GJ* will provide assistance to WP2, WP4, WP5, WP6 and WP7.

Hreinn Stefansson (HS) PhD, head of the CNS division deCODE genetics, Reykjavík, Iceland. This company is a global leader in human genetics with expertise in generating, analysing and managing large data sets. *HS* has extended experience in coordinating international research projects through his role as the PI of several collaborative ventures, including SGENE (EU-FP6) and PSYCH GENE (EU-FP7). His fields of expertise fall within in genomics, population genetics and identification of disease genes. *HS* will be responsible for WP3. Moreover he will provide services to other WPs and

synergise with them, in particular the pharmacological WP (provision of genetic data and conduction of statistical analyses) and the metabolomics WP (provision of genetic data).
Thomas Hankemeier (*THH*), MSc, PhD, director of the Netherlands Metabolomics Centre, full professor of analytical biosciences, Leiden/Amsterdam Center for Drug Research, Leiden University. *THH* is the PI of several large research projects on the use of metabolomics for discovery of biomarkers including a project with a budget of 53 M Euro to identify clinically predictive biomarker fingerprints for early disease diagnosis. *THH* will be responsible for the metabolomics WP. He will interact and synergise with the drug and lipid metabolite WP (provision of lipid metabolite data), the pharmacokinetic WP (acquisition of pharmacokinetic data) and the genetic WP (acquisition of genetic data). The Netherlands Metabolomics Centre is a member of a network headed by the Pharmacometabolomics Center, Duke University Medical Center, Durham, USA, which is the home of a multi-institutional interdisciplinary research consortium funded by the NIH and consists of centres of excellence in metabolomics and metabolomic bioinformatics, together with centres for molecular pharmacologic and pharmacogenomic science. Director of the Pharmacometabolomics Center is Rima Kaddurah-Daouk. One of the pioneers of metabolomics and a leading player in this field, Rima Kaddurah-Daouk has built programmes that bridge genetic and biochemical global -omics approaches to bring a deeper understanding of the pathways implicated in disease and in drug response. We are connected to the Pharmacometabolomics Center, through the Netherlands Metabolomics Center. Rima Kaddurah-Daouk and the Pharmacometabolomics Center will be participating in the project.
Søren Brunak (SB), physicist, PhD, DSc, professor and director, of the Center for Biological Sequence Analysis, Technical University of Denmark, one of the largest academic bioinformatics clusters in Europe with more than 100 employees. *SB* is also professor of disease systems biology, The Novo Nordisk Foundation Center for Protein Research, University of Copenhagen and primary investigator of several international research projects. *SB* will be the principal investigator of WP5.
Olivier Taboureau (OT), MSc, PhD, associate professor at the Center for Biological Sequence Analysis, Technical University of Denmark. *OT* has expertise in the integration of large-scale chemical and biological data to examine the interplay between small molecules and systems biology. He has been involved in the development of *in-silico* chemical biology including QSAR systems for toxicity prediction and chemical toxicology networks. *OT* has participated in several EU projects, i.e. Psychgene, DEER, Etox and Danish programme (SMVs) and the Innovative Medicines Initiative (IMI) EU programme. He will be responsible for the bioinformatics in WP1 and WP2 as well as WP5. He will also have responsibility for the exchange of knowledge and cooperation with the Pharmacometabolomics Center at Duke University and its outreach into Europe, e.g. the Netherlands Metabolomics Centre.
Tine Houmann (TH), MD, associate professor in child and adolescent psychiatry at the Medical School, University of Copenhagen and senior hospital physician at Glostrup University Hospital. *TH* is the head of the in-patient unit for preschool children and the ADHD Clinic. She is conducting a preschool ADHD study, and is a co-investigator in the Copenhagen Child Cohort, CCC 2000 Study, from which she has experience in the daily clinical management of scientific studies. *TH* is teaching pre- and postgraduate courses in ADHD, PDD and psychopharmacology, and is a pre- and postgraduate scientific supervisor of medical students and trainees. *TH* will be responsible for the ADHD WP. She will also synergise and interact with the pharmacokinetic WP (acquisition of data and development of guidelines for genotype-based dose adjustments) and the genetic WP (receiving results from genotyping of patients).
Anne Mette Skovgaard (AMS) MD, DMSc, associate professor, chief consultant and head of the research unit at Child- and Adolescent Psychiatric Centre Glostrup, University Hospital of Copenhagen. *AMS* is head of the Copenhagen Child Cohort, CCC 2000 and heads a research group

of three PhD students and three postdocs. She is head of the Infant Mental Health Screening and Intervention Study and the Infant-toddler Clinical Database of the Capital Region as well as supervisor for four PhD students in the field of developmental psychopathology. *AMS* will supervise the WP4 PhD student jointly with *TH*.
Peter Riis Hansen (PRH), DMSc, PhD and associate professor at the Medical School, University of Copenhagen is an invasive cardiologist and consultant at the Department of Cardiology P, Gentofte University Hospital. *PRH* is involved in various areas of cardiovascular research and currently supervises 6 PhD students. *PRH* will be the primary person responsible for WP7. He will also synergise and interact with the pharmacological WP (acquisition of data and development of guidelines for genotype-based dose adjustments) and the genetic WP (receiving results from genotyping of patients).

Project management
The following means will serve to coordinate INDICES and create synergy between the participating parties:

1) Regular contact between the members of the management group through email and telephone, including conference calls.
2) Regular quarterly meetings will take place with key individuals in order to coordinate the overall objectives of INDICES. For those WPs particularly dependent upon each other there will be additional meetings with key people and younger scientist for exchange of results and ideas.
3) Three working meetings during the project period with presentations by the key investigators and their PhD students. These meetings will be held to up-date project participants on the activities in the different WPs and create synergy between participants from different WPs. The working meeting will include key scientists from international research institutions offering study visits for the project's PhD students. This will serve to promote the collaboration and active involvement of external partners in the project and provide opportunities to discuss the study visits and the project as a whole. Working meetings will last 1½ days each.
4) Mid-term evaluation with an advisory panel consisting of two or three internationally recognised specialists. These specialists will in due time receive a report of the achieved results and remaining goals before arriving in Copenhagen, where the evaluation will take place.

In the event of project mismanagement or lack of ability of a participant to achieve the desired milestones within the predetermined period the project leader is authorized to discontinue the grant of this participant and seek for a replacement.
The head of each WP is responsible for developing and carrying out education activities for the PhD associated with the WP in question. For WP2 (pharmacokinetic study) and WP6 (clinical study A – ADHD) the key persons *KD* and *TH* will be assisted by *GJ* and *AMS*, respectively. Contact and collaboration with national and international research groups and networks will primarily be mediated and promoted by the persons heading the individual WP.

12. KEY REFERENCES

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Nominating an assessment committee – statement from the principal supervisor

Responsibilities of the principal supervisor:

- ✓ Make sure that the committee members agree to participate in the assessment committee.
- ✓ Plan the defence together with the PhD student and the assessment committee.
- ✓ Assist the committee with travel arrangements, when the PhD thesis has been approved for defence.

Name of PhD student	Kristine Kaalund-Brok

1. Has the PhD study been satisfactory? Yes ■ No ☐	
2. Has the PhD student gained experience of teaching activities or other form of knowledge dissemination which is related to the student's PhD project? (In publications, at symposiums, conferences, lectures, etc.) Yes ■ No ☐	
3. Do you confirm that the PhD thesis or parts of it does *not* form the basis of any other academic degree awarded to the PhD student? Yes ■ No ☐	
4. Does the assessment committee meet the requirements for the composition of members? Yes ■ No ☐	

Signature

Date: September 15, 2020

Signature, principal supervisor Pia Jeppesen

Full name, principal supervisor: Pia Jeppesen