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Effects of prenatal nutrient supplementation and early life exposures on neurodevelopment at age 10: a randomised controlled trial - the COPSYCH study protocol

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ABSTRACT

Introduction Nutrient deficiency and immune and inflammatory disturbances in early life may compromise neurodevelopment and be implicated in the aetiology of psychiatric disorders. However, current evidence is limited by its predominantly observational nature. COpenhagen Prospective Study on Neuro-PSYCHiatric Development (COPSYCH) is a research alliance between Copenhagen Prospective Studies on Asthma in Childhood (COPSAC) and Center for Clinical Intervention and Neuropsychiatric Schizophrenia Research with the overall aim to investigate effects of prenatal and early life exposures on neurodevelopment at 10 years. COPSYCH will investigate the impact of prenatal n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA) and high-dose vitamin D supplementation on neurodevelopment reflected by brain development, neurocognition and psychopathology. Moreover, the neurodevelopmental impact of early life exposures such as infections, low grade inflammation and the gut microbiome will be scrutinised.

Methods and analysis COPSYCH is based on the prospective and ongoing $COPSAC_{2010}$ birth cohort of 700 mother–child pairs. Randomised controlled trials of supplementation with n-3 LCPUFA and/or high-dose vitamin D or placebo in the third trimester were embedded in a factorial 2×2 design (ClinicalTrials.gov: *NCT01233297 and NCT00856947*). This unique cohort provides deep phenotyping data from 14 previous clinical follow-up visits and exposure assessments since birth. The ongoing 10-year visit is a 2-day visit. Day 1 includes a comprehensive neurocognitive examination, and assessment of psychopathological dimensions, and assessment of categorical psychopathology. Day 2 includes acquisition of brain structural, diffusion and functional sequences using 3 Tesla MRI. Study outcomes are neurocognitive, psychopathological and MRI measures.

Ethics and dissemination This study has been approved by the Danish National Committee on Health Research Ethics and The Danish Data Protection Agency. The study is conducted in accordance with the guiding principles of the Declaration of Helsinki. Parents gave written informed consent before enrolment.

Strengths and limitations of this study

- The Copenhagen Prospective Studies on Asthma in Childhood 2010 cohort consists of 700 children and is a randomised, placebo-controlled trial on effects of prenatal nutrient supplementation on prenatal n-3 long-chain polyunsaturated fatty acids and high-dose vitamin D supplementation on child development.
- The COpenhagen Prospective Study on Neuro-PSYCHiatric Development (COPSYCH) study comprises an extensive 2-day 10-year clinical visit and focuses on brain development, neurocognition and psychopathology.
- Deep phenotyping is available from the previous 14 clinical visits, and a limited selection of neurodevelopmental measures have been continuously assessed.
- The randomised controlled trial design allows for unique, causal inferences on effects of prenatal nutrient supplementation on neurodevelopment at age 10.
- The planned COPSYCH study would be strengthened by longitudinal neurodevelopmental data, thus future similar evaluations of the cohort will be applied for.

INTRODUCTION

Mounting evidence suggests that health and disease are programmed in early life, and that a wide range of prenatal and early life exposures affect brain development.[1] Early

life may therefore represent a window of opportunity for promoting a healthy neurodevelopment. Nevertheless, the evidence regarding early life exposures and their effects on neurodevelopment are limited by observational study designs. Neurodevelopmental disorders, for example, autism spectrum disorder, attention-deficit/hyperactivity disorder (ADHD) and intellectual disability are all highly heritable with complex, polygenetic contributions[2–5] and have all been associated with brain structural and functional aberrations and neurocognitive impairment. Prenatal exposures in the form of supply of micronutrients, like folic acid, are shown to be pivotal to decrease risk of congenital neural tube defects, including anencephaly, spina bifida and encephalocele[6].

Our previous study on the Copenhagen Prospective Studies on Asthma in Childhood 2010(COPSAC$_{2010}$) cohort showed that boys born to mother receiving n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA) supplementation had improved motor development, improved global neurocognitive function at age 2½ years of age as assessed with the neurocognitive part of Bayley Scales of Infant and Toddler development, third edition[7] and reduced functional impact of emotional and behavioural problems by age 6 years according to parents' replies to the Strengths and Difficulties Questionnaire (SDQ).[8–11] No effect on neurodevelopment in the first 6 years of life was observed in the offspring born to high-dose vitamin D3 supplemented mothers.[12]

The risk for manifestation of psychiatric disorders later in life is associated with environmental insults, like maternal infections during pregnancy,[13 14] infections during childhood and adolescence[15–18] or ongoing inflammatory processes like asthma.[19–22] Furthermore, emerging evidence has indicated that n-3 LCPUFA and vitamin D are important for brain development in particular maturation of cerebral white matter myelin and cognition.[23–26]

The beneficial effects of maternal n-3 LCPUFA supplementation on neurocognitive development are not consistent in previous randomised controlled trials (RCTs), and the interpretation is complicated by methodological issues.[27] Still, there is some evidence that n-3 LCPUFA supplement may reduce the prevalence rate of children with lower neurocognitive scores indicative of delayed neurocognitive development at 18 months of age.[28] This may be hypothesised to be due to downregulation of the pro-inflammatory n-6 LCPUFA-derived eicosanoids rather than the more anti-inflammatory eicosanoids since n-3 LCPUFA and n-6 LCPUFA competes for the same enzymes.[29] However, to the best of our knowledge, no RCT of prenatal supplementation with n-3 LCPUFA and high-dose vitamin D in pregnancy has examined the neurocognitive outcome in late childhood.

The composition of the gut microbiome matures within the first years of life and the microbiome may have the ability to affect host inflammatory status at a time, when significant brain development takes place. Perturbation of the microbial and viral homeostasis during this critical period of development may affect the signalling pathways between gut and brain (immune, neural and endocrine pathways, the so-called 'gut-brain axis').[30] Thus, the microbiome may be an intermediary player in the interaction between the host and its environment in the mechanisms that determine the transition from health to disease.[31 32] In the human gut viruses are at least as abundant as bacteria. The viral community (virome) mostly target bacteria (bacteriophages) while only a fraction directly affect humans. Their role in disease development is poorly understood, but may be detrimental in the transition from health to disease in early life, either through manipulation of the bacterial community or through direct virome–host effects.[33–35]

Most of the hypotheses regarding early risk factors are based on studies reporting associations between early life exposures and a later outcome, which could be confounded by other exposures and cannot infer causality.[36] The factorial, randomised controlled design of the current study provide the unique opportunity to unravel causal effects of prenatal n-3 LCPUFA and vitamin D supplementation on neuropsychiatric and neurocognitive status as well as neuroimaging, all reflecting neurodevelopment at age 10. Furthermore, we have extensive information on a broad range of environmental exposures, which were collected through personal interviews with the families and when possible validated against register data, and assessed objectively and longitudinally, including repeated assessment of the airway and gut microbiome from birth throughout childhood.

The main objective of COpenhagen Prospective Study on Neuro-PSYCHiatric Development (COPSYCH) is characterising the neurocognitive, psychopathological and brain structural and functional outcomes, and relating these to the n-3 LCPUFA and vitamin D interventions and early life exposures. Data are collected at a comprehensive, ongoing 2-day 10-year follow-up visit of the COPSAC$_{2010}$ cohort. Comprehensive data on early exposures have been meticulously collected at previous follow-up visits of the COPSAC$_{2010}$ cohort. The ambitious goal is to provide the basis for preventive interventions in prenatal or early life in order to improve neurocognitive development and prevent development of psychiatric disorders.

METHODS AND ANALYSIS

COPSYCH is a 5-year translational research alliance between COPSAC and Center for Clinical Intervention and Neuropsychiatric Schizophrenia Research (CINS) initiated in January 2019.

First clinical examination took place on 11 February 2019. Expected end of clinical examinations is December 1st 2021, followed by data processing and analysis. The COPSAC$_{2010}$ cohort and previous assessments are briefly summarised below and detailed previously.[37]

Table 1 Neurocognitive test battery

Neurocognitive domain	Test	Outcome variable
Speed of processing	Coding (WISC-IV)*	Total number correct.
	Symbol search (WISC-IV)	Sum of total number correct, errors subtracted.
	Trail making test 2—number sequencing (D-KEFS)†	Time to complete in seconds.
Attention/vigilance	Rapid visual information processing (CANTAB)‡	A' (A prime).**
	Reaction time (CANTAB)	Simple and five-choice reaction time.
Motor functioning	Reaction time (CANTAB)	Simple and five-choice movement time.
Fine motor dexterity	Grooved pegboard	Time to complete for the dominant and non-dominant hand.
Verbal memory	Word selective reminding—immediate recall (TOMAL-2)§	Total number of words recalled over six learning trials.
	Object recall (TOMAL-2)	Total number of objects recalled over five learning trials.
Verbal working memory	Digit span (WISC-IV)	Total number of correcr forward and backward digit sequencing.
	Letter-number sequencing (WISC-IV)	Total number of correct sequences.
Visual memory	Paired associates learning (CANTAB)	First trial memory score, total errors (adjusted).
Executive functions		
Flexibility/set shift	Intra–extra dimensional set shift (CANTAB)	Extra-dimensional stage errors.††
	Trail making test 4—number–letter switching (D-KEFS)	Time to complete in seconds.
Spatial working memory	Spatial span task (CANTAB)	Span length.‡‡
	Spatial working memory (CANTAB)	Total errors§§ and strategy formation.
Planning	Stockings of cambridge (CANTAB)	Problems solved in minimum moves.
Behaviour rating of executive functions	BRIEF-2¶	Impulse control, self-monitoring, flexibility, emotional control, initiating, planning/organisation, working memory and task monitoring.
Intelligence	Vocabulary (WISC-IV)	Total number correct.
	Matrices (WISC-IV)	Total number correct.

*WISC-IV = Wechsler Intelligence Scale for Children—fourth edition.
†D-KEFS = Delis-Kaplan Executive Function System.
‡CANTAB = Cambridge Neuropsychological Test Automated Battery.
§TOMAL-2 = Test of Memory and Learning—Second Edition.
¶The Behaviour Rating Inventory of Executive Function—second edition.
**A prime measures target sensitivity.
†† Extra-dimensional stage errors denotes the number of errors made in the extra-dimensional stage of the task, where the child is required to make an extradimensional shift.
‡‡Span length denotes the longest sequence successfully recalled by the child (three attempts at each level).
§§ Total errors comprise the number of times a box is selected that do not have hidden target that must be found and therefore should not have been visited by the child.
BRIEF-2, Behaviour Rating Inventory of Executive Function, Second Edition; CANTAB, Cambridge Neuropsychological Test Automated Battery; D-KEFS, Delis-Kaplan Executive Function System; hs-CRP, High-sensitivity C-Reactive Protein; TNFα, Tumor Necrosis Factor α; TOMAL-2, Test of Memory and Learning, Second Edition; WISC-IV, Wechsler Intelligence Scale for Children.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

The COPSYCH 10-year visit

The ongoing 10-year COPSYCH visit extends over 2 days. Day 1 is a 6–8 hour visit at the COPSAC research clinic. The first part consists of a neurocognitive assessment (table 1) and the second part is a neuropsychiatric assessment, where categorical and dimensional psychopathology is assessed using a diagnostic interview and parental and teacher questionnaire ratings (table 2).

Neurocognition

The *neurocognitive test battery* is based on selected subtests from several neuropsychological tests. The Cambridge Neuropsychological Test Automated Battery (CANTAB)[38] is a neuropsychological test battery developed from a neuroscience approach. The CANTAB battery has been validated for use between the ages 4–80 years[39] and has been extensively used in, for example, lesion studies as well as neuropsychiatric and neurological populations. The CANTAB tests have been used in clinical cohorts of both children[40] and adults with neurodevelopmental disorders including schizophrenia and ADHD, and healthy controls[41]. To assess specific neurocognitive

Table 2 Psychopathological interviews and questionnaires

Psychopathology				
Categorical	Dimensional			
Examinator	Parents	Teachers*	Examinator	Child
K-SADS-PL screening interview and supplements, initially with the parent(s), followed by the child	ADHD -RS SDQ CBCL SRS-2	ADHD-RS SDQ TRF	TOF C- GAS K-SADS-PL Schizophrenia Spectrum and Other Psychotic Disorders Supplement—number of psychotic-like experience (PLE)†	MTQ

The COpenhagen Prospective Study on Neuro-PSYCHiatric Development 10-year psychopathological examination programme at day 1.
*Questionnaires are mailed to the child's teacher.
†The supplement is administered with all the participants to assess the prevalence of PLE.
ADHD-RS, Attention Deficit/Hyperactivity Disorder-Rating Scale; CBCL, child behaviour checklist; C-GAS, Children's Global Assessment Scale (included in K-SADS-PL); CXCL, CXC Chemokine Ligand 8; K-SADS-PL, The Kiddie Schedule for Affective Disorders and Schizophrenia, Present and Lifetime Version; MTQ, Magical Thinking Questionnaire; SDQ, Strengths and Difficulties Questionnaire; SRS-2, Social Responsiveness Scale; TOF, Test Observation Form; TRF, Teacher Report Form.

functions across the selected neurocognitive domains from CANTAB, we have included the following: rapid visual information processing to assess sustained attention; reaction time task to assess both simple and five-choice reaction time and movement time; and paired associates learning to assess visual memory. Executive functions are assessed using the intra–extra dimensional set shifting task examining mental flexibility; the stockings of cambridge examining planning, and the spatial span and spatial working memory tasks, respectively, testing working memory span, and spatial working memory manipulation and strategy formation.

Two subtests (vocabulary and matrices) from the Wechsler Intelligence Scale for Children, fourth edition (WISC-IV)[42] are used to estimate intelligence; additionally other subtests are used to characterise verbal working memory (digit span and letter–number sequencing) and processing speed (coding and symbol search).[40 42] From the Delis-Kaplan Executive Function System, trail making 2 is applied as an additional measure of processing speed, and the trail making 4 to measure flexibility/set shift.[40 43] Moreover, selected subtests (Word Selective Reminding and Object Recall) from Test of Memory and Learning—Second Edition are used for evaluating verbal memory (2011).[40 44] Fine motor dexterity is assessed with Grooved Pegboard and Reaction Time in CANTAB.[45 46] The parent and teacher fill out Behaviour Rating Inventory of Executive Function, Second Edition (see table 1)[47] measuring daily life executive functions in the home environment and school environment, respectively.

All the neurocognitive tests are well validated, reliable and appropriate for this age group.[39 41 48–50] The time for completing the full test battery is approximately 2 hours, but the duration is individualised with breaks when needed. Tests are administered in a fixed order.

Categorical psychopathology

The diagnostic interview Kiddie-Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL)[48] is used to provide information on current and lifetime psychopathology. K-SADS-PL is a semi-structured clinical diagnostic interview containing the same questions for parents and children (except in the Autism Spectrum Supplement that contains extra questions for the parents only). According to the K-SADS-PL guidelines the interview is first completed with a parent/caregiver and afterwards with the child younger than 11 years of age. The K-SADS-PL consists of a general screening interview. If the threshold of a symptom has been reached in the general screening interview, K-SADS-PL contains additional questions in supplements, which target specific disorders. K-SADS-PL Schizophrenia Spectrum and Other Psychotic Disorders Supplement is applied whether or not any psychotic symptoms appear in the screening interview to detect possible psychotic-like experiences (PLE).[51]

To obtain information on potential current and earlier use of psychotropic medicine, questions from the Schedules for Clinical Assessment in Neuropsychiatry[52] are reworded to interview about prescribed psychotropic medication to the child (antidepressants, mood stabilisers, anxiolytics, hypnotics, antipsychotics, central stimulants).

Children's global assessment of functioning[53] (included in K-SADS-PL), is used for assessment of the child's global level of functioning.

Research diagnoses are made according to both International Classification of Diseases 10th Revision (ICD-10)[54] and Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5).[55] Diagnoses are based on information from all available sources (clinical observations during the neuropsychological testing and psychopathological assessments of each participating child). All K-SADS-PL interviews are video recorded. The videos are used for supervision and to reach consensus on diagnostics. Neurocognitive assessments and clinical interviews are supervised by a senior researcher, psychologist with specialty in child and adolescent psychiatry (JRMJ) and consensus diagnoses are made weekly (between JRMJ and

at least two examiners). All diagnoses are furthermore re-evaluated at a monthly conference with a professor of child and adolescent psychiatry (NB). Additionally, cases with an inconclusive diagnosis are supervised by NB.

Ratings of The Test Observation Form (TOF), the verbal subtest from WISC-IV (vocabulary) and Children's Global Assessment Scale are also based on consensus rating with JRMJ. Inter-rater reliability will be estimated based on 10 K-SADS-PL screening interview videos recorded (by JRMJ) during the first half data collection period. Each 20th child that is not assigned a diagnosis will undergo re-evaluation with NB. The four interviewers have supervision on a weekly basis.

Dimensional psychopathology

In order to also quantify psychopathology, which may or may not meet the threshold for a psychiatric diagnosis, dimensional assessments of psychiatric symptoms and behavioural problems are undertaken on all children. For a detailed registration of inattentive and impulsive–hyperactive behaviours in different situations, the ADHD—Rating Scale (ADHD-RS) questionnaire is administered (filled out by parents and teachers, respectively).[56] Similarly, the Social Responsiveness Scale—2 is used for parental ratings of the severity of autism spectrum disorder traits.[57 58] In terms of dimensions of child and adolescent psychopathology, the parents report their evaluation in the Child Behaviour Checklist (CBCL)[59 60]; The teacher's report form (TRF) is completed by the child's teacher. In most aspects the TRF corresponds to the CBCL as most of its items have counterparts in the CBCL. SDQ is a multi-informant screening tool for emotional and behavioural problems in children and adolescents, answered by parents and teachers.[61] TOF will be used to score severity of behavioural and emotional problems observed during the neurocognitive test session. It consists of 125 items, scored on a 4-point Likert scale. TOF is completed by the examiner immediately after the neurocognitive testing.[62]

MRI (day 2)

Day 2 is a 1-hour visit (table 2). MRI is a non-invasive medical scanning technique, which can acquire images of the brain without application of radioactive ligands or X-rays. MRI scans are acquired on a 3T Philips Achieva scanner (Philips Healthcare, Best, the Netherlands). Both brain structural and functional sequences are acquired (table 3). The total duration of MRI acquisition is 35 min, which including breaks effectively corresponds to 40–45 min of scan time.

All children undergo a virtual MRI 'practice scanning' using a pair of three-dimensional (3D) goggles on examination day 1. In order to minimise movement and impatience, all children are given the opportunity to watch an animation movie while inside the MRI scanner, using a mirror and video projection. We extract head motion parameters using the Tracoline system, which is an advanced 3D stereo vision system to monitor and capture head movements using 3D surfaces from structured invisible infrared light, enabling markerless tracking of the subject's head in a clinical MRI setup (https://tracinnovations.com/markerless-technology/). These motion parameters are used to retrospectively correct for scanner head motion.

Table 3 MRI sequences

MR sequence	Abbreviations	Time (min)
Survey		0.5
Structural MRI	T1w	6
T2-weighted imaging	T2w	3.5
Magnetisation transfer imaging	MTI	3
Diffusion weighted imaging (restricted spectrum imaging)	DWI	7.5
Quantitative MRI (strategically acquired gradient echo, STAGE)	T1 map and PD map	4.5
Magnetic resonance spectroscopy	MRS	5
MR angiography and flow	PCM*	5
Total time 35 min		

MR sequences at the COpenhagen Prospective Study on Neuro-PSYCHiatric Development 10-year visit.
*Phase-contrast mapping.

COPSAC$_{2010}$ cohort

COPSYCH is based on the COPSAC$_{2010}$ cohort consisting of 700 mother–child pairs followed prospectively from pregnancy week 24.[37 62] A population-based sample of 736 pregnant women were recruited during 2008–2010 and participated in a double blinded, randomised and placebo controlled study of fish oil, n-3 LCPUFA (2.4 g/day n-3 LCPUFA) and/or high-dose vitamin D (2400 IU/day) supplementation during the third trimester with the primary outcome of persistent wheeze/asthma until age 3, where the trials were unblinded (ClinicalTrials.gov: *NCT00798226 and NCT00856947*). Data validation and quality control followed the guidelines of Good Clinical Practice. Exclusion criteria were chronic cardiac, endocrinological, nephrological or lung disease other than asthma or a daily vitamin D intake above 600 IU.

Neurodevelopmental outcomes were prespecified secondary outcomes specified in the primary study protocol (including milestones, language and The Bayley Scales of Infant and Toddler Development) The parents were informed that they participated in a cohort study lasting at least 3 years, and that we planned to follow the children forward if we succeeded in financing. The clinical visits after 3 years have been planned accordingly for the purpose of gaining as much knowledge in the prespecified fields as possible, and to replicate and continue further research of the previous findings

The mothers and their children have been closely followed prospectively at the COPSAC research unit with 14 completed clinical visits scheduled from pregnancy week 24 until 8 years and with day-to-day diaries from birth capturing all symptoms related to airways, skin and infectious episodes the first 3 years between visits

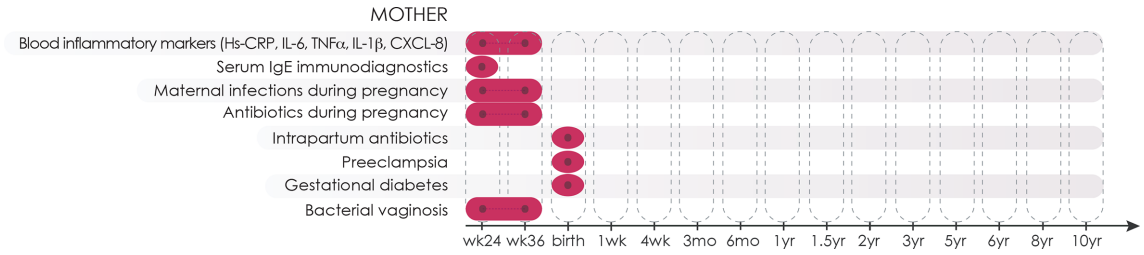

Figure 1 Overview of inflammation and infection markers in COPSAC2010 (mothers) - Modified from: http://copsac.com/home/copsac-cohorts/copsac2010-clinic/)

(figure 1). Retention rate for the previous visit at age 8 years was 92%.

The ongoing neurocognitive and psychopathological 10-year examinations are undertaken by trained medical doctors and experienced nurses, who will remain blinded to the intervention status of the children throughout the data collection and statistical analyses.

Previous visits and examinations

At all previous 14 clinical visits, the parents were interviewed about a variety of socioeconomic factors such as smoking during pregnancy, family size, household pets and so on. Furthermore, information has been collected on delivery mode, Apgar scores, maternal diet during pregnancy 4 weeks prior to randomisation, breastfeeding length, fatty acid composition of breast milk and introduction of solid foods. The child's daily activity level and sleep patterns were also measured at 2 years and 10 years of age by accelerometry (wristband that measures daily physical activity, omnidirectional Actical accelerometer, Philips Respironics, Murrysville, Pennsylvania, USA).[63]

Metabolomics

Global metabolomics profiling of blood was done in the mothers at 24 weeks gestation and 1-week post partum and in the children at age 0.5, 1.5 and 6 years.[64] Further, dry blood spot metabolomics profiling was done in the newborn child and urine metabolomics by age 1 month.[65] These metabolomic assessments allow discovery of yet unknown mechanisms affecting aspects of neurodevelopment.

Genetics

Genomewide scan has been conducted on the cohort using the Illumina HumanOmniExpressExome-8 V1.2 BeadChip, including genetic variants of importance for n-3 LCPUFA and vitamin D levels and metabolism, which will be included in the analyses of the intervention. This data will allow us to analyse to which extent the effect of early exposures are modified by host genetics.

Microbiome

Longitudinal gut microbiome composition profiling has been performed in the cohort using 16S rRNA gene amplicon sequencing[66] at 1 week, 1 month, 1 year, 4 and 6 years and metagenome at 1 year. These early samples together with samples collected at 8 and 10 years will allow us to characterise the microbial development until age 10 and how this may impact components of neurodevelopment.

Inflammation in mothers and children

We measured systemic inflammatory surrogate markers including high-sensitivity C-Reactive Protein (hs-CRP), interleukin (IL)-6, Tumor Necrosis Factor alpha (TNFα), IL-1β and CXC Chemokine Ligand 8 (CXCL-8). Furthermore we assessed a panel of immune mediators in the upper airways related to specific immune signalling pathways in unstimulated upper airway mucosal lining fluid at 1 month, 2 years and 6 years of age and at episodes with acute respiratory symptoms. Finally, at 18 months a deep immunological phenotyping was done with an extensive profiling of immune cell distribution and function including detailed phenotyping of 25 immune cell populations and 8 ligand simulations in freshly collected whole blood (figure 1).[67]

Infections and prenatal information

Mothers were interviewed regarding infectious symptoms and use of medication including all courses of antibiotics in pregnancy at the scheduled visits preintervention and post-intervention. The first 3 years of the child's life the families kept diary cards monitoring signs of infection categorised as common cold, pneumonia, pharyngitis, otitis, fever, gastrointestinal infection and absence from daycare because of illness. The COPSAC physician validated the diary cards entries with the parents at each visit and interviewed them regarding other types of infections. Information was checked against hospital and medication prescription registries. After age 3, data on hospital admissions and information about prescribed medication were gathered at the scheduled visits. Diagnosed preeclampsia, gestational diabetes and pre-pregnancy weight were registered. These data allow

Diagnosis of asthma, allergy and eczema

Asthma/persistent wheeze was diagnosed according to a validated symptom-based quantitative algorithm (five episodes of troublesome lung symptoms, typical asthma symptoms, symptom relief when using inhaled short-acting B_2-agonists and response to a 3-month trial of inhaled corticosteroid).[68 69] Lung function was measured with whole-body plethysmography and spirometry, and

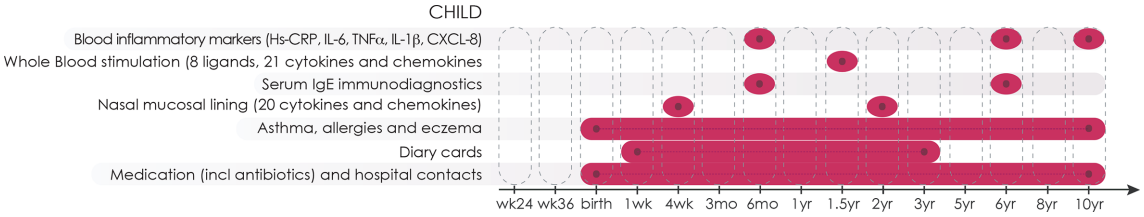

Figure 2 Overview of inflammation and infection markers in COPSAC2010 (children) - Modified from: http://copsac.com/home/copsac-cohorts/copsac2010-clinic/)

bronchial responsiveness was evaluated by methacholine challenge.[70 71]

Allergen specific IgE levels along with skin prick tests have been measured for the most common inhalant and food allergens and allergic rhinitis was diagnosed prospectively.[72 73]

Eczema diagnosis was based on the Hanifin-Rajka criteria[74] and the SCORing Atopic Dermatitis (SCORAD) index was used to measure severity during flares (severity scoring of atopic dermatitis)[73 75–77] (figure 2).

Previous neurodevelopmental and cognitive assessments

The children's neurodevelopmental status[78] has been examined throughout the years. Motor milestone achievements were continuously evaluated during the first years of life by the parents using a registration form based on the Denver Developmental Index,[79] and WHO milestones.[79 80] Language development was evaluated with MacArthur Bates Communicative Developmental Inventory at age 1 and 2 years.[81] By the age of 2½ years global neurocognitive functioning was examined using the neurocognitive part of Bayley Scales of Infant and Toddler development, third edition.[7 81] At age 3 years general neurodevelopment was assessed by the Ages and Stages Questionnaire.[82] At ages 6 and 8 years, parents were asked to fill out a SDQ[8–10] focusing on emotional and behavioural problems. Moreover, at age 8 years, parents were also asked to fill out the ADHD-RS[56 83] which is a questionnaire merely focused on ADHD symptoms and oppositional defiant disorder symptoms (figure 3).

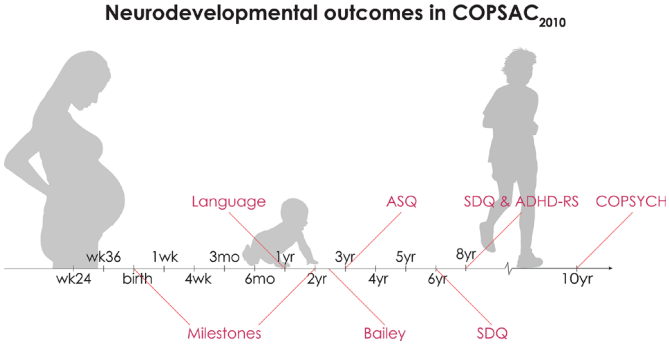

Figure 3 Neurodevelopmental outcomes in COPSAC$_{2010}$. ADHD-RS, Attention Deficit/Hyperactivity Disorder-Rating Scale; ASQ, Ages and Stages Questionnaire; COPSAC$_{2010}$, Copenhagen Prospective Studies on Asthma in Childhood 2010; COPSYCH, COpenhagen Prospective Study on Neuro-PSYCHiatric Development; SDQ, Strengths and Difficulties Questionnaire.

Power calculation

At the last visit at 8 years, the retention rate was 92%, and we anticipate a similar participation rate at the 10-year visit. With a total sample size of N=644 and an alpha level of 0.05, t and a SD of 1.00, this study has a power of 80% to detect an effect size of 0.22 SD. Based on the SD of the psychopathological and global functioning raw scores reported in a large, recent Danish sample of population-based control children 7 years of age,[40] the effect size of 0.22 SD is equivalent to 3.3 raw scores on the child behaviour checklist total problem scale, and 3.0 raw scores on the CGAS, respectively. We assume that the raw score distributions observed in these children with a mean age of 7.8 years are equivalent for 10-year-old children. Finally, this effect size is equivalent to 3.3 scores on the Processing Speed Index and the Working Memory Index using the published Danish WISC-IV norms.[84]

Risks, side effects and potential benefits

There are no known risks of simple MRI scanning. Discomfort during the actual scanning will be minimised with earplugs and hearing protectors.

Children with psychiatric symptoms who have not been evaluated prior to the visit may likely benefit from the thorough COPSYCH evaluation. Early identification of mental challenges or detection of a psychiatric diagnosis is crucial in order to optimise, for example, the child's schooling performance.[85] Children who have psychiatric disorders are referred directly to child and adolescent mental health services (after the parents give their permission and consent).

Main outcomes

Neurocognition

Neurocognitive functioning reflecting intelligence and the following seven neurocognitive domains: speed of processing, attention/vigilance, psychomotor speed, verbal memory, visual memory, executive functions and motor functioning (table 1).

Categorical psychopathology

Categorical psychopathology, that is, child and adolescent disorders according to ICD 10[54] and DSM 5.[55]

Dimensional psychopathology

Dimensional psychopathology scores will be based on ratings in all psychopathological questionnaires (total scores and subscales scores (table 2) and on the number of mental and behavioural symptoms reported in the

K-SADS-PL.[48] Severity is thus reflected in a quantitative composite index of psychiatric symptoms which is the number of symptoms endorsed by a severity score of 'three' in the complete K-SADS-PL interview, that is, total number of symptoms at/over the threshold. Present symptoms will be counted separately from previous symptoms. Additionally, the number of symptoms within different subdomains will be calculated, for example, affective and anxiety disorders; neurodevelopmental and behavioural disorders. Counting symptoms does not interfere with the standard administration of the K-SADS-PL interview.

Using this method we will quantify the symptom load of each child since a dimensional approach may be more sensitive than the categorical diagnostic approach in our context. A similar approach has been applied here where they use symptom composite measure from the K-SADS.[86] The symptom count method will include all children irrespective of their diagnostic status.

PLE

We will examine if the children have experienced PLE.[87 88] PLE will be measured by semi structured interviews using the K-SADS-PL-items on psychotic symptoms (Schizophrenia Spectrum and Other Psychotic Disorders Supplement), each symptom scored as 'not present' (1) 'likely present' (2) or 'definitely present' (3). A similar method was used in a previous Danish Copenhagen Child Cohort study (CCC2000).[89] A score of 2 is reflective of a PLE. PLE will be consensus rated with a medical doctor (MR) with extensive experience from a prior similar study.[89]

Brain structure and function

Structural MRI measures of grey and white matter will be used to disentangle the putative link between early inflammation, psychosocial functioning, neurocognition and psychopathology. We will use conventional software packages to obtain measures of cortical thickness and surface area, curvature and volumes of specific grey matter brain structures (eg, FreeSurfer (http://surfer.nmr.mgh.harvard.edu/): Diffusion data we will used to assess white matter integrity using, for example, Tract-based Spatial Statistics (TBSS) (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/TBSS). Moreover we will use the functional data to assess cerebral blood flow and the spectroscopy data will provide novel data on cerebral lactate metabolism.[90]

ETHICS AND DISSEMINATION

This study is conducted in accordance with the guiding principles of the Declaration of Helsinki and was approved by the Local Ethics Committee: H-B-2008–093 and the Danish Data Protection Agency 2015-41-3696. All families will receive written information about the study prior to participation and both parents will give written informed consent before enrolment.

Acquired data are stored in the secure Research Electronic Data Capture system provided by the Capital Region of Copenhagen. Direct electronic typing of as much data as possible is enforced, and double data entry by independent staff ensures validity of data.

Perspectives

This is the largest study of its kind, with a broad range of exposures, which were validated by personal interviews with the families. Identifying the possible linkage between neurocognitive impairments, psychopathology and early life exposures may become a game changer in future prevention of psychiatric disorders and neurocognitive impairments, for example, in terms of change of recommended guidelines of micronutrient and vitamin supplementation or other exposures during pregnancy. Knowledge delineating the mechanisms involved in linking early life exposure to aberrant neurodevelopment will potentially enable a more targeted therapeutic approach for the prevention or amelioration of structural and functional brain and neurocognitive developmental abnormalities, and the onset of psychopathology. Thereby, the results of this research may have direct, easily accessible and essential impact on children's brain health.

Positive, negative and inconclusive results will be presented at national and international conferences. Papers will be submitted to peer-reviewed journals.

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Contributors HB was overall responsible for designing and conducting the $COPSAC_{2010}$ cohort. HB is the sponsor and principal investigator of the study. HB, KB, BHE and BYG have conceived the idea and promoted the concept of the COPSYCH study with contributions from BC and JS. BF, JRMJ, NB and CP were involved in designing the cognitive and psychopathological part of the study. BHE, JMR, HL, MV and UL were involved in planning the MRI sequences and MRI data processing. MES, DS and NF were key personal in planning and conducting the study, including data handling. JBR and PM were the principal clinical investigators of the project. The manuscript was drafted by PM, JBR, RV, JRMJ and BHE. PM and JBR contributed equally to this paper and share first authorship. BHE and HB share senior authorship. All authors have contributed to the description of the study and have made critical revision of the manuscript. All authors have read and approved the final version of the manuscript.

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Data availability statement The COPSYCH study will record data sets of up to 700 participants. The data set will include demographic data from interviews, self-reported behavioural data from questionnaires, imaging data (MRI), neurocognitive test data and laboratory (biochemical) data. Researchers are blinded prior to analysis to avoid bias in the analysis results of the randomised controlled trial. Data is collected with approval from the Danish Data Protection Agency and the National Scientific Ethical Committee. All research data and associated documentation is available to other researchers and official institutions (Ethical Committee, The National Board of Health and the Good Clinical Practice Unit) affiliated to the COPSYCH study during the intervention. All Case Report forms data will be stored for 5 years after completing the primary analysis and hereafter destroyed.

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