

PROF. HANS BISGAARD (Orcid ID : 0000-0003-4131-7592)

Article type : Regular Article

Determinants of Neurodevelopment in Early Childhood – Results from the Copenhagen Prospective Studies on Asthma in Childhood (COPSAC₂₀₁₀) Mother-Child Cohort

Elín Bjarnadóttir, MD, PhD^{a+b*}; Jakob Stokholm, MD, PhD^{a+b*}; Bo Chawes, MD, PhD, DMSc^a; Jonathan Thorsen, MD, PhD^{a+b}; Anna-Rosa Cecilie Mora-Jensen, MD^{a+b}; Maja Deleuran, MD^{a+b}; Klaus Bønnelykke, MD, PhD^a; Lotte Lauritzen, MSc, PhD^c; Hans Bisgaard, MD, DMSc^a

*These authors contributed equally to the manuscript.

Affiliations: ^aCOPSAC, Copenhagen Prospective Studies on Asthma in Childhood, Herlev and Gentofte Hospital, University of Copenhagen, Copenhagen, Denmark; ^bDepartment of Pediatrics, Naestved Hospital, Naestved, Denmark; and ^cDepartment of Nutrition, Exercise and Sports, University of Copenhagen, Frederiksberg, Denmark

Address correspondence to: Professor Hans Bisgaard, MD, DMSc, Copenhagen Prospective Studies on Asthma in Childhood, Gentofte Hospital, University of Copenhagen, Ledreborg Alle 34, DK-2820 Gentofte, Denmark, (+45)-39777360, Fax: (+45)- 39777129, bisgaard@copsac.com, www.copsac.com

Running title: Determinants of early neurodevelopment

Ethics committee approval: The study was conducted in accordance with the guidelines in the Declaration of Helsinki and approved by the Ethics committee for Copenhagen (H-B-2008-093) and the Danish Data Protection Agency (j.nr. 2008-41-2599).

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/apa.14753

This article is protected by copyright. All rights reserved.

Abbreviations

BSID-III = Bayley Scales of Infant and Toddler Development, Third Edition

CDI= MacArthur Communicative Development Inventory

CI= Confidence interval

COPSAC₂₀₁₀ = Copenhagen Prospective Studies on Asthma in Childhood₂₀₁₀

PCA = Principal Component Analysis

PC = Principal Component

WHO = World Health Organization

Category of study: This is a clinical study

Abstract

Aim: The objective of this study was to identify possible pre- and postnatal factors influencing neurodevelopment of the young child.

Methods: We used data from the Copenhagen Prospective Studies on Asthma in Childhood 2010 (COPSAC₂₀₁₀) mother-child cohort, but excluded those with a neurological diagnosis, born <37 weeks of gestation and birth weights <2500g, resulting in 650 children analyzed. Neurodevelopment was assessed as age of achievement of early milestones, language scores at 1 and 2 years and cognitive score at 2 ½ years of age.

Results: Neurodevelopmental scores were not associated with breastfeeding, persistent wheeze, eczema, and number of sick days ($p>0.05$ in all tests). Early age at milestone achievement was associated with male sex ($p=0.05$), lower maternal age ($p=0.02$), higher gestational age ($p<0.001$) and paternity leave ($p=0.01$). A higher 1-year language score was associated with female sex ($p=0.02$) and maternal smoking during pregnancy ($p=0.01$) and a higher 2-year language score with female sex ($p<0.001$) and being first born ($p=0.01$). A higher cognitive score was associated with female sex ($p=0.02$).

Conclusion: Neurodevelopmental scores were unrelated to breast-feeding, persistent wheeze, eczema, and number of sick days.

Neurodevelopment in early childhood was mostly associated with gender.

Key Notes

Multiple prenatal and early life risk factors can influence neurodevelopment of the child.

Breastfeeding has been shown to increase IQ, whereas asthma and eczema in infancy have been suggested to have a negative effect on neurodevelopment.

This study shows no association between breastfeeding, persistent wheeze and eczema and neurodevelopment of the young child, but identifies gender as the most consistent determinant.

Introduction

Early life exposure may play a role shaping neurodevelopment(1) and be an important indicator of the child's subsequent life achievements(2,3). Neurodevelopment, including cognitive function such as intelligence and language as well as fine and gross motor skills, depends on the pre- and postnatal development of the brain, which is affected by both genetic and environmental factors. During the last trimester of fetal life and the first two years of childhood, the brain undergoes a period of rapid growth(4) where it reaches 80% of its adult weight. Throughout this period the brain development is particularly vulnerable to the pre- and perinatal environment including not just biological factors like lack of oxygen or extreme prematurity, but also other factors like socio-demographic determinants(5), stress and nutrition(2,3). Higher IQ later in life has been associated with a longer duration of breastfeeding(6). Delayed neurodevelopment in childhood has been associated with chronic inflammatory disease such as asthma, eczema and allergic sensitization in infancy (7) and conversely delayed neurodevelopmental scores have also been shown to predate later development of such disorders(8), though in general the studies have shown ambiguous results(9,10).

The aim of this study was to identify prenatal and early life factors associated with achievement of developmental milestones, and scores in language and cognitive tests in the Copenhagen Prospective Studies on Asthma in Childhood 2010 (COPSAC₂₀₁₀) mother-child cohort. We explored whether early neurodevelopment was associated with duration of breastfeeding, sex, being a first born, paternity leave, smoking during pregnancy, gestational age, maternal age, age at start daycare, maternal education and furthermore examined potential effects of persistent wheeze, eczema and number of sick days in the first three years of life.

Methods

Study design and participants

The COPSAC₂₀₁₀ is a population-based clinical prospective mother-child cohort study of 738 pregnant women and their children recruited during 2008-2010 which has previously been described in details(11). The study was conducted in accordance with the guidelines in the Declaration of Helsinki and approved by the Ethics committee for Copenhagen (H-B-2008-093) and the Danish Data Protection Agency (j.nr. 2008-41-2599). Both parents gave informed consent prior to enrollment of the children.

The children were enrolled in the study at 1 week of age, excluding anyone with severe congenital abnormality. The children were followed in the COPSAC clinical research units with 11 planned visits during their first three years of life. Children with a neurological diagnosis, born <37 weeks of gestation and birth weights <2500g were excluded from the present study.

Neurodevelopmental assessment

Milestones

At the child's first visit (1 week of age) the parents received a registration form, with thorough instructions, based on The Denver Development Index(12) and WHO (World Health Organization) milestones registration(13). Dates of achievement of 13 predefined milestones were registered prospectively by the parents. Implementation of the prospective milestone registration started after the first 500 children were born, and some of the milestones of these children were therefore registered retrospectively.

Language development

Language development was assessed with the Danish version of The MacArthur Bates Communicative Developmental Inventory (CDI)(14). The assessment was performed by a web-based questionnaire filled out by parents around the child's 1st (CDI-WG: Words and gesticulation) and 2nd year birthdays (CDI-WS: Words and sentences). The 1-year questionnaire evaluates language comprehension, early word production and gestural communication and the 2-year questionnaire assesses vocabulary, grammatical skills, syntax and morphology.

Cognitive development

Cognitive development was assessed at 2½ years of age, using the cognitive part of the third edition of the Bayley Scales of Infant and Toddler Development (BSID-III)(15). 10 examiners performed examinations under video surveillance, and inter-examiner consistency was all checked by the first author inspecting of every video recordings blinded for the children's score. During the examinations, the examiner presented a series of test materials to the child and observed the child's responses and behavior. Based on its performance, the child was given a composite score, which was standardized by use of a normalization material of age corrected means of 100 and a standard deviation of 15 (range 50-150).

Further methodological details regarding neurodevelopment are described in Appendix S1.

Predictors of neurodevelopmental outcomes

Information regarding factors potentially associated with the child's neurodevelopment was collected prospectively during the scheduled visits to the COPSAC clinics. This included information regarding pregnancy and birth (i.e. sex, ethnicity, anthropometric data, congenital disease, gestational age, maternal smoking during pregnancy and maternal age at delivery), home environment (i.e. older siblings, language spoken), socioeconomic and educational status, paternal leave and age at start in daycare.

Duration of breastfeeding

Information on breastfeeding was obtained longitudinally by interviews and investigated as duration of exclusive breastfeeding and total breastfeeding period. Breastfeeding was evaluated as both continuous variables and grouped: Exclusive breastfeeding (0-1 month, 1-4 months, 4-6 months, >6 months) and total breastfeeding (0-1 month, 1-4 months, 4-6 months, 6-12 months, >12months).

Clinical predictors of neurodevelopment

Respiratory and skin symptoms were recorded by the parents in daily diaries from birth to 3 years. Persistent wheeze was diagnosed according to a previously validated quantitative algorithm(16) requiring all of the following: (1) recurrent troublesome lung symptoms (verified diary recordings of ≥ 5 episodes of troublesome lung symptoms lasting ≥ 3 days within 6 months or continuous troublesome lung symptoms > 4 weeks); (2) typical symptoms of asthma, e.g. exercise induced symptoms, prolonged nocturnal cough, persistent cough outside common cold; (3) need for intermittent bronchodilator; and (4) response to a 3-month trial of inhaled corticosteroids and relapse upon cessation(16).

Eczema diagnosis was based on the criteria of Hanifin and Rajka, which requires the presence of 3 of 4 major criteria and ≥ 3 of 23 minor signs(17).

Diagnoses of persistent wheeze and eczema at any time point before age 3 were used as dichotomized end-points.

Sick days: Infections, categorized as common cold, pneumonia, pharyngitis, otitis, fever, gastro-intestinal infection were monitored in the daily diaries. The number of days with either troublesome lung symptoms and/or infection in the child's first year was used as a continuous variable to define "number of sick days in first year of life".

Statistical analysis

All variables were tested for normal distribution and differences in the population characteristics were determined by Chi-square test, Student's t-test, or Wilcoxon's rank-sum test. Missing data was treated as missing observations, except in the principal component analysis (PCA) of milestones.

Principal component analysis (PCA) was used to extract underlying latent components (principal components, PCs), which describe the systematic part of the variation across the original milestones and language variables in fewer uncorrelated variables. For the milestone data we used probabilistic PCA (R) to generate the PCs, as this analysis can analyze full data set with missing values, assuming that the missing values are at random. No transformation of data was needed for language data as there were no missing values. Linear or logistic regression analysis was used where appropriate to determine significant predictors of neurodevelopmental test scores. All the *a priori* factors identified as possible predictors of neurodevelopment were included in the model (sex, parity, smoking

during pregnancy, gestational age, maternal age at delivery, maternal educational level, breastfeeding duration, paternal leave, and age at start in daycare). Analyses of clinical predictors (persistent wheeze, eczema and sick days) were adjusted for sex.

To examine correlations between neurodevelopmental endpoints, a heatmap was drawn based on Spearman's correlations between all variables, using pairwise complete observations. For presentation, the variables were clustered using hierarchical clustering of euclidean distances, and drawn using the R package "pheatmap".

A significance level of 0.05 was used in all types of analysis. All estimates were reported with 95% confidence intervals (CI). The data processing was conducted using SAS version 9.3 for Windows (SAS Institute Inc., Cary, NC, USA) and R version 3.3.0 (R core team 2016), with the packages "ggplot2", "ggbiplot2" and "ggrepel".

Results

Baseline characteristics

A total of 700 children were included in the COPSAC₂₀₁₀ cohort at birth with a clinical follow-up rate of the cohort of 98% at age 1 year; 95% at age 2 years; and 94% at age 3 years. 34 children were excluded from the neurodevelopmental analysis: 5 because of a neurological diagnosis, 24 because they were born prematurely and 5 because of low birth weight. Furthermore, 16 children did not complete any of the neurodevelopmental tests, leaving 650 eligible children with neurodevelopmental assessment. **Figure 1** shows the flow of participants throughout the study.

Baseline characteristics of the participants is shown in **Table 1**. The children were solely breastfed for 104 days (SD 60) and the total length of breastfeeding was 245 days (SD 155). During the first year of life, the children had a median of 48 (IQR, 28-79) days with either an infection and/or troublesome lung symptoms. By the age of 3 years, persistent wheeze had been diagnosed in 19% (123 children), and eczema in 25% (165 children). There were no significant differences in the baseline characteristics between the children, who completed the neurodevelopmental endpoints and the ones, who did not.

Neurodevelopment

Milestones

At least one of the 13 milestones were registered by 610 of the families and **Figure 2** shows the age of milestone achievement in these children. In the PCA all the milestones were positively correlated in the first principal component (PC1), which explained 37% of the variation and was driven primarily by the late gross-motor milestones (crawling, walking and standing). The second principal component (PC2) explained 16% of the variation and was primarily driven by the early milestones

(smiling, lifting the head, and sitting with support) and the later milestones in opposite directions (Supplemental Figure S1). PC1 and PC2 were used as the main milestone variables in our predictor analysis as they explained the majority of the variation in our dataset.

Early age at milestone achievement was associated with male sex (PC1, $p=0.05$), lower maternal age at birth (PC1, $p=0.02$), as well as higher gestational age (PC1, $p<0.001$; PC2, $p=0.01$) and paternity leave (PC2, $p=0.01$) (**Table 2**).

Analysis of the original milestone variables by conventional statistics using regression analysis confirmed these findings as boys achieved 9 out of 13 milestones at a younger age than the girls, 12 out of 13 milestones showed early attainment correlated with lower maternal age, and 13 out of the 13 milestones showed early attainment correlated with high gestational age and (demonstrated in Supplemental figures S5-S7).

Language development

Complete language data at 1 and 2 years of age was obtained from 323 and 470 participants, respectively. The overall results from the language tests are shown in **Table 3**.

The 1-year language scores were all positively correlated in the PCA model, where PC1 explained 53% of the overall variation in the dataset (Supplemental Figure S2), which was therefore used as a combined measure of 1-year language development. A higher 1-year language score was associated with female sex ($p=0.02$) and maternal smoking during pregnancy ($p=0.01$) (**Table 4**). Analysis of word production as the endpoint (data not shown) confirmed that maternal smoking during pregnancy was the strongest predictor for the 1-year language development (mean difference=5.3 words (95% CI= 2.4, 8.2), $p<0.001$).

The 2-years language scores were all positively correlated in the PCA model, where PC1 explained 60% of the variation in the dataset (Supplemental Figure S3). A higher 2-year language score was associated with female sex ($p<0.001$) and being first born ($p=0.01$) (**Table 4**). Analysis of word production as the endpoint (data not shown) confirmed that female sex was the strongest predictor for 2-year language development (mean difference=63 words (34-91), $p<0.001$).

Cognitive score

601 children completed the BSID-III test. The mean composite score was 104.9 (SD 9.8). There were no differences in scores among the 10 persons performing the tests. Nevertheless, we included test person as a possible confounding factor in our analysis. A higher cognitive score was only

significantly associated with female sex ($p=0.02$); girls scored 1.88 (95% CI: 0.30-3.47) points higher than boys (**Table 4**).

Neurodevelopment and breastfeeding period

None of the neurodevelopmental endpoints was significantly associated with the continuous variables of exclusive or total breastfeeding duration. The same was found when categorizing the exclusive and total breastfeeding periods as <1month, 1-4 months, 4-6 months, >6 months (**Table 5**).

Neurodevelopment and clinical predictors in the first years of life

None of the neurodevelopmental endpoints was significantly associated with any of the clinical predictors: persistent wheeze 0-3yrs, eczema 0-3yrs or number of sick days in the first year of life (**Table 6**).

Correlation between neurodevelopmental endpoints

A heat map with all the original milestones, 1- and 2-year language scores and the BSID-III composite score (Supplemental Figure S4), showed that all the language scores and all the milestone were inter-correlated and that the composite score of the BSID-III was highly correlated with the 2-year language scores.

Discussion

Principal Findings

We found that all neurodevelopmental scores in early childhood was markedly sex determined with boys achieving motor milestones at an earlier age whereas girls had higher language scores and a higher cognitive composite score. None of the neurodevelopmental scores was influenced by persistent wheeze, eczema, or number of sick days in the first years of life. Particularly, neither milestone, language nor cognitive scores were influenced by breastfeeding duration.

Strengths and Limitations

It is a significant strength, that the study was conducted in an unselected population-based cohort, which increases external validity. Furthermore, the longitudinal design, following the children from birth until age 3 allowed neurodevelopmental assessment at 11 visits to the research clinic. The fact that all the assessors were well-known to the children may also have increased test compliance and feasibility.

The specificity of the clinical endpoints is high as the children were followed in a comprehensive clinical program including a daily symptom diary and standardized diagnoses of clinical endpoints. Another advantage of the design is the comprehensive information on factors possibly influencing

neurodevelopment including information on breastfeeding duration, introduction of infant formula, and weaning foods obtained prospectively by interviews.

The PCA approach was a valid method to reduce milestone and language scores to fewer uncorrelated variables, which reduces the risk of multiple testing.

The main limitation of our study is the lack of assessment of parental IQ and parental stimulation of the child. Another possible limitation is the retrospective registration of milestones for some children, increasing the risk of recall bias. However, studies have shown an excellent correlation between gross motor milestones evaluated by a pediatric neurologist and parental recollection of the age of milestone achievement two years later(18).

Finally, the number of research personnel, who performed the cognitive test is a possible disadvantage. However, the same person trained them, and all sessions were video recorded allowing for subsequent review by the first author, where no differences in scores were found.

Interpretation

The COPSAC₂₀₁₀ children achieved gross motor milestones at a later age than children in the WHO Multicenter Growth study, but at a similar age as the Norwegian children in the same study (19), which might indicate a culture-specific care behavior. All the developmental outcomes in the study were within normal range.

Overall the 1 and 2-year language scores in the COPSAC₂₀₁₀ cohort were lower than in other cohort studies(20) and slightly lower than in an another Danish birth cohort(21). It is known that Danish children generally score lower on both the 1 and 2-year language tests(22) and it has been suggested that the delay is related to the nature of Danish sound structure, which presents Danish children with a harder task of segmentation(22). However, it could also be affected by cultural differences and early attendance to daycare in Denmark(22).

The mean cognitive score from the BSID-III test was slightly above average (104.8, SD 9.8), and no children scored less than 85. The average score was similar to other cohort studies of healthy children(7,23) and the lack of participants with scores of less than 85 might be due to a selection bias in the cohort or the exclusion criteria including prematurity and neurological disorders in our study.

Accepted Article

None of the neurodevelopmental outcomes was influenced by breastfeeding duration. More than 90% of the cohort were breastfed for more than 1 month. The children were followed closely from birth and we were able to take multiple social factors into account to strengthen the reliability of our results. The duration of breastfeeding has previously been associated with both increased cognitive function in school age(24) and adult IQ(6). Thereby our study could point towards effects occurring later in life or perhaps more likely that a successful long breastfeeding period may be determined and thereby confounded by social factors. Alternatively, the lack of association could be due to an improved composition of infant formula feeding over the last decades.

Milestones were achieved at an earlier age in boys than in girls, which is in contradiction to a previous large multicenter study(19), but in agreement with another Danish study(25). Both language and cognitive scores were higher for girls than boys, which is in agreement with a number of other studies(5,23) and might be explained by the fact that girls on average mature faster than boys(26). We found higher language scores at 1 year associated with smoking during pregnancy, but did not find any associations with the other neurodevelopmental scores, which may indicate a spurious finding. Maternal smoking during pregnancy has in previous studies been shown to be a potential confounder of psychomotor development, both as a positive(27) and negative factor(28). In contrast to previous studies(5,21,29), we did not find significant associations between any of the neurodevelopmental scores and maternal education. The effects of gestational age, maternal age and being first born were in concordance with previous studies(27,30).

Lastly, we were unable to see any associations between the neurodevelopmental outcomes and persistent wheeze or eczema. These results are contradicting a previous study showing that children with allergic sensitization and especially eczema at 12 months, had lower motor scores at 18 months of age(7). The inconsistencies of these finding might be explained by the age of assessment or the method of evaluation of the neurodevelopmental outcome. Furthermore, our close clinical follow up of the children assures early diagnosis and immediate initiation of the relevant treatment, thus minimizing negative effects of the disease.

Conclusion

Early life neurodevelopment was not influenced by breastfeeding, contradicting previous reports. This could point towards residual confounding in previous studies or enhanced infant formulas over the last decades. This study confirms all neurodevelopmental outcomes being associated with sex, with motor milestones being achieved at an earlier age among boys, whereas higher language and cognitive scores were found among girls. A younger age of milestone achievement was observed with lower maternal age, higher gestational age and paternal leave; a higher one year language score was associated with maternal smoking in pregnancy and a higher two year language score with being first born. We did not find any association between neurodevelopmental outcomes and persistent wheeze, eczema or number of sick days.

Acknowledgements

We gratefully express our gratitude to the children and families of the COPSAC₂₀₁₀ cohort study for all their support and commitment. We acknowledge and appreciate the unique efforts of the COPSAC research team. We thank MD, DMSci, Peter Uldall professor in Child Neurology, Pediatric and Adolescent Medicine, Copenhagen University Hospital, Rigshospitalet, Denmark for his contribution regarding neurodevelopmental assessment. He did not receive any compensation for his contribution.

Conflict of interest

The authors declare no potential, perceived, or real conflict of interest regarding the content of this manuscript.

Financial disclosure

The authors have no financial relationships relevant to this article to disclose.

Role of funding source

The funding agencies did not have any role in design and conduct of the study; collection, management, and interpretation of the data; or preparation, review, or approval of the manuscript. No pharmaceutical company was involved in the study.

Fundings: COPSAC is funded by private and public research funds all listed on www.copsac.com. The Lundbeck Foundation; Danish State Budget; Danish Council for Strategic Research; Danish Council for Independent Research and The Capital Region Research Foundation have provided core support for COPSAC.

Reference List

1. Shonkoff JP, Garner AS, Siegel BS, Dobbins MI, Earls MF, Garner AS, et al. The Lifelong Effects of Early Childhood Adversity and Toxic Stress. *Pediatrics* 2012; 129:e232–46.
2. Monk C, Georgieff MK, Osterholm EA. Research Review: Maternal prenatal distress and poor nutrition – mutually influencing risk factors affecting infant neurocognitive development. *J Child Psychol Psychiatry* 2013; 54:115–30.
3. Nyaradi A, Li J, Hickling S, Foster J, Oddy WH. The role of nutrition in children's neurocognitive development, from pregnancy through childhood. *Front Hum Neurosci* 2013; 7:97.
4. McCann JC, Ames BN. Is docosahexaenoic acid, an n–3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. *Am J Clin Nutr* 2005; 82:281–95.
5. Koutra K, Chatzi L, Roumeliotaki T, Vassilaki M, Giannakopoulou E, Batsos C, et al. Socio-demographic determinants of infant neurodevelopment at 18 months of age: Mother-Child Cohort (Rhea Study) in Crete, Greece. *Infant Behav Dev* 2012; 35:48–59.
6. Victora CG, Horta BL, de Mola CL, Quevedo L, Pinheiro RT, Gigante DP, et al. Association between breastfeeding and intelligence, educational attainment, and income at 30 years of age: a prospective birth cohort study from Brazil. *Lancet Glob Health* 2015; 3:e199–205.
7. Meldrum SJ, D'Vaz N, Dunstan JA, Mori TA, Hird K, Simmer K, et al. Allergic disease in the first year of life is associated with differences in subsequent neurodevelopment and behaviour. *Early Hum Dev* 2012. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22284984>
8. Julvez J, Torrent M, Guxens M, Antó JM, Guerra S, Sunyer J. Neuropsychologic status at the age 4 years and atopy in a population-based birth cohort. *Allergy* 2009; 64:1279–1285.
9. Schmitt J, Buske-Kirschbaum A, Roessner V. Is atopic disease a risk factor for attention-deficit/hyperactivity disorder? A systematic review. *Allergy* 2010; 65:1506–24.
10. Chida Y, Hamer M, Steptoe A. A bidirectional relationship between psychosocial factors and atopic disorders: a systematic review and meta-analysis. *Psychosom Med* 2008; 70:102–16.
11. Bisgaard H, Vissing NH, Carson CG, Bischoff AL, Følsgaard NV, Kreiner-Møller E, et al. Deep phenotyping of the unselected COPSAC2010 birth cohort study. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* 2013; 43:1384–94.
12. Frankenburg W, Dodds J. The Denver developmental assessment (Denver II). Denver: University of Colorado Medical School; 1990.
13. Wijnhoven TM, de Onis M, Onyango AW, Wang T, Bjoerneboe G-EA, Bhandari N, et al. Assessment of gross motor development in the WHO Multicentre Growth Reference Study. *Food Nutr Bull* 2004; 25:S37–45.
14. Bleses D, Vach W, Slott M, Wehberg S, Thomsen P, Madsen TO, et al. The Danish

Communicative Developmental Inventories: validity and main developmental trends. *J Child Lang* 2008; 35:651–69.

15. Bayley, N. Bayley Scales of Infant and Toddler Development (Third edition), Administration Manual. Harcourt Assessment. San Antonio, TX; 2006.
16. Bisgaard H, Hermansen MN, Loland L, Halkjaer LB, Buchvald F. Intermittent inhaled corticosteroids in infants with episodic wheezing. *N Engl J Med* 2006; 354:1998–2005.
17. Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol* 1980; 92:44–7.
18. Majnemer A, Rosenblatt B. Reliability of parental recall of developmental milestones. *Pediatr Neurol* 1994; 10:304–8.
19. WHO Multicentre Growth Reference Study Group. Assessment of sex differences and heterogeneity in motor milestone attainment among populations in the WHO Multicentre Growth Reference Study. *Acta Paediatr Oslo Nor 1992 Suppl* 2006; 450:66–75.
20. Feldman HM, Dollaghan CA, Campbell TF, Colborn DK, Kurs-Lasky M, Janosky JE, et al. Parent-reported language and communication skills at one and two years of age in relation to otitis media in the first two years of life. *Pediatrics* 1999; 104:e52.
21. Lauritzen L, Jørgensen MH, Olsen SF, Straarup EM, Michaelsen KF. Maternal fish oil supplementation in lactation: effect on developmental outcome in breast-fed infants. *Reprod Nutr Dev* 2005; 45:535–47.
22. Bleses D, Vach W, Slott M, Wehberg S, Thomsen P, Madsen TO, et al. Early vocabulary development in Danish and other languages: a CDI-based comparison. *J Child Lang* 2008; 35:619–50.
23. Makrides M, Gibson RA, McPhee AJ, Yelland L, Quinlivan J, Ryan P, et al. Effect of DHA Supplementation During Pregnancy on Maternal Depression and Neurodevelopment of Young Children. *J Am Med Assoc* 2010; 304:1675–83.
24. Kramer MS, Aboud F, Mironova E, et al. Breastfeeding and child cognitive development: New evidence from a large randomized trial. *Arch Gen Psychiatry* 2008; 65:578–84.
25. Engel S, Tronhjem KMH, Hellgren LI, Michaelsen KF, Lauritzen L. Docosahexaenoic acid status at 9 months is inversely associated with communicative skills in 3-year-old girls. *Matern Child Nutr* 2012. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22642227>
26. Lynn R, Kanazawa S. A longitudinal study of sex differences in intelligence at ages 7, 11 and 16 years. *Personal Individ Differ* 2011; 51:321–4.
27. Oken E, Østerdal ML, Gillman MW, Knudsen VK, Halldorsson TI, Strøm M, et al. Associations of maternal fish intake during pregnancy and breastfeeding duration with attainment of developmental milestones in early childhood: a study from the Danish National Birth Cohort. *Am J Clin Nutr* 2008; 88:789–96.
28. Auestad N, Scott DT, Janowsky JS, Jacobsen C, Carroll RE, Montalto MB, et al. Visual,

cognitive, and language assessments at 39 months: a follow-up study of children fed formulas containing long-chain polyunsaturated fatty acids to 1 year of age. *Pediatrics* 2003; 112:e177-183.

29. Fenson L, Bates E, Dale P, Goodman J, Reznick JS, Thal D. Measuring variability in early child language: don't shoot the messenger. *Child Dev* 2000; 71:323-8.

30. Fenson L, Dale PS, Reznick JS, Bates E, Thal DJ, Pethick SJ. Variability in early communicative development. *Monogr Soc Res Child Dev* 1994; 59:1-173; discussion 174-185.

Figures:

Figure 1. Flow chart of the study participants through the trial and main outcome measures

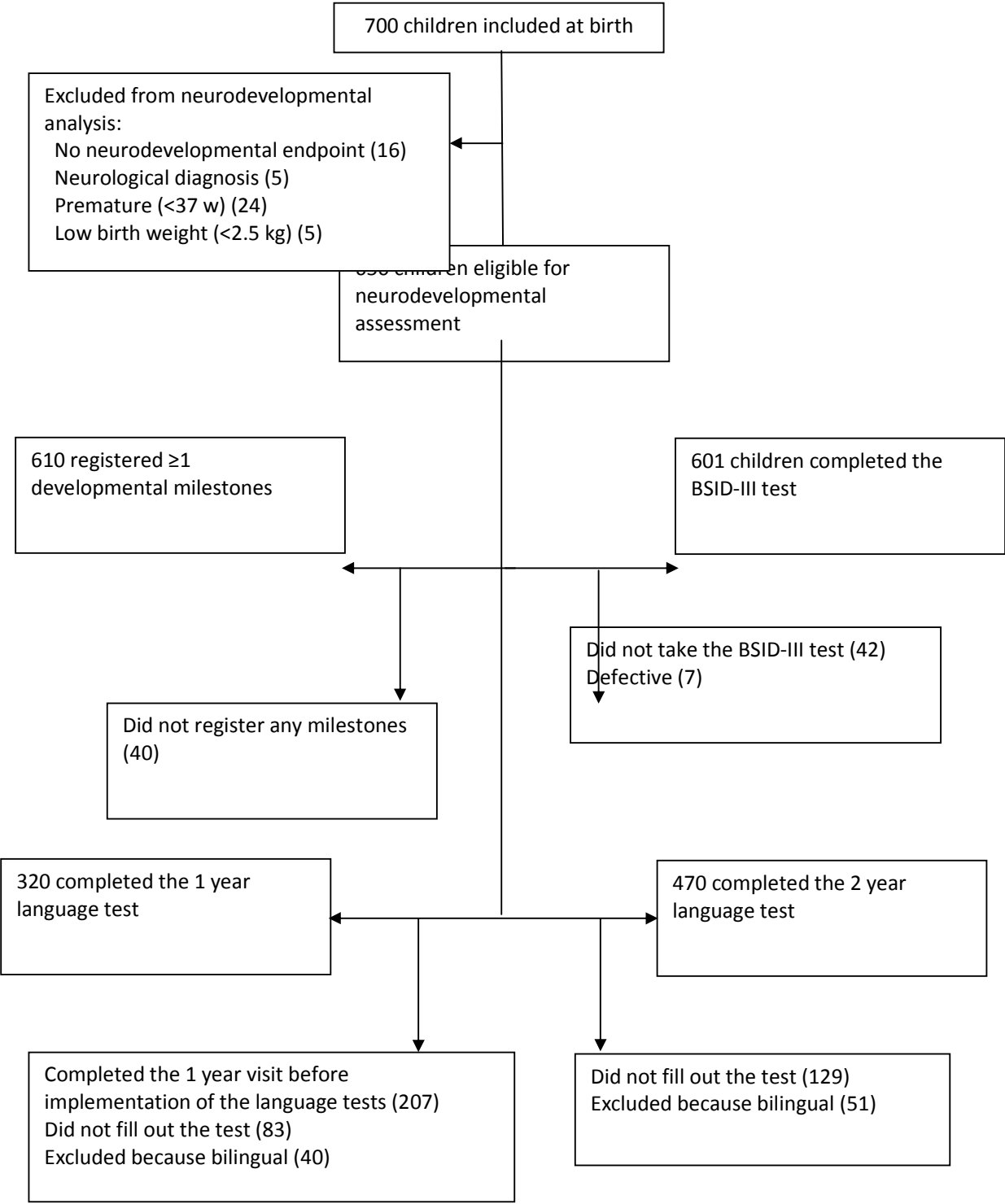
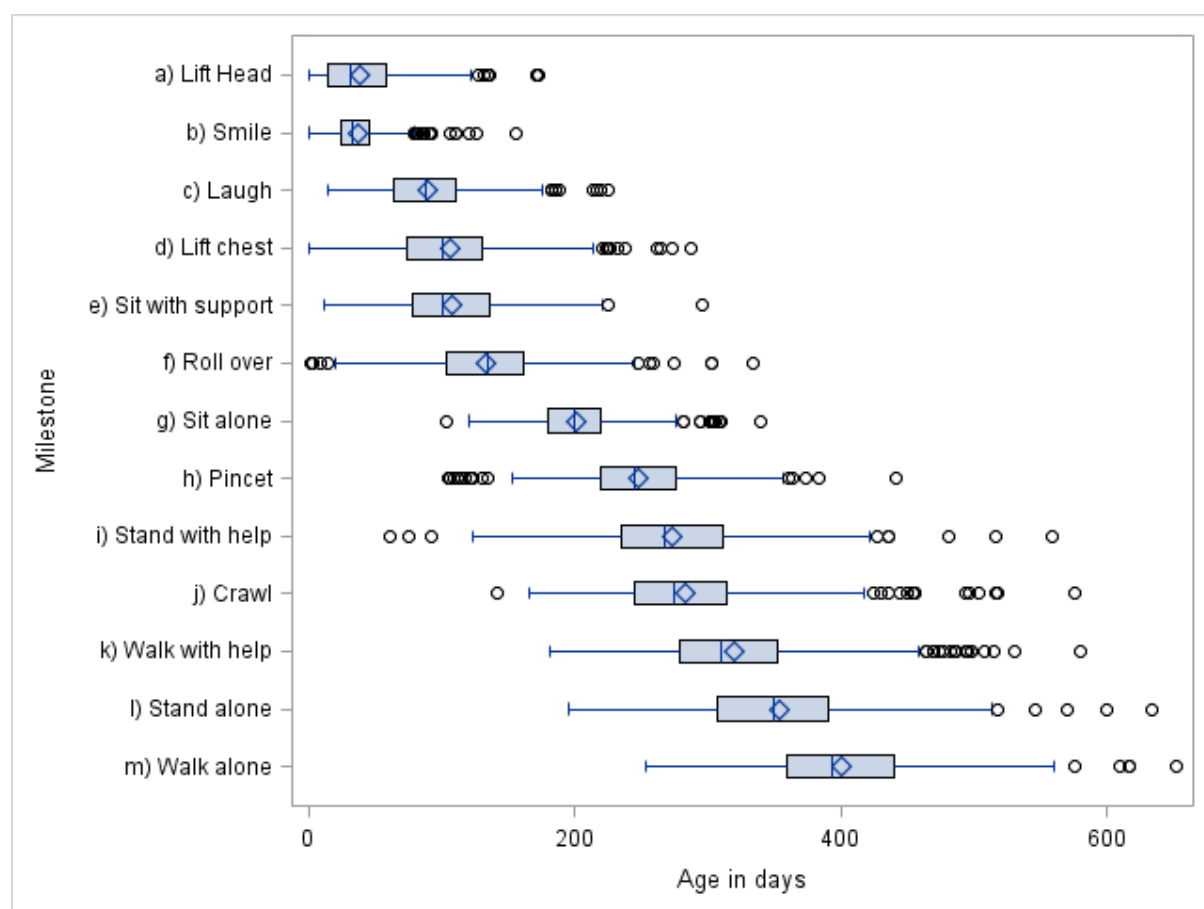


Figure 2. Box-plot of the window of achievement of developmental milestones



Accepted Article

Tables

Table 1. Baseline characteristics of the COPSAC₂₀₁₀ cohort and the children that completed the different neurodevelopmental test outcomes.

	All n=700	Milestones n=610	1 y language n=320	2y language n=470	Bayley n=601
Birth cohort					
Boys, % (n)	51% (360)	51% (297)	50% (160)	51% (236)	51% (293)
Mother's age at birth, mean $\pm$ SD, years	32.3 $\pm$ 4.4	32.3 $\pm$ 4.3	32.3 $\pm$ 4.2	32.3 $\pm$ 4.2	32.3 $\pm$ 4.3
Caucasian % (n)	96% (669)	96% (588)	97% (323)	98% (463)	96% (580)
Pregnancy and birth					
Gestational age, mean $\pm$ SD, weeks	39.9 $\pm$ 1.7	40.1 $\pm$ 1.3	40.1 $\pm$ 1.3	40.1 $\pm$ 1.2	40.1 $\pm$ 1.2
Weight 1 week, mean $\pm$ SD, kg	3.6 $\pm$ 0.5	3.6 $\pm$ 0.5	3.6 $\pm$ 0.5	3.6 $\pm$ 0.5	3.6 $\pm$ 0.5
Length 1 week, mean $\pm$ SD, cm	52.1 $\pm$ 2.2	52.1 $\pm$ 2.1	52.2 $\pm$ 2.1	52.1 $\pm$ 2.2	52.1 $\pm$ 2.1
Head circumference at 1 week, mean $\pm$ SD, cm	35.7 $\pm$ 1.4	35.7 $\pm$ 1.3	35.7 $\pm$ 1.3	35.7 $\pm$ 1.3	35.7 $\pm$ 1.4
Apgar score at 5 min., mean $\pm$ SD	9.9 $\pm$ 0.34	9.9 $\pm$ 0.33	9.9 $\pm$ 0.23	9.9 $\pm$ 0.33	9.9 $\pm$ 0.33
Exposure					
Birth order, % first born, (n)	46% (323)	45% (279)	44% (145)	43% (205)	46% (277)
Smoking during pregnancy, % (n)	7.7%(54)	7.2% (44)	6.0% (20)	6.1% (29)	7.4% (43)
Duration of solely breastfeeding, mean $\pm$ SD, days	104 $\pm$ 60	104 $\pm$ 59	105 $\pm$ 59	107 $\pm$ 58	105 $\pm$ 59
Duration of total breastfeeding, mean $\pm$ SD, days	245 $\pm$ 155	246 $\pm$ 147	248 $\pm$ 140	251 $\pm$ 142	248 $\pm$ 152
Age at start in daycare, mean $\pm$ SD, months	10.8 $\pm$ 3.1	10.8 $\pm$ 3.1	10.9 $\pm$ 3.2	10.7 $\pm$ 3.1	10.8 $\pm$ 3.0
Paternity leave >4 weeks, % (n)	53% (354)	54% (323)	55% (179)	54% (249)	53% (313)
Socioeconomic variables					
Maternal educational level (% Low: Medium: High) ¹	8:64:28	7:65:28	7:64:30	7:64:29	8:65:28
Annual household income (% Low: Medium: High) ²	10:53:37	10:52:38	9:51:40	10:51:40	10:52:38
Clinical predictors³					
Persistent wheeze, % (n)	19% (123)	18% (108)	19% (61)	18% (83)	18% (108)
Eczema, % (n)	25% (165)	26% (153)	26%(85)	26% (121)	26%(154)
Days sick in first year of life, median (25 th -75 th percentile)	48(28-79)	49 (28-80)	47 (28-74)	50 (28-82)	48 (28-80)

¹: Low (elementary school or college graduate), Medium (tradesman or medium length), and High (university candidate)

²: Low (<55.000 Euros/year), High (>110.000 Euros/year)

³ Prevalence of persistent wheeze and eczema in the 660 children who have full follow up to 3 years. Days sick in first year of life is given for the 522 children with a full diary registration (365 days)

Table 2. Multivariable analysis of factors associated with overall age of milestone development¹

Predictor	PC 1 Effect (95% CI) P=0.05	PC 2 Effect (95% CI) P=0.01
Sex (Female/Male)	0.32 (0.00, 0.63) P=0.05	0.02 (-0.20, 0.22) P=0.96
First born (Y/N)	-0.04 (-0.38, 0.30) P=0.83	-0.11 (-0.34, 0.11) P=0.33
Paternity leave (Y/N)	-0.01 (-0.33, 0.31) P=0.96	-0.28 (-0.50, -0.06) P=0.01
Smoking during pregnancy (Y/N)	0.15 (-0.48, 0.78) P=0.64	0.30 (-0.13, 0.72) P=0.17
Gestational age (weeks)	-0.23 (-0.36, -0.11) P<0.001	-0.11 (-0.20, -0.03) P=0.01
Maternal age at birth (years)	0.05 (0.01, 0.09) P=0.02	0.02 (-0.01, 0.05) P=0.22
Age start daycare (months)	-0.02 (-0.07, 0.03) P=0.37	-0.02 (-0.05, 0.01) P=0.26
Mother's education	P=0.60	P=0.56
-High	0.18 (-0.20, 0.55)	-0.06 (-0.31, 0.19)
-Medium (ref)	0	0
-Low	0.16 (-0.46, 0.79)	-0.22 (-0.64, 0.20)

Abbreviation: CI= confidence intervals, PC= Principal Component from PCA in On-line Figure S1

PC1 & PC2 explained 37% and 16% of the overall variation in the data, respectively.

PC1: Mostly driven by the later milestones (crawling, walking and standing)

PC2: Mostly driven by the early milestones (lifting the head, smiling, laughing, sitting with help and lifting the chest) and later milestones (crawling, walking, standing) in opposite directions.

¹The estimate for each predictor is adjusted for all the other predictors using a multiple linear regression model. The effects are expressed as either differences in means (categorical variables) or β (continuous variables) with (95% CI). All bolded values in the tables are significant ($p < 0.05$).

Table 3. Language development at 1 and 2 years of age assessed by MacArthur Bates Communicative Developmental Inventory and cognitive scores at 2½ years of age assessed by Bayley III¹.

1 year language test n=320	
Age at test (months), mean ± SD	12.2 ± 0.3
Starting to talk (labeling and/or imitation) (%)	47.4
Early gestures (n out of 18), mean ± SD	11.2 ± 2.6
Girls - early gestures	11.6 ± 2.6
Boys – early gestures	10.8 ± 2.6
Late gestures (n out of 45), mean ± SD	8.9 ± 5.2
Phrases understood (n out of 26), mean ± SD	9.8 ± 5.2
Vocabulary comprehension (n of words from a list of 409), median (25 th -75 th percentile)	39 (20-66)
Vocabulary production (n of words from a list of 409), median (25 th -75 th percentile)	3 (1-7)
2 year language test n=470	
Age at test (months), mean ± SD	24.2 ± 0.3
Vocabulary production (n of words from a list of 725), median, (25 th – 75 th percentile)	250 (121-364)
Girls – vocabulary production	287 (162-401)
Boys – vocabulary production	197 (79-327)
Use of abstract words (n from a list of 5), median (25 th - 75 th percentile)	5 (4-5)
Uses grammar (%)	67.2
Girls – uses grammar	73.7
Boys – uses grammar	61.0
Irregular words (n from a list of 29), median (25 th – 75 th percentile)	2.0 (0-6)
Girls – irregular words	3 (1-7)
Boys – irregular words	2 (0-5)
Overregularized words (n from a list of 61), median (25 th – 75 th percentile)	0 (0-2)

Length of longest sentences (n of morphemes), mean $\pm$ SD	3.6 $\pm$ 1.6
Girls – longest sentence	4.0 $\pm$ 1.5
Boys – longest sentence	3.2 $\pm$ 1.5
Sentence complexity (n of complex from a list of 33 pairs), median (25 th – 75 th percentile)	3 (0-7)
Girls – sentence complexity	4 (1-9)
Boys – sentence complexity	1 (0-5)
Bayley test at 2 ½ years n=601	
Age at test (months), mean $\pm$ SD	30.5 $\pm$ 0.92
Cognitive composite score (standardized), mean $\pm$ SD	104.9 (9.80)
Girls – composite score	106.0 (10.4)
Boys – composite score	103.8 (9.9)

¹ Sexes are presented separately where there is a statistical difference in the outcomes (p<0.05)

Table 4. Factors influencing 1 and 2 years language development and Bayley scores at 2 ½ years of age¹

	Language 1 year	Language 2 years	Bayley composite score
Predictor	PC1 Effect (95% CI)	PC1 Effect (95% CI)	Composite score Effect (95% CI)
Sex (F/M)	0.50 (0.08, 0.92) P=0.02	0.87 (0.50, 1.23) P<0.001	1.88 (0.30, 3.47) P=0.02
First born (Y/N)	0.21 (-0.23, 0.66) P=0.37	0.48 (0.09, 0.86) P=0.02	1.08 (-0.65, 2.82) P=0.22
Paternity leave (Y/N)	0.01 (-0.42, 0.44) P=0.97	0.09 (-0.29, 0.46) P=0.65	0.66 (-0.97, 2.29) P=0.43
Smoking during pregnancy (Y/N)	1.19 (0.28, 2.09) P=0.01	0.49 (-0.29, 1.28) P=0.22	-0.89 (-4.05, 2.26) P=0.58
Gestational age (weeks)	0.05 (-0.12, 0.23) P=0.56	0.01 (-0.14, 0.16) P=0.91	0.44 (-0.21, 1.08) P=0.18
Maternal age at birth (years)	-0.05 (-0.10, 0.01) P=0.10	-0.04 (-0.09, 0.01) P=0.10	-0.08 (-0.29, 0.13) P=0.47
Age start daycare (months)	0.04 (-0.03, 0.10) P=0.29	0.03 (-0.03, 0.09) P=0.37	-0.03 (-0.29, 0.24) P=0.85
Mother's education	P=0.23	P=0.37	P=0.15
-High	-0.44 (-0.93, 0.06)	-0.01 (-0.44, 0.42)	1.93 (-0.01, 3.87)
-Medium	0	0	0
-Low	-0.04 (-0.94, 0.85)	-0.52 (-1.24, 0.21)	0.36 (-2.83, 3.56)

Abbreviation: CI= confidence intervals; PC= Principal Component from PCA in On-line Figure S2

PC1 at 1 year explained 53% of the variation in the dataset and is driven by all the language scores in the same direction.

PC1 at 2 years explained 58% of the variation in the dataset and is driven by all the language scores in the same direction.

¹The estimate for each predictor is adjusted for all the other predictors using a multiple linear regression model. The effects are expressed as either differences in means (categorical variables) or β (continuous variables) with (95% confidence intervals). All bolded values in the tables are significant ($p<0.05$).

Table 5. Correlations between neurodevelopment and exclusive and total duration of breastfeeding¹.

	Age at milestone (PC1)	Age at milestone (PC2)	Language at 1 year (PC1)	Language at 2 years (PC1)	Bayley composite score
Exclusively breastfeeding (months)	-0.01 (-0.09, 0.08) P=0.86	-0.05 (-0.11, 0.01) P=0.08	-0.05 (-0.17, 0.06) P=0.39	0.00 (-0.10, 0.09) P=0.93	0.05 (-0.37, 0.48) P=0.80
<1 month (N=137)	REF	REF	REF	REF	REF
1-4 months (N=166)	0.21 (-0.26, 0.67) P=0.38	0.15 (-0.16, 0.47) P=0.33	-0.06 (-0.69, 0.58) P=0.86	-0.48 (-1.03, 0.08) P=0.09	0.39 (-2.00, 2.78) P=0.75
4-6 months (N=322)	-0.18 (-0.60, 0.23) P=0.39	-0.12 (-0.41, 0.16) P=0.40	-0.30 (-0.88, 0.27) P=0.30	-0.11 (-0.60, 0.39) P=0.67	0.49 (-1.65, 2.64) P=0.65
6+ months (N=32)	0.71 (-0.11, 1.53) P=0.09	-0.43 (-0.99, 0.13) P=0.13	-0.22 (-1.26, 0.81) P=0.67	0.01 (-0.86, 0.88) P=0.98	0.14 (-3.93, 4.21) P=0.95
Total breastfeeding (months)	-0.03 (-0.07, 0.00) P=0.07	-0.03 (-0.04, 0.00) P=0.09	0.03 (-0.02, 0.08) P=0.22	0.04 (-0.00, 0.08) P=0.05	0.06 (-0.12, 0.24) P=0.54
<1 month (N=46)	REF	REF	REF	REF	REF
1-4 months (N=77)	0.25 (-0.54, 1.03) P=0.54	-0.10 (-0.63, 0.42) P=0.70	-0.22 (-1.29, 0.85) P=0.68	-0.09 (-1.07, 0.89) P=0.86	-1.63 (-5.66, 2.38) P=0.42
4-6 months (N=91)	-0.12 (-0.88, 0.64) P=0.76	0.12 (-0.39, 0.62) P=0.64	-0.34 (-1.36, 0.67) P=0.50	0.20 (-0.74, 1.14) P=0.68	-0.68 (-4.56, 3.20) P=0.73
6-12 months (N=340)	0.27 (-0.40, 0.94) P=0.43	-0.16 (-0.61, 0.29) P=0.49	-0.54 (-1.47, 0.37) P=0.24	0.37 (-0.44, 1.23) P=0.40	-1.32 (-4.81, 2.17) P=0.46
12+ months (N=102)	-0.29 (-1.06, 0.49) P=0.47	-0.10 (-0.62, 0.42) P=0.70	0.35 (-0.69, 1.39) P=0.51	0.79 (-0.17, 1.75) P=0.11	0.77 (-3.21, 4.75) P=0.70

Abbreviation: PC= Principal Component from PCAs in On-line Figure S1 and S2

¹The estimate for each predictor is adjusted for all other predictors using a multiple linear regression model. The effects are expressed as either differences in means (categorical variables) or β (continuous variables) with (95% confidence intervals), using the neurodevelopmental score as the outcome.

Table 6. Correlations between neurodevelopment and persistent wheeze, eczema and sickness in the first years of life¹

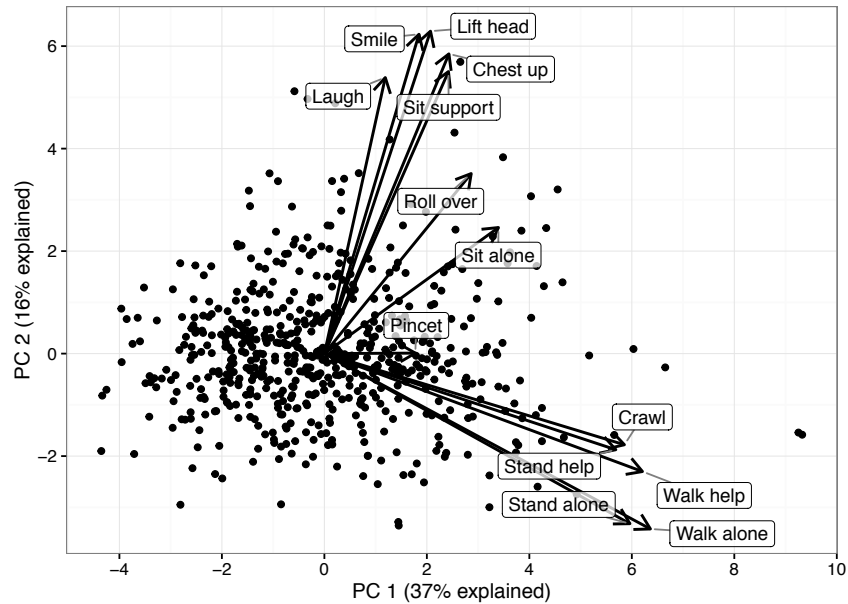
	Age at milestone (PC1)	Age at milestone (PC2)	Language at 1 year (PC1)	Language at 2 years (PC1)	Bayley composite score
Persistent wheeze (yes/no)	0.17 (-0.25, 0.59) p=0.54	0.01 (-0.29, 0.27) p=0.95	0.34 (-0.21, 0.90), p=0.23	0.001 (-0.47, 0.48) p=0.98	-0.55 (-2.61, 1.51) p=0.60
Eczema (yes/no)	0.02 (-0.34, 0.39) p=0.90	0.09 (-0.15, 0.34) p=0.46	-0.28 (-0.77, 0.21) p=0.26	-0.15 (-0.56, 0.26) p=0.48	-0.58 (-2.38, 1.22) p=0.53
Sick days in first year of life (weeks)	-0.03 (-0.06, 0.002) p=0.07	0.002 (-0.02, 0.02) p=0.81	0.04 (-0.01, 0.08) p=0.09	0.02 (-0.01, 0.05) p=0.52	-0.05 (-0.20, 0.09) p=0.47

Abbreviation: PC= Principal Component from PCAs in On-line Figure S1 and S2

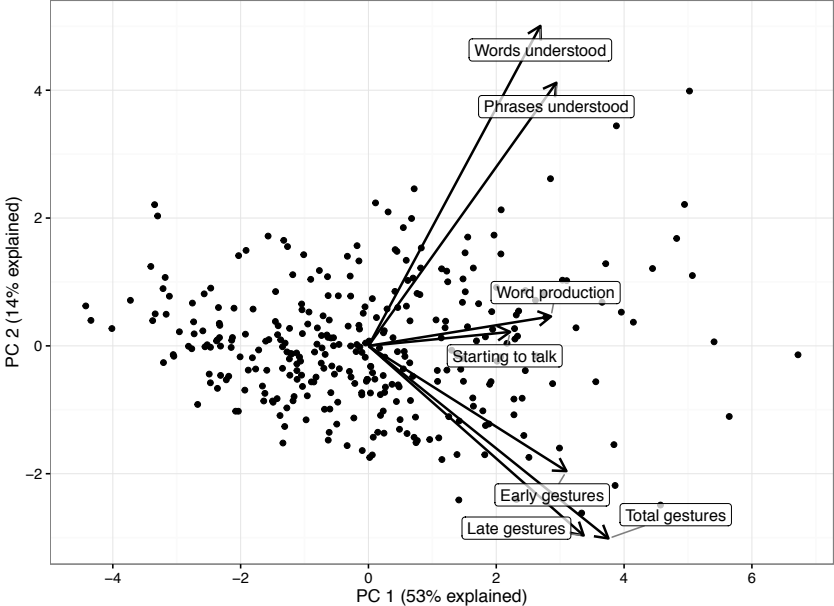
¹The estimate for each predictor is adjusted for sex using a multiple linear regression model. The effects are expressed as either differences in means (categorical variables) or β (continuous variables) with (95% confidence intervals), using the neurodevelopmental score as the outcome.

Supplementary Online Figures

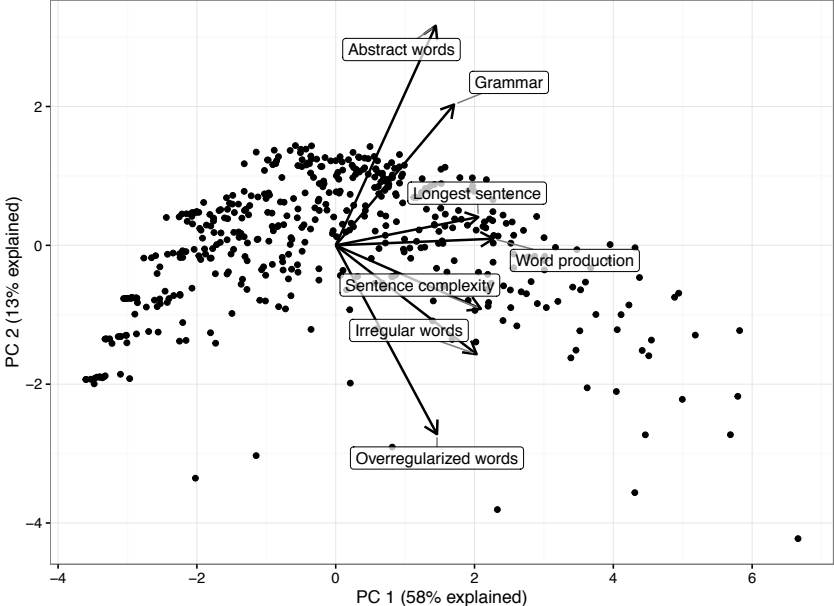
Supplemental Figure S1. Biplot from principal component analysis of all the 13 milestones. Principal component 1 and 2 (PC1 & PC2) explained 37% and 16% of the overall variation in the data, respectively. PC1: Overall later milestone development. PC2: Late early milestones, early late motor milestones.



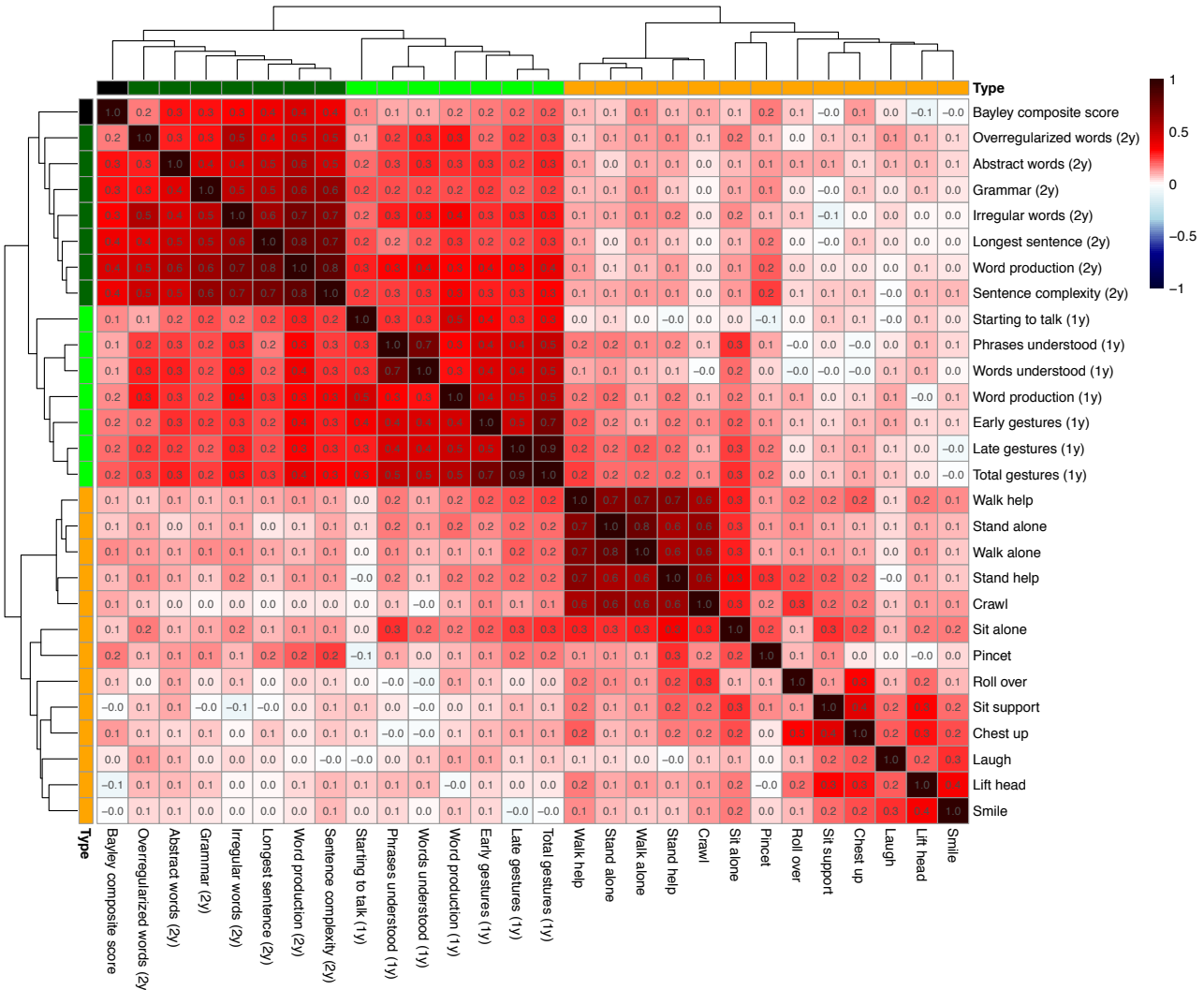
Supplemental Figure S2. Biplot from principal component analyses of all the 1-year language development scores. Principal Component 1 (PC1) explained 53% of the variation in the dataset



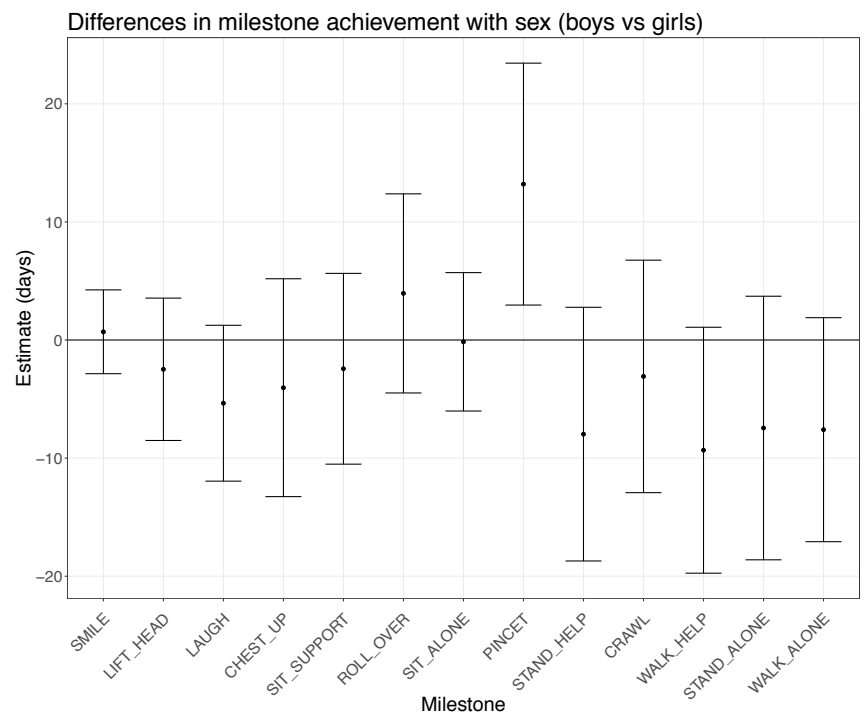
Supplemental Figure S3. Biplot from principal component analyses of all the 2-year language development scores. Principal Component 1 (PC1) explained 58% of the variation in the dataset.



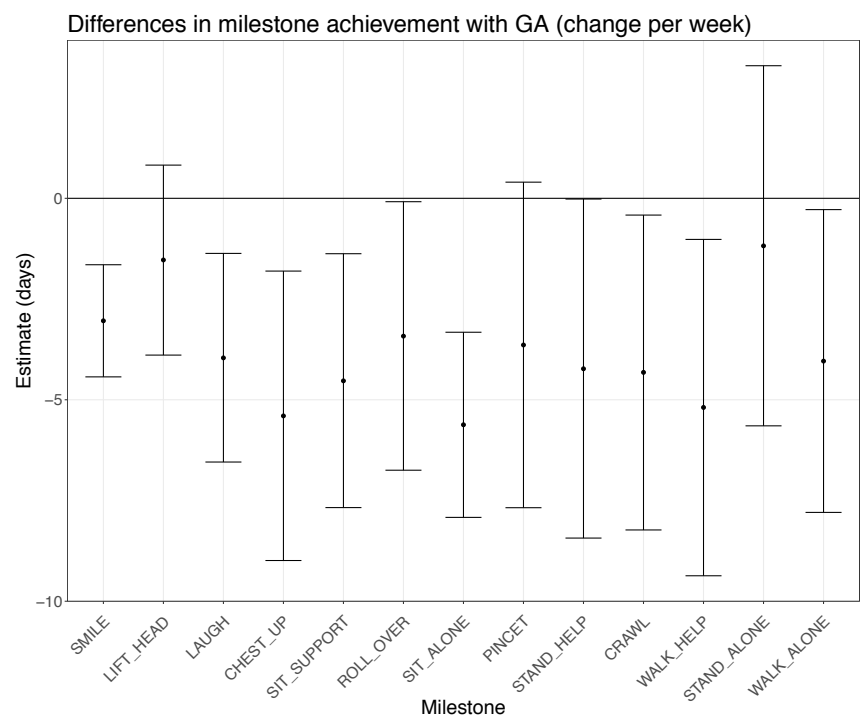
Supplemental Figure S4. Heatmap based on spearman correlations showing the correlation between the neurological endpoints



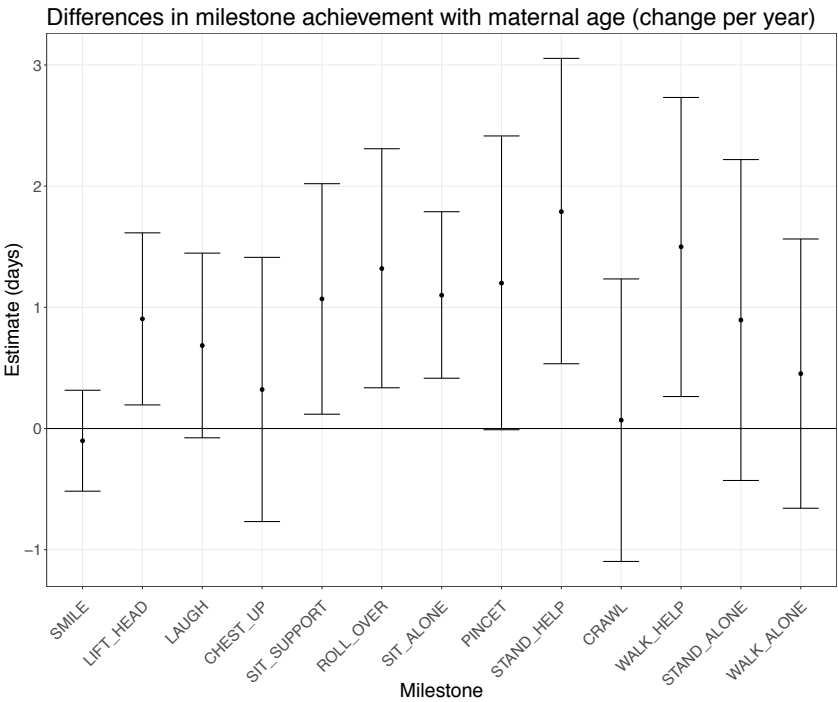
Supplemental Figure S5.



Supplemental Figure S6.



Supplemental Figure S7.



Appendix S1

METHODS

Neurodevelopmental assessment

Milestones

At the child's 1 week visit the parents received a registration form, with thorough instructions, based on The Denver Development Index (1) and WHO (World Health Organization) milestones registration (2). Dates of achievement of 13 predefined milestones were registered by the parents and reviewed at each visit to the research clinic. The registration form contained a description and an illustration of the milestones. Any difficulties in remembering the specific date were registered as “missing”. The clinical staff carefully reviewed the forms with the parents in order to standardize the registration, and thus minimize differences in interpersonal interpretations. Implementation of milestone registration started after the first 500 children were born, thus some of the milestones were registered retrospectively.

Language development

Language development was assessed with the Danish version of The MacArthur Bates Communicative Developmental Inventory (CDI), which is a well-recognized and validated tool to assess monolingual children's lexical growth by a standardized parent reporting system (3). The test was performed as a web-based questionnaire filled out by parents around the child's 1 year (CDI-WG: Words and gesticulation) and 2 years birthdays (CDI-WS: Words and sentences). The 1-year test evaluates language comprehension, early word production and gestural communication.

Language comprehension was assessed by counting the number of words that the parents think the child understands from a list of 409 words which are commonly found in the vocabulary of Danish children around 1 year of age. The assessment of word production was based on the same list and

counts the number of words the child actually says or does a lingual imitation of. Gestural communication was assessed by questions regarding the use of gestures typical of early and later communicative development (numbers used out of 18 and 45, respectively). The 2-year test assesses vocabulary, grammatical skills, syntax and morphology. Vocabulary was assessed by counting the number of words the child pronounces from a list of 725 common words. Grammatical skills were determined from the use of past, future, abstract, plural, possessive and past tense and the number of irregular and over-regularized words. Syntax and morphology was assessed according to whether the child combines words and the mean length of the three longest utterances (M3L: calculated from number of morphemes per utterance in the 3 longest sentences the child has said in the previous two weeks). The CDI was not performed in the first 209 participants as it was implemented in the cohort after they had completed their 1 year visit. Children who were considered bilingual (regularly in contact with another language than Danish at home) were excluded from the language development analysis (40 from the 1 year test and 51 from the 2 years test). Language tests completed when the child was more than 3.5 months older than intended were excluded (a total of 9 children).

Cognitive development

Cognitive development was assessed at 2½ years of age, using the cognitive part of the 3rd edition of the Bayley Scales of Infant and Toddler Development (BSID-III) (4). The test was given on an individual basis lasting 20–60 minutes. The scale includes items that assess abilities such as sensorimotor development, exploration and manipulation, object relatedness, concept formation, memory, and other aspects of cognitive processing. The examiner presented a series of test materials to the child and observed the child's responses and behavior. Based on its performance, the child was given a composite score, which was standardized by use of a normalization material of age corrected means of 100 and standard deviation of 15 (range 50-150). Ten examiners were

involved in performing the test and they were all trained by a single expert in the Bayley test procedure (first author). The sessions were video-recorded and the Bayley expert continuously reviewed the videos and supervised the test-persons in order to achieve consistency in all aspects of the testing procedure.

1. Frankenburg W, Dodds J. The Denver developmental assessment (Denver II). Denver: University of Colorado Medical School; 1990.
2. Wijnhoven TM, de Onis M, Onyango AW, Wang T, Bjoerneboe G-EA, Bhandari N, et al. Assessment of gross motor development in the WHO Multicentre Growth Reference Study. *Food Nutr Bull*. 2004 Mar;25(1 Suppl):S37-45.
3. Bleses D, Vach W, Slott M, Wehberg S, Thomsen P, Madsen TO, et al. The Danish Communicative Developmental Inventories: validity and main developmental trends. *J Child Lang*. 2008 Aug;35(3):651–69.
4. Bayley, N. Bayley Scales of Infant and Toddler Development (Third edition), Administration Manual. Harcourt Assessment. San Antonio, TX; 2006.