Environmental Research 204 (2022) 112049

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Prenatal exposure to neurotoxic metals and micronutrients and neurodevelopmental outcomes in early school age children from Poland

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ARTICLE INFO

Keywords:
Prenatal exposure
Metals
Micronutrients
Developmental neurotoxicity
Emotional and behavioral symptoms
Children

ABSTRACT

Exposure to environmental factors, such as neurotoxic metals and micronutrients, during critical periods of development can contribute to long-term consequences in offspring's health, including neurodevelopmental outcomes. The aim of this study was to evaluate the association between simultaneous prenatal exposure to metals [lead (Pb), cadmium (Cd), mercury (Hg)] and micronutrients [selenium (Se), zinc (Zn), copper (Cu)] and neurodevelopmental outcomes in school-age children from the Polish Mother and Child Cohort (REPRO_PL).

Metals and micronutrients concentrations were measured in cord blood (Pb, Cd, Se, Zn, Cu) and in maternal hair (Hg) collected during the 3rd trimester of pregnancy. Behavioral and emotional problems, as well as children's cognitive and psychomotor development, were assessed in 436 school-age children using the Strengths and Difficulties Questionnaire (SDQ, filled in by the mothers) and the Polish adaptation of the Intelligence and Development Scales (IDS, administered by trained psychologists). Multivariate regression models were applied after imputation of missing values, using two approaches: (i) a joint analysis taking into account all metals and micronutrients simultaneously, and (ii) an ExWAS study (single-exposure model).

In the SDQ, Hyperactivity/Inattention problems and Total difficulties were associated with higher Hg concentrations in maternal hair (0.18, 95% CI: 0.05; 0.3; and 0.14, 95% CI: 0.01; 0.3, respectively), whereas Emotional symptoms were inversely associated with Se and Zn levels in cord blood (−0.13, 95% CI: −0.3; 0.004; and −0.10, 95% CI: −0.2; 0.02, respectively). In the IDS, cord blood Pb levels were found to be negatively associated with Fluid and Crystallized IQ (−0.12, 95% CI: −0.3; 0.02; and −0.14, 95% CI: −0.3; 0.007, respectively) as well as Mathematical skills (−0.15, 95% CI: −0.3; 0.01).

The current research has been able to simultaneously assess the exposure to various interacting chemicals during the prenatal period. We demonstrate that prenatal co-exposures to Pb, Hg, Zn and Se have long-term influences on the neuropsychological outcome of school-age children.

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https://doi.org/10.1016/j.envres.2021.112049
Received 25 May 2021; Received in revised form 16 July 2021; Accepted 6 September 2021
Available online 11 September 2021
0013-9351/

1. Introduction

According to the Developmental Origins of Health and Disease (DOHaD) theory, the exposure to environmental factors such as toxic metals and micronutrients during critical periods of development can contribute to long-term consequences in offspring's health, including their behavioral, cognitive and psychomotor outcomes (Barker, 2007). Within few weeks after conception, the neural plate folds and fuses, forming the neural tube, which is a fundamental step in the development of the fetal brain. Since then, a timely sequence of neurodevelopmental processes, including cell proliferation and migration occur during gestation, while neurogenesis, synaptic formation and myelination continue until late adolescence (Hines, 2018). Thus, identification of associations linking prenatal exposure to a wide range of modifiable environmental factors with child neurodevelopment is of public health and clinical relevance for prevention of mental disorders.

The worldwide prevalence of mental disorders in children and adolescents was reported to be 13.4%, and specific clinical conditions such as anxiety, depressive disorders or Attention-Deficit/Hyperactivity Disorders (ADHD) had a prevalence of 6.5%, 2.6% and 3.4%, respectively (Polanczyk et al., 2015). Subclinical decrements in brain function and decreased intellectual potential are even more common than neurobehavioural and neurodevelopmental disorders (Grandjean and Landrigan 2006, 2014). These conditions can affect children's well-being, academic achievement and social functioning, thus generating substantial costs to families and the society (Polanczyk et al., 2015; Gustavsson et al., 2011).

It is widely acknowledged that overall genetic factors account for about 30% of neurodevelopmental disorders (Grandjean and Landrigan, 2014). Thus, non-genetic, environmental stressors are therefore also involved in causation, in some cases also by interacting with genetically inherited predispositions. Despite robust evidence on the relevant role of environmental chemicals, lifestyle and socio-demographic factors on child neurodevelopment (Bellinger, 2009; De Felice et al., 2015; Grandjean and Landrigan, 2006, 2014), the association between the early life exposome and neurodevelopmental impairment in children is still limited (Braun et al., 2014; Steer et al., 2015; Kim et al., 2018; Calamandrei et al., 2020; Tanner et al., 2020; Skogheim et al., 2021; Maitre et al., 2021; Jedynak et al., 2021). The real exposure scenario during prenatal period consists of simultaneous exposure to different classes of chemicals, usually at low levels, whose effects might be, in turn, modified by other factors such as socioeconomic or socio-demographic (*i.e.* home environment) as well as pregnancy and lifestyle related domains (*i.e.* obstetric and pregnancy complications, diet) (Bellinger, 2009; Vrijheid, 2014; Slama and Vrijheid, 2015; Siroux et al., 2016; Stingone et al., 2017; Haug et al., 2018; Tamayo-Uria et al., 2019). In our previously published meta-analysis (based on REPRO_PL and PHIME cohorts) with simultaneous assessment of several prenatal exposures, some consistent trends were evident, relative to the adverse influence of Pb on children's language and cognition and the positive influence of Se on language abilities in the first two years of life (Calamandrei et al., 2020). Two recently published studies based on longitudinal population-based birth cohorts in Europe have also identified several environmental contaminants and healthy lifestyle habits that may influence behavioral conduct in children (Maitre et al., 2021; Jedynak et al., 2021).

For this purpose, analyses considering simultaneous exposures are needed in order to understand the potential long-term effects of multiple prenatal environmental factors on neurodevelopmental outcomes. This knowledge is fundamental to establish effective preventive measures to protect vulnerable populations such as pregnant women and their children. In such framework, based on our previous assessment on 1- and 2-year-old children (Polańska et al., 2013; Polańska et al., 2016a; Polańska et al., 2017a; Polańska et al., 2018; Calamandrei et al., 2020), we aim to investigate the effects of prenatal exposure to chemicals in the same cohort at school-age, to verify whether the effects already detected during early infancy persist at a later developmental stage.

To this aim we evaluated the association between prenatal exposures to metals [*i.e.* lead (Pb), cadmium (Cd), mercury (Hg)] and micronutrients [*i.e.* selenium (Se), zinc (Zn), copper (Cu)] and neurodevelopmental outcomes, in particular behavioral, cognitive and psychomotor skills, in 7-year old children from the Polish Mother and Child Cohort (REPRO_PL). For this purpose, we applied two different approaches: on the one hand, an Exposome-wide Environmental Study (ExWAS), considering the exposures to the aforementioned metals and micronutrients independently (single-exposure model); and also a joint analysis, taking into account the different exposures simultaneously.

2. Material and methods

2.1. Study population

The study is based on the REPRO_PL cohort, a multicenter prospective cohort that was established in 2007 in Poland. All details regarding the cohort methodology has been provided in previous publications (Polańska et al., 2009; Polańska et al., 2011; Polańska et al., 2016b). Briefly, the following inclusion criteria were defined: single pregnancy up to the 12th week of gestation, without assisted conception, pregnancy complications and chronic diseases, as specified in the study protocol. The women who agreed to participate in the study were followed up three times during the pregnancy period and at delivery (Phase I of the study: 2007–2011). Child's exposure, health status and neurodevelopmental assessments were performed at 1 and 2 years of age (Phase II of the study: 2008–2013) and 7 years of age (Phase III of the study: 2014–2019). For the purpose of this study, neurotoxic metals and micronutrients concentrations were assessed in cord blood (Pb, Cd, Se, Zn, Cu) and in maternal hair (Hg) collected during the 3rd trimester of pregnancy. Child neuropsychological examination was performed at the age of 7 years. In addition, relevant covariates collected during the pregnancy and/or at postnatal periods were taken into account. The final sample included 436 mother-child pairs.

Ethical Committee of the Nofer Institute of Occupational Medicine, Lodz, Poland granted approval for each phase of the study (Decision No. July 2007; No. March 2008 and No. 22/2014) and written informed consent was obtained for the procedures within scheduled phases of the cohort.

2.2. Exposure variables

The following metals and micronutrients were measured in cord blood: Pb, Cd, Se, Zn and Cu. Hg was measured in maternal hair during 3rd trimester of pregnancy. Details regarding the exposure assessment have been published elsewhere. Specifically for metals in cord blood (Pb, Cb) or in maternal hair (Hg), concentrations were presented in Polańska et al. (2013, 2018), while micronutrients' levels in cord blood (Se, Cu and Zn) were reported in Polańska et al. (2016a, 2017a). Briefly, cord blood samples were centrifuged. Zn and Cu were analyzed in plasma by flame atomic absorption spectrometry (FAAS) whereas graphite furnace atomic absorption spectrometry (GFAAS) was used for Se determination (Agarwal and Henkin, 1985; Neve and Molle, 1986; Neve et al., 1987). Pb and Cd were determined by inductively coupled plasma mass spectrometry (ICP-MS) using nitric acid sample deproteinisation (Trzcinka-Ochocka et al., 2016). For the assessment of prenatal exposure to Hg, hair samples clipped close to the scalp from the back of head were collected from women between the 30th and 34th week of pregnancy. Hg in hair was determined using cold vapour atomic absorption spectrophotometry (CVAAS) (Brodzka and Trzcinka-Ochocka, 2009). All the analyses mentioned above were performed in an accredited laboratory according to PN/EN ISO/IEC 17025 at the Nofer Institute of Occupational Medicine, Poland. Moreover, the laboratory successfully participated in the German External Quality Assessment Scheme (G-EQUAS).

2.3. Outcome variables

Behavioral and emotional problems were assessed in children at 7 years of age using the Strengths and Difficulties Questionnaire (SDQ) filled in by the mothers (Jankowska et al., 2019a, 2019b). The SDQ, a widely used tool for the evaluation of child behavior, is a 25-item questionnaire that consists of 5 scales, namely: Conduct problems scale, Emotional problems scale, Hyperactivity scale, Peer relationships problems scale and Prosocial scale (www.sdqinfo.com; Goodman, 1997; Duinhof et al., 2019). For each of the 5 items within each scale, 3 response categories were possible: "not true", "somewhat true", and "certainly true" (with a scoring of 0, 1 and 2, respectively), resulting in a final scoring for the scale falling in a range from 0 to 10. Summary scores were calculated only if at least 3 of the 5 items have been completed. All sub-scales, except the Prosocial scale, were added as a Total difficulties score, ranging from 0 to 40. Higher total, conduct, emotional, hyperactivity and peer relationships SDQ scores are indicative of higher difficulties, whereas higher number of points for Prosocial scale indicates more favorable behavior. In this study, the outcomes were assessed as continuous (score) variables.

To assess children's cognitive and psychomotor development at age of 7 years, the Polish adaptation of the Intelligence and Development Scales (IDS) was administered by trained psychologists (Grob et al., 2009). In this study, the following domains were evaluated: general intellectual ability (Fluid and Crystallized Intelligence), Cognition, Mathematical skills, Language skills and Psychomotor skills (more details can be found in previous publications by Jankowska et al., 2019a; Jankowska et al., 2019b). Fluid intelligence involves being able to think and reason abstractly and solve problems. This ability is considered independent of learning, experience, and education. Crystallized intelligence involves knowledge that comes from prior learning and past experience. Reliability for Fluid and Crystallized Intelligence equals 0.94 and the correlations with analogous scales from Wechsler Intelligence Scale for Children (WISC-R) are 0.80 (Jaworowska et al., 2012; Grob et al., 2009).

2.4. Covariates

Covariates were defined *a priori* based on the literature and considering the availability of data. The following variables were included in the models: maternal age (at child birth), maternal educational level (years of completed education: ≤9; 9–12; >12), socio-economic status (SES) of the family (least affluent: poor and very poor; affluent: good; and most affluent: very good), household status (parents living together; single parent household), maternal pre-pregnancy body mass index (BMI, kg/m^2), cotinine level in saliva (ng/ml) collected during the 1st trimester of pregnancy (details are provided in a previous publication, Polańska et al., 2017b), number of siblings (0, ≥1), child's age (only for SDQ as IDS is standardized for age) and child's sex. As child cognitive and psychomotor development was assessed by seven psychologists, the evaluator was also included as a covariate in the IDS models. Details regarding the covariates are presented in Table 1.

2.5. Statistical analysis

Data analysis and graphics were performed using the statistical software R (R Core Team, 2021) and ggplot2 package (Wickham, 2016). Imputation of missing values in exposure and covariate data was performed using chained equations with the mice package (Van Buuren and Groothuis-Oudshoorn, 2011). Specifically, the imputation models included 100 datasets using 30 iterations each. Missing values ranged between 0 and 4% in covariates and between 34% and 56% in exposure data (see Tables 1 and 2). Before inclusion in the models, exposure variables were transformed into the natural logarithm for normalization. In addition, all the variables were standardized (centered at zero and scaled to two standard deviations) for cross-comparison of the models (Gelman, 2008). Multivariate regression models were applied for each outcome, using all exposure data (joint analysis) or individually for each compound (ExWAS study). In order to assess the linearity between the associations, a generalized additive model was fitted between the SDQ/IDS outcomes (standardized scores) and the concentrations (log-transformed and standardized). The associations are presented in

Table 1
Characteristics of the study population according to participation in the metals and neurodevelopmental assessments (both SDQ and IDS) in the REPRO_PL birth cohort (n = 436).

	Missing	Participants included in the assessment
	N (%)	N (%)
Maternal age at delivery (age)[a]	15 (3%)	29.3 ± 4.0
Maternal educational status	3 (1%)	
<9 years		9 (2%)
9–12 years		128 (30%)
>12 years		296 (68%)
Socio-economic status	6 (1%)	
Least affluent		7 (2%)
Affluent		318 (74%)
Most affluent		105 (24%)
BMI pre-pregnancy (kg/m^2)[a]	6 (1%)	22.3 ± 3.9
Cotinine at 1st trimester (ng/ml)[b]	17 (4%)	1.3 (0.68–2.3)
Parity at child's birth	2 (1%)	
None		251 (58%)
One or more		183 (42%)
Hosehold status	9 (2%)	
Parents living together		378 (89%)
Single parent		49 (11%)
Alcohol consumption	18 (4%)	
No		333 (80%)
Yes		85 (20%)
Sex of the child	0 (0%)	
Female		224 (51%)
Male		212 (49%)
Child's age at examination (years)[a]	0 (0%)	7.5 ± 0.6

[a] Mean ± standard deviation (SD).
[b] Median and interquartile range (IQR).

Table 2
Descriptive statistics of prenatal exposures to micronutrients and metals in the REPRO_PL Birth cohort (n = 436).

	Missing N (%)	Minimum	Percentile 25	Median	Percentile 75	Percentile 90	Maximum
Micronutrients							
Copper in cord blood *(mg/l)*	150 (34%)	0.030	0.33	0.45	0.64	0.88	3.0
Selenium in cord blood *(μg/l)*	156 (36%)	15.0	22.1	30.3	35.4	41.0	66.1
Zinc in cord blood *(mg/l)*	149 (34%)	0.43	0.84	1.0	1.2	1.4	2.4
Metals							
Lead in cord blood *(μg/l)*	233 (53%)	2.9	7.6	9.9	13.5	18.5	70.0
Cadmium in cord blood *(μg/l)*	233 (53%)	0.020	0.045	0.060	0.19	0.40	1.8
Mercury in hair *(μg/g)*	201 (46%)	0.011	0.11	0.18	0.31	0.49	1.3

The reported values for micronutrients and metals are the raw exposures (non-imputed and non-log-transformed).

Figures S1 and S2.

Sensitivity analysis was performed in order to test the robustness of the associations between SDQ/IDS scores and the exposure variables. First of all the models were performed for both joint analysis and ExWAS without imputation and considering only the complete observations. In addition, further models for the joint analysis were prepared imputing not only the covariates included at each model, but also additional exposure measurements available in the REPRO_PL birth cohort (Polańska et al., 2013; Polańska et al., 2017a; Polańska et al., 2018). For this purpose, the concentrations of the studied metals and micronutrients in other time points of the study were taken into account, as follows: for Pb and Cd, blood samples at the 2nd trimester of pregnancy; and for Zn, Se and Cu, blood samples at the 1st, 2nd and 3rd trimesters of pregnancy as well as at delivery. Correlations between the different metals and micronutrients are shown in Figure S3. And finally, sensitivity analysis included the application of the same models adjusted by additional imputed covariates (birth weight and gestational weeks, both of them also standardized).

3. Results

3.1. Characteristics of the study population

The characteristics of the study population are presented in Table 1. Briefly, maternal age at delivery was 29 years of age. Two thirds of the pregnant women had a high level of education (>12 years) and belonged to the affluent socio-economic status (68% and 74%, respectively). For more than half of the women it was the first pregnancy with a healthy body mass index (BMI). About a 20% of the women consumed alcohol, and the median cotinine values at 1st trimester of pregnancy was 1.3 ng/ml. Half of the offspring were females, and the median age at neurodevelopmental assessment was 7.5 years.

Table 3
Distribution of SDQ and IDS scores in the REPRO_PL Birth Cohort (n = 436).

	Mean (±SD)	Range
SDQ		
Conduct problems	1.8 (1.4)	0–7
Emotional symptoms	2.2 (1.9)	0–9
Hyperactivity/Inattention	3.9 (2.5)	0–10
Peer relationships problems	1.3 (1.5)	0–7
Pro-social behavior	8.4 (1.6)	3–10
Total difficulties	9.1 (5.0)	0–26
IDS		
Crystallized IQ	104 (15)	60–142
Fluid IQ	103 (14)	55–136
Language skills	21 (4.7)	6–32
Mathematical skills	11 (3.1)	3–19
Motor skills	30 (5.6)	9–43
Total Cognition	73 (11.2)	17–99

SD: Standard Deviation; Range: Minimum and Maximum values. SDQ: Strengths and Difficulties Questionnaire; IDS: Intelligence and Development Scales.

3.2. Occurrence of micronutrients and metals

The concentrations of micronutrients and metals in cord blood or at the 3rd trimester of pregnancy in the studied population of Poland are shown in Table 2. Median micronutrients cord blood concentrations were 0.45 mg/l for Cu (ranging from 0.030 mg/l to 3.0 mg/l), 30.3 μg/l for Se (ranging from 15.0 μg/l to 66.1 μg/l) and 1.0 mg/l for Zn (ranging from 0.43 mg/l to 2.4 mg/l). Median Cd and Pb cord blood concentrations were 0.060 μg/l and 9.9 μg/l, respectively, ranging from 0.020 μg/l to 1.8 μg/l for Cd, and from 2.9 μg/l to 70 μg/l for Pb (with 3 women exceeding 1 μg/l for Cd and 3 women exceeding 50 μg/l for Pb). The median Hg concentration in hair of pregnant women at the 3rd trimester of pregnancy was 0.18 μg/g, ranging from 0.011 μg/g to 1.3 μg/g. The distribution of these concentrations are shown in supplementary material (Figure S4).

3.3. Child behavioral, cognitive and psychomotor development

The description of child behavioral problems (SDQ) and cognitive as well as psychomotor development (IDS) are presented in Table 3. More details regarding the studied outcomes are provided in our previous publications (Jankowska et al., 2019a, 2019b). Briefly, mean SDQ total difficulties score was 9.1 ± 5.0 points (considering possible 0–40 points range for the score). Some variability in individual test scores evaluating child cognitive and psychomotor functions (IDS) were observed in the studied population (*e.g.* Fluid IQ range 55–136, Crystallized IQ range 60–142) but mean ± SD values (*e.g.* Fluid IQ 103 ± 14, Crystallized IQ 104 ± 15) indicate that the children are within the normal range. The distribution of SDQ and IDS scores are shown in supplementary material (Figure S5).

3.4. Association between prenatal exposures and neurodevelopmental outcomes

Figs. 1 and 2 show the results of both the joint analysis and the ExWAS study for SDQ and IDS, respectively. Briefly, in the joint analysis, in which all the metals and micronutrients were taken into account simultaneously, Zn and Se in cord blood were negatively associated with SDQ emotional symptoms scale (−0.10, 95% CI: −0.22; 0.018; and −0.13, 95% CI: −0.26; 0.0044, respectively), suggesting a lower risk of emotional problems (statistically significance at a 90% level). On the other hand, higher Hg levels in maternal hair collected during the 3rd trimester of pregnancy were associated with higher (worse) scores on the Hyperactivity/Inattention scale (0.18, 95% CI: 0.051; 0.30) and Total difficulties (0.14, 95% CI: 0.0066; 0.26), both of them being statistically significant at a 95% level (Fig. 1 and Table S1). In the analysis of the Intelligence and Development Scales, Pb cord blood levels were found to be negatively associated with Crystallized IQ (−0.14, 95% CI: −0.29; 0.0067), Fluid IQ (−0.12, 95% CI: −0.27; 0.023) and Mathematical skills (−0.15, 95% CI: −0.31; 0.013). The aforementioned associations were significant at a 90% level. Multivariate regression parameters of the joint analysis for SDQ and IDS are shown in Tables S1

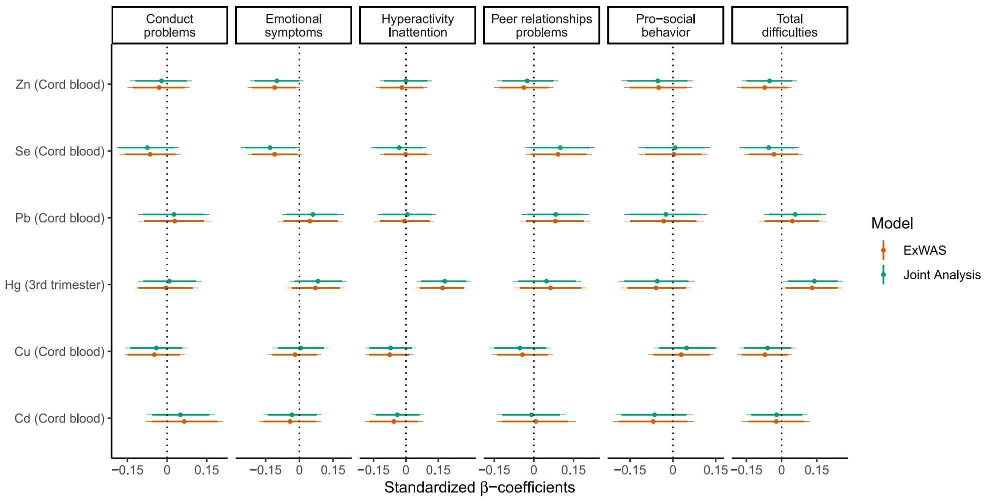

Fig. 1. Standardized beta-coefficients from multivariate linear regression models accounting for metals and micronutrients in cord blood (except for Hg in hair at 3rd trimester of pregnancy) globally (Joint Analysis) and separately (ExWAS) on the behavioral scales (SDQ) in children at 7 years of age. Models are adjusted by child's sex and age at examination, household status, SES, maternal educational level, maternal age, parity at child's birth, pre-pregnancy BMI, alcohol consumption and cotinine values at 1st trimester of pregnancy.

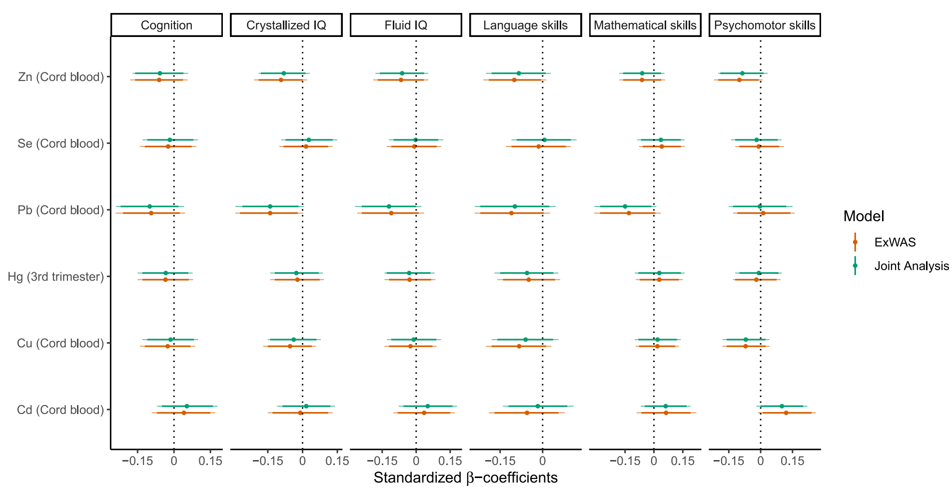

Fig. 2. Standardized beta-coefficients from multivariate linear regression models accounting for metals and micronutrients in cord blood (except for Hg in hair at 3rd trimester of pregnancy) globally (Joint Analysis) and separately (ExWAS) on the intelligence and development scales (IDS) in children at 7 years of age. Models are adjusted by child's sex, household status, SES, maternal educational level, maternal age, parity at child's birth, pre-pregnancy BMI, alcohol consumption, cotinine values at 1st trimester of pregnancy and examiner.

and S2, respectively.

Most of the previous results were also observed in the ExWAS study, in which exposures to each metal and micronutrient were analyzed individually (Figs. 1 and 2, and Tables S1 and S2 for SDQ and IDS, respectively). In addition, other positive associations were found between Psychomotor skills and Cd and Zn levels (0.12, 95% CI: −0.013; 0.26, and −0.10, 95% CI: −0.22; 0.0096, respectively) as well as Language skills and Zn levels (−0.10, 95% CI: −0.21; 0.013). These individual associations were, however, only significant in the ExWAS study at a significance level of 90% (Fig. 2; Table S2).

3.5. Sensitivity analyses

In sensitivity analyses, the previous associations remained, and only few additional relationships between the neurodevelopmental outcomes and environmental exposures appeared. In the case of SDQ, total Hg was not only associated with Hyperactivity/Inattention and Total difficulties, but also with Pro-social behavior, having a negative impact in all of them (Table S3). Zn and Se remained to be associated with Emotional symptoms, although the latter appeared to be also associated with Peer-relationships problems (only in the non-imputed models) (Table S3). Regarding IDS, while lead concentrations in cord blood remained associated with Crystallized IQ, Fluid IQ and Mathematical skills in most of the sensitivity analyses performed, new associations with most of the intelligence scores appeared for Hg. Specifically, higher Hg levels were associated with lower scores in Total Cognition, Crystallized and Fluid IQ, as well as with Language and Psychomotor skills. These associations, however, only appeared in some of the non-imputed models (Table S4).

4. Discussion

The current study has applied a two-fold approach considering on the one hand all prenatal exposures individually [through an ExWAS study], as well as a simultaneous analysis of the studied metals and micronutrients [the so-called joint analysis]. Both approaches have provided similar results. Specifically, we were able to demonstrate some long-term effects of two neurotoxic metals (Hg and Pb) and two micronutrients (Zn and Se) on child behavior and IQ. Cautious interpretation of the results is suggested as none passed the significance levels after controlling for multiple comparisons through the Bonferroni correction (data not shown). In any case, the discussion is focused on the associations that were observed in both the joint analysis and the ExWAS study.

A detailed discussion of the neurotoxic metal and micronutrient concentrations in the biological samples observed in our population has been published elsewhere (Polańska et al., 2013; Polańska et al., 2017a; Polańska et al., 2018). Briefly, the concentrations in the Polish population were comparable to those measured in populations of most European countries and US regions. A recently published analysis by Jedynak et al. (2021) based on 5 European birth cohorts reported median whole blood Pb and Cd levels of 10.4 μg/l and 0.20 μg/l, respectively (compared to 9.9 μg/l and 0.060 μg/l, respectively, in our population). Se levels in Poland suggested a sub-optimal intake of this micronutrient, likely linked to dietary habits commonly found in central Europe. Fish is one of the main sources of Se and Hg intake by diet. Our previously published analysis derived from dietary questionnaires administered to women enrolled in the REPRO_PL cohort confirmed low fish consumptions in Poland (Wesolowska et al., 2019).

We report here that prenatal levels of both micronutrients (Se and Zn) and neurotoxic metals (Pb and Hg) have significant effects on children's neuropsychological development at 7 years of age. Notably, Se and Zn levels in cord blood are negatively correlated with a specific subscale of the SDQ, which include five items describing the child emotional symptoms such as fear, depressed mood, anxiety, tearfulness and psychosomatic complaints. To the best of our knowledge, this is the first study reporting a positive impact of prenatal Se and Zn on emotional development assessed in early school-age children. So far, prospective studies evaluating the relationship between Se status during pregnancy and child neuropsychological development have mainly focused on psychomotor and cognitive abilities in preschool children. In general, the extent of the effects differs among cohort studies (Polańska et al., 2016a; Oken et al., 2016; Amorós et al., 2018a; Močenić et al., 2019) depending on Se concentration during pregnancy, with too low or too high levels associated with detrimental effects on some neuropsychological outcomes in a Spanish cohort (Amorós et al., 2018b). In REPRO_PL cohort we have previously described a positive association between Se level in the 1st trimester of pregnancy and motor skills and child language at one and two years of age, respectively (Polańska et al., 2017a). Se is an essential element as it forms the core of several enzymes, such as glutathione peroxidases (GPx) or thioredoxin reductases (TRXR), which have a pivotal role in antioxidant defences, reactive oxygen species (ROS) scavenging and thyroid hormones metabolism (Duntas, 2020; Roman et al., 2014). Notably, in experimental studies nutritional deficiency of Se is accompanied by decreased GPx activity in the brain associated to altered turnover of specific neurotransmitters (Castaño et al., 1993) and thyroid dysfunction (Hofstee et al., 2019). Although the neurobiological mechanisms by which Se promotes neurodevelopment are not fully understood, both the modulatory effects of Se on thyroid function and the protection of developing neural cells from oxidative damage could exert beneficial effects on cognitive and emotional maturation (Esposito et al., 1997; Sher, 2000). Of note, several human studies have demonstrated the involvement of Se deficiency in mood disorders (Benton, 2002; Hawkes and Hornbostel, 1996). Findings from animal models also support the anxiolytic and antidepressant effects of Se (Casaril et al., 2019; Sousa et al., 2018). Specifically, a preclinical study demonstrated that Se supplementation during pregnancy and lactation promoted a reduction in anxiety-like behavior and lower responsiveness to stress in rat offspring (Laureano-Melo et al. 2015).

As for the beneficial effects of Zn on emotional responses, they have been reported mostly in adults. Some studies reported an inverse correlation between dietary Zn intake and mental illnesses, including anxiety, and some indicators of sleep disorder (Song et al., 2016; Nakamura et al., 2019; Hajianfar et al., 2021). Only a single study in infants associated lower serum Zn levels before the age of six months with socio-emotional impairment (Metwally et al., 2016). Preclinical studies support the role of Zn on emotional behaviors: Zn deficiency induced the parallel increase in anxiety-like behavior and serum corticosterone concentration in mice (Takeda et al., 2007). Interestingly, a potential link between Autism Spectrum Disorder (ASD) and Zn deficiency is currently investigated, hypothesizing the implication of brain glutammate neurotransmission, which is significantly modulated by Zn (Ha et al., 2018). In a murine model of ASD (Shank3−/− mice), maternal Zn supplementation prevents ASD-associated deficits, including anxiety-like behaviors (Vyas Y et al., 2020).

In the IDS no effects of either Se or Zn are evident, with the exception of a moderate negative influence (ExWAS only) of Zn on psychomotor skills and language development. However, this association was not found in any of the additional models performed in sensitivity analyses and might be due to familywise error rate.

In this study, Hyperactivity/Inattention problems and Total difficulties were associated with higher Hg concentrations in maternal hair collected during the 3rd trimester of pregnancy. Our previous assessments based on REPRO_PL (Polish) and PHIME (Slovenia and Croatia) cohorts indicated that Hg positively influenced motor scores within two years of life in the Polish cohort, while it decreased the motor scores in the Slovenia and Croatian sub-cohorts, a discrepancy that might be explained by the different levels of exposure related to fish intake (Calamandrei et al., 2020). In the current assessment, we have not observed an association between Hg concentrations and child motor abilities at age of 7 years in our main ExWAS or joint analysis. Hg, particularly methylmercury (assessed in this study), is an established developmental neurotoxicant (Grandjean and Landrigan, 2006, 2014). Developmental neurotoxicity due to Hg occurs at much lower exposures than the concentrations that affect adult brain function, with the deficits observed not only within the first years of life but also in teenagers (Grandjean and Landrigan, 2014; Vrijheid et al., 2016). The impact of prenatal Hg levels on offspring behavioral outcomes has been evaluated in several previously published studies based on which negative but also positive or no effects were postulated (Myers et al., 2000, 2003; Davidson et al., 2011; Boucher et al., 2012; Sagiv et al., 2012; van Wijngaarden et al., 2013; Golding et al., 2016; Skogheim et al., 2021; Jedynak et al., 2021). Some inconsistencies may be explained by different exposure levels and the fact that Hg levels could be a proxy measure for the intake of polyunsaturated fatty acids and other beneficial nutrients for brain development that is found in seafood (Garí et al., 2013; Avella-Garcia and Julvez, 2014). In this study we were not able to control for fish intake (which, as it was mentioned previously, is generally at low level of consumption by Polish pregnant women).

Lead is another well-established neurodevelopmental toxicant with no known safe exposure level for neurodevelopment (Grandjean and Landrigan, 2014). In this study we conformed that the negative impact of prenatal Pb level on child cognitive development observed shortly after birth still persist at 7-years of age (namely in the form of less points received for Crystallized Intelligence) (Polańska et al., 2013; Polańska et al., 2018). These findings confirm once more that even mean concentrations of Pb in pregnancy lower than the threshold considered as overtly neurotoxic by regulatory agencies have a significant impact on child cognitive abilities.

Our previous analysis based on REPRO_PL data (focused on child neurodevelopmental outcomes assessed within the first two years of life) indicated that the most perturbed metabolic pathways from co-exposure to heavy metals and phthalates were pathways related to the tricarboxylic acid cycle (TCA cycle) and oxidative phosphorylation, suggesting the implication of disrupted mitochondrial respiration (Sarigiannis et al., 2021). Overproduction of ROS, the presence of glutathione peroxidase 3 (GPx3) during pregnancy and presence of glutathione peroxidase 1 (GPx1) in the umbilical cord were linked to developmental problems.

This analysis, together with current results and other newly performed studies in this field proved that in real life, adverse outcomes occur as a combination of environmental and social factors (not covered in this study, although adjusted in the analyses), all of them acting towards the deployment of an observed neurodevelopmental consequences.

The strengths of this study are related to the longitudinal design, which allows for prospective assessment of prenatal exposures (considered as a critical period for brain development), simultaneous assessment of neurotoxic metals and micronutrients, as well as the use of standardized tools for the measurement of child neurodevelopment. Prospective birth cohort designs allow not only for the collection of objective prenatal exposure data (by biomarkers measurements) but also for a variety of covariates included in current analysis (with cotinine level in maternal saliva as a biomarker of active and passive smoking during pregnancy). Moreover, the toxic metal and micronutrient concentrations were measured in one accredited laboratory and used reference materials for validation of results.

The current study has considered a broad spectrum of child neurodevelopment, including behavioral and cognitive as well as psychomotor domains that were assessed based on widely used standardized and validated questionnaires and tools, thus increasing comparability of the obtained results. SDQ is a suitable tool for the evaluation of behavioral problems in children, either showing or not clinical symptoms. Behavioral assessments based on continuous scales (as we have applied in the current study) allow to measure subclinical conditions, which is specially relevant for low-risk community populations, like the one examined here. Further to behavioral measures such as SDQ, used also in other newly published assessments performed on birth cohorts (*e.g.* Jedynak et al., 2021; Maitre et al., 2021; Skogheim et al., 2021), we have considered additional measures of child cognitive and psychomotor development. It also needs to be underlined that the study was restricted to healthy women with non-assisted reproduction technologies' pregnancy. Finally, the use of advanced statistical methods including multiple imputation is a further strength of the study, since it allowed us not only to cover a higher sample size in our analyses, but also to improve our estimates by the additional knowledge of existing biomarker exposures in other time points of the study (*e.g.* samples at 1st, 2nd and 3rd trimesters of pregnancy). Nevertheless, we do not disregard the fact that the application of multiple imputation in case of many missing values might have an impact on the resulting effect estimates (such as those we observed for Hg and Pb on the IDS scores).

The limitations of the study need to be also considered. The analysis was restricted to selected neurotoxic metals and micronutrients (for which, based on our previous assessment, the associations with child neurodevelopmental outcomes at age of 1 and 2 years were observed), however some other chemicals as well as socio-demographic and lifestyle related factors create potential for further simultaneous analyses. Due to limited availability of biological samples (or lack of samples, which is especially relevant to cord blood), some biomarkers of exposure were not assessed in all study participants. Although the models were adjusted for numerous variables, the possibility of residual or unmeasured confounding cannot be excluded. Moreover, we focused only on prenatal exposure and did not assess exposure in early postnatal life, a period also recognized as crucial for brain development. And finally, we should mention that other statistical approaches, such as dimension reduction techniques or penalized regression tools, would have enhanced the results of our assessment. We preferred, however, to employ a basic statistical tool (such as multivariate linear regression) together with multiple imputation as a first insight into the studied associations.

5. Conclusion

This study points out that prenatal exposures to metals and micronutrients might have an effect on behavioral and cognitive development of children. Specifically, early school age children from Poland exposed to neurotoxic metals such as Hg and Pb had poorer scores on behavioral (SDQ) and intelligence (IDS) tests, respectively. The present results confirm that there is no safe level for neurotoxic metals, as exposure to very low levels of Pb and Hg during pregnancy may have negative impacts on children's neuropsychological functions later in life. In addition, our research was able to reveal for the first time a positive association between two micronutrients (Zn and Se) and the Emotional screening sub-scale of the SDQ questionnaire. Identification of environmental risk factors for emotional and behavioral problems in children is a priority issue for promotion of mental health. These results might be useful for protecting children's health by implementing prophylactic measures (*e.g.* maternal diet) during pregnancy.

Credit author statement

KP and WH are the principal investigators of the study and obtained the necessary funds for the study design and development. MeG, KP and GC wrote the main manuscript. AJ, AK, JJ and AMT provided significant contributions to the manuscript. MaG, MK and PK performed fieldwork and participated in data interpretation. BJ and RK were responsible for the laboratory analyses of metals and micronutrients in cord blood and hair samples. DK, MeG, AMT and GC were responsible for data acquisition and interpretation of behavioral and intelligence tests. MeG performed the statistical analyses and data visualization. All authors have revised the study development and approved the submitted version of the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Ministry of Science and Higher Education, Poland (PBZ-MEiN-/8/2//2006; Contract No. K140/P01/2007/1.3.1.1); by the grant PNRF-218-AI-1/07 from Norway through the Norwegian Financial Mechanism within the Polish-Norwegian Research Fund, National Science Centre under the call of JPI HDHL Nutrition and Cognitive Function (2015/17/Z/NZ7/04273), the Polish National Center for Research and Development (ERA-HDHL/01/ALPHABET/1/2017) and the National Science Centre, Poland (DEC-2014/15/B/NZ7/00998). We would like to thank all the psychologists and especially to Marcin Drabek involved in children examinations and interpretation of the results related to child cognitive and psychomotor development. Mercè Garí acknowledges the support from the Joachim Herz Foundation through the Add-on Fellowship for Interdisciplinary Science.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.112049.

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