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# Association of phthalate exposure with autistic traits in children

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ABSTRACT

*Background:* Phthalates are synthetic chemicals with endocrine-disrupting properties. They are reportedly associated with various neurotoxic outcomes. Studies on exposure to phthalates and children's autistic traits have shown inconsistent results with respect to sex and susceptible time periods. We investigated the association of phthalate exposure during the prenatal period and childhood with autistic traits over time using a birth cohort in South Korea.

*Methods:* Five phthalate metabolites were measured during mid-term pregnancy and children's follow-up at ages of 4, 6, and 8 years among a total of 547 mother–child pairs. The social communication questionnaire (SCQ) was used to assess autistic traits of children at each time point. The relationship between phthalate metabolites and SCQ scores were analyzed by exposure windows and sex.

*Results:* A 2.7 fold increase in di-(2-ethylhexyl) phthalate metabolite levels, mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) during pregnancy was associated with increased SCQ scores at 4 years by 8.5% (95% confidence intervals [CI]: 1.9%, 15.5%) and 7.4% (95% CI: 0.3%, 15.0%), respectively, but not at the age of 6 or 8 years. Moreover, MEHHP levels at ages of 4 and 8 years were associated with increased SCQ scores at 8 years by 9.9% (95% CI: 1.8%, 18.6%) and 9.6% (95% CI: 1.3%, 18.6%), respectively. Boys showed stronger associations between phthalate exposure and SCQ scores than girls.

*Conclusion:* The study suggested different susceptible time windows of phthalate exposure: exposure during pregnancy is associated with autistic traits in young children, whereas exposure during early childhood years leads to autistic traits in school-aged children, particularly boys.

## 1. Introduction

Autism spectrum disorder (ASD) is a childhood-onset neurodevelopmental disorder characterized by impairment in social communication, restricted interests, and repetitive behavior (American Psychiatric Association 2013). Autistic traits manifest as early as 18 months and become rather stable after the age of 3 years (Barbaro and Dissanayake 2009). Approximately one in 54 children suffer from ASD, as estimated by the US Centers for Disease and Prevention in 2019 (Baio et al. 2018), and its prevalence has increased rapidly over the past three decades (Hansen et al. 2015). Environmental risk factors may contribute partially to this rapid increase (Modabbernia et al. 2017). However, given the complex interaction of genetic and environmental factors, exact etiological factors remain unknown (Kim et al. 2019).

Phthalates are synthetic chemicals with endocrine-disrupting properties; they are a major health concern due to their ubiquitous sources (Zota et al. 2014). They are widely used in cosmetics, personal care products, food packaging, medical devices, and toys (Pak et al. 2011).

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Phthalates are reportedly associated with various neurotoxic outcomes, including low intelligence quotient (IQ) (Tanner et al. 2020), high attention problems (Praveena et al. 2020), and increased autistic traits (Jeddi et al. 2016). These neurodevelopmental problems may be caused via various pathways, including altered thyroid hormone production and function, disrupted hypothalamic-pituitary-thyroid axis target points, placental development and function, antiandrogenic activity, and disrupted brain dopaminergic activity (Zhu et al. 2020).

Previous studies examined phthalate exposure and autistic traits; however, there were conflicting results (Braun et al. 2014; Day et al. 2021; Kardas et al. 2016; Miodovnik et al. 2011; Oulhote et al. 2020; Shin et al. 2018; Stein et al. 2013; Testa et al. 2012). A recent systematic review that summarized previous findings suggested slight or indeterminate changes in social behaviors associated with phthalate exposure (Radke et al. 2020). Moreover, previous studies had limited access to various time windows of phthalate exposure as none of the prenatal phthalate studies considered postnatal phthalate levels related to autistic traits, and vice versa. Therefore, susceptible time periods of exposure during the life-course have not been identified; exposure to some environmental agents during a certain period may result in a greater effect on a health outcome than during other periods (Sanchez et al. 2011). The developmental origin of health and disease (DOHaD) hypothesis suggests that environmental exposures influence developmental programming during critical periods of early life development through epigenetic modifications, leading to permanent changes in disease susceptibility in later life (Waterland and Michels 2007). Identification of these exposure windows could lead to targeted exposure recommendations that would maximize public health impact (Buckley et al., 2019b).

The objectives of this study were: 1) to investigate the overall relationship of phthalate exposure with autistic traits over time, and 2) to identify susceptible time periods during which phthalate exposure manifests negative effects on autistic traits at multiple time points with regard to the subject's sex. Considering that brain plasticity is dynamic throughout the life span, we hypothesized that there might be more than one sensitive period (Lewis and Maurer 2005; Reh et al. 2020). We used data on phthalate exposure at multiple time points (pregnancy and children at ages of 4, 6, and 8 years) and repeatedly measured autistic traits in children (at ages of 4, 6, and 8 years) from an ongoing prospective birth cohort in South Korea.

## 2. Materials and methods

### 2.1. Study design and participants

This study was part of the Environment and Development of Children (EDC) study, an ongoing prospective birth cohort in South Korea. The protocol of the EDC study has been previously described (Kim et al. 2018). Briefly, 13,484 pregnant women in the second trimester (between 14 and 27 weeks of gestation) were enrolled in the Congenital Anomaly Study from 2008 to 2010. Their individual characteristics (sociodemographic and lifestyle factors, past medical history) and exposure levels of endocrine-disrupting chemicals measured by spot urine or peripheral blood samples, including phthalate metabolites, were measured. After delivery, children's birth information was collected. Subsequently, 2,085 mothers were randomly selected and contacted for follow-up of their offspring, and 726 were finally recruited in the EDC study. The children underwent physical examinations at the Seoul National University Hospital every two years from the age of 2 years. They were asked to fast for breakfast on the day of visit from the age 4 years. Blood and urine samples were drawn to examine environmental exposure levels and clinical markers. Children's characteristics including sociodemographic characteristics, living environments, and health statuses were reported by their mothers using structured questionnaires. Daily dietary intake at age 4, 6 and 8 was assessed by using a validated semi-quantitative food-frequency questionnaire (FFQ) that assesses the frequency of consumption and portion size of 91 food items during the previous year (Kim et al. 2016). The amount of each item was transferred into grams and daily calorie intake was estimated using the Computer Aided Nutritional Analysis Program 4.0 for professionals (CAN-pro 4.0, Korean Society of Nutrition, Seoul, Korea). These food items were classified according to the NOVA food processing classification system into 4 groups (Monteiro et al 2018; Sung et al. 2021 (Buckley et al., 2019a): Table S1). Professional psychologists evaluated children's neurocognitive development (autistic traits, executive function, and attention problems) 4 years onwards and IQ at the age of 6 years. As the IQ level is considered to be stable (Schalke et al. 2013), children's IQ scores were assessed once at age 6 using the Korean Educational Developmental Institute's Wechsler Intelligence Scale for Children (Park et al. 1996). Additionally, mothers' IQ scores were evaluated using the Korean Wechsler Adult Intelligence Scale (Lim et al. 2000).

Of 726 children, we excluded 62 (8.5%) children who did not visit at least once between age 4 and 8 years, and 37 (5.1%) children due to missing information on the social communication questionnaire (SCQ) scores. Additionally, 80 (11.0%) children were excluded due to missing information on phthalate metabolites. A total of 547 (75.3%) children were included in the analyses, and 344 (47.4%), 477 (65.7%), and 440 (60.6%) visited at ages of 4, 6, and 8 years, respectively (Fig. 1).

Written informed consent was obtained from mothers (since pregnancy) and children (aged $\geq$ 8 years). The study protocol was reviewed and approved by the Institutional Review Board of the Seoul National University Hospital (IRB No. C-1201–010-392). This study was performed in accordance with the principles of the Declaration of Helsinki.

### 2.2. Measurement of phthalate metabolites

The protocol for measuring urinary phthalate metabolites has been described elsewhere (Kim et al. 2017). Briefly, maternal phthalate metabolite levels were measured using maternal urine during the second trimester, i.e., between 14 and 27 weeks (mean of 20 weeks) of gestation, and childhood phthalate metabolite levels were measured using the urine of children aged 4, 6, and 8 years. The metabolites of di-(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP) and benzylbutyl phthalate (BBzP) were measured; specifically mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-2-ethyl-5-carboxypentyl phthalate (MECPP), mono-n-butyl phthalate (MnBP), and mono-benzyl phthalate (MBzP) as they are the most widely used phthalates in personal-care products and have been associated with adverse health effects (Hauser and Calafat 2005). Moreover, our previous studies repeatedly found that MEHHP, MEOHP and MnBP were associated with neurodevelopmental outcomes in different cohorts (Cho et al. 2010; Kim et al. 2009; Kim et al 2011). There was missing information on levels of MECPP and MBzP due to shortage of urine volume in some of the samples (available sample size: 232, 349, and 326 at ages of 4, 6, and 8 years, respectively). Therefore, we included MEHHP, MEOHP, and MnBP in the main analyses and provided results of MECPP and MBzP in the supplementary file.

The urine phthalate metabolite concentrations were measured with high-performance liquid chromatography tandem mass spectrometry (TQ4500; AB Sciex, USA). Spot urine was collected in a paper cup from each participant between 0900 and 1100 h and transferred to high-clarity polypropylene Falcon tubes and stored at −20 °C immediately after collection. One blank and one quality control (QC) samples were included in each batch of samples. The QC samples were spiked with pooled urine and a mixture of phthalate monoester standard (100 ng/mL). Analytical procedures followed a strict internal quality assurance protocol by measuring procedural blank and internal quality control (QC) urine samples in each batch of measurement. Internal QC was performed prior to analysis of the whole sample and included tests of linearity, accuracy, precision and detection limit. In the linearity test, the $R^2$ was 0.999 in the calibration curve applied to 7 points of the

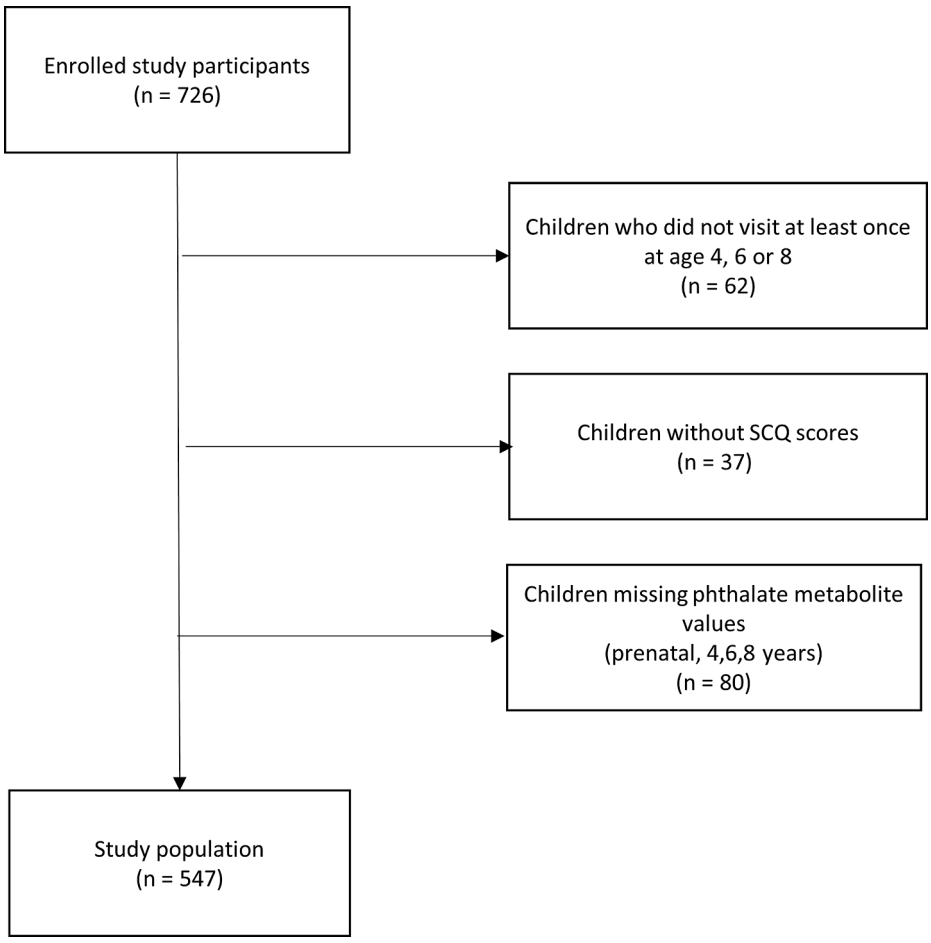


**Fig. 1. Flow chart of the study participants.** Abbreviation: SCQ, social communication questionnaire.

concentration range in pooled urine. The accuracy test was performed by using the standard reference materials (National Institute of Standards & Technology, NIST 3672 – organic contaminants in smoker's urine and NIST 3673 – organic contaminants in non-smoker's urine) and yielded recovery rates of 97.5 – 104.0 % (100.8% and 103.4%; 98.1% and 99.0%; 99.5% and 104.0%; 97.5% and 100.6%; 100.8% and 98.0% for MEHHP, MEOHP, MnBP, MECPP and MBzP, respectively) and coefficient of variations of 2.4 – 11.8% (2.9% and 7.4%; 2.7% and 2.4%; 11.8% and 5.4%; 4.5% and 5.5%; 6.4% and 9.8%; for MEHHP, MEOHP, MnBP, MECPP and MBzP, respectively) in phthalate metabolites. The detection limits of phthalate metabolites were 0.208, 0.487, 0.724, 0.270, and 0.356 μg/L for MEHHP, MEOHP, MnBP, MECPP, and MBzP, respectively (Smartive, Hanam, Republic of Korea). For values below the limit of detection (LOD), LOD $/\sqrt{2}$ was assigned (Hornung and Reed, 1990).

For measuring creatinine, CREA (Roche, Indianapolis, IN, USA) reagent was used with a Hitachi 7600 machine (Hitachi, Tokyo, Japan) with a kinetic colorimetric assay (rate-blanked and compensated). The values of phthalate metabolites (μg/L) per creatinine (g/L) were used for the statistical analyses to correct urine dilution.

### 2.3. Assessment of autistic traits

We evaluated children's autistic traits using the SCQ at ages 4, 6, and 8 years. The SCQ is a parent-report questionnaire consisting of 40 items, from which children's communication skills, social functioning, and repetitive behaviors during the past three months were evaluated. The first item is to evaluate eligibility for the assessment (4 children were excluded as they were not able to talk in short phrases or sentences). The rest of the 39 binary items (1: yes; 0: no) were responded to and summed up for the total SCQ score (Rutter et al. 2003). Higher scores indicate more autistic traits. The SCQ scores were right-skewed, and the distribution of the SCQ scores at each age is shown in Fig S1. A cutoff score of 11 to 18 is recommended (Marvin et al. 2017); however, as the proportion of children exceeding these scores were small, we used a cutoff score of 8 to define subthreshold ASD (Evans et al. 2019).

### 2.4. Covariates

Potential covariates were selected based on previous literature (Miodovnik et al. 2011; Oulhote et al. 2020; Shin et al. 2018; Stein et al. 2013), including maternal characteristics: maternal age at pregnancy (years), maternal education (<or ≥ college education), paternal education (<or ≥ college education), socioeconomic status (monthly family income < $3,500 or monthly family income ≥ $3,500), pre-pregnancy diabetes mellitus (yes or no), and maternal IQ; and the child's characteristics: age (in months), sex, body mass index (BMI) (kg/m$^2$), twin (yes or no), birth order (<or ≥ 2$^{nd}$), gestation age at birth (weeks), breastfeeding (exclusive breastfeeding, mixed feeding, or exclusive formula feeding), current environmental tobacco smoke (ETS) (yes or no), percent of total energy intake from ultra-processed foods among all foods, and IQ (measured at the age of 6 years). Among the potential covariates, we controlled for different sets of covariates for models related to different exposure time windows (prenatal and postnatal) based on exploratory analyses for associations of potential covariates with SCQ scores at ages of 4, 6, and 8 years and phthalate metabolite levels (at pregnancy and ages of 4, 6, and 8 years) (Table S2, S3, and S4).

### 2.5. Statistical analyses

As phthalate metabolite levels were not normally distributed, we used the natural log-transformed values in all subsequent analyses. Pearson correlation coefficients between phthalate levels during pregnancy and at ages of 4, 6, and 8 years were calculated. Univariate Poisson regression models were used to investigate the association between potential covariates and SCQ scores and linear regression models were used to explore the association between those covariates and phthalate levels at different ages. To compare the characteristics of included and excluded mothers, we used independent samples t-tests (for continuous variables) and chi-square tests (for categorical variables). We used descriptive statistics to summarize the characteristics of the children aged 4, 6, and 8 years. Intraclass correlation coefficients (ICCs; two-way mixed models, average rater, absolute agreement option: ICC(A,k)) were computed to examine differences of SCQ scores and phthalate metabolites within a child over time (at ages of 4, 6, and 8 years). Associations between phthalate exposure and SCQ scores were analyzed using two different analytic methods: (1) an overall association in a repeated measured analysis considering within-subject correlation and (2) exposure window-specific associations in Poisson regression analyses and logistic regression analyses, as described below.

To investigate the overall association of phthalate exposure with SCQ scores over time (at ages of 4, 6, or 8 years), we conducted a repeated measured analysis using generalized estimating equation (GEE) models with a log-link function (i.e., Poisson regression models) and an "exchangeable" covariance structure to account for within-subjects (n = 547). In a sensitivity analysis, we examined the average effect of postnatal exposure to phthalates on social development in school-age children by linking postnatal exposures at all three ages (ages of 4, 6, and 8 years) with SCQ scores at 8 years of age using a GEE model (n = 460). We constructed GEE models for all children, for boys and for girls, separately, after adjusting for age, sex (for all children), twin, birth order, maternal education levels, current ETS, and phthalate levels during pregnancy.

We identified susceptible exposure windows by linking possible combinations of exposure periods (pregnancy and ages of 4, 6, and 8 years) and assessments for autistic traits (at ages of 4, 6, and 8 years). We used Poisson regression models and logistic regression models for each natural log-transformed phthalate metabolite (MEHHP, MEOHP, and MnBP) at each time period (pregnancy and ages of 4, 6, and 8 years) as an independent variable and the SCQ total score or subthreshold ASD status (with a cutoff score of 8) at ages of 4, 6, or 8 years as the dependent variable. We controlled for different covariates in prenatal and postnatal exposure models: we adjusted for child's age, sex, twin, birth order, and mutual childhood phthalate metabolite levels at the age of SCQ assessment in the prenatal exposure models (e.g., MEHHP levels at the age of 4 years were adjusted in the analyses on the association between prenatal MEHHP and SCQ at the age of 4 years), whereas we adjusted for child's age, sex, twin, birth order, maternal education, current ETS, and mutual prenatal or childhood phthalate metabolite levels at the age of SCQ assessment for postnatal exposure models (e.g., MEHHP levels at the age of 8 years were adjusted in the analyses on the association between MEHHP at the age of 4 years and SCQ at the age of 8 years). As eligible participants differed by follow-up ages, we included 344, 477, and 440 children aged 4, 6, and 8 years, respectively. Effect modification by sex was examined in sex-stratified analyses.

### 2.6. Sensitivity analyses

In the sensitivity analysis, we adjusted for maternal (or childhood) IQ, breastfeeding, SES, or percent of total energy intake from ultra-processed foods in addition to covariates in the prenatal (or postnatal) exposure models as these characteristics were related to both phthalate exposure and autistic traits (Miodovnik et al. 2011), and inclusion of these covariates resulted in significant reduction in sample size (n = 473 for childhood IQ, n = 452 for maternal IQ, n = 542 for breastfeeding, n = 500 for SES). As the percent of total energy intake from ultra-processed foods was assessed only in the children, we adjusted for the dietary intake in the postnatal models only. We also analyzed MECPP and MBzP in the same manner (n = 417 for MECPP and n = 369 for MBzP).

All statistical analyses were performed using SPSS for Windows software (ver. 26.0; Armonk, NY: IBM Corp.) and R version 4.0.2 (The Comprehensive R Archive Network, Vienna, Austria; http://cran-r-project.org). Statistical significance was defined as a $p < 0.05$ (two-tailed).

## 3. Results

### 3.1. Characteristics of the participants

Mean maternal age at birth was 31.5 years (standard deviation [SD]: 3.5), and most mothers had a college education (82.4%). Excluded mothers (n = 179) had similar characteristics as included mothers (n = 547) (Table 1). The characteristics of the children at ages of 4, 6, and 8 years are presented in Table 2. The mean ages (±SD) were 47.1 ± 1.7 months, 71.1 ± 1.5 months, and 95.1 ± 1.4 months at 4, 6, and 8 years, respectively. The proportion of boys was similar across visits (53.2%, 53.2%, and 53.6% at ages of 4, 6, and 8 years, respectively). The mean SCQ scores (±SD) decreased over time (4.3 ± 3.0, 3.6 ± 2.7, and 3.4 ± 3.1 at ages of 4, 6, and 8 years, respectively), and SCQ scores were higher in boys compared to girls at all ages (4.8 ± 3.1 vs 3.7 ± 2.8, $p < 0.01$ at age 4; 4.1 ± 3.0 vs 3.1 ± 2.4, $p < 0.01$ at age 6; and 3.8 ± 3.2 vs 3.0 ± 3.2, $p < 0.01$ at age 8). The ICC (0.65) showed relatively stable SCQ scores within a child during the follow-up. In regard to subthreshold ASD, 52 (15.1%) at age 4, 44 (9.2%) at age 6, 51 (11.6%) at age 8 surpassed the cutoff of 8.

### 3.2. Phthalate metabolite levels

The distribution of each phthalate metabolite at different ages is

**Table 1**
Characteristics of the mothers of included and excluded children in the study.

| Variables | Included children (n = 547) | Excluded children (n = 179) | P-value |
|---|---|---|---|
| Maternal age at pregnancy, years, mean (SD) | 31.3 (3.5) | 31.6 (3.8) | 0.43 |
| Maternal Education, N (%) | | | 0.11 |
| < College education | 96 (17.6) | 41 (22.9) | |
| ≥ College graduate | 451 (82.4) | 138 (77.1) | |
| Paternal education, N (%) | | | 0.58 |
| < College education | 88 (16.1) | 32 (17.9) | |
| ≥ College graduate | 458 (83.7) | 147 (82.1) | |
| Monthly family income | | | |
| < $3,500, N (%) | 112 (22.4) | 14 (18.7) | 0.47 |
| ≥$3,500, N (%) | 388 (77.6) | 61 (8.13) | |
| Prepregnancy DM, yes, N (%) | 20 (3.7) | 3 (1.7) | 0.19 |
| Maternal IQ, mean (SD) | 116.6 (11.5) | 116.2 (11.6) | 0.82 |
| Phthalate metabolite levels at pregnancy, μg/g creatinine, GM (GSD) | | | |
| MEHHP | 15.6 (2.3) | 15.5 (2.3) | 0.62 |
| MEOHP | 16 (2.1) | 16.1 (2.1) | 0.98 |
| MnBP | 42.2 (2.2) | 41.9 (2.1) | 0.61 |
| MECPP | 22.8 (1.9) | 20.0 (1.9) | 0.11 |
| MBzP | 6.0 (2.5) | 5.3 (2.3) | 0.86 |

Abbreviations: SD, standard deviation; DM, diabetes mellitus; IQ, intelligence quotient; GM, geometric mean; GSD, geometric standard deviation; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MnBP, mono-n-butyl phthalate, MECPP, mono-2-ethyl-5-carboxypentyl phthalate; MBzP, mono-benzyl phthalate
P-value for difference of characteristics between included and excluded children (chi-square test for categorical variables and *t*-test for continuous variables)

**Table 2**
Characteristics of the children at ages of 4, 6, and 8 years.

| Variables | Age 4 (n = 344) | Age 6 (n = 477) | Age 8 (n = 440) |
|---|---|---|---|
| Age, months, mean (SD) | 47.1 (1.7) | 71.1 (1.5) | 95.1 (1.4) |
| Sex, boys, N (%) | 183 (53.2) | 254 (53.2) | 236 (53.6) |
| BMI, kg/m$^2$, mean (SD) | 15.7 (12.0) | 15.8 (1.7) | 16.9 (2.5) |
| Twin, yes, N (%) | 32 (9.3) | 42 (8.8) | 41 (9.3) |
| Birth order, N (%) | | | |
| 1st | 196 (57.0) | 274 (57.4) | 258 (58.6) |
| 2$^{nd}$ or higher | 148 (43.0) | 203 (42.6) | 182 (41.4) |
| Gestational age, weeks, mean (SD) | 38.6 (1.5) | 38.6 (1.6) | 38.6 (1.6) |
| Breastfeeding, N (%) | | | |
| Exclusive breastfeeding | 99 (29.1) | 143 (30.0) | 133 (30.2) |
| Mixed feeding | 230 (67.6) | 32 (67.1) | 296 (67.3) |
| Formula feeding | 11 (3.2) | 1 (2.7) | 11 (2.5) |
| Current ETS, yes, N (%) | 88 (25.6) | 118 (24.7) | 44 (10.0) |
| Dietary contribution of ultra-processed foods to total energy intake (%) | 23.3 (0.1) | 23.6 (0.1) | 24.4 (0.1) |
| SCQ total score, mean (SD) | 4.3 (3.0) | 3.6 (2.7) | 3.4 (3.1) |
| Child's IQ, mean (SD) | 110.3 (12.6) | 109.5 (12.8) | 109.6 (12.9) |
| Phthalate metabolite levels, μg/g creatinine, GM (GSD) | | | |
| MEHHP | 70.2 (2.1) | 57.3 (1.8) | 31.8 (2.0) |
| MEOHP | 55.4 (1.9) | 39.1 (1.8) | 22.3 (2.0) |
| MnBP | 82.4 (1.9) | 71.4 (1.7) | 53.3 (2.1) |
| MECPP | 97.9 (2.3) | 77.0 (1.8) | 46.1 (1.9) |
| MBzP | 8.5 (2.6) | 5.0 (2.8) | 2.4 (3.9) |

Abbreviations: SD, standard deviation; BMI, body mass index; ETS, environmental tobacco smoke; SCQ, social communication questionnaire; IQ, intelligence quotient; GM, geometric mean; GSD, geometric standard deviation; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MnBP, mono-n-butyl phthalate, MECPP, mono-2-ethyl-5-carboxypentyl phthalate; MBzP, mono-benzyl phthalate.

presented in Table S5 (unit: μg/ g creatinine). All phthalate metabolites at each period showed a detection frequency of greater than 99%, except for MBzP at age 6 and MBzP at age 8 (96.3% and 80.1%, respectively). Childhood phthalate levels were higher compared to those measured during pregnancy, and phthalate levels showed a tendency to decrease across different ages (50$^{th}$ percentile of MEHHP: 16.7 at pregnancy, 69.1 at the age of 4 years, 57.5 at the age of 6 years, and 31.8 at the age of 8 years). Boys had higher levels of all phthalate metabolites at all time-points (Table S6). MEHHP and MEOHP levels were highly correlated at each time point, ranging from 0.9 to 0.97. The correlations of other phthalate metabolite pairs were small to moderate at each time point (maternal levels: 0.52–0.54; childhood levels: 0.10–0.54), whereas correlations between prenatal and childhood phthalate metabolites ranged between −0.13 and 0.08 (Fig S2). Similarly, the ICC for childhood metabolites showed moderately stable levels during the follow-up (0.41 for MEHHP, 0.51 for MEOHP, and 0.47 for MnBP).

### 3.3. Overall association between childhood exposure to phthalate and autistic traits

Table 3 shows the results of the repeated measured analysis. Changes in SCQ scores for all children were positive but insignificant after accounting for the correlation within them (1.2%, 95% confidence intervals [CI]: −4.7%, 7.5%; 1.6%, 95% CI: −4.7%, 8.3%; and 5.9%, 95% CI: −0.3%, 12.5% per increase by 2.7 fold of childhood exposure to MEHHP, MEOHP, and MnBP, respectively). The associations for MEHHP and MEOHP were positive for boys and negative for girls, whereas MnBP showed positive estimates for both sexes. An average association between childhood exposure (ages of 4 to 8 years) to phthalate and SCQ scores at 8 years of age showed smaller estimates than overall associations but similar directions of associations with regard to sex (Table S7).

**Table 3**
Overall and sex-specific associations between phthalate metabolite levels and SCQ measured/assessed at ages of 4, 6, and 8 years and accounted for differences within and between children (n = 547).

| | Total (% change [95% CI]) | Boys (% change [95% CI]) | Girls (% change [95% CI]) |
|---|---|---|---|
| MEHHP | 1.2 (−4.7, 7.5) | 3.0 (−5.2, 12.0) | −1.7 (−9.5, 6.7) |
| MEOHP | 1.6 (−4.7, 8.3) | 3.4 (−5.4, 13.1) | −1.7 (−9.9, 6.7) |
| MnBP | 5.9 (−0.3, 12.5) | 4.4 (−3.2, 12.6) | 6.8 (−2.9, 17.6) |

Adjusted for age, sex, twin, birth order, maternal education level, current ETS, and phthalate levels during pregnancy.
Abbreviation: SCQ, social communication questionnaire; CI, confidence interval; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MnBP, mono-n-butyl phthalate.

### 3.4. Identification of susceptible exposure windows

Table 4 shows associations between exposure (with respect to exposure time) to MEHHP, MEOHP, and MnBP and SCQ scores. A 2.7 fold increase in MEHHP and MEOHP levels during pregnancy was associated with 8.5% (95% CI: 1.9%, 15.5%) and 7.4% (95% CI: 0.3%, 15.0%) increase in SCQ scores at 4 years of age. None of the phthalate metabolite levels were associated with SCQ scores at 6 years of age. A 2.7 fold increase in MEHHP and MEOHP levels at 4 years of age was associated with increased SCQ scores at 8 years (9.9% change [95% CI: 1.8%, 18.6%]; 12.9% change [95% CI: 3.6%, 23.1%], respectively). Further, a 2.7 fold increase in MEHHP, MEOHP, and MnBP levels at the age of 8 years was associated with increased SCQ scores at age 8 (9.6% change [95% CI: 1.3%, 18.6%]; 12.3% change [95% CI: 3.8%, 21.5%]; 8.9% [95% CI: 1.2%, 17.1%]; respectively).

The sex-specific associations between exposure to MEHHP and MEOHP and SCQ showed greater estimates among boys compared to girls. MEHHP at pregnancy was significantly associated with SCQ at 4 and 6 years of age in boys but not among girls (8.9% change [95% CI: 0.8%, 17.7%] for boys versus 3.8% change [95% CI: −6.9%, 15.8%] for girls at the age of 4 years; 8.1% change [95% CI: 0.7%, 16.1%] for boys versus −4.8% change [95% CI: −13.0%, 4.2%] for girls at the age of 6 years) (Table 4 and Fig. 2). Exposure to MEHHP at the age of 4 years was significantly associated with SCQ scores at the age of 8 years for boys but not for girls (17.1% change [95% CI: 5.2%, 30.4%] for boys versus 2.7% change [95% CI: −7.4%, 14.0%] for girls). Similar sex-specific associations were observed for MEOHP; however, MnBP showed mixed findings (Table 4 and Fig S3).

In the logistic regression analyses, there were no phthalate metabolites that showed significant associations with subthreshold ASD risk (data not shown).

### 3.5. Sensitivity analyses

Additional adjustment for childhood or maternal IQ, breastfeeding, SES, or percent of total energy intake from ultra-processed foods did not change the associations between exposure to phthalate metabolites over time (Table S8) or exposure window-specific phthalate metabolites and SCQ scores (Fig S4).

Repeated measurements of MECPP and MBzP were not significantly associated with SCQ scores over time (Table S9). Prenatal exposures to MECPP and MBzP showed different directions of association with SCQ

**Table 4**
Associations between exposure to phthalates and SCQ scores by exposure windows.

| Age of SCQ assessment | Exposure windows | Total[a] (% change [95% CI]) | | | Boys[b] (% change [95% CI]) | | | Girls[b] (% change [95% CI]) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | MEHHP | MEOHP | MnBP | MEHHP | MEOHP | MnBP | MEHHP | MEOHP | MnBP |
| Age 4 (n = 344) | Pregnancy | **8.5 (1.9, 15.5)** | **7.4 (0.3, 15.0)** | 1.6 (−5.3, 9.0) | **8.9 (0.8, 17.7)** | 8.1 (−0.8, 17.8) | 2.8 (−6.0, 12.5) | 3.8 (−6.9, 15.8) | 2.8 (−8.6, 15.6) | −2.3 (−12.9, 9.5) |
| | Age: 4 years | −0.1 (−7.0, 7.3) | −0.1 (−8.1, 8.5) | 5.4 (−2.4, 13.9) | 3.7 (−5.8, 14.2) | 4.0 (−6.5, 15.6) | 9.9 (−1.0, 22.0) | −5.9 (−15.0, 4.2) | −7.9 (−19.2, 4.9) | 0.1 (−10.7, 12.2) |
| Age 6 (n = 477) | Pregnancy | 3.3 (−2.3, 9.3) | 0 (−6.2, 6.7) | −2.7 (−8.6, 3.5) | **8.1 (0.7, 16.1)** | 5.2 (−3.0, 14.0) | 0.4 (−7.2, 8.7) | −4.8 (−13.0, 4.2) | −9.4 (−18.7, 1.0) | −7.2 (−16.2, 2.7) |
| | Age: 4 years | −3.9 (−10.2, 2.7) | −2.2 (−9.8, 5.9) | −2.3 (−9.4, 5.3) | 0.1 (−9.2, 10.4) | 1.0 (−9.5, 12.8) | 1.9 (−8.3, 13.1) | −7.6 (−15.6, 1.0) | −6.4 (−16.8, 5.3) | −6.9 (−16.3, 3.5) |
| | Age: 6 years | −5.5 (−13.3, 2.9) | −5.3 (−12.9, 2.9) | 5.9 (−3.5, 16.2) | −3.0 (−13.3, 8.7) | −1.9 (−12.1, 9.4) | 6.3 (−5.7, 19.8) | −9.8 (−21.1, 3.2) | −10.3 (−21.3, 2.2) | 7.8 (−7.1, 25.1) |
| Age 8 (n = 440) | Pregnancy | −1.7 (−7.4, 4.4) | −2.8 (−9.3, 4.2) | −6.3 (−12.5, 0.3) | 1.0 (−6.5, 9.1) | 0 (−8.2, 9.0) | −2.2 (−10.1, 6.5) | −7.0 (−15.6, 2.3) | −9.4 (−19.7, 2.2) | **−13.2 (−22.6, −2.6)** |
| | Age: 4 years | **9.9 (1.8, 18.6)** | **12.9 (3.6, 23.1)** | 6.0 (−2.1, 14.9) | **17.1 (5.2, 30.4)** | **19.6 (6.2, 34.6)** | **14.8 (2.9, 28.0)** | 2.7 (−7.4, 14.0) | 5.8 (−6.8, 20.0) | −2.5 (−13.1, 9.2) |
| | Age: 6 years | 0.2 (−8.8, 10.0) | 0.5 (−8.4, 10.2) | 5.8 (−4.4, 17.1) | 1.5 (−10.1, 14.7) | 1.5 (−10.0, 14.5) | 0.2 (−11.8, 13.9) | 0.3 (−13.6, 16.5) | 1.1 (−12.8, 17.2) | 18.3 (−0.1, 40.0) |
| | Age: 8 years | **9.6 (1.3, 18.6)** | **12.3 (3.8, 21.5)** | **8.9 (1.2, 17.1)** | 8.3 (−2.2, 19.8) | 9.2 (−1.9, 21.7) | 4.7 (−5.2, 15.6) | 7.3 (−5.5, 21.7) | 11.0 (−1.5, 25.1) | **11.9 (0.4, 24.8)** |

[a]Adjusted for child's age, sex, twin, birth order, and phthalate levels at age of SCQ assessment for phthalates measured during pregnancy.
Adjusted for child's age, sex, twin, birth order, maternal education level, current ETS, and phthalate levels at time of SCQ assessment (or phthalate measured at pregnancy) for phthalates measured during childhood.
[b]Adjusted for child's age, twin, birth order, and phthalate levels at age of SCQ assessment for phthalates measured during pregnancy.
Adjusted for child's age, twin, birth order, maternal education level, current ETS, and phthalate levels at time of SCQ assessment (or phthalate measured at pregnancy) for phthalates measured during childhood.
Abbreviations: SCQ, social communication questionnaire; CI, confidence interval; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MnBP, mono-n-butyl phthalate; ETS, environmental tobacco smoke.
Significant results shown in bold.

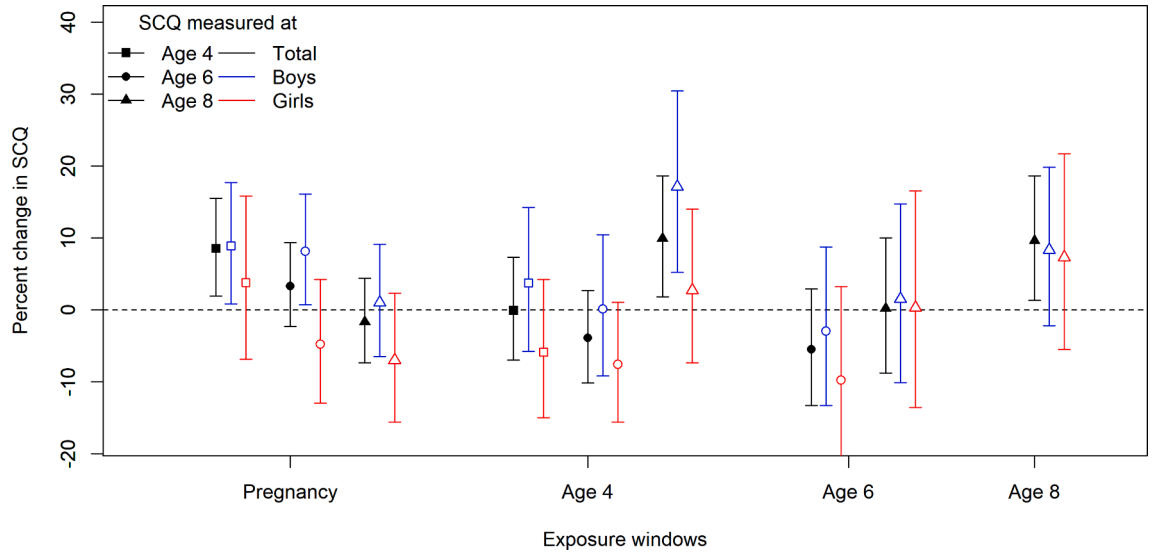


**Fig. 2. Associations between exposure to MEHHP and SCQ measured at ages of 4 (n = 344), 6 (n = 477) and 8 (n = 440) years.** Adjusted for child's age, sex (for total children), twin, birth order, and phthalate levels at age of SCQ assessment for phthalates measured during pregnancy. Adjusted for child's age, sex (for total children), twin, birth order, maternal education level, current ETS, and phthalate levels at time of SCQ assessment (or phthalate measured at pregnancy) for phthalates measured during childhood. The associations of exposures to phthalates with SCQ measured at ages of 4 (square), 6 (circle), and 8 (triangle) years were expressed with 95% confidence intervals in vertical lines. Abbreviations: SCQ, social communication questionnaire; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate.

scores at the age of 4 years (positive and negative, respectively), but the associations were not statistically significant. Autistic traits at the age of 8 years were positively and negatively associated with exposure to MECPP and MBzP, respectively, at the age of 4 years: 11.7% change (95% CI: 1.8%, 22.5%) and −3.0% change (95% CI: −9.4%, 3.8%), respectively (Table S10). Boys showed greater estimates for the association between MECPP exposure and SCQ scores at most ages compared to girls (Table S10).

## 4. Discussion

To our knowledge, this is the first study that investigated the association between exposure to phthalates since pregnancy and autistic traits at multiple time points. Overall childhood exposure to phthalates was not linked to SCQ scores. However, prenatal exposure to MEHHP and MEOHP showed positive association with autistic traits at the age of 4 years, whereas childhood exposure (ages of 4 and 8 years) influenced autistic traits in school-aged children (8 years of age). Moreover, boys showed a stronger association with MEHHP and MEOHPs than girls. MnBP levels at the age of 8 years showed a significant association with SCQ scores at that age, but sex-specific associations were not as evident as that of DEHP metabolites.

Our results were consistent with those of recent studies investigating susceptible exposure windows of environmental toxins related to neurocognitive development. A study on 253 mother–child pairs identified ages of 2–3 years to be most susceptible to the impact of phthalates on IQ development (Li et al. 2019). In the present study, prenatal exposures were related to autistic traits at the age of 4 years, while phthalate exposures at ages of 4 and 8 years were related to increased autistic traits at the age of 8 years (Fig. 3). Our findings may indicate that children's autistic traits are influenced by multiple exposure periods. This may be plausible when reflecting on the dynamic neurodevelopment process that extends from the fetal period to adolescence and early adulthood. Neuroplasticity is a complex process that is heightened during time-sensitive periods of pre- and postnatal brain development (Ismail et al. 2017) during which the brain is most amenable to change (Meredith 2015). Neurogenesis is most prominent during early fetal development, whereas synaptogenesis starts from 27 weeks of gestation and intensifies over the first 2 years of life (Johnston et al. 2009). Synaptic pruning occurs rapidly from the age of 2 years to 10 years (Huttenlocher and Dabholkar 1997). The most susceptible windows of phthalate exposure related to autistic traits were somewhat similar to the critical periods of neurodevelopment.

Environmental chemicals such as phthalate can alter DNA methylation patterns in the developing brain and confer risks to ASD in genetically susceptible individuals (Keil and Lein 2016; Nadeem et al. 2021). The results found in our study can be explained by the methylation process. First, the prenatal period and age 4 were found to be susceptible periods to phthalate exposure. A recent longitudinal genome-wide methylation study that analyzed 783,659 CpG sites found that the methylation status in 110,726 sites changed during the first 5 years after birth, while only 460 CpG sites changed during ages 5–10. (Acevedo et al. 2015; Perez et al. 2019). We can speculate that the methylome would be more prone to environmentally toxic materials in the stages of life when changes are more dynamic (Choi et al. 2020). Second, prenatal exposure affected autistic traits at age 4, and exposure at age 4 affected autistic traits at age 8. Previous studies have reported that early-life methylation changes can affect psychiatric outcomes in later childhood (Walton et al. 2017; Cecil et al 2016). Changes in epigenetic patterns at early-stages of life may trigger downstream developmental consequences resulting in enduring individual differences in brain structure, without the epigenetic signature being maintained over time (Numata et al. 2012). Third, the effect of prenatal exposure disappeared at ages 6 and 8. The influence of prenatal phthalates on DNA methylation may have been mitigated by other biological and sociological factors that determine DNA methylation (Choi et al. 2020).

SCQ scores at age 6 were not associated with any phthalate metabolites exposure. Age 6 is a period of rapid change; as children enter elementary school and spend less time at home, so this may have affected the perception of recent autistic traits by parents. Assessment of autistic traits by teachers may help the improve identification of autistic traits. The cross-sectional effects of phthalate on autistic traits at ages 4 and 8 are in line with previous cross-sectional studies in ASD patients (Kardas et al 2016; Stein et al 2013; Testa et al 2012). The cross-sectional effect may also be due to bias from reverse causation, indicating that children with autistic traits may have elevated phthalate levels due to restricted choices in diet or repetitive behaviors (Sharp et al. 2013).

Autistic traits have been suggested to be relatively stable throughout childhood (Haraguchi et al. 2019); however, others argued that symptoms of autistic traits could be varied throughout childhood (Mandy et al. 2018; Whitehouse et al. 2011). Previous studies have reported that although approximately 70% (ranging from 50 to 90%) of the variance in autistic traits across ages could be accounted for genetic factors, and the remaining variance can be primarily explained by non-shared environmental factors, including dietary intake, smoking, toxin exposure and infection, indicating a modest-to-moderate influence of environment factors (Holmboe et al. 2014; Ronald and Hoekstra, 2011). We speculate that prenatal phthalate exposure may be related to symptom manifestation (around age 4), but postnatal phthalate exposure may be related to symptom variance throughout childhood.

The associations between various exposure windows of DEHP and autistic traits were greater among boys compared to girls in our study. These results were consistent with a recent study on 510 Canadian children, which reported that doubling gestational MnBP concentrations was associated with a 1.0 point increase in Social Responsiveness Scale (SRS) scores in boys aged 3–4 years (Oulhote et al. 2020). However, sex-specific associations have not been consistent; a study by Miodovnik and colleagues did not find any sex-specific associations between phthalate

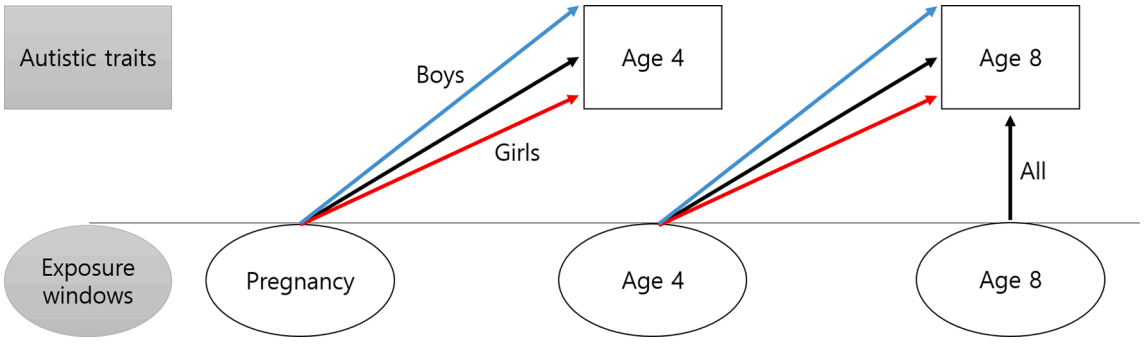


**Fig. 3. Summary of associations between phthalates (MEHHP and MEOHP) and autistic traits across multiple exposure windows.** Abbreviations: MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate.

levels and social development in a cohort of 137 children aged 7–9 years (Miodovnik et al. 2011). A recent study on early pregnancy phthalate mixtures and SRS scores at ages of 4–5 years showed that the association may be dependent on sex. For example, monoethyl phthalate (MEP) and mono (3-carboxypropyl) phthalate (MCPP) showed high mixture weights in girls, whereas MBzP and MECPP were the most contributing in boys (Day et al. 2021). Differences in sample sizes, phthalate measurement methods, and tools for assessing autistic traits make a direct comparison of these study results difficult. Reduction of testosterone production by phthalates in the male fetus is a potential mechanism explaining the sex-specific associations (Howdeshell et al. 2008). Influence of sex hormones on early fetal brain development is believed to be an important early biological risk factor of ASD (Baron-Cohen et al. 2015). Further studies investigating the mediating role of testosterone on the association between phthalates and autistic traits are warranted to confirm these speculations.

The effect sizes of phthalate were small in magnitude. For example, a 2.7 fold increase in MEHHP during pregnancy was associated with an 8.5% increase in SCQ score of age 4, which was equivalent to 0.37 points (mean score 4.3 points). Other studies using SRS scores reported small effect sizes as well; Oulhote et al. 2000 reported that doubling in MnBP and MCPP was associated with 0.6 and 0.5, respectively, increases in total SRS T-scores (1.3% and 1.1% of a mean score of 45.3). Miodovnik et al. 2011 found that a 2.7-fold increase in MEP is associated with 1.4 points increase in SRS T-scores (2.6% of a mean score of 52.9). ASD is a complex genetic disorder caused by interaction between numerous genetic and environmental factors. Therefore, the impact of a single factor may be small (Cheroni et al. 2020). The population burden of a risk factor depends on both its effect size and its distribution (or incidence/prevalence) (Bellinger 2012), and as phthalate exposure is pervasive in children, the small impact may contribute to the population burden. However, as this population was generally healthy, with mean SCQ levels much lower than the cutoff, results must be interpreted cautiously.

Although the proportion of children that surpassed the SCQ cutoff score in our cohort was low, autistic traits are dimensional and have been found to show a continuous distribution in the general population (Constantino et al. 2003; Constantino 2011). The conceptualization of ASD as a discrete phenotype is being increasingly questioned (Lyall et al. 2017) and the NIMH Research Domain Criteria (RDoC) initiative encourages researchers to deconstruct diagnostic categories and rather use tools that measure the range of behavioral dimensions (Cuthbert and Insel, 2013). From a clinical perspective, elevated levels of autistic traits have been found to be related to a higher degree of comorbid psychiatric symptoms, such as anxiety (Kanne et al. 2009), depression, suicidality (Chen et al. 2020) and cognitive inflexibility (Albein-Urios et al. 2018). Furthermore, ASD and autistic traits have been reported to share common etiological genetic/environmental factors, and further studies in ASD patients are warranted to investigate whether our present study's results are replicated in clinical settings (Lundström et al. 2012; Robinson et al. 2011).

The relationship between autistic traits and intelligence is closely interrelated and complex. ASD and intellectual disability frequently co-occur, as ASD with comorbid intellectual disability accounts for 31% of ASD patients and an additional 23% of children lie in the borderline range (Alloway 2010). IQ levels have been associated with symptom presentation and level of adaptation in children with ASD (Kenworthy et al. 2010). Studies have suggested that the type of repetitive behaviors in those with ASDs varied with IQ (Richler et al. 2007; Hus et al. 2007), and also that communication deficits were associated with verbal abilities (Joseph et al. 2002; Nishiyama et al. 2009). Several studies have found that parental IQ can affect ASD risk in the offspring; however, the results have been inconsistent (Crespi 2016; Folstein et al. 1999; Schmidt et al. 2008). The degree of maternal intelligence can also alter the sensitivity of the perception of autistic traits. Although the attenuation was negligible after adjusting for child and maternal IQs in the present study (Figure S3), investigating maternal and child IQs on the association of phthalates and autistic traits is warranted in further studies.

We found significant results with regard to DEHP metabolites (MEHHP, MEOHP, and MECPP) that are known as high-molecular-weight phthalates. Three cross-sectional studies have reported increased mono-(2-ethylhexyl) phthalate (MEHP) levels, a phthalate belonging to the DEHP family, in children diagnosed with ASD compared to typically developing children (Kardas et al. 2016; Stein et al. 2013; Testa et al. 2012) aged 7, 10, or 11 years (Table S11). However, the sum of prenatal exposure to DEHP-family metabolites and ASD diagnosis (Shin et al. 2018) or SRS scores at ages of 3–4 years (Braun et al. 2014; Oulhote et al. 2020) showed null associations in three cohort studies. A previous cohort study in the U.S. found associations of only low-molecular weight phthalates (specifically MEP) with autistic traits at ages of 7–9 years (Miodovnik et al. 2011), while other studies showed that autistic traits at younger ages were significantly related with both prenatal exposure to low- and high-molecular weight phthalates, such as MnBP and MBzP (Day et al. 2021; Oulhote et al. 2020). MnBP and MBzP at age 4 were associated with autistic traits at age 4, and MBzP at age 8 was associated with SCQ scores at age 8. However, these relationships were cross-sectional and MBzP at age 6 showed a protective effect in regard to autistic traits at age 8. Several factors may have caused the discrepancy in study results, including exposure misclassification, period of heightened susceptibility, sex-specific effects, and the effects of phthalate mixtures.

Urine creatinine is affected by age, sex, muscle mass, body mass index, dietary intake, physical activity and comorbidities (Barr et al. 2005). The creatinine-adjusted phthalate metabolite levels tend to decrease with increased age, and the variation in phthalate exposure according to age may be caused by physiological factors (kidney function, body weight, and muscle mass) and behavioral factors (dietary intake and use of personal care products) (Watkins et al. 2014). Therefore, children with low body weight have lower urinary creatinine concentrations and thus have increased creatinine-adjusted urine concentrations of a metabolite compared with that of children with larger body weight (Barr et al. 2005). Cautious interpretation of creatinine-adjusted phthalates is warranted in young children with rapidly-changing body weight (Mian and Schwartz 2017).

The strengths of this study include its longitudinal prospective study design investigating phthalate exposure from the prenatal period to childhood (ages of 4, 6, and 8 years), whereas previous studies have only assessed phthalate exposure either during pregnancy or childhood (Braun et al. 2014; Day et al. 2021; Miodovnik et al. 2011; Oulhote et al. 2020; Shin et al. 2018; Stein et al. 2013; Testa et al. 2012). Although the fetal period has been considered as the most important period of brain development (Modabbernia et al. 2017), repeated metabolite assessments enabled us to compare the effects of prenatal and postnatal exposure, and also to identify susceptible exposure windows at a period critical for neurodevelopment. Sex-specific associations provided insights into the mechanism underlying phthalate exposure and autistic traits association. We were able to adjust for potential covariates related to exposure and outcome, including both maternal and childhood characteristics (e.g., IQ).

There were several limitations to this study. First, the autistic traits were evaluated using a questionnaire. Although the SCQ has high validity and reliability (Corsello et al. 2007), it is primarily used as a screening tool for ASD. Further, a semi-structured interview may better quantify autistic traits and confirm ASD diagnosis. Our study population consisted of community-based children, and the results of this study may differ in a clinical sample of ASD. Phthalate metabolites were measured once at each time period (mid-term pregnancy, and ages of 4, 6, and 8 years); however, phthalate levels may not represent the actual phthalate exposure at specific time-points due to its short half-life of 12–48 h (Hoppin et al. 2002). Moreover, there may be substantial within-day variability in a single participant according to sampling time

(Bastiaensen et al. 2020). Results of the present study should be interpreted cautiously due to the usage of a spot urine sample at each time point; pooled measurements within-subject are desirable for future studies (Oulhote et al. 2020). Within-subject pools have been reported to show less temporal variability and higher reproducibility compared to spot and first-morning voids (Heffernan et al. 2014; Perrier et al. 2016; Shin et al. 2019). Although there are more than 16 phthalates that are quantifiable in urine, only three types of phthalate metabolites were included in the main analyses, and two other metabolites were only included in additional analyses due to their small sample size (MECPP and MBzP), limiting the investigation of potential effects of other phthalates on child autistic traits. Our cohort is overrepresented by mothers with high SES (77.4 % had a monthly family income of ≥$3,500) and high intellectual abilities (mean maternal IQ 116.6 ± 11.5). Therefore, the results of our cohort may not be generalizable to populations with low SES or low cognitive abilities. As multiple testing may have inflated the statistical significance of our results, our findings are suggestive and must be interpreted cautiously. We were unable to account for postnatal factors that have been known to affect brain development, including exposure to chronic stress or novel positive experiences (Tooley et al. 2021). We also did not have any information on the dietary intake of the mothers during pregnancy. Finally, although we controlled for relevant covariates, the results could be confounded by other endocrine-disrupting chemicals (Li et al., 2020) or intake of prenatal vitamins or folic acid (Oulhote et al. 2020).

## 5. Conclusions

We examined 547 children's exposure to phthalates in-utero till the age of 8 years and linked it with their social impairment in a birth cohort in South Korea. The study suggests that phthalate exposure during mid-term pregnancy is related to autistic traits during early childhood, whereas that during childhood is associated more with autistic traits in school-age children than in-utero exposure. It was also suggested that the associations differ with regard to sex. The findings emphasize the importance of reducing phthalate exposure during both pregnancy and early childhood to promote normal social development in children.

## CRediT authorship contribution statement

**Johanna Inhyang Kim:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing - review & editing. **Jung Lee:** Conceptualization, Methodology. **Kyung-Shin Lee:** Data curation, Methodology, Software. **Young Ah Lee:** Conceptualization, Data curation. **Choong Ho Shin:** Conceptualization, Data curation. **Yun-Chul Hong:** Funding acquisition, Writing - review & editing. **Bung-Nyun Kim:** Conceptualization, Supervision, Writing - review & editing. **Youn-Hee Lim:** Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing - review & editing, Supervision.

## 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.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106775.

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