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Neurodevelopment for the first three years following prenatal mobile phone use, radio frequency radiation and lead exposure

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ABSTRACT

Background: Studies examining prenatal exposure to mobile phone use and its effect on child neurodevelopment show different results, according to child's developmental stages.
Objectives: To examine neurodevelopment in children up to 36 months of age, following prenatal mobile phone use and radiofrequency radiation (RFR) exposure, in relation to prenatal lead exposure.
Methods: We analyzed 1198 mother-child pairs from a prospective cohort study (the Mothers and Children's Environmental Health Study). Questionnaires were provided to pregnant women at ≤20 weeks of gestation to assess mobile phone call frequency and duration. A personal exposure meter (PEM) was used to measure RFR exposure for 24 h in 210 pregnant women. Maternal blood lead level (BLL) was measured during pregnancy. Child neurodevelopment was assessed using the Korean version of the Bayley Scales of Infant Development-Revised at 6, 12, 24, and 36 months of age. Logistic regression analysis applied to groups classified by trajectory analysis showing neurodevelopmental patterns over time.
Results: The psychomotor development index (PDI) and the mental development index (MDI) at 6, 12, 24, and 36 months of age were not significantly associated with maternal mobile phone use during pregnancy. However, among children exposed to high maternal BLL *in utero*, there was a significantly increased risk of having a low PDI up to 36 months of age, in relation to an increasing average calling time (p-trend = 0.008). There was also a risk of having decreasing MDI up to 36 months of age, in relation to an increasing average calling time or frequency during pregnancy (p-trend = 0.05 and 0.007 for time and frequency, respectively). There was no significant association between child neurodevelopment and prenatal RFR exposure measured by PEM in all subjects or in groups stratified by maternal BLL during pregnancy.
Conclusions: We found no association between prenatal exposure to RFR and child neurodevelopment during the first three years of life; however, a potential combined effect of prenatal exposure to lead and mobile phone use was suggested.

1. Introduction

Radio frequency radiation (RFR) exposure is widespread in modern life. Seven billion people, comprising 95% of the global population, live in areas covered by a cellular network (International Telecommunication Union, 2016). However, despite the increasing use of cellular phones across the globe, the health implications of increased RFR exposure are largely unknown.

The developing pediatric brain may be more susceptible to environmental exposure, such as electromagnetic radiation, compared to the

Abbreviations: BSID II, Bayley Scales of Infant Development-Revised; BLL, Blood lead level; GEE, Generalized estimating equation; LOD, Limit of detection; MDI, Mental development index; MOCEH, The Mothers and Children's Environmental Health; PEM, Personal exposure meter; PDI, Psychomotor development index; RFR, Radiofrequency radiation

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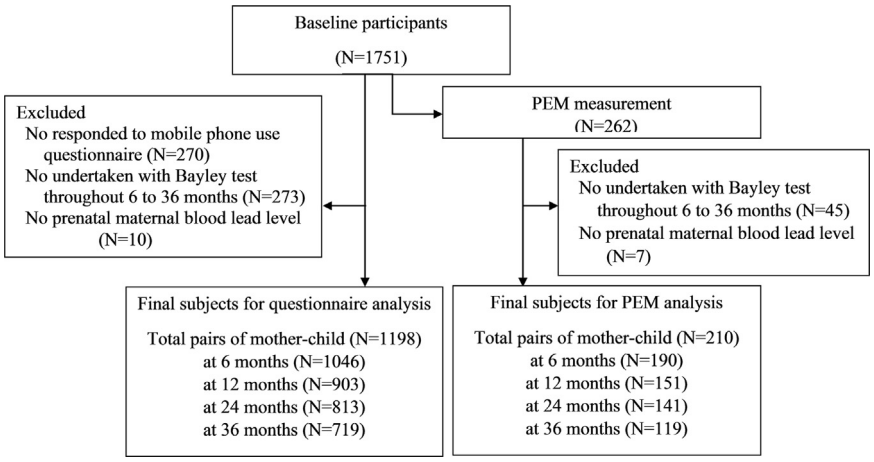

Fig. 1. Selection process of study subjects. PEM: Personal exposure meter, EME Spy100® (Satimo, France).

adult brain (Bellieni and Pinto, 2012). Research derived from animal studies has highlighted positive effects of prenatal RFR exposure in the behavior of animal offspring (Aldad et al., 2012; Haghani et al., 2013; Zhang et al., 2015), although a study reported no adverse effects (Shirai et al., 2017). Epidemiological studies have shown different results regarding maternal mobile phone use during pregnancy and subsequent child neurodevelopment: null findings in earlier ages (Vrijheid et al., 2010; Divan et al., 2011; Guxens et al., 2013) but positive association in later ages (Divan et al., 2008, 2012; Sudan et al., 2016). With the exception of one study, which used a prospective questionnaire (Vrijheid et al., 2010), all studies used questionnaires to retrospectively collect information regarding mobile phone use during pregnancy, which could lead to reporting bias.

On the other hand, lead is a common and environmental neurotoxicant among children (Liu et al., 2014). Even low levels of lead exposure during pregnancy are associated with adverse neurodevelopment outcomes in children (Jedrychowski et al., 2009; Kim et al., 2013; Liu et al., 2014; Shah-Kulkarni et al., 2016; Vigeh et al., 2014). Furthermore, a study showing that child mobile phone use increases the risk of attention deficit hyperactivity disorder (ADHD) in children, particularly among those with high blood lead levels (BLL), suggests an effect modification of blood lead level in association with RFR exposure due to mobile phone use and child neurodevelopment (Byun et al., 2013). However, a possible interaction effect of prenatal RFR exposure and lead on neurodevelopment has not been examined.

Therefore, this study examined the association between prenatal exposure to RFR and child neurodevelopment during the first three years of life in a prospective birth cohort, with consideration of a potential modifying effect arising from prenatal lead exposure.

2. Methods

2.1. Study subjects

The Mothers and Children's Environmental Health (MOCEH) study is a multi-center prospective cohort study that has been conducted in Korea since 2006. Participants were enrolled at ≤20 weeks gestation and the association between prenatal environmental exposure and children's health was evaluated (Kim et al., 2009). The study protocol was approved by the Institutional Review Boards at Ewha Woman's University Hospital in Seoul (a metropolitan city), at Dankook University Hospital in Cheonan (a medium-sized city), and at Ulsan University Hospital in the industrial city of Ulsan. Written informed consent was obtained from each participant prior to enrollment in the study.

Of the 1751 pregnant women enrolled in the MOCEH study, 270 did not respond to the questionnaire concerning mobile phone use, 273 children did not take the neurodevelopment test, and 10 did not provide blood samples for measurement of BLL during pregnancy. Consequently, these mother-infant pairs were excluded from this study. The remaining 1198 mother-infant pairs were included for analysis (Fig. 1).

2.2. Mobile phone questionnaire data

The questionnaire solicited responses regarding average calling frequency (≤2, 3–5, and ≥6 times/day) and average calling time (< 3, 3–10, 10–30, and ≥30 min/day) during pregnancy. Heavy mobile phone use was defined as making ≥6 mobile phone calls per day or using a mobile phone ≥30 min per day. Several months of call data obtained by participants in the present study from their respective telecommunication companies showed moderate-to-high correlations with the questionnaire information about mobile phone use (correlation coefficient, 0.50–0.60) (Choi et al., 2016).

2.3. Twenty-four-hour personal exposure meter monitoring

The EME Spy100® personal exposure meter (PEM) (SATIMO, 2010) was used to measure RFR in 262 study participants who volunteered to be monitored from November 2007 to August 2010. Of the 262 participants, 45 had children who did not undergo a child neurodevelopment assessment, and 7 did not provide blood samples for measurement of BLL during pregnancy; data from these participants was subsequently excluded. Data from 210 subjects were included in the analysis (Fig. 1).

The exposure meter detects 10 different bands of frequency ranging from 88 MHz to 2.17 GHz, such as FM, TV7, TETRA, TV47, uplink and downlink of CDMA, uplink and downlink of PCS, and uplink and downlink of IMT-2000, with electric field strength ranging from 0.05 to 5.0 V/m. Differentiating between uplink and downlink is useful not only for assessment of frequencies contributed by each transmitter, but also for avoiding corruption of the results by phones emitting frequencies close to the dosimeter (SATIMO, 2010). The exposure level was recorded every 15 s for 24 h (5760 measurements in total). For each individual, we calculated the arithmetic mean value for each frequency band. To allow measurements below the limit of detection (LOD) of 0.05 V/m, arithmetic mean values were calculated using the Kaplan-Meier method considering left and right censored data, with LOD/2 used in cases below the LOD (Helsel, 2005). The total exposure index of each subject was calculated as the sum of the square of the arithmetic mean value for each frequency band divided by the guidance level (Korean Ministry of Science, 2013). The exposure index for mobile communication calculated using the same formula with the total exposure index for specific bands of frequency, such as CDMA, PCS,

Table 1
Study subjects' characteristics and distribution of prenatal mobile phone use and neurodevelopment index.

	Subjects	Heavy user[a]	
	N	N (%)	p
< Maternal characteristics >			
All	1198	418 (34.9)	
Year of enrollment			0.007
2006	260	70 (26.9)	
2007	364	125 (34.3)	
2008	245	99 (40.4)	
2009	218	75 (34.4)	
2010	111	49 (44.1)	
Area			0.03
Cheonan	351	118 (33.6)	
Seoul	363	141 (38.8)	
Ulsan	484	159 (32.9)	
Age (years)			0.07
< 30	526	201 (38.2)	
30–34	509	160 (31.4)	
≥35	163	57 (35.0)	
Unknown	–	–	
Education (years)			0.75
≤12 years	318	108 (34.0)	
> 12 years	865	306 (35.4)	
Unknown	15	4 (26.7)	
Household income (10^3 KRW/month)			0.04
< 2000	300	92 (30.7)	
2000–3000	409	136 (33.3)	
≥3000	459	180 (39.2)	
Unknown	30	10 (33.3)	
Occupation			< 0.0001
No	703	197 (28.0)	
Yes	415	193 (46.5)	
Unknown	80	28 (35.0)	
Maternal IQ			0.41
< 100	53	14 (26.4)	
≥100	516	172 (33.3)	
Unknown	629	232 (36.9)	
Urinary cotinine level[b]			0.27
T1	396	125 (31.6)	
T2	396	142 (35.9)	
T3	387	142 (36.7)	
Unknown	19	9 (47.4)	
Prenatal maternal blood lead level[c]			0.63
< 75%	902	312 (34.6)	
≥75%	296	106 (35.8)	
Frequency of using headset			0.06
N/A[d]	8	3 (37.5)	
Always or almost	29	12 (41.4)	
Sometimes	181	77 (42.5)	
Never	949	314 (33.1)	
Unknown	31	12 (38.7)	
< Children's characteristics >			
Sex			0.18
Male	624	204 (32.7)	
Female	574	214 (37.3)	
Gestational age (weeks)			0.99
< 36	49	17 (34.7)	
≥37	1118	387 (34.6)	
Unknown	31	14 (45.2)	
Birth order			0.006
1st	510	200 (39.2)	
2nd or more	532	161 (30.3)	
Unknown	156	57 (36.5)	

MDI: Mental development index, PDI: Psychomotor development index.
P-value estimated using chi-squared test, Kruskal-Wallis test, or Wilcoxon rank sum test.
[a] Heavy user defined as persons using mobile phone with calling frequency more than six times per day or calling time more than 30 min per day.
[b] Tertiles of maternal urinary cotinine levels (ng/mL): T1[0.5,1.3], T2[1.3,2.9], and T3[≥2.9].
[c] The cut-off point of high maternal blood lead level (75%): 1.69 μg/dL.
[d] N/A: not applicable; mobile phone non-user.

and IMT-2000. Based on a previous study (Choi et al., 2016), the operator's log data regarding mobile phone use of the participants showed moderate correlations with the calculated exposure index, as measured by the PEM (correlation coefficient: 0.4–0.5) and with the mobile phone use questionnaire (0.5–0.6). However, a poor correlation between the results obtained from the PEM and the questionnaire (0.05–0.06) was found because the PEM measured not only RFR exposure from mobile phone use, but also various sources of RFR exposure, including TV, radio, working on the internet, and mobile phone base stations.

2.4. Blood lead level

BLL was measured by graphite furnace atomic absorption spectrometry with Zeeman background correction (Perkin Elmer AAS800, Perkin Elmer). Samples were analyzed by a commercial laboratory certified by the Korean Ministry of Health and Welfare. For internal quality assurance and control, commercial reference materials were used (Lyphochek® Whole Blood Metals Control; Bio-Rad, Hercules, CA, USA) and the laboratory passed the German External Quality Assessment Scheme of Friedrich-Alexander University (Germany), both in the occupational and environmental sample ranges, as well as the Quality Assurance Program operated by the Korea Occupational Safety and Health Agency. The LOD for BLL using this method was 2.07 μg/L. None of the blood samples had a BLL below the LOD. A previous study of MOCEH participants reported an association between child neurodevelopment and maternal BLL during late pregnancy (Shah-Kulkarni et al., 2016); therefore, this study used the maternal BLL during late pregnancy for the analysis. If subjects did not have a BLL obtained during late pregnancy, we used the BLL obtained in early pregnancy (n = 235). BLLs were dichotomized, according to the 75th percentile (1.69 and 1.61 μg/dL), for analysis of mobile phone use and RFR by PEM, respectively.

2.5. Child neurodevelopment

The Bayley Scales of Infant Development-Revised (BSID-II) tool is widely used by clinicians for assessing neurodevelopment of children up to 3 years of age (Bayley, 1993). In this study, child neurodevelopment assessed using the Korean version of BSID-II at 6, 12, 24, and 36 months of age. Assessments were performed by trained examiners and lasted 30–45 min (Park and Cho, 2006). The Korean version of the BSID-II was validated by back translation and test–retest stability (Park and Cho, 2006). It is composed of a mental development index (MDI) and a psychomotor development index (PDI) (Bayley, 1993), and produces a composite score that compares the child's developmental performance with the norms for typically developing Korean children of the same age (Park and Cho, 2006). Each test was standardized to produce developmental indices with a mean score of 100 and an SD of 15 (Park and Cho, 2006). Inter-rater reliability (kappa value > 0.8) was confirmed annually through rater training sessions and video monitoring of the examination process. Each measurement was double-checked and confirmed through feedback between the examiners and the central coordinator.

2.6. Confounding factors and covariates

The year of enrollment, center area, and responses to questions concerning maternal age at pregnancy (< 30, 30–34, and ≥35 years), household income (< 2,000, 2000–3,000, and ≥3000 10^3 KRW per month), whether or not the mother is employed, educational level (≤12 or > 12 years), and frequency of headset use (never, sometimes, or often to always) were obtained via a questionnaire at the time of study enrollment. Information on gestational age, sex of the infant, and birth order was obtained from subjects' medical records. The maternal intelligence quotient (IQ) was measured by the Korean version of the

Table 2
Linear relationship between prenatal mobile phone use from the questionnaire and following the child neurodevelopmental index at 6, 12, 24 and 36 months of age.

		6 months (N = 1046)		12 months (N = 903)		24 months (N = 813)		36 months (N = 719)	
	Prenatal mobile phone use	N	β (95% CI)	N	β (95% CI)	N	β (95% CI)	N	β (95% CI)
MDI	Average calling frequency per day								
	≤2[a]	198	−0.87 (−2.82, 1.09)	173	0.23 (−2.54, 3.01)	142	−0.74 (−3.37, 1.89)	137	−0.73 (−3.46, 2.00)
	3–5	500	Ref.	448	Ref.	401	Ref.	346	Ref.
	≥6	320	−0.16 (−1.85, 1.53)	269	−0.33 (−2.75, 2.09)	252	0.54 (−1.65, 2.73)	217	−0.20 (−2.55, 2.16)
	p-trend		*0.61*		*0.72*		*0.37*		*0.73*
	Average calling time (min/day)								
	< 3[a]	167	−1.76 (−3.89, 0.36)	139	0.05 (−3.00, 3.10)	115	−1.52 (−4.42, 1.39)	104	−1.82 (−4.85, 1.21)
	3- < 10	419	Ref.	379	Ref.	349	Ref.	312	Ref.
	10- < 30	307	−0.35 (−2.08, 1.39)	263	0.64 (−1.83, 3.11)	243	−1.08 (−3.32, 1.16)	203	0.67 (−1.76, 3.10)
	≥30	127	0.11 (−2.25, 2.47)	111	−1.62 (−4.97, 1.73)	90	−0.62 (−3.82, 2.58)	83	−0.19 (−3.57, 3.18)
	p-trend		*0.30*		*0.68*		*0.98*		*0.27*
	Heavy user (Yes/No[a])	363/655	0.50 (−1.05, 2.04)	306/584	−0.11 (−2.33, 2.11)	282/513	1.50 (−0.52, 3.51)	245/456	0.59 (−1.55, 2.74)
PDI	Average calling frequency per day								
	≤2[a]	198	−1.08 (−3.45, 1.30)	173	−3.51 (−6.29, −0.73)	142	−0.45 (−2.89, 1.99)	137	−0.24 (−2.77, 2.29)
	3–5	500	Ref.	448	Ref.	401	Ref.	346	Ref.
	≥6	320	−0.97 (−3.02, 1.08)	269	0.59 (−1.84, 3.02)	252	2.47 (0.43, 4.51)	217	0.94 (−1.24, 3.12)
	p-trend		*0.89*		*0.01*		*0.02*		*0.33*
	Average calling time (min/day)								
	< 3[a]	167	−0.82 (−3.40, 1.76)	139	1.35 (−1.71, 4.41)	115	0.03 (−2.68, 2.75)	104	1.13 (−1.68, 3.94)
	3- < 10	419	Ref.	379	Ref.	349	Ref.	312	Ref.
	10- < 30	307	1.39 (−0.71, 3.50)	263	3.60 (1.12, 6.07)	243	1.00 (−1.09, 3.09)	203	0.31 (−1.94, 2.57)
	≥30	127	0.47 (−2.39, 3.33)	111	1.51 (−1.85, 4.87)	90	1.84 (−1.14, 4.83)	83	1.07 (−2.06, 4.20)
	p-trend		*0.18*		*0.14*		*0.20*		*0.91*
	Heavy user (Yes/No[a])	363/655	−0.36 (−2.24, 1.52)	306/584	1.43 (−0.81, 3.66)	282/513	2.63 (0.75, 4.51)	245/456	0.79 (−1.20, 2.78)

MDI; Mental Development Index, PDI; Psychomotor Development Index.
Generalized linear regression model (GLM) performed, adjusted for year of enrollment, area, maternal age, maternal educational level, maternal IQ, household income, maternal occupation, prenatal blood lead level, prenatal urinary cotinine level, infant's sex, birth order, gestational age, and frequency of using a head set.
The category with the largest number of subjects was chosen as the referent.
[a] Heavy user defined as person using mobile phone with calling frequency more than six times per day or calling time more than 30 min per day. Comparison versus non-heavy user including non-user.

Adult Intelligence Scale (K-WAIS) in 572 mothers (47.4%) (Lim et al., 2000) and classified as < 100 (mean) or ≥100. As a biomarker of prenatal secondhand smoke exposure, maternal urinary cotinine levels during late pregnancy were measured by enzyme-linked immunosorbent assay (ELISA).

2.7. Statistical analysis

A chi-square test was performed to evaluate the differences in distribution of heavy mobile phone users according to general characteristics. The Kruskal-Wallis test or Wilcoxon rank-sum test was used to compare distributions of the exposure index according to general maternal characteristics. Regression analysis was performed to evaluate the effects of prenatal exposure to RFR on child neurodevelopment at 6, 12, 24, and 36 months of age adjusted for year of enrollment, center area, maternal age, maternal educational level, maternal IQ, household income, having a maternal occupation, maternal urinary cotinine levels during late pregnancy, infant's sex, birth order, frequency of head set use (only for prenatal mobile phone use), and gestational age. The generalized estimating equation (GEE) was used for repeated measurements of child neurodevelopment from 6 to 36 months of age. Trajectory analysis was performed to classify child neurodevelopment patterns over time, in which a minimum Bayesian Information Criterion (BIC) model was selected (number of latent period = 2), using the lcmm package in the R software (Proust-Lima et al., 2015). Children with low level or decreasing neurodevelopment indices from 6 to 36 months of age were considered as a high-risk group. Odds ratios and 95% confidence intervals for risk groups were estimated using a logistic regression model adjusted for covariates. For a sensitivity analysis of PEM related results, we reanalyzed with GEE and a logistic regression model on the trajectory grouping, after multiple imputation for missing data for PEM among the 1198 subjects. We imputed 10 data sets of RFR exposure index values (total exposure index and mobile communication index) over 50 iterations with the random forest model, using the mice package in the R software (van Buuren and Groothuis-Oudshoorn, 2011). To examine the correlation in the children's neurodevelopment at each time point, we performed a Pearson's correlation analysis. All statistical analyses were performed using R 3.1.2 (R Core Team, 2014) and a p-value ≤ 0.05 was considered significant.

3. Results

Increased mobile phone use (both in calling frequency and calling time) was observed in those less than 30 years of age, enrolled recently in the study, living in Seoul, with higher household income, currently employed, and giving birth to their first child. Maternal education level, maternal IQ, maternal urinary cotinine level, and maternal BLL were not associated with mobile phone use during pregnancy (Table 1). Of the subjects, 30.9% used a mobile phone ≥6 times per day and 12.1% used a mobile phone for ≥30 min per day.

The median (range) PEM measurement in 210 pregnant women was 5.0 $(1.0–122.0)\times10^{-5}$ V/m for the total exposure index, and 2.4 $(0.3–119.3)\times10^{-5}$ V/m for the exposure index of mobile communication, with a skewed distribution to the right (data not shown).

Mobile phone use during pregnancy did not show significant negative linear associations with the child neurodevelopmental index at 6, 12, 24, or 36 months of age. Rather, PDI at 12 and 24 months of age showed positive linear trends with increasing calling frequency per day, and children of heavily mobile phone using mothers had significantly higher PDIs (Table 2). The corresponding unadjusted models showed a similar pattern, although the estimated sizes were somewhat attenuated (Table S1).

Fig. 2 shows the trajectory analysis results of MDI and PDI over time. Results from this analysis show two primary groups differentiated by MDI and PDI. One group maintained a high level, while the other group exhibited decreasing MDIs or maintained low level PDIs (Fig. 2).

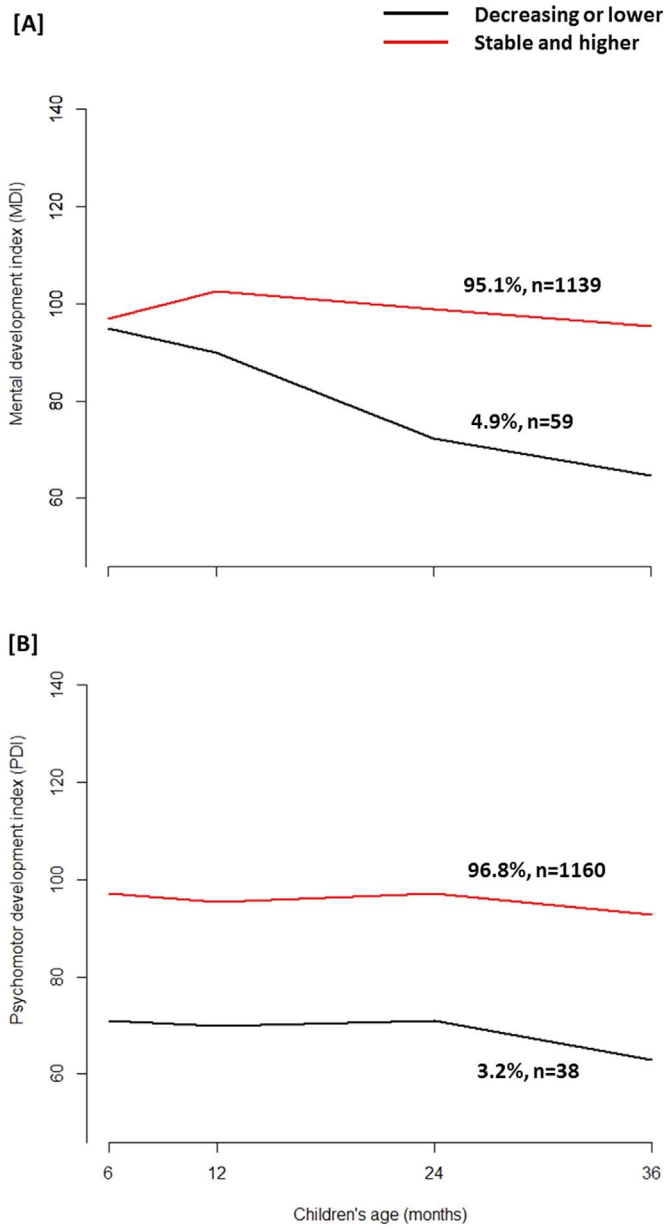

Fig. 2. Trajectory groups in children's neurodevelopment from 6 to 36 months of age.[A] Mental Development Index, [B] Psychomotor Development Index.

The baseline characteristics of the two groups did not differ significantly, with the exception of sex of the child and location (Table S2).

Among children of mothers with higher BLLs during pregnancy, more prenatal maternal mobile phone use was more likely to be associated with having decreasing MDI over time (calling frequency: *p-trend = 0.007*; calling time: *p-trend = 0.05*) or maintaining a low level of PDI over time (calling time: *p-trend = 0.008*). However, children of mothers with lower maternal BLLs during pregnancy showed significantly decreasing patterns of risk, having decreasing MDI over time (*p-trend = 0.01*) or having low PDI over time (*p-trend = 0.04*), in association with an increase of maternal mobile phone use during pregnancy (Table 3). The association between prenatal maternal mobile phone use and the risk of decreasing MDI was modified significantly by prenatal maternal BLL (p-interaction < 0.02). Results from the corresponding unadjusted models (Table S3) and the GEE models using a continuous scale of MDI/PDI scores (Table S4) showed a similar pattern.

MDI and PDI scores at 6, 12, 24, or 36 months of age were not significantly associated with RFR exposure measured by PEM in all children, and in those of mothers with both low and high maternal BLL during pregnancy (Table 4). The corresponding unadjusted models showed a similar pattern (Table S5) and the repeated analysis results in the imputed data for 1198 pairs of subjects did not show significant associations (Table S6). The risk of having a decreasing or low MDI or PDI over time in association with RFR exposure could not be estimated due to the small sample size and the results of imputed data did not show any significant association (Table S7).

4. Discussion

We found no significant association between prenatal exposure to RFR and child neurodevelopment during the first three years of life. However, prenatal maternal lead exposure had a potential modifying effect on the risk of delayed or lower neurodevelopment in association with maternal mobile phone use during pregnancy.

Previous studies on maternal mobile phone use during pregnancy and child cognitive or behavioral problems have shown different results when compared to the developmental stages of children. Adverse effects have been reported in later ages, for example, at 7 years (Divan et al., 2008, 2012), and 11 years (Sudan et al., 2016). However, null findings have reported in earlier ages, for example, at 14 months (Vrijheid et al., 2010), at 6 and 18 months (Divan et al., 2011), and at 5 years (Guxens et al., 2013). The maximum age of children in the present study was 3 years of age, which falls the ages used in previous studies to report null findings. Our findings are consistent in previous studies, and suggests that the effects of prenatal RFR exposure due to maternal mobile phone use may not be apparent before 3 years of age.

In addition, there are differences between studies in term of measurement tools of neurodevelopment and the time of information collection (prospective versus retrospective). A cohort study prospectively collected information on maternal mobile phone use during pregnancy (Vrijheid et al., 2010), and used the BSID II to assess child neurodevelopment. Similar to this study, results from that study showed little difference between users and non-users of mobile phones, and no significant dose-response pattern in neurodevelopment found in children at 14 months of age.

After stratification with prenatal maternal BLL, we found significant contrasting trends between the lower and the higher maternal BLL group; the latter showed a significantly increased risk of having poor or delayed neurodevelopment up to 36 months of age in association with maternal mobile phone use during pregnancy. A previous study reported possible modifying effects of lead exposure in children, although it was not *in utero* exposure, in relation with mobile phone use and the risk of ADHD in children (Byun et al., 2013). In addition, lead is a well-known neurotoxicant in the developing brain (Jedrychowski et al., 2009; Kim et al., 2013; Liu et al., 2014; Vigeh et al., 2014). These studies suggest that prenatal RFR exposure due to maternal mobile phone use and simultaneous exposure to lead have synergistically adverse effects on the neurodevelopment of children. A neuro-protective effect in children of women with low BLL during pregnancy, particularly with regard to calling frequency, may be due to unknown confounding factors, such as the child rearing environment.

However, we found no significant association between prenatal RFR exposure measured by PEM and child neurodevelopment in this study. Although previous studies have reported that RFR exposure may be associated with increased behavioral problems in children and adolescents (Thomas et al., 2010), none of these studies measure the prenatal RFR exposure by PEM, or examine the cognitive or behavioral development in children. The null finding in the present study may be partially due to a lack of statistical power, particularly when considering the non-significant decrease in child neurodevelopment indices among those with high lead exposure *in utero*. The analysis results in the multiple imputed data, to compensate for the small sample size, did not show a significant association. Therefore, if the variable of mobile phone use comprises something more than RFR exposure such as the

Table 3
Association between prenatal mobile phone use and the risk of decreasing or low in child neurodevelopment from 6 to 36 months of age, stratified by prenatal maternal blood lead level.

		All		Maternal blood lead during pregnancy				
				Low (< 75%)		High (≥75%)		*p-interaction*[c]
	Prenatal mobile phone use	Case/Total N.	OR (95% CI)	Case/Total N.	OR (95% CI)	Case/Total N.	OR (95% CI)	
Risk of decreasing MDI[b] (For 6–36 months)	Average calling frequency per day							
	≤2[a]	12/230	0.88 (0.60, 1.31)	12/181	1.17 (0.77, 1.78)	0/49	0 (0, Inf)	
	3–5	33/562	Ref.	25/424	Ref.	8/138	Ref.	
	≥6	13/370	0.73 (0.50, 1.05)	9/274	0.65 (0.42, 0.99)	4/96	0.50 (0.12, 2.08)	
	p-trend		*0.14*		*0.01*		*0.007*	*0.0009*
	Average calling time (min/day)							
	< 3[a]	6/190	0.50 (0.30, 0.83)	6/142	0.71 (0.42, 1.21)	0/48	0 (0, Inf)	
	3- < 10	28/484	Ref.	21/372	Ref.	7/112	Ref.	
	10- < 30	19/345	0.85 (0.60, 1.19)	14/250	0.86 (0.57, 1.28)	5/95	2.11 (0.67, 6.68)	
	≥30	5/145	0.63 (0.37, 1.08)	5/115	0.76 (0.43, 1.34)	0/30	0 (0, Inf)	
	p-trend		*0.86*		*0.48*		*0.05*	*0.02*
	Heavy user[a]	14/418	0.63 (0.44, 0.89)	10/312	0.52 (0.35, 0.78)	4/106	1.78 (0.62, 5.10)	*0.02*
Risk of low PDI[b] (For 6–36 months)	Average calling frequency per day							
	≤2[a]	10/230	1.58 (0.97, 2.56)	9/181	1.92 (1.13, 3.26)	1/49	0.97 (0.53, 1.77)	
	3–5	16/562	Ref.	14/424	Ref.	2/138	Ref.	
	≥6	9/370	0.81 (0.49, 1.33)	7/274	0.80 (0.46, 1.38)	2/96	0.95 (0.58, 1.57)	
	p-trend		*0.06*		*0.04*		*0.54*	*0.37*
	Average calling time (min/day)							
	< 3[a]	3/190	0.47 (0.24, 0.94)	2/142	0.41 (0.19, 0.92)	1/48	0.45 (0.23, 0.89)	
	3- < 10	17/484	Ref.	15/372	Ref.	2/112	Ref.	
	10- < 30	10/345	0.77 (0.49, 1.23)	8/250	0.81 (0.49, 1.35)	2/95	1.10 (0.69, 1.76)	
	≥30	4/145	0.64 (0.32, 1.29)	4/115	0.73 (0.36, 1.48)	0/30	1.56 (0.74, 3.26)	
	p-trend		*0.54*		*0.26*		*0.008*	*0.44*
	Heavy user[a]	10/418	0.72 (0.46, 1.14)	8/312	0.68 (0.41, 1.12)	2/106	0.93 (0.58, 1.47)	*0.47*

MDI; Mental Development Index, PDI; Psychomotor Development Index.
The cut-off point of high and low maternal blood lead level (75%): 1.69 μg/dL.

[a] Heavy user defined as persons using mobile phone with calling frequency more than six times per day or calling time more than 30 min per day. Comparison versus non-heavy user including non-user.

[b] Throughout the 6–36 months of age period, the child neurodevelopmental index was classified into 2 groups, stable and decreasing/low of MDI or PDI score by trajectory analysis (number of latent = 2, minimum BIC model selected). OR and 95% CI were estimated for decreasing/low versus stable group of MDI and PDI using the logistic regression model adjusted for year of enrollment, area, maternal age, maternal educational level, maternal intelligence quotient, household income, maternal occupation, prenatal urinary cotinine level, infant's sex, birth order, gestational age, and frequency of using a head set.

[c] *p value for interaction* estimated using log likelihood ratio test between with and without interaction term (mobile phone use and maternal blood lead) in corresponding logistic regression model. The numbers of children were 46 and 856 of low lead group, 13 and 283 for high lead group, in decreasing and stable group in MDI, respectively, 32 and 870 for low lead group, 6 and 290 for high lead group, in low and stable group in PDI, respectively. The category with the largest number of subjects was chosen as the referent.

rearing environment, future replication studies, alongside for the finding of a modifying effect with lead exposure as investigated in the present study, are needed using larger cohort data.

Multiple biological mechanisms of the effects on the brain of infants exposed to RFR *in utero* have been proposed. First, RFR exposure creates an energy transfer, which increases the permeability of the blood brain barrier (BBB) to macromolecules (Stam, 2010). Even though RFR energy from the mother's mobile phone use or holding a mobile phone near the body would be at a very low level when it reaches the fetal brain (Varsier et al., 2014), the immature fetal BBB may be susceptible. Alongside this, an increased level of lead in cord blood, which is a well-known neuro-toxicant, could transfer to the fetal brain, resulting in adverse neurodevelopmental effects. In addition, lead in maternal blood crosses the blood placenta barrier (BPB) and enters the cord blood (Goyer, 1990). It is also conceivable that more lead crosses the BPB if RFR energy increases the permeability of the BPB as well. However, there have not been studies on RFR effects on the BPB.

Secondly, RFR exposure appears to disrupt the release of melatonin from the pituitary gland, which may affect metabolic and/or sex hormones of the pregnant mother and affect fetal brain development (Hocking, 2009). In the present study, however, we did not observe any significantly different findings between sexes, which does not offer support from this study for a hypothesis of sex hormone involvement (data not shown). Lastly, RFR exposure may also affect fetal stem cells, including future neuronal cells (Bellieni and Pinto, 2012). Although interesting, none of these hypotheses have been confirmed to date (Feychting, 2011).

This study obtained information on mobile phone use from self-administered questionnaires provided to pregnant women upon study enrollment. Consequently, it is likely to be free of recall bias. Furthermore, the correlation coefficient of mobile phone operator log data was 0.5–0.6, indicating moderate to high validity (Choi et al.,

Table 4
Association between RFR exposure measured by the personal exposure meter during pregnancy and child neurodevelopment for 6–36 months of age.

RF exposure index[a] (V/m)	No. of children	MDI		PDI	
		Total exposure β (95% CI)	Mobile communication β (95% CI)	Total exposure β (95% CI)	Mobile communication β (95% CI)
Linear regression models					
6 months	190	1.31 (−0.98, 3.60)	1.67 (−0.13, 3.47)	−0.15 (−3.21, 2.91)	0.26 (−2.17, 2.69)
12 months	151	−0.50 (−3.89, 2.89)	0.18 (−2.47, 2.83)	−2.49 (−6.76, 1.78)	−1.57 (−4.92, 1.78)
24 months	141	−0.51 (−4.08, 3.06)	0.85 (−1.99, 3.69)	−2.78 (−6.09, 0.53)	−1.16 (−3.81, 1.49)
36 months	119	0.46 (−3.64, 4.56)	2.66 (−0.85, 6.17)	−0.51 (−4.21, 3.19)	1.28 (−1.91, 4.47)
Analysis using generalized estimating equation for 6–36 months					
All	210	1.42 (−1.15, 3.99)	1.54 (−0.56, 3.64)	−0.02 (−2.96, 2.92)	−0.20 (−2.65, 2.25)
Maternal blood lead during pregnancy[b]					
Low (< 75%)	157	1.16 (−1.60, 3.92)	1.67 (−0.70, 4.04)	−0.10 (−3.31, 3.11)	−0.29 (−3.11, 2.53)
High (≥75%)	53	−1.35 (−8.41, 5.71)	−3.56 (−8.07, 0.95)	−2.00 (−11.15, 7.15)	−0.61 (−6.25, 5.03)
p-interaction[c]		*0.35*	*0.98*	*0.20*	*0.25*

MDI; Mental Development Index, PDI; Psychomotor Development Index.
The linear regression model or generalized estimating equation were adjusted for year of enrollment, area, maternal age, maternal educational level, maternal intelligence quotient, household income, maternal occupation, prenatal urinary cotinine level, infant's sex, birth order, and gestational age.

[a] RFR exposure index measured by the personal exposure meter (EME Spy100®) and the values were log transformed: Total exposure included measured exposure from the frequency bands of FM, TV7, TETRA, TV47, CDMA, PCS, and IMT-2000. Exposure index for mobile communication included measured exposure from the frequency bands of CDMA, PCS, and IMT-2000.

[b] The cut-off point of high and low maternal blood lead level (75%): 1.61 μg/dL.

[c] *p value for interaction* estimated using log likelihood ratio test between with and without interaction term (RFR exposure index and maternal blood lead level) in the corresponding model.

2016). Unlike previous studies, this study used expert examiners to objectively measure child neurodevelopment rather than use subjective assessments of child development by parental-report (Divan et al., 2008, 2011, 2012; Guxens et al., 2013). This study was also carried out through a period covering 36 months of age, which is important when analyzing a pattern or trend over time. The neurodevelopment of children is variable over time, and this reflects in the correlation results between developing stages (Table S9). Given this, it is reasonable to examine the trajectory of neurodevelopment using multiple-time point measurement values over developmental years as an index of children's neurodevelopment, rather than using one-time point measurement, particularly in earlier ages. Other studies did not consider trends over time, but instead analyzed data at one (Divan et al., 2008, 2012; Guxens et al., 2013; Vrijheid et al., 2010) or two time points (Divan et al., 2011) without considering the repeated measurements.

The distribution between the included and the excluded in the present study differed regarding center area, age, and household income. This may be due to differences in the follow-up rate by area, as well as being due to poor follow-up rates in subjects who were classified within the unknown category for age and household income at enrollment (that is, subjects who did not answer the questions) (Table S8). However, other general characteristics of pregnant women did not differ between those included and excluded from the study. The measurements mostly collected from two areas, Cheonan and Seoul, for the three centers in the PEM analysis; the differences in age and occupation may be due to poor follow-up of the unknown category at enrollment (Table S8). This study had a small sample size, particularly for RFR exposure measurements, which resulted in insufficient statistical power. Therefore, further research warranted in additional large cohort studies.

5. Conclusions

We found no association between prenatal exposure to RFR and child neurodevelopment for the first three years, but a potential combined effect of maternal lead exposure and mobile phone use during pregnancy was suggested.

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Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2017.04.029.

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