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도시지역 녹지 노출과 어린이 인지발달과의 연관성 연구

Exposure to greenness in urban area and cognitive development in children

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서울대학교 대학원

의학과 예방의학 전공

이 경 신

A thesis of Degree of Doctor of Philosophy

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A thesis of Degree of Doctor of Philosophy

Exposure to greenness in urban area and cognitive development in children

by

Kyung-Shin Lee, MPH, M.Sc

A thesis submitted to the Department of Medicine in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Medicine (Preventive Medicine) at Seoul National University College of Medicine

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Approved by Thesis Committee

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Abstract

Exposure to greenness in urban area and cognitive development in children

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Background: It has been reported that residential greenness is definitely linked to the health benefits of children, including improved cognitive ability. A prospective cohort design was conducted on the relationship between the intelligent quotient (IQ) of 6-year-olds in Seoul, South Korea, and surrounding greenness during the pregnancy and postnatal exposure. We also analyzed whether these effects vary depending on the types of natural greenness or built greenness.

Epigenetic change is hypothesized as a regulatory pathway through which greenness exposures influence child development and health. We aimed to investigate the associations between residential surrounding greenness and DNA methylation (DNAm) changes associated with cognitive abilities at age 2. We also assessed the associations between differential methylation and children's IQ at age 6 in a prospective cohort study.

Method: To estimate the associations between residential greenness and children's IQ, we were conducted on 189 mother-child dyads from the Environment and Development of Children Cohort study, who lived in Seoul during the prenatal period and when the child was 6 years old. The surrounding greenness was defined using the data of the Landsat image of the Korean Arirang satellite with buffers within a radius of 100 to 2,000 meters from the participants' residence. We analyzed the two types of greenness including natural and built greenness, separately. Using the Wechsler Intelligence Scale for Children by the Korea Educational Development Institute, the children's IQ was measured.

To investigate the associations between residential surrounding greenness and DNAm changes, which associated with cognitive abilities, we identified cytosine-guanine dinucleotide sites (CpGs) associated with cognitive abilities from epigenome- and genome-wide association studies through a systematic literature review for candidate gene analysis. We estimated the residential surrounding greenness at age 2 using a geographic information system. DNA methylation was analyzed from whole blood using the HumanMethylationEPIC array in 59 children at age 2. We analyzed the association between greenness exposure and DNA methylation at age 2 at the selected CpGs using multivariable linear regression adjusting for mother's age, maternal education level, exposure to environmental tobacco smoke at age 2, child sex, children's body mass index and cell composition. We further investigated the relationship between DNA methylation and children's IQ, including the total IQ and two subsets of IQ.

Result: We found that prenatal exposure to built greenness in 500 m and 1000m buffers was associated with total IQ of children in a full model [Difference in IQ (95% CI): 3.46 (0.68, 6.24) and 3.42 (0.53, 6.31) per interquartile increase in percentages of greenness in buffers]. However, postnatal exposure to built greenness in all buffers was significantly related to the child's total IQ. We also found a stronger association between children's total IQ and built greenness than natural greenness. For epigenetics study, we identified 8,743 CpGs associated with cognitive ability based on the literature review. Among these CpGs, we found that 25 CpGs were significantly associated with greenness exposure at age 2, including cg26269038(Bonferroni-corrected $P \leq 0.002$) located in the body of *SLC6A3*, which encodes a dopamine transporter. DNA methylation at cg26269038 at age 2 was significantly associated with children's performance IQ at age 6.

Conclusion: When six-year-old children live near greenness area, their IQ score tends to increase. The results suggested the health benefits of greening, providing urban planning and public health support to build healthy cities for children and pregnant women.

Exposure to surrounding greenness was associated with cognitive ability-related DNA methylation changes, which was also associated with children's IQ. Further studies are warranted to clarify the epigenetic pathways linking greenness exposure and neurocognitive function.

Keyword: Children, Greenness, Built greenness, Natural greenness, DNA methylation, Intelligence Quotient

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

IQ, Intelligence quotient; NDVI, Normalized difference vegetation index; DNA, DeoxyriboNucleic Acid; EWAS, Epigenome-wide association study; GWAS, Genome-wide association study; GIS, Geographic Information System; NO_2, Nitrogen dioxide; $PM_{2.5}$, Particulate matter 2.5 μm; SES, Socioeconomic status

DNAm, DNA methylation; EDC, Environment and Development of Children; ETS, Environmental tobacco smoke; ANOVA, Analysis of variance; SE, Standard Error; SD, Standard Deviation; IQR, Interquartile range; 95% CI, 95% Confidence Interval; LSMEANS, Least-Squares Means; ND, Not detected; NA, Not analyzed; WISC, Wechsler Intelligence Scale for Children; WAIS-R, Wechsler Adult Intelligence Scale-Revised; CVD, cardiovascular disease; BMI, Body mass index; BMIQ, Beta Mixture Quantile; DAVID, Database for Annotation, Visualization, and Integrated Discovery; SNP, Single nucleotide polymorphisms; UCSC, University of California, Santa Cruz; DRD2, Dopamine Receptor D2.

Chapter 1. Introduction

1.1. Study Background

More than half of the world's population (4.1 billion people) live in urban areas (WHO, 2018). Health risks, including mental illness, are associated with exposure to urban environments characterized by environmental hazards, lack of social support, or inadequate health services (Hystad et al. 2014). Children's mental health is especially important, accounting for a third of the world's population, many mental health conditions begin before adulthood, and appropriate interventions and treatments for children's mental health are cost-effective (Weiss et al. 2020).

Urban greenness is one of the important elements of the urban environment in term of public health (Figure 1). Exposure to greenness in urban areas is estimated to have physiological and psychosocial health benefits in children (Amoly et al. 2014; Casey et al. 2016; Dadvand et al. 2012; Fuertes et al. 2014; Markevych et al. 2014). Urban greenness can contribute to reducing the harmful effects of urbanization (Kabisch et al. 2017), including reducing exposure to environmental toxicants (Cilluffo et al. 2018) and noise (Dzhambov et al. 2019), increasing the level of physical activity (Grigsby-Toussaint et al. 2011; Ward et al. 2016), and enhancing social cohesion (Wan et al. 2021) in children.

Previous studies have shown that greenness exposure has consistently positive effects on mental health. James et al. mentioned the mechanisms for greenness and mental health, which was expected to reduce depression and anxiety through stress reduction, increase in physical activity and social interaction, and mitigation of noise

(James et al. 2015)(Figure 2). Recently, there is evidence suggesting indirect roles such as increased physical activity (Liu et al. 2019; Ward et al. 2016), traffic-related air pollution (Liao et al. 2019), traffic noise reduction (Klompmaker et al. 2019), social contact (Liu et al. 2019; Sugiyama et al. 2008), and stress relief (Liu et al. 2019) in manay previous studies. However, the biological mechanisms underlying the association between greenness exposure and desirable health effects remain unclear.

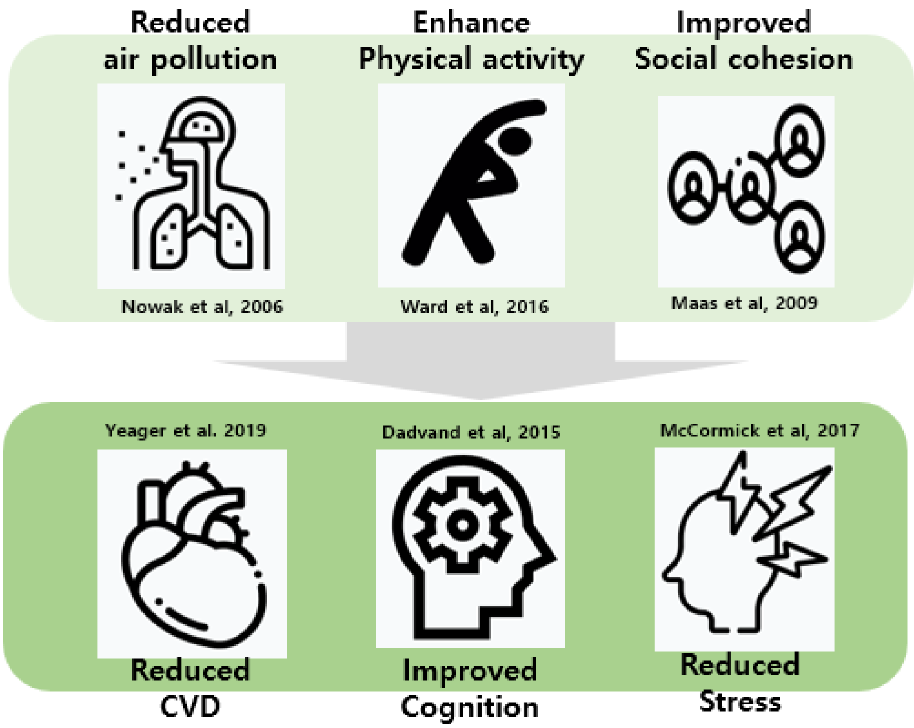

Figure 1. Health impact of greenness in previous studies (ISEE conference, 2020)

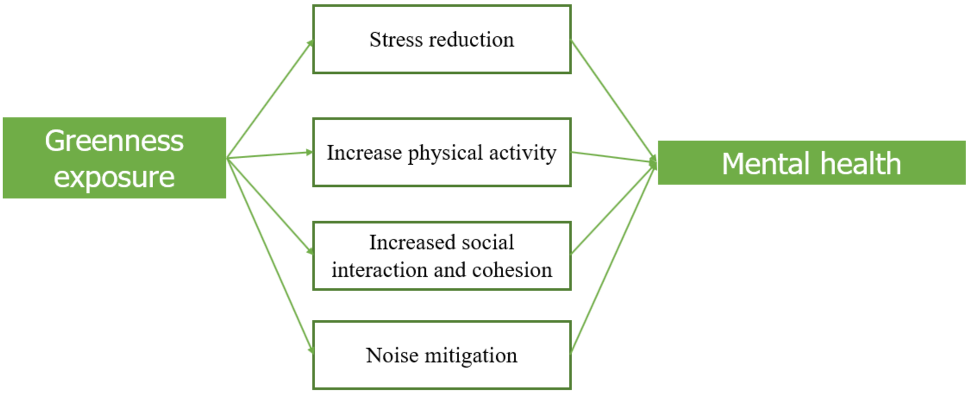

Figure 2. Association between greenness exposure and mental health (James et al., 2015)

1.1.1. Association between greenness and children's cognition

Human intelligence consists of cognitive abilities such as thinking, reading, learning, problem solving, and academic achievement tests (Reuben et al. 2019), and therefore environmental considerations in children's residential areas are largely related to long-term intelligence quotient (IQ) (Reuben et al. 2019). They suggested that raised in residential greener in children showed better cognitive ability at ages 5, 12, and 18, but the positive associations were best accounted for neighborhood socioeconomic factors (Reuben et al. 2019). Recently, Bijnens et al showed that the association between residential greenness and both intelligence and behavior in children with comparing to urban, suburban, and rural area in Belgium. They found that residential greenness was more beneficial for the children's intellectual and the behavioral development of children, who live in urban areas than rural areas (Bijnens et al. 2020). However, most previous studies including Bijnens study used cross-sectional design and few evaluated the association between exposure to greenness during critical periods, such as during pregnancy or early stages of infant and child neurodevelopment. Another study was reported to be associated between greenness exposure at birth and psychomotor development index at age 2 (Liao et al. 2019), and a map of normalized difference vegetation index (NDVI) with limited information on plant type identification and green quality. Previous studies are still lacking because few studies have linked individual perception outcomes to green residential areas in children of Asia.

1.1.2. Association between greenness exposure and children's epigenetics

Many epidemiological studies have shown that an adverse intrauterine environment including smoking (Joubert et al. 2012; Lee et al. 2015; Markunas et al. 2014), chemical exposures (Broberg et al. 2014; Cardenas et al. 2015; Herbstman et al. 2012; Khosla et al. 2001), ambient air pollution (Gruzieva et al. 2017; Kingsley et al. 2016), and stress (Cao-Lei et al. 2016; Vidal et al. 2014) may result in epigenetic perturbations of the developing fetus and can be associated with an increased risk of adverse health outcomes in later life (Figure 3). Additionally, exposure to heavy metal in early childhood (ages 1-4 years) was significantly associated with epigenetic change such as *H19* hypermethylation, which may contribute to growth and metabolic diseases (Goodrich et al. 2016). Hence, DNA methylation may be a possible mechanism by which early-life environmental factors contribute to increased risk of diseases in later life (Bianco-Miotto et al. 2017; Peng et al. 2018). In addition, epigenetic modifications, such as DNA methylation, are susceptible to genetic and environmental factors and may provide insights into individual differences in health outcomes (Marioni et al. 2018). Epigenetic change is hypothesized to be a regulatory pathway through which exposure to greenness in early childhood may influence child development and health.

However, few studies have assessed the association between greenness exposure and changes in DNA methylation. Xu et al. (2021) showed an association between greenness exposure, gene, and their interactions on blood-derived DNA methylation in 479 adult females (Xu et al. 2021). They found greenness-associated DNA methylation changes of cytosine-guanine dinucleotide (CpG) sites at genes related to various human diseases such as mental disorders, neoplasms, nutritional and metabolic diseases (Xu et al. 2021). The *CNP* gene at cg04720477 was strongly associated with greenness exposure and encodes a protein that has been related to low expression in brain tissue of schizophrenic (Peirce et al. 2006) and depressive patients (Rajkowska et al. 2015). These results suggest that high greenness may be related to elevated *CNP* expression due to reduced methylation of this gene in female adults (Xu et al. 2021). However, this study had a cross-sectional design, and so it was unable to determine whether DNA methylation plays a role in the association between improved mental health and exposure to greenness. In addition, they did not estimate the epigenetic impact of greenness on clinical outcomes.

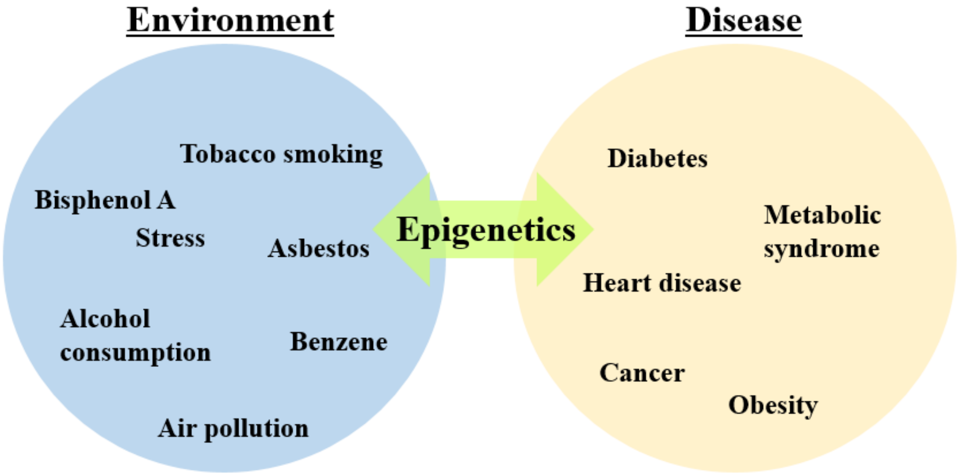

Figure 3. Previous studies in environmental epigenetics and disease in humans (Feil and Fraga 2012; Tzika et al. 2018).

1.2. Review of the Literature

To review previous studies for the association studies between exposure to greenness and cognitive development in children, PubMed were searched on June 30, 2020 for studies with titles or abstracts containing “greenness” or “green space” or “mental health” or “children”. Search results were further limited to those studying humans only. Next, titles and abstracts were screened to select only those that conducted original article. The main findings of each study are presented in a Table 1.

Table 1. Previous study for the association studies between exposure to greenness and mental health in children in the last five years.

No	Title	Country (study design)	Study population	Study design	Variable for greenness exposure	Timing of greenness exposure	Buffer size in greenness exposure	Age at outcome measurement(years)	Outcome variable	Major confounders or mediating factors	Significance
1	The relationship between the natural environment and individual-level academic performance in Portland, Oregon (Donovan et al. 2020)	Spain	17918	Cross-Sectional study (C-S)	Exposure to the natural environment	Grades 3 to 8 and Grade 11	250 m to 2,000 m	Grades 3 to 8 and Grade 11	Individual-level standardized math and reading test scores	Socioeconomic status	Yes (200m buffer)
2	The effect of residential greenness and city park visiting habits on preschool children's mental and general health in Lithuania: a cross-sectional study (Andrusaityte et al. 2020).	Lithuania	1489	C-S	NDVI, and time spent in a park	4-6-year-old children	100m,300m, 500m	4-6-year-old children	Strengths and Difficulties Questionnaire (SDQ) for parents	Children's sex, birth order, breastfeeding, antibiotic usage during the first postnatal year, wheeze during 12 months, clinically diagnosed asthma, allergy, underweight, tobacco smoke, the parents' socio-economic status, birth weight, air pollutants (NO_2, $PM_{2.5}$)	Yes (100m)
3	Community greenness and neurobehavioral health in children and adolescents (Lee et al. 2019)	Korea	1817	C-S	Soil adjusted vegetation index (MSAVI)	7 to 17 years.	50 m up to 3000 m	7 to 17 years.	Child Behavior Checklist (CBCL)	Age, sex, physical activity, monthly family income, exposure to second-hand smoke, exposure to NO_2, and blood lead level.	Yes

4	Residential neighborhood greenery and children's cognitive development. (Reuben et al. 2019)	UK	1658	Cohort	Normalized Difference Vegetation Index (NDVI)	ages 5 to 18	1-mile home radius	ages 5, 12, and 18	Fluid and crystalized intellectual performance	SES, neighborhood, genetic, family	Mixed
5	How is environmental greenness related to students' academic performance in English and mathematics? (Leung et al. 2019)	USA	27493	C-S	Massachusetts Geographic Information System (Mass GIS), NDVI, and green land use area	over 9 years	250–2000 m	over 9 years	Academic performance (i.e. English and Mathematics achievement level) were primarily based on Composite Performance Index (CPI)	Sex, student-teacher ratio, financial status, language ability, and race and ethnicity.	Yes
6	The psychological and social benefits of a nature experience for children: a preliminary investigation. (Dopko et al. 2019)	Canada	80	Experimental study	nature school VS. museum	elementary school	-	elementary school	shortened mood measure	-	mixed
7	The association between lifelong greenspace exposure and 3-dimensional brain magnetic resonance imaging in Barcelona schoolchildren (Dadvand et al. 2018).	Spain	253	Cohort	NDVI	7–10 y old	100m, 500m	7–10 y old	3-dimensional magnetic resonance imaging (3D MRI) (voxel-wise brain volume)	-	Yes
8	Availability, use of, and satisfaction with green space, and children's mental wellbeing at age 4 years in a multicultural, deprived, urban area: results from the Born in Bradford cohort study (McEachan et al. 2018).	UK	2594	Cohort	NDVI	4-year	100m,300m, 500m	4-year	Strengths and Difficulties Questionnaire.	Moderation by ethnicity and SES	Yes
9	Residential landscape as a	Belgiu	172	C-S	Residential	aged	100-m	aged	Stress	Age, sex, and parental	Yes

	predictor of psychosocial stress in the life course from childhood to adolescence (Van Aart et al. 2018).	m		and longit udinal associ ations	landscape	6.7–12.2	to 5-km	6.7–12.2	(standardized behavioral and emotional questionnaires and hair cortisol)	socioeconomic status	
10	The impact of greening schoolyards on the appreciation, and physical, cognitive and social-emotional well-being of schoolchildren: a prospective intervention study (Van Dijk-Wesselius et al. 2018).	Nether lands	700	Prospe ctive interv ention study	greening schoolyard s,	age 7–11	-	age 7–11	Physical, cognitive, and social-emotional well-being	Physical activity	Yes
11	Connection to the natural environment and well-being in middle childhood (Whitten et al. 2018).	Austra lia	268 93	Cohor t	Connection to Nature Index	11 years	-	11 years	Self-satisfaction, Prosocial Behavior: Empathy, Attention. Social supports	-	mixed
12	The Relationship between Surrounding Greenness in Childhood and Adolescence and Depressive Symptoms in Adolescence and Early Adulthood (Bezold et al. 2018a).	USA	137 54	Cohor t	NDVI	18 years	1000m	9–14	Depressive symptom	Household SES, paternal education, race/ethnicity, age and sex, $PM_{2.5}$	Yes
13	Urban Residential Greenspace and Mental Health in Youth: Different Approaches to Testing Multiple Pathways Yield Different Conclusions (Dzhambov et al. 2018).	Bulgar ia	399	C-S	NDVI (NDVI), Self-reported measures of availability, access,	15–25 years	500m	15–25 years	Mental health (General Health Questionnaire)	Restorative quality of the neighborhood, neighborhood social cohesion, commuting and leisure time physical activity, road traffic noise annoyance, and	mixed

					quality, and usage of greenspace					perceived air pollution	
14	Measuring connectedness to nature in preschool children in an urban setting and its relation to psychological functioning (Sobko et al. 2018).	Hong Kong	493	C-S	Connectedness to Nature (CN)	8–10 years	-	8–10 years	'Strength and Difficulties Questionnaire' (SDQ)	-	Yes
15	Lifelong residential exposure to green space and attention: a population-based prospective study (Dadvand et al. 2017).	Spain	888	Cohort	NDVI and vegetation continuous fields	birth, 4–5 y, and 7 y	100m,300m, 500m	4 to 5 y,7y	Conners' Kiddie Continuous Performance Test (K-CPT) at 4–5y(n= 888) and Attentional Network Task (ANT) at 7 y (n= 987)	age, sex, preterm birth, maternal cognitive performance, maternal smoking during pregnancy , and exposure to environmental tobacco smoke, maternal educational attainment at enrollment (individual-level socioeconomic status (SES)), and for the Urban Vulnerability Index, neighborhood SES	Yes
16	The relationship between neighborhood green space and child mental wellbeing depends upon whom you ask: Multilevel evidence from 3083 children aged 12–13 years (Feng and Astell-Burt 2017a).	Australia	3083	C-S	Neighborhood green space quantity and quality	12–13 Years	-	12–13 Years	Strengths and Difficulties Questionnaire	Indicators of area disadvantage and geographic remoteness, maternal education, child age and gender	Yes
17	Impact of urban nature on executive functioning in early	USA	67	Experimental	park-like area vs	4-5 years	-	4-5 years and 7-	Assessments of working memory,	-	Yes

	and middle childhood (Schutte et al. 2017).			study	urban streets	and 7-8years		8years	inhibitory control, and attention		
18	Effectiveness of a playground intervention for antisocial, prosocial, and physical activity behaviors (Mayfield et al. 2017).	USA	1196	Longitudinal, Cluster-randomized design	Peaceful Playgrounds™ (P2)	4-5 elementary grade	-	4-5 elementary grade	Antisocial behaviors (ASB),physical activity (PA) and prosocial behaviors (PSB)	-	mixed
19	The role of public and private natural space in children’s social, emotional and behavioral development in Scotland: a longitudinal study (Richardson et al. 2017).	UK	2909	Cohort	total natural space and parks	4 years	500m	4 years	Strengths and Difficulties Questionnaire (SDQ)	Sex, age, hours of screen time per day, educational attainment, equalized annual income, care’s mental component summary score, Neighborhood-level disadvantage	Yes
20	The Association Between Natural Environments and Depressive Symptoms in Adolescents Living in the United States (Bezold et al. 2018b).	USA	9385	C-S	greenness (NDVI vegetation) and blue space (water	12–18 years	250-m and 1,250-m radius	12–18 years	Depressive symptoms (using self-reported responses to the McKnight Risk Factor Survey.)	Race/ethnicity, grade level, age, and gender. Household income, father's education, and maternal history of depression., income, home value, percent white, and percent college educated, $PM_{2.5}$	Yes
21	Residential Green Space Quantity and Quality and Child Well-Being: A Longitudinal Study (Feng and Astell-Burt 2017b).	Australia	4,968	Cohort	Green space quantity and quality	4–5 years	-	4–5 years	Strengths and Difficulties Questionnaire		Yes
22	Inverse Relationship between	USA	543	Cohor	Multiple	5-12	100m	5-12	Childhood autism	Race, gender and	Yes

	Urban Green Space and Childhood Autism in California Elementary School Districts. (Wu and Jackson 2017)			t	types of green space	years		years	prevalence	socioeconomic status. urban land, stratified models by road density	
23	How is high school greenness related to students' restoration and health? (Akpinar 2016)	Turkey	223	C-S	School greenness	12–20 years	-	12–20 years	Stress, mental health, physical health, and quality of life	Sex, age, accommodation, and income	Yes
24	Horticultural activity program for improving emotional intelligence, prosocial behavior, and scientific investigation abilities and attitudes in kindergarteners (Park et al. 2016).	Korea	336	Experimental study	Indoor and outdoor activities such as planting seeds, transplanting plants so on.	5-7 years	-	5-7 years	emotional intelligence, prosocial behavior, and scientific investigation abilities and attitudes in 24hr program	-	Yes
25	Association of Sociodemographic and Environmental Factors with the Mental Health Status among Preschool Children-Results from a Cross-Sectional Study in Bavaria, Germany (Zach et al. 2016).	Germany	6206	C-S	accessibility of green space (availability of public parks or green spaces).	3–6 years	-	3–6 years	The prevalence of a borderline or abnormal SDQ-TDF and hyperactivity-inattention score	sociodemographic and environmental factors	Yes
26	Urban Natural Environments, Obesity, and Health-Related Quality of Life among Hispanic Children Living in Inner-City Neighborhoods (Kim et al. 2016).	USA	92	C-S	Quality of urban natural environments, landscape spatial patterns.	9 to 11 years old	400m, 800m	9 to 11 years old	health-related quality of life (HRQOL)	age, gender, maternal employment status, PA, BMI	Yes

27	Environmental Determinants of Aggression in Adolescents: Role of Urban Neighborhood Greenspace (Younan et al. 2016).	USA	1287	C-S	NDVI	9 to 10 years	250, 350, 500, and 1000 m	9 to 10 years	aggressive behaviors by the parent-reported Child Behavior Checklist.	age, sex, self-reported race/ethnicity, socioeconomic status (SES), and perceived neighborhood quality.	Yes
28	Adding Natural Areas to Social Indicators of Intra-Urban Health Inequalities among Children: A Case Study from Berlin, Germany (Kabisch et al. 2016).	Germany	30427	cross-sectional survey data	Green and blue areas	g 5- to 6-year-old	300m	g 5- to 6-year-old	language and viso-motoric development	-	Yes
29	The Impact of Children's Exposure to Greenspace on Physical Activity, Cognitive Development, Emotional Wellbeing, and Ability to Appraise Risk (Ward et al. 2016).	New Zealand	108	cross-sectional survey data	GPS receiver	11–14 years	-	11–14 years	Life Satisfaction Scale(LSS), Ten Domain Index of Wellbeing(TDIW), measure of happiness	-	Yes

1.3. Purpose of Research

Therefore, this research had several objectives in two parts.

In first part, to estimate the association between prenatal or postnatal exposure to greenness and children's IQ was observed (Figure 4). There are two purposes in the first part. 1) We investigated the relationship between prenatal and postnatal exposure to residential surrounding greenness and IQ score of 6-year-old in the prospective cohort. 2) We analyzed whether the effects differ depending on the types of natural greenness and built greenness.

In second part, we hypothesized that residential greenness in early childhood may be associated with epigenetic alterations and that these alterations may influence later childhood cognitive outcomes (Figure 5). Using a sub-study of 59 children with DNA methylation data, we sought to evaluate the association between residential greenness exposure and DNA methylation changes, which reported from genome-wide association studies (GWAS) and epigenome-wide association studies (EWAS) of cognitive ability in children in literature reviews. Then, we investigated the association between the DNA methylation changes, which were significantly associated with greenness exposure, and children's IQ in the prospective EDC cohort.

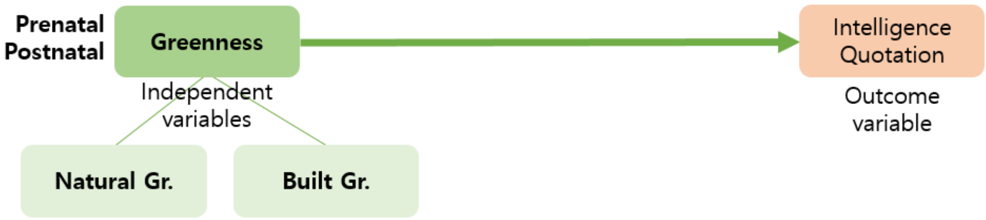

Figure 4. Scheme of the association between greenness and children's IQ

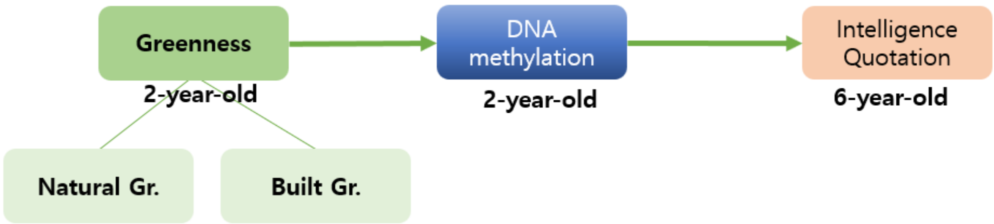

Figure 5. Scheme of the association between greenness, DNAm, and Children' IQ.

Chapter 2. Association between greenness and children's IQ

2.1. Study population.

The EDC (Environment and Development of Children) study utilizes an ongoing prospective study design to evaluate the effect of environmental exposure on development of cognition and physiology in children. A detailed description of participant recruitment has been mentioned previously (Kim et al. 2018). Briefly, a total of 726 women in mid-pregnancy from eight participating hospitals from 2008 to 2010 in Seoul and Incheon cities and Gyeonggi province were recruited. Children who had not congenital defects at birth and had an informed consent signed by the parents were included, but who had congenital defects at birth or invalid contact information were excluded. From 2012 to 2013, a total of 425 children aged 2 years, who were born to these mothers were tracked. Then, we additionally enrolled 81 children at age 4 due to the loss during follow-up. A total of 645 children were recruited and followed up at age 4 years from 2013 to 2015. Among them, 574 children were followed up at 6 years of age from 2015 to 2017(Figure 6). Because our study focused on the effect of urban greenness and children's cognition, the participants were 189 mother-children pairs, who lived in Seoul when the child was 6 years old and during the prenatal period in the EDC cohort. The study protocol was approved by the institutional review board (IRB) of the Seoul National University Hospital (IRB No. 1201-010-392). Written informed consent was obtained from the parents of the participants before enrollment.

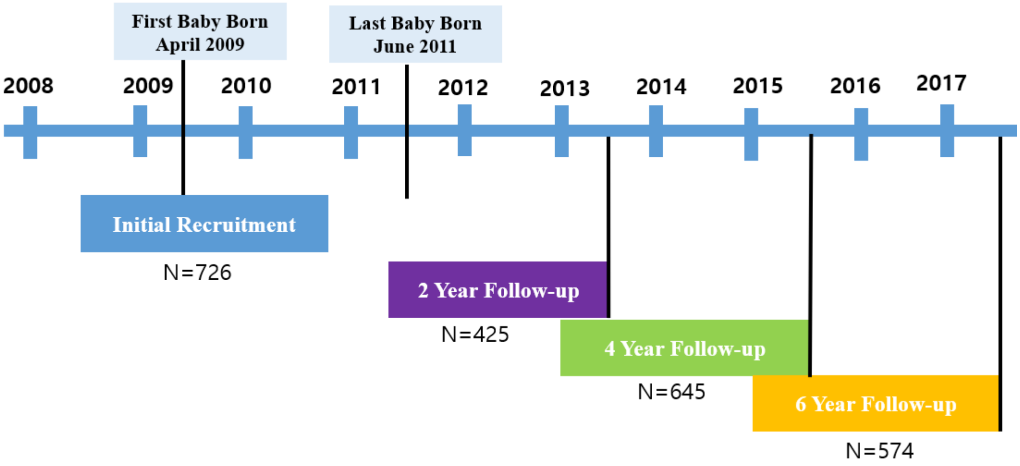

Figure 6. The follow-up scheme of EDC cohort from recruitment to the follow-up in 6-year-olds (2008-2017)

Table 2. The definition of variables (n=189)

Variables	**Source or Categorical**	**Value**	**Year for data collection**	**Period for follow-up**
Study Population	EDC cohort	-	2008-2010	Prenatal
			2015-2017	6y
Greenness	Land cover map (https://egis.me.go.kr/main.do)	Continuous (%)	2010	Prenatal
				6y
IQ	Korean Educational Developmental Institute's Wechsler Intelligence Scale for Children	Continuous (score)	2015-2017	6y
Sex	Questionnaire	Boy, girl	2011-2013	2y
Maternal age at enrollment	Questionnaire	Continuous (yr)	Baseline	Prenatal
Maternal educational level	Questionnaire	College graduates , graduate students	Baseline	Baseline
Exposure to environmental cigarette smoke	Questionnaire	Yes, no	2008-2010 /2015-2017	Prenatal and 6y
Maternal IQ	Korea's Wechsler Adult Intelligence Scale	Continuous (score)	2015-2017	6y
NO_2 concentration	National monitoring data (http://www.airkorea.or.kr/eng)	Continuous (ppb)	2008-2010 /2015-2017	Prenatal and 6y
Deprivation index	Shin et al. 2009	Continuous (score)	2004-2006	Prenatal and 6y
Road density during	Land cover map (https://egis.me.go.kr/main.do)	Continuous (%)	2010	Prenatal and 6y
Subjective noise level at age 6	Questionnaire	very loud, loud, moderate, quiet, very quiet	2015-2017	6y
Household income at age 6	Questionnaire	Under $3,300 per month, more than $3,300	2015-2017	6y
Distance to main road at age 6	Questionnaire	More than 500 m/ 50-499 m/ less than 50 m	2015-2017	6y
Physical activity time in outside at age 6	Questionnaire	Continuous (hr)	2015-2017	6y

2.2. Greenness exposure assessment

Table 2 showed the definition of variables. Prenatal and postnatal residential addresses were collected at the recruitment and the follow-up, when the children were aged 6. The residential surrounding greenness exposure was collected by the Environmental Geographic Information Service, using the Landsat image data from the IKONOS satellite images (Dial et al. 2003) and Korean Arirang satellite images, which were administered by the Ministry of the Environment in South Korea (https://egis.me.go.kr/main.do; Figure 7). We also used the Geocoder-Xr program to transform the participants' addresses to Coordinates (http://www.gisdeveloper.co.kr/?p=4784) (Figure 8). We selected the highest spatial resolution(1m) in the land cover map. In Figure 9 and 10 showed the spatial analysis from the satellite images to calculating the percentages of greenness. The study used Esri ArcGIS 10.5 Desktop (ArcMap) for all spatial data processing and geographic analysis. For spatial analysis, we firstly collected the participant's address for each follow-up period individually. After we transformed the participant's addresses at the followed up including during pregnancy and age 6 to coordinates such as longitude and latitude, we pointed on a map using the XY coordinates in ArcGIS program. Then, we created 100-2000 buffers around input as their addresses using the buffer tool in ArcGIS program (Figure 11). The "Input Features" parameter was assigned the participant's address, and the "Distance" parameter was assigned 100m to 2000m. The optional parameters were defaulted. Then, we computed a geometric intersection both the buffers and the greenness area in land cover map (Figure 12). After then, we opened the intersection table, we added a new field to commute the density of greenness density. Right-click the added field, choose the field calculator. calculated the density of greenness for each participant (Figure 13). To dissolve for aggregating each participants' greenness density, input features parameter was assigned the intersections' datafile after calculating the greenness density in dissolve tool. We selected both participants' id field and the greenness density fields in the dissolve fields (Figure 14).

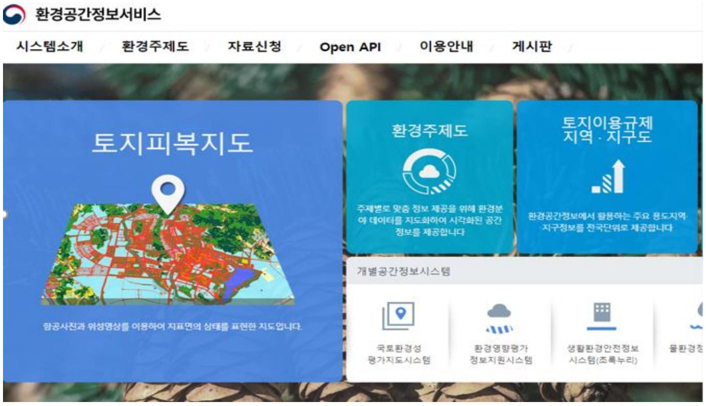

Figure 7. The homepage of Environmental Geographic Information Service, Ministry of the Environment(https://egis.me.go.kr/main.do)

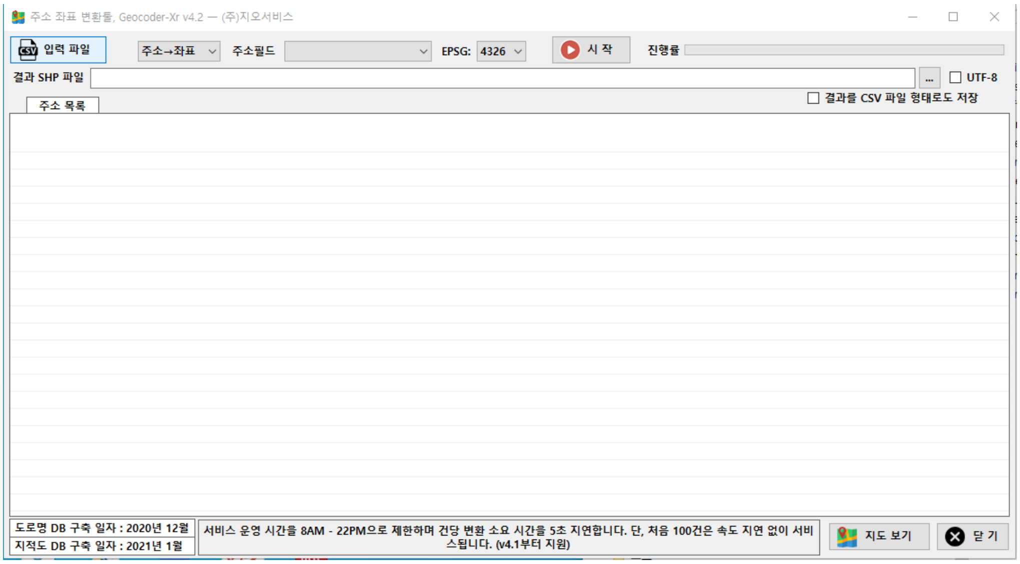

Figure 8. The Geocoder-Xr program for transforming an address to coordinate(http://www.gisdeveloper.co.kr/?p=4784).

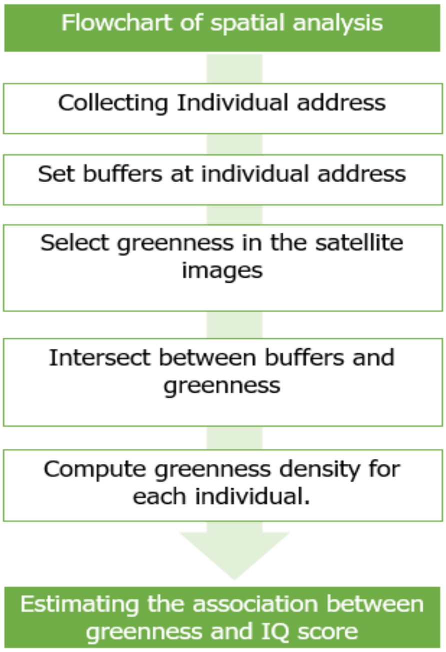

Figure 9. Flowchart of spatial analysis

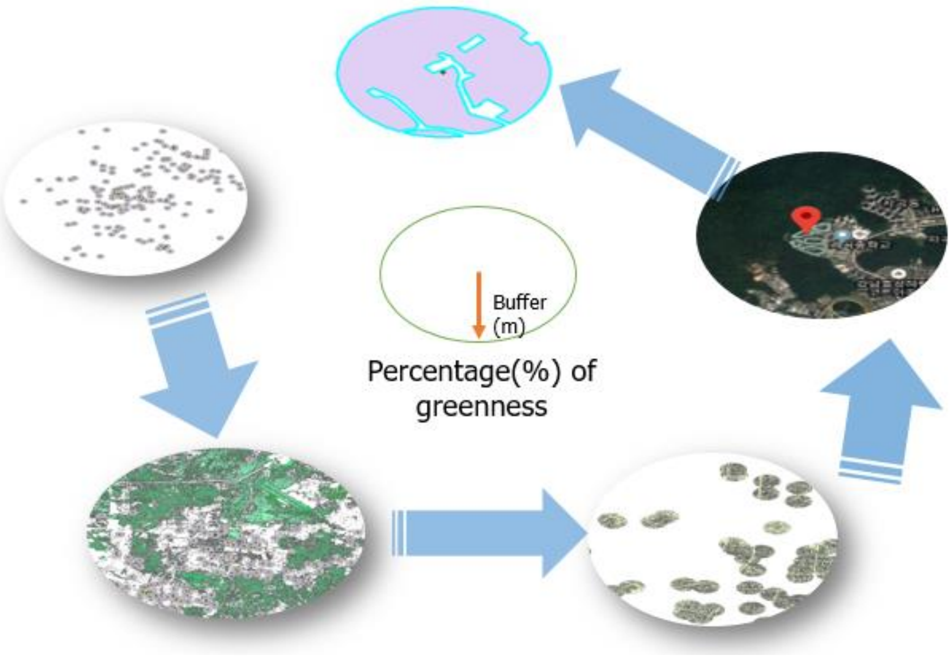

Figure 10. Compose the percentage of greenness in each participant address

To estimate greenness exposure in our study, greenness densities were calculated within buffer radii of 100 m, 500 m, 1000 m, 1500 m, and 2000 m from each mother and child's residential address. Then, we determined the percentage of greenness (density) in each buffer radius. We separately analyzed two types of greenness, namely natural greenness including forest or natural grassland, and built greenness including artificial grassland, urban parks or street trees. More detail definition for the type of greenness was represented in Table 3. Because more than 10% of the participants were not exposed to natural greenness both 100 and 500 m radiuses from their residential address, we did not analyze the effect of natural greenness both 100 and 500 m radiuses. In addition, we conducted to define the built greenness including golf course and cemetery parks, which were provided to greenness from the Landsat image data in a sensitivity analysis. Even though golf courses and cemetery parks were not given to children to play, we thought that these parks are having partial ecological benefits such as reducing urban complexity as well as increasing comfort. Some previous studies also defined greenness exposure including cemetery park or golf course (Lai et al. 2020; Li et al. 2015; Richardson et al. 2012; Wang et al. 2013)

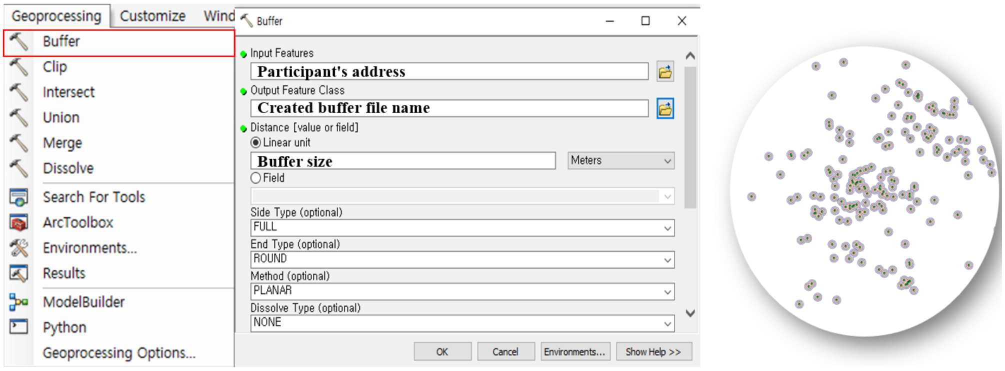

Figure 11. The process of each buffer around the participants' address creating in ArcGIS program.

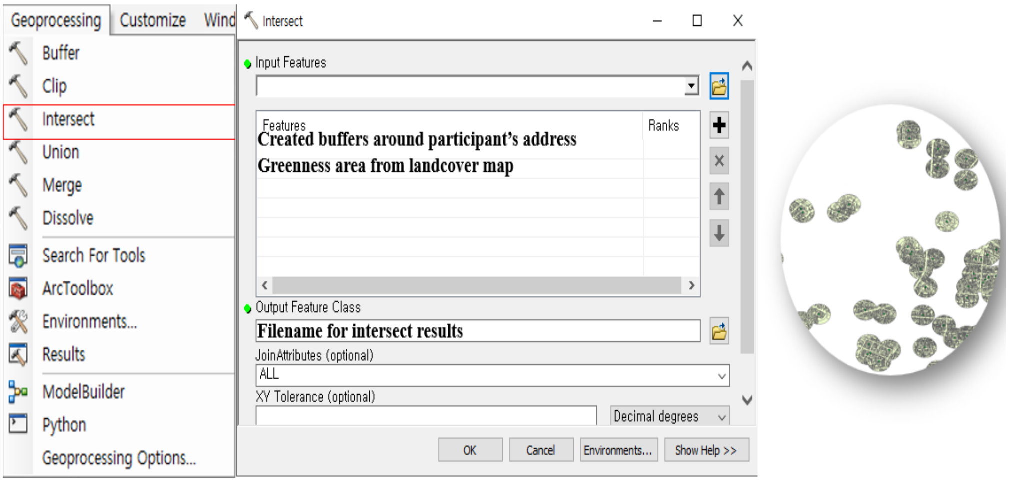

Figure 12. The process of the intersection between buffers and greenness area from landcover map in ArcGIS program.

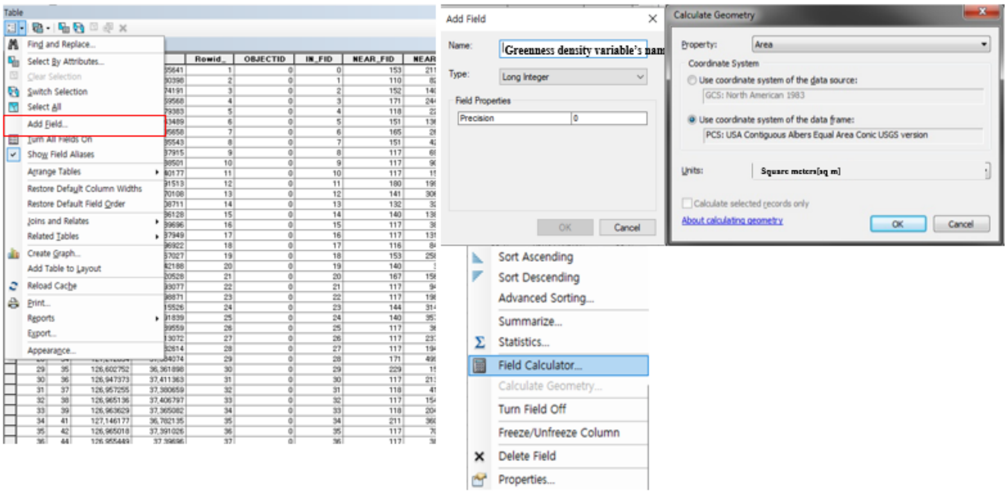

Figure 13. Calculated the greenness densities

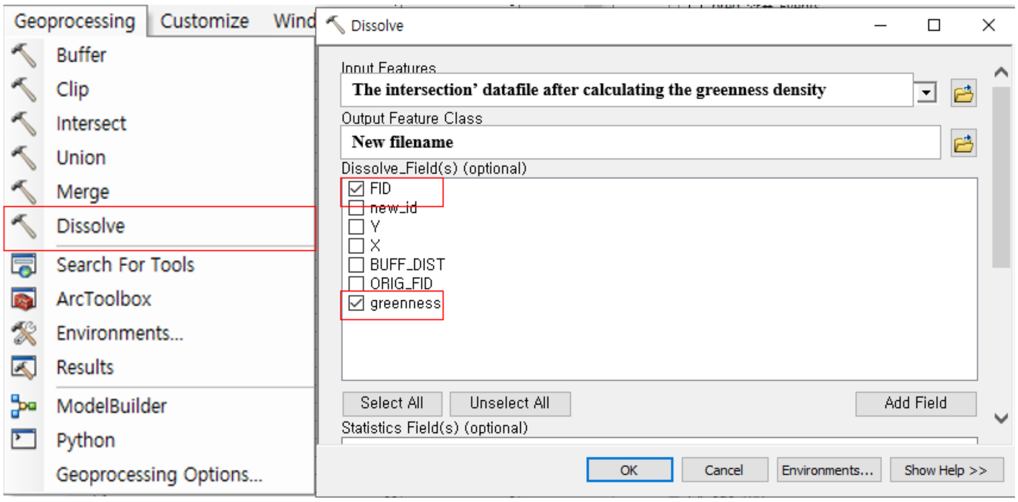

Figure 14. Dissolved for aggregates for each individual's data

Table 3. The definition the type of greenness

Type of Greenness	Definition
Natural greenness	
Broadleaf forest	A broadleaved forest refers to a place where a broadleaf forest accounts for more than 75% of the total forest area.
Coniferous forest	A coniferous forest refers to a place where coniferous forests account for more than 75% of the total forest area.
Mixed forest	A Mixed forest refers to an area where the forest area of a broadleaf forest is mixed with the forest area of a coniferous forest, and the forest area of a coniferous forest is less than 75% or the forest area of a coniferous forest is less than 75%.
Natural grassland	Natural grassland refers to the grassland of the naturally occurring mountain top, the silver grass of the ridge, and the leading part of the river and forest, and if there are trees less than 10% of the total grassland area, they are classified as natural grassland.
Built greenness	
Artificial grassland or street trees	It includes stable cases by creating a grassland for planting feed crops, inside the interchange, cut-off section of the road, and a slope in the construction area. It includes grassland and street trees other than the facilities of farms, farms, ranches, and grazing fields. It includes grasslands created as buffer green areas such as urban parks around roads and apartments.

2.3. Cognitive development in children

The IQ score in 6-year-old children was measured using the Korean Educational Developmental Institute's Wechsler Intelligence Scale for Children (Park et al. 1996). This form of KEDI-WISC has been verified in Korean children and is used to produce global estimates of intellectual function, which were modified version of the Wechsler Intelligence Scale for children between the ages of 5 and 15 years (Park et al. 2015). A licensed clinical neuropsychologist coordinated the IQ tests and supervised the examiners using a standardized procedure (Cho et al. 2010). All the children were given instructions in a loud voice, and most of the children could understand the task. The examiner demonstrated each task to ensure that the children understood the instructions (Park et al. 2015). The test consists of the scales including vocabulary, arithmetic, picture arrangement, and block design tests. The two subsets were performed: verbal IQ, which consisted of sum of scores on tests including vocabulary and arithmetic intelligence, and performance IQ, which consisted of the sum of scores on tests including picture arrangement and block design (Kim et al. 2017). Higher scores indicated greater intelligence.

2.4. Covariates

Based on previous studies, we selected covariates including the average concentration of child's sex (boy or girl), maternal IQ (continuous), exposure to environmental cigarette smoke (ETS) during pregnancy (Yes or No), the percentages of road density in each radius (100 m to 2000 m, continuous), and nitrogen dioxide (NO_2) as traffic-related air pollution, when we estimated the association between prenatal greenness and children's IQ. NO_2 is a local pollutant that can be used to infer urban area and local emissions, including from road traffic (Park et al. 2020). We added some covariates subjective environmental noise levels (very loud, loud, moderate, quiet, very quiet), the distance to the main road from the house (more than 500 m, more than 50 m, less than 50 m), when we estimated the association between postnatal exposure at age 6 and children's IQ. Since one of the important covariates in our study was the socioeconomic state (SES), which we consider to be associated with variables in both exposure and outcome, we included the SES variables as both individuals and neighborhood SES in the analysis. Individual SESs used two variables such as the household income at the age of six (under \$3,300, more than \$3,300 per month), and the mother's education level (over college graduates or graduate students). The residential deprivation index was used as an indicator of neighborhood SES during the mother's pregnancy and during the child's 6 years of age (continuous variable). The residential deprivation index was calculated in the sum of five domains, including unemployment, poverty, housing, labor, and social networks. The range of the indicators is 0–500 and is calculated as the sum of all domain scores (each score ranges from 0–100) after standardization using geometric transformations by region. The higher index score is indicated the expansion of poverty (Shin et al. 2009). The intelligence index of mothers was evaluated using Korea's Wechsler Adult Intelligence Scale (Lim et al. 2000).

2.5. Statistical analysis

We estimated the covariates in the study population using frequencies (for categorical variables) or means (for continuous variables) (Table 4). Distributions of greenness in Seoul area by types of greenness, namely, natural versus built environment were evaluated (Table 5). Then, we examined prenatal and postnatal exposure to greenness as the percentages of residential greenness in each buffer area surrounding the participants' addresses. We used Student's t-test, ANOVA (for category variables), or regression (for continuous variables) to analyze the relationship between greenness and covariates (Table 6). The crude model examined the association between prenatal or postnatal exposure to the total, natural, and built greenness using PROC GENMOD in SAS program. Then, we examined the association between prenatal or postnatal exposure to greenness and children's IQ at age 6 using PROC MIXED in SAS program, adjusting for administrative district as a random effect. We assessed the relationship between prenatal exposure to greenness and children's IQ, adjusting for the confounding factors of mother's age, children's sex, mother's education level, mother's IQ, exposure to ETS, average NO_2 concentration during the previous 3 years in the area of residence during pregnancy, residential deprivation index as an index of neighborhood SES, and the percentage of road density in each radius during pregnancy. The association between postnatal exposure to greenness and children's IQ in the full model was adjusted for mother's age, children's sex, mother's education level, mother's IQ, exposure to ETS at age 6, average NO_2 concentration during the previous 3 years in the area of residence at age 6, household income as an index of individual SES, residential deprivation index as an index of neighborhood SES, a subjective noise level, the distance to main road from the house at aged 6, the percentage of road density in each radius at aged 6, and a physical activity time (hours) at age 6.

We tested the relationship between greenness exposure and children's IQ stratified by maternal education level to identify the role of individual SES in the relationship. We plotted the Least-Squares Means (LSMEANS) of total IQ by the prenatal or postnatal exposure to greenness as three categorical group such as the percentages of greenness below 25% group, between 25% to 75% group, more than 75% group in R package *plotrix* (Duursma et al. 2009). We also tested the significance for the difference of total IQ by the percentages of greenness between 25% to 75% group, more than 75% group versus below 25% group using Dunnett multiple comparison test. In sensitivity analysis, we calculated the averages of greenness exposure using the participant's addresses at the follow-up periods of pregnancy, 2, 4, and 6 years old. Additionally, we calculated the proxy to greenness including total, natural, and built greenness using *NEAR* tool in ArcGIS program. All statistical analyses were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC, USA) and R software (version 3.6.0; https://cran.r-project.org/).

2.6. Results

2.6.1. General characteristics of the participants

Table 4 presents the characteristics of our participants. The mean age of the mothers at mid-pregnancy was 32.05 years (standard deviation [SD]: 3.49). Of the mothers, 84.13% were more than a university undergraduate education. A total of 86.96% of the participants had exposed to ETS during pregnancy. The mean mothers' IQ was 118.23 (SD: 12.13). The composite deprivation index during pregnancy was 150.61 (SD: 63.61). Ambient NO_2 exposure during pregnancy in the area of residence for a 3-year period was 37.09 ppb (SD: 2.85). The percentages of road density in each radius around the residential addresses during pregnancy ranged from 29.42% to 41.05%. The percentage of boys (51.85%) was slightly higher than girls. The percentage of exposed to ETS occurred among 23.81% of the children at age 6. The percentage of monthly household income > $3300 at age 6 was 77.78%. Regarding subjective noise levels reported by our participants, 19.05% of participants answered the residential area to be “loud” and 1.06% of participants reported “very loud.” 20.11% of participants lived near a main road, which was defined as a road with more than two lanes, within 50 m of the house, and 56.08% of those who lived within 500 m or more from a main road. Among the children, 55.56% played outdoors for less than 1 hour per a day, whereas 12.7% played outdoors for 3 hours or more. The percentages of road density in each radius from 100 to 2000 m of the house at age 6 ranged from 28.86% to 37.42%.

Table 4. Characteristics in study population (N=189)

Variables	Categorical	Mean±SD or N(%)
Prenatal covariates		
Maternal age during pregnancy		32.05±3.49
Maternal education level	≤University graduate	159(84.13)
	≥Graduate school	30(15.87)
Prenatal exposure to ETS	Yes	160(86.96)
	No	24(13.04)
Maternal IQ (score)		118.23±12.13
Deprivation index as neighborhood SES during pregnancy(score)		150.61±63.61
The average of ambient NO_2 concentration during pregnancy in residential area among 3 years(ppb)		37.09±2.85
The percentages of road density in each radius during pregnancy (%)	100m	41.05±13.48
	500m	35.26±7.96
	1000m	31.93±7.55
	1500m	30.31±6.76
	2000m	29.42±5.99
Postnatal covariates	**Categorical**	**Mean ± SD or N(%)**
Children's sex	Girls	91(48.15)
	Boys	98(51.85)
Exposure to ETS at aged 6	Yes	45(23.81)
	No	144(76.19)
The average of ambient NO_2 concentration in residential area at age 3 to 6 (ppb)		32.88±2.71
Monthly household income as individual SES (dollars)	≤ $ 3300	42(22.22)
	> $ 3300	147(77.78)
Composite deprivation index as neighborhood SES at age 6		140.44±64.42
Subjective noise level at age 6	Very Loud	2(1.06)
	Loud	36(19.05)
	Moderate	84(44.44)
	Quiet	56(29.63)
	Very quiet	11(5.82)
Proxy to main road from the house at age 6(m)	≥ 500m	106(56.08)
	50m-499m	45(23.81)
	≤ 50m	38(20.11)
Physical activity time in outdoor at age 6(hr)	≥ 3hr	24(12.7)
	1-2hr	60(31.75)
	≤ 1hr	105(55.56)
The percentages of road density in each radius at age 6(%)	100m	37.42±16.09
	500m	33.58±10.59
	1000m	30.53±8.72
	1500m	29.54±7.74
	2000m	28.86±6.98

Abbreviations: NO_2: nitrogen dioxide, IQ: intelligence quotient, ETS: environmental tobacco smoke, SES: Socioeconomic status, SD: Standard Deviation

2.6.2. Exposure to greenness exposure in Seoul

Figure 15 shows the distribution of greenness by type, including natural or built in Seoul area. Natural greenness was predominately distributed along the border of the administrative district, especially on the northern and southern boundary in Seoul area. Built greenness was distributed evenly, and predominately comprised constructed grassland or street trees. Table 5 shows the percentages of participants with prenatal and postnatal exposure to residential greenness in each buffer area. The mean percentages of total greenness within 100 m to 2000 m buffer radii ranged from 15.11% to 23.59%.

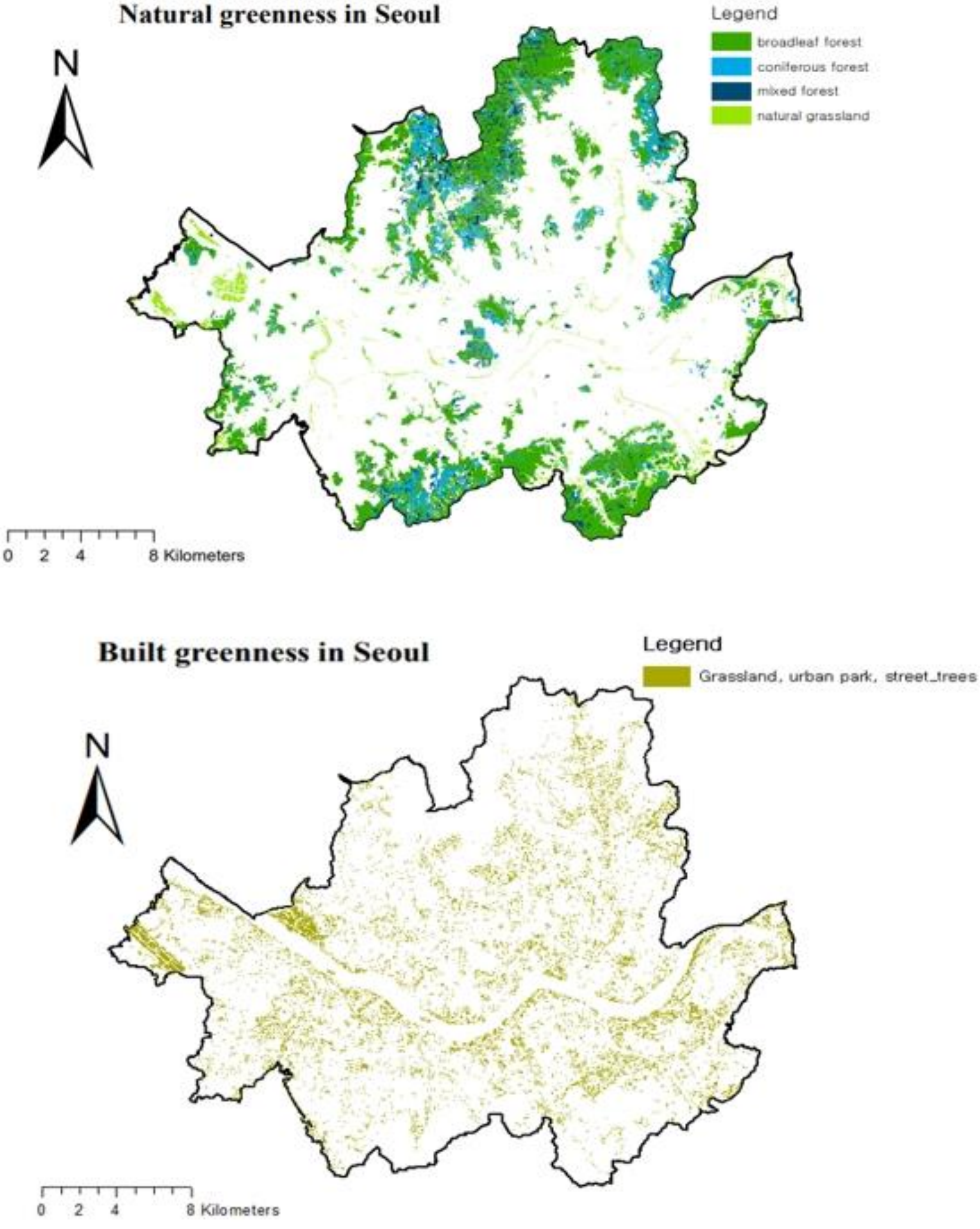

Figure 15. Distribution greenness in Seoul across to the type of greenness.

Table 5. The percentages (%) of pre- and postnatal exposure to residential greenness in each buffer area(N=189).

Buffer	Exposure to greenness during pregnancy														
	Total greenness					Natural greenness					Built greenness				
	Mean	SD	Min	Max	ND(%)	Mean	SD	Min	Max	ND(%)	Mean	SD	Min	Max	ND(%)
100m	15.11	14.94	0.00	91.31	7.9	NA	NA	NA	NA	76.7	12.71	12.48	0.00	49.93	9.0
500m	18.81	14.79	1.02	83.75	-	NA	NA	NA	NA	14.3	9.92	6.96	0.05	32.48	
1000m	21.45	13.32	2.03	78.04	-	12.65	13.07	0.01	75.55	-	8.92	4.99	2.02	35.68	
1500m	22.72	11.98	5.47	70.66	-	14.59	12.39	0.64	66.98	-	8.45	4.12	2.06	32.37	
2000m	23.59	11.45	7.87	66.44	-	16.24	12.20	0.74	62.99	-	7.86	3.13	2.51	23.03	
	Exposure to greenness at age 6														
Buffer	Total greenness					Natural greenness					Built greenness				
	Mean	SD	Min	Max	ND(%)	Mean	SD	Min	Max	ND(%)	Mean	SD	Min	Max	ND(%)
100m	18.37	17.45	0.00	80.48	6.3	NA	NA	NA	NA	65.1	13.87	12.93	0.00	51.64	7.4
500m	23.26	16.32	1.24	83.30	-	NA	NA	NA	NA	5.3	10.91	6.88	0.19	35.58	-
1000m	25.19	15.65	2.19	79.22	-	15.86	16.12	0.01	76.75	-	9.40	5.02	2.18	39.36	-
1500m	26.05	14.46	7.45	72.33	-	17.40	15.49	0.64	68.84	-	8.78	3.99	1.75	32.66	-
2000m	26.81	13.66	9.42	70.72	-	18.73	14.86	1.49	68.65	-	8.33	3.07	2.18	22.59	-

Abbreviations: ND (Not detected), NA (Not analyzed)

2.6.3. Associations between exposure to greenness exposure and covariates

Table 6 showed that the association between prenatal and postnatal exposure to greenness and covariates. Figure 16-34 were constructed using generalized additive models (GAMs) and boxplot to investigate the relationship between exposure to greenness and covariates. In Table 6 showed that the percentages of total greenness exposure during pregnancy were significantly associated with maternal age (Figure 17), the average of NO_2 concentration during pregnancy (Figure 18), residential deprivation index (Figure 19), the percentage of road density (Figure 20), Maternal education level (Figure 21), and children's sex (Figure 21). In the GAM models, an inverted U shape relationship between greenness exposure in 500m-2km buffer radii during pregnancy and maternal IQ (Figure 16) was observed. We found that the negative associations between greenness exposure during pregnancy, and both NO_2 concentration during pregnancy (Figure 18) and the percentage of road density (Figure 20) were observed. The groups of boys (Figure 21), higher maternal education level (Figure 21), and non-exposed ETS during pregnancy (Figure 21) had higher exposed the percentage of greenness during pregnancy than the groups of girls, exposed ETS, and lower maternal educational level. We also found significant associations between residential deprivation index (Figure 26), the percentage of road density (Figure 27), children's sex (Figure 28), maternal education level (Figure 29), and postnatal greenness exposure (Table 6). In the GAM models, there was a non-linear relationship between maternal IQ (Figure 23), maternal age (Figure 24), the average of NO_2 concentration at age 6 (Figure 25), and the percentage of postnatal greenness exposure.

Table 6. The association between (a) prenatal and (b)postnatal total greenness and covariates
(a)

Covariates	Buffer size	100m		500m		1000m		1500m		2000m	
	Categorical	Mean(%)(SD) or beta (SE)	*P*-value	Mean(%)(SD) or beta (SE)	*P*-value	Mean(%)(SD) or beta (SE)	*P*-value	Mean(%)(SD) or beta (SE)	*P*-value	Mean(%)(SD) or beta (SE)	*P*-value
Children's sex	Girls	13.75(13.78)	0.229	**16.59(13.21)**	**0.041**	20.59(12.69)	0.256	22.82(12.22)	0.659	24.23(12.01)	0.918
	Boys	16.37(15.90)		**21.01(16.07)**		22.85(14.38)		23.62(12.65)		24.05(11.83)	
Maternal age during pregnancy		**0.63(0.30)**	**0.040**	**1.03(0.30)**	**0.000**	**0.75(0.27)**	**0.007**	**0.54(0.25)**	**0.033**	**0.55(0.24)**	**0.026**
Maternal education level	≤ university graduate	**14.00(15.08)**	**0.019**	18.46(15.23)	0.368	21.81(14.02)	0.911	23.28(12.58)	0.894	24.51(11.84)	0.323
	≥ graduate school	**20.95(12.89)**		21.13(12.89)		21.51(11.33)		22.95(11.70)		22.17(12.14)	
Maternal IQ		**0.19(0.09)**	**0.049**	0.04(0.09)	0.659	-0.03(0.08)	0.682	-0.02(0.07)	0.779	-0.01(0.07)	0.863
Exposure to ETS during pregnancy	No	20.45(20.02)	0.161	20.45(18.61)	0.615	22.45(16.88)	0.838	23.27(15.06)	0.968	22.85(14.29)	0.551
	Yes	14.33(14.00)		18.79(14.47)		21.83(13.28)		23.38(12.19)		24.42(11.66)	
Average of NO_2 concentration during previous 3 years in residential area during pregnancy		**-0.91(0.38)**	**0.018**	-0.65(0.38)	0.089	-0.46(0.35)	0.187	-0.46(0.32)	0.145	-0.46(0.3)	0.135
Residential deprivation index as neighborhood SES during pregnancy		**-0.03(0.01)**	**0.022**	-0.02(0.01)	0.083	-0.00(0.01)	0.693	0.00(0.01)	0.495	**0.02(0.01)**	**0.036**
Road density in each radius during pregnancy		-0.04(0.08)	0.655	**-0.74(0.12)**	**<.0001**	**-1.08(0.10)**	**<.0001**	**-1.28(0.09)**	**<.0001**	**-1.49(0.09)**	**<.0001**

(b)

Covariates	Buffer size	100m		500m		1000m		1500m		2000m	
	Categorical	Mean(%)(SD) or beta (SE)	*P*-value	Mean(%)(SD) or beta (SE)	*P*-value	Mean(%)(SD) or beta (SE)	*P*-value	Mean(%)(SD) or beta (SE)	*P*-value	Mean(%)(SD) or beta (SE)	*P*-value
Children's sex	Girls	16.38(18.68)	0.068	**20.60(15.81)**	**0.010**	**22.99(14.09)**	**0.017**	24.63(13.41)	0.066	25.81(13.00)	0.118
	Boys	21.38(18.69)		**27.04(17.89)**		**28.56(17.71)**		28.69(16.45)		29.07(15.32)	
Maternal age during pregnancy		0.18(0.39)	0.646	0.45(0.35)	0.203	0.26(0.34)	0.436	0.17(0.31)	0.591	0.13(0.29)	0.643
Maternal education level	≤ university graduate	18.76(19.57)	0.659	24.44(17.82)	0.351	**26.76(16.81)**	**0.037**	27.45(15.45)	0.135	28.22(14.24)	0.110
	≥ graduate school	20.11(14.28)		21.24(13.26)		**21.20(12.21)**		22.93(13.10)		23.66(14.31)	
Maternal IQ		0.08(0.12)	0.507	-0.05(0.11)	0.651	-0.12(0.10)	0.226	-0.11(0.09)	0.255	-0.10(0.09)	0.287
Exposure to ETS at age 6	No	19.95(19.00)	0.200	24.45(17.34)	0.468	26.20(16.84)	0.625	26.84(15.60)	0.862	27.55(14.68)	0.933
	Yes	15.83(18.01)		22.31(16.77)		24.84(14.41)		26.39(13.82)		27.34(13.19)	
Average of NO_2 concentration during previous 3 years in residential area at age 6		0.68(0.51)	0.178	0.84(0.46)	0.069	0.59(0.44)	0.182	0.21(0.41)	0.616	-0.04(0.39)	0.927
Household income as personal SES	Low	15.13(16.61)	0.134	22.18(15.22)	0.454	25.05(14.51)	0.711	25.46(14.47)	0.538	26.73(13.76)	0.694
	High	20.07(19.29)		24.44(17.72)		26.11(16.77)		27.10(15.38)		27.72(14.50)	
Residential deprivation index as neighborhood SES at age 6		-0.00(0.02)	0.945	0.00(0.01)	0.889	0.01(0.01)	0.322	**0.03(0.01)**	**0.028**	**0.05(0.01)**	**0.000**
Subjective	Very Loud	18.80(14.47)	0.949	**39.00(12.72)**	**0.022**	**44.43(18.64)**	**0.000**	**39.44(17.00)**	**0.011**	**35.91(15.15)**	**0.032**

noise level	Loud	20.38(17.94)		**25.08(15.78)**		**25.68(15.46)**		**27.37(15.14)**		**28.73(14.41)**	
	Moderate	17.76(20.52)		**21.07(17.37)**		**23.76(14.91)**		**25.42(13.85)**		**26.57(13.29)**	
	Quiet	19.51(18.08)		**23.92(18.20)**		**24.55(15.98)**		**23.88(14.89)**		**24.09(14.01)**	
	Very quiet	21.54(2.116)		**29.85(15.59)**		**42.35(31.21)**		**45.30(33.13)**		**47.21(33.45)**	
Distance to main road at age 6	≥ 500m	19.88(18.43)	0.662	23.39(17.46)	0.639	25.33(16.83)	0.775	26.19(15.35)	0.830	27.01(14.32)	0.849
	50m-499m	16.83(20.78)		23.22(17.41)		25.76(14.96)		27.02(14.04)		27.79(13.22)	
	≤ 50m	18.97(17.63)		26.30(16.36)		27.53(16.46)		27.90(16.18)		28.51(15.77)	
Physical activity time in outdoor at age 6	≤ 1hr	18.94(18.23)	0.688	24.35(16.99)	0.644	26.53(16.72)	0.416	27.94(15.76)	0.280	28.87(14.98)	0.221
	1-2hr	20.14(21.28)		24.44(19.19)		26.38(16.94)		26.28(15.40)		26.71(14.30)	
	≥ 3hr	16.20(14.64)		20.85(12.35)		21.76(11.97)		22.55(11.04)		23.50(10.33)	
Road density in each radius at age 6		**-0.32(0.08)**	**<.0001**	**-1.06(0.08)**	**<.0001**	**-1.42(0.08)**	**<.0001**	**-1.60(0.08)**	**<.0001**	**-1.74(0.07)**	**<.0001**

Abbreviations: NO_2: nitrogen dioxide, IQ: intelligence quotient, ETS: environmental tobacco smoke, SES: Socioeconomic status, SE: Standard Error, SD: Standard Deviation

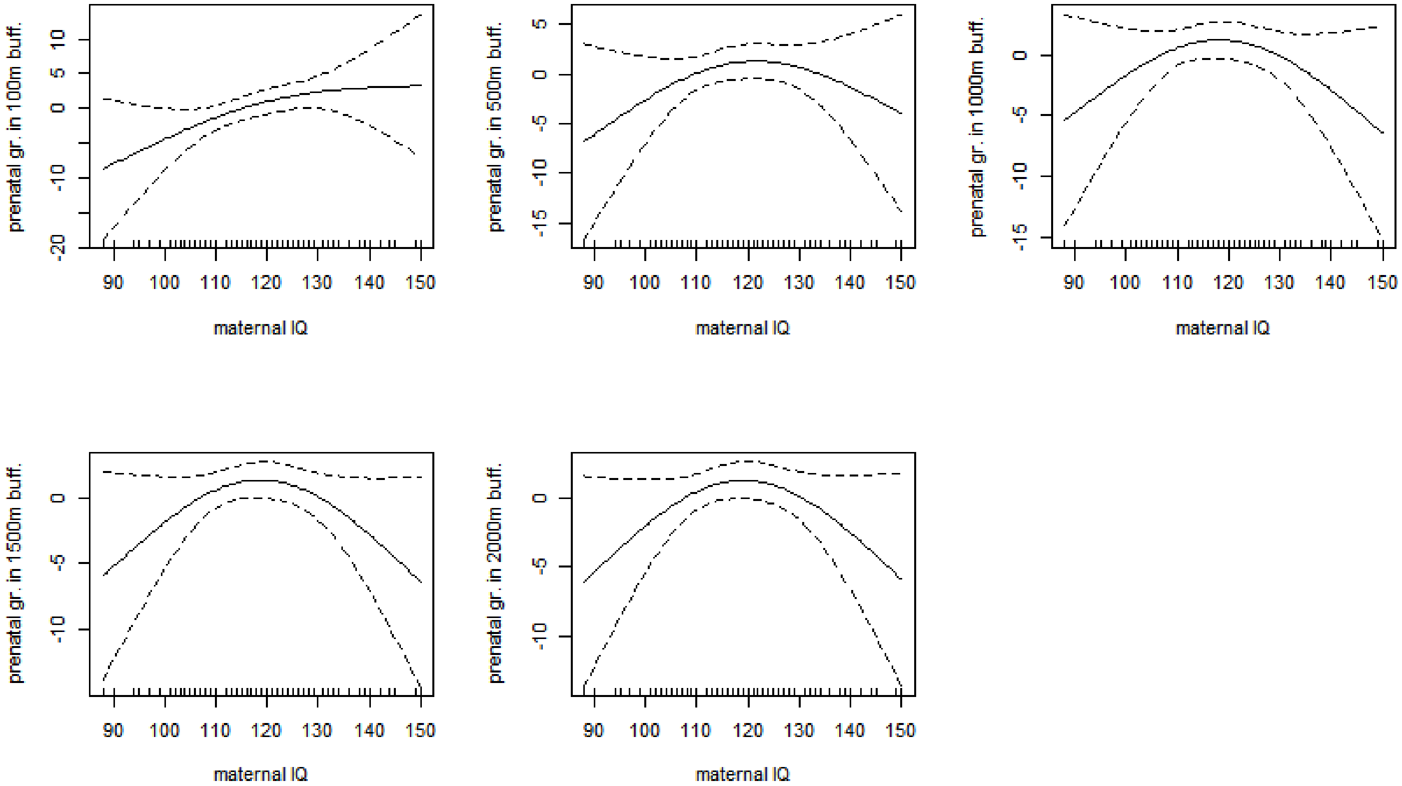

Figure 16. Association between prenatal greenness (%) and maternal IQ (score)

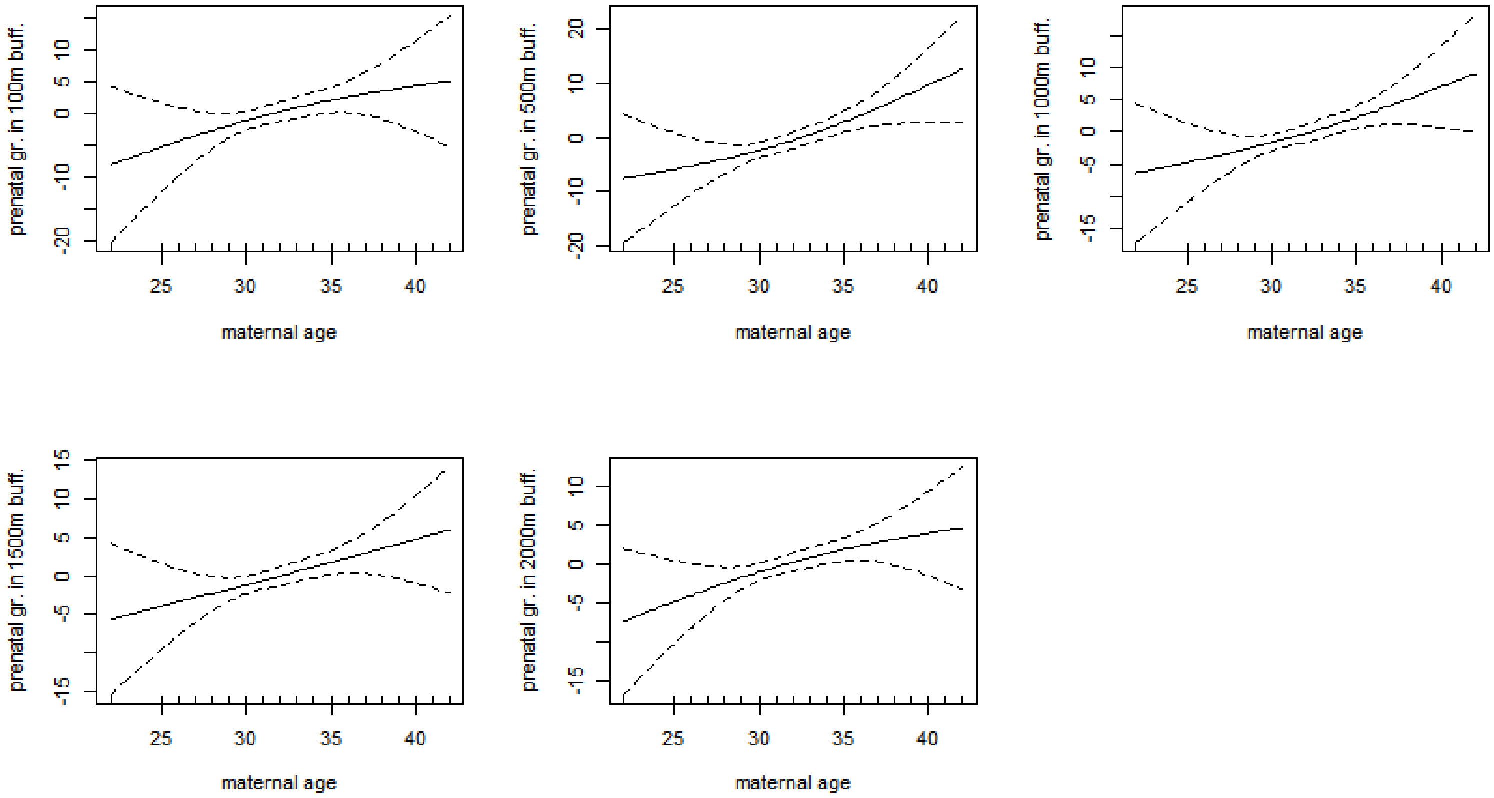

Figure 17. Association between prenatal greenness (%) and maternal age(year)

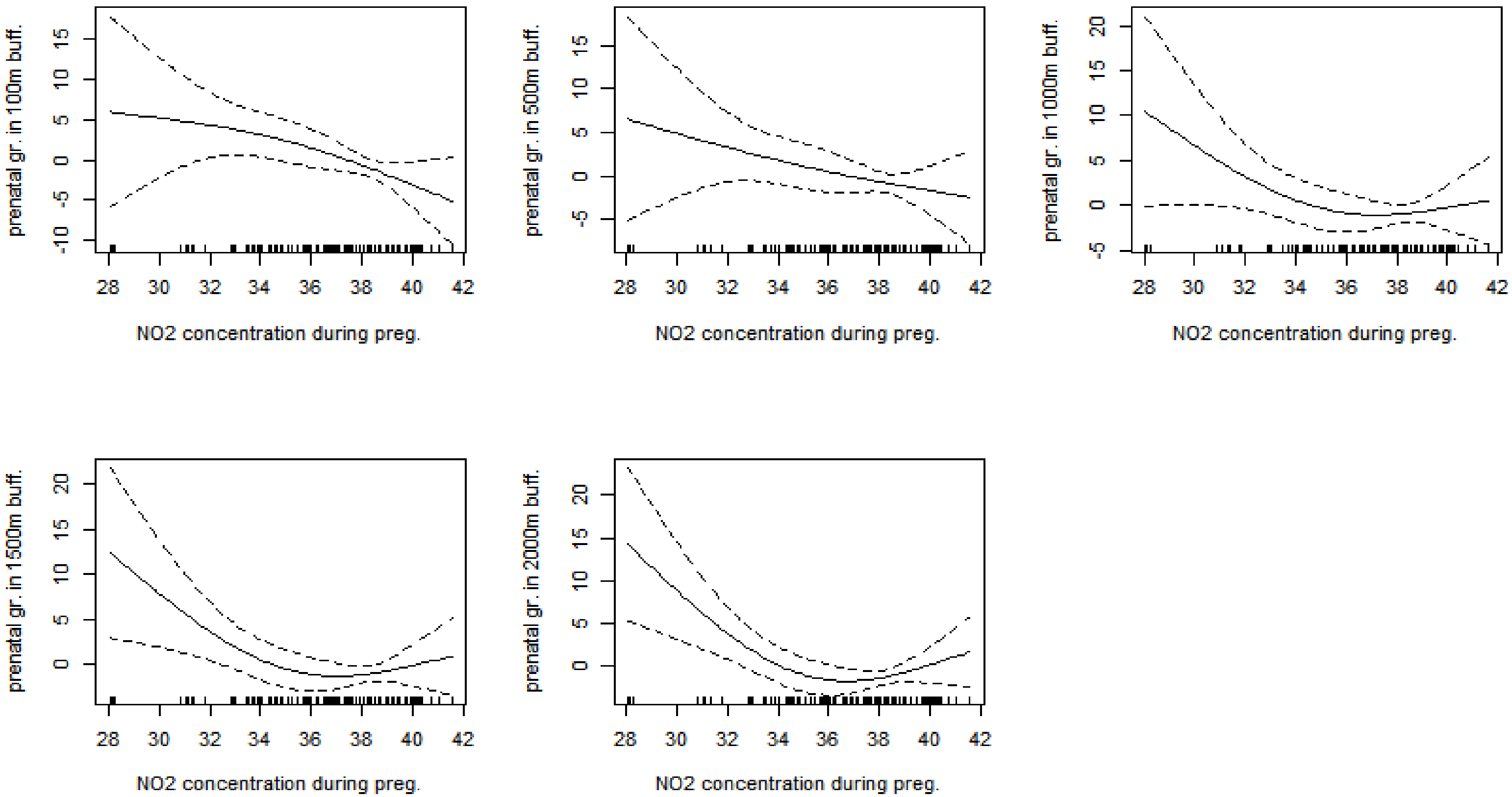

Figure 18. Association between prenatal greenness (%) and NO_2 concentration(ppb) during pregnancy

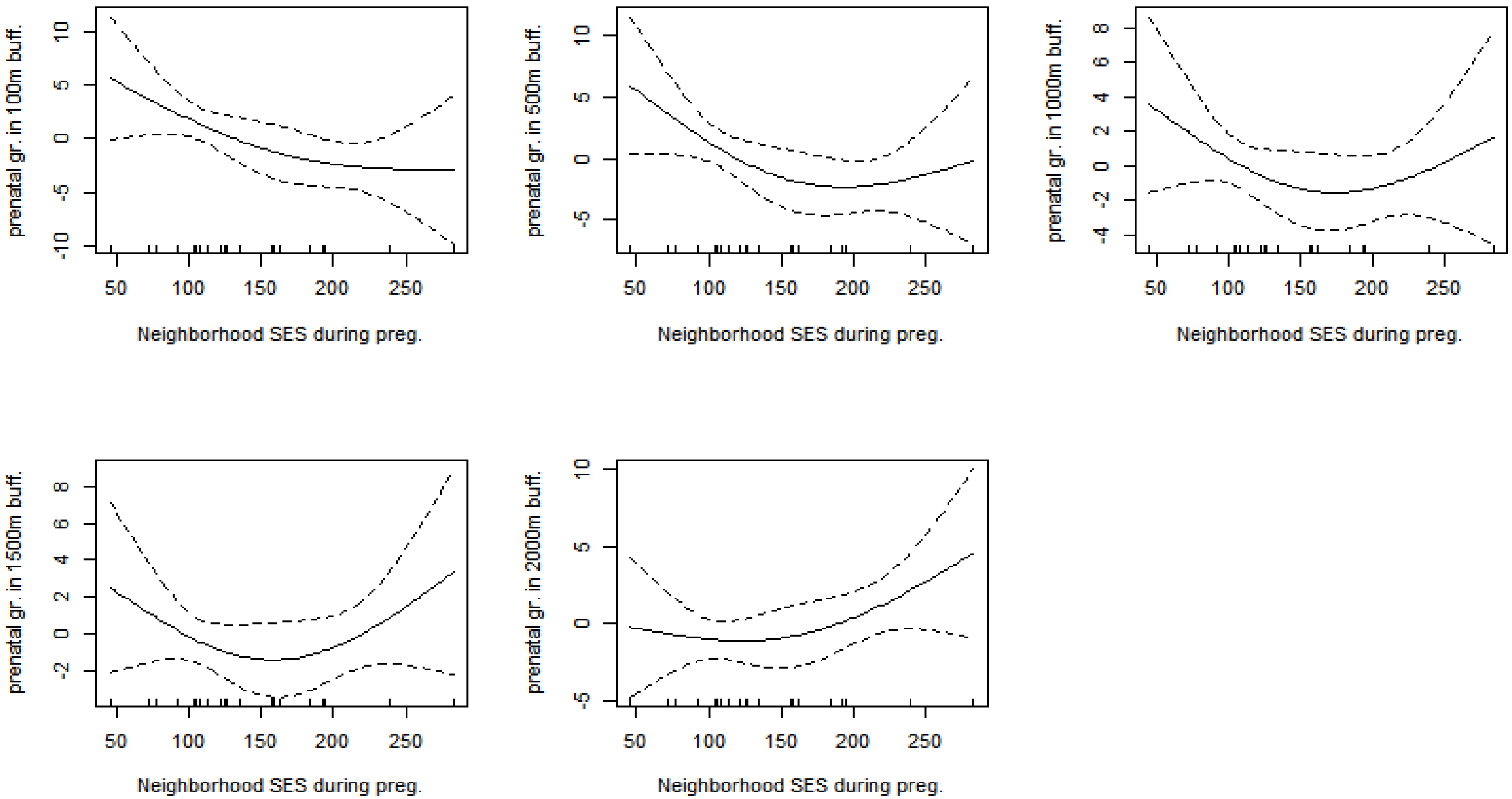

Figure 19. Association between prenatal greenness (%) and neighborhood SES (DI index; score) during pregnancy

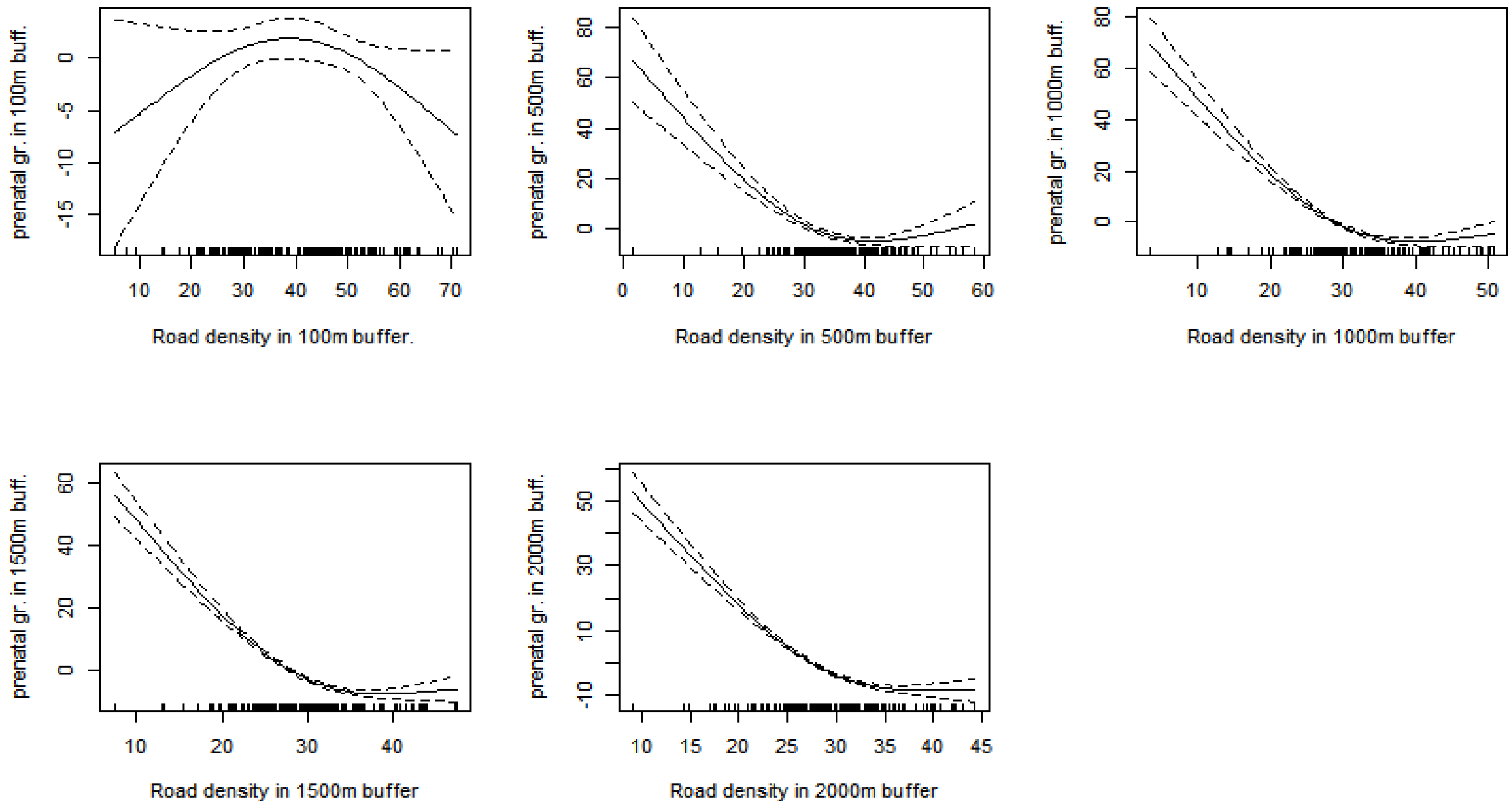

Figure 20. Association between prenatal greenness (%) and percentages of road density (%)

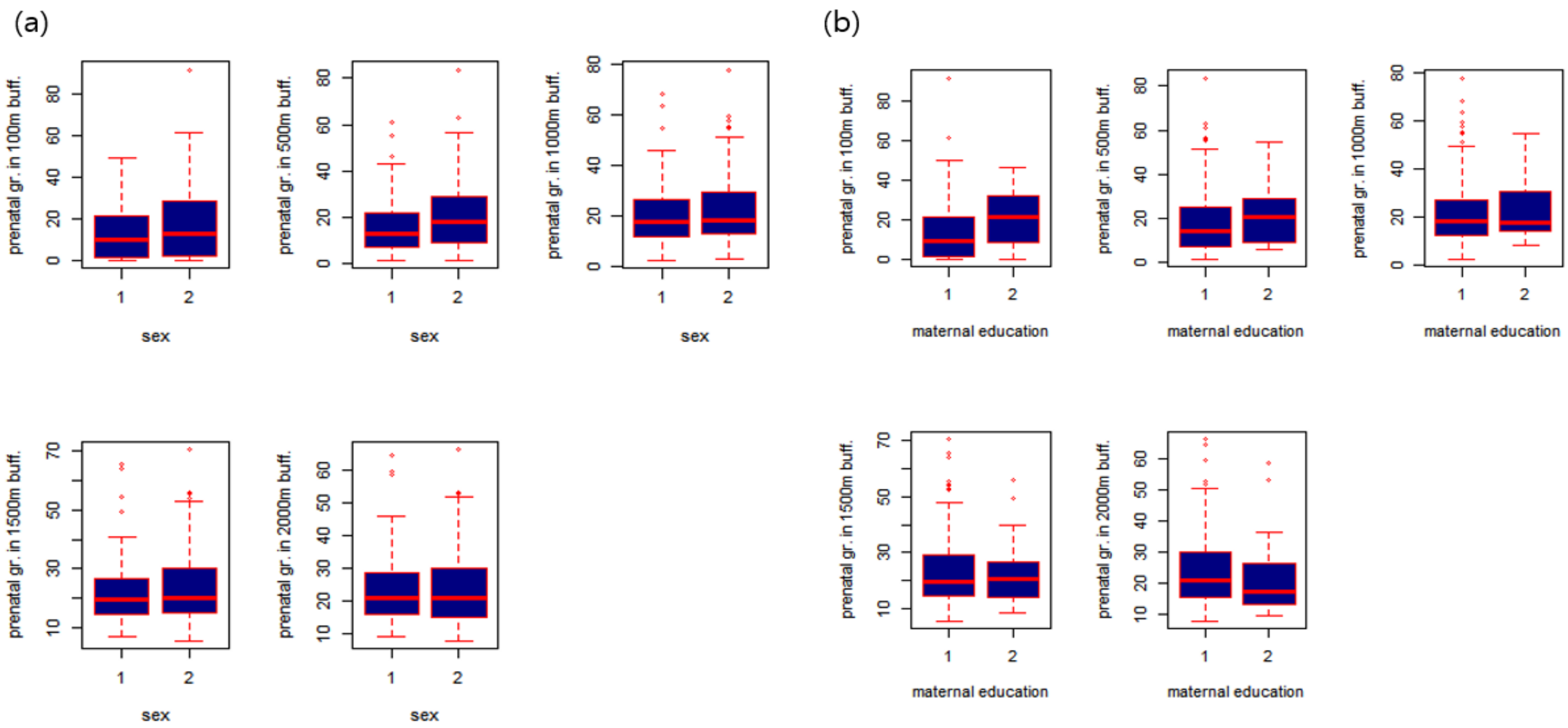

Figure 21. Association between prenatal greenness and (a)children's sex, (b)maternal education level
(Note: Y axis is representing the percentage of greenness during pregnancy in each buffer, X axis is representing 'sex' variable;1: girls, 2: boys, 'maternal education level' variable; 1: low, 2: high)

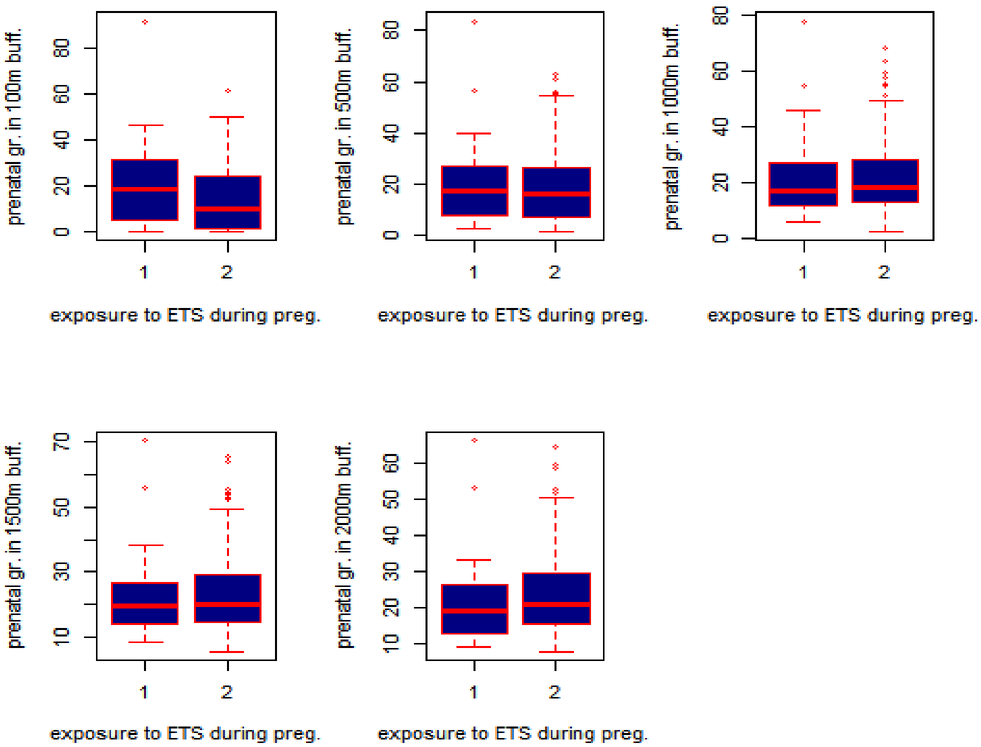

Figure 22. Association between prenatal greenness and exposure to ETS during pregnancy.
(Note: Y axis is representing the percentage of greenness during pregnancy in each buffer, X axis is representing 1: No, 2: Yes)

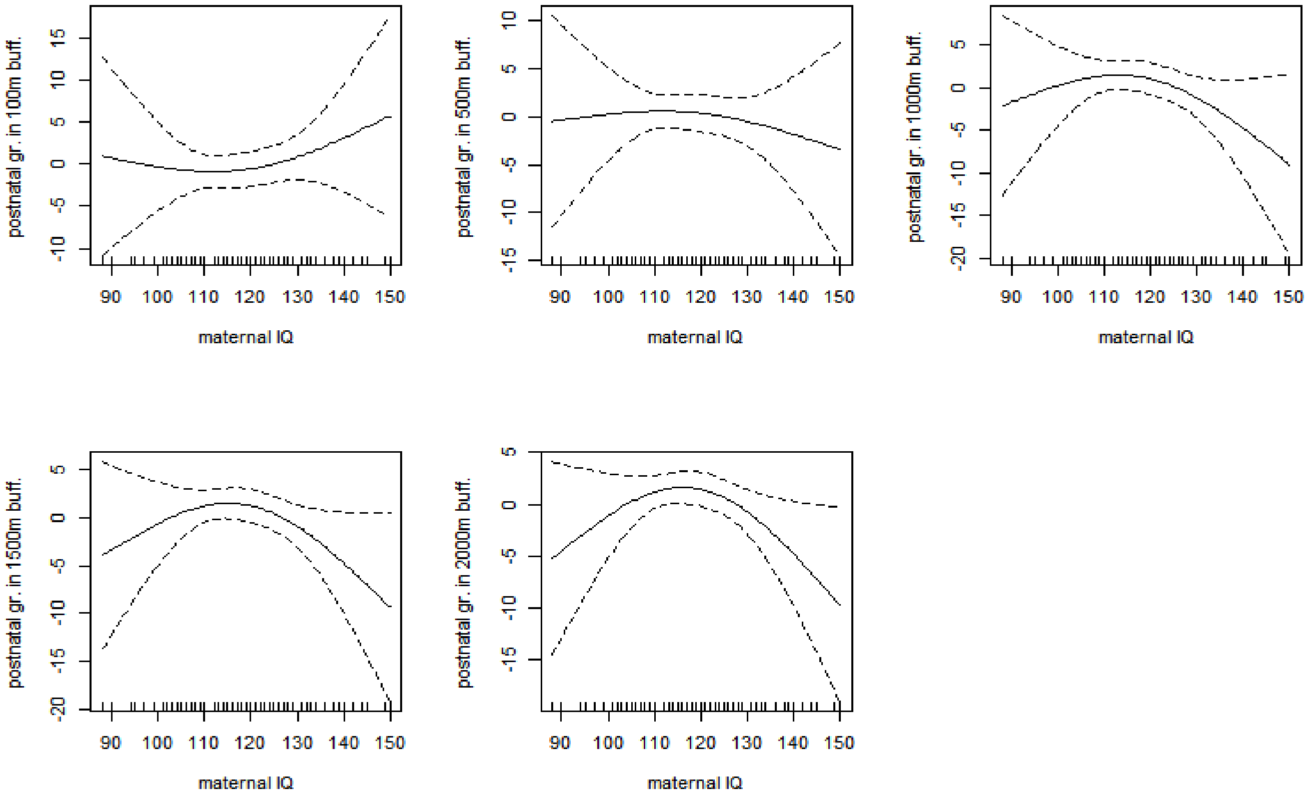

Figure 23. Association between postnatal greenness (%) and maternal IQ (score)

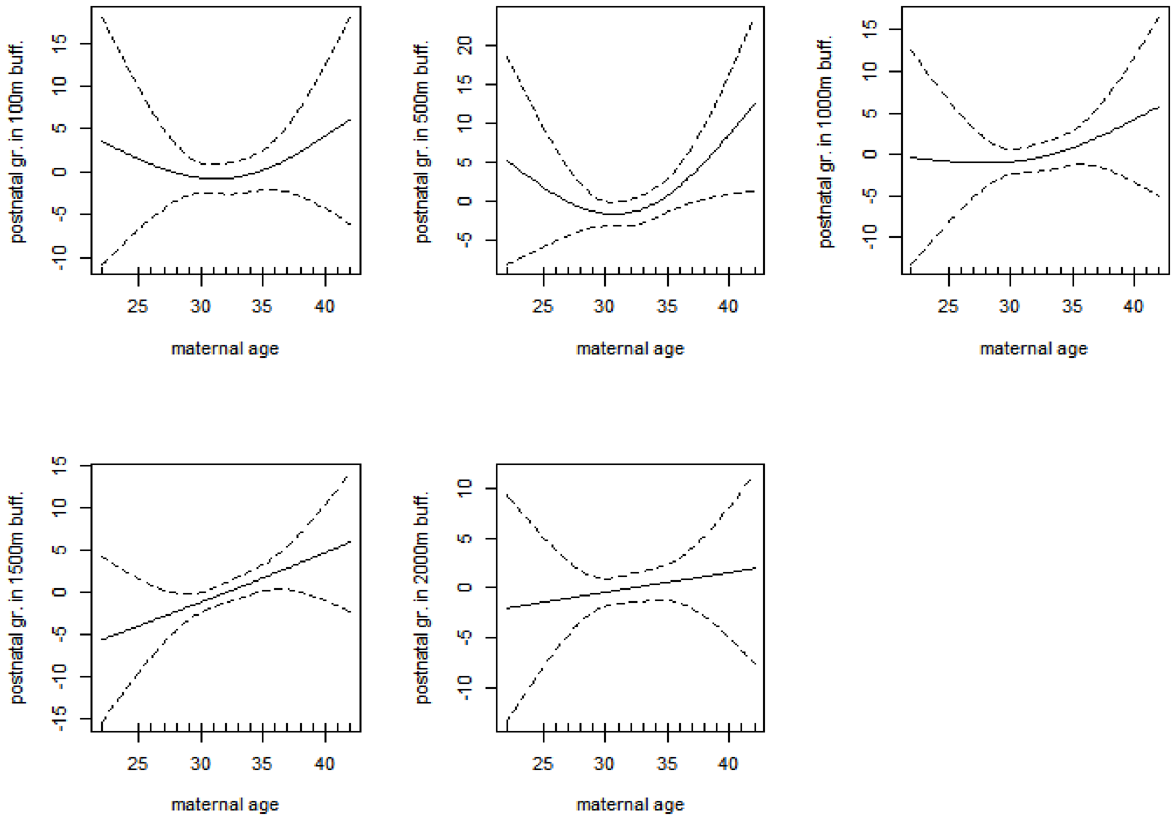

Figure 24. Association between postnatal greenness (%) and maternal age(year)

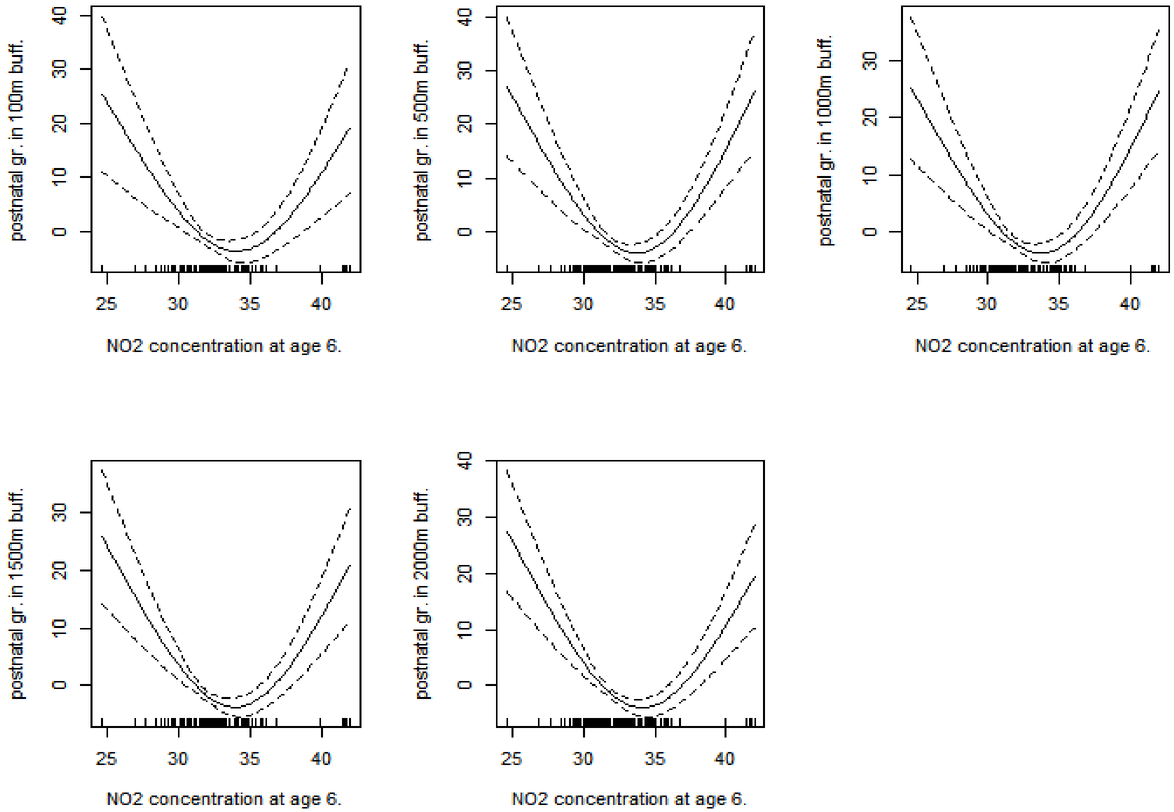

Figure 25. Association between postnatal greenness (%) and NO_2 concentration(ppb) at age 6

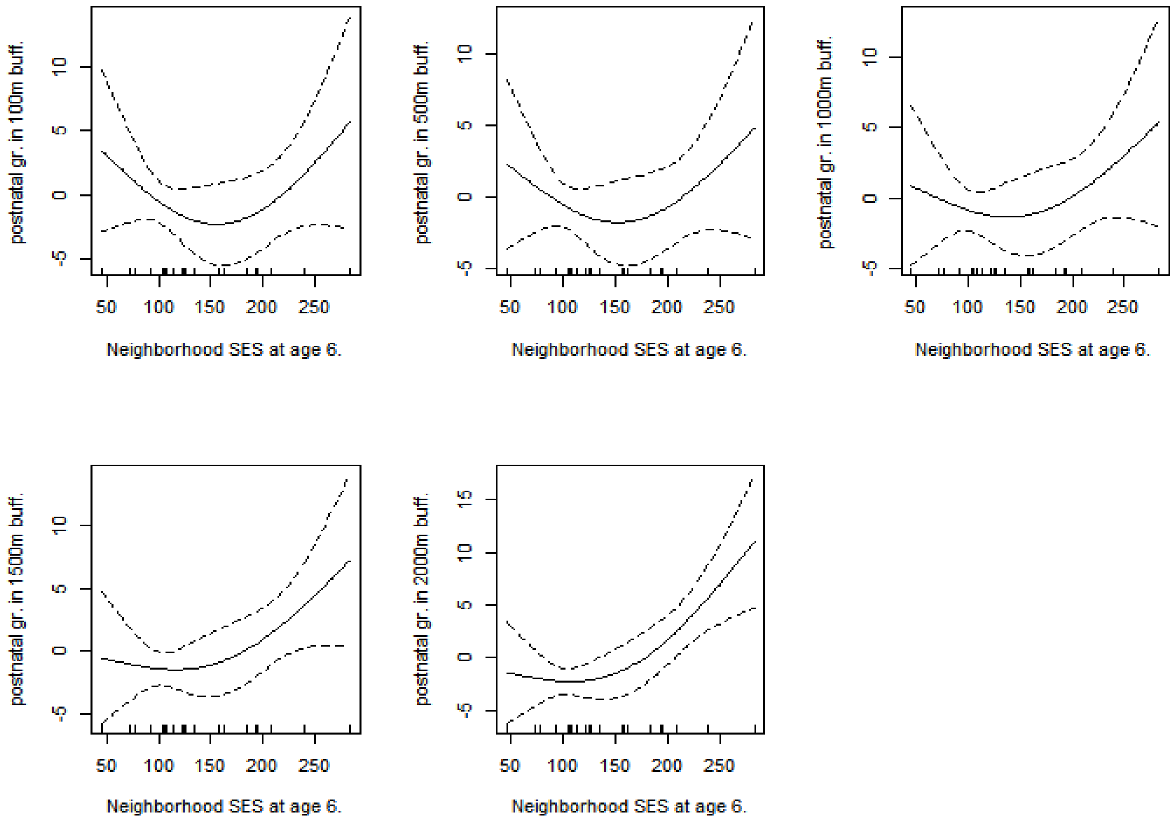

Figure 26. Association between postnatal greenness (%) and neighborhood SES (DI index: score) at age 6

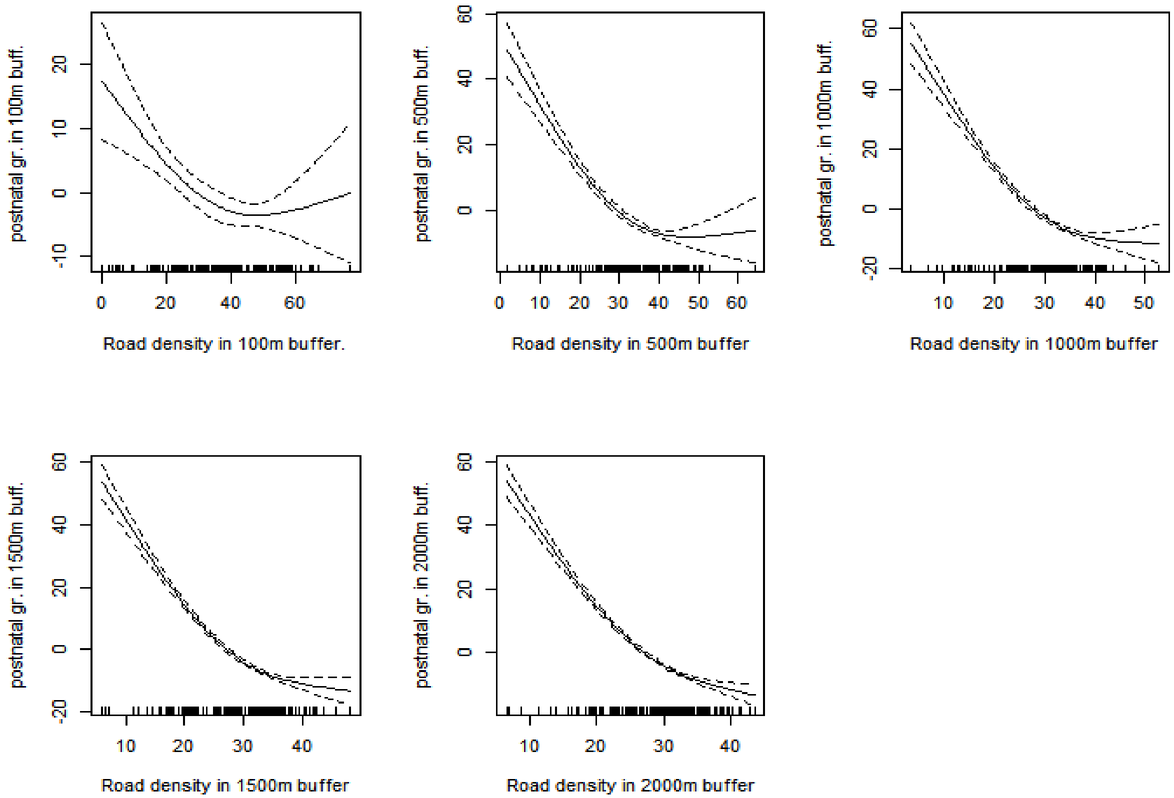

Figure 27. Association between postnatal greenness (%) and the percentage of road density (%) at age 6.

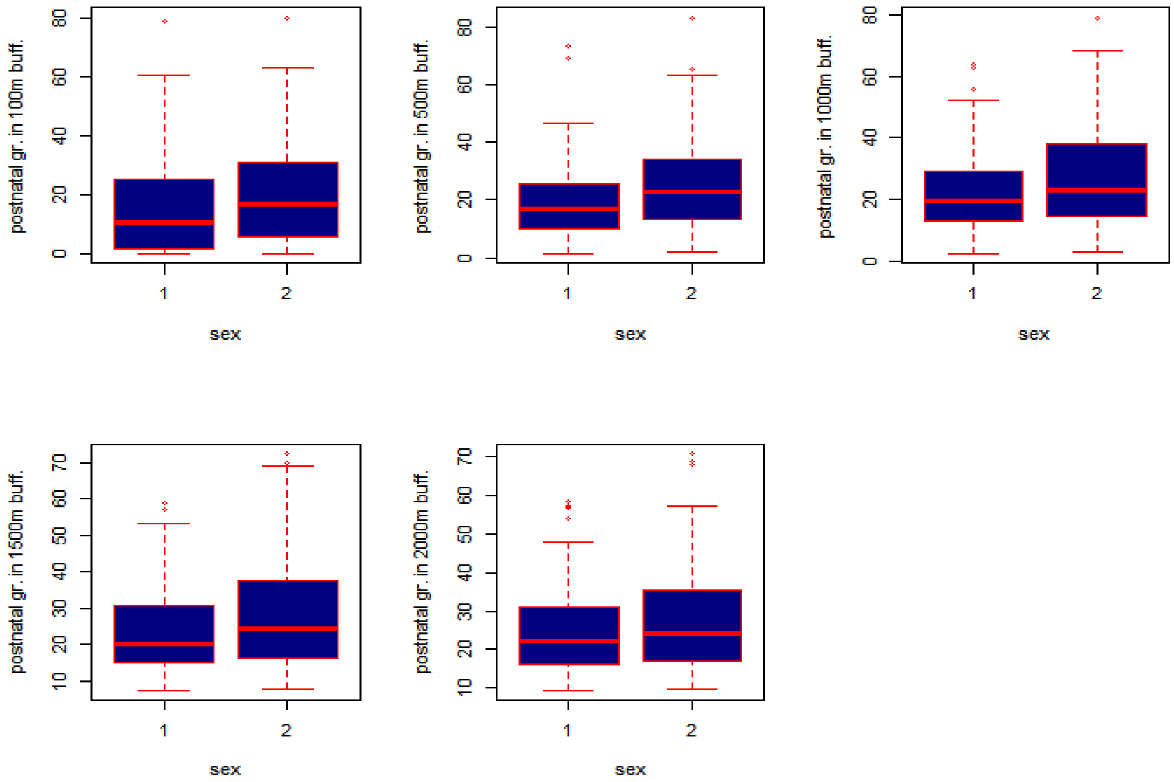

Figure 28. Association between postnatal greenness exposure and children's sex
Note: Y axis is representing the percentage of greenness at age 6 in each buffer, X axis is representing 1: girls, 2: boys)

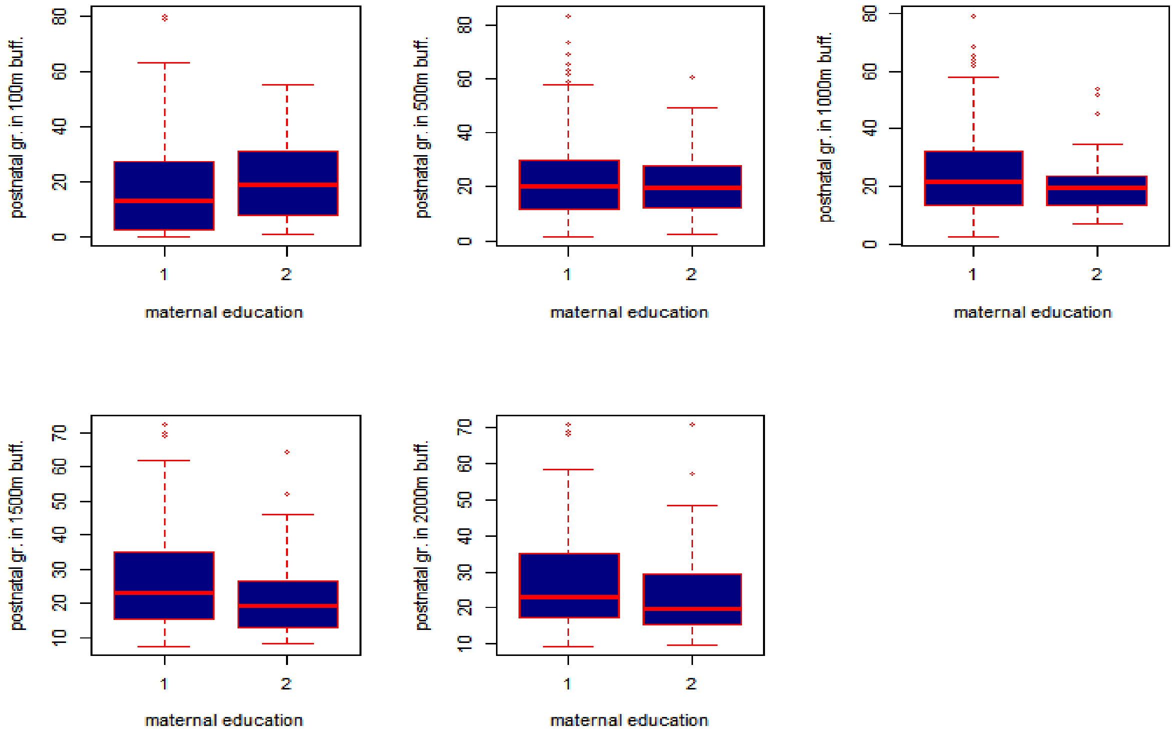

Figure 29. Association between postnatal greenness and maternal education level
(Note: Y axis is representing the percentage of greenness exposure at age 6 in each buffer, X axis is representing 1: low, 2: high)

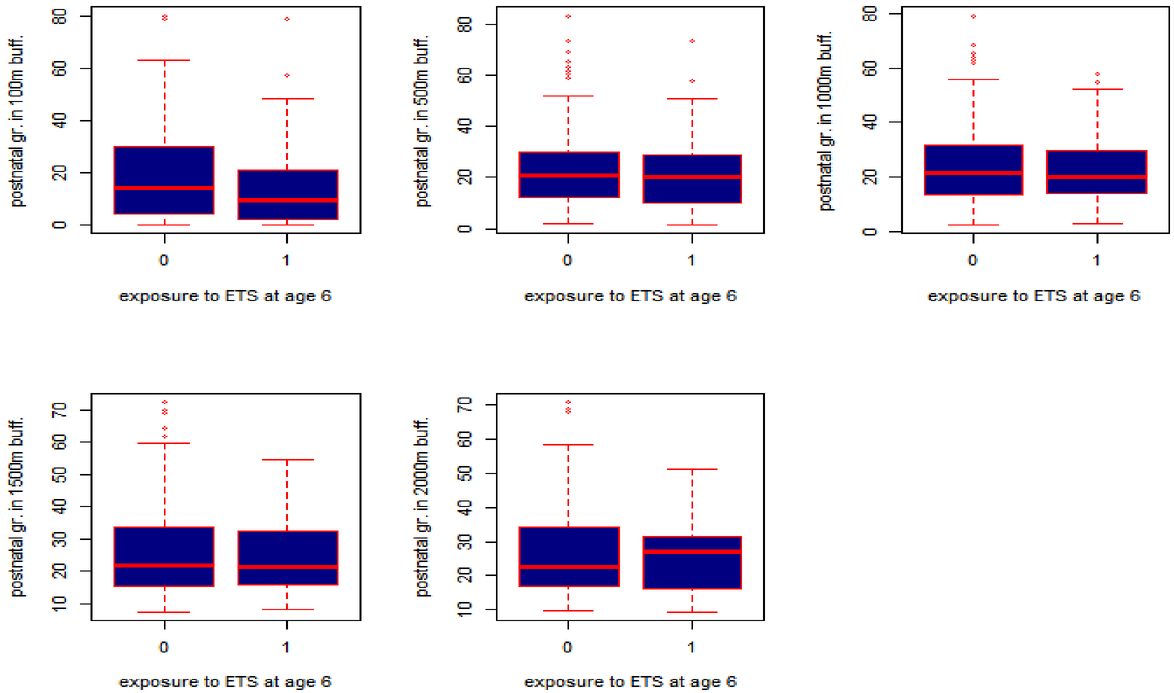

Figure 30. Association between postnatal greenness exposure and exposure to ETS at age 6.
(Note: Y axis is representing the percentage of greenness exposure at age 6 in each buffer, X axis is representing 0: No, 1: Yes)

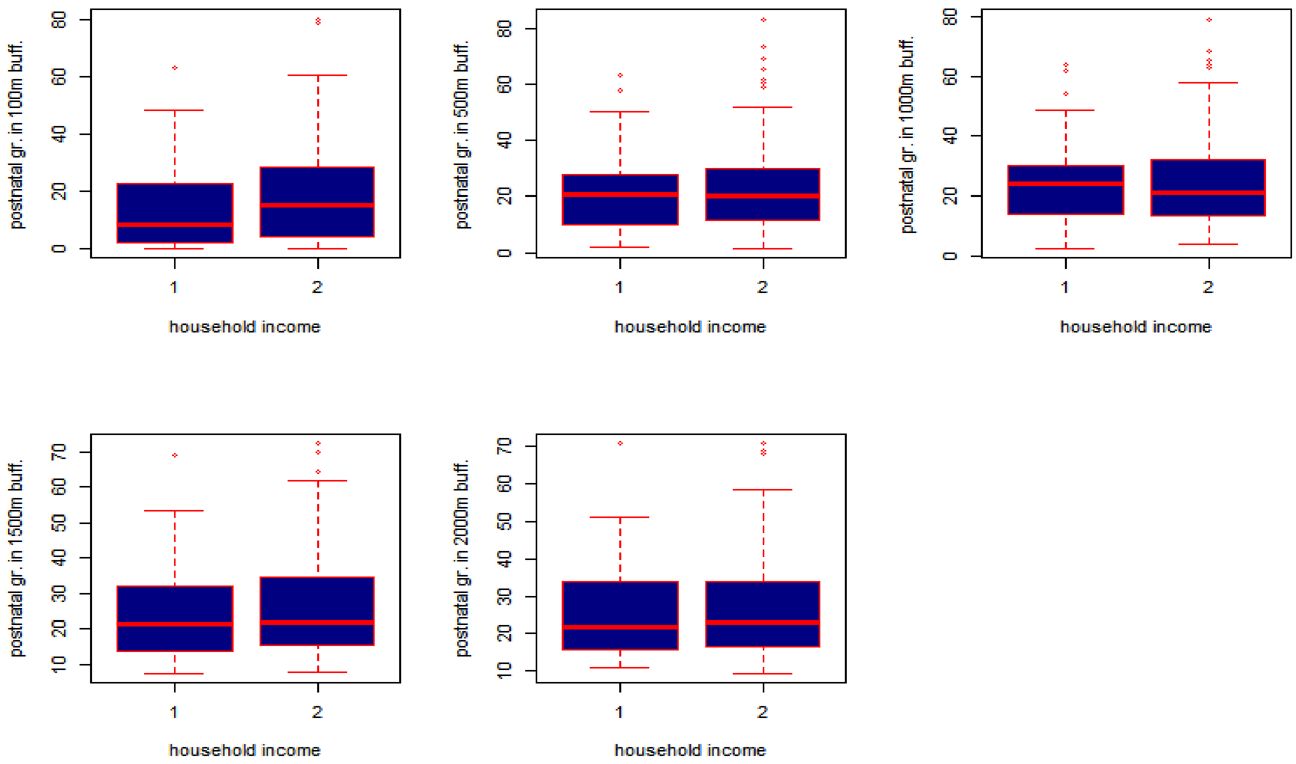

Figure 31. Association between postnatal greenness exposure and household income at age 6.
(Note: Y axis is representing the percentage of greenness exposure at age 6 in each buffer, X axis is representing 1: low, 2: high)

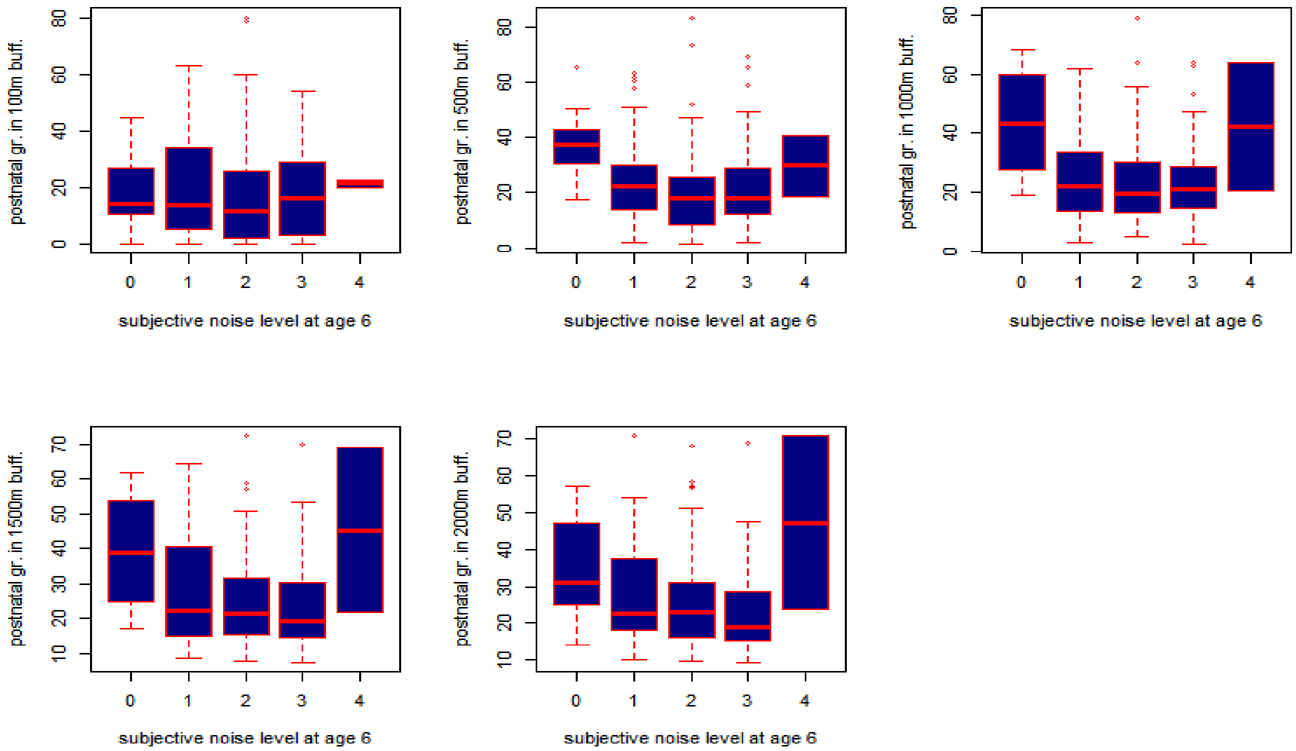

Figure 32. Association between postnatal greenness exposure and subjective noise level at age 6.
(Note: Y axis is representing the percentage of greenness exposure at age 6 in each buffer, X axis is representing 0: Very loud, 1: loud, 2: moderate, 3: quiet, 4: very quiet)

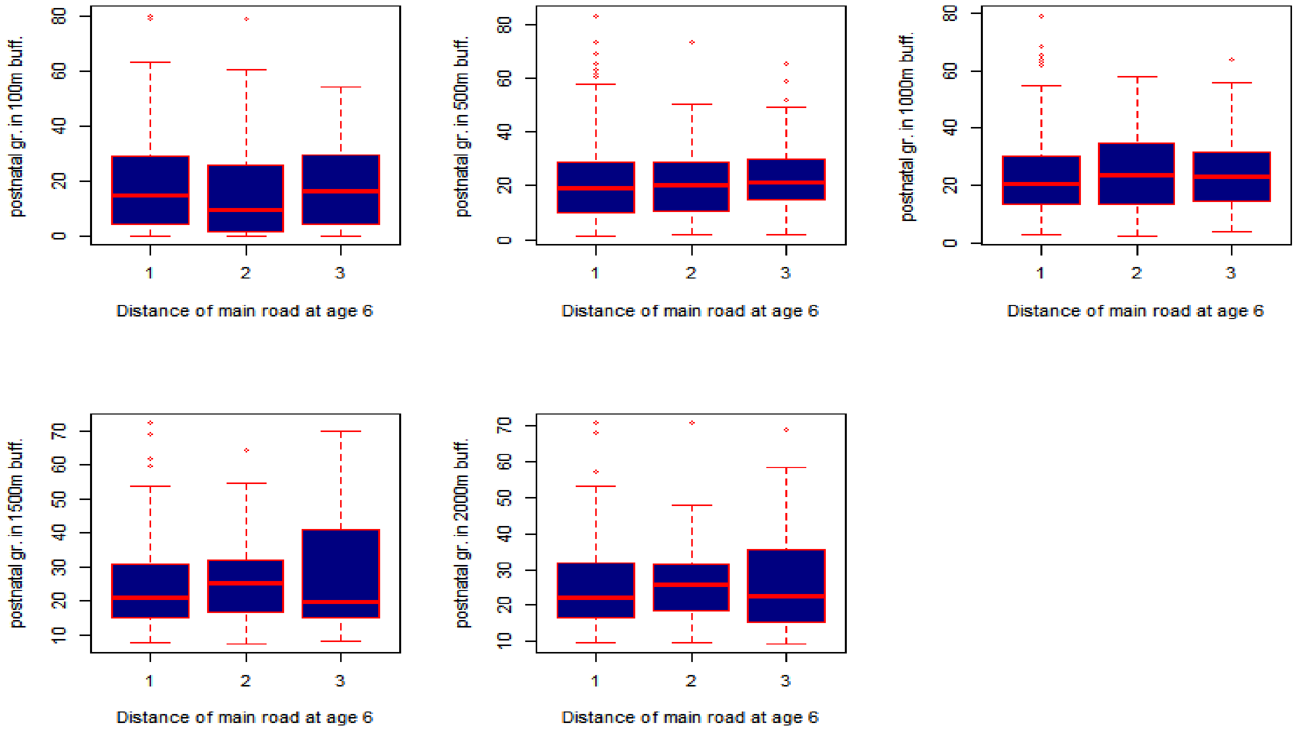

Figure 33. Association between postnatal greenness exposure and distance to main road at age 6.
(Note: Y axis is representing the percentage of greenness exposure at age 6 in each buffer, X axis is representing 1: <50m, 2: 50-499m, 3: 500m)

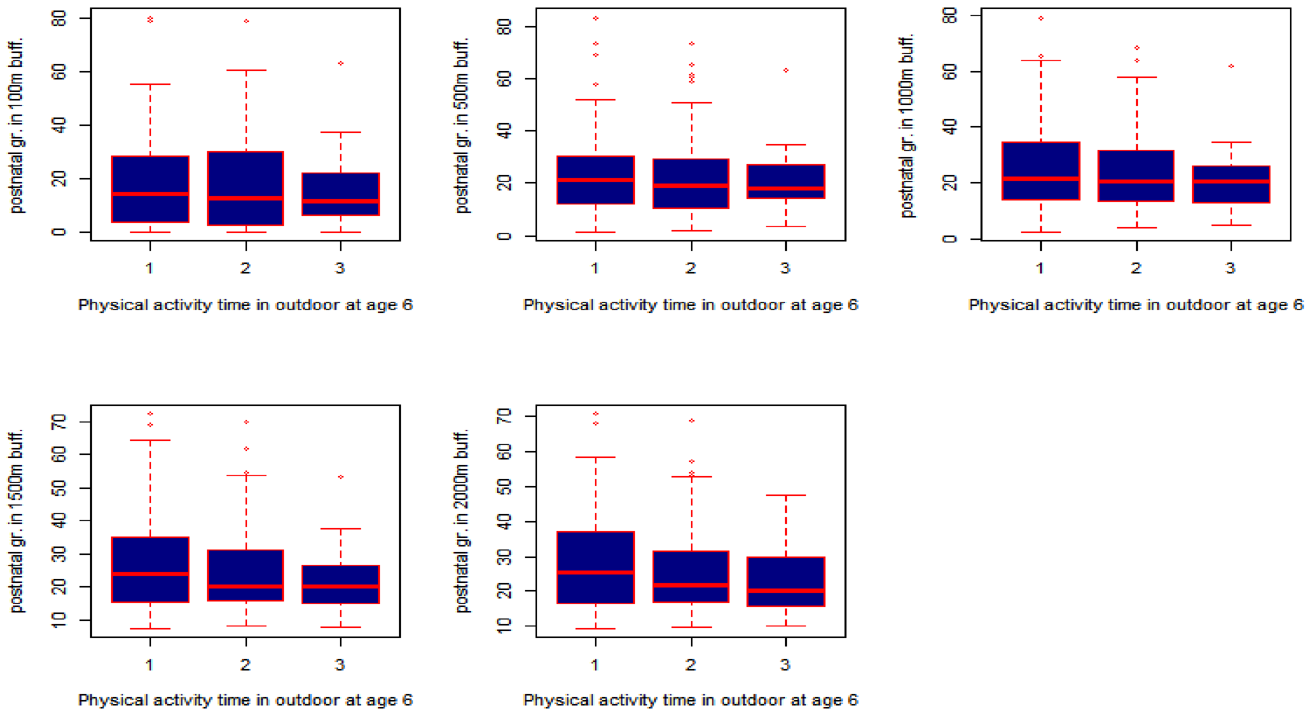

Figure 34. Association between postnatal greenness exposure (%) and physical activity time in outdoor at age 6.
(Note: Y axis is representing the percentage of greenness exposure at age 6 in each buffer, X axis is representing 1: <1hr, 2: 1-2hr, 3: 3hr)

2.6.4. Associations between covariates and children's IQ

Table 7 showed the association between covariates and children's IQ. The mean of children's IQ whose was high group of maternal education level, was higher than lower group (116.0 vs. 109.0; *P*-value=0.004). Maternal IQ was significantly positive association with children's IQ (*P*-value=0.001). The mean of children's IQ whose exposed to ETS at age 6, was higher than non-exposed group (111.10 vs. 106.9; *P*-value=0.049). Residential deprivation index as neighborhood SES at age 6 was negatively associated with children's IQ (*P*-value=0.017). Figure 35-39 were constructed using generalized additive models (GAMs) and a boxplot to investigate the relationship between covariates and children's IQ.

To summarize the association between exposure to greenness, covariates, and children's IQ, we visualized the directions and significance among them (Figure 40, 41). Prenatal greenness was positively associated with the maternal age, and maternal educational level. These covariates were also positively associated with children's IQ. The percentages of road density were negatively associated with exposure to greenness, and children's IQ. Postnatal greenness exposure was associated with the percentage of road density, maternal educational level, children's sex, deprivation index as neighborhood SES, and subjective noise level. Several variables of these covariates such as the percentages of road density, and maternal educational level were also associated with children's IQ at age 6. Further mediation studies for potential mechanisms should be investigated.

Table 7. The association between the covariates and children's IQ

Covariates	Buffer size	Children's IQ	
	Categorical	Mean (SD) or beta (SE)	*P*-value
Children's sex	Girls	109.00(10.18)	0.210
	Boys	111.20(13.39)	
Maternal age during pregnancy		0.23(0.26)	0.375
Maternal education level	**≤ university graduate**	**109.00(11.59)**	**0.004**
	≥ graduate school	**116.00(12.39)**	
Maternal IQ		**0.24(0.07)**	**0.001**
Exposure to ETS during pregnancy	No	114.00(12.20)	0.102
	Yes	109.70(11.91)	
Average of NO_2 concentration during previous 3 years in residential area during pregnancy		0.49(0.36)	0.175
Residential deprivation index as neighborhood SES during pregnancy		-0.02(0.01)	0.205
Road density in each radius during pregnancy	100m	0.13(0.07)	0.048
	500m	0.05(0.11)	0.689
	1000m	-0.04(0.12)	0.735
	1500m	-0.02(0.13)	0.895
	2000m	0.00(0.15)	0.984
Exposure to ETS at age 6	**No**	**111.10(11.73)**	**0.049**
	Yes	**106.90(12.37)**	
Average of NO_2 concentration during previous 3 years in residential area at age 6		-0.02(0.01)	0.205
Road density in each radius at age 6	100m	0.04(0.06)	0.474
	500m	0.03(0.09)	0.737
	1000m	-0.09(0.10)	0.360
	1500m	-0.07(0.12)	0.524
	2000m	-0.07(0.13)	0.595
Household income as personal SES	Low (<$3300)	107.60(12.21)	0.131
	High (≥$3300)	110.90(11.86)	
Residential deprivation index as neighborhood SES at age 6		**-0.03(0.01)**	**0.017**
Subjective noise level	Very Loud	112.70(10.59)	0.257
	Loud	111.19(11.06)	
	Moderate	109.01(12.91)	
	Quiet	109.82(11.01)	
	Very Quiet	126.50(3.54)	
Distance to main road at age 6	≥ 500m	109.69(11.98)	0.705
	50m-499m	111.56(10.79)	
	≤ 50m	109.92(13.28)	
Physical activity time in outdoor at age 6	≤ 1hr	110.00(12.41)	0.963
	1-2hr	110.52(10.73)	
	≥ 3hr	109.90(13.54)	

Abbreviations: NO_2: nitrogen dioxide, IQ: intelligence quotient, ETS: environmental tobacco smoke, SES: Socioeconomic status, SE: Standard Error, SD: Standard Deviation

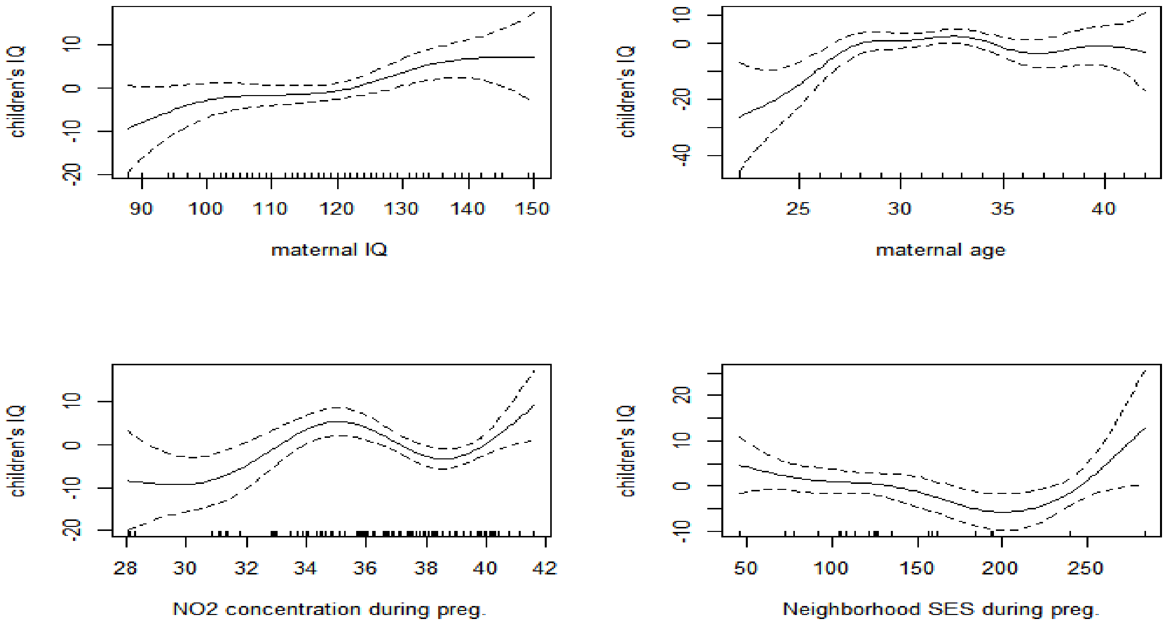

Figure 35. Association between covariates and children's IQ at age 6
(Note: Y axis is representing the score of IQ, X axis is representing maternal IQ (score); maternal age(year); NO_2 concentration during pregnancy(ppb), neighborhood SES during pregnancy (DI index: score))

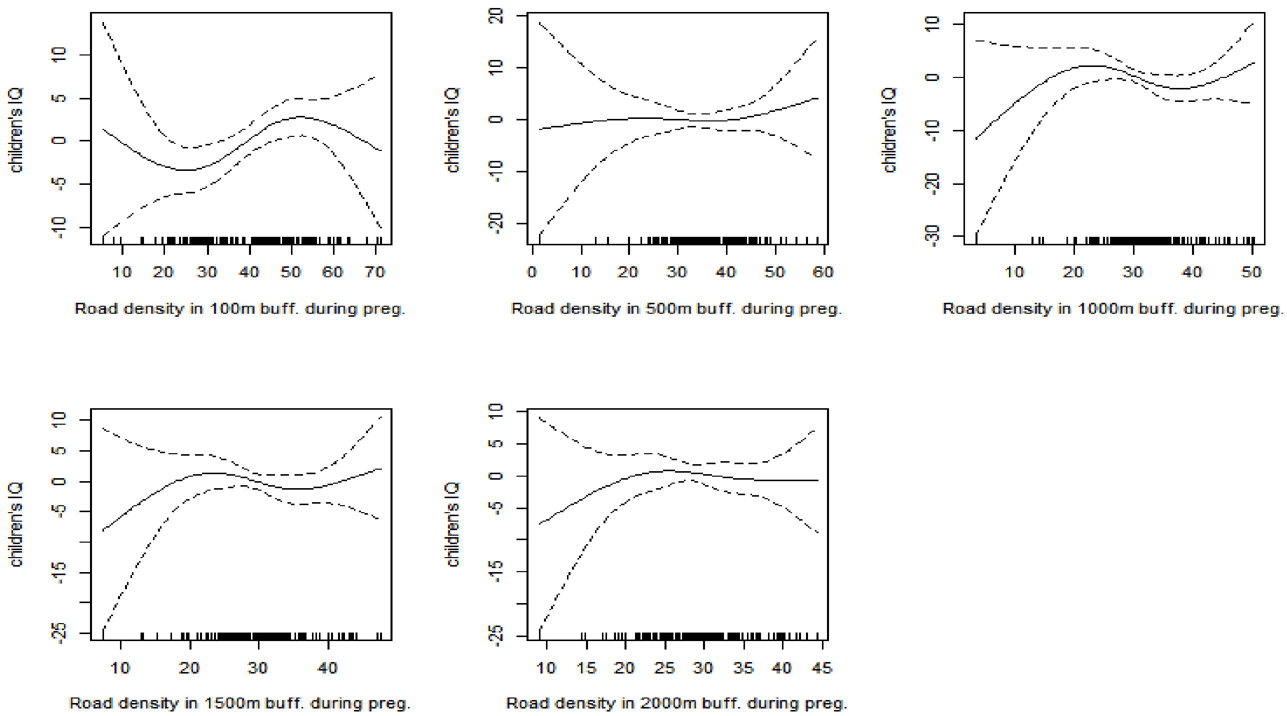

Figure 36. Association between covariates and children's IQ at age 6
(Note: Y axis is representing the score of IQ, X axis is representing the percentage of road density (%) in each buffer during pregnancy)

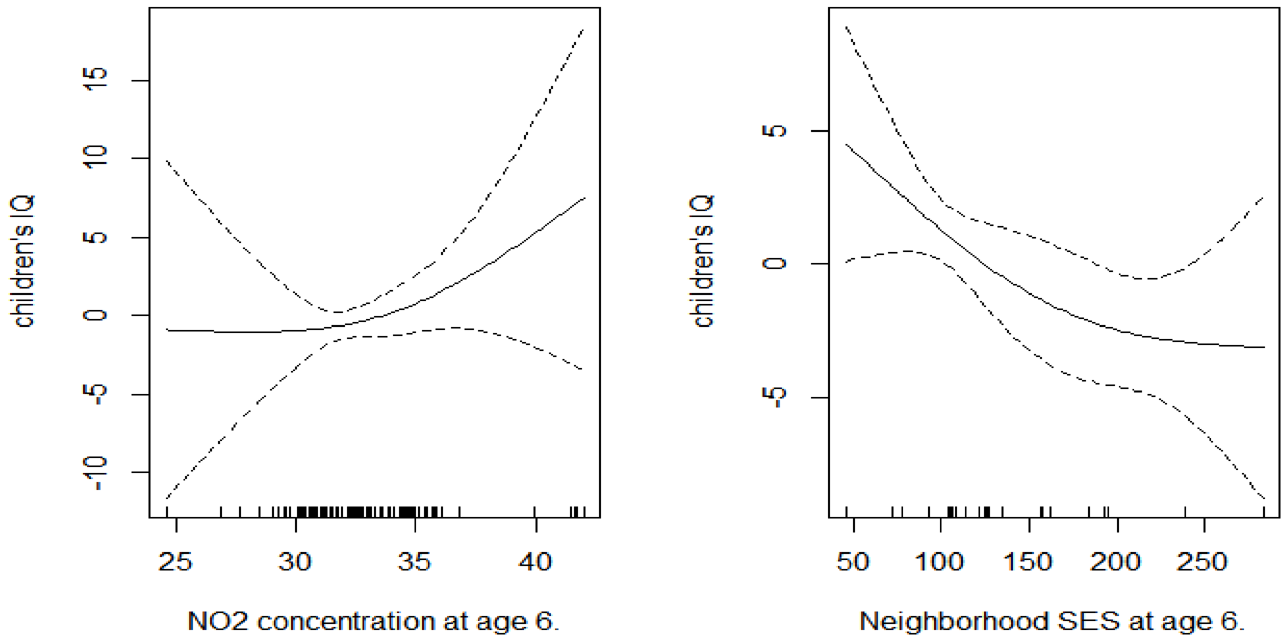

Figure 37. Association between covariates and children's IQ at age 6
(Note: Y axis is representing the score of IQ, X axis is representing NO_2 concentration at age 6(ppb), neighborhood SES at age 6(DI index: score))

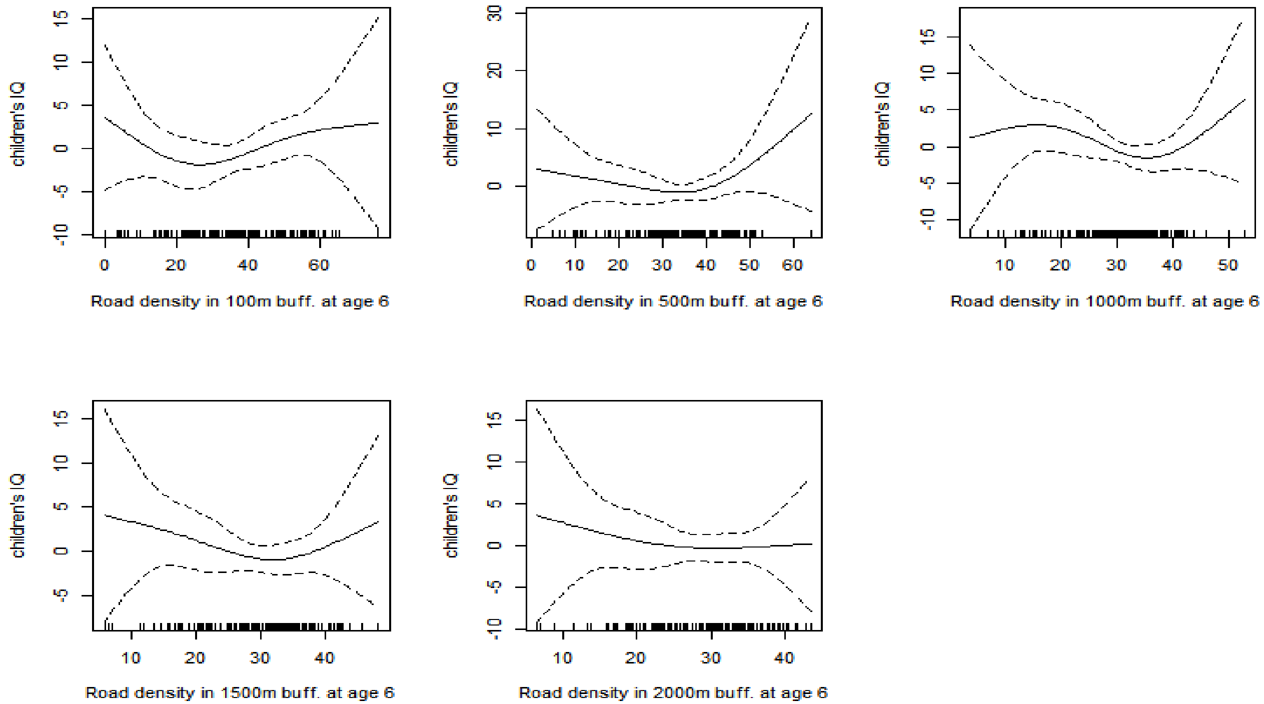

Figure 38. Association between covariates and children's IQ at age 6
(Note: Y axis is representing the score of IQ, X axis is representing the percentage of road density (%) in each buffer at age 6)

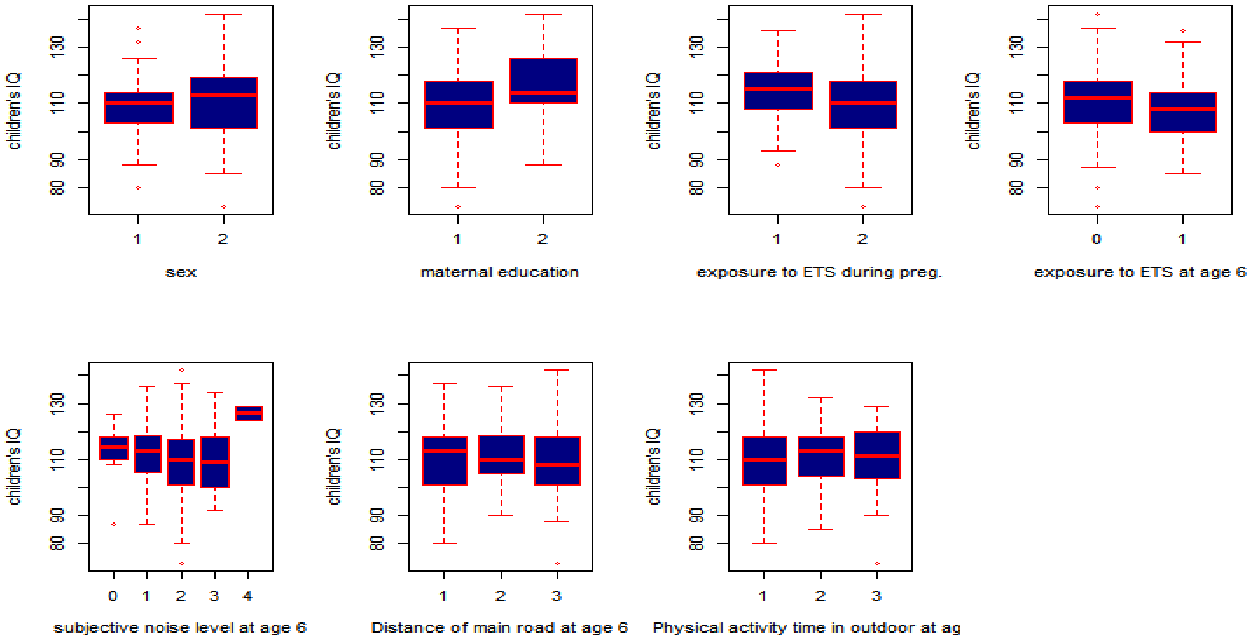

Figure 39. Association between categorical covariates and children's IQ at age 6
(Note: Y axis is representing the score of IQ, X axis is representing sex; 1: girls, 2: boys; maternal education; 1:low, 2: high; exposure to ETS during pregnancy; 1: No, 2: Yes; exposure to ETS at age 6l; 0: No, 1: Yes; subjective noise level at age 6; 0: vary loud, 1:loud, 2: moderate, 3:quiet, 4: very quiet; distance to main road at age 6; 1: <50m, 2: 50-499m, 3: 500m; physical activity time in outdoor at age 6; 1: >1hr, 2: 2-3hr, 3: 3hr)

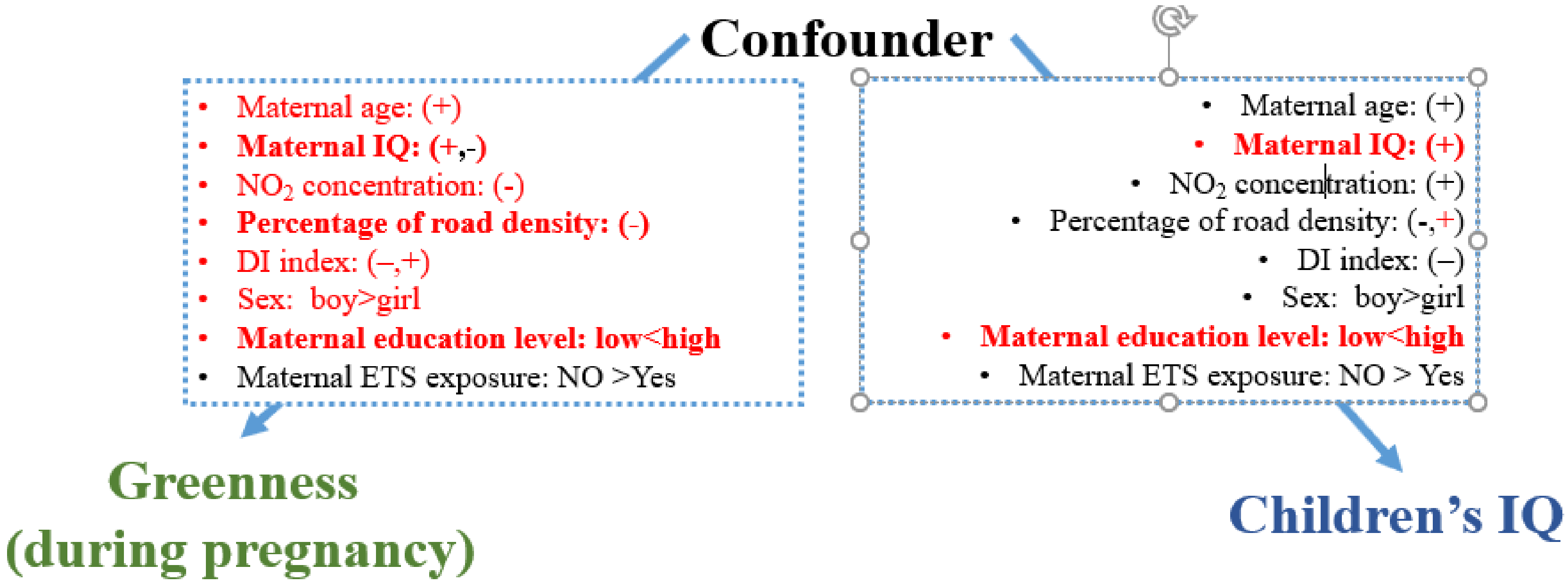

Figure 40. Summary of the association between greenness exposure during pregnancy, covariate, and children's IQ at age 6

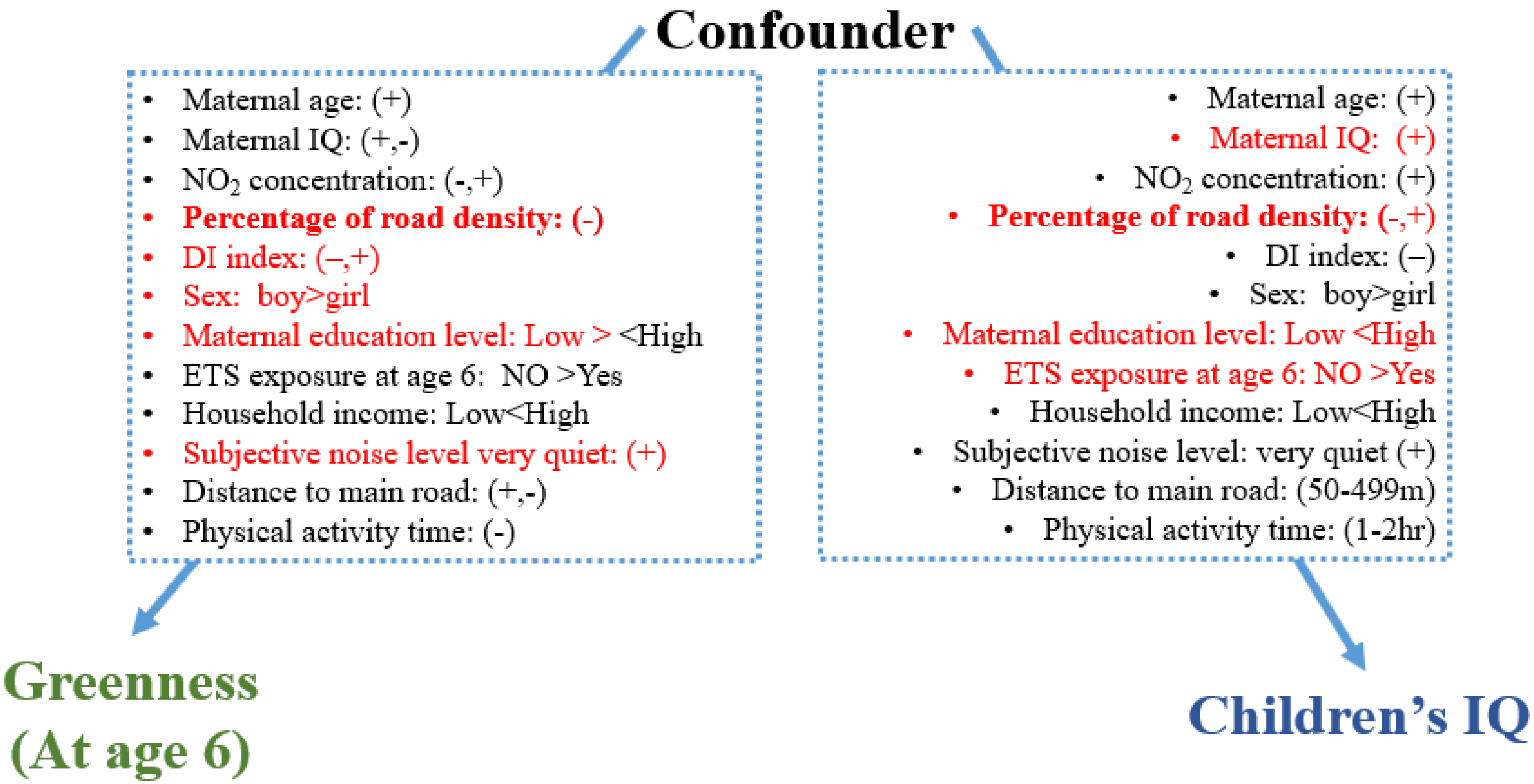

Figure 41. Summary of the association between greenness exposure at age 6, covariates, and children's IQ at age 6

2.6.5. Association between greenness and IQ

Prenatal exposure to built greenness within 500 m and 1000m buffers was positively associated with children's total IQ in an adjusted model [difference in IQ (95% CI): 3.46 (0.68, 6.24) and 3.42 (0.53, 6.31) per IQR increase in greenness percentage] (Table 8). Interestingly, we found a stronger association between total IQ and postnatal exposure to greenness while the child was aged 6 than prenatal exposure to greenness. In addition, we found that built greenness affected children's IQ more effectively than did natural greenness. Table 9 shows the association between verbal IQ and greenness. Both prenatal and postnatal exposure to built greenness within 1000 m and 1500 m buffers were associated with verbal IQ in an adjusted model. Table 10 showed the association between performance IQ and greenness. Prenatal exposure to total greenness within both 100 m and 500 m buffers, and built greenness within 500 m buffer was positively associated with performance IQ. Both total and built greenness in postnatal exposure within 500 m buffer were positively associated with performance IQ. There was no significance in the associations between natural greenness and two subsets including verbal IQ, and performance IQ.

Table 8. Difference (95% confidence interval) in total intelligence quotient per IQR increase in the exposure to residential greenness percentages in buffer area during pregnancy or 6-year-old.

(a)

During pregnancy	Crude			Adjusted[1]		
	Total	Natural	Built	Total	Natural	Built
Buffer	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)
100m	**3.13(0.58, 5.68)***	NA	**3.17(0.52, 5.83)***	2.39(-0.63, 5.41)	NA	1.59(-1.33, 4.51)
500m	**2.54(0.37, 4.72)***	NA	**4.45(2.05, 6.84)***	2.31(-0.47, 5.1)	NA	**3.46(0.68, 6.24)***
1000m	0.83(-1.15, 2.8)	-0.22(-2.14, 1.70)	**3.81(1.36, 6.25)***	-0.12(-2.89, 2.64)	-1.39(-4.42, 1.64)	**3.42(0.53, 6.31)***
1500m	0.16(-1.91, 2.22)	-0.75(-2.84, 1.34)	**3.12(0.93, 5.32)***	-0.65(-3.76, 2.46)	-2.04(-5.52, 1.45)	2.84(0.15, 5.52)
2000m	-0.71(-2.96, 1.54)	-1.21(-3.33, 0.91)	**2.53(0.34, 4.71)***	-1.79(-5.54, 1.96)	-2.62(-6.38, 1.14)	2.24(-0.56, 5.04)

(b)

At age 6	Crude			Adjusted[2]		
	Total	Natural	Built	Total	Natural	Built
Buffer	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)
100m	2.18(-0.29, 4.65)	NA	**3.73(0.89, 6.58)***	1.70(-0.81, 4.22)	NA	**3.15(0.28, 6.03)***
500m	1.53(-0.43, 3.48)	NA	**4.01(1.49, 6.54)***	2.92(0.21, 5.64)	NA	**3.43(0.78, 6.07)***
1000m	1.38(-0.63, 3.40)	0.51(-1.57, 2.59)	**3.35(1.11, 5.60)***	2.49(-1.10, 6.08)	0.10(-3.82, 4.02)	**3.59(1.18, 6.01)***
1500m	0.89(-1.29, 3.06)	0.06(-2.03, 2.15)	**3.39(1.19, 5.59)***	1.17(-3.24, 5.58)	-2.45(-6.89, 2.00)	**4.28(1.78, 6.77)***
2000m	0.33(-1.93, 2.58)	-0.32(-2.59, 1.95)	**3.44(0.93, 5.96)***	-0.67(-5.78, 4.44)	-4.67(-9.76, 0.41)	**5.32(2.35, 8.29)***

1. Adjusted for maternal age, children's sex, maternal education level, maternal IQ, exposure to ETS, average of NO_2 concentration for 3 years in residential area during pregnancy, residential deprivation index as neighborhood SES, the percentages of road density in each radius during pregnancy and the administrative district as random effect.

2. Adjusted for maternal age, children's sex, maternal education level, maternal IQ, exposure to ETS at age 6, average of NO_2 concentration for 3 years in residential area at age 6, household income as personal SES, residential deprivation index as neighborhood SES, subjective noise level, distance to main road from the house at age 6, percentage of road density in each radius at age 6, physical activity time at age 6 and the administrative district as random effect.

**P*-value < 0.05, Abbreviations: IQR; Interquartile range, NA; Not analyzed

Table 9. Difference (95% confidence interval) in verbal intelligence quotient per IQR increase in the exposure to residential greenness percentages in buffer area during pregnancy or 6-year-old.

(a)

During pregnancy	Crude			Adjusted[1]		
	Total	Natural	Built	Total	Natural	Built
Buffer	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)
100m	0.59(-0.31, 1.50)	NA	0.75(-0.24, 1.74)	0.49(-0.51, 1.49)	NA	0.56(-0.56, 1.67)
500m	0.05(-0.76, 0.86)	NA	**1.26(0.36, 2.15)***	0.01(-1.00, 1.03)	NA	0.88(-0.18, 1.94)
1000m	-0.20(-0.92, 0.53)	-0.59(-1.29, 0.12)	**1.44(0.54, 2.34)***	-0.34(-1.61, 0.92)	-0.90(-2.02, 0.23)	**1.19(0.09, 2.29)***
1500m	-0.26(-1.02, 0.50)	-0.63(-1.40, 0.13)	**1.28(0.47, 2.08)***	-0.31(-1.85, 1.22)	-0.89(-2.19, 0.41)	**1.13(0.12, 2.14)***
2000m	-0.47(-1.30, 0.35)	-0.68(-1.46, 0.09)	**1.12(0.32, 1.92)***	-0.71(-2.44, 1.01)	-0.96(-2.37, 0.45)	0.90(-0.15, 1.95)

(b)

At age 6	Crude			Adjusted[2]		
	Total	Natural	Built	Total	Natural	Built
Buffer	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)
100m	0.45(-0.35, 1.24)	NA	**1.23(0.18, 2.28)***	0.29(-0.50, 1.08)	NA	1.10(-0.04, 2.23)
500m	0.03(-0.72, 0.78)	NA	**1.13(0.20, 2.07)***	0.57(-0.50, 1.64)	NA	0.77(-0.28, 1.81)
1000m	0.04(-0.57, 0.66)	-0.29(-1.06, 0.47)	**1.34(0.52, 2.17)***	0.76(-0.65, 2.16)	-0.17(-1.70, 1.35)	**1.27(0.31, 2.22)***
1500m	-0.02(-0.63, 0.59)	-0.35(-1.12, 0.41)	**1.51(0.71, 2.31)***	0.84(-0.89, 2.56)	-0.58(-2.32, 1.15)	**1.63(0.65, 2.61)***
2000m	-0.10(-0.78, 0.58)	-0.37(-1.20, 0.47)	**1.39(0.47, 2.32)***	0.18(-1.82, 2.17)	-1.14(-3.14, 0.86)	**1.75(0.56, 2.93)***

1. Adjusted for maternal age, children's sex, maternal education, maternal IQ, exposure to ETS, average of NO_2 concentration for 3 years in residential area during pregnancy, residential deprivation index as neighborhood SES, percentage of road density in each radius during pregnancy and the administrative district as random effect.
2. Adjusted for maternal age, children's sex, maternal education, maternal IQ, exposure to ETS at age 6, average of NO_2 concentration for 3 years in residential area at age 6, household income as personal SES, residential deprivation index as neighborhood SES, subjective noise level, distance to main road from the house at age 6, percentage of road density in each radius at age 6, physical activity time at age 6 and the administrative district as random effect.

**P*-value < 0.05, Abbreviations: IQR: Interquartile range, NA: Not analyzed

Table 10. Difference (95% confidence interval) in performance intelligence quotient per IQR increase in the exposure to residential greenness percentages in buffer area during pregnancy or 6-year-old.

(a)

During pregnancy	Crude			Adjusted[1]		
	Total	Natural	Built	Total	Natural	Built
Buffer	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)
100m	**1.31(0.40, 2.22)***	NA	**1.28(0.28, 2.28)***	**0.94(-0.04, 1.93)***	NA	0.69(-0.43, 1.80)
500m	**1.52(0.72, 2.32)***	NA	**1.50(0.59, 2.41)***	**1.36(0.37, 2.35)***	NA	**1.27(0.22, 2.33)***
1000m	0.66(-0.08, 1.41)	0.41(-0.32, 1.13)	0.92(-0.01, 1.86)	0.18(-1.09, 1.45)	-0.07(-1.22, 1.07)	0.84(-0.28, 1.95)
1500m	0.35(-0.42, 1.13)	0.15(-0.64, 0.94)	0.69(-0.15, 1.53)	-0.21(-1.75, 1.33)	-0.41(-1.74, 0.92)	0.55(-0.48, 1.59)
2000m	0.05(-0.80, 0.90)	-0.06(-0.86, 0.74)	0.46(-0.37, 1.30)	-0.59(-2.32, 1.14)	-0.63(-2.07, 0.80)	0.39(-0.68, 1.46)

(b)

At age 6	Crude			Adjusted[2]		
	Total	Natural	Built	Total	Natural	Built
Buffer	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)
100m	0.73(-0.08, 1.55)	NA	**1.09(0.01, 2.17)***	0.56(-0.22, 1.33)	NA	0.88(-0.24, 1.99)
500m	**0.92(0.16, 1.68)***	NA	**1.32(0.36, 2.27)***	**1.18(0.15, 2.21)***	NA	**1.16(0.14, 2.17)***
1000m	**0.63(0.01, 1.25)***	0.57(-0.21, 1.35)	0.74(-0.12, 1.60)	0.59(-0.75, 1.93)	0.04(-1.43, 1.51)	0.82(-0.11, 1.75)
1500m	0.44(-0.18, 1.06)	0.39(-0.39, 1.18)	0.60(-0.25, 1.44)	-0.38(-2.02, 1.27)	-1.07(-2.74, 0.60)	0.84(-0.13, 1.81)
2000m	0.28(-0.42, 0.97)	0.18(-0.68, 1.04)	0.75(-0.21, 1.71)	-0.88(-2.76, 1.01)	-1.83(-3.75, 0.09)	**1.34(0.18, 2.50)***

1. Adjusted for maternal age, children's sex, maternal education, maternal IQ, exposure to ETS, average of NO_2 concentration for 3 years in residential area during pregnancy, residential deprivation index as neighborhood SES, percentage of road density in each radius during pregnancy and the administrative district as random effect.

2. Adjusted for maternal age, children's sex, maternal education, maternal IQ, exposure to ETS at age 6, average of NO_2 concentration for 3 years in residential area at age 6, household income as personal SES, residential deprivation index as neighborhood SES, subjective noise level, distance to main road from the house at age 6, percentage of road density in each radius at age 6, physical activity time at age 6 and the administrative district as random effect.

*P-value < 0.05, Abbreviations: IQR: Interquartile range, NA: Not analyzed

A comparison of total IQ between the three groups of the percentages of greenness was observed in Figure 42 and 43. There was no significant association between the prenatal exposure to greenness and total children’s IQ among the three groups (Figure 42). On the other hand, both the group between 25% and 75% in 500m buffer, and more than 75% in 500m to 2000m buffer of postnatal exposure to built greenness were significantly higher total IQ compared to the reference group (below 25% group) (Figure 43).

In a sensitivity analysis, we conducted the built greenness adding golf course, and cemetery parks in Table 11. Prenatal exposure to built greenness in 500m buffer was associated with children’s total IQ. postnatal exposure to total greenness in 500m buffer was also associated with children's total IQ [difference in IQ (95% CI): 4.06 (1.41, 6.70) per IQR increase in greenness percentage].

We also estimated an average percentage of exposure to greenness from pregnancy to 6 years old, then we investigated the association between the average percentage of exposure to greenness and children’s IQ (Table 12). We found that the positive associations between the percentage of both total greenness within 100-500m buffers and built greenness within 500 - 2km buffers, and children’s IQ in adjusted model.

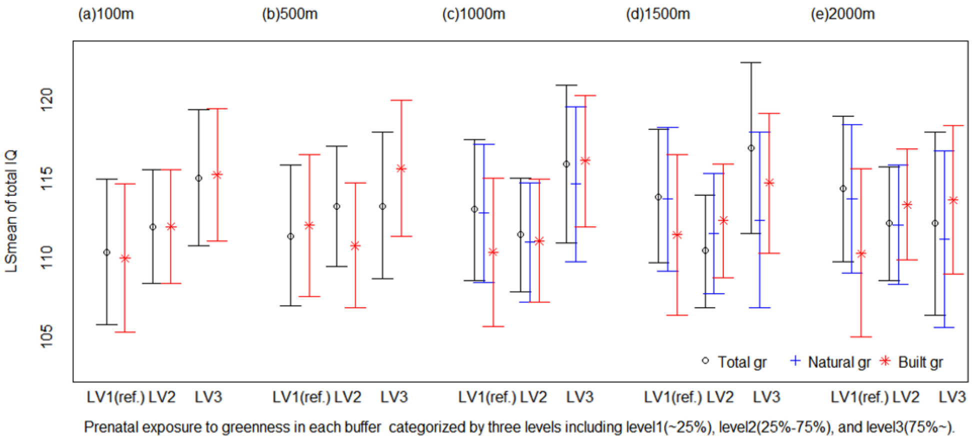

Figure 42. The plot of the LSMEANS of total intelligence quotient by prenatal exposure to greenness as a categorical variable. (Note: Greenness percentage in each buffer radius; LV1: below 25%(reference), LV2: between 25 and 75%, LV3: more than 75%).

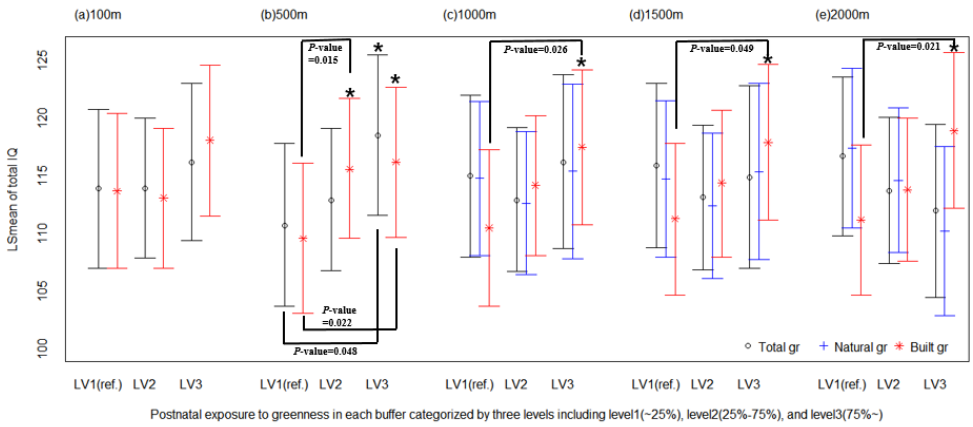

Figure 43. The plot of the LSMEANS of total intelligence quotient by postnatal exposure to greenness as a categorical variable. (Note: Greenness percentage in each buffer radius; LV1: below 25%(reference), LV2: between 25 and 75%, LV3: more than 75%. **P*-value <0.05)

Table 11. Difference (95% confidence interval) in **total intelligence quotient** per IQR increase in the exposure to residential greenness percentages in buffer area during pregnancy or 6-year-old.

(a)

During pregnancy	Crude			Adjusted[1]		
	Total	Natural	Built	Total	Natural	Built
Buffer	Difference (95%CI)	Difference (95%CI)	Difference (95%CI)	Difference (95%CI)	Difference (95%CI)	Difference (95%CI)
100m	**3.14(0.58, 5.70)***	NA	**3.18(0.52, 5.85)***	2.53(-0.41, 5.48)	NA	1.87(-0.99, 4.72)
500m	**2.52(0.36, 4.68)***	NA	**4.43(2.03, 6.83)***	2.48(-0.23, 5.20)	NA	**3.44(0.64, 6.23)***
1000m	0.71(-1.26, 2.68)	-0.22(-2.14, 1.70)	**3.31(0.91, 5.72)***	0.02(-2.71, 2.76)	-1.39(-4.42, 1.64)	2.66(-0.11, 5.42)
1500m	-0.12(-2.23, 1.98)	-0.75(-2.84, 1.34)	**2.2(0.03, 4.38)***	-0.99(-4.13, 2.16)	-2.04(-5.52, 1.45)	1.32(-1.17, 3.80)
2000m	-0.96(-3.30, 1.38)	-1.21(-3.33, 0.91)	1.6(-0.53, 3.74)	-2.12(-5.92, 1.69)	-2.62(-6.38, 1.14)	0.74(-1.76, 3.23)

(b)

At age 6	Crude			Adjusted[2]		
	Total	Natural	Built	Total	Natural	Built
Buffer	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)
100m	**2.42(0.11, 4.74)***	NA	**3.54(1.08, 6.00)***	2.35(-0.11, 4.80)	NA	**3.27(0.75, 5.80)***
500m	1.69(-0.18, 3.55)	NA	**3.17(1.15, 5.20)***	**4.06(1.41, 6.70)***	NA	**2.80(0.68, 4.91) ***
1000m	1.28(-0.70, 3.25)	0.51(-1.57, 2.59)	**2.37(0.41, 4.34)***	2.57(-0.88, 6.03)	0.10(-3.82, 4.02)	**2.53(0.48, 4.57) ***
1500m	0.64(-1.67, 2.95)	0.06(-2.03, 2.15)	2.01(-0.14, 4.16)	0.41(-4.16, 4.97)	-2.45(-6.89, 2.00)	**2.66(0.42, 4.89) ***
2000m	-0.02(-2.35, 2.31)	-0.32(-2.59, 1.95)	1.61(-0.89, 4.12)	-2.00(-6.95, 2.94)	-4.67(-9.76, 0.41)	**3.01(0.35, 5.66) ***

1. Adjusted for maternal age, children's sex, maternal education level, maternal IQ, exposure to ETS, average of NO_2 concentration for 3 years in residential area during pregnancy, residential deprivation index as neighborhood SES, the percentages of road density in each radius during pregnancy and the administrative district as random effect.

2. Adjusted for maternal age, children's sex, maternal education level, maternal IQ, exposure to ETS at age 6, average of NO_2 concentration for 3 years in residential area at age 6, household income as personal SES, residential deprivation index as neighborhood SES, subjective noise level, distance to main road from the house at age 6, percentage of road density in each radius at age 6, physical activity time at age 6 and the administrative district as random effect. *Bold represented *P*-value < 0.05, Abbreviations: IQR: Interquartile range, NA; Not analyzed

Table 12. Difference (95% confidence interval) in **total intelligence quotient** per IQR increase in the average percentage of exposure to residential greenness percentages in buffer area from during pregnancy to 6 years old.
(a)

The average of greenness	Crude			Adjusted[1]		
	Total	Natural	Built	Total	Natural	Built
Buffer	Difference (95%CI)	Difference (95%CI)	Difference (95%CI)	Difference (95%CI)	Difference (95%CI)	Difference (95%CI)
100m	**3.59(0.96, 6.23)***	NA	**4.01(1.22, 6.80)***	**3.44(0.46, 6.41)***	NA	2.74(-0.17, 5.65)
500m	**2.17(0.12, 4.22)***	NA	**4.35(1.90, 6.80)***	**3.29(0.77, 5.82)***	NA	**3.25(0.54, 5.96)***
1000m	1.17(-0.95, 3.29)	0.05(-1.70, 1.81)	**4.05(1.56, 6.55)***	2.29(-0.53, 5.11)	0.82(-1.65, 3.28)	**3.40(0.62, 6.18)***
1500m	0.38(-1.74, 2.50)	-0.47(-2.60, 1.65)	**3.71(1.24, 6.18)***	1.86(-1.16, 4.88)	0.66(-2.53, 3.85)	**3.25(0.41, 6.09)***
2000m	-0.29(-2.48, 1.90)	-0.90(-3.07, 1.27)	**3.58(0.94, 6.22)***	1.02(-2.17, 4.20)	0.02(-3.27, 3.31)	**3.47(0.26, 6.67)***

Adjusted for maternal age, children's sex, maternal education level, maternal IQ, exposure to ETS, average of NO_2 concentration for 3 years in residential area during pregnancy, residential deprivation index as neighborhood SES, the percentages of road density in each radius during pregnancy and the administrative district as random effect.
*Bold represented *P*-value < 0.05, Abbreviations: IQR; Interquartile range, NA; Not analyzed

2.6.6. Association between proxy to greenness and children's IQ

In a sensitivity analysis, we conducted the association between proxy to greenness and children's IQ. The distribution of greenness presented in Figure 44. We found that a positive association between proxy greenness exposure and children's IQ was observed (Figure 45). There were a negative association between the proxy to greenness and children's IQ in adjusted model (Table 13). So, we suggested that both the greenness density and proxy to greenness have beneficial association for children's cognition.

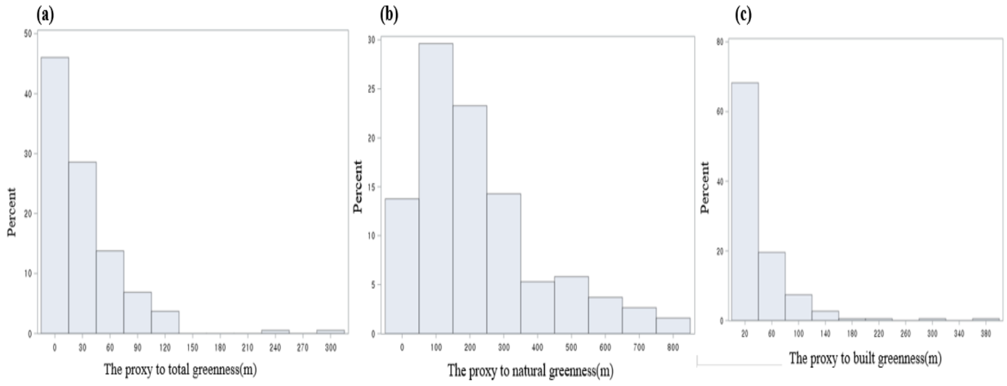

Figure 44. The distribution of the proxy to greenness.
(Note: (a) x axis represents the proxy to total greenness(m), (b) x axis represents the proxy to natural greenness(m), (c) x axis represents the proxy to built greenness(m)

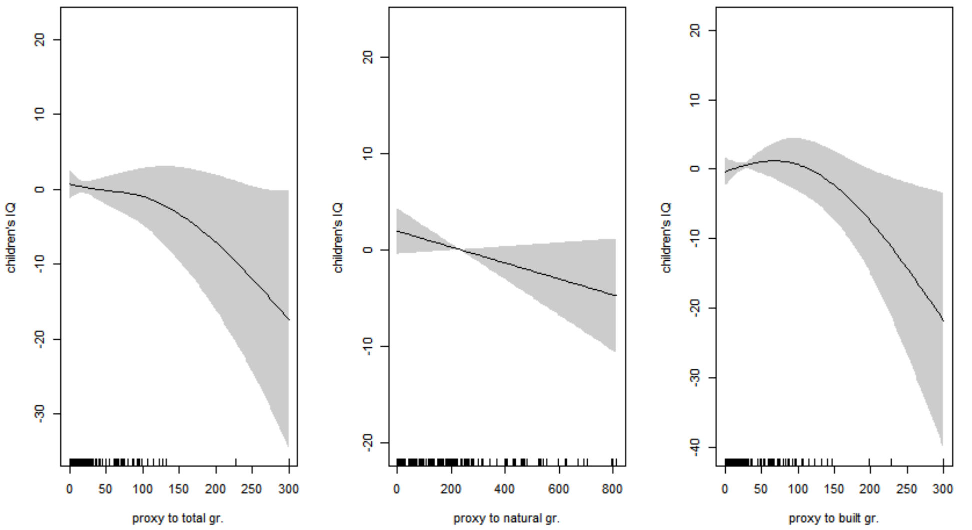

Figure 45. Association between proxy to greenness and children's IQ
(Note: the models adjusted for maternal age, children's sex, maternal education level, maternal IQ, exposure to ETS, average of NO_2 concentration for 3 years in residential area during pregnancy, residential deprivation index as neighborhood SES, the percentages of road density in each radius during pregnancy)

Table 13. Association between proxy to greenness and children's IQ

	Crude model		Adjusted model	
Proxy to greenness	Difference (95%CI)	*P*-value	Difference (95%CI)	*P*-value
Total greenness	**-1.93(-3.62, -0.24)**	**0.027**	-1.41(-3.19, 0.36)	0.122
Natural greenness	-0.86(-2.78, 1.06)	0.381	-1.46(-3.52, 0.60)	0.168
Built greenness	-1.54(-3.19, 0.11)	0.069	-1.44(-3.47, 0.59)	0.167

Adjusted for maternal age, children's sex, maternal education level, maternal IQ, exposure to ETS, average of NO_2 concentration for 3 years in residential area during pregnancy, residential deprivation index as neighborhood SES, the percentages of road density in each radius during pregnancy and the administrative district as random effect.
*Bold represented *P*-value < 0.05, Abbreviations: IQR: Interquartile range

2.7. Discussion

To the best of our knowledge, this is the first study to explore the relationship between exposure to greenness and children’s IQ in South Korea. Our results indicated that more greenness exposure during pregnancy, as well as at age 6 was associated with higher IQ among the children. We also examined the association by type of greenness and found that children’s IQ was significantly associated with built greenness, but not with natural greenness. In addition, postnatal exposure to greenness at age 6 was more strongly associated with children’s IQ than prenatal exposure to greenness.

Our findings concur with those of previous studies, which have consistently revealed beneficial associations between greenness and mental health in children (Table 1). Relatively few previous studies reported relationships between exposure to greenness and intelligence test among children. Reuben et al. analyzed an association between residential greenness exposure and intellectual performance at ages 5, 12, and 18 years in a longitudinal study in England (Reuben et al. 2019). Greater exposure to residential greenness in 1-mile buffers was associated with IQ scores at ages 5, 12, and 18. However, they found no significant association between greenness exposure and IQ in children after adjusting for family or neighborhood socioeconomic status. The researchers also found that families in better economic circumstances tended to receive more exposure to greenness in urban areas of England (Reuben et al. 2019). Thus, it is important to control for socioeconomic status in the analyses of the effect of greenness on health outcomes, and there should be additional consideration of unequal exposure to greenness according to socioeconomic status. We adjusted for socioeconomic status using maternal education level and household income as individual SES, and deprivation index as neighborhood SES. We also conducted the stratification by individual SES in Table 14. There is positively significant association between children's IQ and postnatal exposure to built greenness in both low and high SES group, and prenatal exposure to built greenness in high SES group. A number of studies has investigated the contributions of socioeconomic status in the association between greenness and health outcome (Dadvand et al. 2014; Asta et al. 2019; Pinault et al.2021). There is a growing evidence has reported that both individual SES or neighborhood SES can modify the health benefit of greenness. We tried to control SES in the model, and we found that subjects with the lower maternal education level, or the higher residential deprivation index was lower exposed greenness during pregnancy and their early childhood period. Especially, these associations were more highlighted in the relationship between built greenness and covariates (Table S4). Due to the linkage between SES indicator and greenness may be associated with health outcome, we recommend further studies on this association in considering both greenness and SES with careful explanation and investigating the possible mechanism underlying this association.

Possible mechanisms underlying the beneficial impact of exposure to greenness on mental health have been mentioned in previous studies (Dadvand et al. 2015; James et al. 2015). Exposure to greenness may have a directly restorative effect on children's cognition (James et al. 2015). Better mental health has been linked to a greater chance of social interaction (Berkman et al. 2014), increasing social cohesion (Liu et al. 2019), and access to physical activities (Browning and Lee

2017; Liu et al. 2019; Sugiyama et al. 2008; Toftager et al. 2011; Ward et al. 2016). In addition, improved health outcomes may be related to reduced exposure to environmental toxic agents, such as air pollution (Dadvand et al. 2012a; Dadvand et al. 2015; Dzhambov et al. 2019; Klompmaker et al. 2019) or noise (Dzhambov et al. 2019; Klompmaker et al. 2019). In this study, we found that the lower percentage of road density, the higher maternal age, the higher maternal IQ, the lower NO_2 concentration level, and the higher group of maternal education level were associated with higher percentage of greenness exposure during pregnancy. Some of these covariates such as maternal education level, and maternal IQ were also consistantly related to children's IQ (Figure 40). We suggested that maternal educational level and intelligence was strongly related to these associations. On the other hand, the percentage of road density, maternal educational level, and subjective noise level were significantly associated with greenness exposure at age 6. The higher maternal IQ, the lower residential deprivation index at age 6, the higher group of maternal education level, and unexposed ETS exposure group were associated with increased children's IQ. However, the directions of some covariates were inconsistent, so further research should be more required for exploring potential mechanisms in these associations.

Previous studies have limitations in how they assessed time and quality of contact between children and greenness, and types of greenness, as well as parental mental health (Vanaken and Danckaerts et al. 2018). NDVI was the most frequently used measurement of greenness, which has limitations due to its inability to distinguish different type of greenness or to evaluate the quality of greenness (Putra et al. 2020). To overcome these limitations, we compared the associations between children's IQ and the type of greenness. However, our study also has the limitation that it did not consider how the children used residential greenness. There is the mean of 16 m^2 for urban park space per 1 citizen in Seoul area (which was higher than Paris(10.7m^2) or Tokyo(4.5m^2), was lower than London(33.4m^2) (The Seoul Institute). In addition, there are 1296 children's parks for their leisure in Seoul, even though depending on the participants have different numbers of urban parks in the surrounding house. Investigation of health effects considering the equity of greenness use for children may be necessary in the future.

Our results showed that postnatal exposure to greenness was more beneficial to children's IQ than was prenatal exposure to greenness. Previous studies have been inconsistent regarding the effects of prenatal and postnatal environmental exposure. A systematic review by Gonzales-Alzga et al. (González-Alzaga et al. 2014) estimated the effects of pre- and postnatal exposure to organophosphates (OPs) on neurodevelopment. Most extant studies considered the neurodevelopmental effects of prenatal exposure to OPs compared to postnatal exposure. Differences between the effects associated with pre- and postnatal exposure may be linked to the existence of a critical period of developmental neuroplasticity (González-Alzaga et al. 2014). However, both Huang et al. (Huang et al. 2015) and Kim et al. (Kim et al. 2017) showed an inverse association between postnatal exposure to phthalate and children's IQ scores. There was no significant association between prenatal phthalate exposure and children's IQ scores. We could not locate any previous study by which to compare pre- and post-natal beneficial effects of greenness (Akpinar et al. 2016). Therefore, studies to determine susceptible periods in fetal or postnatal exposure to greenness are required. Based on the well-known association between lead exposure and IQ reduction (Desrochers-Couture et al. 2018; Lanphear et al. 2005; Needleman and Gatsonis 1990; Tatsuta et al. 2020), further investigation is also needed among children between lead exposure, greenness and IQ.

Table 14. Difference (95% confidence interval) in total intelligence quotient per 1 % increase in the exposure to residential greenness percentage in buffer area during pregnancy or 6-year-old by stratifying the individual SES[1].

(a)

During pregnancy	Maternal education level=Low			Maternal education level=High		
	Total	Natural	Built	Total	Natural	Built
Buffer	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)	Difference(95%CI)	Difference (95%CI)	Difference(95%CI)
100m	0.06(-0.08, 0.20)	NA	0.04(-0.11, 0.20)	**0.57(0.18, 0.95)***	NA	**0.63(0.20, 1.05)***
500m	0.09(-0.07, 0.24)	NA	0.18(-0.14, 0.49)	0.41(-0.09, 0.91)	NA	**1.10(0.45, 1.76)***
1000m	0.05(-0.15, 0.24)	-0.02(-0.25, 0.20)	0.36(-0.10, 0.83)	-0.23(-0.81, 0.36)	-0.64(-1.29, 0.01)	**1.66(0.22, 3.09)***
1500m	0.07(-0.18, 0.32)	-0.02(-0.28, 0.24)	0.49(-0.09, 1.08)	-0.43(-0.96, 0.09)	**-0.73(-1.28, -0.18)***	1.52(-0.42, 3.46)
2000m	-0.01(-0.29, 0.27)	-0.07(-0.36, 0.21)	0.48(-0.29, 1.25)	-0.49(-1.05, 0.08)	**-0.65(-1.25, -0.05)***	1.68(-1.38, 4.73)

(b)

At age 6	Maternal education level=Low			Maternal education level=High		
	Total	Natural	Built	Total	Natural	Built
Buffer	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)	Difference(95%CI)	Difference(95%CI)	Difference (95%CI)
100m	**0.38(0.05, 0.72)***	NA	**0.45(0.08, 0.81)***	-0.003(-0.12, 0.11)	NA	0.08(-0.07, 0.23)
500m	0.42(-0.08, 0.91)	NA	**1.03(0.14, 1.93)***	0.11(-0.05, 0.27)	NA	0.26(-0.03, 0.54)
1000m	0.05(-0.57, 0.67)	-0.09(-0.83, 0.65)	0.55(-0.83, 1.93)	0.08(-0.13, 0.29)	-0.06(-0.28, 0.16)	**0.59(0.16, 1.03)***
1500m	-0.06(-0.93, 0.82)	-0.10(-1.01, 0.81)	0.16(-1.47, 1.78)	-0.05(-0.30, 0.20)	-0.21(-0.46, 0.05)	**0.93(0.34, 1.52)***
2000m	0.08(-0.82, 0.98)	-0.05(-0.98, 0.89)	0.59(-1.32, 2.5)	-0.10(-0.39, 0.19)	-0.24(-0.52, 0.04)	**1.25(0.45, 2.04)***

1. Using linear regression analysis
2. Adjusted for maternal age, children’s sex, maternal IQ, exposure to ETS during pregnancy, average of NO_2 concentration for 3 years in residential area during pregnancy, residential deprivation index as neighborhood SES, and road density in each radius.
3. Adjusted for maternal age, children's sex, maternal education, maternal IQ, exposure to ETS at age 6, average of NO_2 concentration for 3 years in residential area at age 6, residential deprivation index as neighborhood SES, subjective noise level, distance to main road, road density in each radius, and physical activity time.

*P-value < 0.05, Abbreviations: NA: Not available

We focused on the types of greenness, namely natural versus built environment (e.g., artificial grassland, urban parks, and street trees). Most previous studies assumed that those who live closer to greater quantities of greenness may have better mental and general health compared to those closer to smaller quantities of greenness. However, few previous studies considered the differences between types of greenness. Akpinar et al. (Akpinar et al. 2016) explored whether mental health was related to the size or type of green space, such as forest, rangeland, agricultural land, wetland, and urban greenness. Forest exposure and forest size were significantly associated with fewer days of mental health complaints, but no significant relationships were found with other types of greenness. The rich complexity of forests can facilitate a variety of activities and forests per se can be a source of fascination (Van den Berg et al. 2014). In addition, forests provide various components of restorative environments (Kaplan and Kaplan 1989). However, our results demonstrated that built greenness was more positivity associated with children's IQ than were natural greenness. The reason for this might be convenience and easiness for children to access built greenness than natural greenness. Additionally, further study is needed to see that the different types of vegetation based on the composition of plant species may affect differently the emissions of biogenic volatile organic compounds(bVOCs) (Malik et al. 2018). The bVOCs had the positive neurological role in mouse models (Antonelli et al. 2020), suggesting that bVOCs emissions from the different types of vegetation influence mental health in children differently.

2.8. Conclusion

We found that 6-year-old children who lived in greener neighborhoods tended to exhibit higher IQ scores than did children with less exposure to greenness. The results provide further evidence for the health benefits of greenness and provide support for urban planning and public health to build healthy urban cities for children and pregnant women. Future studies are warranted to further evaluate the relationship between children's exposure to greenness and their health outcomes, considering factors such as children's utilization and accessibility of greenness. To do so, we need to include measurement such as physical activity survey or use of portable devices in the future.

Chapter 3. Association between greenness and epigenetics among children

3.1. Study population

Our research was based on a subset of the EDC study cohort, an ongoing prospective cohort study designed to evaluate the association between prenatal and postnatal environmental exposures and physical or cognitive development. Detailed information on the study design has been described elsewhere (Kim et al. 2018). Briefly, a total of 726 eligible pregnant women from eight local hospitals in Seoul and Gyeonggi province of South Korea were enrolled from August 2008 to July 2010. We collected urine and blood samples to estimate exposure to environmental factors during the second trimester of pregnancy. A total of 425 children aged 2 years and 574 children aged 6 years at enrolment were followed up. DNA methylation analysis was conducted in a subset of 59 participants using blood samples collected at the age of 2 years (Figure 46). Informed consent was obtained from the parents after sufficient explanation of the study. The study protocol was approved by the Institutional Review Board of Seoul National University College of Medicine (IRB No. 1201-010-392).

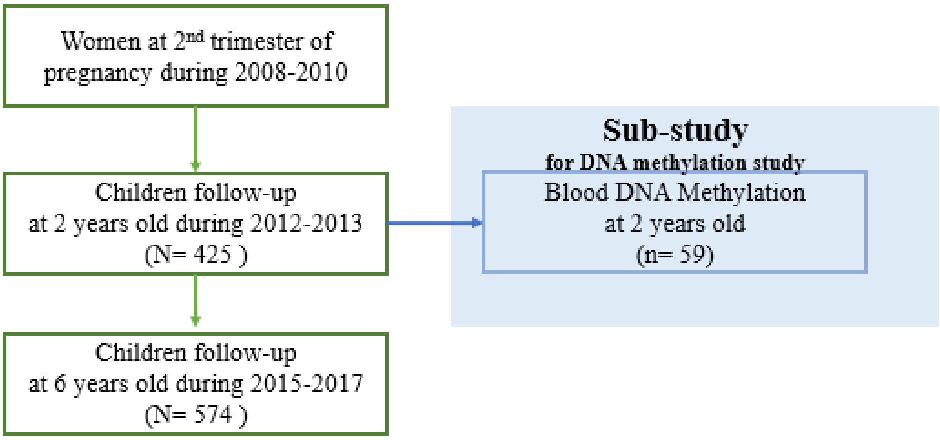

Figure 46. The flow of participants through the study

3.2. Greenness exposure assessment

To estimate exposure to greenness, the residential addresses were collected at the age of 2 years. The surrounding greenness was recorded using Landsat image data from the IKONOS satellite images (Dial et al. 2003) and Korean Arirang satellite images taken by the Environmental Geographic Information Service of the Ministry of the Environment (https://egis.me.go.kr/main.do). To estimate exposure to greenness, the densities of greenness were calculated within buffer radii of 100, 500, 1000, 1500, and 2000 m from each child's residential address. Then, we determined the percentage of greenness (density) from the area within each buffer. We separately analyzed two types of greenness, namely natural greenness, forest or natural grassland, and built greenness, including artificial grassland, urban parks, and street trees. We did not analyze the effect of natural greenness within buffer radii of both 100 and 500 m because natural greenness was barely observed within these ranges. We described in detail for the spatial analysis in Section 2.2.

3.3. DNA methylation changes in children at ages 2

3.3.1. Systematic review of literature and selection of candidate cytosine-guanine dinucleotide sites

As we were specifically interested in the question of whether DNA methylation mediates the effects of exposure to greenness on children's IQ, we targeted CpG sites that were more likely to be involved in cognitive ability instead of scanning the whole epigenome. For the selection of previous EWAS or GWAS on association with cognitive abilities, we searched PUBMED and EMBASE on April 1, 2021, using keywords ("epigenome-wide association study" or "genome-wide association study") and ("intelligence" or "cognitive ability" or "cognitive development") from titles or abstracts. The selection criteria were EWAS or GWAS regarding cognitive ability in healthy children or adults. From previous EWAS or GWAS that investigated the association between DNA methylation and cognitive ability in healthy children or adults, we identified CpG sites associated with cognitive ability-related parameters (Figure 47). In the GWAS, single nucleotide polymorphisms (SNPs) associated with cognitive ability were identified, and then the genes annotated to these SNPs were identified. The CpG sites associated with these genes were pooled using the Database for Annotation, Visualization, and Integrated Discovery (DAVID, http://david.abcc.ncifcrf.gov/home.jsp).

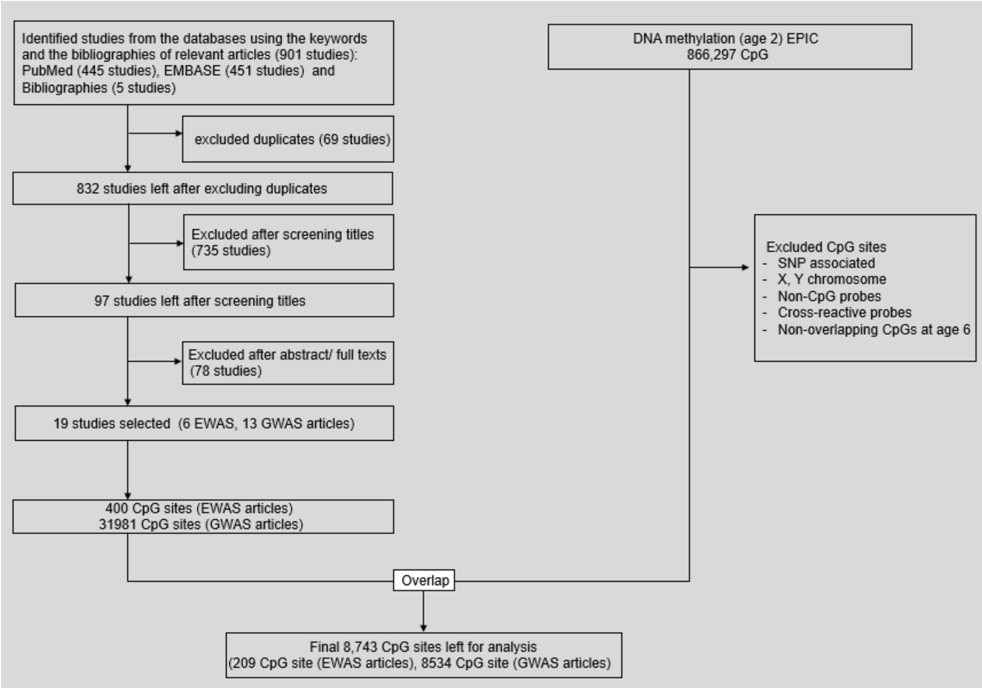

Figure 47. Workflow for model building for selecting cognitive abilities based on a systematic literature review.

3.3.2. Assessment of DNA methylation at age 2 in the EDC cohort.

We performed genome-wide DNA methylation analyses using the whole blood samples of 59 at age 2 as described in an earlier study (Choi et al. 2020). Briefly, DNA samples were tested for quality using a NanoDrop® ND-1000 UV-Vis spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). Electrophoresis was performed using 1% agarose gel, and samples with genomic DNA (gDNA) were diluted to 50 ng/μL based on Quanti-iT Picogreen quantification (Thermo Fisher Scientific, Wilmington, DE, DE). The diluted gDNA samples (minimum 500 ng) were diluted, then bisulfite-converted using the Zymo EZ DNA methylation kit (Zymo Research, Irvine, CA, USA), and the DNA was then amplified to be used on the DNA BeadChip. At age 2, we used the Illumina Infinium Human MethylationEPIC BeadChip, which yielded 850,000 CpG sites (Illumina, San Diego, CA, USA). Images were read by the Illumina BidArray Reader, and the image intensities were extracted using the Illumina GenomeStudio software. Microarrays were handled by Macrogen Co. (Seoul, South Korea). For functional annotation analysis, we used the DAVID, http://david.abcc.ncifcrf.gov/home.jsp) tool.

3.3.3. Quality control of methylation data

Filtered data were normalized using the Beta Mixture Quantile (BMIQ) method [35]. With the Human MethylationEPIC BeadChip (850K), a total of 866,297 CpG sites were extracted for the raw data, and 609 CpG sites (0.07%) which had detection p-value ≥ 0.05 across more than 25% of all samples were excluded from analysis. Thus, 865,688 CpG sites were left for analysis. We also filtered CpG sites according to the following exclusion criteria: (a) SNP-associated CpG sites defined as 0 or 1 base pair near SNP loci or minor allele frequency (MAF) > 5% (213,660 CpG sites); (b) CpG sites that corresponded to the X or Y chromosome (19,627 CpG sites); (c) CpG sites corresponding to non-CpG loci (3627 CpG sites).; (d) cross-reactive CpG sites (42,558 CpG sites). We were finally left with 256,866 CpG sites which overlapped with the available epigenome data of 6-year-old children for further analysis. We also excluded multimodal CpG sites if they appeared in statistically significant CpG sites, which were identified using the dip test statistic for multimodality, which was calculated using the R package *diptest* module (Maechler, 2013).

3.4. Measurement of intelligence quotient in children

The IQ of the 6-year-old children was measured using the Korean Educational Developmental Institute's Wechsler Intelligence Scale for Children (Park et al. 1996). Higher scores indicated higher IQ. Two subsets were measured: verbal IQ, based on the sum of the test results for vocabulary and arithmetic intelligence, and performance IQ, based on the sum of the tests for picture arrangement and block design (Kim et al. 2017).

3.5. Covariates

We collected demographic information on the children and their mothers by means of interviews using structured questionnaires. Covariates were selected based on a literature review (Lee et al. 2021; Xu et al. 2021). The covariates used to analyze the association between greenness and DNA methylation were mother's age at pregnancy (years), mother's educational level (middle school graduate, high school graduate, college graduate, or graduate school attendance), children's exposure to environmental tobacco smoke (ETS) at age 2 (yes or no), children's sex (boy or girl), children's age at follow-up (months, continuous variable), children's body mass index (BMI) (kg/m^2, continuous variable), and cell type fractions (continuous variables). The cell type fractions in blood samples were calculated using the R package *minfy* module (Aryee et al. 2014). To estimate the percentage of CD8+T cells, CD4+T cells, natural killer cells, B cells, monocytes, and neutrophils, adults' leukocyte reference dataset was used (Houseman et al. 2012). We also used the covariates for analyzing the association between DNA methylation and children's IQ at age 6, including children's age at follow-up, children's BMI, maternal age during pregnancy, maternal education level, exposure to ETS at age 2, maternal IQ, and children's sex. The short form of the Korean Wechsler Adult Intelligence Scale was used to assess maternal IQ at the time of their children's follow-up visit at the age of six (Lim et al. 2000).

3.6. Statistical analysis

We compared the demographic and clinical characteristics of the subset population to the population of the EDC study that was not included in our study using the Student's t-test (for continuous variables) or chi-square test (for categorical variables) (Table 15). We used batch effect-adjusted DNA methylation data obtained using the R package *ComBat* module to adjust different distributions according to chips and positions from the array data (Johnson et al., 2007). This process uses an empirical Bayes method to adjust batch effects in small sample sizes. We performed multivariable linear regression to determine the relationship between exposure to greenness at age 2 and cognitive ability-related DNA methylation at age 2, adjusting for monthly age at follow-up, BMI at age 2, maternal education level, cell type fractions (CD8+T cells, CD4+T cells, natural killer cells, B cells, monocytes, and neutrophils), exposure to ETS, maternal age at delivery, and child's sex. Using the CpG sites significantly associated with exposure to greenness at age 2, we tested the association of these sites with total, verbal, and performance IQ scores at age 6, adjusting for children's age at follow-up, children's BMI, maternal age during pregnancy, maternal education level, exposure to ETS at age 2, maternal IQ, children's sex, and cell type fractions. To account for multiple testing, a significant difference was defined as a site with a Bonferroni-corrected *P*-value ≤ 0.05/the numbers of selected CpGs (Weisstein et al., 2004). Pathway enrichment analysis was performed using the *ReactomePA* R package (Yu et al., 2016). Enrichment analysis of functional terms revealed the *Reactome* pathway enriched in the genes identified as significant from their association between greenness exposure and cognitive ability-related CpG sites

(Bonferroni-corrected $P \leq 0.05$/ the numbers of selected CpGs). All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and R software version 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

3.7. Results

3.7.1. General characteristics of the participants

Table 15 presents the participant characteristics. The mean maternal age at delivery was 31.10 years (standard deviation [SD]: 3.79 years). The mean age and BMI of children were 23.32 months (SD: 0.77 months) and 16.57 kg/m^2 (SD: 1.20 kg/m^2), respectively. The mean age and BMI of children were 23.32 months (SD: 0.77) and 16.57 kg/m^2 (SD: 1.20 kg/m^2), respectively. The mean maternal IQ was 117.8 (SD: 11.5). The percentages of mothers who received less than a high school education and more than a graduate school education were 15.25% and 13.56%, respectively. A total of 23.73% of the participants were in a group with positive exposure to ETS during pregnancy. There were similar numbers of girls and boys in the study (30 and 29, respectively). The percentage of greenness exposure at age 2 within 100–2000 m was ranged from 17.67% to 25.29%. The mean total, verbal, and performance IQ scores at age 6 were 107.40 (SD: 13.70), 21.08 (SD: 5.03), and 23.47 (SD: 5.10), respectively. In addition, we found that the characteristics of subset were not significantly different from those of the participants in the entire EDC cohort, except for exposure to greenness at age 2 in 1000 m buffer of residential address (Table 15).

Table 15. Characteristics of participants at age 2 in sub-study compare to total EDC population.

Variables		Study Population (n = 59)	EDC population excluded from the study (n = 366)	*P*-value
		n (%) or Mean ± SD	n (%) or Mean ± SD	
Maternal age at pregnancy (yr)		31.10±3.79	31.68±3.60	0.256
Children's age(month)		23.32±0.77	23.31±0.76	0.922
Children's BMI at age 2		16.57±1.20	16.48±1.44	0.666
Maternal IQ		117.8±11.5	115.8±11.1	0.248
Maternal education level	High school graduate	9(15.25)	70(19.13)	0.669
	College graduate	42(71.19)	257(70.22)	
	Graduate school	8(13.56)	39(10.66)	
Prenatal exposure to ETS	Yes	14(23.73)	89(24.32)	0.922
	No	45(76.27)	277(75.68)	
Children's sex	Girl	30(50.85)	172(46.99)	0.582
	Boy	29(49.15)	194(53.01)	
Percentage of total greenness in home address at age 2	100m	17.67±12.8	19.82±14.0	0.276
	500m	18.71±11.1	21.36±13.9	0.108
	1000m	19.95±11.2	23.61±13.6	0.028
	1500m	23.63±12.8	24.77±12.9	0.529
	2000m	25.29±13.4	26.32±12.7	0.569
IQ at age 6	Total IQ	107.4±13.7	110.6±12.5	0.088
	Verbal IQ	21.08±5.03	20.81±7.06	0.732
	Performance IQ	23.47±5.10	22.91±7.26	0.487

Abbreviations: EDC, Environment and the Development of Children; SD, standard deviation; ETS, environmental tobacco smoke; 3-PBA, 3-phenoxybenzoic acid; ADHD, attention-deficit/hyperactivity disorder; IA, inattention; HI, hyperactivity-impulsivity

3.7.2. Systematic literature review

We found a total of 896 studies (445 studies in PubMed and 451 studies in EMBASE) after applying the keywords search strategy described in Section 3.3.1. Five studies were included in the bibliographic search (Table S1). After excluding duplicated studies (n=69), 97 studies were included for screening by title, and 735 studies were excluded because they were studies of cognitive aging or cognitive disease or were not the primary study result. We further excluded irrelevant articles such as invalid study designs or cognitive outcomes, such as mathematics, school performance, or memory, finally leaving a total of 19 articles. (Figure 47).

A total of 400 CpG sites were selected from 6 EWAS (Caramaschi et al., 2020; Caramaschi et al., 2017; Krushkal et al., 2014; Linnér et al., 2017; Marioni et al., 2018; Paquette et al., 2016). Additionally, a total of 31,981 CpG sites selected, which were annotated to 835 genes reported from 13 GWAS after excluding duplicate genes (Benyamin et al., 2014; Coleman et al., 2019; Hill et al., 2014; Jansen et al., 2020; Kong et al., 2013; Kornilov et al., 2019; Luciano et al., 2006; Savage et al., 2018; Smajlagić et al., 2018; Sniekers et al., 2017; Zabaneh et al., 2018; Zhao et al., 2014; Zhu et al., 2018). These CpG sites were filtered according to the quality control method introduced earlier. As a result, 209 CpG sites from the EWAS and 8,534 CpG sites from the GWAS were finally selected (Table S2 and Table S3).

3.7.3. Association between greenness exposure and DNA methylation

A total of 209 CpG sites from the EWAS and 8,534 CpG sites from the GWAS were analyzed in our study. We found that 25 cognitive ability-related CpG sites were significantly associated with greenness exposure at age 2 (8 CpG sites from EWAS and 17 CpG sites from GWAS) in total greenness in buffers of 100–2000 m, natural greenness in buffers of 1000–2000 m, and built greenness in buffers of 1000 m and 1500 m, with a significance criterion for Bonferroni-corrected *P*-values < 2.3×10^{-4} for the EWAS, $P < 5.8 \times 10^{-6}$ for the GWAS) (Table 16).

Table 16. The significant relationship between greenness exposure and selected DNAm at aged 2[†].

Origin Study	greenness type	Buffer	CpG	Gene	Difference[§]	*P*-value
EWAS Study	Total	100m	cg13092901	*TYMP*	0.021(0.012, 0.029)	2.0×10^{-5}
		500m	cg04789403	-	0.031(0.015, 0.047)	1.1×10^{-4}
		500m	cg07266431	*CDK6*	0.028(0.015, 0.040)	1.9×10^{-4}
		500m	cg13599020	*SAMD3*	0.026(0.014, 0.039)	1.9×10^{-4}
		500m	cg27492942	*CISD3*	0.029(0.013, 0.045)	1.7×10^{-4}
		1000m	cg00252813	*GAPDH*	0.013(0.006, 0.020)	6.1×10^{-5}
	Natural	1000m	cg00252813	*GAPDH*	0.013(0.007, 0.019)	5.8×10^{-5}
		1000m	cg04789403	-	0.020(0.010, 0.030)	7.7×10^{-5}
	Built	1000m	cg16594502	-	0.015(0.008, 0.022)	9.4×10^{-5}
		1500m	cg25189904	*GNG12*	0.028(0.013, 0.044)	2.3×10^{-4}
GWAS Study	Total	100m	cg26269038	*SLC6A3*	-0.011(-0.015, -0.007)	3.2×10^{-8}
			cg14464361	*AGAP1*	-0.023(-0.032, -0.016)	2.2×10^{-6}
			cg21175642	*CELSR3*	0.013(0.007, 0.016)	3.4×10^{-6}
		1000m	cg23651585	*AUTS2*	-0.039(-0.056, -0.024)	9.9×10^{-7}
			cg27636559	*EFTUD1*	0.007(0.004, 0.009)	1.2×10^{-6}
			cg27609819	*PLCL1*	-0.027(-0.038, -0.015)	2.3×10^{-6}
			cg16296679	*WBP2NL*	0.015(0.009, 0.022)	2.9×10^{-6}
		1500m	cg17146029	*AUTS2*	0.010(0.007, 0.013)	1.0×10^{-7}
			cg00809988	*ELAVL2*	-0.006(-0.009, -0.003)	1.5×10^{-7}
		2000m	cg17146029	*AUTS2*	0.009(0.006, 0.012)	3.9×10^{-8}
			cg00809988	*ELAVL2*	-0.004(-0.008, -0.002)	3.2×10^{-7}
			cg03367519	*PDE4D*	-0.005(-0.008, -0.002)	3.3×10^{-6}
			cg27609819	*PLCL1*	-0.029(-0.039, -0.020)	3.7×10^{-8}
			cg23651585	*AUTS2*	-0.043(-0.059, -0.027)	7.4×10^{-8}
			cg27636559	*EFTUD1*	0.007(0.005, 0.009)	2.2×10^{-7}
	Natural	1500m	cg23651585	*AUTS2*	-0.041(-0.055, -0.025)	2.6×10^{-7}
			cg23159678	*NOVA1*	0.009(0.004, 0.014)	1.9×10^{-6}
			cg05016953	*SLC6A4*	-0.004(-0.006, -0.001)	2.2×10^{-6}
			cg27609819	*PLCL1*	-0.025(-0.036, -0.016)	2.2×10^{-6}
			cg03367519	*PDE4D*	-0.005(-0.007, -0.002)	2.9×10^{-6}
			cg00809988	*ELAVL2*	-0.005(-0.007, -0.002)	5.3×10^{-6}
		2000m	cg17146029	*AUTS2*	0.010(0.006, 0.012)	1.8×10^{-6}
			cg23651585	*AUTS2*	-0.046(-0.064, -0.026)	1.9×10^{-6}
			cg11176256	*BAIAP2*	0.016(0.010, 0.023)	3.5×10^{-6}
			cg05897638	*PROS1*	-0.007(-0.010, -0.003)	5.1×10^{-6}
			cg00809988	*ELAVL2*	-0.005(-0.009, -0.002)	5.5×10^{-6}
			cg12414502	*BTN2A1*	0.010(0.006, 0.012)	5.6×10^{-6}
	Built	1500m	cg19258882	*ERBB3*	0.024(0.015, 0.032)	4.6×10^{-6}
			cg18311871	*PTPRN2*	0.081(0.047, 0.115)	3.2×10^{-6}

Note: CpG site location- based on Illumina annotation, derived from the University of California, Santa Cruz (UCSC), Adjusted for children's age, children's BMI, maternal education level, cell count (CD8+T cells, CD4+T cells, natural killer cells, B cells, monocytes and neutrophils), ETS, maternal age, children's sex, and batch effect. The list was significantly associated for multiple comparison-corrected *P*-value (*) by Bonferroni method (The EWAS study was $P<2.3x10^{-4}$, the GWAS study was $P<5.8x10^{-6}$). † Analyzed using a linear regression model. § Change in DNA methylation level by 1 interquartile range increases of greenness within each buffer.

3.7.4. Pathway enrichment analysis

We investigated potential biological functions by performing pathway enrichment analysis with the cutoff p-value set to 0.1. We found the top 20 pathways, including transmission across chemical synapses, opioid signaling, and neuronal systems pathway (Figure 48). Notably, a single pathways of neurotransmitter clearance associated with the *SLC6A3* and *SLC6A4* genes were significantly related to greenness exposure, of which *SLC6A3* also showed significant associations with children's IQ in this study (adjusted *P*-value: 0.009; Table S4). In addition, the pathway enrichment analysis identified transmission across chemical synapses, opioid signaling, and neuronal systems pathway as playing an important role in the association between greenness and DNA methylation and contains genes associated with the nervous system (Figure 49).

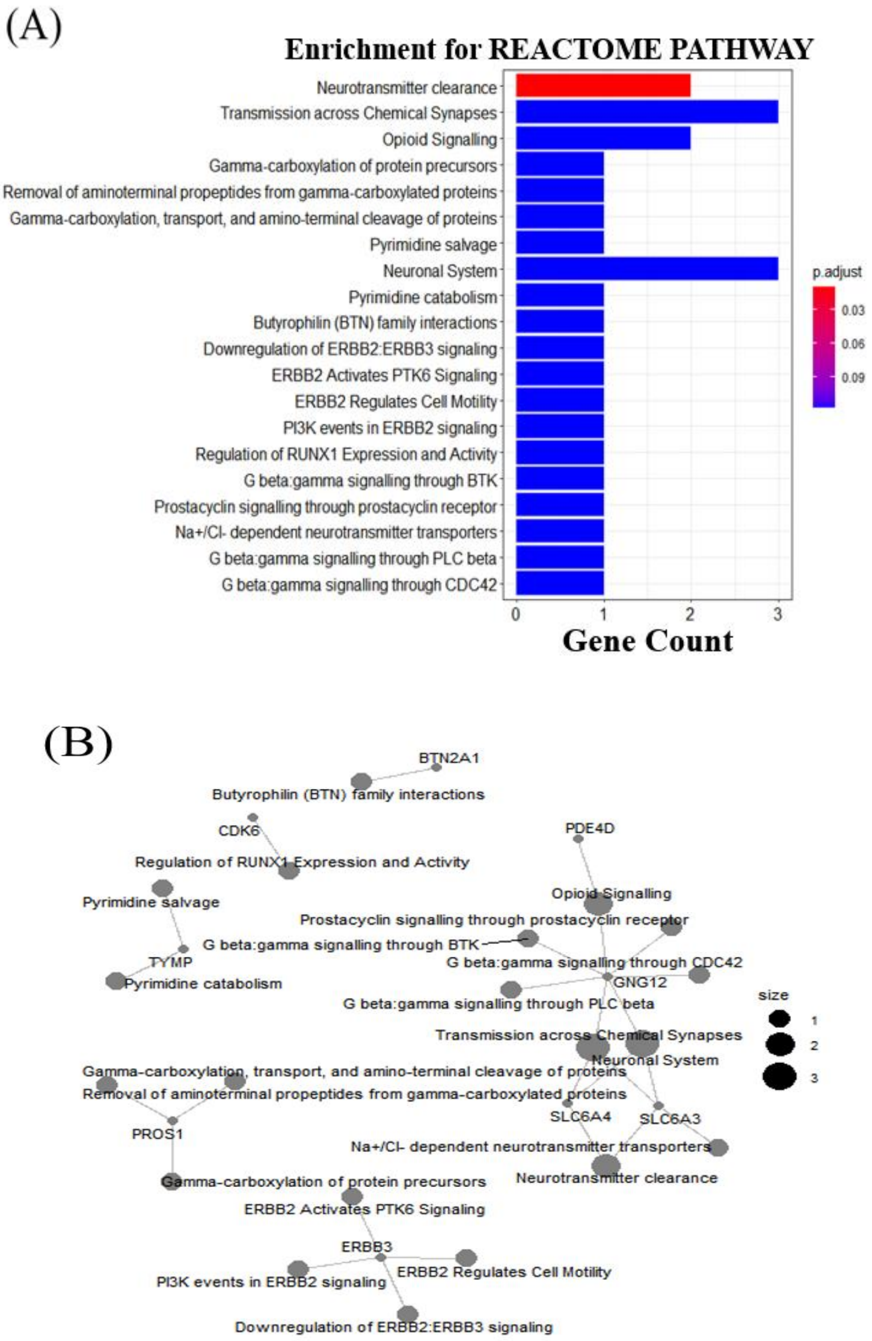

Figure 48. *Reactome* pathway enrichment analysis of the greenness-associated genes, which were showed top 20 pathways. (A) The enrichment scores in the *Reactome* pathway analysis of the greenness exposure-related genes. (B) The network of most enriched pathways of the greenness exposure-related genes. Larger nodes represent larger enrichment scores represent different enrichment modules.

3.7.5. Association between DNA methylation and children's intelligence quotient

We showed the association of the methylation levels of the 25 CpG sites at age 2 and performance IQ scores at age 6 in Table 17. Notably, the methylation level at cg26269038 was significantly associated with increased performance IQ score at age 6 (ß: 164.5; Standard Error: 48.4) in adjusted models after Bonferroni adjustment (*P* <0.002). However, there was no significance in total IQ and verbal IQ with DNA methylation (not shown). The enrichment analysis from *Reactome* pathway analysis has shown that most of the differentially expressed in several pathways, such as neurotransmitter clearance, transmission across chemical synapses, and neuronal system (Figure 48).

We plotted the least-squares means of the methylation level at cg26269038 by exposure to greenness as the quartile group. The percentages of greenness in the 100 m buffer of residential address for each participant were divided into quartiles and were then performed to determine whether individuals in the three higher quartiles differed significantly from those in the lowest quartile. The highest quartile of DNA methylation level at cg26269038 was significantly different from the lowest quartile (Figure 49).

Table 17. Association between selected CpG sites and children's Performance IQ (n=59) †

CpG sites	Chr	Gene	Gene group	Relation to CpG island	Difference (95% CI)§	*P*-value
cg14464361	2	*AGAP1*	Body	Island	0.79 (-0.88, 2.46)	0.353
cg23651585	7	*AUTS2*	Body	N_Shelf	-0.20 (-0.44, 0.03)	0.088
cg17146029	7	*AUTS2*	Body	-	-0.11 (-1.82, 1.59)	0.895
cg11176256	17	*BAIAP2*	Body	S_Shore	-0.86 (-2.42, 0.69)	0.278
cg12414502	6	*BTN2A1*	3'UTR	-	1.86 (-0.07, 3.79)	0.059
cg07266431	7	*CDK6*	Body	N_Shelf	-0.33 (-2.59, 1.94)	0.778
cg21175642	3	*CELSR3*	TSS1500	S_Shore	-1.10 (-2.57, 0.37)	0.141
cg27492942	17	*CISD3*	TSS1500	N_Shore	-0.77 (-2.38, 0.85)	0.352
cg27636559	15	*EFTUD1*	Body	-	-0.80 (-4.10, 2.50)	0.634
cg00809988	9	*ELAVL2*	TSS1500	N_Shore	0.16 (-1.80, 2.12)	0.873
cg19258882	12	*ERBB3*	TSS1500	Island	-1.53 (-2.76, -0.3)	0.015
cg00252813	12	*GAPDH*	TSS1500	N_Shore	1.40 (-1.15, 3.95)	0.281
cg25189904	1	*GNG12*	TSS1500	S_Shore	-1.75 (-3.73, 0.24)	0.084
cg23159678	14	*NOVA1*	Body	N_Shelf	-1.15 (-2.59, 0.30)	0.120
cg03367519	5	*PDE4D*	Body	Island	-0.43 (-2.21, 1.35)	0.638
cg27609819	2	*PLCL1*	Body	-	-0.47 (-0.93, 0.00)	0.049
cg05897638	3	*PROS1*	TSS200	S_Shore	-1.35 (-3.09, 0.39)	0.128
cg18311871	7	*PTPRN2*	Body	-	-0.12 (-1.49, 1.26)	0.868
cg13599020	6	*SAMD3*	Body	-	-0.81 (-3.70, 2.08)	0.585
cg26269038	**5**	***SLC6A3***	**Body**	**-**	**2.94 (1.22, 4.66)***	**0.001**
cg05016953	17	*SLC6A4*	1stExon	Island	-0.91 (-2.44, 0.62)	0.244
cg13092901	22	*TYMP*	Body	Island	-0.62 (-2.78, 1.54)	0.576
cg16296679	22	*WBP2NL*	TSS1500	N_Shore	-0.13 (-1.70, 1.43)	0.867
cg04789403	15	-	-	-	3.27 (0.10, 6.44)	0.043
cg16594502	15	-	-	-	-0.19 (-4.02, 3.64)	0.922

* Bold was significant association using Bonferroni correction $P< 0.002$

† Analyzed using a linear regression model. § Difference (95% CI) was calculated by 1 interquartile range change in DNA methylation level at each CpG site. Adjusted for children's age, sex, children's BMI, maternal education level, exposure to ETS, maternal age, maternal IQ, and cell type fractions.

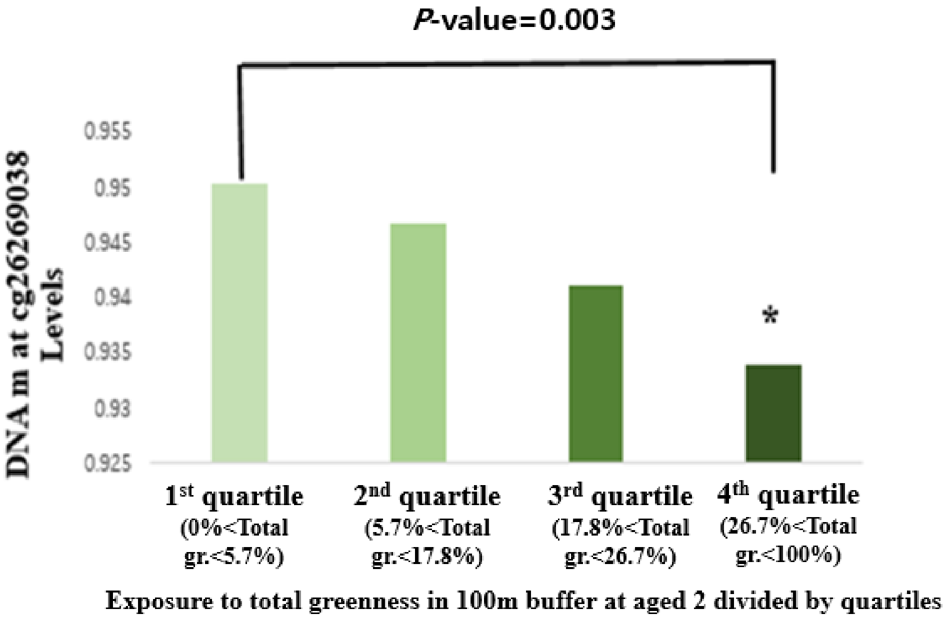

Figure 49. The LSMEANS of DNA m at cg26269038 levels in four quartiles of exposure to greenness among children

* Represents significant differences between a quartile and the lowest quartile, with *P*-values < 0.05 considered statistically significant. Adjusted for children's age, BMI, maternal education level, children's ETS exposure, maternal age, maternal IQ, children's sex, and cell counts.

3.8. Discussion

We found that the methylation levels at 25 cognitive ability-related CpG sites at age 2 were significantly associated with greenness exposure during early childhood and that the methylation level at cg26269038 at age 2 (*SLC6A3*) was significantly associated with the performance IQ score at age 6.

In our study, several genes significantly associated with greenness exposure, including *PDE4D*, *PLCL1*, *GNG12*, *SLC6A4*, and *SLC6A3*, were also linked to neurotransmitter clearance in the pathway enrichment analysis results. Signaling in the central nervous system is terminated by the clearance of neurotransmitters from the synapse via high-affinity transporter molecules in the presynaptic membrane (Figlewicz, 1999). Recently, a review article reported that disruption in the clearance of neurotransmitters and increased amyloid β and tau from astrocytes appeared to be involved in neurotoxicity in Alzheimer's disease patients (González-Reyes et al., 2017). A CpG site at cg05016953 (*SLC6A4)* as a serotonin transporter, which was significantly associated with greenness exposure in our study, was reported to be regulated by a *5HTTLPR* functional polymorphism, which was significantly associated with IQ scores in a previous study (Volf et al., 2015). However, our results showed no significant association between DNA methylation changes at cg05016953 (*SLC6A4*) at age 2 and children's IQ at age 6. Because there is a wide distribution of the *5HTTLPR* genotype by race and ethnicity (Gelernter et al., 1997; Kunugi et al., 1997; Nakamura et al., 2000), further studies should be conducted among Asian children.

The most significant DNA methylation change at cg26269038 is located in the body, intron between the 3rd and 4th exon, of the gene solute carrier family 6, the member 3 (*SLC6A3*) on chromosome 5. The gene encodes a dopamine transporter (*DAT*), which is a member of the sodium- and chloride-dependent neurotransmitter transporter family, and provides rapid clearance of dopamine (Chen et al., 2004; Fuke et al., 2001), which mediates the reuptake of dopamine from the synaptic cleft (Vandenbergh et al., 2000). Cómbita et al. (2017) determined whether *SLC6A3/DAT1* gene contributed to individual differences in children's self-regulation skills (Cómbita et al., 2017). They evaluated self-regulation skills and cognitive tasks such as conflict processing, inhibitory control, and intelligence assessments in 127 children at ages 4 and 6 in Spain. They found that the presence of the 10 alleles of the *SLC6A3* gene was related to a declining function of the dopaminergic transmission system, which was associated with poorer performance in self-regulation. Dopaminergic neurotransmission related to the SLC6A3 and DRD2 genes is reportedly associated with cognitive capacities, such as IQ, in previous studies (Kaminski et al., 2018; Montague et al., 2004; Schlagenhauf et al., 2013). Further study should be investigated that epigenetic regulation of gene expression is modulated by environmental factors such as exposure to stress and level of physical activity (Kaminski et al., 2018; Knab et al., 2010; McGowan et al., 2009).

Epigenetic markers, such as DNA methylation, are dynamically reprogrammed during gametogenesis and early embryo preimplantation (Feil et al., 2012; Reik, 2007). Experimental evidence suggests that the epigenome of mammalian embryonic cells is more susceptible to environmental stimulation than other differentiated cells (Khosla et al., 2001; Young et al., 2001) because of the abundance of de novo DNA methyltransferases in these rapidly dividing pluripotent cells (Feil et al., 2012; Reik, 2007). In line with previous findings, we found that greenness exposure in early childhood is a modifiable factor related to DNA methylation change, which was found to be associated with cognitive ability in a previous study. The *SLC6A3* gene, which accumulates cytotoxic dopamine or other toxins in dopamine neurons, is a risk factor for Parkinson's disease (Zhai et al., 2014). Because these neurodegenerative disorders are related to increased environmental stressors, toxins, and oxidative stress in adults (Herman et al., 2018), brain development in children may also be linked to oxidative stress, which is reduced by greenness exposure. Similarly, oxidative stress is widely related to brain development. Recently, greenness exposure was significantly associated with reduced oxidative stress in Italian children (De Petris et al., 2021). Our results show that children with higher greenness exposure had lower DNA methylation levels of the *SLC6A3* gene. This region might be linked to greenness exposure and neurological development in children. However, further studies are needed to understand how these cognitive ability-related CpG sites are linked to greenness exposure.

Exposure to different types of greenness has been shown to have different effects on children's health. A previous study found a strong association between exposure to built greenness, but not natural greenness, and children's IQ at age 6 (Lee et al., 2021). However, DNA methylation changes were more significantly associated with natural greenness at age 2. There have been no previous studies of the association between type of greenness and DNA methylation change, and further studies are needed to investigate the effect of exposure to various types of greenness and intelligence in children. The DNA methylation changes and greenness exposure still need to be analyzed in the EDC entire cohort rather than sub-study (Lee et al., 2021).

3.9. Conclusion

Residential surrounding greenness exposure at age 2 was associated with DNA methylation changes, further associated with cognitive abilities. Further studies are warranted to clarify the epigenetic pathways linking greenness exposure and neurocognitive functions in children.

Chapter 4. Discussion

4.1. Main findings

Our results indicated that greater exposure to greenness during pregnancy, as well as the exposure while children were aged 6 was associated with higher IQ among the children. We examined the association by type of greenness and found that children's IQ was associated with built greenness, but not with natural greenness. In addition, postnatal exposure to greenness at age 6 was more strongly associated with children's IQ than prenatal exposure to greenness.

In addition, we investigated whether the DNA methylation change was mediated for the association between greenness exposure and children’s IQ. We identified 209 CpGs and 8534 CpGs associated with cognitive ability from literature reviews. We found that the methylation levels at 25 cognitive ability-related CpG sites at age 2 were significantly associated with greenness exposure during early childhood and that the methylation level at cg26269038 at age 2 (*SLC6A3*) was significantly associated with the performance IQ score at age 6.

4.2. Strength and limitations

Our study has some strengths. First, a longitudinal analysis of prospective data was performed, namely of exposure to greenness during prenatal and postnatal periods. In addition, we analyzed greenness exposure and DNA methylation level at age 2, and then estimated the effect on children's IQ at age 6 in the EDC cohort population. Second, we estimated the percentages of greenness in various buffer sizes. In previous studies, larger buffer sizes (up to 2000 m) were associated with better physical health than were smaller buffers (Browning and Lee 2017). As there remains no definitive answer regarding the optimal distance to greenness from the child in terms of promoting the child's mental health, further studies should be conducted using buffers of various sizes, rather than considering only one buffer size. Third, we adjusted for various confounding factors, including noise, air pollution, time spent outdoors, and neighborhood or individual SES level, when we estimated the association between greenness exposure and children's IQ. In addition, we adjusted maternal IQ as a major determinant of children's IQ, which was directly or indirectly associated with the child's neurodevelopment (Lean et al. 2018; Ronfani et al. 2015). Finally, it is the first study to explore the interaction of DNA methylation change and greenness on children's IQ based on our knowledge. Our study might provide a clue to explain the causal role of greenness in neurodevelopment in children.

However, our study was also subject to some limitations. First, we used satellite-derived land maps to measure surrounding greenness. This objective measurement of greenness allowed us to measure small-scale green spaces in a standardized way, but the map captured a single period in the year 2010. However, this might not be a major concern because the rate of change in forested areas of Seoul is typically modest (0.2% decrease between 2004 and 2009) (Kil et al. 2016). Second, because we focused on green spaces surrounding residential areas, the exposure to greenness of women at work during pregnancy was not reflected in the data and could have caused exposure misclassification. Third, we did not have available data on the accessibility of green spaces to our participants, which could be a factor underlying the association between exposure to greenness and IQ (Dadvand et al. 2012b). Fourth, we couldn't estimate the greenness exposure considering each participant's moving information, but we tried to estimate the averages of greenness exposure, which were calculated by the mean percentage of greenness exposure using addresses at ages pregnancy, 2 ,4, and 6 follow-up period. Fifth, this study was based on observational data that limit statements on causality. In the future, a randomized clinical trial should be conducted to evaluate the effect of the greenness exposure and neurodevelopment in children. Finally, we only had DNA methylation data on 59 children, not the EDC entire cohort, even though we found no significant differences in the characteristics of the children in the study sub-cohort and the full EDC cohort; thus, our findings need to be further evaluated in a larger cohort.

4.3. Future research directions

Recently, positive associations between greenness and mental health have been reported that were mediated by microbiome (Pearson et al. 2020). Nielsen showed that the influence of a natural environment within 500m of a residential address in urban context on 355 infants' gut microbiota at age 4 months. When the infants live near natural environments, their gut microbiota tends to have a high diversity (Nielsen et al. 2020). Recently, Pelley et al showed that there was different variation depending on height above ground (Pelley 2021). The community composition of bacterial genera that occurred in both air and soil tended to decline with height from the ground. They suggested that further research is needed microbiome diversity in children, because they are different condition such as exposure to microbial communities compared to adults due to their height. Furthermore, this study suggests that greenness which can sit or lie on the ground, can promote more biodiverse exposure to microbes (Pelley 2021). Microbial biodiversity within children's gut may be a potential indirect pathway in the association between exposure to greenness and mental health (Prescott et al. 2016). In addition, Liddicoat et al. suggested a new hypothesis, linking biodiverse soil exposure, gut microbiome, and mental health (Liddicoat et al. 2020). Increased relative microbial abundance has been associated with reduced anxiety-like behavior among the most anxious mice in animal studies, and a rich composition of butyrate-producing bacteria may help explain the beneficial effects of biodiverse green space on human mental health (Liddicoat et al. 2020). However, fewer study still reported that the association between exposure to greenness and cognitive ability, which was modified by the diversity or composition of gut microbiome in children. So further research is needed to replicate and better interpret these interactions.

Since our study has shown that the association between exposed to greenness and DNA methylation changes, further study should be needed to explain the possible mechanism such as reducing air pollution or enhancing physical activity in the association between greenness exposure and developing cognitive function, which mediating DNA methylation changes. Possible mechanism that exposure to greenness was associated with enhancing physical activity and reducing air pollution as we mentioned, their beneficial effects may be impact on cognitive ability in children, mediating DNA methylation changes as gene-environmental interaction factors. So further research should be needed to have evidence for linking to epigenetic changes, exposure to greenness, and cognitive ability in children.

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초록

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배경: 거주지 주변 녹지의 노출은 인지 능력 향상을 포함해서 아동에게 정신 건강상의 이익을 가져온다고 보고되었다. 본 연구에서는 임신 중 녹지 노출과 아이의 6세 시점에서의 녹지 노출이 아이의 6세 시점에서의 아이큐 점수와 연관성이 있는지 코호트 연구디자인으로 서울지역 189명의 아동에게서 분석되었다. 이러한 녹지 노출은 자연 녹지와 인공 녹지로 구분하여 녹지의 유형에 따른 아이큐 점수와의 연관성 분석을 수행하였다. 또한, 이러한 인지기능의 발달에 영향에 대한 생물학적 메커니즘을 설명하기 위해 녹지 노출에 따른 후생 유전자(DNA 메틸화; DNAm)의 변화를 확인함으로써, 녹지 노출이 아동의 인지발달에 미치는 간접 경로에 대한 분석을 수행하였다.

방법: 집 주변 녹지 노출과 아동의 IQ의 연관성을 분석하기 위해 임신중 및 6세 때 서울에 거주한 전향적 코호트의 엄마-아이 189명을 대상으로 실시하였다. 거주지 주변 녹지는 집주소 주변 반경 100~2000m 이내에 있는 녹지를 평가하였고, 이는 환경공간정보서비스에서 제공되는 토지피복지도인 위성 이미지 데이터를 활용해 분석되었다. 녹지는 전체 녹지, 자연 녹지와 인공 녹지로 나누어 분석하였다.

또한, 녹지 노출과 DNAm 변화 사이의 연관성을 조사하기 위해, 문헌 연구를 통해 인지기능과 관련된 후보 유전자를 선택해서, 2세 아동 59명을 대상으로 녹지 노출에 따라서 DNAm의 변화의 유의한 차이가 있는 CpG sites를 확인하였다. 유의한 CpG site를 가지고 6세 IQ(총 아이큐

점수, 언어성 아이큐점수, 동작성 아이큐점수)와의 연관성을 분석하였다.
결과: 서울지역 내 임신 중 및 6세 당시의 녹지 노출과 아이큐와의 연관성 분석을 수행한 결과, 임신 시점에서 거주지 주변 반경 500m 및 1000m에 녹지 비율이 증가될수록 아이의 아이큐점수와 양의 상관성을 보이는 것을 확인 하였다. 그러나 6세 시점에서의 녹지 노출은 100-2000m에서 모두 유의하게 아이큐와 연관성이 있었다. 특히, 아이들의 IQ점수는 자연녹지보다는 인공 녹지와 더 강한 연관성을 보였다. 녹지 노출에 따른 DNA메틸화의 변화가 있는지 확인하기 위해 문헌 리뷰에서 인지 능력과 관련된 209 CpGs와 8534 CpGs를 확인했다. 이 CpG site를 가지고 2세 시점의 녹지 노출과의 연관성을 분석했을 때, 25 개의 CpGs들은 거주지 주변 녹지 노출 정도와 유의한 연관성을 보였고, 이 CpG site 중 알려진 유전자는 총 22개였다. 또한, 2세 녹지 노출에 따라 차이를 보이는 25개의 CpG들과 6세 때의 IQ와 통계적 유의성이 있는지 확인하였는데, 이중에 *SLC6A3* at cg26269038에서 통계적으로 유의하게 6세의 동작성 아이큐 점수와 관련이 있는 것으로 확인 되었다 (Bonferroni correction $P < 0.002$).
결론: 본 연구결과는 거주지 주변의 녹지 노출정도가 증가할 수록 어린이 인지발달의 긍정적 효과를 가져온다는 연구결과이며, 2세 아동에서의 녹지 노출이 DNA메틸화에 영향을 주어, 6세 아동의 인지기능발달에도 영향을 미칠 수 있음을 시사한다. 이 연구결과를 통해 어린이 및 임신부를 위한 녹지조성 계획이 주는 건강상의 이점을 확인할 수 있었고, 이 자료를 바탕으로 도시 계획 및 공공 건강 지원계획에 근거자료로 활용될 수 있다.

주요어: 어린이, 녹지, 인공녹지, 자연녹지, DNA메틸화, 아이큐

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Conflict of Interest

The authors declare no conflict of interest.

Appendix

Supplementary table 1. Previous EWAS or GWAS studies for the association between DNA methylation and IQ.

No	Author	Title	Measurement Of cognitive ability	Age for methylation data (years)	Number of study participants	Methylation analysis method
EWAS						
1	Krushkal et al. 2014	Epigenetic analysis of neurocognitive development at 1 year of age in a community-based pregnancy cohort	Full-scale IQ score	1	168	Humanmethylation27 BeadChip
2	Paquette et al. 2016	Regions of variable DNA methylation in human placenta associated with newborn neurobehavior	Neurobehavioral assessments	Newborn	335	HumanMethylation450 BeadChips
3	Linnér et al. 2017	An epigenome-wide association study meta-analysis of educational attainment	Educational attainment	Range 26.6-79.1	10767	HumanMethylation450 BeadChips
4	Caramaschi et al. 2017	Exploring a causal role of DNA methylation in the relationship between maternal vitamin B12 during pregnancy and child's IQ at age 8, cognitive performance and educational attainment: a two-step Mendelian randomization study	Total IQ, Verbal IQ, Performance IQ	8.6	1557	HumanMethylation450 BeadChips
5	Marioni et al. 2018	Meta-analysis of epigenome-wide association studies of cognitive abilities	Memory, Digit Symbol test, Verbal Fluency, MMSE	56.2-79.1	Range 219-2307	HumanMethylation450 BeadChips
6	Caramaschi et al. 2020	Meta-analysis of epigenome-wide associations between DNA methylation at birth and childhood cognitive skills	Full-scale IQ score	4-9	2196-3798	HumanMethylation450 BeadChips
GWAS						
1	Kong et al. 2013	IQdb: an intelligence quotient score-associated gene resource for human intelligence	IQ	Not mentioned	Not mentioned	HumanMethylation450 BeadChips
2	Zhao et al. 2014	A systems biology approach to identify intelligence quotient score-related genomic regions, and pathways relevant to potential therapeutic treatments	IQ	Not mentioned	Not mentioned	Not mentioned
3	Benyamin et al. 2014	Childhood intelligence is heritable, highly polygenic and associated with FNBP1L	IQ or Cognitive ability	Range 6–18 years	17 989	Not mentioned
4	Coleman et al. 2019	Biological annotation of genetic loci associated with intelligence in a meta-analysis of 87,740	Performance on the SAT or ACT	6–18 years	87,740	Illumina HumanHap550 (ALSPAC) Illumina

		individuals				Human610_Quadv1 chip (LBC1936) Illumina Human660W Quad Array(Raine) Illumina 610K array(GenR)
5	Hill et al. 2014	Human cognitive ability is influenced by genetic variation in components of postsynaptic signaling complexes assembled by NMDA receptors and MAGUK proteins	Crystallized cognitive ability, memory and processing speed	Middle-aged and older	3511	UK BiLEVE Axiom array, Illumina Infinium OmniExpress
6	Jansen et al. 2020	Genome-wide meta-analysis of brain volume identifies genomic loci and genes shared with intelligence	Brain volume & IQ overlapping	Not mentioned	47,316	Not mentioned
7	Kornilov et al. 2019	Genome-Wide Homozygosity Mapping Reveals Genes Associated With Cognitive Ability in Children From Saudi Arabia	General cognitive ability	7.34 to 18.71 years	7,186	UK BiLEVE AxiomTM array, UKBTM Axiom array
8	Luciano et al. 2006	Genome-wide Scan of IQ Finds Significant Linkage to a Quantitative Trait Locus on 2q	Multidimensional Aptitude Battery subtests and WAIS-R Digit Symbol subtest, and two word-recognition tests	15.7-22.2 years	361 families	Illumina, HumanCoreExome array
9	Smajlagić et al. 2018	Moderating effect of mode of delivery on the genetics of intelligence: Explorative genome-wide analyses in ALSPAC	WISC	8.5 years	2,421	Not mentioned
10	Zabaneh et al. 2018	A genome-wide association study for extremely high intelligence	IQ	12 years and adults	1238cases / 8172controls	Illumina Human Hap 550 quad
11	Zhu et al. 2018	Multi-level genomic analyses suggest new genetic variants involved in human memory	Long-term, and short-term memory	Mean age 18	1522-1623	Illumina Human Omni-2.5 Quad Beadchip
12	Sniekers et al. 2017	Genome-wide association meta-analysis of 78,308 individuals identifies new loci and genes influencing human intelligence	IQ	Children(under 13) to adult(18-78years)	78,308	HumanOmniZhongHua-8 Beadchip v1.1
13	Savage et al. 2018	Genome-wide association meta-analysis in 269,867 individuals identifies new genetic and functional links to intelligence	IQ	Range 5-98	269,867	Not mentioned

Supplementary Table 2. List of selected 25 CpG sites, which were associated with greenness in children (n=59)

No	CpG site	UCSC RefGene Name	Description*	SNP distance	Minor allele frequency	Study
1	cg14464361	AGAP1	ArfGAP With GTPase Domain, Ankyrin Repeat And PH Domain 1	36	0.000200	Savage et al. 2018
2	cg23651585	AUTS2	Activator Of Transcription And Developmental Regulator AUTS2	27	0.000200	Savage et al. 2018
3	cg17146029	AUTS2	Activator Of Transcription And Developmental Regulator AUTS2	2	0.000399	Savage et al. 2018
4	cg11176256	BAIAP2	BAR/IMD Domain Containing Adaptor Protein 2	43	0.003594	Savage et al. 2018
5	cg12414502	BTN2A1	Butyrophilin Subfamily 2 Member A1	51	0.000200	Savage et al. 2018
6	cg07266431	CDK6	Cyclin Dependent Kinase 6	38	0.003794	Marioni et al. 2018
7	cg21175642	CELSR3	Cadherin EGF LAG Seven-Pass G-Type Receptor 3	47	0.000200	Savage et al. 2018
8	cg27492942	CISD3	CDGSH Iron Sulfur Domain 3	33	0	Marioni et al. 2018
9	cg27636559	EFTUD1	Elongation Factor Like GTPase 1	4	0.004593	Savage et al. 2018
10	cg00809988	ELAVL2	Embryonic Lethal, Abnormal Vision, Like RNA Binding Protein 2	3	0.000200	Savage et al. 2018
11	cg19258882	ERBB3	Erb-B2 Receptor Tyrosine Kinase 3	32	0.000200	Savage et al. 2018
12	cg00252813	GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase	4	0.003794	Marioni et al. 2018
13	cg25189904	GNG12	G Protein Subunit Gamma 12	3	0.0002	Marioni et al. 2018
14	cg23159678	NOVA1	NOVA Alternative Splicing Regulator 1	47	0.000399	Savage et al. 2018
15	cg03367519	PDE4D	Phosphodiesterase 4D	12	0.002097	Savage et al. 2018
16	cg27609819	PLCL1	Phospholipase C Like 1	47	0.000599	Savage et al. 2018
17	cg05897638	PROS1	Protein S	38	0.002995	Savage et al. 2018
18	cg18311871	PTPRN2	Protein Tyrosine Phosphatase Receptor Type N2	11	0.005591	Savage et al. 2018
19	cg13599020	SAMD3	Sterile Alpha Motif Domain Containing 3	7	0.001597	Marioni et al. 2018
20	cg26269038	SLC6A3	Solute Carrier Family 6 Member 3	10	0.027356	Kong et al. 2013
21	cg05016953	SLC6A4	Solute Carrier Family 6 Member 4	29	0.000399	Savage et al. 2018
22	cg13092901	TYMP	Thymidine Phosphorylase	43	0.006589	Marioni et al. 2018
23	cg16296679	WBP2NL	WBP2 N-Terminal Like	11	0.000599	Savage et al. 2018
24	cg04789403	NA	-	39	0.000399	Marioni et al. 2018
25	cg16594502	NA	-	44	0.0002	Marioni et al. 2018

*The information was obtained by 'https://www.genecards.org/'.

Supplementary Table 3. List of 20 top-ranked genes in *Reactome* pathway analysis

ID	Description	GeneRatio	BgRatio	p.adjust	qvalue	geneID	Count
R-HSA-112311	**Neurotransmitter clearance**	**2/14**	**10/10654**	**0.009**	**0.006**	***SLC6A3/SLC6A4***	**2**
R-HSA-8939243	RUNX1 interacts with co-factors whose precise effect on RUNX1 targets is not known	1/14	38/10654	0.117	0.075	*AUTS2*	1
R-HSA-8878171	Transcriptional regulation by RUNX1	2/14	239/10654	0.117	0.075	*AUTS2/CDK6*	2
R-HSA-5663213	RHO GTPases Activate WASPs and WAVEs	1/14	36/10654	0.117	0.075	*BAIAP2*	1
R-HSA-8851680	Butyrophilin (BTN) family interactions	1/14	12/10654	0.117	0.075	*BTN2A1*	1
R-HSA-8934593	Regulation of RUNX1 Expression and Activity	1/14	17/10654	0.117	0.075	*CDK6*	1
R-HSA-2559585	Oncogene Induced Senescence	1/14	33/10654	0.117	0.075	*CDK6*	1
R-HSA-1358803	Downregulation of ERBB2:ERBB3 signaling	1/14	13/10654	0.117	0.075	*ERBB3*	1
R-HSA-8847993	ERBB2 Activates PTK6 Signaling	1/14	13/10654	0.117	0.075	*ERBB3*	1
R-HSA-6785631	ERBB2 Regulates Cell Motility	1/14	15/10654	0.117	0.075	*ERBB3*	1
R-HSA-1963642	PI3K events in ERBB2 signaling	1/14	16/10654	0.117	0.075	*ERBB3*	1
R-HSA-1250196	SHC1 events in ERBB2 signaling	1/14	22/10654	0.117	0.075	*ERBB3*	1
R-HSA-8863795	Downregulation of ERBB2 signaling	1/14	29/10654	0.117	0.075	*ERBB3*	1
R-HSA-70263	Gluconeogenesis	1/14	34/10654	0.117	0.075	*GAPDH*	1
R-HSA-8964315	G beta:gamma signalling through BTK	1/14	18/10654	0.117	0.075	*GNG12*	1
R-HSA-392851	Prostacyclin signalling through prostacyclin receptor	1/14	19/10654	0.117	0.075	*GNG12*	1
R-HSA-418217	G beta:gamma signalling through PLC beta	1/14	20/10654	0.117	0.075	*GNG12*	1
R-HSA-8964616	G beta:gamma signalling through CDC42	1/14	20/10654	0.117	0.075	*GNG12*	1
R-HSA-500657	Presynaptic function of Kainate receptors	1/14	21/10654	0.117	0.075	*GNG12*	1
R-HSA-392170	ADP signalling through P2Y purinoceptor 12	1/14	22/10654	0.117	0.075	*GNG12*	1
R-HSA-428930	Thromboxane signalling through TP receptor	1/14	23/10654	0.117	0.075	*GNG12*	1
R-HSA-1296041	Activation of G protein gated Potassium channels	1/14	25/10654	0.117	0.075	*GNG12*	1
R-HSA-1296059	G protein gated Potassium channels	1/14	25/10654	0.117	0.075	*GNG12*	1
R-HSA-392451	G beta:gamma signalling through PI3Kgamma	1/14	25/10654	0.117	0.075	*GNG12*	1
R-HSA-418592	ADP signalling through P2Y purinoceptor 1	1/14	25/10654	0.117	0.075	*GNG12*	1
R-HSA-997272	Inhibition of voltage gated Ca2+ channels via Gbeta/gamma subunits	1/14	25/10654	0.117	0.075	*GNG12*	1
R-HSA-202040	G-protein activation	1/14	28/10654	0.117	0.075	*GNG12*	1
R-HSA-400042	Adrenaline,noradrenaline inhibits insulin secretion	1/14	28/10654	0.117	0.075	*GNG12*	1
R-HSA-1296065	Inwardly rectifying K+ channels	1/14	31/10654	0.117	0.075	*GNG12*	1

R-HSA-392518	Signal amplification	1/14	32/10654	0.117	0.075	*GNG12*	1
R-HSA-397795	G-protein beta:gamma signalling	1/14	32/10654	0.117	0.075	*GNG12*	1
R-HSA-451326	Activation of kainate receptors upon glutamate binding	1/14	32/10654	0.117	0.075	*GNG12*	1
R-HSA-456926	Thrombin signalling through proteinase activated receptors (PARs)	1/14	32/10654	0.117	0.075	*GNG12*	1
R-HSA-163359	Glucagon signaling in metabolic regulation	1/14	33/10654	0.117	0.075	*GNG12*	1
R-HSA-420092	Glucagon-type ligand receptors	1/14	33/10654	0.117	0.075	*GNG12*	1
R-HSA-977444	GABA B receptor activation	1/14	39/10654	0.117	0.075	*GNG12*	1
R-HSA-991365	Activation of GABAB receptors	1/14	39/10654	0.117	0.075	*GNG12*	1
R-HSA-180024	DARPP-32 events	1/14	26/10654	0.117	0.075	*PDE4D*	1
R-HSA-111885	Opioid Signalling	2/14	91/10654	0.117	0.075	*PDE4D/GNG12*	2
R-HSA-159740	Gamma-carboxylation of protein precursors	1/14	10/10654	0.117	0.075	*PROS1*	1
R-HSA-159782	Removal of aminoterminal propeptides from gamma-carboxylated proteins	1/14	10/10654	0.117	0.075	*PROS1*	1
R-HSA-159854	Gamma-carboxylation, transport, and amino-terminal cleavage of proteins	1/14	11/10654	0.117	0.075	*PROS1*	1
R-HSA-140875	Common Pathway of Fibrin Clot Formation	1/14	22/10654	0.117	0.075	*PROS1*	1
R-HSA-140877	Formation of Fibrin Clot (Clotting Cascade)	1/14	39/10654	0.117	0.075	*PROS1*	1
R-HSA-163841	Gamma carboxylation, hypusine formation and arylsulfatase activation	1/14	39/10654	0.117	0.075	*PROS1*	1
R-HSA-76002	Platelet activation, signaling and aggregation	2/14	262/10654	0.117	0.075	*PROS1/GNG12*	2
R-HSA-442660	Na+/Cl- dependent neurotransmitter transporters	1/14	19/10654	0.117	0.075	*SLC6A3*	1
R-HSA-112315	Transmission across Chemical Synapses	3/14	272/10654	0.117	0.075	*SLC6A3/SLC6A4/GNG12*	3
R-HSA-112316	Neuronal System	3/14	413/10654	0.117	0.075	*SLC6A3/SLC6A4/GNG12*	3
R-HSA-73614	Pyrimidine salvage	1/14	11/10654	0.117	0.075	*TYMP*	1
R-HSA-73621	Pyrimidine catabolism	1/14	12/10654	0.117	0.075	*TYMP*	1
R-HSA-8956321	Nucleotide salvage	1/14	23/10654	0.117	0.075	*TYMP*	1
R-HSA-8956319	Nucleobase catabolism	1/14	37/10654	0.117	0.075	*TYMP*	1
R-HSA-69231	Cyclin D associated events in G1	1/14	44/10654	0.120	0.077	*CDK6*	1
R-HSA-69236	G1 Phase	1/14	44/10654	0.120	0.077	*CDK6*	1
R-HSA-381676	Glucagon-like Peptide-1 (GLP1) regulates insulin secretion	1/14	42/10654	0.120	0.077	*GNG12*	1
R-HSA-6814122	Cooperation of PDCL (PhLP1) and TRiC/CCT in G-protein beta folding	1/14	42/10654	0.120	0.077	*GNG12*	1
R-HSA-432040	Vasopressin regulates renal water homeostasis via	1/14	43/10654	0.120	0.077	*GNG12*	1

	Aquaporins						
R-HSA-977606	Regulation of Complement cascade	1/14	47/10654	0.126	0.080	*PROS1*	1
R-HSA-418597	G alpha (z) signalling events	1/14	48/10654	0.127	0.081	*GNG12*	1
R-HSA-1227986	Signaling by ERBB2	1/14	50/10654	0.130	0.083	*ERBB3*	1
R-HSA-445717	Aquaporin-mediated transport	1/14	52/10654	0.132	0.084	*GNG12*	1
R-HSA-8848021	Signaling by PTK6	1/14	54/10654	0.133	0.085	*ERBB3*	1
R-HSA-9006927	Signaling by Non-Receptor Tyrosine Kinases	1/14	54/10654	0.133	0.085	*ERBB3*	1
R-HSA-977443	GABA receptor activation	1/14	56/10654	0.136	0.086	*GNG12*	1
R-HSA-1236394	Signaling by ERBB4	1/14	58/10654	0.136	0.087	*ERBB3*	1
R-HSA-166658	Complement cascade	1/14	58/10654	0.136	0.087	*PROS1*	1
R-HSA-2029482	Regulation of actin dynamics for phagocytic cup formation	1/14	61/10654	0.141	0.090	*BAIAP2*	1
R-HSA-4086398	Ca2+ pathway	1/14	62/10654	0.141	0.090	*GNG12*	1
R-HSA-70171	Glycolysis	1/14	71/10654	0.158	0.101	*GAPDH*	1
R-HSA-2219530	Constitutive Signaling by Aberrant PI3K in Cancer	1/14	75/10654	0.164	0.104	*ERBB3*	1
R-HSA-9009391	Extra-nuclear estrogen signaling	1/14	77/10654	0.164	0.104	*GNG12*	1
R-HSA-422356	Regulation of insulin secretion	1/14	78/10654	0.164	0.104	*GNG12*	1
R-HSA-418594	G alpha (i) signalling events	2/14	406/10654	0.164	0.104	*PDE4D/GNG12*	2
R-HSA-416482	G alpha (12/13) signalling events	1/14	80/10654	0.166	0.105	*GNG12*	1
R-HSA-2029480	Fcgamma receptor (FCGR) dependent phagocytosis	1/14	86/10654	0.173	0.110	*BAIAP2*	1
R-HSA-425366	Transport of bile salts and organic acids, metal ions and amine compounds	1/14	85/10654	0.173	0.110	*SLC6A3*	1
R-HSA-418346	Platelet homeostasis	1/14	88/10654	0.174	0.111	*GNG12*	1
R-HSA-4420097	VEGFA-VEGFR2 Pathway	1/14	99/10654	0.177	0.113	*BAIAP2*	1
R-HSA-9006934	Signaling by Receptor Tyrosine Kinases	2/14	473/10654	0.177	0.113	*BAIAP2/ERBB3*	2
R-HSA-2219528	PI3K/AKT Signaling in Cancer	1/14	101/10654	0.177	0.113	*ERBB3*	1
R-HSA-6811558	PI5P, PP2A and IER3 Regulate PI3K/AKT Signaling	1/14	103/10654	0.177	0.113	*ERBB3*	1
R-HSA-70326	Glucose metabolism	1/14	91/10654	0.177	0.113	*GAPDH*	1
R-HSA-373080	Class B/2 (Secretin family receptors)	1/14	95/10654	0.177	0.113	*GNG12*	1
R-HSA-390466	Chaperonin-mediated protein folding	1/14	95/10654	0.177	0.113	*GNG12*	1
R-HSA-1296071	Potassium Channels	1/14	99/10654	0.177	0.113	*GNG12*	1
R-HSA-391251	Protein folding	1/14	101/10654	0.177	0.113	*GNG12*	1
R-HSA-5619102	SLC transporter disorders	1/14	99/10654	0.177	0.113	*SLC6A3*	1
R-HSA-15869	Metabolism of nucleotides	1/14	101/10654	0.177	0.113	*TYMP*	1

R-HSA-194138	Signaling by VEGF	1/14	107/10654	0.180	0.115	*BAIAP2*	1
R-HSA-2559582	Senescence-Associated Secretory Phenotype (SASP)	1/14	110/10654	0.180	0.115	*CDK6*	1
R-HSA-199418	Negative regulation of the PI3K/AKT network	1/14	110/10654	0.180	0.115	*ERBB3*	1
R-HSA-163685	Integration of energy metabolism	1/14	108/10654	0.180	0.115	*GNG12*	1
R-HSA-2559580	Oxidative Stress Induced Senescence	1/14	125/10654	0.201	0.128	*CDK6*	1
R-HSA-114608	Platelet degranulation	1/14	129/10654	0.205	0.130	*PROS1*	1
R-HSA-76005	Response to elevated platelet cytosolic Ca2+	1/14	134/10654	0.210	0.134	*PROS1*	1
R-HSA-202733	Cell surface interactions at the vascular wall	1/14	137/10654	0.212	0.135	*PROS1*	1
R-HSA-3858494	Beta-catenin independent WNT signaling	1/14	146/10654	0.222	0.142	*GNG12*	1
R-HSA-453279	Mitotic G1-G1/S phases	1/14	149/10654	0.224	0.143	*CDK6*	1
R-HSA-5619115	Disorders of transmembrane transporters	1/14	176/10654	0.258	0.164	*SLC6A3*	1
R-HSA-72163	mRNA Splicing - Major Pathway	1/14	183/10654	0.265	0.168	*ELAVL2*	1
R-HSA-72172	mRNA Splicing	1/14	191/10654	0.272	0.173	*ELAVL2*	1
R-HSA-2559583	Cellular Senescence	1/14	195/10654	0.274	0.175	*CDK6*	1
R-HSA-112314	Neurotransmitter receptors and postsynaptic signal transmission	1/14	206/10654	0.285	0.182	*GNG12*	1
R-HSA-416476	G alpha (q) signaling events	1/14	217/10654	0.296	0.188	*GNG12*	1
R-HSA-8939211	ESR-mediated signaling	1/14	223/10654	0.300	0.191	*GNG12*	1
R-HSA-72203	Processing of Capped Intron-Containing Pre-mRNA	1/14	244/10654	0.321	0.204	*ELAVL2*	1
R-HSA-5673001	RAF/MAP kinase cascade	1/14	249/10654	0.323	0.206	*ERBB3*	1
R-HSA-425407	SLC-mediated transmembrane transport	1/14	251/10654	0.323	0.206	*SLC6A3*	1
R-HSA-5684996	MAPK1/MAPK3 signaling	1/14	255/10654	0.324	0.207	*ERBB3*	1
R-HSA-1257604	PIP3 activates AKT signaling	1/14	264/10654	0.331	0.211	*ERBB3*	1
R-HSA-9607240	FLT3 Signaling	1/14	267/10654	0.331	0.211	*ERBB3*	1
R-HSA-5683057	MAPK family signaling cascades	1/14	294/10654	0.354	0.225	*ERBB3*	1
R-HSA-71387	Metabolism of carbohydrates	1/14	295/10654	0.354	0.225	*GAPDH*	1
R-HSA-9006931	Signaling by Nuclear Receptors	1/14	299/10654	0.355	0.226	*GNG12*	1
R-HSA-9006925	Intracellular signaling by second messengers	1/14	305/10654	0.357	0.228	*ERBB3*	1
R-HSA-195258	RHO GTPase Effectors	1/14	327/10654	0.375	0.239	*BAIAP2*	1
R-HSA-195721	Signaling by WNT	1/14	331/10654	0.376	0.239	*GNG12*	1
R-HSA-5663202	Diseases of signal transduction	1/14	377/10654	0.413	0.263	*ERBB3*	1
R-HSA-194315	Signaling by Rho GTPases	1/14	455/10654	0.473	0.301	*BAIAP2*	1
R-HSA-	GPCR ligand binding	1/14	468/10654	0.479	0.305	*GNG12*	1

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R-HSA-2262752	Cellular responses to stress	1/14	479/10654	0.480	0.305	*CDK6*	1
R-HSA-6798695	Neutrophil degranulation	1/14	480/10654	0.480	0.305	*PTPRN2*	1
R-HSA-8953897	Cellular responses to external stimuli	1/14	493/10654	0.485	0.309	*CDK6*	1

Supplementary Table 4. The association between (a) prenatal and (b)postnatal built greenness and covariates

(a)

Covariates	Buffer size	100m		500m		1000m		1500m		2000m	
	Categorical	Mean (SD) or beta (SE)	*P*-value	Mean (SD) or beta (SE)	*P*-value	Mean (SD) or beta (SE)	*P*-value	Mean (SD) or beta (SE)	*P*-value	Mean (SD) or beta (SE)	*P* -value
Children's sex	Girls	12.27(12.67)	0.642	8.93(6.39)	0.060	**8.11(4.46)**	**0.032**	**7.75(3.52)**	**0.024**	**7.27(2.82)**	**0.012**
	Boys	13.12(12.36)		10.84(7.36)		**9.67(5.35)**		**9.09(4.53)**		**8.41(3.31)**	
Maternal age during pregnancy		0.21(0.26)	0.423	0.28(0.14)	0.053	0.18(0.10)	0.080	0.11(0.09)	0.217	0.09(0.07)	0.184
Maternal education level	≤ university graduate	**11.45(12.12)**	**0.001**	**8.97(6.34)**	**<.0001**	**8.37(4.59)**	**0.005**	**8.03(3.76)**	**0.012**	**7.62(3.03)**	**0.014**
	≥ graduate school	**19.38(12.46)**		**14.96(8)**		**11.85(6.03)**		**10.67(5.17)**		**9.14(3.37)**	
Maternal IQ		**0.18(0.08)**	**0.029**	**0.12(0.04)**	**0.006**	**0.08(0.03)**	**0.006**	**0.06(0.02)**	**0.024**	**0.04(0.02)**	**0.030**
Exposure to ETS during pregnancy	No	15.82(13.39)	0.199	11.36(7.31)	0.265	9.75(4.43)	0.352	9.34(3.39)	0.248	8.47(2.62)	0.319
	Yes	12.29(12.39)		9.65(6.93)		8.72(5.12)		8.29(4.26)		7.78(3.23)	
Average of NO_2 concentration during previous 3 years in residential area during pregnancy		**-0.74(0.32)**	**0.020**	-0.32(0.18)	0.077	-0.18(0.13)	0.151	-0.15(0.11)	0.158	-0.08(0.08)	0.309
Residential deprivation index as neighborhood SES during pregnancy		**-0.03(0.01)**	**0.019**	**-0.03(0.01)**	**0.0001**	**-0.03(0.01)**	**<.0001**	**-0.03(0.00)**	**<.0001**	**-0.02(0.00)**	**<.0001**
Road density in each radius during pregnancy		0.12(0.07)	0.074	**0.21(0.06)**	**0.001**	**0.19(0.05)**	**<.0001**	**0.20(0.04)**	**<.0001**	**0.20(0.04)**	**<.0001**

(b)

Covariates	Buffer size	100m		500m		1000m		1500m		2000m	
	Categorical	Mean(%)(SD) or beta (SE)	*P*-value	Mean(%)(SD) or beta (SE)	*P*-value	Mean(%)(SD) or beta (SE)	*P*-value	Mean(%)(SD) or beta (SE)	*P*-value	Mean(%)(SD) or beta (SE)	*P* -value
Children's sex	Girls	12.74(12.86)	0.248	10.15(6.65)	0.141	8.93(4.46)	0.220	8.64(3.47)	0.648	8.28(2.90)	0.840
	Boys	14.91(12.97)		11.62(7.04)		9.83(5.48)		8.91(4.43)		8.37(3.24)	
Maternal age during pregnancy		0.28(0.27)	0.309	0.26(0.14)	0.073	0.13(0.10)	0.206	0.10(0.08)	0.238	0.05(0.06)	0.453
Maternal education level	≤ university graduate	13.32(13.14)	0.182	**10.35(6.36)**	**0.040**	9.06(4.47)	0.129	8.51(3.6)	0.114	8.17(2.89)	0.187
	≥ graduate school	16.76(11.55)		**13.89(8.67)**		11.17(7.15)		10.2(5.48)		9.16(3.84)	
Maternal IQ		0.07(0.08)	0.434	0.07(0.04)	0.131	0.04(0.03)	0.199	0.03(0.02)	0.195	0.02(0.02)	0.251
Exposure to ETS at age 6	No	14.65(13.23)	0.138	11.25(7.23)	0.171	9.5(5.28)	0.606	8.89(4.12)	0.480	8.34(3.05)	0.894
	Yes	11.37(11.7)		9.84(5.54)		9.06(4.12)		8.41(3.56)		8.27(3.17)	
Average of NO_2 concentration at age 6		0.03(0.35)	0.936	**0.46(0.18)**	**0.012**	**0.36(0.13)**	**0.007**	**0.29(0.11)**	**0.006**	**0.2(0.08)**	**0.015**
Household income	Low	12.22(13.99)	0.351	9.57(5.73)	0.151	8.86(4.23)	0.437	8.13(3.63)	0.231	7.89(3.00)	0.299
	High	14.34(12.62)		11.3(7.14)		9.55(5.23)		8.96(4.08)		8.45(3.09)	
Residential deprivation index		-0.01(0.01)	0.739	**-0.03(0.01)**	**0.001**	**-0.02(0.01)**	**<.0001**	**-0.02(0.004)**	**<.0001**	**-0.02(0.003)**	**<.0001**
Subjective noise level	Very Loud	11.26(12.64)	0.801	10.64(5.43)	0.608	9.15(4.65)	0.398	9.02(3.86)	0.651	8.51(2.72)	0.724
	Loud	13.97(13.67)		11.16(7.50)		9.28(5.99)		8.75(4.73)		8.26(3.37)	
	Moderate	13.29(12.85)		10.17(6.37)		8.92(4.28)		8.44(3.46)		8.09(2.94)	
	Quiet	15.50(12.61)		12.38(7.56)		10.86(5.16)		9.62(4.00)		8.95(2.96)	
	Very quiet	20.16(2.06)		10.07(3.45)		7.70(0.81)		7.35(3.96)		7.93(5.12)	
Distance to main road at age 6	≥ 500m	14.70(13.42)	0.371	10.61(6.66)	0.260	9.31(5.25)	0.853	8.89(4.23)	0.903	8.46(3.06)	0.789
	50m-499m	11.50(12.91)		10.26(6.92)		9.25(4.60)		8.67(3.36)		8.17(2.72)	
	≤ 50m	14.32(11.47)		12.53(7.35)		9.81(4.95)		8.59(4.09)		8.13(3.51)	
Physical activity time in outdoor at age 6	≤ 1hr	13.85(12.88)	0.993	10.29(6.31)	0.339	8.66(4.25)	0.073	**8.15(3.45)**	**0.043**	**7.78(2.96)**	**0.021**
	1-2hr	13.99(13.09)		11.48(7.99)		10.18(6.23)		**9.37(4.80)**		**8.90(3.32)**	
	≥ 3hr	13.60(13.31)		12.24(6.19)		10.65(4.37)		**10.03(3.59)**		**9.27(2.46)**	
Road density within each buffer at age 6		0.02(0.06)	0.775	0.09(0.05)	0.061	**0.13(0.04)**	**0.002**	**0.17(0.04)**	**<.0001**	**0.18(0.03)**	**<.0001**

Abbreviations: NO_2: nitrogen dioxide, IQ: intelligence quotient, ETS: environmental tobacco smoke, SES: Socioeconomic status, SE: Standard Error, SD: Standard Deviation

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We love because he first loved us − 1 John 4: 19-