Effect of prenatal antioxidant intake on infants' respiratory infection is modified by a *CD14* polymorphism

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Background: **Prenatal maternal diet may influence disease susceptibility in offspring with specific genetic backgrounds. We hypothesized that interactions between prenatal antioxidant intake and polymorphisms in immunity genes influence respiratory tract infection (RTI) susceptibility in infants at 12 months of age.**

Methods: **This study included 550 infants. In the Cohort for Childhood Origin of Asthma and Allergic Diseases (COCOA) birth cohort study, prenatal maternal diet was assessed by administering a food frequency questionnaire. Infants' cord blood was genotyped for *CD14* (rs2569190), *TLR4* (rs1927911), and *GSDMB* (rs4794820) polymorphisms by the TaqMan method.**

Results: **Higher prenatal intake of total fruit and vegetables (FV) was associated with the decreased risk of RTI in offspring (*P*-trend=0.0430). In children with TT genotype at rs2569190, a higher prenatal intake of vitamins A and C, fruits, and total FV decreased RTI risk (*P*-trend <0.05), while in infants with TC+CC genotype, a higher prenatal intake of fruit increased RTI risk (*P*-trend <0.05). When analyzing the 3 genotypes, children with TT genotype at rs2569190 were more protected against RTIs compared with those with CC genotype with respect to vitamin C and fruits [odds ratio (OR)=5.04 and OR=10.30, respectively]. In children with CC genotype at rs1927911, RTI risk showed a dose–response association with a higher prenatal intake of vitamin C (*P* for interaction<0.05). A higher prenatal intake of fruits and total FV reduced RTI risk in infants with GA+AA genotype of rs4794820 (*P* for interaction<0.05).**

Conclusion: **Prenatal antioxidant intake may reduce RTI risk in infants and this relationship may be modified by *CD14*, *TLR4*, and *GSDMB* polymorphisms.**

World J Pediatr 2017;13(2):173-182

Key words: **antioxidants;**
***CD14*;**
fruit;
polymorphism;
respiratory tract infection

Original article

Introduction

The Developmental Origins of Health and Disease hypothesis[1] suggests that environmental exposure during prenatal and early infancy influences the development of the immune system.[2] In addition, a large number of studies have proven that diet can influence the development of inflammatory diseases.[3] Considering the link between early exposure to certain dietary factors and inflammatory diseases in later life, maternal diet during pregnancy is a topic of increasing interest, as it is a modifiable cause of disease in offspring. Deficiencies in specific nutrients, in particular, during pregnancy may increase the susceptibility to and severity of infections in offspring; this may be caused by modulation of immune functions as well as induction of epigenetic or genetic variations in offspring.[2,4]

Specifically, prenatal maternal intake of antioxidants such as vitamins A, C, and E may be particularly important, as they are essential for fetal growth and can influence disease susceptibility later in life. This

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doi: 10.1007/s12519-016-0054-6
Online First, October 2016

may be due to the fact that prenatal antioxidant intake shapes the fetal antioxidant status[5] and influences the development of the immune system,[6-8] oxygen radical absorbance capacity,[9] and epigenetic changes.[10,11] For example, antioxidants, such as vitamin E, decrease lipid peroxidation and superoxide production,[12] protect immune cells from reactive oxygen species (ROS), and enhance macrophage function and production of antibodies such as immunoglobulin A.[13]

Respiratory tract infection (RTI) during infancy imposes a large disease burden because of its high prevalence and morbidity.[14] Susceptibility to RTI is shaped by complex interactions between host genetic factors, pathogenic virulence, and environmental factors.[15] Since airway epithelial cells are continuously in direct contact with the environment, the respiratory tract is a major site of local oxidative injuries caused by respiratory infection.[16]

Nutritional status and diet affect the development of innate immune systems as well as lung.[17] Diet patterns have influence on the production of ROS,[18] which is involved in the signaling cascade of inflammatory gene expression.[19] In addition, ROS activates the transcription of Nuclear factor (erythroid-derived 2)-like 2 (*Nrf2*), which subsequently causes the transcription of Glutathione S-transferase pi 1 (*GSTP1*).[20] The expression of these genes contributes to the capacity of ROS detoxification. Polymorphisms of the above mentioned antioxidant-related genes might contribute to the susceptibility to RTI, in that increased production of ROS in response to RTI augments respiratory epithelial injury in addition to RTI *per se* and affects the immune system, and thereby increases disease severity.

Moreover, polymorphisms in the innate immune system such as *CD14* (rs2569190) and Toll-like receptor 4 (*TLR4*) (rs1927911) have been known to be associated with the development of RTI because they play an important role in the recognition of respiratory viruses and influence the development of RTI.[21] For instance, polymorphisms in *CD14* affect the levels of soluble *CD14*, which affect the mobilization of pathogen components to the immune system and the extent of cytokine production.[22,23] However, to our knowledge, there is no study on the interactions between prenatal antioxidant intakes and polymorphisms of immunity related genes on the risk of RTI during early life.

Therefore, we assessed the influence of prenatal antioxidant intake on the risk of RTI and its interaction with genetic factors in 12-month-old infants in the birth cohort study. Furthermore, considering polymorphism dependent increases of *GSDMB* expression and increases in inflammation in response to viral infection,[24] we analyzed the interactions between *GSDMB* (rs4794820) polymorphisms and RTI risk at 12 months of age in the context of maternal antioxidant intake during pregnancy.

Methods

Sample and study design

The Cohort for Childhood Origin of Asthma and Allergic Diseases (COCOA) study is an ongoing prospective longitudinal study involving women recruited in the 26th week of pregnancy from four antenatal clinics and seven public health centers for antenatal care in Seoul, Republic of Korea and their children. The data in this study was collected from November, 2007 to June, 2012.

The study protocol was approved by the institutional review boards of the Asan Medical Center (IRB No. 2008-0616), Samsung Medical Center (IRB No. 2009-02-021), Yonsei University (IRB No. 4-2008-0588), and CHA Medical Center (IRB No. 2010-010). Informed consent forms were confirmed by each IRB and were obtained from the parents of each infant.

The study methods have been described in detail elsewhere.[25] Among a total of healthy pregnant women enrolled during the study period, 983 mother–infant pairs were followed until the infants were 12 months of age. Of the 12-month-old children, 550 infants were included in the analysis after excluding participants lacking information on maternal diet during pregnancy (n=166), genotypes from cord blood collected at delivery (n=102), and RTI at 12 months of age (n=165). The major reasons of no visit at 12 months of age were mostly due to limited time to visit and cumbersomeness of travel among working mothers, spouse's dissent, and no need to visit due to child in being healthy, etc. There were no significant differences between infants included and those excluded in this study except seasons of birth (Supplementary Table 1).

Outcome and exposure variables, as measured by food frequency questionnaire (FFQ)

The primary outcome variable of interest was the prevalence of RTI that was diagnosed by physicians and pediatric allergy-pulmonology specialists at the 12-month follow-up visits. RTI included common cold, rhinosinusitis, acute otitis media, croup, pneumonia, tracheobronchitis, or bronchiolitis. Risk of RTI was defined as an occurrence of more than one RTI until 12 months of age. To assess maternal dietary intake, a semi-quantitative FFQ was self-administered at 26 weeks of pregnancy. This questionnaire has been validated previously and includes 113 food items with nine non-overlapping intake frequencies during the preceding year (ranging from "rarely eaten" to "eaten more than three times per day") and three portion sizes (small, average, or large).[26] The dietary intake was assessed using a computerized nutrient-intake assessment software program (CAN-Pro 3.0; Korean Nutrition Society, Seoul, Korea).

Genotyping analysis

Cord blood samples obtained from the infants were screened for polymorphisms in *CD14* (rs2569190), *TLR4* (rs1927911), *GSDMB* (rs4794820), *Nrf2* (rs6726395), *NAT2* (rs4271002), and *GSTP1* (rs1695) using the TaqMan fluorogenic 5'-nuclease assays (ABI, Foster City, CA, USA). The assay identification numbers were C_16043997_10, C_11722141_10, C_29609729_20, C_155538_10, C_31028511_10, and C_3237198_20, respectively. All PCRs were performed in 384-well plates using a 384-Well Veriti thermal cycler (ABI, Foster City, CA, USA). The endpoint fluorescent readings were performed on an ABI PRISM 7900 HT Sequence Detection System (ABI, Foster City, CA, USA). Duplicate samples and negative controls were included to ensure the accuracy of genotyping. The distribution of polymorphisms was in Hardy-Weinberg equilibrium (P>0.1).

Statistical analysis

The associations between RTI risk and the general characteristics of the study populations were assessed by chi-square test or t-test, as appropriate. The effect of various doses of nutrients or foods on the risk of RTI and the combined effect of nutrients/food and genetic polymorphisms on RTI risk were investigated by multiple logistic regression. Nutrients/food intake was divided into three levels, and genetic polymorphisms were classified according to whether the major or minor alleles were present either in homozygous or heterozygous states. The minor alleles were considered as risk alleles. The interactions between nutrient/food intakes and genetic polymorphisms were evaluated through the logistic regression models added with an "interaction term". P values of less than 0.05 (two-sided probability) were interpreted as statistically significant. P value of the additional interaction term was derived from the logistic model, representing the P interaction between genetic polymorphisms and nutrients/food intake on RTI risk. Statistical analyses were conducted with SAS for Windows (version 9.2).

Results

Comparison of the COCOA cohort children with and without RTI

The prevalence of RTI in the 12-month-old infants was 58.7%. Table 1 compares the children with and without RTI in terms of their general characteristics. The infants without RTI were more likely to have been born in autumn compared to those with RTI (P=0.0258).

Table 1. General characteristics of study population (n=550) with and without respiratory tract infection

Variables	No RTI (n=227)		RTI (n=323)		P value
	N	Mean±SD or (%)	N	Mean±SD or (%)	
Mother's characteristics					
Maternal age at birth (y)	227	32.2±3.5	323	32.5±3.4	0.2464
Gestation (wk)	216	39.1±1.3	300	39.1±1.3	0.9492
Education (≥university graduation, %)	192/224	(85.7)	284/319	(89.0)	0.2477
Passive smoking during pregnancy (yes, %)	130/215	(60.5)	187/309	(60.5)	0.9903
Multivitamin use during pregnancy (yes, %)	49/213	(23.0)	84/310	(27.1)	0.2910
Normal delivery (yes, %)	131/206	(63.6)	194/282	(68.8)	0.2288
Maternal history of allergic diseases (yes, %)	80/219	(36.5)	101/313	(32.3)	0.3073
Paternal history of allergic diseases (yes, %)	56/208	(26.9)	96/295	(32.5)	0.1765
Maternal nutrient* and food group intake					
Total energy (kcal/d)	227	1901.6±638.7	323	2005.1±723.3	0.0772
Vitamin A (μg/d)	227	856.6±333.7	323	835.1±302.0	0.4318
Vitamin C (mg/d)	227	110.4±40.7	323	109.2±47.1	0.7555
Vitamin E (mg/d)†	227	17.8±4.3	323	17.6±4.6	0.6563
Folate (μg/d)	227	279.3±75.9	323	273.7±73.1	0.2542
Zn (mg/d)	227	10.0±1.3	323	9.9±1.4	0.1974
Vegetables (frequency/d)	227	5.4±2.7	323	5.7±3.5	0.1803
Fruits (frequency/d)	227	3.9±3.3	323	4.0±3.4	0.7970
Total fruits and vegetables (frequency/d)	227	9.2±4.8	323	9.7±5.9	0.3461
Infant's characteristics					
Season of birth (%)					0.0258
Spring (March to May)	44/226	(19.5)	82/314	(26.1)	
Summer (June to August)	31/226	(13.7)	61/314	(19.4)	
Autumn (September to November)	90/226	(39.8)	93/314	(29.6)	
Winter (December to February)	61/226	(27.0)	78/314	(24.8)	
Sex (male, %)	128/227	(56.4)	171/320	(53.4)	0.4947
Low birth weight (<2.5 kg, %)	12/220	(5.5)	21/306	(6.9)	0.5112

*: Nutrient intake was adjusted for total energy intake by using a residual method; †: Vitamin E includes all isoforms of vitamin E. RTI: respiratory tract infection; SD: standard deviation.

However, the two groups did not differ significantly with regard to any other variables, namely, maternal age, gestational age, education level, passive smoking, multivitamin consumption during pregnancy, delivery mode, infant's gender, or low birth weight. In addition, the two groups were similar in the aspect of prenatal maternal nutrient and food intake.

Association between prenatal maternal antioxidant intake and risk of RTI

Table 2 shows the association between prenatal maternal intake of antioxidant nutrients and fruits and/or vegetables and risk of RTI. Despite the weak associations between higher prenatal maternal intake of vitamin C and folate and RTI risk in the aspects of food groups, a higher prenatal maternal intake of total fruits and vegetable (FV) apparently showed the reduced risk of RTI [adjusted odds ratio (aOR)=0.54, 95% CI=0.31-0.94, *P*-trend=0.0430].

Table 2. Adjusted odds ratios (aOR) and 95% confidence intervals (95% CI) for the respiratory tract infection according to the tertiles of nutrients and food groups rich in antioxidants among infants (*n*=550)

Variables	Median (min, max)	RTI		
		n/N¶	aOR** (95% CI)	*P*-trend†
Nutrients from FFQ*				
Vitamin A				
T1	567.20 (179.6, 684.3)	71/183	1.00	
T2	793.50 (685.2, 923.9)	74/184	0.88 (0.55, 1.42)	0.1236
T3	1098.60 (925.5, 2728.6)	82/183	0.69 (0.43, 1.11)	
Vitamin C				
T1	71.80 (16.1, 89.1)	72/183	1.00	
T2	103.40 (89.3, 119.8)	79/184	0.82 (0.51, 1.32)	0.1085
T3	139.90 (119.9, 491.2)	76/183	0.68 (0.42, 1.09)	
Vitamin E‡				
T1	13.70 (4.2, 15.6)	67/183	1.00	0.2581
T2	17.60 (15.7, 19.2)	88/184	0.44 (0.27, 0.71)	
T3	21.60 (19.2, 44.5)	72/183	0.72 (0.44, 1.17)	
Folate				
T1	207.60 (56.0, 239.9)	71/183	1.00	
T2	275.00 (240.6, 302.1)	77/184	0.63 (0.39, 1.02)	0.0585
T3	343.40 (302.4, 635.6)	79/183	0.63 (0.39, 1.02)	
Zn				
T1	8.80 (3.2, 9.4)	79/183	1.00	
T2	10.00 (9.4, 10.5)	69/184	1.19 (0.74, 1.91)	0.4744
T3	11.00 (10.5, 16.1)	79/183	0.83 (0.52, 1.33)	
Food group (frequency/d) from FFQ				
Total vegetable§				
T1	2.74 (0.0, 3.9)	79/183	1.00	
T2	5.01 (4.0, 6.1)	78/184	0.94 (0.58, 1.51)	0.6799
T3	8.08 (6.2, 26.4)	70/183	0.89 (0.51, 1.54)	
Total fruits‖				
T1	1.18 (0.0, 2.0)	85/183	1.00	
T2	2.83 (2.0, 4.4)	69/184	1.43 (0.89, 2.31)	0.7960
T3	6.57 (4.4, 28.2)	73/183	1.17 (0.70, 1.94)	
Total fruits‖ & vegetables§				
T1	4.86 (0.0, 6.4)	71/183	1.00	0.0430
T2	8.44 (6.5, 10.7)	84/184	0.59 (0.36, 0.97)	
T3	13.84 (10.7, 40.1)	72/183	0.54 (0.31, 0.94)	

*: Nutrient intake was adjusted for total energy intake by using a residual method; †: *P* for trend was calculated by logistic regression models using a variable comprising the median value within each nutrient group; ‡: Vitamin E includes all isoforms of vitamin E; §: Vegetables consisted of raw, cooked, canned, frozen or dried forms of cabbages, radishes, cucumbers, carrots, peppers, garlics, onions, spinach, squash, tomatoes, eggplants, soy bean sprouts, balloon flower roots, other vegetable leaves and roots, seaweeds, and mushrooms; ‖: Fruits included raw, dried or canned forms of strawberries, apples, tangerines/oranges, pears, bananas, watermelons, oriental melons/melons, grapes, peaches/prunes, and fruit juices. ¶: N is number of children in the tertile with data on the outcome variable, and *n* is number of children with "yes" for outcome variable; **: All values were adjusted for maternal age at birth (y), maternal body mass index (kg/m^2), maternal education (≥university graduation, <university graduation), passive smoking during pregnancy (y, number), child birth weight (kg), child's gender (male, female), season of birth (spring, summer, autumn, or winter), total energy intake (kcal/d) and multivitamin consumption (yes, no).

Association between *CD14* (rs2569190), *TLR4* (rs1927911), and *GSDMB* (rs4794820) polymorphisms and prenatal antioxidant intake on the risk of RTI

Polymorphisms of each gene were analyzed according to their dominant genotypes. Infants were stratified according to whether they had TT or TC+CC genotype at *CD14* rs2569190, CC or CT+TT genotypes at *TLR4* rs1927911, and GG or GA+AA genotypes at GSDMB rs4794820 (Table 3). There were interactions between polymorphisms of each gene and prenatal antioxidant intake levels on the risk of RTI. For *CD14*, in offspring with TT genotype at rs2569190, the risk of RTI was reduced by higher prenatal intake of vitamin A (aOR=0.33, 95% CI=0.14-0.80, *P*-trend=0.0114) and C (aOR=0.32, 95% CI=0.13-0.80, *P*-trend=0.0148), folate (aOR=0.37, 95% CI=0.14-0.94, *P*-trend=0.0475), fruits (aOR=0.31, 95% CI=0.12-0.83, *P*-trend=0.0113), and total FV (aOR=0.20, 95% CI=0.06-0.64, *P*-trend=0.0041), while in those with the TC+CC genotypes, higher fruits intake showed an increased risk of RTI (aOR=2.35, 95% CI=1.18-4.65, p-trend=0.0339) with significant interactions (*P* for interaction <0.050). Analysis according to the 3 genotypes in *CD14* rs2569190, the effects of prenatal maternal antioxidants intake were more remarkable and the significant interaction still remained in all but vitamin A (Fig. 1). Higher prenatal maternal intake of vitamins A and C, folate, fruits, and total FV reduced the risk of RTI in infants with TT genotype of *CD14*, whereas in those with CC genotype, a higher prenatal intake of vitamin C (aOR 5.04, 95% CI 1.32–19.30, p-trend=0.0181) and fruits (aOR=10.30, 95% CI=2.06-52.00, *p*-trend=0.0077) was associated with an increased risk of RTI.

With regard to *TLR4* rs1927911, a significant interaction of *TLR4* genotypes was only found with vitamin C (*P* for interaction=0.0302). In terms of GSDMB rs4794820, the risk of RTI was reduced by a higher prenatal intake of fruits (aOR=2.34, 95% CI=1.07-5.13 in infants with GG genotype and aOR=0.50, 95% CI=0.23-1.10 in those with GA+AA genotype) and total FV (aOR=0.27, 95% CI=0.11-0.67, p-trend=0.0043) in infants with the GA+AA genotypes in GSDMB; moreover, there was a significant interaction between nutritional intake levels and genetic polymorphisms (*P* for interaction<0.05).

Table 3. The adjusted odds ratios (aOR) and 95% confidence intervals (95% CI) of antioxidant nutrients, fruits and/or vegetables on the risk of respiratory tract infection according to genotypes (CD14 rs2569190, TLR4 rs1927911, and GSDMB rs4794820)

Variables		*CD14* rs2569190 (*n*=540)					*TLR4* rs1927911 (*n*=535)					*GSDMB* rs4794820 (*n*=545)				
		TT (*N*=191)		TC+CC (*N*=349)		*P* for interaction	CC (*n*=202)		CT+TT (*n*=333)		*P* for interaction	GG (*n*=293)		GA+AA (*n*=252)		*P* for interaction
		n	aOR‖ (95% CI)	*n*	aOR (95% CI)		*n*	aOR (95% CI)	*n*	aOR (95% CI)		*n*	aOR (95% CI)	*n*	aOR (95% CI)	
Nutrients from FFQ*																
Vitamin A	T1	61	1.00	119	1.00		66	1.00	112	1.00		101	1.00	80	1.00	
	T2	61	0.82 (0.33, 2.04)	121	0.92 (0.49, 1.72)		68	1.32 (0.57, 3.033)	111	0.56 (0.29, 1.09)		91	0.71 (0.34, 1.47)	92	0.79 (0.39, 1.64)	
	T3	69	0.33 (0.14, 0.80)	109	1.12 (0.58, 2.16)	0.0327	68	1.09 (0.46, 2.57)	110	0.58 (0.29, 1.14)	0.6742	101	0.60 (0.29, 1.25)	80	0.67 (0.32, 1.42)	0.7921
	P-trend¶		0.0114		0.7057			0.8746		0.1424			0.1821		0.2997	
Vitamin C	T1	62	1.00	119	1.00		64	1.00	116	1.00		92	1.00	89	1.00	
	T2	64	0.61 (0.25, 1.49)	116	0.91 (0.48, 1.71)		73	0.95 (0.40, 2.25)	103	0.59 (0.30, 1.16)		104	0.71 (0.34, 1.47)	78	0.77 (0.36, 1.66)	
	T3	65	0.32 (0.13, 0.80)	114	1.19 (0.64, 2.23)	0.0165	65	0.43 (0.18, 1.02)	114	0.82 (0.43, 1.57)	0.0302	97	0.73 (0.35, 1.53)	85	0.56 (0.27, 1.18)	0.5296
	P-trend¶		0.0148		0.5521			0.0466		0.6251			0.4418		0.1264	
Vitamin E†	T1	64	1.00	118	1.00		60	1.00	119	1.00		96	1.00	86	1.00	
	T2	55	0.24 (0.09, 0.65)	126	0.46 (0.24, 0.86)		72	0.81 (0.34, 1.92)	107	0.26 (0.13, 0.53)		95	0.34 (0.16, 0.76)	86	0.41 (0.20, 0.85)	
	T3	72	0.50 (0.21, 1.17)	105	0.93 (0.48, 1.83)	0.3215	70	1.05 (0.44, 2.50)	107	0.48 (0.24, 0.95)	0.5880	102	0.54 (0.26, 1.12)	80	0.74 (0.35, 1.59)	0.4228
	P-trend¶		0.1707		0.8792			0.8607		0.0576			0.1546		0.4359	
Folic acid	T1	54	1.00	126	1.00		61	1.00	115	1.00		92	1.00	90	1.00	
	T2	73	0.37 (0.15, 0.95)	107	0.81 (0.43, 1.54)		71	0.42 (0.17, 1.02)	110	0.62 (0.31, 1.21)		96	0.54 (0.25, 1.15)	86	0.67 (0.33, 1.37)	
	T3	64	0.37 (0.14, 0.94)	116	0.78 (0.42, 1.47)	0.5194	70	0.51 (0.21, 1.27)		0.54 (0.28, 1.04)	0.6645	105	0.50 (0.24, 1.07)	76	0.59 (0.29, 1.22)	0.9408
	P-trend¶		0.0475		0.4441			0.1659		0.066			0.0818		0.1503	
Zn	T1	65	1.00	117	1.00		64	1.00	114	1.00		96	1.00	85	1.00	
	T2	63	0.69 (0.28, 1.70)	115	1.34 (0.71, 2.56)		65	1.74 (0.72, 4.22)	114	0.91 (0.47, 1.75)		89	0.70 (0.32, 1.52)	93	1.62 (0.79, 3.33)	
	T3	63	0.54 (0.23, 1.26)	117	0.92 (0.49, 1.72)	0.5647	73	1.44 (0.62, 3.35)	104	0.54 (0.28, 1.06)	0.2073	108	0.47 (0.22, 0.99)	74	0.90 (0.432, 1.87)	0.5404
	P-trend¶		0.1542		0.8320			0.3874		0.0841			0.0450		0.9264	
Food group (frequency/d) from FFQ																
Total vegetable‡	T1	61	1.00	120	1.00		61	1.00	118	1.00		93	1.00	89	1.00	
	T2	63	1.16 (0.48, 2.78)	117	0.87 (0.45, 1.66)		73	0.69 (0.29, 1.63)	105	1.12 (0.57, 2.22)		100	1.34 (0.64, 2.84)	82	0.78 (0.372, 1.62)	
	T3	67	0.51 (0.18, 1.47)	112	1.22 (0.58, 2.56)	0.1276	68	0.63 (0.22, 1.80)	110	1.20 (0.56, 2.57)	0.6671	100	1.00 (0.43, 2.31)	81	0.72 (0.30, 1.72)	0.8408
	P-trend¶		0.2146		0.5437			0.4053		0.6551			0.9149		0.4724	
Total fruits§	T1	62	1.00	117	1.00		56	1.00	124	1.00		99	1.00	81	1.00	
	T2	63	0.93 (0.37, 2.33)	118	2.07 (1.08, 3.95)		78	1.07 (0.44, 2.60)	100	1.69 (0.87, 3.29)		94	2.46 (1.18, 5.13)	89	0.82 (0.39, 1.73)	
	T3	66	0.31 (0.12, 0.83)	114	2.35 (1.18, 4.65)	0.0028	68	1.04 (0.39, 2.78)	109	1.08 (0.55, 2.13)	0.7947	100	2.34 (1.07, 5.13)	82	0.50 (0.23, 1.10)	0.0260
	P-trend¶		0.0113		0.0339			0.9704		0.928			0.0835		0.0727	
Total fruits§	T1	59	1.00	121	1.00		58	1.00	121	1.00		96	1.00	86	1.00	
& vegetables‡	T2	69	0.92 (0.36, 2.31)	111	0.53 (0.27, 1.03)		75	0.35 (0.14, 0.90)	104	0.73 (0.37, 1.46)		94	0.73 (0.34, 1.57)	88	0.64 (0.30, 1.36)	
	T3	63	0.20 (0.06, 0.64)	117	0.87 (0.42, 1.80)	0.0029	69	0.37 (0.13, 1.10)	108	0.57 (0.27, 1.23)	0.5091	103	0.84 (0.36, 1.96)	78	0.27 (0.11, 0.67)	0.0393
	P-trend¶		0.0041		0.9750			0.1505		0.1611			0.8018		0.0043	

*: Nutrient intake was adjusted for total energy intake by using a residual method; †: Vitamin E includes all isoforms of vitamin E; ‡: Vegetables consisted of raw, cooked, canned, frozen or dried forms of cabbages, radishes, cucumbers, carrots, peppers, garlics, onions, spinach, squash, tomatoes, eggplants, soy bean sprouts, balloon flower roots, other vegetable leaves and roots, seaweeds, and mushrooms; §: Fruits included raw, dried or canned forms of strawberries, apples, tangerines/oranges, pears, bananas, watermelons, oriental melons/melons, grapes, peaches/prunes, and fruit juices; ‖: All values were adjusted for maternal age at birth (years), maternal body mass index (kg/m^2), parental history of allergic diseases (asthma, allergic rhinitis or atopic dermatitis: yes, no), maternal education (≥university graduation, <university graduation), passive smoking during pregnancy (year, number), child birth weight (kg), child's gender (male, female), season of birth (spring, summer, autumn, or winter), total energy intake (kcal/day) and multivitamin consumption (yes, no); ¶: P for trend is from logistic regression models using a variable comprising the median value within each nutrient group.

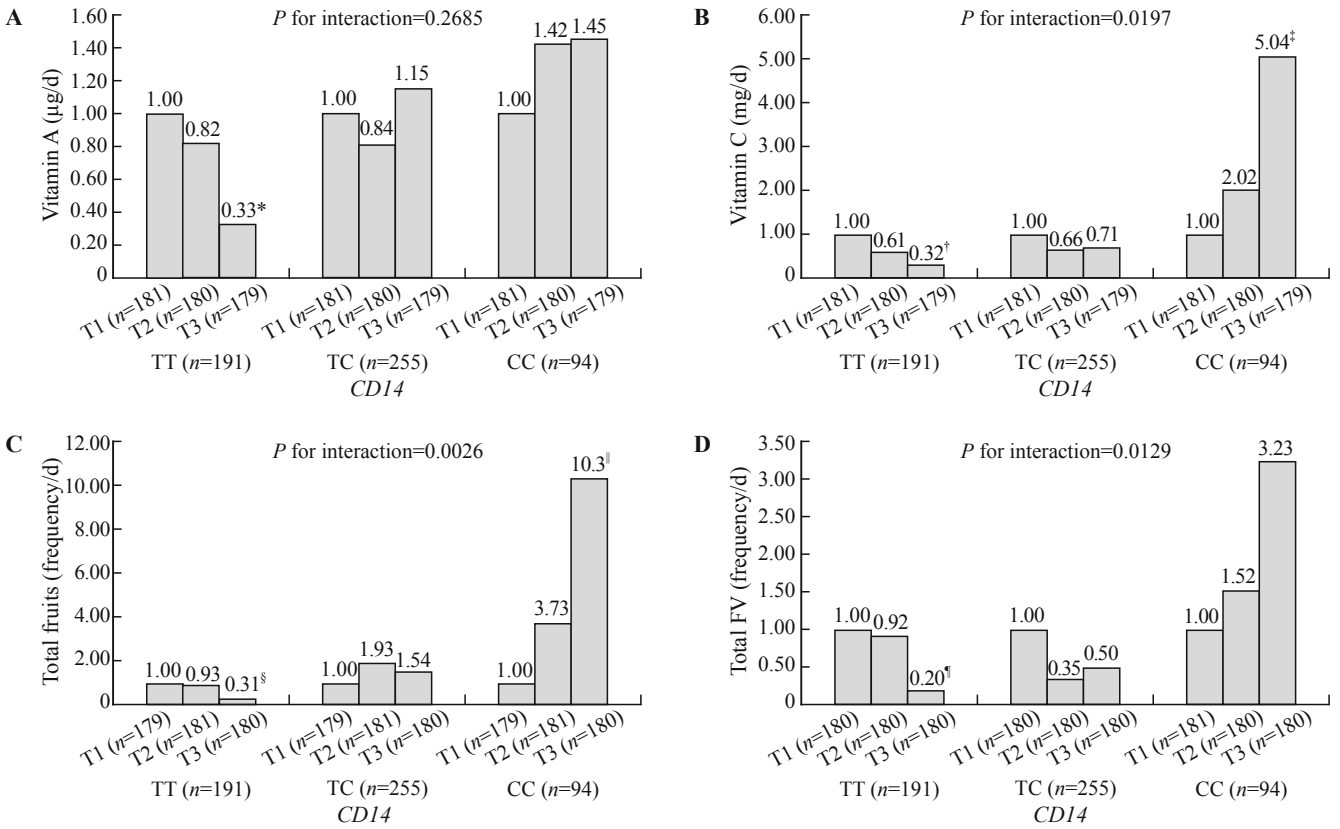

Fig. Interactions between intake of vitamins A and C, fruits, and total fruits and vegetables and the *CD14* rs2569190 polymorphisms (n=540) in the risk of respiratory tract infection. All values were adjusted for maternal age at birth (years), maternal body mass index (kg/m^2), parental history of allergic diseases (asthma, allergic rhinitis or atopic dermatitis: yes, no), maternal education (≥university graduation, <university graduation), passive smoking during pregnancy (year, number), child birth weight (kg), child's gender (male, female), season of birth (spring, summer, autumn, or winter), total energy intake (kcal/day), and multivitamin consumption (yes, no). FV: fruits & vegetables; aOR: adjusted odds ratio. *: aOR=0.33, 95% CI=0.14-0.80; †: aOR=0.32, 95% CI=0.13-0.80; ‡: aOR=5.04, 95% CI=1.32-19.30; §: aOR=0.31, 95% CI=0.12-0.83; ‖: aOR=10.30, 95% CI=2.06-52.00; ¶: aOR=0.20, 95% CI=0.06-0.64.

Association between *Nrf2* (rs6726395), *NAT2* (rs4271002), and *GSTP1* (rs1695) polymorphisms and prenatal antioxidant intake on the risk of RTI

The risk of RTI at 12 month of age was not consistently associated with the interactions between polymorphisms of *Nrf2* (rs6726395), *NAT2* (rs4271002), and *GSTP1* (rs1695) and levels of prenatal antioxidant intake (Supplementary Table 2).

Discussion

The present study showed the importance of maternal intake of antioxidants in the form of vitamin C, fruits, and total FV during pregnancy in order to protect their infants against RTI at 12 months of age. Moreover, there was a significant interaction between maternal nutritional intake status and polymorphisms in immunity–related genes in the infants in terms of the risk of RTI at 12 months of age. The effect of antioxidants was particularly remarkable in the interaction with polymorphisms of *CD14* (rs2569190). A higher prenatal intake of antioxidants was protective against RTI in infants with the TT genotype of *CD14* rs2569190, whereas a higher prenatal maternal intake of vitamin C and fruits was associated with an increased risk of RTI in those with the CC genotype. This suggested that gene-environment interactions between prenatal maternal diet and genetic polymorphisms in immune-related genes are associated with the development of RTI in infants during early life. To our knowledge, no study has elucidated the association between maternal antioxidant intake during pregnancy and risk of RTI in offspring in terms of gene-environment interactions.

Several studies have suggested that maternal diets during pregnancy might be associated with the susceptibility to infectious diseases in offspring. In addition, maternal nutritional levels during pregnancy have implications for developmental programming and the risk of non-communicable chronic diseases such as cancer and cardiovascular disease.[27] Differences in susceptibility

to RTI depending on the state of prenatal maternal diets might be attributed to the altered immune responses to respiratory infection according to genetic polymorphisms, based on the interactions between prenatal antioxidant intake levels and genetic factors.[7]

Until now, most studies have focused on the effect of maternal antioxidant intakes during pregnancy on allergic diseases, rather than on RTI in the offspring. Although the underlying association with allergic diseases remains controversial,[28] many studies have indicated that a higher maternal intake of folate,[28] vitamins A, C, and E,[29-31] Zn,[31] and fruits and/or vegetables[27,30] is associated with the decreased risk of asthma, wheezing, allergic rhinitis, and/or eczema. Although the protective effects of antioxidant nutrients against RTI was weak in this study, the antioxidants rich food groups such as fruits and total FV showed an inverse dose response association on the risk of RTI. It may suggest that vitamin C and vitamin C-rich foods such as fruits and total FV play a role on the development of RTI during critical periods. A possible contribution of FV to the protection against RTI may also come from various polyphytochemicals, such as flavonoids and a subgroup of polyphenolic compounds, which are effective antioxidants.[32]

Although vitamin E is a member of antioxidants nutrients, we found no association between prenatal maternal intake state of total vitamin E and RTI risk, which might be partially attributable to the conflicting effect of vitamin E on inflammation according to its isoforms.[33] Although α-tocopherol and γ-tocopherol have similar capacity to scavenge ROS, γ-tocopherol has additional role in response to reactive nitrogen species, and thereby increases inflammation,[33,34] whereas α-tocopherol has a protective role for lung inflammation.[35] Since we did not evaluate intake dose of each isoform of vitamin E in the present study, there are limitations in the assessment for the effect of each isoform of vitamin E on RTI.

Antioxidant imbalance influences the developing immune system, and thereby, affects the susceptibility to infections.[36] Although some antioxidants do not markedly affect the risk of RTI, its impact might be prominent with the gene-environment interaction in that antioxidants can modulate signal transduction and gene expression in immune cells[37] and inflammation-associated gene transcription.[19,38] This has been demonstrated by several studies which showed phytochemicals can cause epigenetic changes such as alterations in DNA methylation, histone acetylation, and histone deacetylation,[39] although the underlying mechanisms still need to be fully elucidated. On the basis of the above findings, imbalance of ROS might affect the programming of immunity, and thereby, affects the susceptibility to RTI, although further studies are required.

A variety of micronutrients such as copper and zinc act as essential cofactors for or themselves acting as antioxidants.[40] Because the expression of enzymatic antioxidants genes are affected by the balance of oxidative stress,[20] the interactions between antioxidant genes and micronutrients also might influence the degree of oxidative stress. Furthermore, these interactions affect fetal immune programming and immunity in the offspring through diverse mechanisms,[29] and thereby cause influence on the susceptibility to RTI. Further studies are needed to evaluate the effect of interactions between micronutrients and antioxidants genes on immunity and health.

Several considerations should be given when interpreting the findings of this study. Firstly, the diagnosis of RTI was made by physicians without laboratory evaluations for the identification of RTI-related pathogens. Despite some limitations of this study, the protective effects of vitamin C, fruits and total FV against RTI were more clearly observed when stratified according to the infants' genetic factors.

Moreover, the results of this study could have been confounded by other factors such as exposure to environmental tobacco smoking or duration and amount of breastfeeding or multivitamin supplementation. In this cohort, mothers who had ever smoked and who smoked during their pregnancies were relatively few, accounting for 8.3% and 0.4%, respectively. Maternal asthma rate was also low (2.1%) and there was no difference between infants with and without RTI in this context (P value=0.3441 by Fisher's exact test). We could not adjust for the effect of breastfeeding because questions on breastfeeding were introduced only during the course of this study. In addition, because we did not measure any biomarkers for the oxidant and antioxidant levels during pregnancy, it was impossible to correlate the prenatal maternal antioxidant intake with oxidant/antioxidant levels in serum. Although the findings of studies on the relationship between antioxidant intake and antioxidant levels in serum are inconclusive, some studies have found a positive correlation between antioxidant intake and antioxidant levels in serum during pregnancy and those at delivery.[5] In addition, we did not consider the duration and type of multivitamin supplementation in the analysis, which may affect maternal antioxidant levels. This non-differential misclassification in antioxidant intake may bias the results. Further studies considering the effect of postnatal factors, such as breast feeding and complementary feeding practices and the associations between dietary antioxidant intake and serum antioxidant levels are required to elucidate the relationship between prenatal

antioxidant intake and the risk of RTI.

Among the 983 infants recruited at baseline, 433 were excluded because of unavailable information on maternal diet, genotypes, or RTI. However, comparison of the demographics of the included and excluded infants did not show any significant differences except in the season of birth. The seasonal difference in birth may not be related to the maternal intake during the pregnancy. Because, although prenatal dietary intake by FFQ was assessed only at the study baseline (26 weeks of gestational age), the FFQ generally reflects average long-term intake over a specific period,[41] which in the case of this study was carried out by asking subjects in the study recall their diet from 12 months prior to the time of the interview rather than intake on a few specific days.

The overproduction of free radicals during inflammation or in the context of antioxidant deficiencies, which is enhanced by certain alleles in antioxidant genes, may lead to increased oxidative stress.[42] Although polymorphisms in antioxidant genes affect disease susceptibility by altering the host's ability to detoxify ROS,[43] the present study did not find any remarkable interactions between polymorphisms in *Nrf2* (rs6726395), *NAT2* (rs4271002), or *GSTP1* (rs1695) and maternal antioxidant intake during pregnancy on the development of RTI in the offspring. This might be associated, at least in part, with significant down-regulation of the airway antioxidant system during viral respiratory infection.[44] Further studies are needed to elucidate the associations between polymorphisms in antioxidant genes, maternal antioxidant intake during pregnancy, and the development of RTI in offspring, and its underlying pathophysiology.

Although little is known about the function of *GSDMB* in the development of RTI, several studies have revealed that viral infection increases the expression of *GSDMB*, and thereby, augments the unfolded protein response, which is associated with increases in airway inflammation.[24] In addition, polymorphisms in *GSDMB* influence the efficiency of viral infection or replication.[45] To date, although there have been a few studies on the gene–environment interactions between *GSDMB* polymorphisms and virus-induced wheezing illness,[45] no study to date has investigated the associations between *GSDMB* polymorphisms and RTI risk in the offspring of mothers with a higher prenatal antioxidant intake. Additional studies are needed to elucidate whether genetically susceptible infants with recurrent RTI during early life, born of mothers with higher prenatal antioxidant intakes are protected against the development of childhood asthma later in life and whether the *GSDMB* rs4794820 polymorphism influences this pattern.

Dose-response association between nutrition and risk of infections has been evaluated since the 1960s.[46] Moreover, nutritional status may affect the risk of infection differently according to the timing of intake. Although little is known about the underlying mechanisms, prenatal maternal nutritional status influences the development of immune system, such as thymus development and the proportion of $CD8^+$, $CD56^+$, and $CD4^+$ cells,[47,48] and thereby, affects the susceptibility of the infant to infection. Furthermore, nutritional status may exert a transgenerational effect on the programming of immune functions.[49,50] Thus, nutritional deficiencies can modulate the prenatal programming of immune function, partially through epigenetic mechanisms.

In conclusion, the present study demonstrated that maternal intake of antioxidants determined by total FV intake during a critical period may reduce the risk of RTI at 12 months of age. In addition, this effect may be modulated by polymorphisms in *CD14* (rs2569190), *TLR4* (rs1927911), and *GSDMB* (rs4794820). This study highlights the importance of prenatal maternal diet and supports the future development of specific health intervention strategies that are tailored according to the presence of certain genetic polymorphisms.

Acknowledgements

We would like to gratefully and sincerely thank Dr. Ja-Young Kwon, Suk-Joo Choi, Kyung-Ju Lee, Hee Jin Park, Hye-Sung Won, Mi-Jin Kang, Ho-Sung Yu, Hyung Young Kim, Ju-Hee Seo, Byoung-Ju Kim, Hyo-Bin Kim, and So-Yeon Lee for their participation in this study.

Funding: This research was supported by fundings (2008-E33030-00, 2009-E33033-00. 2011-E33021-00, 2012-E33012-00) from Research of Korea Centers for Disease Control and Prevention.

Ethical approval: This study was performed according to the guideline of Asan Medical Center, Samsung Medical Center, Yonsei University and CHA Medical Center, which abides by the Helsinki Declaration on ethical principles for medical research.

Competing interest: The authors declare that they have no competing interests.

Contributors: Hong SJ and Oh SY designed and supervised the execution of the study, and Hong SA and Lee E performed the analyses and wrote the manuscript. Kwon SO, Kim KW, Shin YH, Ahn KM, Kim EJ, and Lee JG participated in the interpretations. Hong SA and Lee E contributed equally to this work as co-first authors.

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Received September 18, 2015
Accepted after revision November 16, 2015

(Supplementary information is linked to the online version of the paper on the journal's website)