

Prenatal maternal distress affects atopic dermatitis in offspring mediated by oxidative stress

Hyoungh Yoon Chang, MD, PhD, MPH,^{a,b,c,*} Dong In Suh, MD,^{d,*} Song-I. Yang, MD, PhD,^e Mi-Jin Kang, MS,^f So-Yeon Lee, MD, PhD,^g Eun Lee, MD,^g In Ae Choi, MS,^h Kyung-Sook Lee, PhD,ⁱ Yee-Jin Shin, MD, PhD,^j Youn Ho Shin, MD,^k Yoon Hee Kim, MD,^l Kyung Won Kim, MD, PhD,^l Kangmo Ahn, MD, PhD,^m Hye-Sung Won, MD, PhD,ⁿ Suk-Joo Choi, MD, PhD,^o Soo-Young Oh, MD, PhD,^o Ja-Young Kwon, MD, PhD,^p Young Han Kim, MD, PhD,^p Hee Jin Park, MD, PhD,^q Kyung-Ju Lee, MD, PhD,^q Jong Kwan Jun, MD, PhD,^r Ho-Sung Yu, MS,^f Seung-Hwa Lee, MS,^f Bok Kyoung Jung, MS,^f Ji-Won Kwon, MD,^s Yoon Kyung Choi, PhD,^t Namhee Do, PhD,^t Yun Jin Bae, PhD,^t Ho Kim, PhD,^u Woo-Sung Chang, PhD,^v Eun-Jin Kim, PhD,^v Jeom Kyu Lee, PhD,^v and Soo-Jong Hong, MD, PhD^{f,g}

Suwon, Seoul, Anyang, Osan, Seongnam, and Cheongju, Korea

Background: Recent evidence suggests that prenatal maternal distress increases the risk of allergic diseases in offspring. However, the effect of prenatal maternal depression and anxiety on atopic dermatitis (AD) risk remains poorly understood.

Objective: We investigated whether prenatal maternal distress is associated with AD risk in offspring and whether the mechanism is mediated by reactive oxygen species.

Methods: Two general population-based birth cohorts formed the study. One cohort (Cohort for Childhood Origin of Asthma and Allergic Diseases [COCOA]) consisted of 973 mother-baby dyads, and the other (Panel Study on Korean Children [PSKC]) consisted of 1531 mother-baby dyads. The association between prenatal distress and AD was assessed by using Cox proportional hazards and logistic regression models. In COCOA placental 11 β -hydroxysteroid dehydrogenase type 2 and glutathione levels and serum IgE levels in 1-year-old children were measured.

Results: In COCOA and PSKC AD occurred in 30.6% (lifetime prevalence) and 11.6% (1 year prevalence) of offspring, respectively. Prenatal maternal distress increased the risk of AD in offspring, both in COCOA (hazard ratio for depression, 1.31 [95% CI, 1.02-1.69]; hazard ratio for anxiety, 1.41 [95% CI, 1.06-1.89]) and PSKC (odds ratio for distress, 1.85 [95% CI, 1.06-3.25]). In COCOA both prenatal maternal depression and anxiety scores were positively related to the predicted probability of AD ($P < .001$ in both). Prenatal distress decreased placental glutathione to glutathione disulfide ratios ($P = .037$) and, especially in those who later had AD, decreased placental 11 β -hydroxysteroid dehydrogenase type 2 levels ($P = .010$) and increased IgE levels at 1 year of age ($P = .005$).

Conclusion: Prenatal maternal depression and anxiety promote risk of AD in offspring. Maternal distress increases the predicted probability of AD. The mechanism might involve chronic stress, abnormal steroid levels, and reactive oxygen species. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: Anxiety, depression, atopic dermatitis, prenatal, psychological stress, cohort, reactive oxygen species

From ^athe Department of Psychiatry, Ajou University School of Medicine, Suwon; ^bthe Sunflower Center of Southern Gyeonggi for Women and Children Victims of Violence, Suwon; ^cthe Center for Traumatic Stress, Ajou University Medical Center, Suwon; the Departments of ^dPediatrics and ^eObstetrics and Gynecology, Seoul National University College of Medicine, Seoul; ^fthe Department of Pediatrics, Hallym Sacred Heart Hospital, Hallym University College of Medicine, Anyang; ^gAsan Institute for Life Sciences, University of Ulsan College of Medicine, Seoul; ^hthe Department of Pediatrics, Childhood Asthma Atopy Center, Research Center for Standardization of Allergic Diseases, Environmental Health Center, University of Ulsan College of Medicine, Seoul; ⁱthe Department of Obstetrics and Gynecology, Asan Medical Center, University of Ulsan College of Medicine, Seoul; ^jSewon Infant Child Development Center, Seoul; ^kthe Department of Rehabilitation, Hanshin University, Osan; ^lthe Department of Psychiatry, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul; the Departments of ^mPediatrics and ⁿObstetrics and Gynecology, Yonsei University College of Medicine, Seoul; ^othe Department of Pediatrics, CHA Gangnam Medical Center, CHA University School of Medicine, Seoul; the Departments of ^pPediatrics and ^qObstetrics and Gynecology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul; ^rthe Department of Obstetrics and Gynecology, CHA Gangnam Medical Center, CHA University School of Medicine, Seoul; ^sthe Department of Pediatrics, Seoul National University Bundang Hospital, Seongnam; ^tKorea Institute of Child Care and Education, Seoul; ^uthe Graduate School of Public Health, Seoul National University, Seoul; and ^vthe Division of Allergy and Chronic Respiratory Diseases, Center for Biomedical Science, Korea National Institute of Health, Cheongju.

*These authors contributed equally to this work as co-first authors.

Supported by a fund (2008-E33030-00, 2009-E33033-00, 2011-E33021-00, 2012-E33012-00, 2013-E51003-00, and 2014-E51004-00) from the Research of Korea Centers for Disease Control and Prevention. This study was also funded by the Panel Study of Korean Children (PSKC) of the Korea Institute of Child Care and Education (KICCE) and developed based on the PSKC data set of 2008-2013.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication August 1, 2015; revised December 15, 2015; accepted for publication January 20, 2016.

Corresponding author: Soo-Jong Hong, MD, PhD, Department of Pediatrics, Childhood Asthma Atopy Center, Research Center for Standardization of Allergic Diseases, Environmental Health Center, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea. E-mail: sjhong@amc.seoul.kr.

0091-6749/\$36.00

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<http://dx.doi.org/10.1016/j.jaci.2016.01.020>

Atopic dermatitis (AD) is a leading cause of distress and an important element in health care expenditures in the developed world because of its increasing prevalence and chronic disease course.^{1,2} Review articles suggest that prenatal maternal distress might increase the risk of allergic disease in children,^{3,4} but most evidence focuses on its association with wheezing or asthma in offspring.^{4,5} Recently, a birth cohort study showed that prenatal maternal psychological distress also increases the risk of AD.⁶ This was supported by 2 more recent birth cohort studies that showed that when mothers experienced stressful life events during pregnancy, their children had an increased risk of eczema.^{7,8}

Abbreviations used

AD:	Atopic dermatitis
CESD:	Center for Epidemiological Studies-Depression
CLDN1:	Claudin 1
COCOA:	Cohort for Childhood Origin of Asthma and Allergic Diseases
GSSG:	Glutathione disulfide
HPA:	Hypothalamic-pituitary-adrenal
11 β -HSD2:	11 β -Hydroxysteroid dehydrogenase type 2
ISAAC:	International Study of Asthma and Allergies in Childhood
K6:	Kessler Six-question Psychological Distress Scale
NR3C1:	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
PSKC:	Panel Study on Korean Children
ROS:	Reactive oxygen species
STAI:	State-Trait Anxiety Inventory

However, several problems with the methodological methods adopted in these 3 studies⁶⁻⁸ have reopened the debate about the relationship between prenatal maternal distress and AD. First, offspring's AD was assessed by asking the mother,⁶⁻⁸ which is a method that can be affected by maternal psychological status.⁹ Second, only major life events^{7,8} were considered. These events only occur rarely and thus are a less suitable measure of everyday distress than self-reported questionnaires. Lastly, 2 of the studies did not adjust for the effect of postnatal maternal distress,^{6,8} even though this could blunt the association between prenatal maternal distress and offspring outcomes.

Oxidative stress is hypothesized to be one of the mechanisms through which distress affects physical health. Reactive oxygen species (ROS) are also reported to participate in the pathogenesis of AD.¹⁰ The detoxifying system of the fetus is extremely underdeveloped, which means that the detoxifying capacity of the placenta is critical.¹¹ If the mother produces very high ROS levels during pregnancy, this could overwhelm the placental detoxification capacity, expose the fetus to oxidative stress, and thereby promote susceptibility to AD after birth.

Given the heavy medical and psychological burden of AD, further investigation into the association between prenatal maternal distress and subsequent AD in offspring is warranted. It would also be of interest to evaluate whether prenatal distress is associated with placental oxidative stress and the subsequent development of AD in offspring. The Cohort for Childhood Origin of Asthma and Allergic Diseases (COCOA) is the first prospective birth cohort study that was hypothesized and designed purposely to test the aforementioned hypotheses.¹² Its main objectives were to investigate the association between prenatal maternal psychological distress and AD in offspring and whether this mechanism is mediated by altered steroid and ROS levels.

METHODS**Participants**

We separately analyzed 2 sets of birth cohorts. The first was that of the COCOA study and the second was that of the Panel Study on Korean Children (PSKC). COCOA is a prospective birth cohort that aims to identify early risk factors for childhood allergic diseases.^{12,13} On the other hand, PSKC was designed to survey the longitudinal characteristics of demographics and child development, child care behavior, and education.¹⁴ For detailed information

on the study population, including the criteria, characteristics, and current status of each cohort, see the [Methods](#) section and [Fig E1](#) in this article's Online Repository at www.jacionline.org. The institutional ethics committee of each institution approved this protocol.

Prenatal maternal psychological distress measurement

Depression, anxiety, and distress were collectively designated as psychological distress. Although depression and anxiety were measured in COCOA, distress was measured in PSKC. Prenatal distress scores were exposure variables, whereas postnatal scores served as potential confounders in the adjusted models.

In COCOA, prenatal maternal distress was evaluated by using 2 self-reported questionnaires at 36 weeks' gestation, namely the Center for Epidemiological Studies-Depression (CESD) 10,¹⁵ which measures depression, and the State-Trait Anxiety Inventory-Trait subscale (STAI-T),^{16,17} which measures anxiety. CESD has been validated for use during pregnancy. The score ranges from 0 to 30, with higher scores indicating more severe depressive symptoms. A cutoff of 10 was used to identify women with depression.¹⁵ The internal consistency of CESD-10 in this study indicated excellent reliability (intraclass correlation coefficient, 0.785). STAI-T is a 20-item questionnaire that is scored on a 4-point Likert-type scale that reflects a general tendency to be anxious. The score ranges from 20 to 80, with a higher score indicating a more severe anxiety level. In this study subjects with scores of greater than the 25th percentile were identified as being anxious. The Korean version of STAI has previously been shown to exhibit excellent psychometric properties.¹⁷ In terms of internal consistency, it has been reported as having a Cronbach α coefficient of 0.91. In this study the reliability coefficient of STAI-T was 0.92. The 2 questionnaires were also provided 6 months postpartum to measure postnatal maternal distress.

In PSKC the Kessler Six-question Psychological Distress Scale (K6),¹⁸ in which mothers were asked to recount their psychological state during the last 30 pregnancy days retrospectively, was completed immediately after delivery. The K6 was developed for the redesigned US National Health Interview Survey. Six items are answered with a Likert scale (0-4), yielding a score range of 0 to 24. Subjects were divided into 2 groups at the threshold of a K6 score of 13 or greater; thus one group was normal or had mild-to-moderate distress, and the other group had severe distress.¹⁸ K6 was readministered 6 months after delivery to determine postpartum distress.

Ascertainment of AD

In COCOA, AD was ascertained by pediatric allergy specialists who made a clinical diagnosis after a detailed history and physical examination on the basis of the criteria of Hanifin and Rajka¹⁹ at 6 months and 1, 2, 3, and 4 years of age. A child was deemed to have AD if he or she was given a diagnosis of AD at any stage of the study. In PSKC, on the other hand, interviewers applied the modified Korean version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire, which is identical to the one that is actually used in international ISAAC studies in Korea,^{20,21} at the fifth-year routine follow-up survey. In this study we used answers to the following question: "Did the child participant receive treatment for atopic dermatitis in the last 12 months?" This allowed us to identify children with AD with a minimal false-positive inclusion.

Measurement of placental 11 β -hydroxysteroid dehydrogenase type 2, placental glutathione, and child serum IgE levels at 1 year of age

To find clues for the involvement of stress, steroids, and ROS in the development of AD, we analyzed placental levels of 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2), which converts active cortisol to an inactive form,²² and the antioxidant glutathione,²³ as well as total serum IgE levels in 1-year serum samples of offspring in the COCOA repository.

Placental levels of 11 β -HSD2 and glutathione were determined by using the 11 β -HSD2 ELISA kit (USCN Life Science, Wuhan, China) and the

TABLE I. Demographic characteristics of the 2 birth cohort samples

Characteristics	COCOA (n = 973)	PSKC (n = 1531)
Maternal age		
≤32 y	540 (55.5)	1007 (65.8)
>32 y	433 (44.5)	524 (34.3)
Maternal educational level		
≤12 y	65 (6.7)	460 (30.2)
12-16 y	685 (70.4)	982 (64.6)
>16 y	223 (22.9)	79 (5.2)
Maternal history of allergic diseases		
No	675 (69.4)	993 (65.8)
Yes	298 (30.6)	538 (35.1)
Delivery methods		
Vaginal delivery	614 (63.1)	839 (54.8)
Cesarean section	359 (36.9)	692 (45.2)
Birth season		
Spring	225 (23.1)	1531 (100)
Summer	212 (21.8)	0 (0)
Autumn	249 (25.6)	0 (0)
Winter	287 (29.5)	0 (0)
Child's sex		
Girl	477 (49.0)	790 (51.6)
Boy	496 (51.0)	741 (48.4)
Birth weight (kg)	3.18 ± 0.42	3.27 ± 0.40
Birth height (cm)	49.30 ± 2.12	50.25 ± 2.51
GA (wk)	39.15 ± 1.19	39.28 ± 1.12
Prematurity (GA <37 wk)	0/973 (0)	51/1531 (3.3)

Values are presented as numbers (percentages) or means ± SDs.
GA, Gestational age.

glutathione assay kit (BioVision, Mountain View, Calif), respectively. Four groups of subjects were selected from COCOA subjects who did not have a family history of allergic disease (asthma, AD, and allergic rhinitis), namely children with AD whose mothers did (n = 5) or did not (n = 5) have prenatal distress and children without AD whose mothers did (n = 21) or did not (n = 7) have prenatal distress. The placentas of these children were subjected to assays that measured total 11β-HSD2 and total glutathione levels. The placental tissue was homogenized and centrifuged, and the supernatant was tested with an ELISA kit, according to the manufacturer's instructions. Finally, the absorbance of each sample was analyzed with a SpectraMax190 (Molecular Devices, Sunnyvale, Calif).

The total serum IgE level of each child at age 1 year was measured by using a fluorescent enzyme immunoassay (AutoCAP System; Phadia AB, Uppsala, Sweden).

Statistical analysis

The Cox proportional hazards model was used with COCOA, and the multivariate logistic regression model was used with PSKC. The association between prenatal distress and AD was examined in various ways. In COCOA 4 models were used to fully examine the association: (1) unadjusted; (2) adjusted for maternal age, maternal educational level,²⁴ delivery method,²⁵ birth season,²⁶ parental history of allergic diseases, and child's sex; (3) additionally adjusted for postnatal depression; and (4) additionally adjusted for postnatal anxiety. In PSKC, 3 models were used: (1) unadjusted; (2) adjusted for maternal age, maternal educational level, delivery method, parental history of allergic diseases, and child's sex; and (3) additionally adjusted for postnatal distress.

The groups were compared with respect to their placental 11β-HSD2 and glutathione levels by using ANOVA with least significant difference *post hoc* tests. All statistical analyses were performed with SAS (version 9.3; SAS Institute, Cary, NC) and SPSS (version 22; SPSS, Chicago, Ill) software.

TABLE II. Distribution of AD and prenatal maternal distress in the cohorts

Characteristics	COCOA (n = 973)	PSKC (n = 1531)
AD at:		
6 mo	167/901 (18.5)	NA
1 y	132/801 (16.5)	NA
2 y	113/639 (17.7)	NA
3 y	77/460 (16.7)	NA
4 y	32/229 (14.0)	NA
5 y	10/86 (11.6)	178/1531 (11.6)*
Ever in 5 y	298/973 (30.6)	NA
Prenatal maternal		
Depression	237/970 (24.4)	NA
Anxiety	178/801 (22.2)	NA
Distress	NA	88/1531 (5.8)
Postnatal maternal		
Depression	125/507 (24.7)	NA
Anxiety	154/506 (30.4)	NA
Distress	NA	345/1531 (22.5)

Values are presented as numbers (percentages).

NA, Not available.

*AD treatment in the last 12 months.

RESULTS

Participants

Table I summarizes the demographic distributions of the COCOA and PSKC cohorts, which consisted of 973 and 1531 mother-baby pairs, respectively. The characteristics of the mothers who participated in the COCOA and PSKC studies were similar with respect to most demographic distributions, although the mothers in the COCOA study were slightly older and had a higher educational level. The offspring who were included in the COCOA or PSKC analyses generally did not differ from the offspring who were excluded from the analyses, except for maternal age (PSKC), education level, and birth season (COCOA; see Tables E1 and E2 in this article's Online Repository at www.jacionline.org).

Prevalences of AD and prenatal maternal distress

Table II shows the prevalence of AD in offspring and prenatal maternal distress. In COCOA, AD was ascertained at 6 months of age and around every birthday thereafter. The prevalence was highest at 6 months of age (18.5%), after which it decreased slowly. During the whole follow-up period, 30.6% were given a diagnosis of AD. In PSKC, AD was only ascertained in 5-year-olds. The AD prevalence was 11.6%. COCOA 5-year-old children had an identical prevalence (11.6%).

In COCOA, 24.4% and 22.2% of mothers had prenatal depression and anxiety, respectively, as well as a slightly higher prevalence of postnatal depression and anxiety (24.7% and 30.4%, respectively). In PSKC, 5.8% of the mothers reported having had prenatal maternal distress, and 4-fold more reported having had postnatal distress (22.5%).

Association between prenatal maternal distress and AD

In both cohorts, children whose mothers had prenatal distress were more likely to have AD. In COCOA, after adjustment for various factors, prenatal depression and anxiety were associated

TABLE III. Associations between prenatal maternal distress and AD in offspring

Maternal status	Unadjusted		Adjusted*		Additionally adjusted for:					
	No.	HR/OR (95% CI)	No.	HR/OR (95% CI)	Postnatal depression		Postnatal anxiety		Postnatal distress	
COCOA† Prenatally depressed	942	1.35 (1.05-1.73)§	942	1.31 (1.02-1.69)§	499	1.68 (1.18-2.39)§	498	1.48 (1.04-2.11)§	NA	
Prenatally anxious	775	1.49 (1.12-1.99)§	775	1.41 (1.06-1.89)§	414	1.79 (1.20-2.68)§	413	1.59 (1.03-2.45)§	NA	
PSKC‡ Prenatally distressed	1521	1.89 (1.09-3.29)§	1521	1.85 (1.06-3.25)§	NA		NA		1521	1.86 (1.06-3.26)§

HR, Hazard ratio; NA, not available; OR, odds ratio.

*Adjusted for maternal age, educational level, delivery method, birth season (only in COCOA), maternal history of allergic diseases, and child's sex.

†The Cox proportional hazards model was used. Results shown are hazards ratios (95% CIs).

‡The logistic regression model was used. Results shown are odds ratios (95% CIs).

§ $P < .05$.

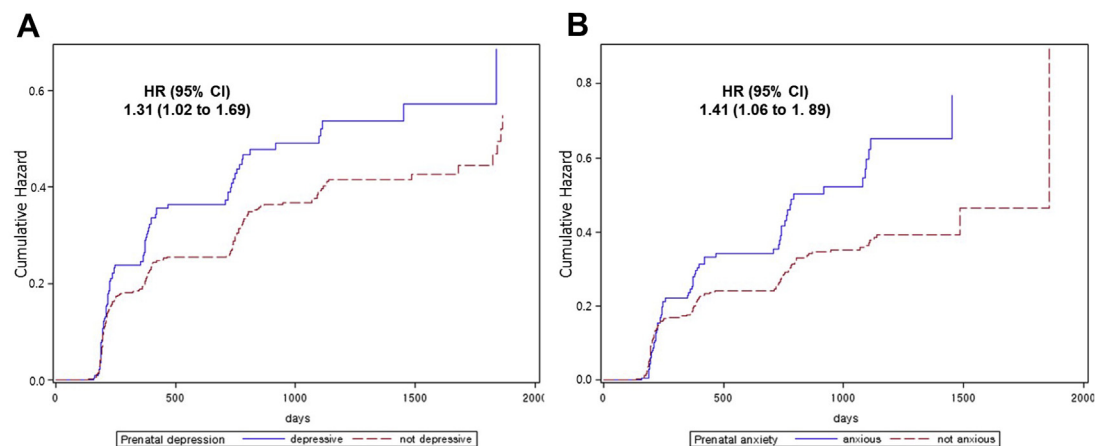


FIG 1. Cumulative hazard for a physician's diagnosis of AD in the child when the mother had prenatal depression (**A**) or anxiety (**B**) in COCOA. Values were adjusted for maternal age, educational level, delivery method, birth season, maternal history of allergic diseases, and child's sex. HR, Hazard ratio.

with higher hazard ratios of the child having AD (depression, 1.31 [95% CI, 1.02-1.69]; anxiety, 1.41 [95% CI, 1.06-1.89]; [Table III](#)). After additional adjustment for postnatal maternal distress, the associations remained significant: prenatal depression and anxiety were associated significantly after adjustments for postnatal depression (depression, 1.68; [95% CI, 1.18-2.39]; anxiety, 1.79 [95% CI, 1.20-2.68]) and for postnatal anxiety (depression, 1.48 [95% CI, 1.04-2.11]; anxiety, 1.59 [95% CI, 1.03-2.45]; [Table III](#)). Both prenatal maternal depression and anxiety scores were positively related to the predicted probability of AD ($P < .001$ in both, [Figs 1 and 2](#)).

In PSKC, prenatal maternal distress increased the adjusted odds ratios of AD in the child (1.85; 95% CI, 1.06-3.25). This association remained significant after additional adjustment for postnatal distress (1.86; 95% CI, 1.06-3.26; [Table III](#)).

Placental glutathione levels, glutathione to glutathione disulfide ratios, 11 β -HSD2 levels, and serum IgE levels in children with or without AD whose mothers did or did not have prenatal distress

The 4 groups did not differ significantly in terms of placental total glutathione levels ([Fig 3, A](#)), but the placentas of children without AD whose mothers had distress had lower glutathione to glutathione disulfide (GSSG) ratios than the placentas of children with AD whose mothers did not have

distress ($P = .042$; [Fig 3, B](#)). Analysis of placental glutathione levels and glutathione/GSSG ratios of the 4 groups revealed no differences in total glutathione levels ([Fig 3, C](#)), but the placentas had lower glutathione/GSSG ratios when the mothers were distressed than when they were not ($P = .037$; [Fig 3, D](#)). Whether the child had AD was not apparent based on glutathione/GSSG ratios.

For placental 11 β -HSD2 levels, children without either AD or a prenatally distressed mother had significantly higher levels than children with prenatally distressed mothers, regardless of whether they did ($P = .010$) or did not ($P = .005$) have AD. These differences in children with distressed mothers were not observed in children who lacked a distressed mother but had AD. Children with prenatally distressed mothers had similar 11 β -HSD2 levels, regardless of whether the child later had AD ([Fig 4, A](#)).

One-year-old children with both maternal distress and AD had significantly higher total serum IgE levels than children with maternal distress alone ($P = .007$) or those with neither maternal distress nor AD ($P = .005$; [Fig 4, B](#)).

DISCUSSION

This study showed that prenatal maternal distress, including depression and anxiety, significantly increased the risk of AD in offspring, even after adjustment for postnatal psychological distress. The relationship was not only observed in COCOA,

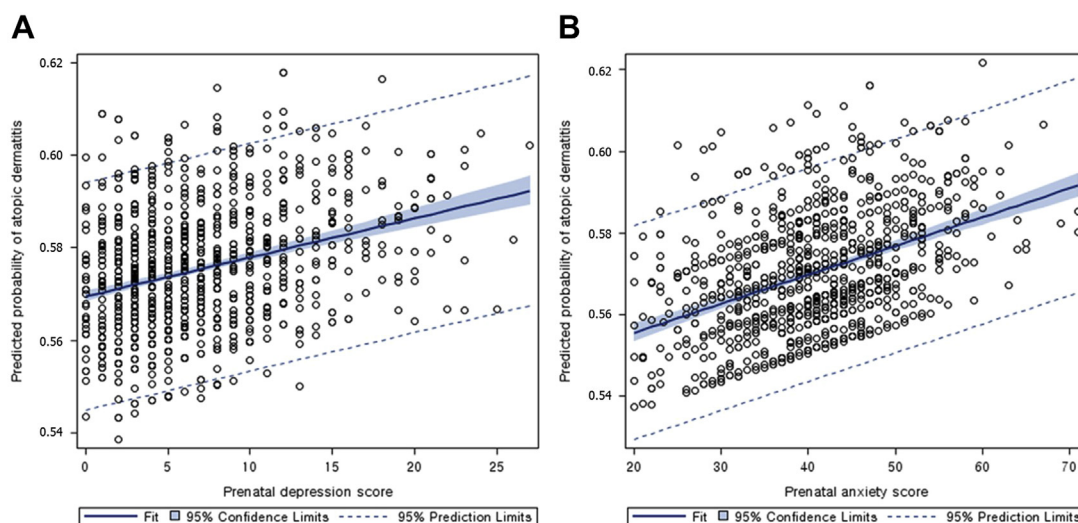


FIG 2. Relationship between prenatal maternal depression (A) and anxiety scores (B) and predicted probability of AD in COCOA. Values were adjusted for maternal age, educational level, delivery method, birth season, maternal history of allergic diseases, and child's sex. For both Fig 2, A and B, the *P* value was less than .001.

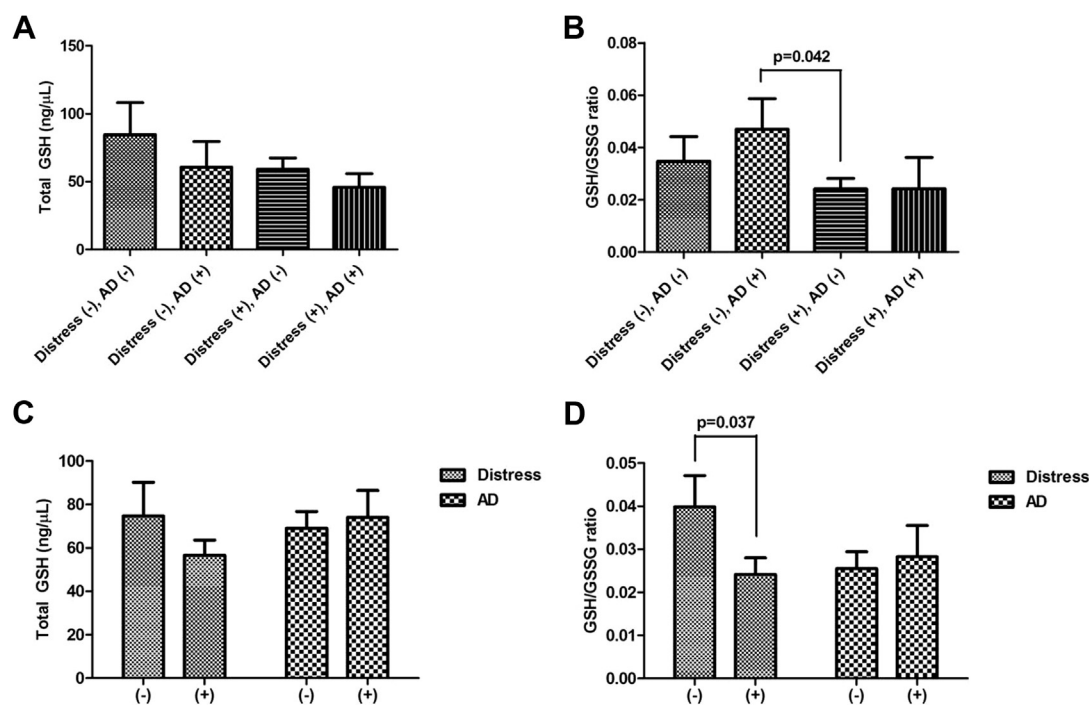


FIG 3. Total glutathione (GSH) levels (A and C) and GSH/GSSG ratios (B and D) in the placentas of children who did or did not have AD and whose mothers were prenatally distressed or unstressed. Data are shown as means (SEs).

the purposely designed Korean birth cohort, but also replicated in PSKC, another independent birth cohort in Korea. On further analyses in a subgroup of COCOA participants, we found clues suggesting that the fetuses of prenatally distressed mothers were chronically exposed to glucocorticoids and increased oxidative stress. The placental analyses showed that children with distressed mothers had lower placental HSD2 activity, which could expose the fetus to excessive cortisol levels, and lower glutathione/GSSG ratios, which indicate glutathione depletion and

oxidative stress induction.²³ In addition, we also found that 1-year-old children with distressed mothers and AD had the highest serum total IgE levels. These findings collectively suggest that maternal prenatal distress could affect fetal immune development, thereby predisposing offspring to AD, and that higher glucocorticoid and oxidative stress levels in fetal life could be contributory factors.

In human pregnancy, severe maternal stress inhibits 11 β -HSD2 activity, which exposes fetuses to excessive cortisol.²²

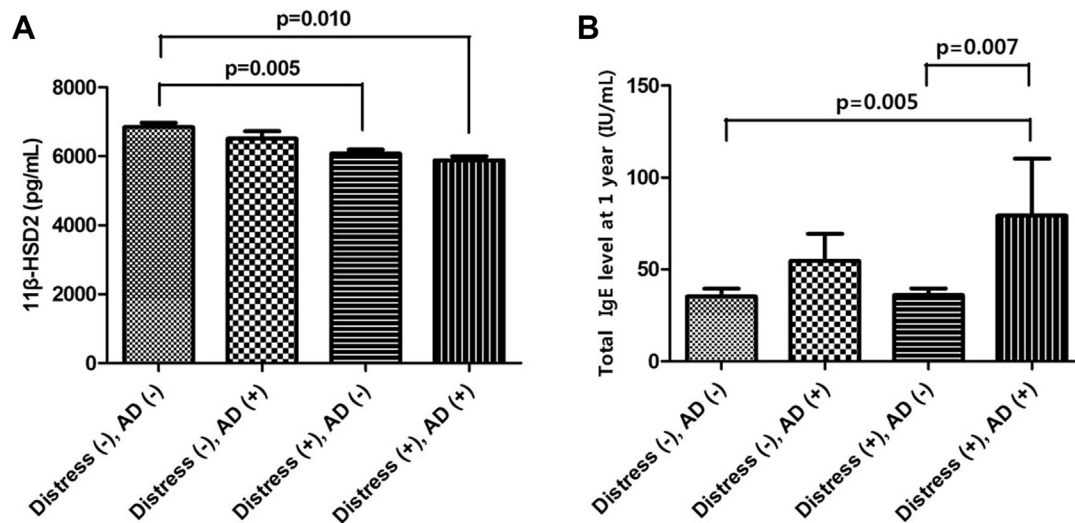


FIG 4. Total 11 β -HSD2 levels in placentas (A) and serum total IgE levels in 1-year-old children with and without AD whose mothers were prenatally distressed or unstressed (B). Data are shown as means (SEs).

We observed that children with unstressed mothers had significantly higher 11 β -HSD2 activity than those with distressed mothers. Excessive glucocorticoid levels activate ROS production by inhibiting glucose use; they also sustain oxidative stress by downregulating various antioxidant enzymes.^{27,28} Indeed, the placental glutathione and glutathione/GSSG analyses showed that children who did not have AD and/or lacked distressed mothers tended to have higher glutathione and glutathione/GSSG levels than those with either or both factors. Moreover, the children with distressed mothers had overall significantly lower placental glutathione/GSSG levels than those without distressed mothers. Because glutathione depletion associates closely with oxidative stress induction,²³ these observations suggest that the offspring of distressed mothers were exposed to more oxidative stress than those without distressed mothers.

How these maternal distress-related changes in the fetal environment promote the susceptibility of the child to AD is not yet clear. We already know that high active cortisol levels disrupt the fetal hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system, which in turn yields a persistently activated HPA axis in the child.^{3,22} Because the HPA axis modulates immune responses, persistent activation of the HPA axis might upregulate immune susceptibility to AD.²⁸ This is supported by the fact that 1-year-old children with distressed mothers and AD had significantly higher serum IgE levels. Unfortunately, the placental analyses lacked a sufficient number of stratifying participants to adjust for possible confounders; this needs to be verified in further studies with larger samples. Another possibility is that the increased oxidative stress in the fetal environment might damage keratinocytes,²⁹ which would render the offspring vulnerable to AD later in life.

In additional genetic analyses, we tried to identify vulnerabilities that might interact with prenatal stress to shape the risk of AD (see the Results section in this article's Online Repository at www.jacionline.org). Polymorphisms in 2 genes were suspected to marginally interact with prenatal distress to increase the possibility of AD in offspring. The GG genotype of nuclear receptor subfamily 3, group C, member

1 (glucocorticoid receptor; *NR3C1*) had a tendency to interact with prenatal anxiety to promote AD development, and the AA genotype of claudin 1 (*CLDN1*) interacted with prenatal depression (interaction $P = .054$ and $.077$, respectively; see Table E3 in this article's Online Repository at www.jacionline.org). This might indicate the involvement of other genes in these pathways or the existence of other pathways, such as those involving epigenetic mechanisms. However, when the interactions were reanalyzed by using multiple comparisons, none of the adjusted P values were significant, which suggested that the interactions could have been due to coincidence. Prospective birth cohort studies that examine AD-prone genotypes and their interaction with distress have not been performed before and are now warranted.

Three birth cohort studies have robustly assessed the effect of maternal prenatal distress on AD susceptibility in the child.⁶⁻⁸

Compared with those studies, the present study has several strengths. First, previous studies only analyzed 1 birth cohort. The present study analyzed 2 independent general population-based cohorts, and both yielded the same findings despite having disparate demographic distributions in age, educational level, and season of birth.

Second, the previous studies assessed maternal distress by counting life events^{7,8} or analyzing answers from general health questionnaires⁶ and AD by assessing mothers' replies to a telephone survey⁶ or questionnaire.^{7,8} By contrast, because the present study was designed purposely to test whether maternal distress during pregnancy affects the risk of AD in offspring and was conducted in close collaboration with allergy specialists, child psychiatrists, and developmental psychologists, more valid measures of maternal prenatal distress (validated questionnaires) and AD (diagnosis by pediatric allergists) were used.

Third, we adjusted the data for not only common confounding factors but also postnatal distress, which is a known confounder because prenatal and postnatal distress correlate closely. A recent trend in analyzing the effect of prenatal distress includes adjusting for postnatal distress.^{5,7}

Fourth, this is the first prospective study using validated psychological measurements of anxiety and depression to examine the effect of prenatal distress on offspring health outcomes in an Asian population.

Lastly, our study demonstrated prenatal distress–associated changes in placental 11 β -HSD2 and glutathione levels, suggesting that chronic excessive cortisol levels and oxidative stress might mediate the AD-promoting effect of maternal distress. None of the previous studies provided any clues about the mechanism underlying the association between prenatal maternal stress and AD in offspring.

This study also has several limitations. First, prenatal maternal distress was assessed based on self-report^{12,14}; structured interviews were not conducted.

Second, approximately one third of the COCOA participants were excluded from the analysis because of missing data (see Fig E1, A). However, the included and excluded participants did not differ significantly in terms of exposure and outcome distribution (see Table E1).

Third, the measures of distress used in COCOA were different from those used in PSKC, which might have been the cause of the differences in the proportions of prenatally distressed mothers between the 2 studies (COCOA vs PSKC: 22.2% to 24.4% vs 5.8%; Table II). However, we believe it is also important to demonstrate that consistent conclusions and statistically significant results can be drawn by using psychometrically different measures of distress.

Lastly, in the replication birth cohort (PSKC), maternal distress was assessed retrospectively immediately after birth with a different tool (K6), and AD was only evaluated by maternal self-reporting with the ISAAC questionnaire. Nonetheless, the PSKC subjects were enrolled randomly from throughout South Korea and followed up with a relatively low dropout rate. These qualities ensured that PSKC would be useful for replicating COCOA results. Moreover, good consistency between the 2 studies was ensured by the fact that the principal investigator of COCOA participated in designing the evaluation of allergic diseases in PSKC.

In conclusion, our findings suggest that prenatal maternal distress, including depression and anxiety, is associated with a higher risk of AD in offspring. Moreover, higher scores of maternal distress directly increased the predicted probability of AD development. Finally, the association between changes in placental 11 β -HSD2 and glutathione levels and prenatal stress might point to a chronic stress–steroid–ROS mechanism for the development of AD in children. Further studies should be designed to evaluate whether early interventions in mothers exhibiting prenatal distress prevent AD development in their offspring.

We appreciate the voluntary participation of all children and their parents, without whom the study would not have been possible. We appreciate the help of members of the KCDC, PSKC, and COCOA teams. We especially thank the scientific advisory committee (Jung Yeon Shim, Woo-Kyung Kim, Gwang Cheon Jang, Dae Jin Song, Hyo Bin Kim, Ju-Hee Seo, Hyung Young Kim, and Bong Seong Kim), collaborating pediatricians and obstetricians (Myung Hyun Sohn, Pil Ryang Lee, Soo Hyun Kim, and Yeon Kyung Cho), collaborating epidemiologists (Yean-Jung Choi, Kyung-Shin Lee, Kil-Yong Choi, Si Hyeon Lee, Jinah Park, Yoojin Lee, and Hye Lim Shin), laboratory team (Hee-Suk Kim), collaborating statisticians (Sung Ho Won and Kim Clara Tammy), collaborating nutrition experts (Hyo Jeong Hwang, Sung-Ok Kwon, and

Se-Young Oh), and environmental analysis team (Cheol Min Lee, Hwan-Cheol Kim, and Jong-Han Leem).

Key messages

- In each of 2 independent birth cohort studies, maternal prenatal psychological distress is associated with AD in offspring after adjustment for postnatal distress.
- Maternal distress increases the predicted probability of AD.
- 11 β -HSD2 and glutathione levels in the placenta were lowest among those who were exposed to prenatal maternal distress and later had AD.

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METHODS

Participants

In COCOA, women in the third trimester of pregnancy were recruited from 5 tertiary hospitals and 8 public health centers for prenatal care; all were located in Seoul, Korea.^{E1} Recruitment took place between August 2007 and August 2014. A woman was recruited if she (1) lacked high-risk conditions that could affect the development of allergic diseases in the child (eg, diabetes, preeclampsia, anemia, and severe infection), (2) planned to deliver at an affiliated medical center, and (3) was a resident of Seoul city. The eligibility of the baby was determined soon after delivery by the collaborating obstetrician and pediatrician. Babies were excluded at birth if (1) their gestational age was less than 37 weeks or (2) they had any major congenital anomalies or birth asphyxia that required oxygen supplementation. Of the 2021 women who were recruited, 333 withdrew consent, and 110 were lost to follow-up. Another 39 were then excluded because the birth was preterm or the child had a congenital anomaly. Finally, 184 women with missing data on AD, 147 women with missing data on prenatal maternal psychological distress, and 235 women with missing data on demographic variables were excluded, leaving 973 mother-baby pairs for analysis (Fig E1, A).

In PSKC, subjects were enrolled from 30 obstetric hospitals nationwide in Korea by using 2-step stratified random sampling.^{E2} Exclusion criteria were as follows: (1) mothers younger than 18 years, (2) mothers who could not communicate in Korean, (3) presence of serious health problems after delivery, (4) baby or mother with a significant disease, (5) plans to adopt baby, and (6) twins. Of the 2150 enrolled mother-child pairs, 1573 pairs completed questionnaires for allergic diseases for this study. An additional 42 pairs were excluded because of missing data on exposure or demographic variables. Thus 1531 pairs were included in the final analysis (Fig E1, B).

Measurement of genetic polymorphisms in offspring

Genomic DNA was extracted from the buffy coat of the COCOA cord blood or peripheral blood samples at age 1 year by using the Gentra Puregene Blood Kit (Qiagen, Germantown, Md), as recommended by the manufacturer. Genotyping was conducted on cord blood genomic DNA by using a TaqMan assay (ABI, Foster city, Calif), and the predesigned assay IDs were C_155538_10 and C_2057740_10 for nuclear factor, erythroid 2-like 2 (*NRF2*; rs6726395) and *CLDN1* (rs9290929), respectively. Genotyping for

NR3C1 (rs41423247) was performed with Custom TaqMan SNP Assays. 5-Hydroxytryptamine (serotonin) transporter gene-linked polymorphic region (*5HTTLPR*) was analyzed by using PCR.^{E3} Duplicate samples and negative controls were included to ensure the accuracy of genotyping. Distributions of these polymorphisms were in Hardy-Weinberg equilibrium.

Interaction between genetic polymorphisms and prenatal distress

Subjects were divided into 2 groups according to the threshold depression and anxiety scores described above and classified according to the major/minor allele for each genetic polymorphism to analyze the combined effect of distress and genetic factors. Gene-environment interactions were examined by using the Cox proportional hazards model with the interaction term.

RESULTS

Gene-environment interactions between gene polymorphisms and prenatal maternal distress

Table E3 shows how representative gene polymorphisms interacted with prenatal maternal distress to shape the risk of AD. The GG genotype of *NR3C1* increased the AD-promoting effect of prenatal anxiety with marginal significance (interaction $P = .054$). The AA genotype of *CLDN1* interacted with prenatal depression with a similar outcome (interaction $P = .077$). However, no significant interaction was observed between both genotypes (*5HTTLPR* and *NRF2*) and the 2 types of distress (depression or anxiety).

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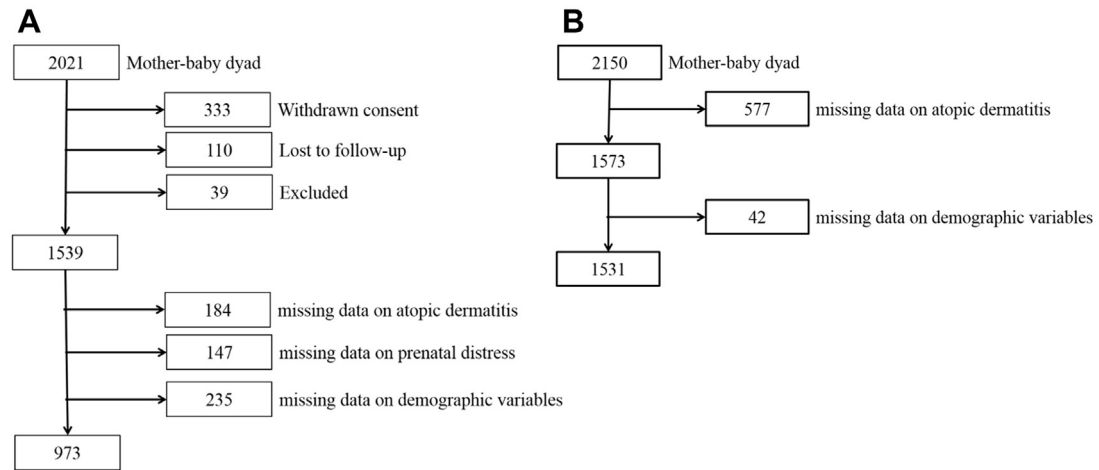


FIG E1. Flow chart of the study participants. **A**, COCOA. **B**, PSKC.

TABLE E1. Comparison of subjects who were included and excluded from analysis in COCOA

Characteristics	Included (n = 973)	Excluded (n = 676)	P value*
Prenatal maternal depression	237 (24.4)	93 (20.5)	.100
Prenatal maternal anxiety	386 (48.2)	201 (46.1)	.482
AD at 6 mo of age	167 (18.5)	46 (13.9)	.059
Maternal age			
≤32 y	540 (55.5)	243 (50.3)	.062
>32 y	433 (44.5)	240 (49.7)	
Maternal educational level			
≤12 y	65 (6.7)	27 (4.3)	.012
12-16 y	685 (58.9)	478 (76.4)	
>16 y	223 (22.9)	121 (19.3)	
Maternal history of allergic diseases			
No	675 (69.4)	344 (66.5)	.263
Yes	298 (30.6)	173 (33.5)	
Delivery methods			
Vaginal delivery	614 (63.1)	341 (66.7)	.166
Cesarean section	359 (26.9)	170 (33.3)	
Birth season			
Spring	225 (23.1)	178 (26.4)	.037
Summer	212 (21.8)	174 (25.8)	
Autumn	249 (25.6)	148 (21.9)	
Winter	287 (29.5)	175 (25.9)	
Child's sex			
Girl	477 (49.0)	311 (46.4)	.286
Boy	496 (51.0)	360 (53.7)	
Birth weight (kg)	3181.8 ± 421.1	3168.0 ± 475.7	.583
Birth height (cm)	49.3 ± 2.12	49.4 ± 2.03	.394
Gestational age (wk)	39.1 ± 1.19	39.1 ± 1.22	.983

Values are presented as numbers (percentages) or means ± SDs.

*Subjects who were included and excluded from the study were compared by using the χ^2 test (categorical variables) or Student *t* test (continuous variables).

TABLE E2. Comparison of subjects who were included and excluded from analysis in PSKC

Characteristics	Included (n = 1531)	Excluded (n = 619)	P value*
Prenatal maternal distress	88/1531 (5.8)	30/546 (5.5)	.826
AD at 5 y of age	178/1531 (11.6)	8/34 (19.1)	.142
Maternal age			
≤32 y	1007/1531 (65.8)	322/547 (58.9)	.004
>32 y	524/1531 (34.3)	225/547 (41.1)	
Maternal educational level			
≤12 y	460/1531 (30.2)	183/541 (33.3)	.092
12-16 y	982/1531 (64.6)	322/541 (59.5)	
>16 y	79/1531 (5.2)	36/541 (6.7)	
Maternal history of allergic diseases			
No	993/1531 (64.9)	46/63 (73.0)	.183
Yes	538/1531 (35.1)	17/63 (27.0)	
Delivery methods			
Vaginal delivery	839/1531 (54.8)	295/546 (54.0)	.756
Cesarean section	692/1531 (45.2)	251/546 (46.0)	
Child's sex			
Girl	790/1581 (51.6)	269/547 (49.2)	.331
Boy	741/1581 (48.4)	278/547 (50.8)	
Birth weight (kg)	3.3 ± 0.4	3.3 ± 0.4	.399
Birth height (cm)	50.2 ± 2.6	50.3 ± 2.5	.524
Gestational age (wk)	39.2 ± 1.3	39.3 ± 1.1	.158

Values are presented as numbers (percentages) or means ± SDs.

*The subjects who were included and excluded from the study were compared by using the χ^2 test (categorical variables) or Student *t* test (continuous variables).

TABLE E3. Gene-environment interactions: Gene polymorphisms that modified the AD-promoting influence of prenatal maternal distress

	Prenatal depression	No.	HR (95% CI)	Prenatal anxiety	No.	HR (95% CI)
NR3C1						
GG	Not depressed	341	Reference	Not anxious	179	Reference
GC + CC	Not depressed	239	1.23 (0.91-1.66)	Not anxious	140	1.14 (0.80-1.62)
GG	Depressed	116	1.55 (1.09-2.19)	Anxious	187	1.80 (1.22-2.67)
GC + CC	Depressed	67	1.23 (0.78-1.95)	Anxious	102	1.01 (0.56-1.83)
Interaction <i>P</i> value*			.647	.054		
5HTTLPR						
SS	Not depressed	350	Reference	Not anxious	197	Reference
SL + LL	Not depressed	195	1.04 (0.76-1.44)	Not anxious	99	1.09 (0.75-1.59)
SS	Depressed	103	1.23 (0.84-1.80)	Anxious	169	1.28 (0.82-1.98)
SL + LL	Depressed	63	1.62 (1.06-2.46)	Anxious	102	2.10 (1.28-3.44)
Interaction <i>P</i> value*			.445	.243		
CLDN1						
AA	Not depressed	507	Reference	Not anxious	279	Reference
AG + GG	Not depressed	73	1.04 (0.66-1.64)	Not anxious	42	1.13 (0.68-1.87)
AA	Depressed	167	1.41 (1.06-1.88)	Anxious	253	1.55 (1.11-2.17)
AG + GG	Depressed	17	0.39 (0.10-1.56)	Anxious	35	0.56 (0.14-2.28)
Interaction <i>P</i> value*			.077	.138		
NRF2						
GG	Not depressed	213	Reference	Not anxious	114	Reference
GA + AA	Not depressed	365	1.09 (0.80-1.49)	Not anxious	205	1.39 (0.95-2.03)
GG	Depressed	66	1.21 (0.75-1.95)	Anxious	101	1.88 (1.09-3.22)
GA + AA	Depressed	117	1.45 (0.98-2.14)	Anxious	187	1.64 (1.02-2.65)
Interaction <i>P</i> value*			.762	.186		

All data are from the COCOA study.

5HTTLPR, 5-Hydroxytryptamine (serotonin) transporter gene-linked polymorphic region; *HR*, hazard ratio; *NRF2*, nuclear factor, erythroid 2-like 2.

*Adjusted for maternal age, maternal education level, delivery method, birth season, parental history of allergic diseases, and child's sex.