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FETAL PROGRAMMING AND SYSTEMIC SCLEROSIS

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Condensation

Low birthweight and small for gestational age are risk factors for the development of systemic sclerosis in adulthood.

Short version of title

Developmental origins of systemic sclerosis.

Abstract

OBJECTIVES: this study investigates whether birthweight is linked to an increased risk of developing systemic sclerosis.

STUDY DESIGN: this was a multicenter case-control study with perinatal data obtained from 332 cases with systemic sclerosis and 243 controls. Birthweight was treated as a dichotomous variable (<2500 g vs. ≥2500 g); low birthweight was defined as a weight less than 2500 g and small for gestational age was defined as birthweight below the 10th percentile for gestational age adjusted for gender. The relationship between systemic sclerosis and both low birthweight and small for gestational age was expressed with the crude (univariate analysis) and adjusted (multivariate analysis) odds ratio (OR).

RESULTS: significantly increased ORs were observed in the univariate analysis for low birthweight (OR = 2.59, 95% confidence interval, CI: 1.39-5.05) and small for gestational age (OR = 2.60, 95% CI: 1.34-5.32) subjects. Similarly increased risks were confirmed for both conditions in the multivariate analysis (OR = 3.93, 95% CI: 1.92-8.07) and (OR = 2.58, 95% CI: 1.28-5.19), respectively.

CONCLUSION: Low birthweight and small for gestational age at birth are risk factors for the adult onset of systemic sclerosis.

Keywords

Fetal programming, scleroderma, epigenetics, birthweight, autoimmune disease.

Introduction

Systemic sclerosis (SSc), also known as scleroderma, is a chronic autoimmune disease characterized by vascular obliteration, excessive extracellular matrix deposition and fibrosis of the connective tissues.[1,2] Estimates of the disease's prevalence and incidence range from 50 to 300

cases per million people and 2.3 to 22.8 cases per million people per year, respectively.[3] Women are more likely to develop SSc than men, with reported ratios ranging from 3:1 to 14:1; a slightly increased susceptibility has also been reported among blacks.[4]

The cause of SSc has remained elusive despite intense investigation, although there is convincing evidence that genetic factors contribute to its onset and development. Genetics studies suggest that SSc is a complex polygenic disease. Candidate gene studies have identified critical immunoregulatory genes and gene regions (in particular, the human leukocyte antigen (HLA) region) as susceptibility genes for the development of the disease.[5] However, even though the genetic contribution to the disease has been shown, it now seems that environmental agents also play a critical role.[6] The link between genes and environment is represented by the new field of research of epigenetics: the study of heritable changes in genes and gene expression that do not involve DNA nucleotide sequences. Epigenetic modifications include DNA methylation, histone modifications and microRNA expression.[7] In humans, cytosine methylation and its modifications in response to maternal diet is one of the most widely studied epigenetic modifications and is a sign of adverse exposure *in utero*. Interestingly, differentially methylated regions dependent upon the mother's diet have been identified in the liver of female offspring, representing potential marks of developmental programming that may link the intrauterine environment to metabolic health later in life.[8]

This is an epidemiological study inspired by the robust work of David Barker and others, who postulated that the environment can modify the developmental trajectory of an individual even during the first stages of life, laying the foundations for disease in adulthood (the so-called fetal programming theory).[9] In fact, it is possible that adverse environmental conditions during fetal growth could alter developmental processes, explaining how a single genotype can give rise to different phenotypes ("developmental plasticity").[10] This approach has been shown to be particularly valid for chronic diseases such as cardiovascular disease[11], metabolic diseases (including diabetes)[12], osteoporosis and some forms of cancer.[13] In line with this theory, several

studies have shown that low birthweight, which can be indicative of exposure to an adverse fetal environment during specific stages of gestation (so-called "critical" periods, when rapid cell division takes place), led to an increased risk of chronic diseases in adulthood due to programming of the neuro-endocrine setting, antioxidant defenses, inflammation and the immune system itself.[14,15]

This study is based on the hypothesis that the immune system is subject to developmental plasticity during its maturation, with a real possibility that disruptors in the early fetal environment may impair its function by epigenetic mechanisms, thereby increasing the onset of chronic autoimmune diseases.[16,17] The main goal was to evaluate whether a significant correlation exists between birthweight and/or gestational age and the subsequent development of SSc.

Material and methods

A multicenter case-control study was conducted from June 2012 to November 2013 with 332 consecutive prevalent cases of SSc enrolled from the rheumatologic outpatient clinics of the following hospitals: Careggi University Hospital of Florence, La Sapienza University Hospital of Rome, the IRCCS Foundation and San Matteo University Hospital of Pavia, and the University Hospital of Ancona. The study was approved by the Ethics Committee of the Meyer Children's Hospital, University of Florence. Cases were defined as patients affected by systemic sclerosis according to the recent classification developed by the American College of Rheumatology/European League.[18] Two hundred forty-three consecutive control subjects were recruited from the surgical outpatient clinic of the Careggi University Hospital of Florence during the same period. Controls were matched to cases using a frequency matching method to obtain a similar age/gender distribution in both groups.

In order to collect demographic and perinatal information, a standardized questionnaire was created and the patients were interviewed by trained medical personnel. The following information was collected: patient identification, gender, age, birthweight, gestational age at birth, their mother's age

at birth, whether they were breastfed, and the mother's smoking habit. Birthweight and gestational age were recorded as continuous variables; when subjects did not recall exact values they were asked to categorize birthweight as <2500 g, 2500-3999 g, or ≥4000 g, and gestational age as <37 weeks, 37-41 weeks, or >41 weeks. With regard to the occupational histories of patients and controls, particular attention was given to exposure to crystalline silica, organic solvents, welding fumes, epoxy resins, and pesticides.[19] In order to minimize recall bias and ensure the validity of the approach, the data collected were compared with the data recorded in the clinical charts, even if this was only possible in 40 (12%) of the patients and 24 (10%) of the controls due to a lack of available records, especially in the case of older subjects. Moreover, the interview was repeated one month later in a randomized group of 40 patients and 40 controls to assess the level of uncertainty attributed to recall.

The following disease characteristics were obtained directly from the hospital databases for all patients diagnosed with SSc: auto-antibody pattern (antinuclear antibodies (ANA), anticentromere antibodies (ACA), anti-Scl-70), age at disease onset, type of disease (diffuse cutaneous (dcSSc), limited cutaneous (lcSSc)), organ involvement (articular, cardiac, gastrointestinal, pulmonary), and the presence of pulmonary hypertension and digital ulceration. The exclusion criteria for both patients and controls were refusal to participate, the presence of chronic diseases (such as coronary heart disease and related disorders, stroke, hypertension, and type 2 diabetes[14]), and occupation-related scleroderma risk factors (in particular organic solvents, silica, white spirit, welding fumes and epoxy resins)[19]. Furthermore, the presence of other autoimmune diseases was an exclusion criterion for patients with scleroderma, while the presence of any autoimmune disease was an exclusion criterion for controls. The Regional Center of Rare Diseases of the Meyer Children's Hospital, University of Florence, developed the research protocol, supervised the data collection and performed the statistical data analysis.

Low birthweight was defined as a weight at birth of less than 2500 g, as per the International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10). The term small for gestational age (SGA) refers to infants whose birthweights and/or lengths are at least two standard deviation (SD) units less than the mean for gestational age.[20] In clinical practice SGA is commonly defined as a birthweight less than the 10th percentile for gestational age and gender relative to the population standard: it is used as a measurable proxy for intrauterine growth restriction (IUGR) and later health risks.[21]

The study population was divided according to birthweight (BW) (<2500 g, ≥2500 g, or ≥4000 g) and gestational age (GA) (preterm <37 weeks, at term 37-41 weeks and postterm >41 weeks). The association between low birthweight (LBW) and SSc and between small for gestational age (SGA) and SSc was expressed with the odds ratio (OR) calculated with a univariate analysis that considered two birthweight groups (<2500 g and ≥2500 g). Two multivariate analyses were then performed to test the associations between all three BW groups (<2500 g, 2500-3999 g, and ≥4000 g) as well as both SGA conditions, with the risks for SSc adjusted for other confounding factors. A sample size of 318 cases and 212 controls was estimated, assuming a 5% exposure among controls and a minimum appreciable OR of 3 (alpha error = 5% and power = 90%). The statistical analysis was performed using the Stata 10 software. Eleven percent of subjects in both groups (66/575) were excluded due to uncertainty about their BW (15.4% of case studies and 6.2% of controls), while the GA was uncertain in 4.5% of case studies and 0.0% of the control group.

Results

The clinical characteristics of the study population are presented in Table 1. The expected gender distribution of disease and frequency matching adopted for the enrolled controls demonstrated a high prevalence of females in the study population compared to males. The age comparison between case studies and controls showed that the mean age of SSc patients was slightly higher in

the control group. Mothers of patients with scleroderma were slightly older than the control subjects at the time of delivery.

It was observed that LBW and SGA were more prevalent in subjects with SSc than in the controls (13.9% vs. 6.9% and 12.0% vs. 5.8%, respectively), with a less pronounced difference regarding preterm births (2.7% vs. 1.6%). Table 2 contains the OR of SSc evaluated as a univariate analysis for LBW, SGA, maternal age at birth, gestational age, breastfeeding and the mother's smoking behavior during pregnancy. SSc was more prevalent in LBW infants than in non-LBW infants (OR 2.59, 95% CI 1.39-5.05). When the analysis was limited to female subjects only, OR rose to 2.90 (95% CI 1.51-5.89). A similar result was found when exposure to SGA was considered (OR 2.60, 95% CI 1.34-5.32, increasing to OR 2.73, 95% CI 1.38-5.73 in female subjects). Both estimated risks were statistically significant ($p < 0.05$). The results showed that advanced maternal age (>34 years) was associated with an increased risk of SSc (OR 2.28, 95% CI 1.34-3.88). The mother's age at the time of delivery can therefore be considered an additional, independent risk factor for the disease. Conversely, the breastfeeding and smoking habits of the mothers of patients with scleroderma were not associated with a significantly increased risk of SSc.

Table 3 illustrates the multivariate analysis limited to female subjects which, through two different mathematical models, shows the independent effect of BW grouped into different classes: birthweight (overweight ≥4000 g, underweight <2500 g, and normal weight 2500 g - 4000 g), GA (term, preterm and postterm), age of cases and controls, and maternal age at birth. The multivariate analysis shows that the odds of disease in the female low BW group are approximately four times higher than in the normal BW group. No additional risk was observed in subjects with birthweight >4000 g as compared to subjects of normal weight at birth (2500-3999 g). SGA status increases the OR of disease approximately 2.6-fold in the multivariate analysis that includes the age of cases and controls and maternal age at delivery. In both models, the patient's age is an independent risk factor for disease with a risk of 4% (model 1, Table 3) and 5% (model 2, Table 3) for each additional year.

Table 4 shows several disease features related to LBW and SGA status. No correlation was found between LBW and SGA with the age at the onset of lung, gastro-intestinal, articular, or cardiac involvement, or the presence of ulcers and pulmonary hypertension in SSc patients. Within the context of the high correlation between LBW and adult-onset SSc, there is an increased risk of the limited form compared to the diffuse form of the disease (OR = 0.36, 95% CI 0.10-0.97). The compliance between the questionnaire data and the data recorded in the clinical charts was high and did not differ between cases (93%) and controls (95%). Furthermore, the repetition of the interview resulted in an accuracy of 95%, with 76 out of 80 subjects reporting exactly the same data in both interviews.

The incidence of missing data was always higher for cases than controls due to a lower percentage of survival of subjects' parents (an important source of information). Simulating a situation in which the missing data proved to be opposed to the study's primary hypothesis (LBW and SGA vs. SSc), we attributed a high weight (>2500 g) to all patients of the case group with missing weight data and still obtained a statistically significant OR (OR = 2.1).

Comment

The principal finding of the study is that LBW and SGA status represent risk factors for developing SSc at an adult age, with a higher incidence for LBW. However, it is interesting to note that no additional risk was observed in subjects with high birthweight, in contrast with other epidemiological studies that have demonstrated a significant correlation between birthweight ≥4000 g and adult-onset rheumatologic autoimmune diseases such as Sjogren's syndrome[22] and rheumatoid arthritis.[23,24] An increased risk was also observed for systemic lupus erythematosus for both high BW and premature birth.[25] These findings seem to indicate that both conditions (low birthweight (<2500 g) and high birthweight (≥4000 g)) may influence fetal developmental plasticity, resulting in a chronic autoimmune disease in adulthood.

From a clinical standpoint, this study allows low birthweight to be considered as an additional risk factor with respect to those already known for the development of SSc.[19] It is clear that prematurity or intrauterine growth retardation are not limited to conditioning health in the short-term, but can cause various diseases over the long-term including hypertension, diabetes, obesity, and heart disease; we can add SSc, part of the broader spectrum of autoimmune rheumatic diseases, to this list.

The mechanisms linking birthweight, gestational age and autoimmune disease can be inserted in a growing body of evidence that highlights the importance of intrauterine insults in programming the developing immune system. An adverse intrauterine environment seems to cause thymus dysfunction and subsequent long-term immunological deregulation[26], and it was significantly more likely to find incomplete thymus involution in SSc and rheumatoid arthritis (RA) patients than in a non-autoimmune control group.[27] Several studies have demonstrated that caesarean section (CS), often associated with stressful intrauterine conditions, is linked to short-term consequences for the newborn and increased risk of asthma, allergies and type 1 diabetes mellitus in adulthood.[28,29] CS seems to perturb the neonate's microbiome and the lack of a labor-induced stress response negatively affects immune activation; these mechanisms lead to epigenetic changes and therefore a predisposition to immune-related disorders.[30] For example, DNA hyper-methylation was found in hematopoietic stem cells of infants born by CS at term.[31] Another postulated programming mechanism involves activation of the maternal hypothalamic-pituitary-adrenal axis (HPA) in response to nutritional stress with consequent high fetal exposure to glucocorticoids. This hormone elevation could give rise to an early shift from cell proliferation to cell differentiation in the immune system, with an inappropriate pattern of growth for the stage of development and possible adverse consequences much later in life.[32,33] Altered HPA activity was reported to be a possible underlying mechanism in an animal model study that found a trans-generational effect of maternal treatment with dexamethasone, a common clinical practice in pregnancies at risk of preterm birth: F1 and F2 ewe lamb offspring showed an increased baseline but reduced stimulated HPA activity.[34] Another

pathway through which maternal nutrition has broad relevance for immune-mediated diseases is represented by the link between a lack of omega-3 fatty acids during pregnancy and the suppression of interleukin 13 (IL-13) cytokine production, which seems to alter Th2/Th1 balance at birth with a pronounced Th2 deviation. This mechanism could be the basis for a predisposition to allergic diseases in adult life.[35] In addition to nutritional shortage, early alterations to the immune system could also result from nutritional excesses; for example, regulators related to inflammatory and cytokine signaling were found to be significantly activated in obese women. Furthermore, mother obesity seems to upregulate genes implicated in the development of the hippocampus, cerebral cortex and amygdala, resulting in neurodegeneration and decreased survival of sensory neurons that might influence neurodevelopment.[36] Entringer and colleagues assessed leukocyte telomere length (LTL) in cord blood peripheral cells and observed a significant, independent, linear effect of pregnancy-specific stress on newborn LTL. These findings indicate that psychological stress during pregnancy may program the developing telomere biology system, which is one of the predictors of age-related diseases and mortality.[37]

Furthermore, there is persuasive evidence that supports a significant contribution of epigenetic dysregulation to the origin of SSc: environmental agents may conduce epigenetic modifications to genes involved in the immune system and therefore break tolerance, induce self-antigen abnormality and finally trigger immune reactions.[38] As proof of this, alterations in DNA methylation, histone code modifications and changes in microRNA expression levels have been observed in different cell lines (fibroblasts, lymphocytes, and endothelial cells) of SSc patients.[39,40] One of the main recognized environmental risk factors for SSc remains exposure to silica but other occupational factors[19] and viral agents[41] could also contribute to SSc pathogenesis. Finally, it is now well known that endothelial cell (EC) injury is an early event in SSc pathogenesis, and abnormal vasoreactivity is thought to result from EC malfunction, with an imbalance favoring vasoconstriction.[42] Endothelial progenitor cells (EPCs) are immature cells that derive from bone marrow and proliferate, migrate, and home to sites of neovascularization, differentiating into mature

endothelial cells *in situ*; EPCs play a critical role in vascular repair and new blood vessel formation. A recent study demonstrated that LBW preterm neonates present an alteration of EPC function with subsequent impaired angiogenic capacity as compared to full term neonates. Furthermore, the angiogenic defect of LBW endothelial colony-forming cells (ECFCs) was confirmed in mice by their inability to form robust capillary networks.[43]

Regarding the research implications of the study, further research is needed to clarify the pathogenic links that could explain the correlation between birthweight and the development of this autoimmune disease, in particular the mechanisms that result from a complex interplay among the factors of genetic susceptibility, environmental exposure and epigenetic modifications. On the basis of the identification of specific epigenetic mechanisms, it would be possible to develop appropriate diagnostic and therapeutic strategies as part of a personalized/precision medicine approach, improving the clinical outcome of patients with SSc.

This is the first study that aims to find a link between SSc and early life events in the context of epigenetics. While the study has the strength of having been conducted with a large enrolled study population, the potential existence of several inaccuracies in the data collected cannot be ruled out, as they come from a cohort of births in the nineteen-fifties. However, the inaccuracies of this study were attenuated by cross-checking the interview responses with perinatal data in clinical charts and by performing a repeat interview, methods that have been validated in other studies.[44,45,46]

There is extensive evidence that subjects with low birthweight are at a greater risk of developing many non-communicable, chronic, adult diseases. The results described here show a strong association between low birthweight and adult-onset of SSc.

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Table 1. Study population

		Cases (n=332)	Controls (n=243)	p	Total (n=575)
Mean age years (SD)		59.1 (13.2)	56.1(6.2)	<0.05	58.1 (11.5)
Maternal age at delivery years (SD)		29.3 (6.0)	27.9 (5.8)	<0.05	28.7 (5.9)
Sex	males	15 (4.5%)	18 (7.4%)	0.07	33 (5.7%)
	females	317 (95.5%)	225 (92.6%)		542 (94.3%)
Birthweight	missing	51 (15.4%)	15 (6.2%)		66 (11.5%)
	< 2500 g	46 (13.9%)	16 (6.6%)	<0.05	62 (10.8%)
	≥2500 g	235 (70.8%)	212 (87.2%)		447 (77.7%)
Gestational age at birth	missing	15 (4.5%)	2 (0.9%)		15 (2.6%)
	pre term	9 (2.7%)	4 (1.6%)	0.66	13 (2.3%)
	term newborn	299 (90.1%)	230 (94.6%)		531 (92.3%)
	post term	9 (2.7%)	7 (2.9%)		16 (2.8%)
Small for gestational age	missing	57 (17.2%)	15 (6.2%)		72 (12.5%)
	yes	40 (12.0%)	14 (5.8%)	<0.05	54 (9.4%)
	no	235 (70.8%)	214 (88.1%)		449 (78.1%)
Breastfeeding	missing	108 (32.5%)	4 (1.7%)		112 (19.5%)
	yes	202 (60.8%)	217 (89.3%)	0.41	419 (72.9%)
	no	22 (6.7%)	22 (9.0%)		44 (7.6%)
Mother's smoking habit	missing	107 (32.2%)	7 (2.9%)		114 (19.8%)
	yes	9 (2.7%)	12 (4.9%)	0.29	21 (3.6%)
	no	216 (65.1%)	224 (92.2%)		440 (76.5%)
Disease subtype	missing	13 (3.9%)			
	lcSSC	246 (74.1%)			
	dcSSC	73 (22.0%)			
Antibodies	missing	96 (28.9%)			
	ACA	133 (40.1%)			
	Scl70	100 (30.1%)			
	ACA+Scl70	3 (0.9%)			

Table 2. Odds ratio of systemic sclerosis for birthweight, small for gestational age, maternal age at birth and gestational age exposure. Univariate analysis.

		Cases	Controls	OR (CI 95%)
Birthweight	< 2500 g	46	16	2.59 (1.39 – 5.05)
	≥2500 g	235	212	1.00
Birthweight (females only)	< 2500 g	46	14	2.90 (1.51 – 5.89)
	≥2500 g	224	198	1.00
SGA	yes	40	14	2.60 (1.34 – 5.32)
	no	235	214	1.00
SGA (females only)	yes	40	13	2.73 (1.38 – 5.73)
	no	224	199	1.00
Maternal age at birth (females only)	< 25 years	69	68	1.00
	25-29 years	100	79	1.25 (0.80 – 1.95)
	30-34 years	74	46	1.58 (0.96 – 2.61)
	> 34 years	74	32	2.28 (1.34 – 3.88)
Gestational age (females only)	37-41 weeks	284	213	1.0
	< 37 weeks	9	3	2.28 (0.61 - 8.53)
	> 41 weeks	9	6	1.14 (0.40 - 3.25)
Breastfeeding	yes	202	217	0.93 (0.50 – 1.73)
	no	22	22	1.0
Breastfeeding (females only)	yes	189	202	1.07 (0.54 – 2.14)
	no	20	23	1.0
Mother's smoking habit	yes	9	12	0.78 (0.28 – 2.06)
	no	216	224	1.0
Mother's smoking habit (females only)	yes	8	11	0.74 (0.25 – 2.07)
	no	203	207	1.0

SGA: small for gestational age
OR: odds ratio
CI: confidence interval

Table 3. Odds ratio of systemic sclerosis for birthweight group, small for gestational age, gestational age and maternal ages at birth exposure. Multivariate analysis (females only).

Model 1: Case by BW group in grams, age in years, gestational age in weeks and maternal age at birth in years (females only).		
Parameters	Odds Ratio	CI 95%
BW: 2500-3999 g	1.00	
Birthweight: <2500 g	3.93	1.92 – 8.07
Birthweight: ≥ 4000 g	1.06	0.57 – 1.98
Age (by year)	1.04	1.02 – 1.06
Gestational age: 37-41 weeks	1.00	
Gestational age: <37 or >41 weeks*	2.22	0.88 - 5.59
Maternal age at birth: <25 years	1.00	
Maternal age at birth: 25-29 years	1.23	0.71 – 2.14
Maternal age at birth: 30-34 years	1.53	0.83 – 2.83
Maternal age at birth: >34 years	2.23	1.16 – 4.26
Model 2: Case by SGA, age in years, maternal age at birth in years.		
Parameters	Odds Ratio	CI 95%
SGA: No	1.00	
SGA: Yes	2.58	1.28 – 5.19
Age (by year)	1.05	1.03 – 1.07
Maternal age at birth: <25 years	1.00	
Maternal age at birth: 25-29 years	1.18	0.72 – 1.93
Maternal age at birth: 30-34 years	1.46	0.84 – 2.56
Maternal age at birth: >34 years	2.01	1.11 – 3.63

* Cases with gestational age <37 and >41 weeks were grouped together on the basis of the results of the univariate analysis (Table 2), which showed no statistical difference between each of these groups and the reference category.
BW: birth weight
SGA: small for gestational age
CI: confidence interval

Table 4. Odds ratio (CI 95%) of age at onset and organ involved due to low birthweight and small for gestational age exposure. Females and cases only.

		LBW/no-LBW OR (CI 95%)	SGA/no-SGA OR (CI 95%)
Type of disease	Limited cutaneous (lcSSC)	1.0	1.0
	Diffuse cutaneous (dcSSC)	0.36 (0.10-0.97)	0.43 (0.12-1.18)
Age of disease onset	< 30 years	1.0	1.0
	30-59 years	1.68 (0.61-4.58)	1.81 (0.60-5.47)
	> 59 years	1.71 (0.47-6.19)	2.37 (0.61-9.29)
Pulmonary hypertension	no	1.0	1.0
	yes	1.22 (0.34 – 3.68)	1.54 (0.42 – 4.73)
Ulcers	no	1.0	1.0
	yes	0.62 (0.26 – 1.35)	0.67 (0.27 – 1.53)
Pulmonary involvement	no	1.0	1.0
	yes	1.10 (0.53 – 2.32)	1.38 (0.63 – 3.15)
Gastrointestinal involvement	no	1.0	1.0
	yes	1.53 (0.69 – 3.59)	1.67 (0.71 – 4.25)
Cardiac involvement	no	1.0	1.0
	yes	1.53 (0.15 – 8.67)	1.84 (0.17 – 10.52)
Articular involvement	no	1.0	1.0
	yes	0.56 (0.24 – 1.23)	0.61 (0.25 – 1.39)

OR: odds ratio
CI: confidence interval
LBW: low birthweight
SGA: small for gestational age