

# Separate Influences of Birth Order and Gravity/Parity on the Development of Systemic Sclerosis

TONYA COCKRILL,<sup>1</sup> DEBORAH J. DEL JUNCO,<sup>1</sup> FRANK C. ARNETT,<sup>1</sup> SHERVIN ASSASSI,<sup>1</sup>  
FILEMON K. TAN,<sup>1</sup> TERRY McNEARNEY,<sup>2</sup> MICHAEL FISCHBACH,<sup>3</sup> MARILYN PERRY,<sup>1</sup> AND  
MAUREEN D. MAYES<sup>1</sup>

**Objective.** Birth order has been valuable in revealing the role of environmental influences on the risk of developing certain diseases such as allergy and atopy. In addition, pregnancy has profound effects on the immune system such as short-term effects that permit fetal survival as well as longer-term effects that could influence late-onset diseases. In order to better evaluate these influences, we studied the association of birth order and gravity/parity as risk factors for systemic sclerosis (SSc; scleroderma).

**Methods.** Data regarding SSc cases and their unaffected sibling controls were obtained from the Scleroderma Family Registry and DNA Repository. The case-sibling design was used to minimize confounding due to differences in age, race, ethnicity, or calendar time. The gravity/parity analysis was based on sibships with at least one SSc-affected and one unaffected sister.

**Results.** Birth order was examined in 974 sibships, comparing SSc cases (n = 987) with their unaffected siblings (n = 3,088). The risk of scleroderma increased with increasing birth order (odds ratio [OR] 1.25, 95% confidence interval [95% CI] 1.06–1.50 for birth order 2–5; OR 2.22, 95% CI 1.57–3.15 for birth order 6–9; and OR 3.53, 95% CI 1.68–7.45 for birth order 10–15). Gravity/parity was analyzed in 168 sibships (256 unaffected sisters, 172 SSc cases). We found an association between a history of one or more pregnancies and SSc (OR 2.8).

**Conclusion.** Birth order and pregnancy were independently associated with a higher risk of developing SSc. These findings suggest that immune development in early childhood and/or pregnancy-associated events, including but not limited to microchimerism, plays a role in SSc susceptibility.

## INTRODUCTION

The cause of systemic sclerosis (SSc; scleroderma) is unknown. Evidence suggests that both environmental and genetic factors play a role, but the nature and magnitude of each has yet to be determined. The hygiene hypothesis states that lack of early childhood exposure to infectious agents modulates immune system development and predisposes to diseases such as allergy and atopy (1). Regarding rheumatic diseases, only 3 studies have addressed

disease development in the context of birth order: one in systemic lupus erythematosus (2), one in rheumatoid arthritis (3), and one in juvenile idiopathic arthritis (4). None of these studies found an association between birth order and disease occurrence, but only the juvenile idiopathic arthritis study was adequately powered and designed to address the question. Studies of birth order as a risk factor in the development of SSc have not been previously reported.

The role of gravity and parity in SSc is particularly relevant because microchimerism (the persistence of one individual's cells in another individual) is proposed as

Supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (grants N01-AR-02251, R01-AR-055258, IP50-AR-44888, and IP50-AR-054144), The University Clinical Research Center by the University of Texas Health Science Center at Houston (grants M01-RR-02558 and UL1-RR-024148), The University of Texas Medical Branch (grants M01-RR-00073 and U54-RR-026141), and the Clinical and Translational Science Award (grants M01-RR-01346 and UL1-RR-025767).

<sup>1</sup>Tonya Cockrill, MD, Deborah J. del Junco, PhD, Frank C. Arnett, MD, Shervin Assassi, MD, Filemon K. Tan, MD, PhD, Marilyn Perry, BS, Maureen D. Mayes, MD, MPH; University of Texas Health Science Center at Houston; <sup>2</sup>Terry McNearney, MD; University of Texas Medical Branch at

Galveston; <sup>3</sup>Michael Fischbach, MD; University of Texas Health Science Center at San Antonio.

Dr. Mayes has received consulting fees, speaking fees, and/or honoraria from Actelion, United Therapeutics, and Gilead (less than \$10,000 each).

Address correspondence to Maureen D. Mayes, MD, MPH, The University of Texas–Health Science Center at Houston, 6431 Fannin Street, MSB 5.270, Houston, TX 77030. E-mail: maureen.d.mayes@uth.tmc.edu.

Submitted for publication June 15, 2009; accepted in revised form October 15, 2009.

an etiologic factor (5–8). Iatrogenic microchimerism during hematopoietic cell transplantation manifests as graft-versus-host disease with distinct clinical complications similar to scleroderma (9,10). However, natural microchimerism, which is cell transfer between mother and fetus during pregnancy resulting in the presence of small populations of genetically distinct cells in each individual, is a newer concept. Decades-long persistence of these retained foreign cells has been proposed as a contributing factor in autoimmune disease pathogenesis (6,11). Microchimerism has been well documented in both SSc patients and healthy individuals, which obscures its direct role in SSc pathogenicity (7,8). Previous studies of the role of microchimerism in the development of SSc provide conflicting data, with some studies proposing that pregnancy is protective against SSc (6,12,13), while others report that pregnancy-related phenomena may contribute to SSc development (14,15).

Based on the theories of the hygiene hypothesis, microchimerism, and other pregnancy-related immune effects, we hypothesized that birth order and parity would impact the development of SSc. To better understand this impact, we studied the association of birth order and gravidity/parity with disease development and clinical manifestations in a large cohort of SSc subjects and sibling controls.

## PATIENTS AND METHODS

**Patients.** Patients (i.e., index probands) and their first-degree relatives were members of the Scleroderma Family Registry and DNA Repository (Scleroderma Registry) as well as the Genes versus Environment in Scleroderma Outcome Study cohort. Probands were recruited from the clinical practices of the investigators and other rheumatologists in the US and from appeals to the Scleroderma Foundation patient support groups. Only siblings of the index probands were eligible for this study. Not all first-degree relatives were captured in the registry since family members tended to be scattered geographically and may not have been as motivated to participate as the index case. Demographic characteristics, family history, birth order, number of pregnancies, and number of live births were determined by a questionnaire. Subjects, including index probands and first-degree relatives, provided written informed consent, and the study was approved by the Institutional Review Board of the University of Texas Health Science Center at Houston.

Medical records were obtained on all SSc cases (i.e., all index probands and their SSc-affected siblings) to verify the diagnosis and to characterize the disease. Cases either met the 1980 American College of Rheumatology (formerly, the American Rheumatism Association) preliminary classification criteria (subcommittee for SSc) (16,17) or had at least 3 of the 5 CREST syndrome features (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasias) (16,17). Both multicase and singleton SSc families were included. The database included clinical disease characteristics such as extent of skin involvement (limited cutaneous SSc [lcSSc] versus

diffuse cutaneous SSc [dcSSc]), disease manifestations, and date of disease onset.

**Autoantibody determination.** Autoantibodies, including antinuclear antibodies (ANAs), anti-topoisomerase I (anti-topo I), and anti-RNA polymerase III (anti-RNAP III), were determined on SSc cases by the rheumatology laboratory at the University of Texas Health Science Center at Houston. ANAs were detected by indirect immunofluorescence using HEp-2 cells (Antibodies, Davis, CA) and were considered positive at a titer of  $\geq 1:80$ . Anticentromere antibodies (ACAs) were determined by the pattern of immunofluorescence. Anti-topo I antibodies were determined by immunodiffusion against calf thymus extract with commercial kits (Inova Diagnostics, San Diego, CA), and anti-RNAP III antibodies were determined by enzyme-linked immunosorbent assay (MBL, Nagoya, Japan).

**Study design and statistical analysis.** The case-sibling design, a variation of the case-control approach (18), was applied. Each SSc case was compared with one or more unaffected siblings (as controls) within the same family. Sibship was defined as a group of individuals having the same mother. Index probands without siblings ( $n = 112$ ) were excluded from our analysis as required by the case-sibling design. This design minimizes possible confounding due to case-control differences in age, race, ethnicity, calendar time, or sibship size. The primary independent variables of interest were birth order among all siblings and gravidity and parity for the female siblings in the study.

Gravidity for SSc cases and their unaffected sisters was classified into 4 categories: never pregnant (reference group; odds ratio [OR] 1), one or more pregnancy losses without any live births, one or more pregnancy losses with one or more live births, or one or more live births without pregnancy loss. Pregnancy loss was defined as either a spontaneous abortion (miscarriage) or an elective termination. The data were modeled with conditional logistic regression analysis using each index proband as the conditioning unit, which is the standard method for the case-sibling design.

We also examined covariates (i.e., sex, birth year, and mother's and father's ages at participant's birth) as potential confounders of the association between birth order and SSc using multivariable conditional logistic models. Because female sex is a strong risk factor for SSc, and because birth order is so strongly correlated with maternal/paternal age at birth (19), we examined whether the magnitude of association between birth order and SSc changed by  $>15\%$  after adjusting for these covariates (20). The potential modifying effect of covariates was assessed by including appropriate cross-product interaction terms (e.g., sex  $\times$  birth order) in the logistic modeling. The magnitude of association between SSc development and birth order and gravidity/parity were estimated from the logistic regression models using ORs, their corresponding 95% confidence intervals (95% CIs), and *P* values. Age at the time of the questionnaire was included as a covariate for the analyses of gravidity and parity.

**Table 1. Demographic features of the study population**

	Birth order analysis	Pregnancy analysis
SSc-affected subjects, no.	987	172
Sibships, no.*	974	168
Unaffected siblings, no.	3,088	256
Women, %		
SSc cases	89.2	100
Unaffected siblings	50.5	100
SSc disease onset, mean/median $\pm$ SD years	43.71/44.35 $\pm$ 13.63	41.24/42.20 $\pm$ 12.89
Birth year, mean/median $\pm$ SD years	1951/1950 $\pm$ 13.57	1952/1951 $\pm$ 12.72
Race, %		
White	87	91
African American	9	7
>1 race	2	1
Other	2	1
Ethnicity, %		
Hispanic	11	5
Non-Hispanic	89	95

\* Sibship number differs from number of probands due to the presence of 12 multicase families, including one family with 3 sibling systemic sclerosis (SSc) cases.

Sibships were subgrouped by scleroderma-specific auto-antibody status (ACAs, anti-topo I antibodies, and anti-RNAP III antibodies) and extent of skin involvement (lcSSc versus dcSSc) of the index SSc probands and were evaluated in stratified analyses to test whether the association between SSc and birth order was modified by the differences among probands in these clinical features. We tested the homogeneity of the association between birth order and SSc development across subgroups defined by the SSc proband's antibody status (positive or negative) and extent of skin involvement. The same subgroup analysis strategy and tests for homogeneity were applied to examine the association between SSc and gravidity/parity. Analyses were conducted with STATA statistical software, version 10 (StataCorp, College Station, TX).

## RESULTS

We identified 974 sibships with 4,075 siblings, 987 of which had SSc. There were 12 multicase families. Birth order was examined in the 974 sibships comparing the 987 SSc cases with their unaffected siblings ( $n = 3,088$ ). Demographic characteristics of SSc probands and their sibling controls for both the birth order and pregnancy analyses are shown in Table 1. Most families included in the analysis were white and non-Hispanic. In the birth order part of the study, the average age of SSc onset was 43.71 years for the cases, and mean year of birth for all subjects (cases and sibling controls) was 1951. As expected for SSc, women comprised 89.2% of the SSc cases but only 50.5% of the sibling controls in the birth order analyses.

Sibships with at least one female SSc case and one unaffected sister (168 sibships, 172 SSc cases, 256 unaffected sisters) with complete pregnancy history data were included in the analyses of pregnancies and live births among sisters. Average age of SSc onset was 41.24 years for the cases that were included in the pregnancy analysis, with mean year of birth of 1952 for the cases and sister controls.

**Birth order.** Family size ranged from 2–15 siblings with a mean of 3.2 siblings. As expected, most families had a sibship size between 2 and 5 ( $n = 772$  families). However, 166 families had sibship sizes between 6 and 9, and 36 families had sibship sizes between 10 and 15. This substantial proportion of larger families is more typical of family sizes seen in the 1940s and 1950s, reflecting the presence of older individuals in this population (mean year of birth in the birth order analysis 1952, mean age 56 years). We found that the risk of scleroderma increases with increasing birth order as shown in Table 2 (OR 1.25 for birth order 2–5, OR 2.22 for birth order 6–9, and OR 3.53 for birth order 10–15). For example, 257 SSc probands were first born, and 635 SSc probands had a birth order between 2 and 5. First-born children were used as the reference group.

As expected, male sex was significantly protective against SSc (OR 0.12, 95% CI 0.09–0.15), i.e., female sex was a significant risk factor for SSc (reciprocal OR 8.33). However, inclusion of the sex covariate in the conditional logistic regression model had negligible influence on the magnitude of association between SSc and birth order, suggesting no confounding by sex. Sex was not an effect modifier (i.e., the interaction term, sex  $\times$  birth order was very close to 1.0 and not significant) (data not shown), suggesting that higher birth order increased the risk of SSc in male as well as in female siblings.

Neither maternal nor paternal ages at the participants' births were significant covariates in the multivariable logistic models, and neither covariate appeared to act as a confounder of the association between SSc and birth order (data not shown).

We also addressed the association of scleroderma-specific antibodies and/or the extent of skin involvement (lcSSc versus dcSSc) between SSc and higher birth order. The ORs did not differ between positive and negative antibody subgroups ( $P > 0.05$  for all homogeneity tests). In addition, extent of skin involvement was not significant in

**Table 2. Association between birth order and SSc prevalence\***

Birth order	Unaffected siblings (controls)		OR	P	95% CI
	SSc probands (n = 987)	(n = 3,086)			
1 (n = 974)	257	717	1 (ref.)		
2–5 (n = 2,560)	635	1,925	1.25	0.010	1.06–1.50
6–9 (n = 469)	82	387	2.22	< 0.001	1.57–3.15
10–15 (n = 72)	15	57	3.53	0.001	1.68–7.45

\* SSc = systemic sclerosis; OR = odds ratio; 95% CI = 95% confidence interval.

modifying the influence of birth order (OR 1.36, 95% CI 1.15–1.61;  $P > 0.05$  for lcSSc and OR 1.34, 95% CI 1.10–1.63;  $P > 0.05$  for dcSSc). Not all SSc cases had all antibodies tested, so total numbers varied between antibody subset groups. Families were classified by the antibody status of the SSc index proband. For example, if the index proband was centromere positive, the entire sibship was classified as centromere positive as well. Effect modification by these clinical features was not apparent.

**Gravidity and parity.** In the gravidity and parity analyses (restricted to the subset of female siblings who provided complete pregnancy histories), there was a significant association between history of one or more pregnancies and SSc (Table 3). Because the ORs did not vary across increasing levels of gravidity or parity (increasing numbers of pregnancies or births), results shown in this table are collapsed across the levels of increasing gravidity and parity.

We then addressed the association of fetal loss with the development of SSc as shown in Table 3. A history of one or more pregnancy losses without any live births had the strongest association with SSc development (OR 9.56), although the numbers of affected subjects are quite small for this comparison (7 SSc probands and 3 unaffected sisters). Having at least one fetal loss as well as at least one successful pregnancy resulting in a live birth was also associated with the development of SSc and the magnitude of the OR was slightly greater than the OR of those with at least one birth without fetal loss.

In multivariable models that included birth order as a covariate, the adjusted ORs for gravidity and parity were the same as those estimated from the corresponding univariate models, suggesting that the SSc association with gravidity and parity was independent of birth order.

We also considered possible bias that could have occurred if the unaffected sisters used as the comparator

group in the pregnancy analysis had less opportunity to become pregnant than their SSc-affected sisters. In fact, the unaffected sisters in the pregnancy analysis were younger than their SSc-affected sisters by a mean age difference of  $-0.90$  years. Adjusting for age differences between the unaffected sisters and their affected sisters (by including an age covariate in the logistic modeling) did not change the observed magnitude of association for gravidity or parity (data not shown).

We then investigated the association between SSc development and gravidity according to autoantibody subgroups. Unlike the birth order analysis, we did detect a statistically significant difference in the magnitude of association across the autoantibody subgroups. The topoisomerase-negative subgroup had a much stronger association between SSc development and pregnancy than the topoisomerase-positive subgroup (for homogeneity  $\chi^2[1] = 5.53$ ,  $P = 0.015$ ). After applying Bonferroni adjustment for multiple comparisons, this association remained significant. ORs did not differ between subgroups of skin involvement (OR 3.67 for lcSSc, OR 1.98 for dcSSc,  $P > 0.05$ ). Families with multiple SSc cases that were discordant on antibody status or extent of skin involvement were also excluded in the analyses shown in Table 4, although when such families were included (treating them as concordant with the index proband's status), no effect on OR point estimates or statistical significance was observed.

## DISCUSSION

This study included a large number of SSc cases (987 cases and 3,088 sibling controls) and used a case-sibling design to minimize possible confounding due to case-control differences in age, race, ethnicity, calendar time, or sibship size. Our findings of separate and independent associations of higher birth order and gravidity with SSc suggest

**Table 3. Association between pregnancy outcome (fetal loss, with/without live births) and development of SSc\***

Pregnancy history	SSc cases	Unaffected sisters	OR	P	95% CI
Never pregnant (reference)	41	98	1.00		
≥1 pregnancy loss, no live birth	7	3	9.56	0.003	2.12–43.15
≥1 pregnancy loss, ≥1 live birth	39	40	3.27	0.001	1.62–6.61

\* SSc = systemic sclerosis; OR = odds ratio; 95% CI = 95% confidence interval.

**Table 4. Relationship of scleroderma-specific antibody positivity in pregnancy\***

	SSc probands/ unaffected siblings, no.	OR†	95% CI	P
Centromere-positive	45/63	4.17	1.17–14.87	> 0.05
Centromere-negative	124/189	2.41	1.33–4.37	
Topo I-positive	40/58	1.14	0.48–2.70	0.01
Topo I-negative	129/193	4.43	2.12–9.23	
RNAP III-positive	36/57	10.76	1.38–83.92	> 0.05
RNAP III-negative	133/193	2.26	1.28–3.99	
None of 3 SSc-specific antibodies	51/78	3.39	1.22–9.45	> 0.05

\* SSc = systemic sclerosis (scleroderma); OR = odds ratio; 95% CI = 95% confidence interval; topo I = topoisomerase I; RNAP III = RNA polymerase III.  
† Measures the association between SSc and ever pregnant versus never pregnant as the reference group.

that immune changes from infectious exposure in early life and/or pregnancy-related events may play a role in the pathogenesis of this disease. These findings support the possible relevance of the hygiene hypothesis and the proposed mechanisms of microchimerism as distinct influences in the development of SSc.

We found that the risk of scleroderma increases with increasing birth order, which is the reverse of the reported risk associated with allergic and atopic disorders. Higher birth order is associated with a reduced risk of asthma and atopy, presumably through a beneficial effect of higher infection exposure from the other siblings (1). However, this study found consistent evidence for an association between SSc and higher birth order. How early childhood exposure to multiple infectious agents and/or other mechanisms predisposes to autoimmunity are not well understood. The latent period between early life exposures and the onset of SSc, typically in the 4th to 5th decade of life (21), suggests a necessary requirement for additional external triggers to cause disease. This might be similar to mechanisms linking exposure to viruses such as the human T lymphotropic virus type I (HTLV-I) and development of adult T cell lymphoma in 5% of the HTLV-I seropositive individuals decades later (22). However, this viral exposure in the absence of other factors would not explain the importance of birth order.

Pregnancy has profound effects on the immune system resulting in tolerance to the semi-allograft fetus, which is essential to fetal survival (23–26). In terms of long-term immune effects, it is known that multiparous women have circulating antibodies to HLA antigens, which are believed to promote the increased rate of rejection of solid organ transplants in multiparous versus nulliparous women (23–26). However, there are no studies that address more subtle, persistent, and long-lasting changes of pregnancy that could alter immune tolerance and result in autoimmunity. One of the mechanisms proposed for autoimmunity in general, and SSc in particular, is that of microchimerism.

Microchimerism is proposed to occur because a greater HLA class II compatibility of the microchimeric cells transferred to the maternal (or fetal) host can potentially disrupt immunoregulatory mechanisms, which in turn results in autoimmunity (5,7,8). Male microchimerism has been demonstrated in SSc patients who have never given birth to a son, suggesting that sibling microchimerism may

also occur (27). Higher birth order, compared with lower birth order, could result in increased exposure to chimeric cells from previous pregnancies, which supports our observed finding of association between higher birth order and SSc.

Launay et al found that lcSSc and pulmonary fibrosis were associated with higher parity and had shorter intervals to disease onset based on observations of 100 consecutive women with SSc (15). Also, a recent retrospective Canadian study of live birth rates in women found that those women with SSc had higher live birth rates before disease onset than expected (14).

In our study, we found an association between SSc development and gravidity/parity in female sibships, but the magnitude of this association did not increase with higher gravidity or parity, as would be expected with multiple pregnancies. This finding does not support a straightforward microchimerism mechanism. However, we had limited statistical power given the smaller sample size of these restricted sibships (160 SSc cases and 227 unaffected sisters). More interestingly, it suggests two distinct pregnancy-related mechanisms for SSc development that involve microchimerism: one involving the transmission of microchimeric cells from previous pregnancies in the mother to SSc offspring and the other from a pregnancy in the female SSc case herself.

Other studies in SSc have not supported our finding that pregnancy is associated with an increased risk of SSc. Artlett et al (6) suggested increasing gravidity was protective in a retrospective cohort study of SSc patients (n = 111) by demonstrating that diffuse disease and worsening lung involvement were more common in nulliparous patients. In this study, the age of onset of SSc in nulliparous patients was 32 years versus 46 years in patients with 1–2 pregnancies ( $P < 0.0001$ ), and 51.3 years in patients with 5–7 pregnancies ( $P < 0.0005$ ). However, no comparison cohort was used in this study, so prior pregnancies could only be evaluated as a predictor of one type of SSc over another (e.g., diffuse versus limited).

In an Italian hospital-based case-control study of 46 SSc cases and 153 female controls, parous women had a reduced risk of SSc (OR 0.3, 95% CI 0.1–0.8) compared with nulliparous women (13). This risk was found to decrease with increasing parity. However, the limited number of SSc cases in this study and the use of hospitalized inpa-

tients without SSc for a control group impose significant limits on the interpretation of these results.

A retrospective study of 2,149 patients with a discharge diagnosis of SSc in a population-based registry from Sweden also failed to demonstrate an increased risk of SSc with pregnancy (12). In this study, Lambe et al found that nulliparity was associated with an increased risk of SSc (OR 1.37, 95% CI 1.22–1.55) (12). In addition, in parous women, the risk decreased with increasing parity. However, this study may be limited because it was unable to distinguish lcSSc, dcSSc, or localized SSc in its analysis due to the imprecision of hospital discharge codes as well as the fact that it used the current population as a control group. In all of these studies, it is also possible that patients with severe disease at a young age elect not to get pregnant compared with their older counterparts whose symptoms develop at or toward the end of their reproductive years.

The significant findings in our study (compared with previous epidemiologic studies of gravidity and parity) may be due to the case-sibling design, which increases validity (e.g., reduces population stratification bias) relative to traditional case-control studies. The use of case-sibling design better controls for factors such as age, race, ethnicity, calendar time, and sibship size and might explain our conflicting results of gravidity and SSc risk with previous studies. Other strengths of our study are its size, given the greater number of SSc index probands, and the inclusion of both live births and unsuccessful pregnancies.

We found that a history of pregnancy loss (with or without any live births) increased the risk of SSc more than having a live birth without any pregnancy losses. However, there was quite a bit of overlap in the respective 95% CIs. The small number of individuals with  $\geq 1$  pregnancy loss with no live births (7 cases and 3 controls) makes the interpretation of this finding problematic. Although statistically significant, we believe that additional studies need to be done before this finding can be considered firm. Subtle immunologic or vascular effects in preclinical SSc may decrease the likelihood of conception or successful gestation (12). Although we were unable to report infertility problems or the exact age of SSc probands at conception, 81% of our SSc probands were age  $>30$  years at onset of SSc, which is above the average age at first pregnancy in the US. This suggests that 81% of our probands are likely to have had at least one pregnancy prior to the onset of symptoms. Infertility and lower birth rates in SSc have been reported and may have influenced studies, which suggests pregnancy is protective because SSc patients could not conceive (14). Other studies have also indicated that SSc patients may have higher rates of infertility and delays in conception. Silman and Black reported infertility problems being 3 times higher in SSc patients before diagnosis versus healthy controls (28). Similar findings with infertility were reported by Englert et al (29), although they did not find increased risk of fetal loss in SSc patients.

SSc is known to have several subtypes whose antibodies are usually mutually exclusive. We tested this theory by looking at antibody subtypes and extent of skin involvement in the context of birth order and gravidity/parity. We were unable to detect any significant heterogeneity in the

birth order analysis. However, in the pregnancy analyses, the development of SSc was influenced by the topo I antibody status. No difference was seen between the subgroups defined by limited or diffuse skin involvement, but results of the pregnancy analyses are somewhat limited by our smaller sample size. The significance of these findings is unclear, but again supports distinct mechanisms in SSc development and expression. It is also possible that if we had a larger sample size in the pregnancy analysis, we may have seen greater differences between antibody subtypes and skin involvement.

In summary, this study found that increasing birth order was associated with a higher risk of developing SSc, suggesting that immune responses to early childhood infections provides a predisposition to SSc. In addition, pregnancy increased the risk of SSc, although we were unable to demonstrate enhanced risk with increasing gravidity or parity. This association supports previous work suggesting that microchimerism or other pregnancy-related immune events also play a role in SSc susceptibility.

## ACKNOWLEDGMENTS

The authors wish to thank the following individuals for their work in coordinating the study and performing laboratory evaluations: Julio Charles, Andrew Karnavas, William Babu, and Yasamin Salehi. We also gratefully acknowledge the cooperation of the subjects, without whom this work could not have been done.

## AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Dr. Mayes had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study conception and design.** Cockrill, del Junco, Mayes.

**Acquisition of data.** Arnett, Assassi, Tan, McNearney, Fischbach, Perry, Mayes.

**Analysis and interpretation of data.** Del Junco, Assassi, McNearney, Mayes.

## REFERENCES

- Romagnani S. The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both? *Immunology* 2004;112:352–63.
- Lifshay A, Siegel M. Birth order and the occurrence of systemic lupus erythematosus. *Arthritis Rheum* 1963;6:70–4.
- Sayeeduddin S, Ishaq M, Rao UR. Birth order effect in rheumatoid arthritis. *Br J Rheumatol* 1994;33:598–9.
- Prahalad S, Fraser AM, O'Brien E, Kerber RA, Mineau GP, Bohnsack JF. Lack of association between birth order and juvenile idiopathic arthritis. *Arthritis Rheum* 2003;48:2989–90.
- Adams KM, Nelson JL. Microchimerism: an investigative frontier in autoimmunity and transplantation. *JAMA* 2004;291:1127–31.
- Artlett CM, Rasheed M, Russo-Stieglitz KE, Sawaya HH, Jimenez SA. Influence of prior pregnancies on disease course and cause of death in systemic sclerosis. *Ann Rheum Dis* 2002;61:346–50.
- Nelson JL, Furst DE, Maloney S, Gooley T, Evans PC, Smith A,

- et al. Microchimerism and HLA-compatible relationships of pregnancy in scleroderma. *Lancet* 1998;351:559–62.
8. Nelson JL. HLA relationships of pregnancy, microchimerism and autoimmune disease. *J Reprod Immunol* 2001;52:77–84.
  9. Chosidow O, Bagot M, Vernant JP, Roujeau JC, Cordonnier C, Kuentz M, et al. Sclerodermatous chronic graft-versus-host disease: analysis of seven cases. *J Am Acad Dermatol* 1992; 26:49–55.
  10. Claman HN, Jaffee BD, Huff JC, Clark RA. Chronic graft-versus-host disease as a model for scleroderma. II. Mast cell depletion with deposition of immunoglobulins in the skin and fibrosis. *Cell Immunol* 1985;94:73–84.
  11. Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc Natl Acad Sci U S A* 1996;93:705–8.
  12. Lambe M, Bjornadal L, Neregard P, Nyren O, Cooper GS. Childbearing and the risk of scleroderma: a population-based study in Sweden. *Am J Epidemiol* 2004;159:162–6.
  13. Pisa FE, Bovenzi M, Romeo L, Tonello A, Biasi D, Bambara LM, et al. Reproductive factors and the risk of scleroderma: an Italian case-control study. *Arthritis Rheum* 2002;46:451–6.
  14. Bernatsky S, Hudson M, Pope J, Vinet E, Markland J, Robinson D, et al. Assessment of reproductive history in systemic sclerosis. *Arthritis Rheum* 2008;59:1661–4.
  15. Launay D, Hebbard M, Hatron PY, Michon-Pasturel U, Queyrel V, Hachulla E, et al. Relationship between parity and clinical and biological features in patients with systemic sclerosis. *J Rheumatol* 2001;28:509–13.
  16. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum* 1980;23:581–90.
  17. Leroy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988;15: 202–5.
  18. Gauderman WJ, Kraft P. Family-based case-control studies. In: Elston RC, Olson JM, Palmer L, editors. *Biostatistical genetics and genetic epidemiology*. New York: John Wiley & Sons; 2002. p. 268–9.
  19. Haukka JK, Suvisaari J, Lonnqvist J. Family structure and risk factors for schizophrenia: case-sibling study. *BMC Psychiatry* 2004;4:41.
  20. Hosmer DW, Lemeshow S. *Applied logistic regression*. 2nd ed. Hoboken (NJ): John Wiley & Sons; 2000.
  21. Mayes MD. Scleroderma epidemiology. *Rheum Dis Clin North Am* 1996;22:751–64.
  22. Fujino T, Nagata Y. HTLV-I transmission from mother to child. *J Reprod Immunol* 2000;47:197–206.
  23. Koch CA, Platt JL. T cell recognition and immunity in the fetus and mother. *Cell Immunol* 2007;248:12–7.
  24. Koga K, Aldo PB, Mor G. Toll-like receptors and pregnancy: trophoblast as modulators of the immune response. *J Obstet Gynaecol Res* 2009;35:191–202.
  25. Saito S, Nakashima A, Myojo-Higuma S, Shiozaki A. The balance between cytotoxic NK cells and regulatory NK cells in human pregnancy. *J Reprod Immunol* 2008;77:14–22.
  26. Seavey MM, Mosmann TR. Immunoregulation of fetal and anti-paternal immune responses. *Immunol Res* 2008;40:97–113.
  27. Lambert NC, Pang JM, Yan Z, Erickson TD, Stevens AM, Furst DE, et al. Male microchimerism in women with systemic sclerosis and healthy women who have never given birth to a son. *Ann Rheum Dis* 2005;64:845–8.
  28. Silman AJ, Black C. Increased incidence of spontaneous abortion and infertility in women with scleroderma before disease onset: a controlled study. *Ann Rheum Dis* 1988;47:441–4.
  29. Englert H, Brennan P, McNeil D, Black C, Silman AJ. Reproductive function prior to disease onset in women with scleroderma. *J Rheumatol* 1992;19:1575–9.