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Analysis of Systemic Sclerosis in Twins Reveals Low Concordance for Disease and High Concordance for the Presence of Antinuclear Antibodies

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Objective. **To examine concordance for systemic sclerosis (SSc) in monozygotic (MZ) and dizygotic (DZ) twins.**

Methods. **MZ and DZ twins were recruited nationwide. Zygosity was confirmed by DNA fingerprint analysis. The presence of antinuclear antibodies (ANAs) was determined using indirect immunofluorescence with HEp-2 cells as substrate. Identification of SSc-associated serum autoantibodies was performed by immunoprecipitation and double immunodiffusion. Major histocompatibility complex class II alleles were identified by polymerase chain reaction–restriction fragment length polymorphism analysis.**

Results. **Concordance for SSc was found to be similar in MZ and DZ twins. Overall concordance for SSc was low in the twins (4.7%). Concordance for the presence of ANAs was significantly higher in MZ twins compared with DZ twins. SSc-associated serum autoantibodies occurred exclusively in patients with SSc. The distribution of SSc-associated serum autoantibodies was similar to that observed in our large database of SSc patients. Increased HLA allele sharing was detected in DZ twins, irrespective of disease concordance.**

Conclusion. **These results indicate that inherited genetic factors are not sufficient to explain the development of SSc. Rather, these data indicate that inheritance may play a role in the development of serum autoantibodies in the "healthy" twin sibling of an SSc patient.**

Systemic sclerosis (SSc) is an autoimmune condition with an incidence of 10–19 new cases/million/year (1). The cause of SSc has not been identified, but environmental factors have been implicated in some cases. Studies examining the relative contribution of inherited (genetic) factors and/or environmental factors in the pathogenesis of SSc have been few and inconclusive. In a recent report on familial SSc in 3 US cohorts, the disease occurred in 0.4% of siblings of SSc patients (2).

The classic approach for assessing the role of genetic versus environmental factors in disease pathogenesis is the study of twins. Monozygotic (MZ) twins provide a unique study opportunity, since they share an identical genetic background. The study of such twins allows us not only to control for genotype, but also to control for age, family background/history, and, to some extent, environmental exposures and socioeconomic variables.

Thus far, publications on twins with SSc have been limited to case reports, describing a total of 10 twin pairs. One report described a pair of 5-year-old twins concordant for SSc (3) and 2 other reports each described one pair of adult twins concordant for SSc (4,5). An additional disease-concordant twin pair was recently reported in Belgium (6). Dubois et al described a twin pair in which one twin had SSc and the other had possible systemic lupus erythematosus (SLE) (7). Dustoor et al reported a twin pair discordant for SSc (8). Cook et al described a twin pair in which one twin had SSc and the other twin had possible SSc *sine* scleroderma (9). A 1995 study by McHugh et al included 3 MZ twin pairs discordant for SSc (10). Neither antinuclear antibodies (ANAs) nor SSc-associated autoantibodies were

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detected in the "healthy" twin siblings of these 3 SSc patients (10).

Twin studies in several other autoimmune diseases have been published, with widely varying concordance rates. For rheumatoid arthritis (RA), frequency of twin concordance has ranged from 12.3% to 21% in MZ twins and from 0% to 3.6% in DZ twins (11–14). In SLE, concordance has ranged from 10% to 69% in MZ twins and from 0% to 2% in DZ twins (14–17). In these studies, the overall higher concordance rate in the MZ twins as compared with the DZ twins suggests that a genetic component plays an important role in disease development.

To determine the relative contribution of inheritance to the development of SSc, we have undertaken a study of MZ and DZ twins concordant or discordant for SSc.

PATIENTS AND METHODS

Twin recruitment. Twins were recruited over a period of 5 years through announcements in the newsletters of 2 scleroderma patient support organizations (prior to their merger), the Scleroderma Federation's "The Beacon" and the United Scleroderma Foundation's "Scleroderma Spectrum," and through announcements at support group meetings nationwide. We also solicited MZ and DZ twins concordant or discordant for SSc by announcements sent to 3,826 US rheumatologists.

Twins volunteering for the study were initially interviewed by telephone. They were asked to complete a questionnaire and to provide a blood sample. The questionnaire included detailed personal, family, occupational, exposure, and medical history questions. Medical records of all participants were reviewed after receiving informed consent.

Classification of patients. Patients were considered to have SSc with limited cutaneous (lcSSc) involvement if, throughout their illness, skin thickening was either absent or restricted to the distal extremities (not proximal to the elbows or knees). Patients were classified as having SSc with diffuse cutaneous (dcSSc) involvement if, at any time during the course of their illness, they had skin thickening proximal to the elbows or knees (upper arms, thighs, anterior chest, abdomen) (18).

Evaluation of zygosity. For confirmation of self-reported zygosity and the determination of zygosity in uncertain cases, DNA fingerprint analysis was used as previously described (19,20), with some modifications. Briefly, genomic DNA was isolated from 3 ml of whole blood using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). Five micrograms of DNA was digested with *Hinf* I, *Hae* III, and *Mbo* I (New England Biolabs, Beverly, MA), and fractionated on a 0.7% agarose gel at 30V for 46 hours. The gel was then denatured, neutralized, and hybridized in situ with a ^{32}P end–labeled synthetic oligonucleotide. First, the gel was hybridized to a radiolabeled $(CAC)_5$ oligonucleotide probe for 4 hours at 44°C, and then washed and subjected to autoradiography. Results were confirmed by stripping the gel of the bound probe and rehybridizing to a radiolabeled 22-mer fragment (5′-TGGAGGAGGGCTGGAGGAGGGC-3′) based on the core motif of the 33.6 DNA fingerprinting probe of Jeffreys et al (20).

The band pattern observed upon autoradiography was used to determine zygosity status. An identical band pattern from both hybridizations indicated monozygosity, while a different DNA pattern was interpreted as dizygosity. Zygosity was confirmed by DNA fingerprinting in 33 of the 38 same-sex twin pairs. In 5 pairs, self-reported zygosity was also obtained from physician notes in the medical records received.

ANA detection. ANAs were identified by indirect immunofluorescence testing in which HEp-2 cells (DiaSorin, Stillwater, MN) were used as substrate (21). Serum dilutions of 1:40 were initially examined, and the pattern of immunofluorescence was identified. Sera containing ANAs were serially diluted to determine the end-point titer.

Identification of SSc-associated autoantibodies. The presence of anticentromere antibodies was confirmed by Western blotting as previously described (22). The identification of other SSc-associated autoantibodies was done using immunoprecipitation assays as previously described (21,23). Briefly, immunoglobulins in sera were allowed to adhere to protein A agarose beads (Invitrogen Life Technologies, Carlsbad, CA). For the detection of antibodies against Scl-70 (topoisomerase I), RNA polymerases I, II, and III, PM-Scl, SSA, and SSB, immunoglobulin-bound beads were used to immunoprecipitate antigens from ^{35}S-methionine–labeled HeLa and K562 cell extracts. Immunoprecipitated antigens were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and autoradiography. For the detection of autoantibodies against small nuclear ribonucleoproteins and small cytoplasmic RNPs, Th/To, and anti–transfer RNA synthetases, antigens were prepared from nonradiolabeled cells. Autoantigens were immunoprecipitated and separated by electrophoresis on denaturing acrylamide gels and detected by silver staining. Sera from healthy individuals were used as negative controls, and all autoantibodies were identified by comparison with standard sera.

Identification of major histocompatibility complex (MHC) class II alleles. MHC class II genotyping for HLA–DRB1, DQB1, and DPB1 was done using polymerase chain reaction–restriction fragment length polymorphism analysis, as previously described (24–26). Briefly, genomic DNA was isolated from peripheral blood mononuclear cells, and 100–200 ng of DNA was amplified using allele-specific DNA primers. The products were examined on 12% acrylamide gels prior to and after digestion with a panel of restriction endonucleases. DNA fragments were visualized by ethidium bromide staining.

Organ system involvement. The definitions used for determining SSc-associated organ system involvement have been previously reported (27).

Statistical analysis. Data were analyzed using Fisher's exact test and the Wilcoxon signed rank test.

RESULTS

Characterization of study participants. We recruited 55 pairs of twins in which at least one of the twins

Table 1. Demographic and clinical features of systemic sclerosis (SSc) twin pairs

	Monozygotic (n = 24)	Dizygotic (n = 18)	Total (n = 42)
Race, no.			
White	22	16	38
African American	2	1	3
Hispanic	0	1	1
Sex, no.			
Female/female	23	13	36
Male/male	1	1	2
Opposite sex	0	4	4
Disease duration, mean years (range)			
From onset of first symptom	11.1 (1–25)	12.9 (5–35)	–
From first physician diagnosis	7.1 (0–21)	8.0 (2–20)	–
SSc variant, no.			
Diffuse	9	8	17
Limited	13	5	18
Overlap	1	4	5
Not confirmed	1	1	2
SSc concordance, no. (%)	1/24 (4.2)	1/18 (5.6)	2/42 (4.7)

was diagnosed as having SSc. Five pairs in which the index patient had localized scleroderma were excluded. Information was incomplete on 8 twin pairs. Demographic and disease-related data for the remaining 42 twin pairs were gathered from telephone interviews and responses to a questionnaire. All available medical records were reviewed by two of us (CF-B and TAM). The diagnosis of SSc was made from the medical records if the patient had Raynaud's phenomenon, acrosclerosis, and either a typical internal organ manifestation or an SSc-associated serum autoantibody (18,27). Complete medical histories and physical examinations were completed on 19 twin pairs, by one of us (TAM or TMW).

Demographic and clinical information on the 42 twin pairs is summarized in Table 1. The mean age at evaluation of the MZ twins was 47.9 years and of the DZ twins was 48.5 years (age range for both 28–69 years). Zygosity of the same-sex twin pairs was confirmed by DNA fingerprint analysis (data not shown). The degree of concordance between self-assignment of zygosity and the DNA typing was 97.4% (one set of twins believed that they may be DZ and were determined to be MZ by DNA fingerprinting). HLA typing correlated with the zygosity status, in that all twins determined to be MZ shared the same HLA–DR, DQ, and DP alleles, whereas none of the DZ twins shared all 6 alleles. Twenty-four twin pairs were MZ and 18 were DZ. Thirty-eight twin pairs were white, 3 were African American, and 1 was Hispanic. There were 36 female/female twin pairs, 2 male/male twin pairs, and 4 opposite-sex twin pairs.

We obtained clinical information from review of the medical records and from the history and physical examination. As shown in Table 1, disease duration from disease onset (first symptom attributable to SSc) ranged from 1 year to 35 years. In this cohort, 17 sets of twins had dcSSc, 18 had lcSSc, and 5 had either dcSSc or lcSSc in overlap with another connective tissue disease. The extent of cutaneous involvement was not confirmed in 2 twins.

Concordance for SSc. Of the 42 twin pairs, only 2 were concordant for SSc, and both were female/female pairs. One concordant pair was DZ and both of these twins had lcSSc. The second concordant twin pair was MZ and consisted of 1 twin with dcSSc and 1 with lcSSc. In the latter pair, the onset of disease in the first twin (with dcSSc) was ~15 years after the onset of disease in her sister with lcSSc. These data show a similar concordance rate in MZ twins (4.2%) and DZ twins (5.6%) (P not significant) and an overall cross-sectional concordance rate of 4.7%.

Clinical features in SSc twins. Organ system involvement in the 19 SSc twin pairs who were examined at the University of Pittsburgh consisted of Raynaud's phenomenon in 19 twin pairs (100%), arthritis or tenosynovitis in 11 (57.9%), myositis in 5 (26.3%), gastrointestinal in 11 (57.9%), pulmonary in 6 (31.6%), cardiac in 3 (15.8%), and renal crisis in 4 (21.1%). In 1 DZ twin pair, the nonSSc twin had ulcerative colitis of 25 years' duration and was negative for ANAs. In 1 MZ twin pair, the nonSSc twin had Graves' disease. In 6 female twin pairs (4 MZ and 2 DZ), the "healthy" twin had Raynaud's phenomenon of several years' duration. Three of these 6 MZ twins were positive for ANAs, and 1 had cytoplasmic staining with anti-SSA antibodies. The 2 "healthy" DZ twins with a history of Raynaud's phenomenon were negative for ANAs. Two of the 13 MZ twins and 1 of the 6 DZ twins examined had abnormal nailfold capillaries. Reports from a previous study (28) suggested that microscopic findings of abnormal nailfold capillaries are detected in 82–86% of patients with SSc, in 9% of patients with Raynaud's disease, and in 54% of patients with mixed connective tissue disease. In our study, 50% (3 of 6) of the nonSSc twins with Raynaud's phenomenon had abnormal nailfold capillaries.

Concordance for autoantibodies. As shown in Figure 1, there was a significantly higher concordance for ANA positivity (titer of ≥1:40) in the MZ twins (19 of 21; 90%) versus the DZ twins (4 of 10; 40%) ($P < 0.05$). Even with a higher cut-off value of 1:80, concordance for the presence of ANAs was greater in the MZ twins (71%) compared with the DZ twins (40%), but the

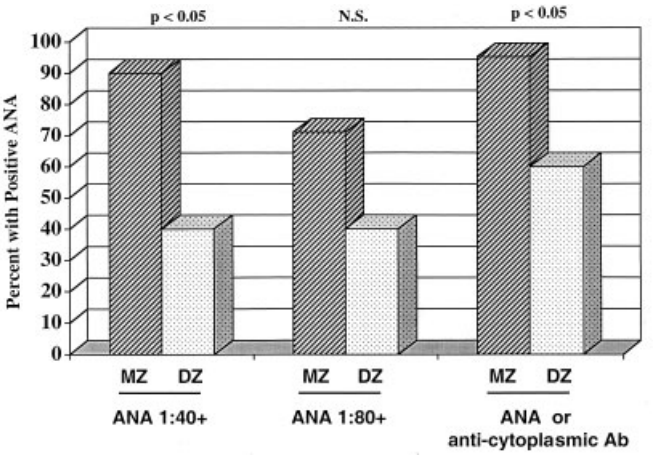

Figure 1. Concordance for the presence of antinuclear antibodies (ANAs) in twins discordant for systemic sclerosis. Serial serum dilutions were analyzed from 21 monozygotic (MZ) twin pairs and 10 dizygotic (DZ) twin pairs. ANAs and anticytoplasmic antibodies (Ab) were detected by indirect immunofluorescence. Data were analyzed using Fisher's exact test. N.S. = not significant.

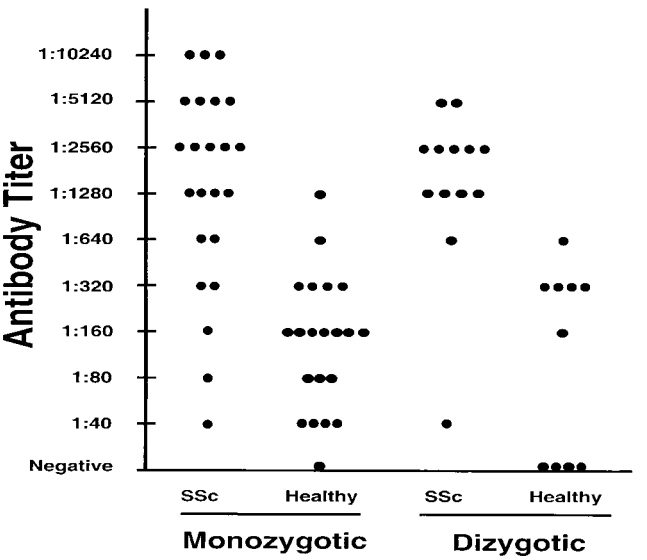

Figure 2. Titers of antinuclear antibodies in the sera of systemic sclerosis (SSc) patients and their healthy twin controls. Significant differences were found using the Wilcoxon signed rank test.

difference was not significant. The MZ twins had a higher concordance rate for the presence of either ANAs or anticytoplasmic autoantibodies as compared with the DZ twins (95% versus 60%; $P < 0.05$). "Healthy" twins with ANA positivity had a variety of nuclear staining patterns, including speckled, nucleolar, and homogeneous staining.

Autoantibody (ANA and anticytoplasmic) titers in these twins ranged from 1:40 to 1:10,240, and the range of titers was comparable in MZ and DZ twins (Figure 2). Two "healthy" twins with cytoplasmic staining had anti-SSA antibodies. Neither of these twins had clinical features of Sjögren's syndrome, but 1 had Raynaud's phenomenon and abnormal nailfold capillaries. Autoantibody titers were significantly higher in the SSc patients compared with the "healthy" twins, in both the MZ and the DZ groups ($P = 0.0001$ for MZ, $P = 0.007$ for DZ, and $P = 0.0001$ for both groups combined).

The SSc-associated serum autoantibodies detected are listed in Table 2. In the MZ twin pair concordant for disease, the twin with lcSSc had anti–U3 RNP and anti-SSA antibodies, while the twin with dcSSc was positive for ANAs and for an autoantibody of unknown specificity. In the DZ twin pair concordant for lcSSc, both twins with positivity for ANAs had anticentromere antibodies in comparable titers (1:1,280 and 1:2,560).

All SSc patients with anti–topoisomerase I antibodies had HLA–DR2 and/or DR5, in accordance with previously reported associations (29). All SSc patients with anticentromere antibodies had HLA–DQB1*0301, 0402, or 0501. They also had HLA–DR1, DR4, or DR8, similar to previously described associations (22,30–33). SSc patients with anti–U1 RNP antibodies had HLA–DR2 or DR4, in addition to having HLA–DPB1*0401, in agreement with reports linking anti–U1 RNP antibodies with these HLA alleles (34–36). SSc patients with anti–U3 RNP (fibrillarin) antibodies had HLA–DQB1*0301, 0602, or 0604, and 3 of 4 had DR6*1302, which are associations that have been previously described for antifibrillarin antibodies (37).

Table 2. Distribution of systemic sclerosis (SSc)–associated serum autoantibodies in SSc patients*

Autoantibody	Monozygotic	Dizygotic	Total
Anti–topoisomerase I	2	4	6 (14.3)
Anticentromere	5	4	9 (21.4)
Anti–RNA polymerases	5	2	7 (16.7)
Anti–U1 RNP	3	1	4 (9.5)
Anti–U3 RNP	4	1	5 (11.9)
Anti–U11/U12 RNP	1	0	1 (2.4)
Anti–PM-Scl	1	0	1 (2.4)
Anti–PL-7	1	0	1 (2.4)
Unknown†	2	0	2 (4.7)
No serum available	1	5	6 (14.3)
Total	25	17	42 (100)

* Values are the no. (%) of patients.
† Autoantibodies did not recognize known autoantigens for which reference sera were available.

Table 3. HLA allele sharing in 10 dizygotic twin pairs*

Twin	HLA–DQB1	HLA–DRB1	HLA–DPB1
1	0302/**0402**	**DR4*0404/DR8*0801**	**0301/0401**
2	**0402**/0501	**DR4*0401/DR8*0801**	**0301/0401**
3	0301/**0302**	**DR4*0401/DR5*1201**	**0401/0401**
4	**0302**/0501	**DR4*0401/DR5*1201**	**0401/0401**
5†	0301/0602	**DR2*1501/DR6*1301**	**0401/0401**
6†	0501/0501	**DR2*1501/DR6*1301**	**0401/0401**
7	0301/0402	DR5*1104/DR8*0801	1301/1301
8	0201/05031	DR3*0301/DR7	0301/0401
9	**0501**/0602	DR2*1501/**DR5*1101**	0101/**1301**
10	0201/**0501**	DR3*0301/**DR5*1101**	1001/**1301**
11	**0302**/0504	**DR4*0401**	**0401/0401**
12	**0302**/0501	**DR4*0401**	**0401/0401**
13	0402/0602	DR2*1501/**DR8*0801**	**0401/0401**
14	0501/0501	DR3*0301/**DR8*0801**	**0401/0401**
15	0301/0302	DR4*0402/DR6*1303	0501/1301
16	0201/0502	DR2*1601/DR7	0201/0401
17	**0201**/0604	**DR3*0301/DR6*1302**	**0201**/0601
18	**0201**/0501	**DR3*0301/DR6*1302**	**0201**/0201
19	**0201**/0201	DR8*080X	ND
20	**0201**/0604	DR6*130X	ND

* Shared alleles are indicated in boldface. ND = not determined.
† Dizygotic twins concordant for systemic sclerosis.

HLA allele sharing. The frequency of HLA allele sharing has been reported to be increased in female/female DZ twins discordant for RA and to be higher than observed for nontwin sibling pairs (38). This increase in HLA sharing may be a phenomenon of DZ twinning, but seems to be specific to female/female DZ twins (38). In our study, HLA sharing of at least one allele occurred in 8 (80%) of 10 DZ female/female twin pairs in whom determination of HLA–DRB1, DPB1, and DQB1 was done (Table 3). Sharing of 5 of 6 alleles occurred in 3 of the twin pairs. These twins shared both HLA–DPB1 alleles and HLA–DRB1 alleles and one HLA–DQB1 allele; all were ANA negative. Sharing of 4 of 6 alleles at the MHC class II locus occurred in 3 twin pairs. No DZ twin pairs shared all 6 MHC class II alleles. The DZ twin pair concordant for SSc shared both HLA–DP1 alleles and HLA–DRB1 alleles, but differed at the HLA–DQB1 locus.

DISCUSSION

We have evaluated 42 twin pairs with one or both members having SSc. The estimated frequency of twinning in the US population is 10 of every 1,000 births (39). Based on the reported prevalence of SSc in the US of between 4 and 253 per million (40) and a national population of 275 million, we estimate the prevalence of twin pairs concordant or discordant for SSc in the US to be 11–696.

The majority of our study participants were female, as expected in SSc. It has been reported that MZ twinning is more common in females than in males (41), with 3 of every 10 female/female pairs being MZ twins and 7 of every 10 being DZ twins (42). Of the DZ twins, it is expected that nearly half will be same-sex twins and half will be opposite-sex twins. In contrast to the above-noted DZ:MZ twinning rate of 2:1 (43), we recruited a greater number of MZ twins as compared with DZ twins (DZ:MZ ratio 0.75:1). A similar ratio has been observed by others who have examined twin disease concordance for various autoimmune conditions (13), which may reflect recruitment bias, particularly when participants are ascertained via a volunteer-based approach (43) or through public advertising and/or patient support organizations (14). Another possibility is that genomic imprinting and/or X-inactivation may occur in MZ twinning in females, e.g., the Wiedemann-Beckwith syndrome (44).

The concordance rate for a disease in MZ twins reflects the contribution of inherited genetic factors to disease development. The overall concordance rate for SSc in our study was low (4.7%) in comparison with the rates of SLE and RA observed in twins (14). This is especially true considering that we identified twins through advertisement rather than through random sampling from a population-based twin registry. The latter approach usually favors recruitment of disease-concordant twins. Thus, factors other than inheritance must play a role in the development of SSc. The concordance rate for DZ twins was similar to that for MZ twins, also suggesting that the triggers for SSc are probably environmental agents or acquired genetic alterations rather than inherited genetic factors.

These findings are consistent with the currently accepted paradigm that inherited factors prime an individual for autoimmunity, which may develop if acquired genetic or environmental factors provide the costimulation necessary for expression of the autoimmune phenotype. With heritability being expressed as 2(rMZ − rDZ), where "r" is the correlation (45), heritability for SSc in our twin cohort would be 0.008, a very low value. One pair of MZ twins was concordant for SSc, but the twins were diagnosed with different SSc variants. This is consistent with the findings of MacGregor et al, who reported a lack of similarity of clinical features in MZ twins concordant for RA (46).

In our twin cohort, ANA positivity in index cases and their "healthy" twin siblings was more frequent among the MZ twins than among the DZ twins, suggesting that inherited genetic background is important for

the development of autoantibodies but that development of these antibodies and the presence of the underlying genetic background are themselves not sufficient for disease occurrence. Positivity for ANAs was found in 40% of "healthy" DZ twins. ANAs have been detected in the sera of healthy individuals, with frequencies varying from 4% in young individuals to 18% in the elderly (47). In a 1997 study conducted by 15 laboratories worldwide using HEp-2 cells as substrate, 31.7% of healthy individuals between the ages of 20 and 60 years were found to be ANA positive at a titer of ≥1:40 (48). Therefore, the concordance of ANA positivity in our SSc twin pairs cannot be explained by the expected rate of positivity for ANAs in the normal population. In a Finnish cohort study, the presence of ANAs was examined in 76 MZ and 86 DZ twin pairs, and concordance for ANAs was rare (49). In studies examining the presence of ANAs in first-degree relatives of SSc patients, ANAs were detected in 12–27% of blood relatives when HEp-2 cells were used as substrate in indirect immunofluorescence (50). Thus, our concordance rate for the presence of ANAs in DZ twins, and especially in MZ twins, is much higher than that previously reported for relatives (including siblings and progeny) of patients with SSc.

Six of the "healthy" twins participating in our study had Raynaud's phenomenon of several years' duration and did not have any detectable SSc-specific autoantibodies. This is consistent with a previous report suggesting that the longer a patient has Raynaud's phenomenon with no associated underlying condition, the less likely he or she is to develop a connective tissue disease such as SSc in the future (51). The occurrence of Raynaud's phenomenon in these twins may also be genetic in etiology, since familial Raynaud's disease is not uncommon (52) and may be unrelated to SSc. The frequency of Raynaud's phenomenon in "healthy" twins of SSc patients in our study was 14% (6 of 42). In a population-based study of Raynaud's phenomenon in Charleston, South Carolina, and Tarentaise, Savoie, France, the prevalence of Raynaud's phenomenon in females was 5.7% and 20.1%, respectively (53).

Our study was a cross-sectional analysis of disease concordance. Although it is possible that some discordant twins may become concordant over time, new twin pairs discordant for SSc may also be identified and concordance rates may not change significantly. For example, a 7.5-year longitudinal followup of twins with multiple sclerosis showed only a minor increase in concordance in MZ twins, from 25.9% to 30.8%, and in DZ twins, from 2.4% to 4.4% (54). In addition, concordant twins may be overrepresented relative to the total number of twins, due to the bias that a twin is more aware of a disease because his or her co-twin has the disease. As noted above, the volunteer-based method of ascertainment that we used is reported to lead to the overrepresentation of MZ twins and to the preferential recruitment of concordant twins (14).

Since no population-based twin study of SSc has been published, we cannot determine if concordance for SSc in twins is comparable with that in nontwin siblings. Familial SSc is rare. Only a few case reports of familial SSc are available (2,5,55), and no conclusions can be drawn from them regarding the role that genetic or environmental factors may play in disease development. The rarity of SSc in families suggests that SSc is more likely to develop following an environmental trigger in a genetically predisposed individual.

In summary, our results suggest that inherited genetic factors are not sufficient for the development of SSc, but that such factors may play a significant role in the susceptibility of individuals to produce autoantibodies. One or a combination of environmental factors and/or acquired genetic alterations may trigger SSc in genetically predisposed individuals. Such a "predisposing" background can partially explain the clustering of autoimmune diseases observed in some families. Different triggers may result in different autoimmune phenotypes, such as SSc, SLE, RA, or thyroiditis, in the context of an "autoimmune" genetic predisposition.

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