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Heritability of Autoantibody Levels in a Twin Population

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HERITABILITY OF AUTOANTIBODY LEVELS IN A TWIN POPULATION

A thesis submitted in partial fulfillment of the requirements for the degree of MSD at Virginia Commonwealth University.

by

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Table of Contents

List of Tables

Page

List of Figures

Page

Abstract

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By AMAL RASTOGI, DMD, PHD

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University.

Virginia Commonwealth University, 2009

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AIM: This study aims to determine what portion of specific autoantibody phenotypes are genetically determined by using a twin model. **METHODS:** This study specifically examines Anti-Ro(SSA), Anti-La (SSB), Anti-Sn/RNP, Anti-Sm, Anti-Jo-1, Anti-Scl-70, Anti-Tg & Anti-TPO, Anti-dsDNA, Anti-PS, and Anti-cardiolipin antibodies for their heritability. This study examined 104 same-sex adult twins (66 monozygous, 38 dizygous) for the above mentioned autoantibody values. The serum autoantibody values in each subject were quantified using automated ELISA. Descriptive statistics including, distributions, quantiles, and moments were calculated by zygosity for continuous antibody values, subject ages, gender, race and smoking status. Categorical antibody levels were

used to determine twin pair concordance rates. Continuous and rank ordered autoantibody values were used to determine the presence and portion of a genetic component. To evaluate how strongly the antibody values in each twin group resembled each other, the intraclass correlation was calculated for each antibody by zygosity. The genetic variances, environmental variances, and heritability were estimated using path models with maximum likelihood estimation techniques. The phenotypic variance was modeled as a linear function of underlying additive genetic (A), dominant genetic (D), common environmental (C), and random environmental (E) effects. **RESULTS:** Several antibodies demonstrated a genetic component in our study population. Anti-cardiolipin had a genetic component with an estimated 69% heritability. Anti-dsDNA yielded a genetic component with a heritability estimate of 55-62%. Anti-Jo-1 presented a genetic component with the heritability estimate to be 41-51%. Anti-SCL-70 demonstrated a genetic component with a heritability estimate of 42-59%. Anti-PL had a genetic component with a heritability estimate of 52-54%. Several antibodies did not have a measurable genetic component. These included anti-Sm, anti-Ro(SSA), anti-La(SSB), anti-sn/RNP, anti-Tg, and anti-TPO. Some possibilities for the lack of a measureable genetic component may be due to the limited number of discordant twin pairs and/or the small number of subjects with higher levels of antibodies. **CONCLUSION:** The results of this study suggest several clinically relevant markers of auto-immunity may be partially genetically determined. These include: anti-cardiolipin, anti-dsDNA, anti-Jo-1, anti-SCL-70, and anti-phospholid.

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CHAPTER 1 INTRODUCTION

Autoimmunity is the failure of an organism to recognize its own constituent parts as self, which results in an immune response against its own cells and tissues. Numerous diseases result from such an aberrant immune response. Prominent examples include Celiac disease, diabetes mellitus type 1 (IDDM), systemic lupus erythematosus (SLE), Sjögren's syndrome, Churg-Strauss Syndrome, Hashimoto's thyroiditis, Graves' disease, idiopathic thrombocytopenic purpura, and rheumatoid arthritis.

Most autoimmune diseases have evidence of a genetic and environmental component. Studies have found familial aggregation of autoimmune diseases such as SLE and rheumatoid arthritis. An analysis of a cohort of 1,177 SLE patients found an increased prevalence of SLE and rheumatoid arthritis in siblings[1]. They also found an increased familial aggregation of autoimmune diseases in this SLE cohort. Other diseases with evidence of a strong genetic component include autoimmune thyroid diseases. Twin studies and familial aggregation indicate that autoimmune thyroid diseases have a complex genetic component. These studies implicate major histocompatability complex and the single nucleotide polymorphism Fc receptor-like 3 as genetic factors[2].

Some autoimmune diseases do not have strong evidence for a specific genetic component. An example includes Sjögren's syndrome. The prevalence of primary Sjögren's syndrome in siblings of affected individuals has been estimated to be 0.09% while the reported general prevalence of the disease is approximately 0.1%[3]. However, there has been an observed aggregation of other autoimmune diseases in families of patients with Sjögren's syndrome.

For the majority of these diseases and their specific markers, it is not clear what portion is heritable. Understanding the genetic and environmental component of autoimmune markers is the first step in developing novel treatments for autoimmune diseases. This study examines what portion of specific autoimmune markers are genetically determined by using a twin model. The subject sample utilized in this study is an existing set of monozygous and dizygous twins with cross sectional assays of autoantibodies. This unique population of twins was originally recruited as part of another study examining genetic risk for periodontal disease[4]. The assays conducted included anti-nuclear, anti-thyroid, and anti-phospholipid antibodies with diagnostic or clinical relevance.

Anti-Nuclear antibodies

Anti-Ro(SSA), Anti-La (SSB), Anti-Sn/RNP, Anti-Sm, Anti-dsDNA, Anti-Jo-1, Anti-Scl-70

Several unique autoantibodies are associated with autoimmune diseases and are frequently used as serological markers for diagnosis and monitoring. A common

serological indicator of autoimmunity in rheumatic disease is the Ro(SSA) antigen[5]. Over half of patients with Sjögren's syndrome (SS), one-third of patients with systemic lupus erythematosus (SLE), and 3-5% of patients with rheumatoid arthritis (RA) have antibodies to Ro(SSA). Other diseases which have been associated with these antibodies include: Sjögren's syndrome-lupus overlap disease, subacute cutaneous lupus erythematosus, lupus with complement component deficiencies, neonatal lupus syndrome, multiple myeloma, polymyositis, progressive systemic sclerosis and primary biliary cirrhosis[6]. Anti-Ro(SSA) antibodies are also found in healthy women of reproductive age with an incidence of 1%[7, 8]. Anti-La(SSB) antibodies are typically found in conjunction with anti-Ro(SSA)[9, 10]. Small RNA molecules called hy-RNAs are the target protein antigens of anti-Ro(SSA)[11]. Complexes of the anti-RO(SSA) with hy-RNAs are termed Ro-ribonucleoproteins (Ro-RNPs) exist but it is not yet clear what their biological function is[12]. Both anti-Ro/SSA and anti-La/SSB antibodies are directed against extractable nuclear antigens.

Another group of nuclear proteins, known as the Sm proteins, were first discovered as antigens targeted by anti-Sm antibodies in a patient with SLE. Other proteins with very similar structures were subsequently discovered and named LSm proteins. New members of the LSm protein family continue to be identified and reported. The Sm autoantigens, named after patient Smith's prototype serum, were isolated in the 1960's by immunodiffusion[13]. As in the case with autoimmune syndrome, anti-Sm antibodies are often found concurrent with other autoantibodies, including, SSA(Ro), SSB(La), DNA, histone, and RNP. Anti-Sm is, however, a highly specific marker for SLE since there is a 25% prevalence of anti-Sm in SLE patients[14].

Sm autoantigen is a class of antibodies against the group of nuclear Sm antigens. There are currently nine unique proteins which are classified as Sm antigens. These nine polypeptides comprise the core proteins of small nuclear ribonucleoprotein (snRNP) particles. The typical biological function of SnRNPs is involvement in pre-mRNA-splicing[15]. Anti-Sm antibodies bind to a broad variety of snRNAs[16]. This is due to the fact that they interact with proteins common to different subunits of snRNP particles (B, D, E, F and G subunits). The D subunit reacts to the majority of Sm autoantigen, however several of the subunits share at least one epitope. An explanation for the immunologic cross-reactivity of Sm proteins is the evolutionarily conserved structural sequence motifs. Sequence comparison has shown that all of the known Sm proteins share two evolutionarily conserved structural sequence motifs[17].

The snRNP particles are also known to elicit an autoantibody response in certain individuals. Anti-sn/RNP antibodies were first observed in SLE patients[16]. Later, anti-sn/RNP antibodies were also associated with mixed connective tissue disease (MCTD) patients. Studies have found that high levels of anti-sn/RNP in the absence of Sm to be a reliable marker for MCTD[18]. Anti-sn/RNP also occurs in other rheumatic diseases such as progressive systemic sclerosis, rheumatoid arthritis, discoid lupus erythematosus, Sjögren's syndrome, and overlapping conditions.

Another potential antigenic component in the nucleus is DNA itself. In some circumstances, double stranded DNA can create an autoimmune response. Anti-double stranded DNA (Anti-dsDNA) is an IgG or IgM anti-double stranded DNA antibody that binds to nuclei or nuclear components. Anti-dsDNA is associated with several conditions

including, systemic lupus erythematosus (SLE), rheumatoid arthritis, mixed connetictive tissue disease, Sjögren's syndrome, and necrotizing vasculitis. Serum anti-dsDNA levels correlate with SLE disease activity and are highly specific to SLE[19]. High levels are associated with kidney and blood vessel damage.

The tRNA synthetase group of enzymes is another target for anti-nuclear antibodies. The histidyl-tRNA synthetase (HRS) enzyme is the target for anti-Jo-1. The HRS enzyme catalyses the following chemical reaction.

$$\text{ATP + L-histidine + tRNAHis} \rightleftharpoons \text{AMP + diphosphate + L-histidyl-tRNAHis}$$

HRS specifically catalyses the coupling of L-histidine to tRNAHis before transport to the ribosome[20]. At the ribosome, L-histidyl-tRNAHis is incorporated into a polypeptide chain during protein synthesis. Anti-Jo-1 is the most common autoantibody in polymyositosis (PM) and dermatomyositis (DM)[21]. PM and DM are inflammatory myopathies with proximal muscle weakness, and elevated muscle enzyme activities. PM and DM patients have a reported an auto-antibody prevalence rate up to 89%, indicating a likely autoimmune pathology[21]. The specific prevalence rate for anti-Jo-1 is 15-20% of myositosis patients and about 30% of adult PM patients[22]. Anti-Jo-1 antibodies are capable of inhibiting HRS activity, may immunoprecipitate labeled enzyme, and may precipitate tRNAhis[23].

Another anti-nuclear antibody is the anti Scl-70. The antigen of Anti Scl-70 was recognized as topoisomerase I in 1986[24]. Topoisomerase I assists in DNA reproduction, by cutting one strand of a DNA double helix, allowing relaxation, and subsequent

reannealing of the cut strand. Topoisomerase I solves the problem caused by tension generated by the winding/unwinding of DNA[25]. Anti Scl-70 is recognized as one of two major classes of autoantibodies in scleroderma. Scleroderma, also known as progressive systemic sclerosis, is a chronic autoimmune disease characterized by a hardening or sclerosis in the skin or other organs[26]. Scleroderma's clinical manifestations include collagen deposition leading to connective tissue destruction of the skin, blood vessels, and some internal organs. A study has estimated the prevalence of scleroderma to be 1 per 5000[27]. The prevalence of anti-Scl-70 in scleroderma patients is approximately 25%[28]. Those scleroderma patients with more skin lesions tend to have the highest rates of Anti-Scl-70 antibodies in their sera[29].

Anti-Thyroid antibodies

Anti-Tg & Anti-TPO

Detection of autoantibodies to the two major thyroid antigens thyroglobulin (Tg) and thyroid peroxidase (TPO) is valuable in the diagnosis of patients with thyroid disease. Over 98% of thyroiditis patients have autoantibodies directed to either or both of these antigens.

The presence of anti-Tg autoantibodies has been shown to be a strong indicator of chronic autoimmune thyroid diseases such as Hoshimoto's thyroiditis and Graves disease[30, 31]. While many patients elaborate antibodies to both thyroid antigens, several cases have been shown to be anti-Tg positive and anti-TPO negative, or vice versa. Therefore,

combined determination of both anti-Tg and anti-TPO antibodies provides the most accurate diagnostic tool for thyroid autoimmunity.

Anti-Phospholipid antibodies

Anti-PS & anti-cardiolipin

Anti-phospholipid antibodies are antibodies against specific phospholipids found in cell membranes[32]. Anti-phospholipid antibodies react against proteins that bind to anionic phospholipids on plasma membranes. Anti-phospholipid antibodies are associated with primary and secondary antiphospholipid syndromes. Primary antiphospholipid syndrome is a disorder of coagulation, that may cause thrombosis, miscarriage, preterm delivery, and severe pre-eclampsia. Secondary antiphospholipid syndrome occurs with other auto-immune diseases such as SLE. Anti-phosphatidylserine antibody (Anti-PS) is a common antibody associated with both antiphospholipid syndromes[33].

A specific antiphosholipid antibody is anti-cardiolipin. Cardiolipin is a mitochondrial membrane phospholipid. Anti-cardiolipin is associated with antiphospholipid syndrome, livedoid vasculitis, myocardial infarction, Behçet's syndrome, SLE, rheumatoid arthritis, and scleroderma[34-36]. Anti-cardiolipin has been associated with cardiovascular sequelae such as atherosclerosis and stroke[37]. Anti-cardiolipin has been linked to adverse pregnancy outcomes such as idiopathic spontaneous abortion, fetal involution, prematurity, and low birth weight[38]. Antibodies to cardiolipin require a co-factor called □ -2-glycoprotein I for binding[39]. □ -2-glycoprotein I is a phospholipid-

binding protein that functions as a anticoagulant[40]. Studies suggest that bound □ -2-glycoprotein I forms the antigen to which antibodies are directed. It is also hypothesized that the anti-cardiolipin and □ -2-glycoprotein I complex may have several inhibitory effects on the coagulation pathway and platelet aggregation[39].

This study aims to determine what portion of specific autoantibody phenotypes are genetically determined by using a twin model. This study specifically examines Anti-Ro(SSA) antibodies, Anti-La (SSB) antibodies, Anti-Sn/RNP, Anti-Sm antibodies, Anti-Jo-1 antibody, Anti-Scl-70 antibody, Anti-Tg & Anti-TPO antibodies, Anti-dsDNA antibody, Anti-PS antibodies, and Anti-cardiolipin antibodies for their heritability.

CHAPTER 2 METHODS

Sample Population & Examination Protocol

This cohort included one-hundred four (104) same-sex adult twin pairs that were originally part of a study examining periodontal disease and autoimmunity. The subjects were recruited from the Virginia twin registry. The registry contained the names of over 20,000 twins born in Virginia between 1915 and 1975. Informed consent was obtained from all subjects with approval from the Virginia Commonwealth University's Institutional Review Board. The zygosity of all twins was classified using a biographical questionnaire designed to assess physical similarities during youth[41].

Assays of antibodies

All subjects had one blood sample taken and processed for serum, which was then stored at -20°C until utilized. Automated cross sectional enzyme linked immuno-sorbent assays (ELISA) of cotinine and autoantibodies were conducted on these serum samples. Serum samples were assayed by Dyn-BioShaf, LTD, Tel Hannan, Israel. The antibodies assayed included the following:

Anti-Ro(SSA) antibodies (IgG)

Anti-La SSB antibodies (IgG)

Anti-Sn/RNP (IgG)

Anti-Sm antibodies (IgG)

Anti-Jo-1 antibody (IgG)

Anti-Scl-70 antibody (IgG)

Anti-Tg & Anti-TPO antibodies (IgG, IgM, IgA)

Anti-dsDNA antibody (IgG)

Anti-PS antibodies (IgG, IgM, IgA)

Anti-cardiolipin antibody (IgG, IgM, IgA)

Statistical Methods

Descriptive statistics including distributions, quantiles, and moments were calculated by zygosity for continuous antibody values, subject ages, gender, race and smoking status. The antibody data was categorized as high or low by comparing to standard curves established with 100 healthy subjects. The categorical antibody data was used to calculate percent concordance for each twin pair. Descriptive statistics and concordance were calculated using statistical analysis (JMP® Statistical Discovery Software Ver 8.0) and spreadsheet software (Microsoft Office Excel ® 2003).

The one-way random effects model was used to calculate intraclass correlations with 95% confidence intervals for each antibody by zygosity. Due to the distribution of the auto-antibody values, the values were adjusted by ranking from lowest to highest. The ranked antibody values also had intraclass correlation coefficients calculated by zygosity.

Intraclass correlations adjusted by significant covariates were additionally determined. Significant covariates used for adjusting intraclass correlations were selected by using a multivariate step-wise regression technique. Intraclass correlations were calculated using statistical analysis software (PASW Statistics Ver 17.0).

In order to determine what portion of specific autoantibody phenotype is genetically determined, genetic variances, environmental variances, and heritability was estimated using path models with maximum likelihood estimation techniques. The phenotypic variance was modeled as a linear function of underlying additive genetic (A), dominant genetic (D), common environmental (C), and random environmental (E) effects. Univariate path models coupled with maximum likelihood estimation coefficient models were used to estimate these parameters for each antibody. Both antibody values and antibody rank values were analyzed using path modeling. Variance/covariance matrices were used with the structural equation modeling methods contained in an on-line module (http://statgen.iop.kcl.ac.uk/bgim/twinfit.html) and with Windows Mx GUI (Version 1.54a, Virginia Commonwealth University, Richmond, Virginia) to estimate model parameters using maximum likelihood techniques. The fit of the model was evaluated from log likelihood computations with an alpha of 0.05. The full ADCE model could not be tested since D and C are confounded when data are available only from reared together twins. The fits of reduced models were compared to the full model (e.g., ACE or ADE) by inspecting changes in -2*log likelihood values relative to differences in the degrees of freedom between models[42]. The module returned the maximum-likelihood estimates for the ACE, ADE, nested sub-models, determined the best-fitting, most parsimonious model,

and gave standardized estimates of the model parameters. A statistically significant estimate of the A or D parameter signified the presence of significant broad sense heritability. Broad sense heritability estimates for each antibody were calculated as the ratio of genetic variance to total variance.

CHAPTER 3 RESULTS

This study examined a monozygous-dizygous twin population to evaluate the heritability of eleven autoantibodies. Autoantibodies were measured once in the serum of the study population. The level of autoantibodies was quantified using ELISA. The distribution of each of the eleven antibodies proved to be skewed (Table 1). The majority of subjects tended to have minimal values and a few subjects exhibited higher values, however, there were not enough subjects with higher values to show a true bimodal distribution. The autoantibody values were examined three ways, categorically, continuously, and by ranking the continuous values. The categorical antibody values were used to determine concordance rates between twin pairs. The continuous and ranked antibody values were examined using intraclass correlations and ADCE path modeling. These multiple analysis methods were chosen in order to correct for the skewed distributions and to thoroughly evaluate the antibody values for evidence of heritability.

Descriptive statistics are shown in Table 1. Monozygous and dizygous groups were evaluated for statistically significant differences at an $\alpha < 0.05$ with χ^2 testing on gender, smoking, and racial data. Student's t test ($\alpha < 0.05$) was used to evaluate statistically significant differences for age and autoantibody values. The monozygous (n=66) and dizygous (n=38) twins were statistically similar in age and percent of smokers. The mean ages were 44 and 47 years for the monozygous and dizyogous twins respectively. The percentage of current smokers was 30% for the monozygous group and

26% for the dizygous group. The monozygous twins were more likely to be African American and female, however, these differences were not statistically significant. There were 20% more females and 8% more African Americans in the monozygous group. The mean values, standard deviation, minimum, maximum, and median for each autoantibody are presented in Table 1. The autoantibody means were not significantly different between the monozygous and dizygous groups.

Categorical analysis provided a method to compare the subjects with higher values to those with lower values. The antibody values were categorized as high or low by comparison to standard curves. The categorized values were used to determine concordance between twin pairs (Figure 1). The monzygous twin pair concordance was compared to the dizygous twin pair concordance for each of the 11 antibodies. There were no significant differences between the concordance of monzygous versus dizygous twin pairs for any of the 11 antibodies evaluated. The categorical method minimizes variance in the data, but at a cost of statistical power. There were not enough subjects to detect a significant difference in categorical conocordance rates. Categorizing the auto-antibody values as high or low is also dependent on the cut-off point selected for high values.

In order to determine how strongly the antibody values in each twin group resembled each other, the intraclass correlation coefficients were calculated. The intraclass correlation coefficients and 95% confidence intervals are presented in Table 2 for each antibody by twin group. To provide the greatest power to detect evidence of heritability, the continuous autoantibody values were examined. Higher correlation coefficients represent greater similarities within the twin group. The intraclass correlation coefficients

were compared between the monzygous and dizygous twin groups to determine evidence of a genetic effect. If there is a significant genetic component to an autoantibody value, one would expect the monozygous intraclass correlation coefficient to be greater than that of the dizygous group. Anti-SCL-70 and anti-PS antibodies had significant differences ($\alpha < 0.05$) in their intraclass correlation coefficients between the monozygous and dizygous groups. Both anti-SCL-70 and anti-PS antibodies had a greater intraclass correlation coefficient in the monozygous group. Examining intraclass-correlation in the continuous data of our study population finds anti-SCL-70 and anti-PS antibodies to potentially have a genetic component. Table 2 includes both the unadjusted and adjusted intraclass correlation coefficients. Smoking status, race, gender, and age were evaluated for significant confounding using stepwise regression. Race, gender, and age did not prove to be significant confounders for any antibody. Only anti-cardiolipin and anti-SCL-70 values were significantly related to smoking.

The options to examine data that has a skewed distribution include categorizing, excluding outliers, adjustment (i.e. logarithmic or exponential), or ranking. A decision was made to rank the antibody values in order to maintain the full distribution of the data but minimizing the range and variance. Antibody values were ranked from the lowest to highest for each twin group. The intraclass correlation was calculated for each antibody (Table 3). The intraclass correlation coefficients and 95% confidence intervals are presented in Table 3 for each antibody by twin group. Table 3 includes both the unadjusted and adjusted intraclass correlation coefficients. Smoking status, race, gender, and age were evaluated for significant confounding using stepwise regression. Race,

gender, and age did not prove to be significant confounders for any antibody. As with the non-ranked values, only anti-cardiolipin and anti-SCL-70 values were confounded by smoking. Also similar to the non- ranked values, only anti-SCL-70 and Anti-PS antibodies had significant differences ($\alpha < 0.05$) in their intraclass correlation coefficients between the monozygous and dizygous groups. Both anti-SCL-70 and anti-PS antibodies had a greater intraclass correlation coefficient in the monozygous group. All intraclass correlation coefficients were equal to or greater than those found using continuous autoantibody values. This was to be expected, since the ranked values were more closely correlated than the continuous values. Examining intraclass correlation in the ranked data of our study population found anti-cardiolipin, anti-dsDNA, anti-SCL-70, and anti-PS antibodies to potentially have a genetic component. When both the continuous and ranked intraclass correlation were taken into consideration, anti-SCL-70 and anti-PS antibodies were the most likely to have a genetic component.

In order to determine what portion of specific autoantibody phenotype is genetically determined, genetic and environmental variances, and heritability were estimated using path models with maximum likelihood estimation techniques. Path models coupled with maximum likelihood estimation methods have an advantage over conventional ANOVA methods in that the fit of full and reduced models can be evaluated and compared using the data[43]. There are several basic assumptions of path model fitting to twin data. The variances in the monzygous and dizygous groups must be approximately equal. If they are not, a poor fit or a failure of convergence will result[42]. The covariances must be less than the variances, which is a basic property of all properly-constructed

covariance matrices. The dizygous covariance must be less than the MZ covariance which is an implication of the genetic model. The ACE model will never fit well if this is not true, although it is possible to observe this given the nature of random sampling. Some antibodies violated the basic assumptions of the path model, and were noted as non-convergent.

The results of the convergent path models using continuous antibody values are shown in Table 4. Table 4 presents the most parsimonious model and parameter estimates for each antibody. The most parsimonious model for anti-cardiolipin as well as anti-TPO was CE, indicating no genetic component. The most parsimonious model for anti-dsDNA and anti-SCL-70 was AE, indicating an additive genetic component. The most parsimonious model for anti-Jo-1 and anti-PS was DE, indicating a dominant genetic component.

The ratio of genetic variance to total variance (broad sense heritability) was calculated from the path models and presented in Table 5. The broad sense heritability is more meaningful then the narrow sense additive or dominant genetic effects, since the power was insufficient to distinguish between broad sense and narrow sense heritability. Table 5 lists the heritability estimates for the most parsimonious model, the full ACE model, and the full ADE models. The heritability estimate was 55% for anti-dsDNA, 41% for anti-Jo-1, 42% for anti-SCL-70, and 52% for anti-PS.

Heritability was estimated using path models on the ranked antibody data as well. The results of the convergent path models using antibody value ranking are shown in Table 6. Table 6 presents the most parsimonious model and parameter estimates for each

antibody. The most parsimonious model for anti-Sm, anti-Ro(SSA), and anti-n-RNP was CE, indicating no genetic component. The most parsimonious model for anti-cardiolipin, anti-dsDNA, anti-Jo-1, and anti-SCL-70 was AE, indicating an additive genetic component. The most parsimonious model for anti-PS was DE, indicating a dominant genetic component.

Table 7 lists the heritability estimates using the ranked antibody values for the most parsimonious model, the full ACE model, and the full ADE models. The heritability estimate was 69% for anti-cardiolipin, 62% for anti-dsDNA, 51% for anti-Jo-1, 59% for anti-SCL-70, and 54% for anti-PS.

TABLE 1 Descriptive Statistics

	MZ (n=66)					DZ (n=38)				
	Mean	SD	Minimum	Maximum	Median	Mean	SD	Minimum	Maximum	Median
Age (years)	43.7	3.9	39.1	52.9	42.5	46.6	4.5	38.7	55.3	47.0
Cardiolipin	18.7	25.8	0.0	156.5	9.8	13.6	12.1	0.4	51.2	9.3
dsDNA	28.3	28.6	2.5	176.8	21.6	26.6	18.3	4.4	87.4	22.5
Jo-1	10.9	11.3	2.3	73.6	7.2	9.3	9.5	1.9	60.4	6.9
Sm	17.7	18.6	1.7	87.6	11.9	13.2	14.2	0.6	84.9	10.2
Ro(SSA)	33.0	149.0	1.3	1209.0	7.6	15.5	16.6	0.7	59.0	5.1
La(SSB)	20.4	78.6	1.2	642.4	6.2	10.1	18.1	0.9	107.4	5.1
Sn/RNP	16.4	32.7	0.7	255.7	8.1	8.7	9.1	0.5	37.4	4.5
SCL-70	9.5	9.3	2.2	55.8	6.3	8.2	6.2	1.8	23.9	5.5
PS	17.9	14.2	0.7	69.8	14.2	20.3	18.7	2.8	86.7	15.1
Tg	25.4	20.9	10.2	151.6	19.0	32.7	32.6	8.6	198.3	22.6
TPO	24.9	76.0	1.0	517.4	3.3	33.9	93.9	1.1	416.5	4.5
	Number	%				Number	%			
Female	50	67%				18	47%			
Current Smokers	20	30%				10	26%			
African American	16	24%				6	16%			

TABLE 2 Intraclass Correlation Coefficients

	ICC (95% CI)						
antibody	MZ	lower bound	upper bound	DZ	lower bound	upper bound	adjusted for
Cardiolipin	0.663	0.422	0.818	0.562	0.167	0.804	-
dsDNA	0.495	0.191	0.713	0.413	-0.028	0.722	-
Jo-1	0.424	0.103	0.666	-0.002	-0.438	0.439	-
Sm	0.321	-0.016	0.594	0.519	0.107	0.781	-
Ro(SSA)	0.004	-0.332	0.341	0.105	-0.347	0.522	-
La(SSB)	-0.015	-0.349	0.324	0.046	-0.398	0.477	-
Sn/RNP	0.144	-0.202	0.459	0.368	-0.081	0.696	-
SCL-70	0.312	-0.026	0.587	0.251	-0.208	0.623	-
PS	0.535	0.243	0.739	0.002	-0.434	0.443	-
Tg	-0.038	-0.368	0.303	-0.002	-0.438	0.439	-
TPO	0.611	0.347	0.786	0.555	0.157	0.800	-
Cardiolipin	0.660	0.426	0.805	0.671	0.257	0.844	smoking
SCL-70	0.294	-0.014	0.558	-0.328	-0.688	0.114	smoking

TABLE 3 Intraclass Correlation Coefficients for Ranked Autoantibody Values

	ICC (95% CI)						
antibody	MZ	lower bound	upper bound	DZ	lower bound	upper bound	adjusted for
Cardiolipin	0.688	0.458	0.832	0.409	-0.032	0.720	-
dsDNA	0.608	0.342	0.784	0.371	-0.078	0.698	-
Jo-1	0.505	0.204	0.719	0.324	-0.130	0.669	-
Sm	0.676	0.440	0.825	0.594	0.214	0.820	-
Ro(SSA)	0.458	0.144	0.689	0.443	0.009	0.739	-
La(SSB)	0.390	0.063	0.643	0.456	0.026	0.747	-
SnRNP	0.714	0.498	0.847	0.657	0.310	0.851	-
SCL-70	0.566	0.285	0.759	0.341	-0.112	0.679	-
PS	0.546	0.258	0.746	0.047	-0.397	0.478	-
Tg	0.294	-0.046	0.575	0.364	-0.086	0.693	-
TPO	0.716	0.501	0.848	0.722	0.418	0.882	-
Cardiolipin	0.683	0.456	0.830	0.540	0.026	0.782	smoking
SCL-70	0.562	0.284	0.758	0.242	-0.161	0.622	smoking

TABLE 4 Path Models for Continuous Antibody Values

Antibody	Model	A	D	C	E	-2log likelihood	df	p value
Cardiolipin	CE			0.67	0.33	2.53	4	0.28
dsDNA	AE	0.55			0.44	0.77	4	0.68
Jo-1	DE		0.41		0.59	0.46	4	0.79
Sm	non-convergent							
Ro(SSA)	non-convergent							
La(SSB)	non-convergent							
Sn/RNP	non-convergent							
SCL-70	AE	0.42			0.58	0.01	4	0.99
PS	DE		0.52		0.48	0.78	4	0.68
Tg	non-convergent							
TPO	CE			0.61	0.39	1.27	4	0.53

TABLE 5
Heritability Estimates of Continuous Antibody Values

Outcome	Most Parsimonious Model	full ACE	full ADE
Cardiolipin	0%	30%	72%
dsDNA	55%	29%	55%
Jo-1	41%	38%	41%
Sm			
Ro(SSA)			
La(SSB)			
Sn/RNP			
SCL-70	42%	38%	42%
PS	52%	50%	52%
Tg			
TPO	0%	25%	66%

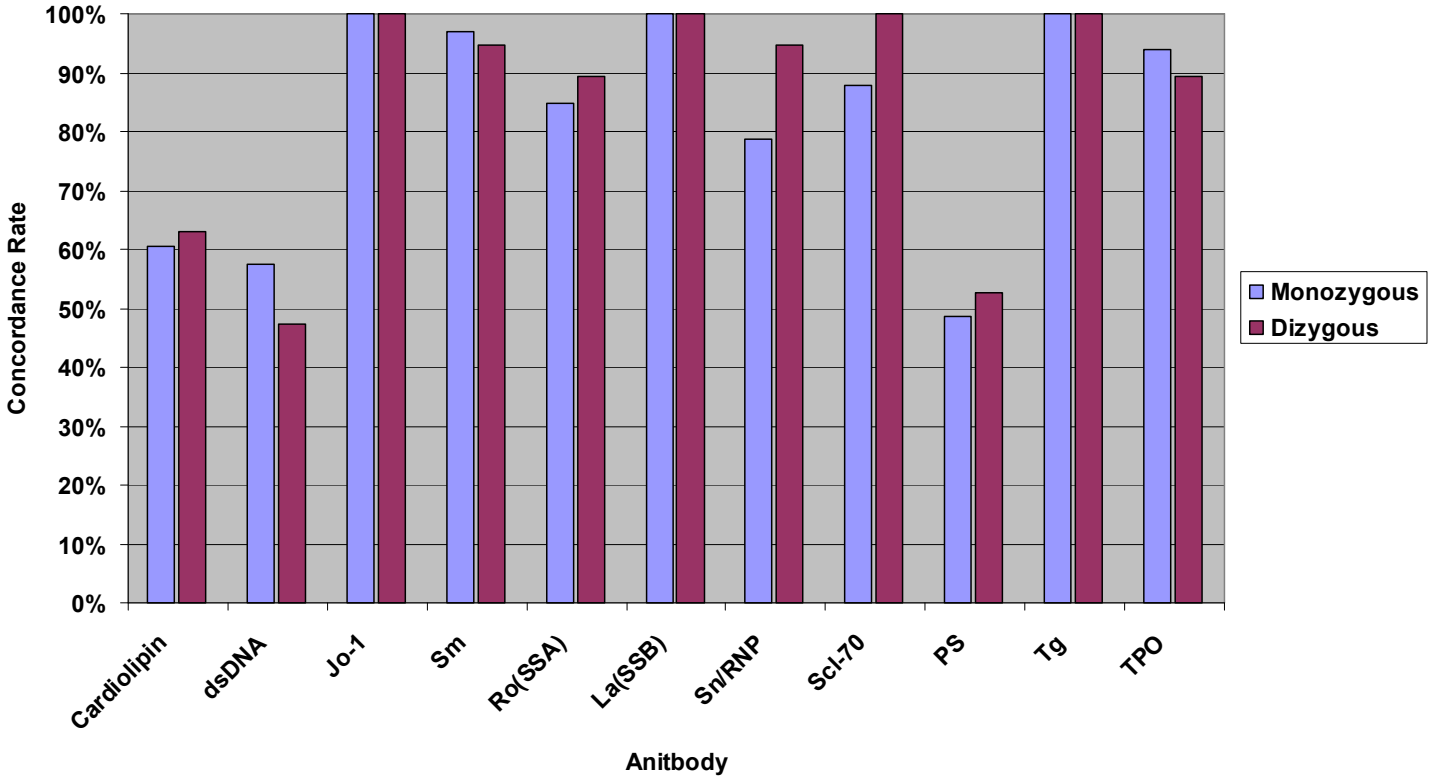
TABLE 6 Path Models for Ranked Autoantibody Values

Antibody	Model	A	D	C	E	-2log likelihood	df	p value
Cardiolipin	AE	0.68			0.31	0.60	4	0.74
dsDNA	AE	0.61			0.38	0.08	4	0.96
Jo-1	AE	0.51			0.49	0.22	4	0.90
Sm	CE			0.64	0.36	0.72	4	0.70
Ro(SSA)	CE			0.44	0.56	0.03	4	0.98
La(SSB)	non-convergent							
Sn/RNP	CE			0.69	0.31	0.42	4	0.81
SCL-70	AE	0.59			0.41	0.05	4	0.98
PS	DE		0.54		0.47	0.51	4	0.77
Tg	non-convergent							
TPO	non-convergent							

TABLE 7
Heritability Estimates of Ranked Autoantibody Values

Outcome	Most Parsimonious Model	full ACE	full ADE
Cardiolipin	69%	48%	68%
dsDNA	61%	53%	61%
Jo-1	51%	36%	51%
Sm	0%	18%	67%
Ro(SSA)	0%	5%	48%
La(SSB)			
SnRNP	0%	12%	69%
SCL-70	59%	52%	59%
PS	54%	51%	54%
Tg			
TPO			

FIGURE 1
Concordance Rates by Antibody



CHAPTER 4 DISCUSSION

This study investigated the heritability of eleven autoantibodies. Five antibodies demonstrated a genetic component in our study population of monozygous and dizygous twin pairs. Heritability estimates were 69% for anti-cardiolipin, 55-62% for anti-dsDNA, 41-51% for anti-Jo-1, 42-59% for anti-SCL-70, and 52-54% for anti-phospholid.

The most parsimonious path models for anti-cardiolipin using the ranked values included an additive genetic component. This finding expands on the partial genetic determination for anti-cardiolipin found in a previous study[44]. The study by Hunnangkul et al examined a cohort of families affected by SLE (n=1,037) and found significant associations in anti-cardiolipin values between siblings. Our study found significant differences in intraclass correlation differences between monozygous and dizygous twins for anti-cardiolipin values. This significant difference supports the hypothesis of a genetic component for anti-cardiolipin values with an estimated heritability for anti-cardiolipin expression of 69%.

The most parsimonious path model for anti-dsDNA using both continuous and ranked values included an additive genetic component. Studies examining anti-dsDNA are mixed. A study examining the genetic contributions to the autoantibody profile in a rabbit model of SLE found higher levels of anti-dsDNA among some full siblings and the presence of higher immune responder ancestors in their pedigrees [45]. Puliyath et al's findings suggest an additive mode of inheritance with high heritability. However, Niewold

et al, found anti-dsDNA to be associated with high interferon alpha levels in SLE patients, interferon alpha to be a complex heritable trait, and anti-dsDNA to be rare in healthy family members[46]. The findings of the present study parallel Puliyath et al's results by finding intraclass correlation suggestive of a genetic effect, path models suggesting additive genetic components, and heritability estimates of 55% and 62% by continuous and ranked analysis, respectively.

Path modeling of anti-Jo-1 suggested a dominant genetic component using continuous data and an additive genetic component using categorical data. This study estimated the broad sense heritability to be 41% and 51% by continuous and ranked analysis, respectively. Furthermore, the study found intraclass correlation to be suggestive of a genetic component in the continuous data. O'Hanlon et al determined certain genetic markers to be associated with anti-Jo-1 antibodies[47]. Although the literature is limited on anti-Jo-1 heritability, the O'Hanlon et al study provides evidence that anti-Jo-1 may have a genetic component. Further studies on anti-Jo-1 heritability should be conducted on other populations to validate our findings.

Both continuous and ranked values of anti-SCL-70 had similar results. The most parsimonious path models included an additive genetic component. The broad sense heritability was 42% and 59%, for continuous and ranked values, respectively. Additionally, the intraclass correlations suggested a genetic component. The literature supports the finding in this study of a genetic component of anti-SCL-70[48]. Takehara et al, examined anti-nuclear antibodies in the relatives (n=35) of patients (n=21) with systemic sclerosis. The frequency of anti-nuclear antibodies reported in relatives was 26% and anti-

SCL-70 was detected in the mother of a systemic sclerosis subject. Although the literature is limited and examines only a small population, it supports our findings of a genetic component of anti-SCL-70.

The continuous and ranked values of anti-PS had comparable results. The most parsimonious path models included dominant genetic components. The broad sense heritability was 52% and 54%, for continuous and ranked values, respectively. Furthermore, the intraclass correlations suggested a genetic component. The literature supports the findings of this study by providing evidence of linkage for anti-PS to chromosome 13q14[49].

Several antibodies did not have a measurable genetic component. These included anti-Sm, anti-Ro(SSA), anti-La(SSB), anti-sn/RNP, anti-Tg, and anti-TPO. Path analysis of the ranked anti-Sm, anti-Ro(SSA), and anti-sn/RNP values indicated the best fitting model had no genetic component. The covariance matrices of anti-La(SSB), anti-Tg, and anti-TPO did not fit the assumptions of the path models and consequently the models were non-convergent. The anti-Tg values had 100% categorical concordance for both monozygous and dizygous twins and this lack of discordance minimized the potential for meaningful analysis. The intraclass correlations for anti-Sm, anti-Ro(SSA), anti-La(SSB), anti-sn/RNP, anti-Tg, and anti-TPO implied that there is not a genetic component detectable in our sample.

The literature contains several studies which suggest the possibility of a genetic component for anti-Sm, anti-Ro(SSA), anti-La(SSB), anti-sn/RNP, anti-Tg, and anti-TPO. Puliyath et al, in a rabbit model of SLE found genetic contributions to the autoantibody

profile of anti-Sm[45]. A study of familial aggregation and linkage analysis of autoantibody traits in pedigrees for SLE reported evidence for linkage to anti-Sm on chromosome 3q27[49]. Ferreira et al, found IgM anti-Ro to have a uniquely high degree of heritability in a study of SLE families[50]. A study of familial aggregation and linkage analysis of autoantibody traits in pedigrees for SLE found potential evidence for linkage to anti-Ro(SSA) or anti-La(SSB) on chromosome 4q34-q35[49]. Several studies have found nuclear antibodies to be associated between relatives[45, 48]. The linkage study by Ramos et al, also reported evidence that anti-sn/RNP linkage may be associated with chromosome 3q21[49]. Complex segregation analysis in a population of Amish families suggested that transmission of anti-TPO is consistent with a mixed model with a major gene transmitted in an autosomal dominant pattern[51]. Outschoorn et al demonstrated that anti-Tg and anti-TPO exhibited positive heritability in patients with Graves disease, but not in chronic lymphocytic or Hashimoto's thyroiditis patients[52, 53] As the literature suggests, anti-Sm, anti-Ro(SSA), anti-La(SSB), anti-sn/RNP, anti-Tg, and anti-TPO production are likely genetically complex traits, however, we did not find a significant genetic component for any of these antibodies in our twin population.

The differences between findings in the literature and the present study may be due to the limited number of discordant twin pairs and/or the small number subjects with higher levels of auto-antibodies. This study was limited primarily by the rarity of elevated auto-antibody levels in the study population, which resulted in two specific complications. The first was that the distributions for the antibody values tended to be skewed rather than bimodal. Since there were relatively few subjects with elevated values, the distribution

presented as normal with a few outliers, skewing the data to the maximum. If there had been more subjects with higher antibody values, the outliers would likely have created a second normal distribution. The options to examine data that exhibited a skewed distribution include categorizing, excluding outliers, adjustment (i.e. logarithmic or exponential), or ranking. We chose to rank the antibody values so that we would maintain the full distribution of the data but minimize the range and variance.

The second complication that arises from a lack of subjects with elevated antibody values is the inherent lack of statistical power. The lack of power probably led to the inability to detect the presence of genetic components of several of the antibodies. This is even more likely for the antibodies which had strong literature support for a genetic component.

Several of the studies reported in the literature examined linkage and associated antibodies with certain regions of chromosomes. There is a possible explanation of the discordant findings between the linkage studies and our results. The linkage studies examined a diseased population (i.e. SLE) with a potentially rare genetic variation that did not occur in our population. Without this genetic variation, it may be possible to lose the genetic component of the autoantibody expression.

Another point to consider is the entitiy being measured. The autoantibody assays evaluate the presence of autoantibodies, not the likelihood of expressing these antibodies. A genetic component may predispose an individual to create an auto-antibody, however, unless we are dealing with a purely genetic effect, expression of these antibodies may not occur in the absence of an environmental trigger. Since these samples were taken from

twins at one point in time, we are limited to the environmental effects present at that moment. It is possible that different phenotypic expressions of antibodies may occur at various points in time.

A second measurement issue relates to the precision, sensitivity, and specificity of the ELISA assays used to quantify the autoantibody values. The majority of antibody values are near zero, leading to the potential of false positives. Tan et al, examined the precision, sensitivity, and specificities of several commercial enzyme based immunoassays for the detection of anti-nuclear auto antibodies[54]. They found the most evident lack of sensitivity and specificity in the anti-dsDNA and anti-Sm kits. Anti-Ro(SSA), anti-RoSSB, anti-SCL-70, anti-Jo-1 kits generally perform well. Several false positives were observed in sera containing multiple myeloma cryoprecipitates. The replicate precision had a wide variability between manufacturers. The results of this precision, sensitivity, and specificity analysis suggests a possible source of variability in the auto-antibody values and, hence, the entire heritability analysis.

CHAPTER 5 CONCLUSION

This study aimed to determine what portion of specific autoantibody phenotypes are genetically determined by using a twin model. This study specifically examined Anti-Ro(SSA) antibodies, Anti-La (SSB) antibodies, Anti-Sn/RNP, Anti-Sm antibodies, Anti-Jo-1 antibody, Anti-Scl-70 antibody, Anti-Tg & Anti-TPO antibodies, Anti-dsDNA antibody, Anti-PS antibodies, and Anti-cardiolipin antibodies for their heritability.

Several antibodies demonstrated a genetic component in our study population. Anti-cardiolipin had a genetic component with an estimated 69% heritability. Heritability estimates were 55-62% for anti-dsDNA, 41-51% for Anti-Jo-1, 42-59% for anti-SCL-70, and 52-54% for anti-PL.

Several antibodies did not have a measurable genetic component. These included anti-Sm, anti-Ro(SSA), anti-La(SSB), anti-sn/RNP, anti-Tg, and anti-TPO. Possibilities for the lack of a measureable genetic component include the limited number of discordant twin pairs, and/or the small number subjects with higher levels of antibodies.

The results of this study suggest several clinically relevant markers of auto-immunity may be partially genetically determined. These include: anti-cardiolipin, anti-dsDNA, anti-Jo-1, anti-SCL-70, and anti-PL. These findings provide a basis for understanding the mechanisms involved in the various autoimmune diseases associated

with these autoantibodies. Additionally, the presence of a genetic component provides a new paradigm for possible therapeutics for autoimmune diseases.

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VITA

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