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Preferential Transmission of Genetic Risk Variants of Candidate Loci at 6p21 from Asymptomatic Grandparents to Mothers of Children with Neonatal Lupus

Amit Saxena[1], **Erin McDonnell**[1], **Paula S. Ramos**[2], **Satria Sajuthi**[2], **Miranda C. Marion**[2], **Carl D. Langefeld**[2], **Jill P. Buyon**[1], and **Robert M. Clancy**[1]

[1]Department of Medicine, New York University School of Medicine, Division of Public Health Sciences, Wake Forest School of Medicine

[2]Center for Public Health Genomics and Department of Biostatistical Sciences, Division of Public Health Sciences, Wake Forest School of Medicine

Abstract

Background—Neonatal lupus (NL) occurs in fetuses exposed to maternal anti-SSA/Ro and/or SSB/La antibodies, although the mothers may not manifest any clinical disease. A focus on transmission of risk factors for NL from maternal grandparents to mothers may yield dividends towards understanding the aggregation of autoantibodies and genetic factors in families.

Methods—51 mothers of children with cardiac and/or cutaneous NL, 48 maternal grandmothers and 35 maternal grandfathers in the Research Registry for Neonatal Lupus were interrogated for clinical symptoms (questionnaire) and laboratory assessments including anti-SSA/Ro and SSB/La antibodies (ELISA) and genotype at rs1800629 (*TNF*α-308) and rs7775397 (*C6orf10*) (allelic discrimination). The transmission disequilibrium test (TDT) was computed to test for nonrandom transmission from maternal grandparents to the NL mothers.

Results—The phenotypic feature held in common in NL mothers was the autoantibody, and not the clinical profile; 7 had lupus, 14 had Sjogren's syndrome, 7 had both, and 23 were asymptomatic. NL mothers were significantly enriched in variant allelic frequencies at both *TNFα-308* and *C6orf10*. The grandparents of NL children carried minimal burden for autoimmune disease or abnormal antibody production and were not enriched in the genetic risk factors. However, the TDT analysis showed significant excess transmission of the risk alleles at both *TNFα-308* (P=3.93×10^{-4}, OR=6.67) and *C6orf10* (P=3.74×10^{-5}, OR=35) to NL mothers.

Conclusion—NL mothers are enriched for the *TNFα-308* and *C6orf10* variant alleles, which are preferentially inherited from the asymptomatic maternal grandparents. These findings support the hypothesis that the development of NL and genetic etiology are multigenerational.

Introduction

Neonatal Lupus (NL) is a model of passively acquired autoimmunity linked to the transplacental passage of anti-SSA/Ro and anti-SSB/La antibodies from the maternal to the fetal circulation (1). Research to date has focused on the affected children and their manifestations of the syndrome, including transient abnormalities of the skin, liver and blood elements, as well as irreversible damage to the heart. The cardiac manifestations of NL (cardiac NL) most often include advanced heart block without structural abnormalities,

Corresponding author: Amit Saxena, MD, Department of Medicine, NYU School of Medicine, Mail Code T 4-407, 560 First Avenue, New York, NY 10016, 212 263 0743 (office), 212 263 0759 (Fax).

and more rarely an isolated cardiomyopathy. The reported mortality rate of children with cardiac NL has ranged from 11 to 29%, and the need for permanent pacemaker placement from 63 to 93% in several studies (2–7).

Despite the strong association between maternal autoantibodies and cardiac and cutaneous NL, the penetrance of disease in an anti-SSA/Ro positive mother who is either primigravida or has had healthy children is roughly 2% (1). These data strongly imply that the presence of the autoantibodies, although necessary, is not sufficient to cause disease in the fetus (8, 9) and that maternal, fetal, and environmental components contribute to full expression. The relationship of the autoimmune phenotype and genetic background in multiple generations of cardiac and cutaneous NL affected families has not been previously studied. Based on the consistent finding of an inflammatory cellular infiltrate in the hearts of fetuses dying shortly following the detection of cardiac NL, a variant in the *TNF*α promoter (rs1800629) in which a substitution of guanine (G) to adenine (A) at position 308 associates with high cytokine production (10, 11) was considered a potential candidate for risk of disease. In a study of 40 children with cardiac NL, 17 with cutaneous NL, 31 unaffected siblings, and their 74 mothers, the frequency of the A allele at rs1800629 was greatest in the mothers and affected siblings, although the frequency was significantly increased in all family members compared to population controls (12). Further evidence supporting a focus on *TNF*α is that higher serum levels have been demonstrated in Caucasians with the HLA-DR3 allele group, which is frequently enriched in mothers of children with cardiac NL (14). The latter is expected since HLA-DR3 is strongly associated with anti-SSA/Ro and SSB/La antibodies (13–15). There is also an association of disease with several HLA SNPs, including rs7775397, a missense variant within the *C6orf10* gene, which codes for an uncharacterized protein and lies between *NOTCH4* and *butyrophilin-like protein 2* (*BTNL2*) in the Class III – Class II boundary (16). In a recent genome wide association study (GWAS) of Caucasian children, the G allele at rs7775397 was significantly associated with cardiac NL (17).

Although mothers of children with NL are often themselves asymptomatic, their autoantibodies are required for the development of fetal/neonatal disease. In this regard, NL mothers have an autoimmune phenotype, regardless of any clinical manifestations of rheumatic disease. Accordingly, this study was initiated to determine the role of NL maternal grandparents in the development of the autoimmune phenotype of NL mothers. The clinical, serologic, and genetic status of maternal grandparents and NL mothers were studied, as well as the transmission pattern of the candidate SNPs associated with NL. The maternal ancestral transmission of these risk variants have never been studied for NL and may provide important information for understanding the aggregation of autoantibodies and clinical disease in NL families.

Methods

Study group

Given the unclear pathogenesis, clinical importance, and absence of effective or prophylactic treatments in NL, the Research Registry for Neonatal Lupus (RRNL) was established in 1994 to provide a source of well documented cases, inclusive of mothers of NL children and their entire families (4). The RRNL served as the source of patients for the current study. Details of the RRNL have been described elsewhere (4). Briefly, a mother is enrolled in the RRNL if her child has any manifestation of NL (heart block, cardiomyopathy, characteristic skin rash, or hematologic or hepatic abnormalities) and if the mother herself has anti-SSA/Ro and/or anti-SSB/La antibodies, irrespective of her clinical status. Cardiac NL is documented by *in utero* echocardiogram or postnatal electrocardiogram, echocardiogram, cardiac biopsy, or pacemaker placement. Cutaneous NL is documented by a photograph demonstrating the characteristic annular lesions, a clear

description of the rash in the child's medical records, and/or findings on skin biopsy. In addition, immediate and extended family members of the affected child are offered enrollment in the RRNL, including the maternal grandparents.

A family was included in the study if the following criteria were met: 1) The mother of an NL affected child was enrolled in the RRNL and 2) Clinical data, blood samples and/or saliva samples were available from one or both maternal grandparents. Clinical diagnosis was ascertained and assigned as previously described (4). Subjects were classified as having systemic lupus erythematosus (SLE) if they met at least 4 of the American College of Rheumatology criteria (18). Subjects were categorized as having Sjögren's syndrome (SS) if they had anti-SSA/Ro and/or SSB/La antibodies, as well as 1) both dry eyes and dry mouth, or 2) either dry eyes or dry mouth along with objective documentation of salivary or lacrimal gland hypofunction or lymphocytic infiltration of these glands. An undifferentiated autoimmune syndrome (UAS) was diagnosed in subjects with features of a rheumatic disease who did not meet the criteria for SLE or SS. Asymptomatic subjects were those who had no clinical evidence of any rheumatic illness.

Questionnaire

In order to evaluate the health status of the mothers and maternal grandparents of NL affected children, subjects that consented to enrollment in the RRNL were sent a questionnaire consisting of 41 items that specifically focused on criteria and symptoms of rheumatic and autoimmune diseases.

The first group of questions addressed symptoms characteristic of autoimmune diseases, mainly SS and SLE: sicca symptoms, hair loss, skin rash, photosensitivity, arthralgias, arthritis, morning stiffness, oral ulcers, nodules, Raynaud's syndrome, pleuritis, pericarditis, renal disease, or central nervous system involvement (seizure, stroke). The next group of questions focused on a history of laboratory data: abnormal findings on urinalysis, low white blood cell count, and low platelet count. The final group of questions specifically inquired about any previous diagnosis of autoimmune diseases: SLE, SS, rheumatoid arthritis, ankylosing spondylitis, scleroderma, polymyositis, dermatomyositis, inflammatory bowel disease, diabetes, primary biliary cirrhosis, or thyroid disease. Information on past and present medications was also obtained. All positive answers were followed up by requesting and reviewing medical records from the subjects' physicians and/or telephone interviews with the patient.

Detection of anti-SSA/Ro and SSB/La antibodies

A standard protocol to determine reactivity of human sera against 60kD SSA/Ro, 52kD SSA/Ro and 48kD SSB/La recombinant proteins was used, as previously described (19, 20). Briefly, nickel column affinity-purified recombinant proteins were diluted in phosphate buffered saline (PBS) to a final concentration of 10 ug/ml, a condition that is optimal for absorption of protein to Immulon 2 microtiter plates (Dynatech, Alexandria, VA). Sequential additions included blocker (gelatin, 0.1% (Sigma)), human sera (1:1,000), alkaline-phosphatase–conjugated goat anti-human IgG (Sigma, 1:3,000) and detection reagent. Each sample was analyzed in triplicate. The cutoff value designating a positive reaction was three standard deviations above the mean optical density (OD) in five normal sera. All samples were confirmed for anti 60kD SSA/Ro and SSB/La antibodies by commercial ELISA from the NYU-Hospital for Joint Diseases immunology laboratory.

DNA isolation and Allelic Discrimination Assay

DNA was isolated from whole blood and from saliva using the manufacturer's guidelines of Qiagen (QIAamp DNA Blood Mini Kit, Valencia, CA) and Oragene kits, respectively. One

hundred nanograms of DNA (in duplicate) was incubated with TaqM genotyping master mix (#4371355, Applied Biosystems) and the 1× probes for *TNFα-308* rs1800629 (#C__7514879, Applied Biosystems) and *C6orf10* rs7775397 (#C__25747007, Applied Biosystems). Standard PCR was performed and assignments in genotyping made based on a post read of the amplified genomic patient DNA. The allele calling rate of 111 DNA samples was 98% for rs7775397 and 97% for rs1800629. For verification of the assignments, PCR amplification and direct sequencing were performed as previously described (12). Briefly, for amplification of genomic DNA to genotype the *TNFα-308* region, the probes were: 5'-AGGCAATAGGTTTTGAGGGCCAT-3' (F-169) and 5'-ACACTCCCCATCCTCCCTGCTC-3'(R-285) and to genotype the *C6orf10* region forward primer, 5'TGGACCTCTTGTTCCTTTGG and reverse primer, 5'-AGATGGGTGTGCCAAGAAGA-3'. PCR products were purified and directly sequenced (commercial service).

Genotyping and Statistical Analysis

Both rs1800629 (*TNFα-308A*) and rs7775397 (*C6orf10*) were tested for Mendelian errors and departure from Hardy-Weinberg Equilibrium expectations. To test for differential transmission of alleles (i.e., linkage in the presence of association) from the grandparents to the NL mother, a transmission/disequilibrium test (TDT) was computed (21). Odds ratios and 95% confidence intervals were computed by adding 0.5 to each cell given complete separation (i.e., to prevent dividing by zero). Given that some of the trios were incomplete (i.e., grandmother or grandfather not present), missing data were accounted for via the likelihood method implemented in the program UNPHASED (22). To test for genotypic differences between the NL mother and maternal grandparents, we computed a chi-squared test of symmetry for matched data. The risk alleles are A for rs1800629 and G for rs7775397.

Results

Patient demographics, clinical assessment and maternal autoantibody status

Fifty-one families met the inclusion criteria of having a RRNL enrolled mother and data on one or both of the NL child's maternal grandparents (Table 1). In 42 families at least one child had cardiac NL and in 9 families the affected child had cutaneous NL. The demographic characteristics, clinical assessment and antibody status are shown in Tables 1 and 2. The mean age at enrollment in the RRNL was 32.8 for NL mothers, 63.2 for grandmothers and 66.5 for grandfathers. Most of the families were Caucasian. There were significant differences between the mothers and maternal grandparents regarding clinical assessment ($P<0.0001$) and antibody status ($P<0.0001$). Specifically, 28 (55%) of the mothers had either SLE and/or SS, while the remainder were asymptomatic or had UAS. In contrast, the majority of grandparents were asymptomatic. Only 4 (8%) of the grandmothers were diagnosed with a rheumatic disease, as follows: 1 with SS, 1 with SLE, 1 with ankylosing spondylitis, and 1 with rheumatoid arthritis. Two (6%) of the grandfathers had a diagnosed rheumatic disease, 1 with SLE and 1 SLE and SS.

Reactivity to SSA/Ro and SSB/La antigens was evaluated in the sera of 51 mothers, 41 grandmothers and 27 grandfathers (Table 2). The general absence of anti-SSA/Ro and SSB/La in the grandparents paralleled their overall asymptomatic clinical status. In contrast to the uniform presence of autoantibodies in the mothers, only 2 (5%) of grandmothers and 1 (4%) of grandfathers were positive for antibodies to any of the SSA/Ro (60kD or 52kD) or SSB/La (48 kD) ribonucleoproteins.

*TNF*α-*308* promoter and *C6orf10* risk allele frequencies in NL mothers and maternal grandparents

The frequencies of the *TNF*α-*308* risk variant (rs1800629, G > A allele) and the *C6orf10* risk variant (rs7775397, T > G allele) are presented in Table 3. There was significant linkage disequilibrium ($r^2 = 0.58$) between rs1800629 (*TNF*α-*308*) and rs7775397 (*C6orf10)* despite the 800kb physical distance between these SNPs. The allele frequency of *TNF*α-*308*A was 38% in the NL mothers and 21% in maternal grandmothers (*P*=0.02); frequency in the CEU samples from the HapMap was 22%, similar to the maternal grandmothers (23). The allele frequency of *TNF*α-*308*A in the grandfathers was lower, but not statistically different from that of the NL mothers (32% vs. 38%, *P* = 0.59). The allele frequency of the *C6orf10* variant in the NL mothers was 32% and 12% in the maternal grandmothers (*P*=0.0065); the maternal grandmothers and CEU HapMap samples had comparable frequencies (12% vs. 9%) (23). In parallel with the results for *TNF*α-*308*, the frequency of the *C6orf10* variant in the maternal grandfather was lower, but was not statistically different from the NL mother (23% vs. 32%, respectively, *P*=0.35). Although there was a trend toward higher median titers of anti-SSA/Ro antibodies in the NL mothers carrying the variant allele at rs7775397 (*C6orf10*) than those without the variant, these results did not reach statistical significance (15,360 +/− 45,594 vs. 9,984 +/− 13,590, respectively, p = 0.21, Mann-Whitney). Higher anti-SSA/Ro titers were not present in the mothers with the allele variant at rs1800629 (*TNF*α-*308*) (11,264 +/− 43,526 vs. 12,160 +/− 14,419, respectively, p = 0.85, Mann-Whitney).

Preferential transmission of risk alleles

There was strong evidence of excess transmission of the risk alleles from grandparents to the NL child's mother at both loci (Table 4 and 5). The TDT analysis of the *TNF*α promoter SNP rs1800629 (*TNF*α-308) showed extreme preferential transmission of the A allele in all complete trios (OR=6.67, $P=3.93\times10^{-4}$), all complete trios with cardiac NL in the child (OR=8.00, $P=9.67\times10^{-4}$) and all Caucasian complete trios with cardiac NL in the child (OR=8.00, $P=9.67\times10^{-4}$). Unbiasedly inferring missing grandparental genotypes allowed an additional 17 pedigrees to be considered and yielded consistent and trivially more significant results (Table 4). There was even stronger evidence of excess transmission of the risk allele from the grandparents to the mother at the *C6orf10* SNP rs7775397. In every case the G allele was transmitted. This pattern was again consistent across the groups: all complete trios (OR=35.0, $P=3.74\times10^{5}$), all complete trios in which the child had cardiac NL (OR=31.0, $P=1.08\times10^{-4}$) and all Caucasian complete trios with cardiac NL in the child (OR=31.0, $P=1.08\times10^{-4}$). Increasing the statistical power by including the 17 incomplete pedigrees and unbiasedly inferring the missing grandparental genotypes (and excluding the patterns that lead to bias), increased the statistical significance to $P=1.42\times10^{-7}$ (Table 5). A formal parent of origin analysis found no evidence of significant parent of origin effects for either of the two variants (Supplemental Tables 1–6).

Discussion

This study represents a novel approach to assessment of the transmission of clinical and genetic risk factors to NL mothers, a group of subjects with a unique autoimmune phenotype that does not necessarily manifest in clinical disease. To our knowledge this is the first attempt to evaluate the upstream role of the grandparents in genetically influencing the phenotype in a passively acquired autoimmune disease. A family study of anti-SSA/Ro and SSB/La antibodies, associated clinical conditions, and the identification of autoimmune susceptibility genes in NL mothers and their parents was utilized to identify the familial aggregation of risk factors for this disease. Neither the clinical symptoms of rheumatic illness nor anti-SSA/Ro and SSB/La antibodies, which were present in the NL mothers,

occurred with increased frequency in the maternal grandparents of NL affected children. There was an increased frequency of the two candidate genetic variants in the NL mothers compared to HAPMAP controls. These frequencies were not observed in the maternal grandmothers, and were significantly different from the NL mothers. Allelic frequencies of the risk variants were more similar between the maternal grandfathers and the NL mothers. Although these data may suggest a differential pattern between grandmother and grandfather, a formal analysis found no evidence of significant parent of origin effects. The clustering of genetic variants was related to a preferential skewing of inheritance from grandparents to the NL mother. These results, based on a two-generational resource of available clinical, laboratory and genetic assessments, imply that mothers accumulate environmental determinants specific to NL, which are not present in the maternal grandparents.

While the majority of the NL mothers were diagnosed with SLE, SS, or a combination of the two diseases, the maternal NL grandparents were almost uniformly free of these conditions. These findings are consistent with unpublished literature regarding first degree relatives of mothers enrolled in the RRNL. Based on a review of enrollment questionnaires completed by 386 mothers, rheumatologic disease was only reported in 5% of the NL grandparents. In a multiplex study of 119 SLE patients, 8% had a first degree relative with SLE (24). A case control study of 876 SS patients revealed that 7.3% had a first degree relative with an autoimmune disease, vs. 3.9% in matched controls (25). Within the pedigrees reported herein, no transmission pattern of clinical disease was apparent as three of the six maternal symptomatic grandparents were related to an NL mother who herself did not meet criteria for either SS or SLE. This observation reinforces the widely held experience that the phenotypic feature held in common in NL mothers is the autoantibody, and not the clinical profile. Moreover, NL mothers are often asymptomatic at the time of detection of the NL child. The clinical data demonstrate that maternal grandparents of NL affected children are not at any greater likelihood of developing a rheumatic illness than would be expected in relatives of patients with other autoimmune diseases.

In addition to the assessment of clinical phenotype, serological evaluation of anti-SSA/Ro and SSB/La autoantibodies may identify a population with candidate factors that transition risk into clinical disease (26). By definition, 100% of the NL mothers had anti-SSA/Ro and/or SSB/La autoantibodies. Only 5% of the maternal grandmothers and 4% of the maternal grandfathers had these antibodies. In contrast, it has been reported that 59% of relatives of patients with antiphospholipid syndrome (APS) generate antibodies to B2-Glycoprotein 1 (27) and 21% of first degree relatives of patients with SS or SLE with anti-SSA/Ro positivity also generate the same antibody responses (28). Thus, the general absence of anti-SSA/Ro and anti-SSA/La antibodies in the NL grandparents is an observation which may distinguish the familial aggregation of NL from other autoimmune diseases (SLE, SS and APS) in which the frequency of similar antibody profiles is relatively higher in blood relatives.

Genome wide association studies have identified several genetic variants contributing to the risk of developing SLE and other autoimmune diseases (29, 30). The initial genetic approach to NL exploited a candidate strategy (12, 17, 31, 32). A focus on variation at the MHC was a logical choice for these early studies. This region spans 7.05 Mb and contains risk alleles for inflammation and certain autoimmune diseases residing within an extended HLA-A1;B8;DR3 haplotype block (33). The earlier described association with a high producing variant of the *TNFα* gene was essentially confirmed and extended in the GWAS focused only on the children with cardiac NL. In consideration of the clues generated by the identification of candidate genes and significant associations by a genome wide assessment, the current study approached the maternal and grandparental contribution to the disease.

Two variants, at *TNFα-308* rs1800629 and *C6Orf10* rs777539, were found to be significantly more frequent in the NL mothers compared to HAPMAP controls, mirroring the associations observed with these variants in the fetus (12,17). These findings highlight a putative "double hit," one in the mother and one in her NL affected offspring. The HLA portion of the extended haplotype DQB1*02; DRB1*03 provides the genetic predisposition for the generation of the candidate autoantibodies, which cross the placenta and bind apoptotic fetal cardiocytes and neonatal keratinocytes, thus initiating an inflammatory response. The *TNFα* portion of the extended haplotype in the children may further contribute to tissue injury by amplifying the inflammatory cascade, a notion supported by the limited in situ hybridization of TNFα transcripts (12) and immunohistochemistry (31). Of the six grandparents with a rheumatic disease, five were genotyped. The grandmothers with ankylosing spondylitis and SLE (both negative for SSA/Ro antibodies) did not have the variants at either candidate locus. However, the grandmother with rheumatoid arthritis (no SSA/Ro antibodies) and both grandfathers (one with SLE and no anti-SSA/Ro and one who had SS/SLE with SSA/Ro antibodies)) were positive for both variants. Based on TDT, there was an overall highly significant preferential transmission of the risk alleles from grandparents to the NL mothers. These findings remained significant when limited to the study groups involved in the previous genetic evaluations: families with a cardiac NL affected child, as well as only Caucasian families with a cardiac NL child. Further subject enrollment and study will be needed to evaluate the risk allele transmission from grandparents to anti-SSA/Ro and SSB/La negative maternal aunts of the NL children.

This analysis is limited in power due to the small number of patients available for clinical, serologic, and genetic studies. However, the TDT and PDT analyses on these samples were strongly statistically significant despite the number of trios tested because of the magnitude of the effect associated with these HLA-region SNPs. Given the rarity of this disease and the challenges in collecting samples, the strength of the study is that it is the largest and most thoroughly documented cohort of maternal grandparents of NL children, the majority of whom have the signature cardiac manifestation associated with maternal anti-SSA/Ro antibodies, advanced heart block. Given the findings in a military population that autoantibodies identify a vulnerable population of asymptomatic subjects who develop into full blown clinical disease (26), another limitation is that the current study is a cross-sectional analysis of clinical and serological data. However, the advanced mean age of the grandparents would presumably have allowed enough time to develop antibodies and disease, and the mothers are within the ages most often susceptible for SLE (albeit not SS). In addition, it is a limitation that clinical data were based on self reported symptoms, however most cases were confirmed by review of medical records. These data cannot distinguish whether transmission of genetic risk variants at 6p21 loci are directly contributory to the pathogenesis of NL or solely restricted to the generation of the maternal autoantibody response. While testing of mothers with high titer anti-SSA/Ro antibodies that had never given birth to an NL child would be a reasonable approach, this analysis would need to be restricted to mothers past childbearing age since we have observed families in the RRNL in whom an NL child was born after 5 healthy children. Even more challenging would be to study both living grandparents if the analysis were restricted to women who had completed child bearing years. Finally, only two genetic markers were studied and we cannot rule out that the two markers are merely in linkage disequilibrium with the true causal genetic factors. Further studies are necessary to confirm the association and fine map the associated region in additional samples and ethnicities. Ultimately, functional work will be needed to identify the contribution of these variants to the fetal pathology.

This is the first study to describe familial aggregation in NL through identification of clinical disease, autoantibody profiles and genetic variation among mothers of affected children and the NL maternal grandparents. These data demonstrate that grandparents of NL

children are at no higher risk for autoimmune disease or abnormal antibody production than would be expected for any relative of a patient with a rheumatic disease. The grandmothers are not enriched in the genetic risk factors associated with the disease, which along with their lack of autoantibodies did not allow for an environment conducive for development of NL in the mother herself. The significant transference of variant risk alleles from grandparents to mothers merits more in depth future study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Patient demographics and clinical assessment

	Mother (N=51)	Grandmother (N=48)	Grandfather (N=35)
Age at RRNL Enrollment, Mean (SD)	32.8 (5.5)	63.2 (7.5)	66.5 (8.5)
Race/ethnicity			
Caucasian	47 (92%)	44 (92%)	33 (94%)
Black	3 (6%)	3 (6%)	1 (3%)
Hispanic	1 (2%)	1 (2%)	1 (3%)
Asian	0 (0%)	0 (0%)	0 (0%)
Other/NA	0 (0%)	0 (0%)	0 (0%)
Affected NL child			
Cardiac-NL	42 (82%)	39 (81%)	30 (86%)
Rash-NL	9 (18%)	9 (19%)	5 (14%)
Clinical Assessment			
Asym/UAS	23 (45%)	44 (92%)	33 (94%)
SS	14 (27%)	1 (2%)	0 (0%)
SLE	7 (14%)	1 (2%)	1 (3%)
SS/SLE	7 (14%)	0 (0%)	1 (3%)
Other	0 (0%)	2 (4%)*	0 (0%)

SD = standard deviation

Asym = asymptomatic

UAS = undifferentiated autoimmune syndrome

SS = Sjogren's syndrome

SLE = systemic lupus erythematosus

* 1 GM with ankylosing spondylitis; 1 GM with rheumatoid arthritis

Table 2

Autoantibody Status

	Mother (N=51)	Grandmother (N=41)	Grandfather (N=27)
Autoantibody positive	51 (100%)	2 (5%)	1 (4%)
Anti-Ro60+/Ro52+/La+	36 (70%)	1 (2.5%)	0 (0%)
Anti-Ro60+/Ro52+/La−	5 (10%)	0 (0%)	1 (4%)
Anti-Ro60+/Ro52−/La+	9 (18%)	0 (0%)	0 (0%)
Anti-Ro60+/Ro52−/La−	1 (2%)	0 (0%)	0 (0%)
Anti-Ro60−/Ro52+/La−	0 (0%)	1 (2.5%)	0 (0%)
Anti-Ro60−/Ro52−/La+	0 (0%)	0 (0%)	0 (0%)
Autoantibody negative	0 (0%)	39 (95%)	26 (96%)

Table 3

Frequency of risk alleles in families with neonatal lupus

	Risk Allele (%)	Comparison with Mother (*P*-value)
***TNFα* at rs1800629 (risk allele A)**		
Mothers (Proband) (n=41)	38	-
Maternal grandmother (n=38)	21	0.02
Maternal grandfather (n=29)	32	0.59
***C6orf10* at rs7775397 (risk allele G)**		
Mothers (Proband) (n=41)	32	-
Maternal grandmother (n=36)	12	0.0065
Maternal grandfather (n=32)	23	0.35

Table 4

Transmission of risk allele (A) for *TNFα-308* promoter SNP (rs1800629) in neonatal lupus (NL) trio cohort

Transmission Disequilibrium Test												
	Mothers with NL Child				**Mothers with Cardiac NL Child**				**Caucasian Mothers with Cardiac NL Child**			
		Nontransmitted				**Nontransmitted**				**Nontransmitted**		
	Allele	**A**	**G**		**Allele**	**A**	**G**		**Allele**	**A**	**G**	
Transmitted	A	3	20	23	A	3	16	19	A	3	16	19
	G	3	24	27	G	2	21	23	G	2	21	23
		6	44	50		5	35	42		5	35	42
	OR=6.67 CI=(1.98–35.03)				OR=8.00 CI=(1.88–71.72)				OR=8.00 CI=(1.88–71.72)			
	$P_{TDT}=3.93\times10^{-4}$				$P_{TDT}=9.67\times10^{-4}$				$P_{TDT}=9.67\times10^{-4}$			
Pedigree Disequilibrium Test												
	Mothers with NL Child				Mothers with Cardiac NL Child				Caucasian Mothers with Cardiac NL Child			
	Allele	Transmitted	Nontransmitted		Allele	Transmitted	Nontransmitted		Allele	Transmitted	Nontransmitted	
	A	31	9.0		A	25	7.4		A	24	7.3	
	G	51	72.0		G	43	60.6		G	40	56.7	
		$P_{Marginal}=1.52\times10^{-4}$				$P_{Marginal}=5.44\times10^{-4}$				$P_{Marginal}=7.52\times10^{-4}$		
		Number of Complete trios		25	Number of Complete trios			21	Number of Complete trios			21
		Number of Pedigrees		43	Number of Pedigrees			35	Number of Pedigrees			32

Table 5

Transmission of risk allele (G) for *C6orf10* SNP (rs7775397) in neonatal lupus (NL) trio cohort

Transmission Disequilibrium Test												
	Mothers with NL Child				**Mothers with Cardiac NL Child**				**Caucasian Mothers with Cardiac NL Child**			
		Nontransmitted				**Nontransmitted**				**Nontransmitted**		
	Allele	**G**	**T**		**Allele**	**G**	**T**		**Allele**	**G**	**T**	**16**
Transmitted	G	1	17	18	G	1	15	16	G	1	15	28
	T	0	34	34	T	0	28	28	T	0	28	44
		1	51	52		1	43	44		1	43	
	OR=35.0 CI=(4.12–infinity)				OR=31.0 CI=(3.59–infinity)				OR=31.0 CI=(3.59–infinity)			
	$P_{TDT}=3.74\times10^{-5}$				$P_{TDT}=1.08\times10^{-4}$				$P_{TDT}=1.08\times10^{-4}$			
Pedigree Disequilibrium Test												
	Mothers with NL Child				Mothers with Cardiac NL Child				Caucasian Mothers with Cardiac NL			
	Allele	Transmitted	Nontransmitted		Allele	Transmitted	Nontransmitted		Allele	Transmitted	Nontransmitted	
	G	26	1.4		G	22	1.3		G	21	1.2	
	T	56	80.6		T	46	66.7		T	43	62.8	
		$P_{Marginal}=1.42\times10^{-7}$				$P_{Marginal}=1.35\times10^{-6}$				$P_{Marginal}=2.11\times10^{-6}$		
		Number of Complete trios		26	Number of Complete trios			22	Number of Complete trios			22
		Number of Pedigrees		43	Number of Pedigrees			35	Number of Pedigrees			32