PREFERENTIAL TRANSMISSION OF GENETIC RISK VARIANTS OF CANDIDATE LOCI AT 6P21 FROM ASYMPTOMATIC GRANDPARENTS TO MOTHERS OF CHILDREN WITH NEONATAL LUPUS

A Saxena, E McDonnell, PS Ramos, SP Sajuthi, MC Marion, CD Langefeld, JP Buyon, RM Clancy

NYU Medical Center

NEONATAL LUPUS (NL): PATHOLOGIC READOUT OF PASSIVELY ACQUIRED AUTOIMMUNITY

ENHANCED FETAL CIRCULATION

CLINICAL FEATURES

• Birth 6 wks, UV-provoked
• Resembles adult SCLE
• Transient, rare scarring
• Risk 7-15%


Maternal/Fetal genetic and environmental components contribute to NL

FAMILIAL AGGREGATION IN RHEUMATIC DISEASES

• No formal studies of the genetic and environmental interplay in NL families

Rheumatic Disease Aggregation in Family Studies

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Degree of Relative</th>
<th>Antigen(s)</th>
<th>Rheumatic Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL Mdm (n=386)</td>
<td>First</td>
<td>Anti-Ro</td>
<td>10%</td>
</tr>
<tr>
<td>SLE (n=1214)</td>
<td>First</td>
<td>Anti-Ro</td>
<td>10%</td>
</tr>
<tr>
<td>SS (n=876)</td>
<td>First</td>
<td>Anti-Ro</td>
<td>7%</td>
</tr>
</tbody>
</table>

Autoantibody Aggregation in Family Studies

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Degree of Relative</th>
<th>Antibody Tested</th>
<th>%Antibody Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>APLA (A) (n=44)</td>
<td>First</td>
<td>Anti-β2GPI</td>
<td>59%</td>
</tr>
<tr>
<td>RA (n=354)</td>
<td>First</td>
<td>Anti-CCP</td>
<td>39%</td>
</tr>
<tr>
<td>Antiphospholipid Syndrome (n=93)</td>
<td>Unselected</td>
<td>Anti-β2GPI</td>
<td>99%</td>
</tr>
</tbody>
</table>

THE ROLE OF VARIATIONS AT CANDIDATE GENES IN NEONATAL LUPUS

• TNFα promoter 308 (rs 1800629) variant A allele
  – Associates with high cytokine production
  – Frequency greatest in NL Mothers and affected children compared to unaffected siblings
  – Higher TNFα levels found in Caucasians with HLA-DR3

• C6orf10 (rs 7775397) variant G allele
  – Significantly associated with Cardiac NL in GWAS of Caucasian children
  – Codes for an uncharacterized protein
  – Lies in the HLA Class III – Class II boundary

FAMILIAL AGGREGATION IN NEONATAL LUPUS

• What is the relationship between genetic background and autoimmune phenotype in multiple generations of NL affected families?

• What is the role of Grandparents in the development of the autoimmune phenotype of NL Mothers?

DISCLOSURES

• Amit Saxena: None
• Erin McDonnell: None
• Paula S. Ramos: None
• Satria P. Sajuthi: None
• Miranda C. Marion: None
• Carl D. Langefeld: None
• Jill P. Buyon: None
• Robert M. Clancy: None
Subjects and Study Questions

Subjects 

- Grandmother (N=48) 
- Grandfather (N=35) 
- Mother (N=51) 
- Father (N=2) 

Relation to NL Child

- N=1 (2%) 
- N=0 (0%) 
- N=0 (0%) 

Rheumatic Disease?

- 53% SS and/or SLE 
- 100% anti-Ro/La positive 

Genetic Frequency and transmission of risk alleles?

- Risk allele frequency 

Methods

- Questionnaire 
  - 41 items focused on clinical diagnosis of rheumatic disease 
  - Positive responses followed with medical record review 

- Serology 
  - Standard protocol of human sera against 60kD SSA/Ro, 52kD SSA/Ro and 48kD SSB/La 
  - Commercial ELISA for SSA/Ro and SSB/La 

- DNA Isolation and Allelic Discrimination 
  - DNA isolated from whole blood and saliva using commercially available kits 
  - Standard PCR and genotype assignments based on post-read of amplified genomic DNA 

Statistical Analysis

- Chi squared test of symmetry for matched data 
  - Comparing genotypic differences between NL Mother and Grandparents 

- Transmission Disequilibrium Test (TDT) 
  - Tests for differential transmission of alleles from Grandparents to NL Mother 

- Pedigree Disequilibrium Test (PDT) 
  - Accounts for missing data if one Grandparent is not available 

Grandparents of NL Affected Children Are Asymptomatic

<table>
<thead>
<tr>
<th></th>
<th>Mother (N=51)</th>
<th>Grandmother (N=41)</th>
<th>Grandfather (N=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at RRNL Enrollment, Mean (SD)</td>
<td>32.8 (5.5)</td>
<td>40.2 (7.1)</td>
<td>45.4 (8.8)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>47 (92%)</td>
<td>44 (92%)</td>
<td>33 (94%)</td>
</tr>
<tr>
<td>Black</td>
<td>3 (6%)</td>
<td>3 (6%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Affected NL Child</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac NL</td>
<td>42 (82%)</td>
<td>39 (91%)</td>
<td>32 (96%)</td>
</tr>
<tr>
<td>Rash NL</td>
<td>9 (19%)</td>
<td>9 (19%)</td>
<td>5 (14%)</td>
</tr>
<tr>
<td>Clinical status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asym/UAS</td>
<td>23 (46%)</td>
<td>44 (98%)</td>
<td>32 (94%)</td>
</tr>
<tr>
<td>SS</td>
<td>14 (27%)</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>SLE</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>SLE/SLE</td>
<td>7 (14%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0%)</td>
<td>2 (4%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

*1 GM with Ankylosing Spondylitis, 1 GM with Rheumatoid Arthritis

Autoantibody positive

- Anti-Ro60+Ro52+La+ 51 (100%) 1 (2.5%) 0 (0%)
- 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%)

*P< 0.0001 for Mother vs. Grandmother and Mother vs. Grandfather
GRANDPARENTS OF NL AFFECTED CHILDREN HAVE LOWER RISK ALLELE FREQUENCIES THAN NL MOTHERS

<table>
<thead>
<tr>
<th>Trait</th>
<th>Risk allele</th>
<th>P value compared to NL Mothers</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α A allele at rs1800629</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAPMAP CEU</td>
<td>22%</td>
<td>-</td>
</tr>
<tr>
<td>NL Mothers (n=41)</td>
<td>38%</td>
<td>-</td>
</tr>
<tr>
<td>Grandmother (n=38)</td>
<td>21%</td>
<td>0.02</td>
</tr>
<tr>
<td>Grandfather (n=29)</td>
<td>32%</td>
<td>0.59</td>
</tr>
<tr>
<td>C6orf10 G allele at rs7775397</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAPMAP CEU</td>
<td>9%</td>
<td>-</td>
</tr>
<tr>
<td>NL Mothers (n=41)</td>
<td>32%</td>
<td>-</td>
</tr>
<tr>
<td>Grandmother (n=36)</td>
<td>12%</td>
<td>0.0065</td>
</tr>
<tr>
<td>Grandfather (n=32)</td>
<td>23%</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Pairwise LD between SNPs intermediate (r^2=0.58) despite 800 kb span

PREFERENTIAL TRANSMISSION OF THE TNF-α RISK ALLELE A

<table>
<thead>
<tr>
<th>rs1800629</th>
<th>Nontransmitted</th>
<th>Transmitted</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>G</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>44</td>
</tr>
</tbody>
</table>

OR=6.7, 95% CI = (1.96, 23.03), P_{TDT} = 3.93 x 10^-4
P_{MARGINAL} = 1.52 x 10^-4

- Similar results when limited to complete trios with cardiac NL in the child (OR=8.00, P=9.67 x 10^-4)
- A formal parent of origin analysis found no evidence of significant parent of origin effects

PREFERENTIAL TRANSMISSION OF THE C6ORF10 RISK ALLELE G

<table>
<thead>
<tr>
<th>rs7775397</th>
<th>Nontransmitted</th>
<th>Transmitted</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>T</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>51</td>
</tr>
</tbody>
</table>

OR = 35.0, 95% CI = (4.13, infinity), P_{TDT} = 3.74 x 10^-5
P_{MARGINAL} = 1.42 x 10^-7

- Similar results when limited to complete trios with cardiac NL in the child (OR=31.0, P=1.08 x 10^-5) and Caucasian trios with cardiac NL in the child (OR=31.0, P=1.08 x 10^-5)
- A formal parent of origin analysis found no evidence of significant parent of origin effects

SUMMARY

- The majority of Grandparents of affected NL children are asymptomatic, with disease prevalence similar to relatives of patients with other rheumatic disease
- Ro/La antibodies are absent in Grandparents, distinguishing familial aggregation in NL from other rheumatic diseases
- Variant allele frequencies were lower in Grandparents compared to the NL Mother, but a highly significant preferential transmission of risk alleles from Grandparents to the NL Mothers was observed
- No significant parent of origin effects between Grandmothers and Grandfathers

TRANSLATIONAL IMPLICATIONS

- These results imply that Mothers accumulate genetic determinants specific to NL, which are not enriched in the maternal Grandparents
- The preferential transmission of risk alleles represents a selection pattern which demonstrates the “perfect storm” of events that leads to NL
- Further study is required to distinguish whether transmission of these risk variants directly contribute to pathogenesis of NL and investigate the autoimmune phenotype of NL motherhood

ACKNOWLEDGMENTS

NYU School of Medicine
Jill P. Buyon
Robert M. Clancy
Erin McDonnell

Wake Forest Medical Center
Carl D. Langefeld
Miranda C. Marion
Paula S. Ramos
Satria P. Sajuthi

To all families in The Research Registry for Neonatal Lupus
Funded by NIAMS