JM News from the NIH: Genetic and Environmental Studies

Cure JM Meeting- Seattle, WA June 2011

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Bethesda, MD
Possible Mechanisms for the Development of Myositis
Genetics Plays a Role in Many Autoimmune Diseases

- Increased frequency of autoimmune diseases in certain families and ethnic groups
- Closer the relationship to the patient, the more likely another autoimmune disease: identical twins > fraternal twins > other relatives
- Associations with specific genes: some are shared among many autoimmune diseases

Lettre 2008 Human Mol Genet; Fernando 2008 PLoS Genet
Autoimmune Diseases are Increased in Close Relatives of Myositis Patients

OR = 7.9, P<0.001

Ginn 1998 Arthr Rheum
Single nucleotide polymorphisms (SNPs) are present about every 300 nucleotides.
Human Leukocyte Antigens (HLA) Important in Immune Response to Environmental Agents

- HLA molecules bind proteins and present them to the immune system
- HLA genes determine how people respond to environmental agents, including infections, toxins and other antigens
- HLA genes have been associated with many autoimmune diseases
Phenotype-Genotype Associations in Myositis

Clinical Phenotypes
- All Myositis
- Polymyositis
- Dermatomyositis
- Juvenile DM

Risk Genotypes in Caucasians
- HLA 8.1 ancestral haplotype
  A1, B8, Cw7, DRB1*0301, DQA1*0501
- HLA A*68
- HLA B*50
- HLA DRB1*0701
- HLA DRB1*15
- HLA DQA1*0104
- HLA DQA1*0301

Autoantibody Phenotypes
- Anti-synthetase
- Anti-SRP
- Anti-Mi-2
- Anti-PM/Scl
- Anti-p155

Other Pro-Inflammatory Variants are Risk and Severity Factors for JDM

<table>
<thead>
<tr>
<th>Allele</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>TNFα-308 A*</td>
<td>↑ TNFα levels</td>
</tr>
<tr>
<td>IL1β+3953 T</td>
<td>↑ IL-1 levels</td>
</tr>
<tr>
<td>IL-1α+4845 T</td>
<td></td>
</tr>
<tr>
<td>IL1α-889 C*</td>
<td></td>
</tr>
<tr>
<td>Gm 13</td>
<td>↑ IgG levels</td>
</tr>
<tr>
<td>Gm 3 23 5, 13</td>
<td>Alter Ab binding affinities</td>
</tr>
<tr>
<td>Gm 1,3,17 5,13,21</td>
<td></td>
</tr>
<tr>
<td>PTPN22 R620W</td>
<td>Alters T cell signaling, ↑ autoreactive T cells</td>
</tr>
</tbody>
</table>

* Associated with calcinosis
Some Genes Shared Between Juvenile and Adult DM, Some Differ

Risk or protective factors are shown in **bold-face** or *italics*, respectively. TNFα -238G, IL-1RN VNTR A1, IL-1α +4845T, IL-1β +3953CT are additional risks for JDM that have not been evaluated in Adult DM; HLA*A68 is an additional risk for Adult DM that has not been evaluated in JDM.
The candidate gene approach
The genome wide association approach
Genome Wide Association Studies Show Novel Genes for Rheumatoid Arthritis

5539 cases, 20169 controls

Courtesy Peter Gregersen
More Than 35 Risk Loci Now Identified For Rheumatic Arthritis

Gregersen 2010 Bull NYU Hosp Joint Dis
### Candidate Genes for SLE Involve Immune Pathways and Cell Processes

<table>
<thead>
<tr>
<th>Cells</th>
<th>Pathways</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dendritic cells</td>
<td>TLR/IFN signaling</td>
<td>IRF5, STAT4, SPP1, IRAK1, TREX1</td>
</tr>
<tr>
<td>Macrophages</td>
<td>TNF/NFkB signaling</td>
<td>TNFAIP3</td>
</tr>
<tr>
<td>Autoreactive T cells</td>
<td>T Cell signaling</td>
<td>HLA-DR, PTPN22, STAT4, PDCD1, IRAK1, TNFSF4</td>
</tr>
<tr>
<td>Autoreactive B cells</td>
<td>B Cell signaling</td>
<td>HLA-DR, BLK, BANK1, FCGR2B, LYN</td>
</tr>
<tr>
<td>Macrophages Neutrophils</td>
<td>Phagocytosis</td>
<td>FCGR3A, FCGR3B, CRP, ITGAM</td>
</tr>
<tr>
<td></td>
<td>Complement</td>
<td>C4A, C4B, C2, C1q</td>
</tr>
<tr>
<td>Other</td>
<td>Apoptosis</td>
<td>ATG5, STAT4</td>
</tr>
<tr>
<td></td>
<td>Ubiquitination</td>
<td>UBE2L3, TNFAIP3</td>
</tr>
<tr>
<td></td>
<td>DNA methylation</td>
<td>MECP2</td>
</tr>
<tr>
<td></td>
<td>Cellular adhesion</td>
<td>ITGAM</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>PXK, ICA1, SCUBE1, NMNAT2, XKR6, KIAA1542</td>
</tr>
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</table>

Harley 2009 Nature Rev Genetics; Moser 2009 Genes Immunity
Myositis Genetics Consortium (MYOGEN)

- Led by Frederick Miller, in collaboration with Peter Gregersen, over two dozen centers in North America and Europe have access to genetic material from over 2500 myositis patients and large numbers of controls
  - Centers contributing JDM patients include Lauren Pachman (Chicago), Ann Reed (Mayo), Lucy Wedderburn (London), Katalin Danko (Hungary), Jiri Vencovsky (Prague) and Lisa Rider (Bethesda)
  - Cure JM has funded 281 US JDM samples
- A first genome-wide association study of 1100 adult and juvenile Caucasian DM patients has been completed
- Goal is to discover genetic factors that will improve our understanding of the disease process and lead to novel treatments
A first genome-wide association study of 1100 adult and juvenile Caucasian DM patients has been completed and suggests that:

- The MHC region is the strongest risk factor
- Genes involved with other autoimmune diseases that are important in immune responses are also risk factors for JDM/DM

Confirmatory studies in additional patients could possibly lead to identification of novel mechanisms and potential new targets for therapy.
Of Genes Shared Between RA, SLE and SSc and Other AIDs, Are They Also Seen in DM/JDM?
MYOGEN – Where Do We Go From Here?

• HLA typing
  ▪ To understand major signal and where coming from

• GWAS
  ▪ Need more samples to find GWAS associations

• Functional studies
  ▪ Discover new pathways important to cause of disease and develop new therapies
Evidence for the Role of the Environment in the Pathogenesis of Autoimmune Diseases

- Less than 50% identical twins (who share the same genes) both develop autoimmune disease
- Timing of exposure shortly before onset of disease
- Disease improvement after agent removal (dechallenge), Disease relapse after re-exposure (rechallenge)
- Seasonality in birth dates and disease onset
- Increasing disease frequency (incidence, prevalence) over time
- Genes determining responses to exposures are major risk factors
- Laboratory and animal studies provide data on mechanisms
- Case-controlled epidemiologic studies show associations between exposures and diseases

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Comments</th>
<th>Odds Ratio or Relative Risk (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>Parvovirus , Enterovirus</td>
<td>- No association with B19 or Coxsackie B (JDM)</td>
<td></td>
</tr>
<tr>
<td>Common Cold (1 year prior)</td>
<td>Questionnaire, case – sibling control (PM, DM)</td>
<td></td>
</tr>
<tr>
<td>Group A Streptococcus (household exposure)</td>
<td>- Case control (JPM, JDM)</td>
<td></td>
</tr>
<tr>
<td>Physical Exertion</td>
<td>- Case – sibling control, 104 cases (PM, DM)</td>
<td></td>
</tr>
<tr>
<td>Collagen Implants</td>
<td>- Cohort study DM&gt;PM</td>
<td></td>
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<tr>
<td>Vaccinations (PM/DM)</td>
<td>- Case – sibling control, 104 cases (PM, DM)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Any vaccine one year prior to diagnosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Influenza vaccination</td>
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Gourley and Miller 2007 Nature Clin Prac Rheum
Ultraviolet Radiation (UVR) May Play a Role in the Development of Dermatomyositis

- Anecdotally, UVR is associated with disease onset and flares in DM and lupus
- UVR increases the expression of the DM autoantigen Mi-2 in cells in the lab
- Global UVR intensity predicts the proportion of DM and anti-Mi-2 autoantibodies
  - Confirmed in US - 7 regions
  - Associated in women - not in men and not in African-Americans

A Number of Environmental Exposures Noted Before Onset of JM, Vary by Phenotype

- 60% of JM patients with documented exposure within 6 months of diagnosis
  - 62% 1 exposure, 26% 2 exposures, 12% 3-5 exposures

- Exposures differ in different subgroups
  - Infections: younger age, antibody negative, polycyclic course
  - Stress, drugs: older age
  - Multiple exposures: SRP antibody

<table>
<thead>
<tr>
<th>Exposure</th>
<th>%</th>
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<tbody>
<tr>
<td>Infection</td>
<td>45</td>
</tr>
<tr>
<td>Medication</td>
<td>18</td>
</tr>
<tr>
<td>Immunization</td>
<td>11</td>
</tr>
<tr>
<td>Stressful Life Events</td>
<td>10</td>
</tr>
<tr>
<td>Sun Exposure</td>
<td>7</td>
</tr>
<tr>
<td>Chemical Exposure</td>
<td>3</td>
</tr>
<tr>
<td>Weight-Lifting</td>
<td>2</td>
</tr>
<tr>
<td>Dietary Supplement</td>
<td>1</td>
</tr>
</tbody>
</table>
Seasonality in Birth Distributions Suggest Perinatal Environmental Exposures in JM

- Seasonal associations suggest seasonal environmental factors
- Seasonal patterns in birth dates of patients with other autoimmune diseases (Type 1 diabetes, celiac disease, Crohn’s, and multiple sclerosis) suggest perinatal exposures may have a role and have long-lasting effects
- Some immune functions and susceptibility to infections vary by season
- Seasonality in birth dates in JM subgroups
  - Hispanic: peak onset in fall, compared to controls (uniform)
  - p155: uniform, compared to p155- (summer peak)
Studies in Families with Twins or Siblings Discordant for Systemic Rheumatic Disorders

Doctors at the National Institutes of Health are conducting pioneering research in understanding the genetic and environmental risk factors that may result in autoimmune diseases. The goal of study 03-E-0099 is to assess why one twin or sibling developed disease and why the other brother or sister did not. The study consists of a blood draw, urine collection and completing surveys. There is no charge for study-related evaluations and medical tests at the NIH. Compensation provided for both participants and referring physician.

You may qualify if:
• You have rheumatoid arthritis/juvenile rheumatoid arthritis, lupus, scleroderma or myositis.
• You were diagnosed within the last 4 years.
• You have a twin or sibling of the same gender within 4 years of age.
• Both children and adults are eligible.
• You have a letter from your referring physician.

Duration of study:
• Five years with an annual questionnaire.

Location of the Study:
• Locally with the help of your physicians or at the NIH Clinical Center in Bethesda, MD.
Similar Gene Expression Profiles in Affected Twins with SAIDs

- Identified 92 differentially expressed genes in affected twins vs. unaffected vs. controls peripheral blood immune cells
  - 74% were under-expressed; 26% were over-expressed
  - These include genes involved in immune function, cell signaling, transcription/translation factors, metabolic functions
    - Interferon response genes were over-expressed in patients vs. controls
- Affected twins with different AIDs share similar gene expression profiles, suggesting common disease mechanisms

O'Hanlon 2011 Arthr Res Ther
Epigenetic Changes Alter Gene Functions and Are Associated with Disease

DNA hypomethylation → Transcription Activation

Associated with certain cancers, Multiple Sclerosis, Rheumatoid Arthritis

Epigenetic Changes Seen in a Number of Immune Response Genes of SLE Discordant Twins

Javierre 2010 Genome Res
Further Evaluation of Environmental Factors
In Ongoing and New Studies

FAMILIES WITH TWINS OR SIBLINGS DISCORDANT
FOR SYSTEMIC RHEUMATIC DISORDERS

Overview of This NIH Study

The Environmental Autoimmunity Group of the National Institutes of Environmental Health Sciences is currently enrolling families in which an adult or child meets criteria for Rheumatoid Arthritis/Polyarticular Juvenile Rheumatoid Arthritis, Systemic Lupus Erythematosus, Systemic Sclerosis or Myositis— and in which a twin or sibling of the same gender, who is within 4 years of age, does not have any of these 4 illnesses or another autoimmune disease. Patients remain under the care of their personal physicians while participating in the study. Compensation is available. There is no charge for study-related evaluations and medical tests at the NIH.

The goal of the study is to understand the genetic and environmental factors that may result in systemic rheumatic diseases.

The study will perform evaluations to assess why one twin or sibling developed disease and why the other brother or sister did not.

Subjects must be diagnosed within 4 years and may enroll at the NIH Clinical Center in Bethesda, Maryland or their local doctor’s office.

Medical records, questionnaires and blood and urine samples will be collected at enrollment.

For each subject, annual questionnaire follow-ups will be collected by mail from up to 4 years after enrollment.

The development of new autoimmune diseases during the study will be tracked.

For Further Information About the Study, Please Call:
Drs. Lisa Rider: (301) 451-6272 or Email: riderl@mail.nih.gov or
Frederick Miller: (301) 451-6280 or 1/(888) 271-6207

or Visit Our Website at http://www.nih.gov/research/clinical/rg/index.cfm

THE GEORGE WASHINGTON UNIVERSITY MEDICAL CENTER
WASHINGTON DC

Factors for Disease Flare in Adult and Juvenile Dermatomyositis and Polymyositis
Genetic and Environmental Factors for Juvenile Myositis - Sum

- A number of genetic risk factors for juvenile myositis have been identified
  - HLA region is the strongest risk factor, with other immune response and cell signaling genes possibly also involved
  - Genes for DM/JDM are shared with other systemic autoimmune diseases

- Environmental risk factors are beginning to be identified for myositis
  - For juvenile myositis, these may include infectious agents, ultraviolet light, perinatal factors
  - Epigenetic changes in immune response genes, resulting from environmental exposures, may also be important and alter their functions
  - Common immune response pathways exist in systemic autoimmune diseases
Summary

Understanding genetic and environmental risk factors for myositis will help identify disease pathways, lead to new treatments, and enable future prevention.
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Cure JM
AutoCure - curing autoimmune diseases