



National Institute of Environmental Health Sciences
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Genetic and Environmental Risk Factors for Myositis Phenotypes Across the Life Span

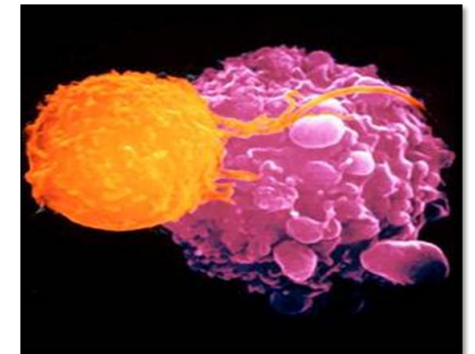
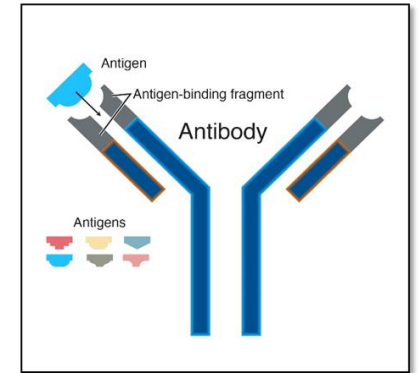
National Advisory Environmental Health Sciences Council
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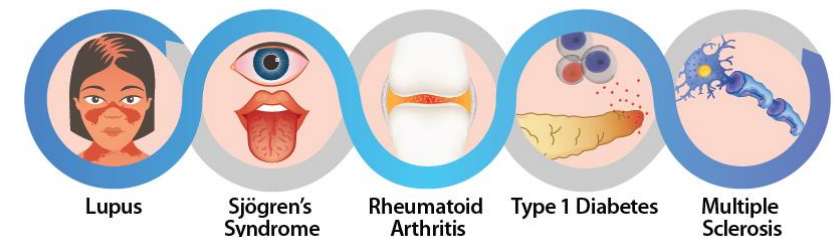


Autoimmune Diseases are a Large Public Health Problem

- Defined by pathologic inflammation associated with autoantibodies or self-directed T lymphocyte responses. Pathogenesis involves chronic immune activation after environmental exposures in genetically susceptible individuals.
- High and growing public health burden, affecting ~8% of the population
 - Over 140 acquired disorders, up to 25 million persons affected in U.S.
 - Chronic, incurable lifelong illnesses with significant morbidity, disability, secondary health consequences
 - May be life or organ threatening
 - U.S. annual cost for rheumatoid arthritis alone > \$50 billion
 - Female predominance, 80% with AiD are women
- Recognized by National Academies of Sciences as understudied, with recent formation of NIH Office of Autoimmune Disease Research to coordinate NIH research



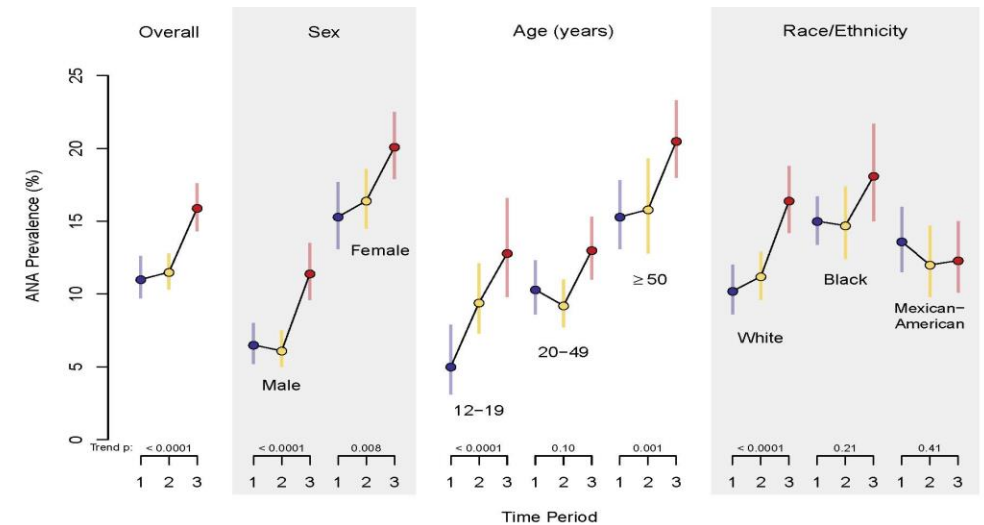
Five Common Autoimmune Diseases



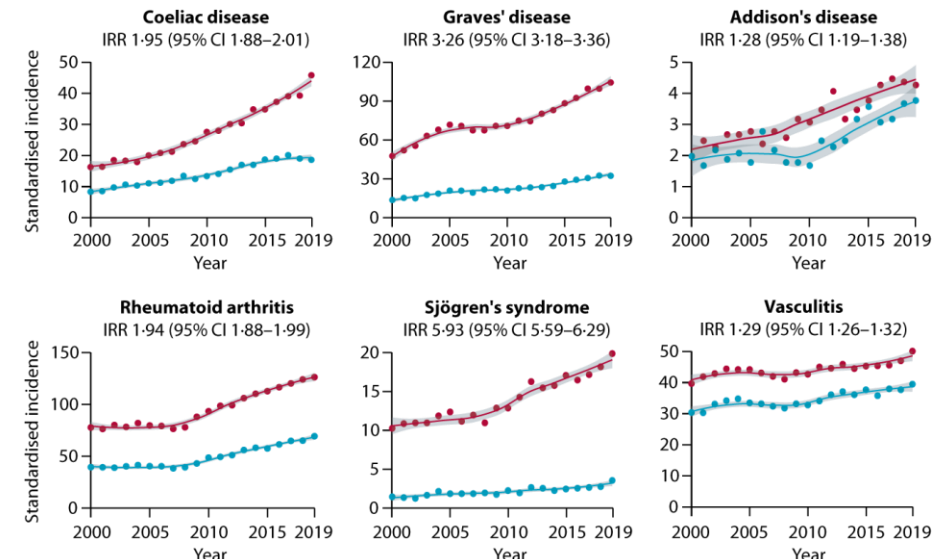
Autoimmunity and Autoimmune Diseases: High Prevalence and Rapidly Increasing

- Using U.S. population-representative data from a national CDC study, anti-nuclear antibody (ANA) prevalence increased from 11% to 16% over several decades: 1988-1991 (1), 1999-2004 (2), 2011-2012 (3)
 - ↑ ANA: in men, adolescents, older adults, and non-Hispanic whites
 - 32% of adults 60+ years of age had ≥ 1 disease-specific autoantibody (rheumatoid factor, thyroid, celiac)
- In UK, 10% of population affected with autoimmune disease, 13-19% of women
 - Incidence rates of autoimmune disease increased over past 20 years
 - Increases in both organ specific and systemic AiDs
 - Largest increases in celiac disease, Graves disease, Sjogren's syndrome

NHANES – ANA Prevalence

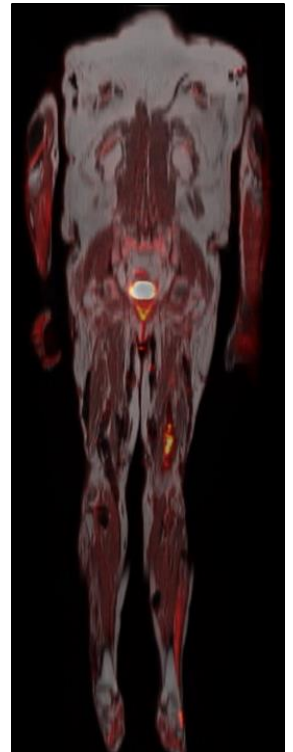


UK – Autoimmune Incidence



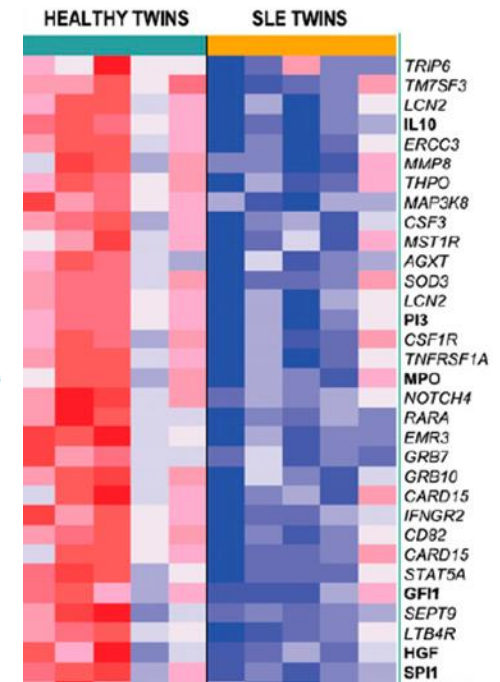
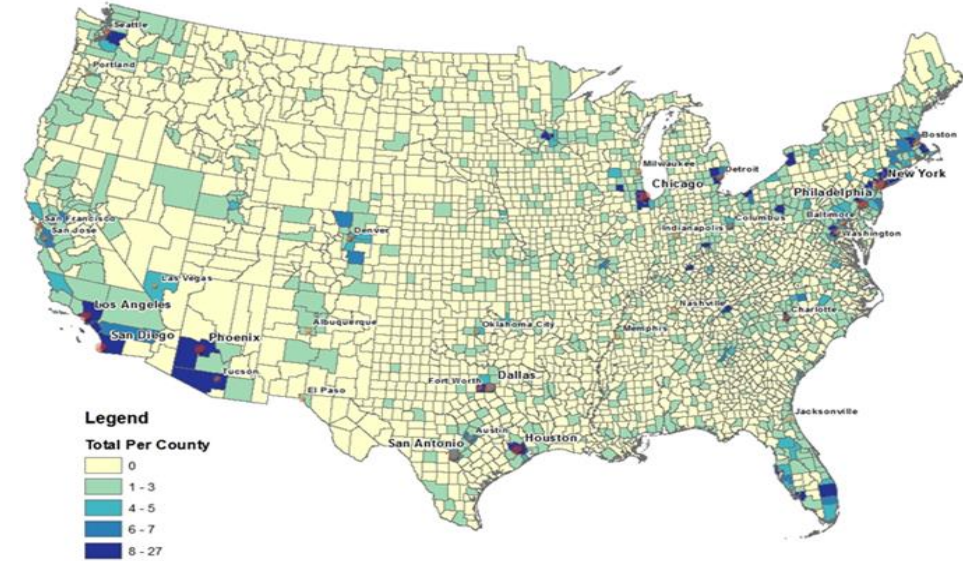
Environmental Autoimmunity Group

- The only NIEHS scientific group in Bethesda, established to use the unique resources in the NIH Clinical Center
- Focused on understanding the role of environment and genes and mechanisms in autoimmune diseases with the aim of disease prevention
- Developed largest myositis databases and biorepository in the world, with >3500 clinically well-characterized patients
- Conduct myositis natural history studies; genetic and environmental risk factor studies; therapeutic trials (rituximab, infliximab, IV sodium thiosulfate)



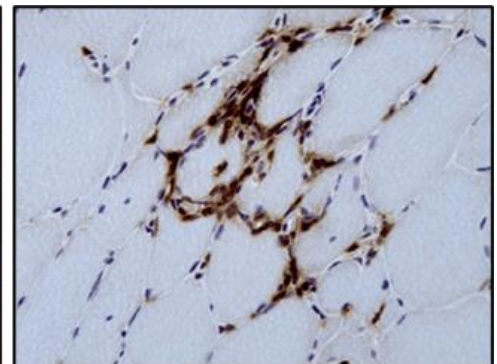
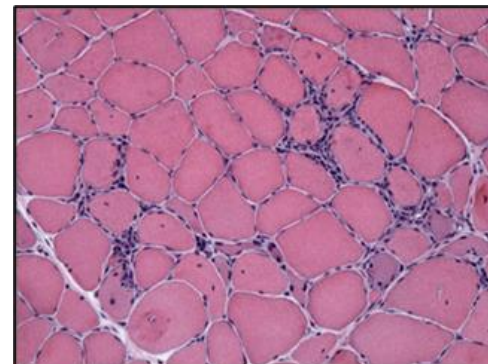
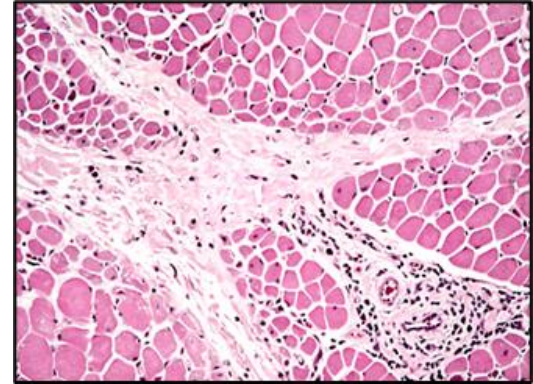
EAG Environmental Studies

- **MYOVISION National Myositis Patient Registry**
 - **Goal:** Examine risks for myositis phenotypes
 - ~2000 patients studied; partnership with TMA, CCHMC
- **Twin-Sibling Study of Systemic Autoimmune Disease (SAiD)**
 - **Goal:** Examine genetic/environmental risks and mechanisms
 - Adult and juvenile rheumatoid arthritis, lupus, myositis, scleroderma
 - Same sex twins or siblings within 5 years of age and diagnosis, discordant for SAiD, and parents (265 sib pairs, ~1000 enrolled)
- **MYORISK Study of Anti-Synthetase Syndrome**
 - **Goal:** Examine risks for myositis phenotype associated with ILD
 - Anti-Synthetase Syndrome (ASS) $\leq$ 2 years from diagnosis vs. non-ASS Myositis vs. Matched controls (150 each)
- **Military Myositis Study**
 - **Goal:** Examine risks for myositis in active-duty personnel
 - Mining of military databases for exposures



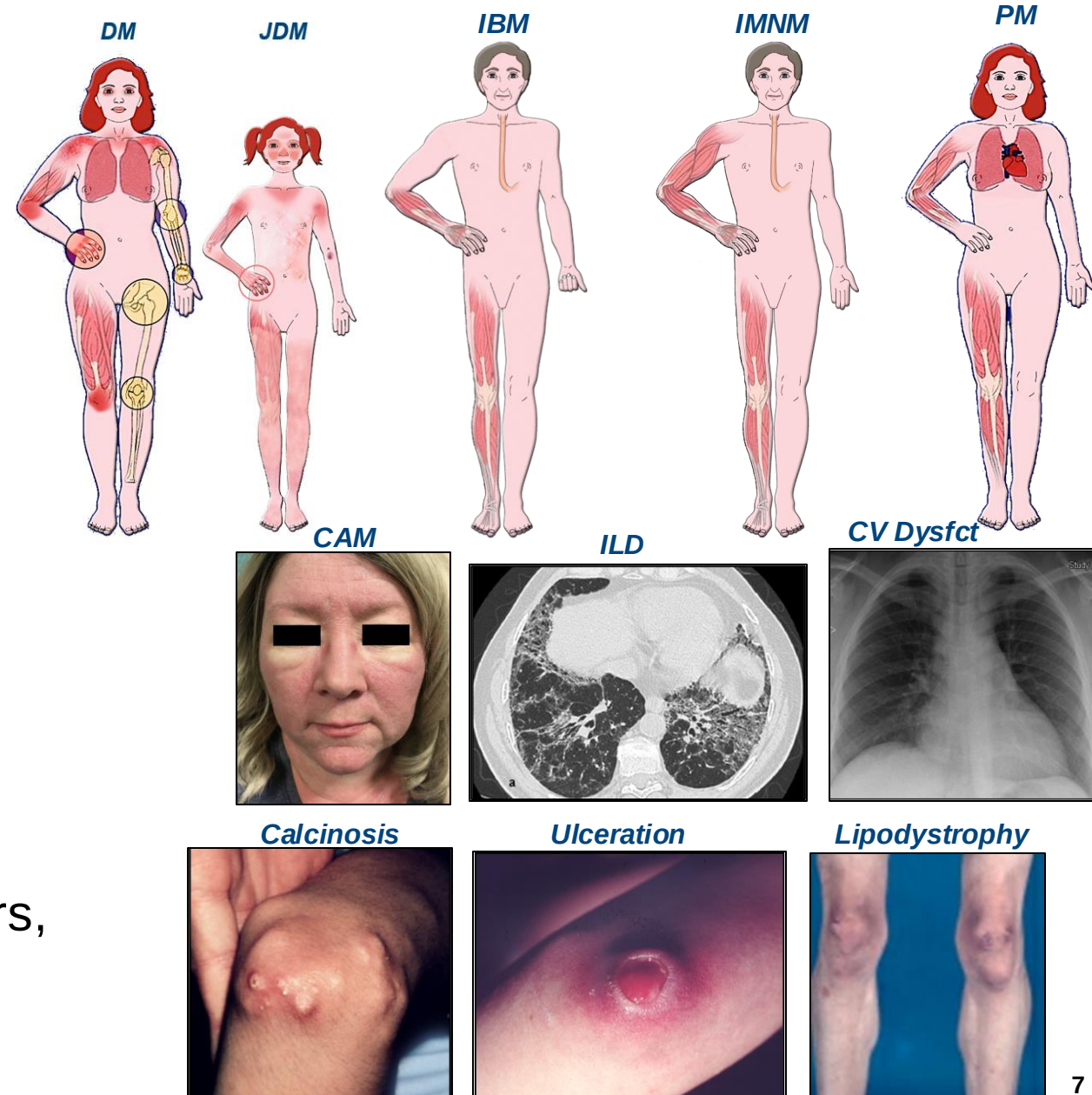
Autoimmune Muscle Disease (Myositis)

- Heterogeneous group of systemic autoimmune diseases with chronic muscle inflammation and unique autoantibodies
- Most common acquired chronic muscle disease in the U.S., but rare: prevalence ~10-34/100,000; female predominance
- Clinical forms include dermatomyositis (DM) with diagnostic rashes, polymyositis (PM), overlap myositis, inclusion body myositis (IBM)
- Debilitating conditions with chronic functional disability, weakness and other complications
- More costly per capita than many autoimmune diseases, with estimated annual national burden of \$575 - \$800M



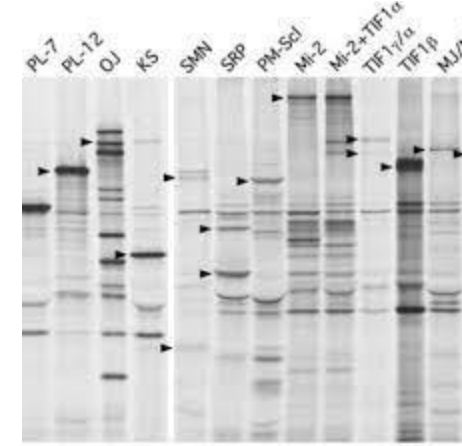
Adult and Juvenile IIM – Similarities and Differences

- Same phenotypes represented in adult and juvenile myositis, but different frequencies:
 - DM ~30% of adults, JDM 80% of juvenile
 - IBM, IMNM, PM more frequent in adults
- Share many clinical features with some important differences
 - Adults with DM- ↑ risk of malignancy, ↑ ILD and cardiac dysfunction in DM/PM
 - Children with JDM - ↑ calcinosis, vasculopathy/ulceration, lipodystrophy
 - Adult DM/PM - ↑ functional disability, chronic illness, ↑ mortality, less response to treatment
- Share many genetic risk factors, with many shared and some distinct environmental factors, which vary by phenotype

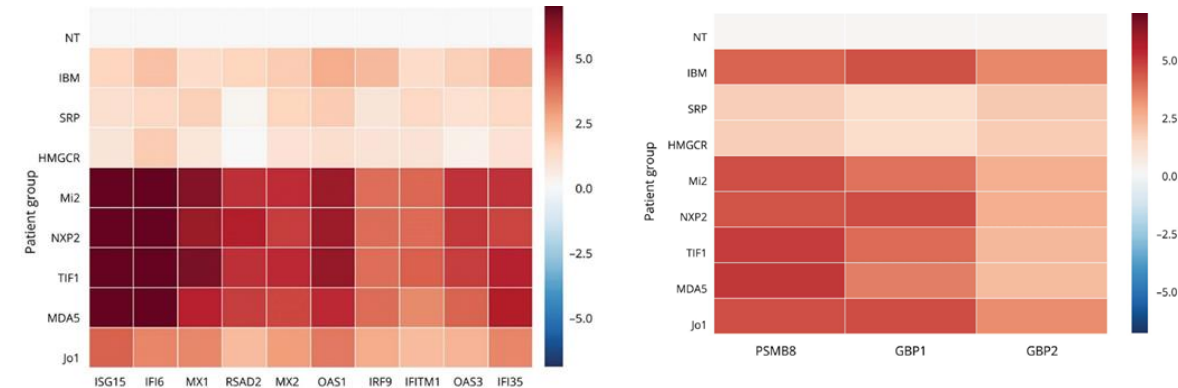


Myositis Specific Autoantibodies Define Distinct Subgroups of Myositis

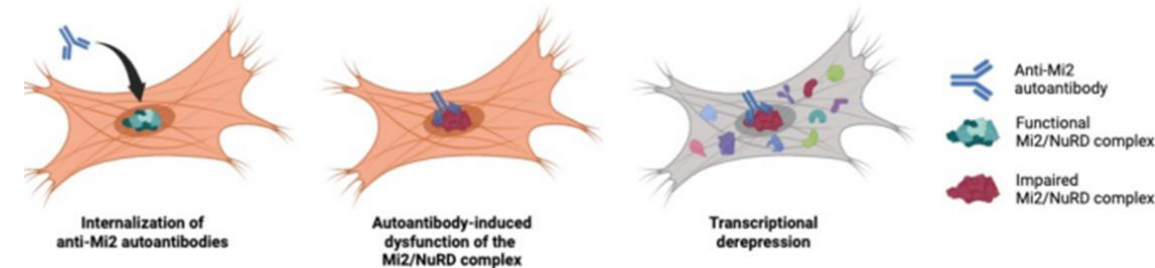
- Present in ~50 - 70% of myositis patient sera
- Occur exclusively in myositis patients
 - Directed against **cellular proteins** involved in **translation or transcription in all cells**, ↑ed in regenerating myoblasts
 - **Antigen-driven:** arise months prior to myositis onset, vary in titer with disease activity
 - **Mutually exclusive**
- Emerging role in pathogenesis
 - Pathogenically distinct
 - ↑ IFN-I increased in muscle of DM Abs
 - ↑ Type II IFN signature in DM Abs, Jo1
 - Abs enter myofibers, inhibit enzyme function (Mi2)
 - Mouse models of autoantigen immunization (TIF1γ, Jo1, MDA5) - features of human disease



Type I and II IFN Genes in Myositis Muscle

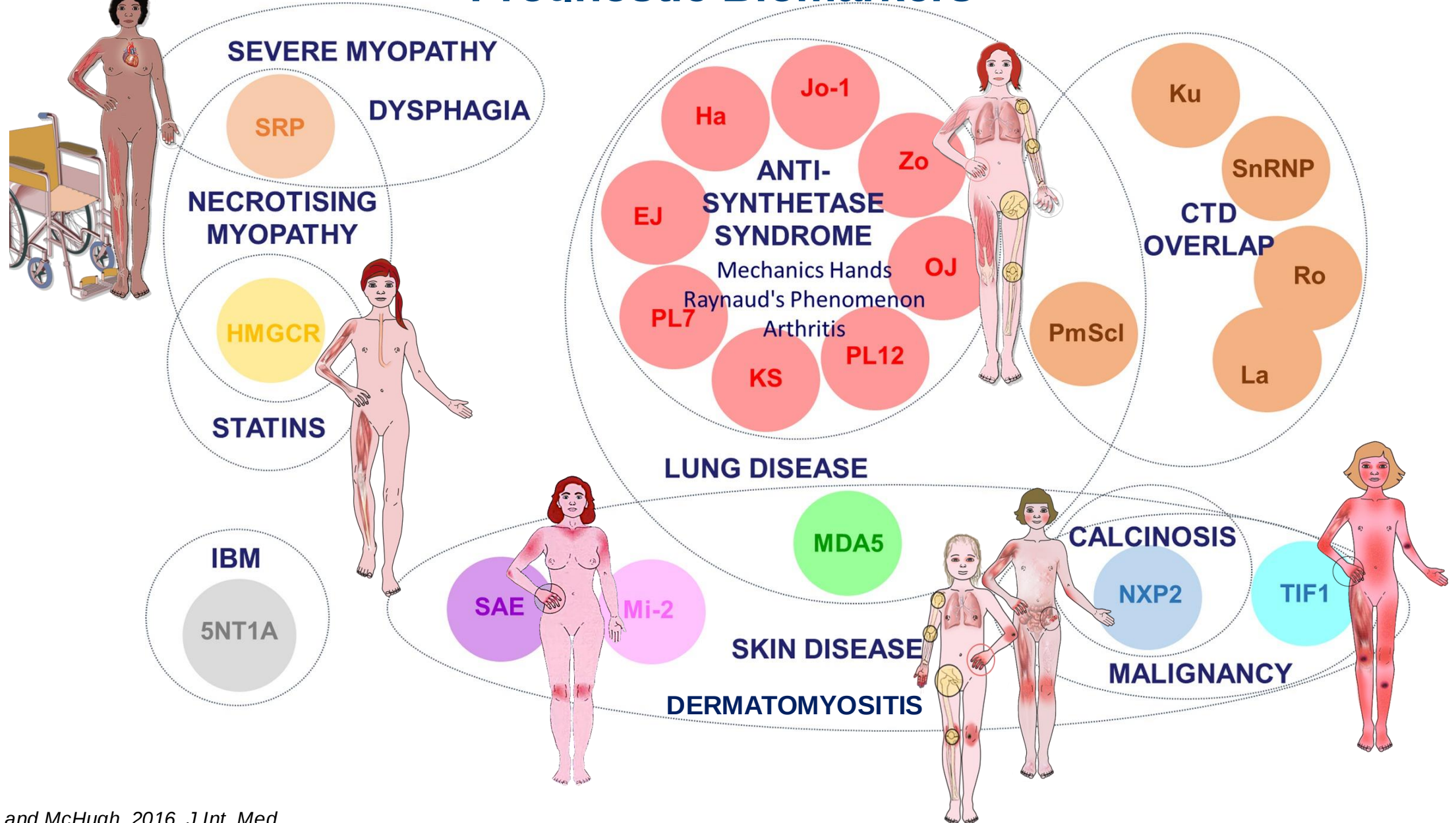


Pinal-Fernandez et al, 2019 Ann Neurol

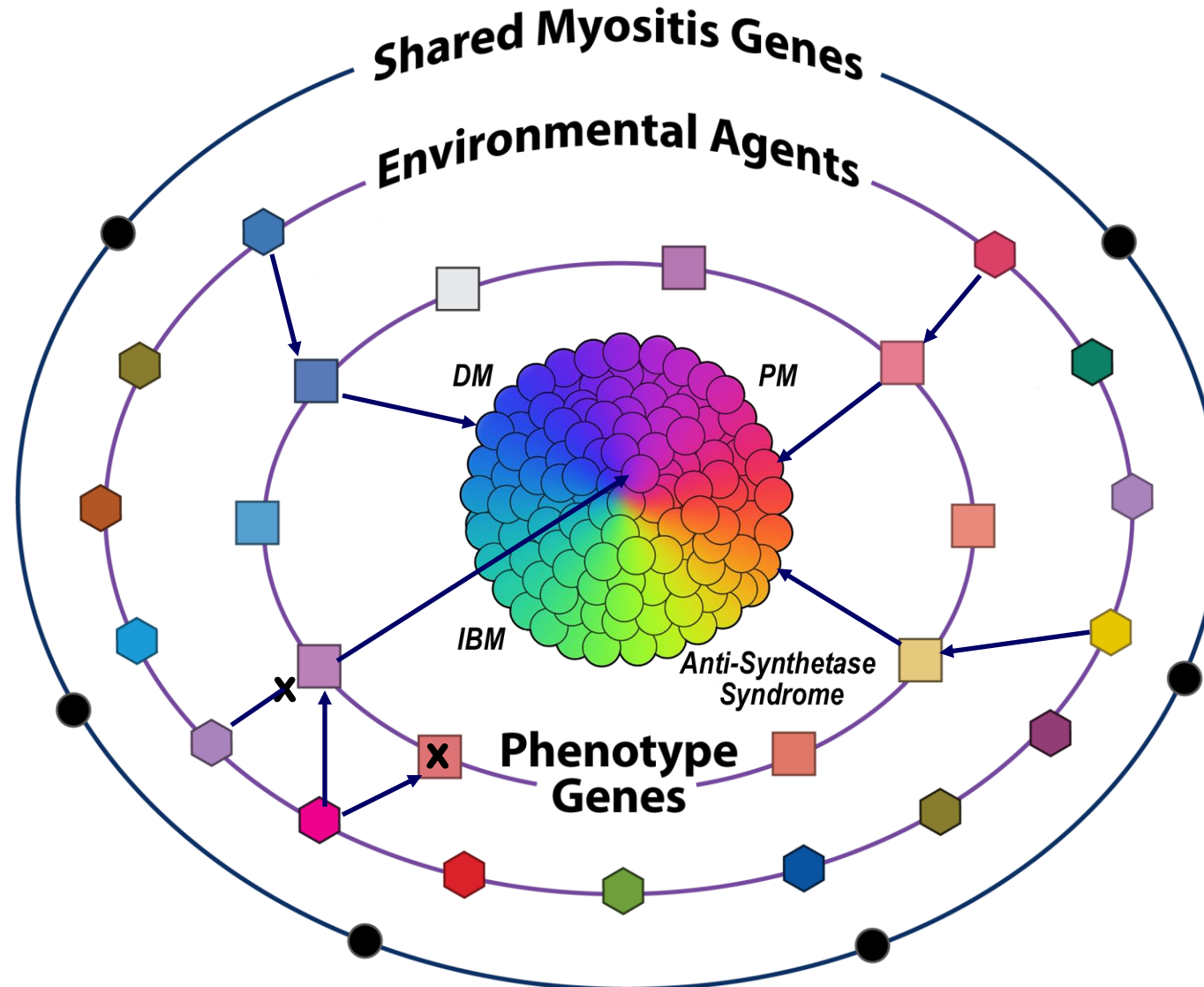


Pinal-Fernandez et al, 2024 Ann Rheum Dis

Myositis Autoantibodies Define Distinct Phenotypes and are Prognostic Biomarkers

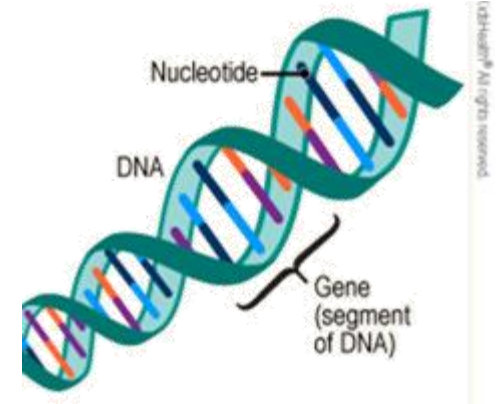


Possible Gene-Environment Interactions Resulting in Myositis Phenotypes

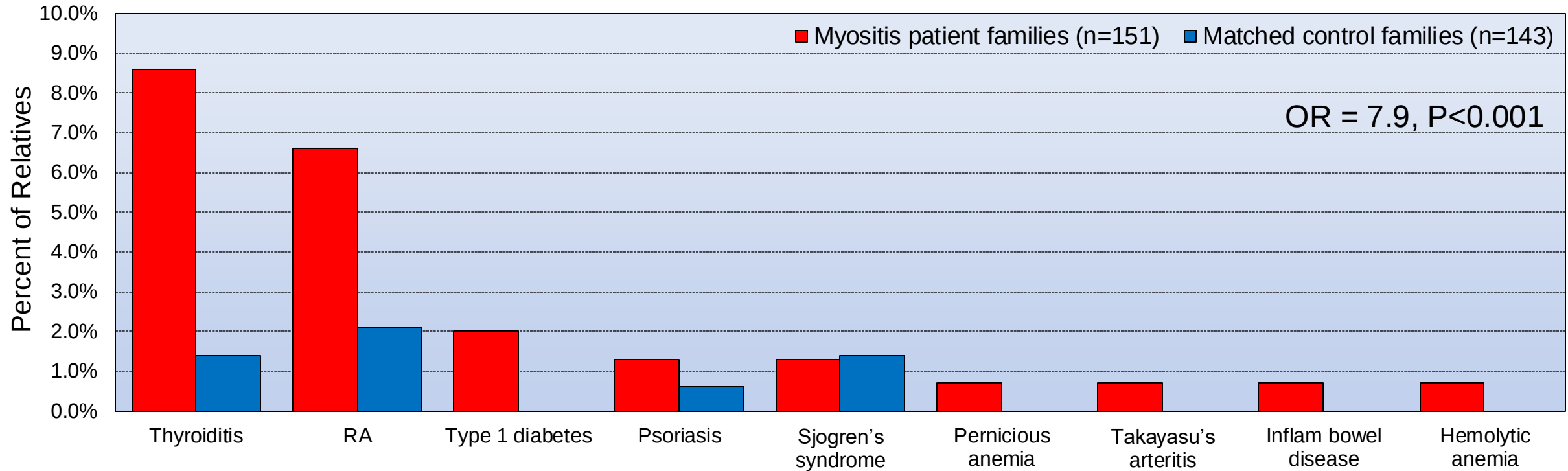


Why Identify Genetic Risk Factors for Myositis?

- Identify genetic risk factors for myositis and similarities/differences among subgroups
- Identify genetic similarities or differences to other autoimmune or inflammatory diseases
- Insights into biological pathways of disease
- May suggest new therapies, and identify patients more likely to respond to different treatments for more targeted management
- Identify diagnostic or prognostic biomarkers

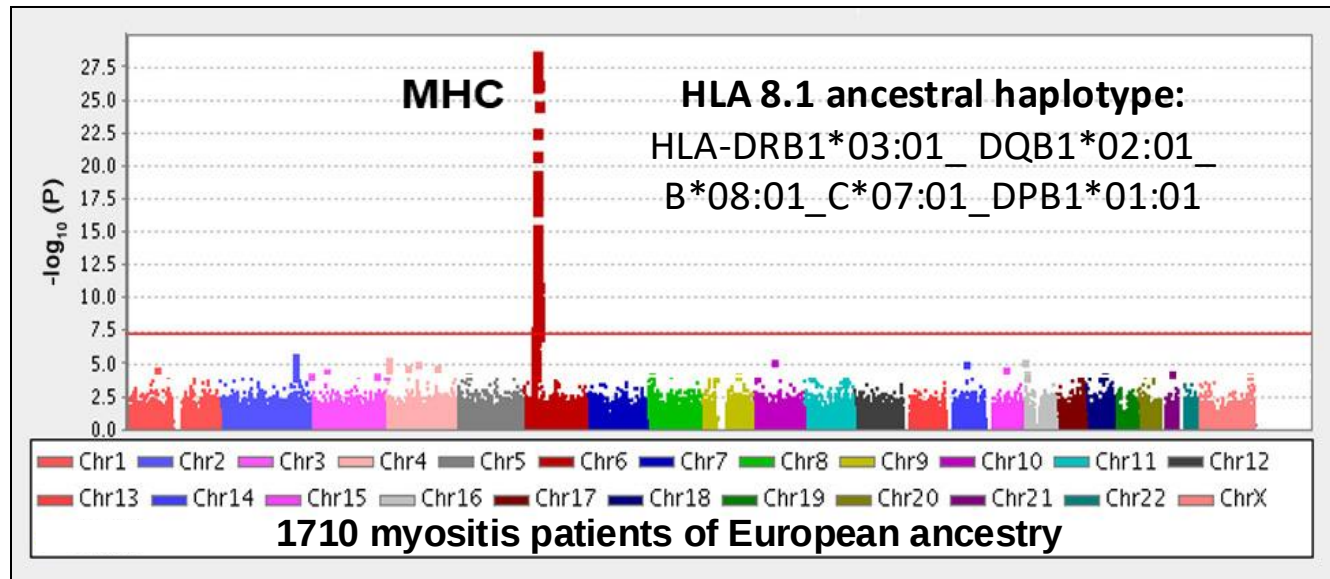


Autoimmune Diseases are Increased in First Degree Relatives of Myositis Patients



- Confirmed in US JDM registry and Swedish national healthcare registry studies. SLE, celiac disease also increased in relatives of IIM patients
- Individuals with IIM 4.3x more likely to have ≥ 1 first degree relative with myositis compared to people without myositis
- Individuals with IIM $\sim 2.7x$ more likely to have a sibling with myositis
- Heritability of myositis was 22-24%

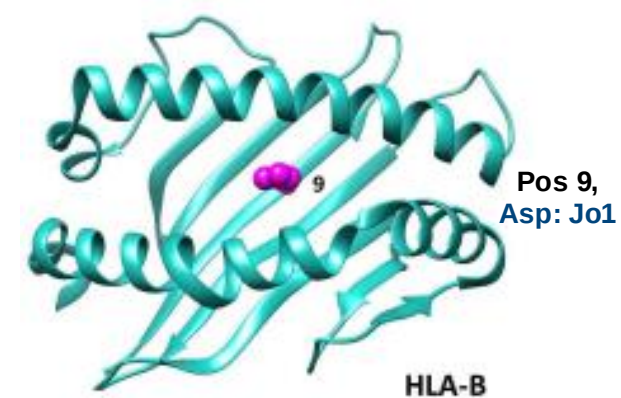
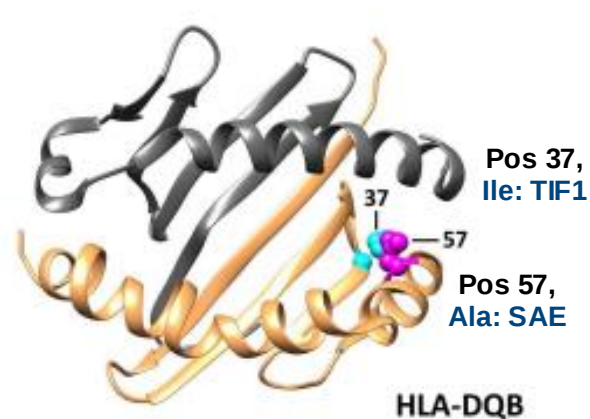
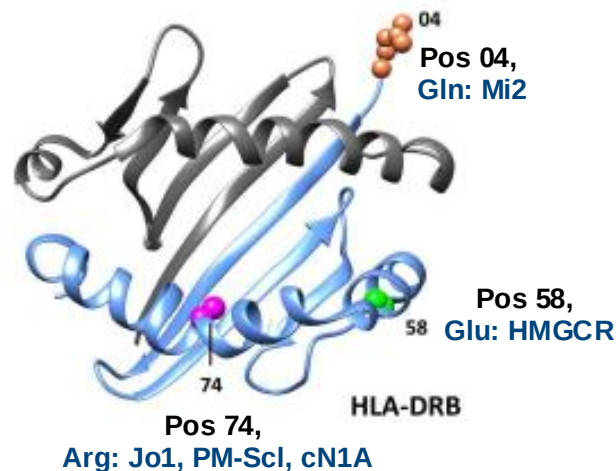
GWAS/ Immunochip Studies Show HLA 8.1 Haplotype is Major Risk Factor for Myositis Clinical Phenotypes, but HLA Risks are Distinct for MSAs



- The HLA 8.1 ancestral haplotype is the major risk factor for myositis, including major clinical subgroups, DM, JDM, PM, IBM
- HLA risk factors are strong risk factors for myositis autoantibodies
 - Different risks for each MSA, and risks for one MSA are protective for others
 - HLA risk factors differ slightly between adult and juvenile patients: (DM vs. JDM, TIF1γ: DQB1*02:01 in DM, *02:02 in JDM)

Miller 2015 Genes Immunity; Deakin 2022 Hum Molec Genetics

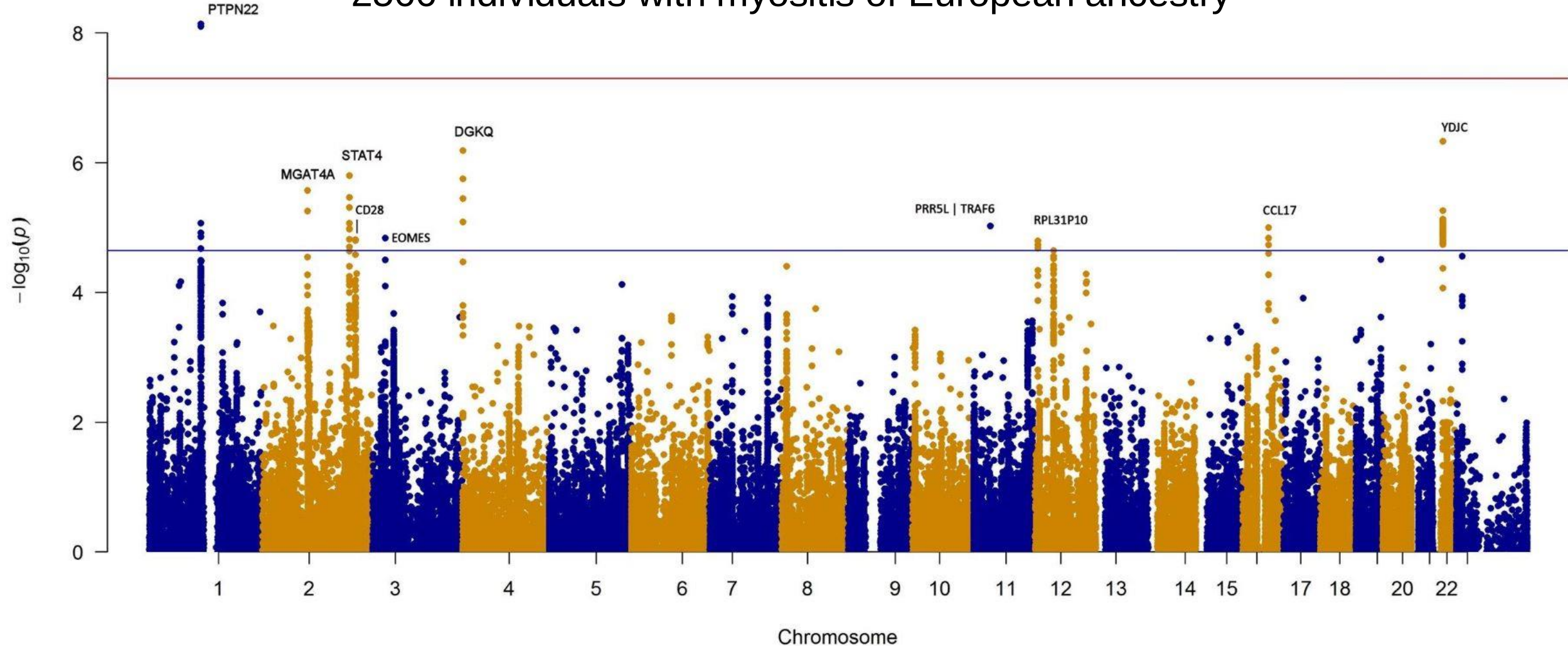
Key Amino Acid Positions within HLA confer risk for MSAs



Rothwell 2019 Ann Rheum Dis

Number of Genetic Risk Variants Identified Outside HLA Region Increases with Sample Size

2566 individuals with myositis of European ancestry



GWAS-ImmunoChip Studies Define New Genetic Risk Loci in Myositis Shared with Other Autoimmune Diseases

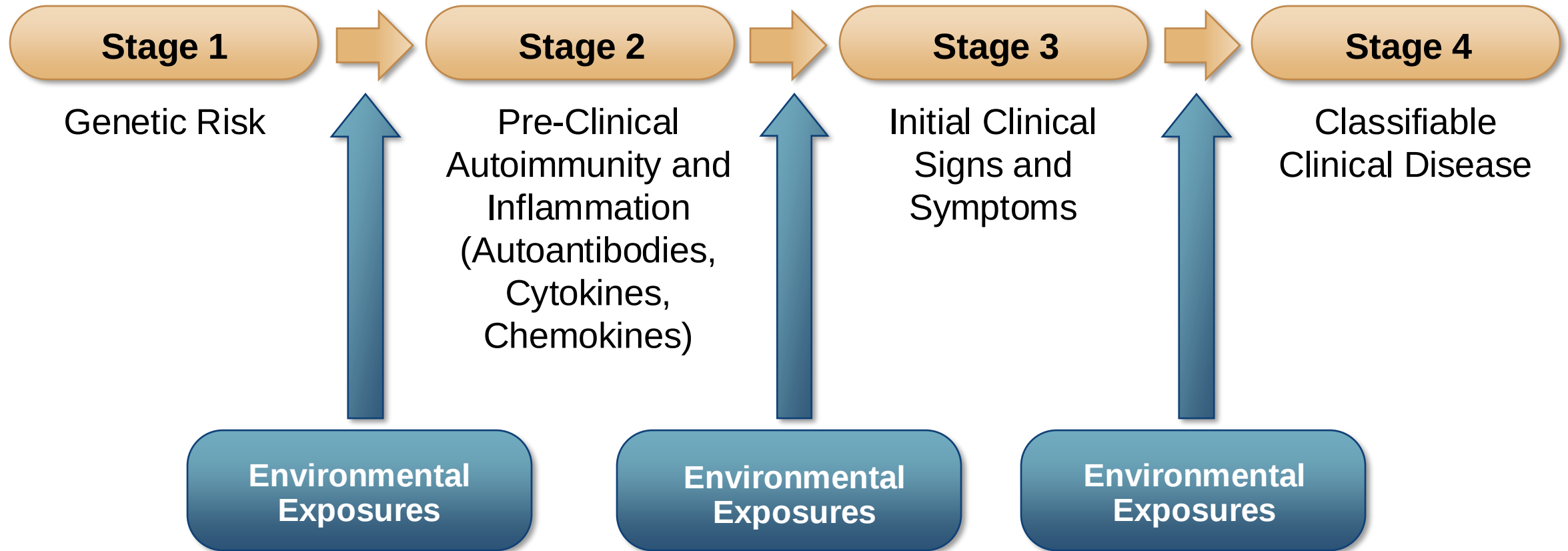
Gene: Name	Protein Function	IIM Phenotype*	SNP Disease Associations*
<i>PTPN22</i>	Alters the responsiveness of T and B cell receptors and TCR signaling	IIM, PM	RA, JIA, SLE, SSc, ATD, T1D
<i>STAT4</i>	Th1 cell development and IFN-γ production	IIM, PM	RA, JIA, SLE, SSc
<i>NAB1</i>	Transcription regulator/repressor for zinc-finger transcription factors; Protective factor	IIM, PM	Seropos RD, RA, SLE, SSc
<i>DGKQ</i>	Cell signaling	IIM	Seropos RD, RA, SLE, SSc
<i>UBE2L3-YDJC</i>	Protein degradation	IIM	RA, JIA, SLE, SSc
<i>IRF5</i>	Regulates transcription of type I IFN and IFN-stimulated genes	IIM	RA, SLE, SSc
<i>PLCL1</i>	Cell signaling	DM/JDM	SLE
<i>GSDMB</i>	Promotes inflammatory programmed cell death	DM/JDM	RA, T1D, IBD, MS
<i>BLK</i>	B cell receptor signaling and B cell development	DM/JDM, PM	RA, SLE, SSc
<i>CCL21</i>	Dendritic cell and T cell migration and proliferation and type I IFN signature generation	DM/JDM	RA, Sjogren's

* PM, polymyositis; DM, dermatomyositis; RA, rheumatoid arthritis; JIA, juvenile idiopathic arthritis; RD; rheumatic disease; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; ATD, autoimmune thyroid disease; T1D, type 1 diabetes

Evidence for Environmental Influences in Autoimmune Diseases

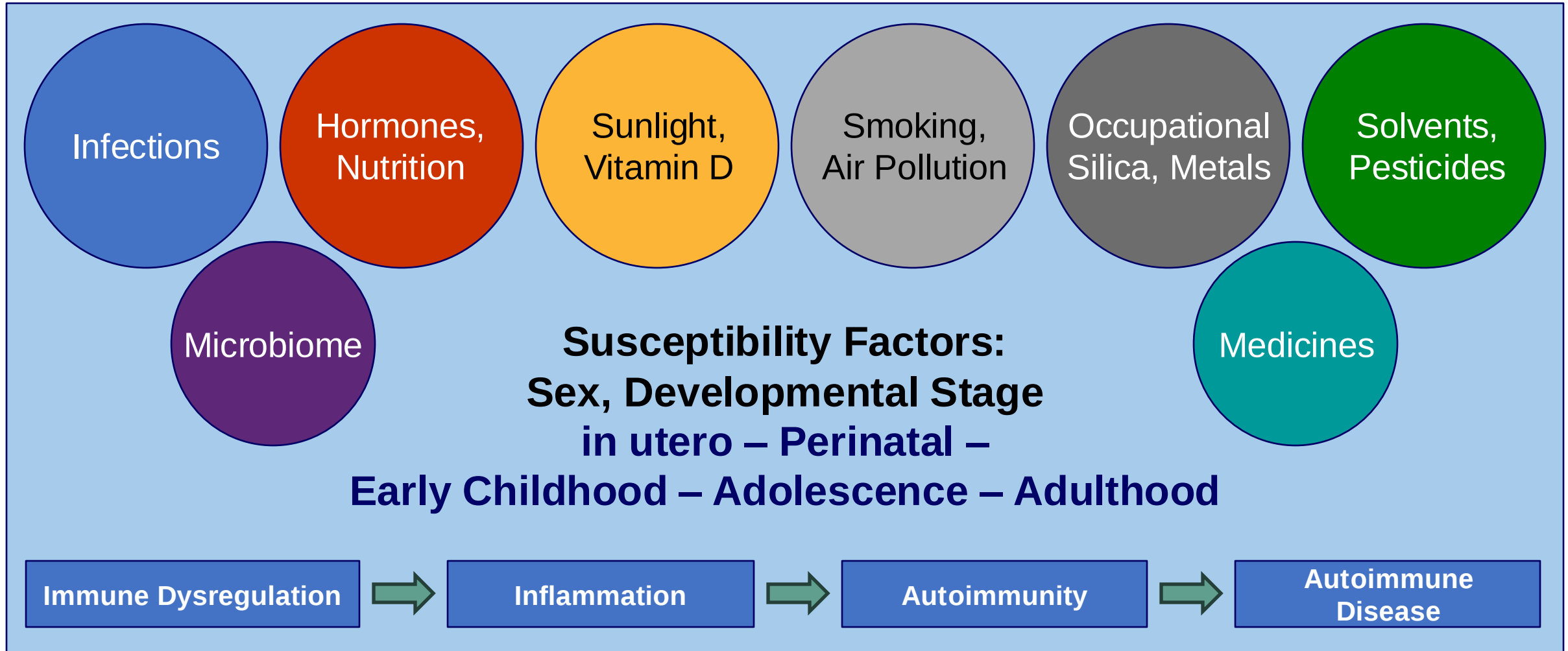
- Low to moderate disease concordance in monozygotic twins
- Major genetic risk factors are environmental response genes
- Biologic plausibility from in vitro and animal studies
- Strong temporal associations with some exposures and disease onset
- Seasonal and geographic clusterings in time and space with disease onset
- Changes in incidence over time
- Examples of dechallenge (improvement after agent removal) and rechallenge (recurrence after agent re-exposure), esp. for drugs, biologic therapies
- Epidemiologic associations (case-control studies) between exposures and certain diseases

Possible Stages of Disease Development and Environmental Influences During the Evolution of Autoimmune Disease



Environmental agents may initiate, advance, sustain or flare disease

Environmental Influences on the Development of Autoimmune Diseases Across the Lifespan

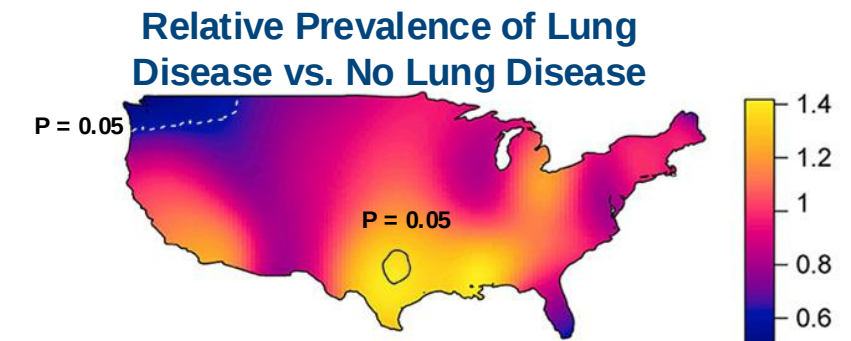
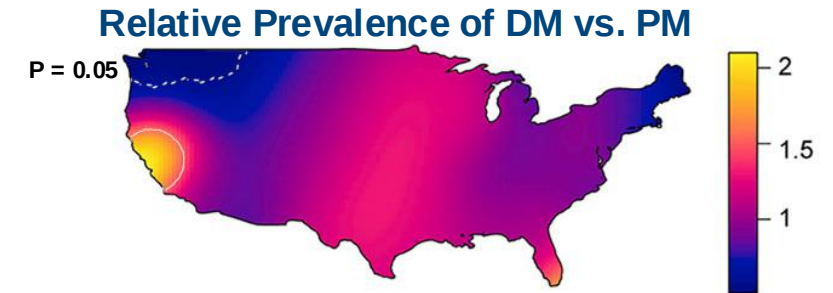


Geospatial Distribution of Myositis in the United States from MYOVISION Patient Registry Suggests Clustering by Phenotype



- Used spatial modeling to examine spatial characteristics and possible risk factors (484 DM, 358 PM, 318 IBM)
- More myositis cases in East/Northeast, including DM and IBM; PM ↑ in South
 - Clustering of cases with differences by subgroup (IBM vs. DM/PM)
- Relative prevalence of DM vs. PM –
↑ West, ↓ NW
- Relative prevalence of lung disease vs. no lung disease – ↑ SW, ↓ NW
- Trend of higher prevalence of IIM, esp. IBM, living within 50 m of major roadway

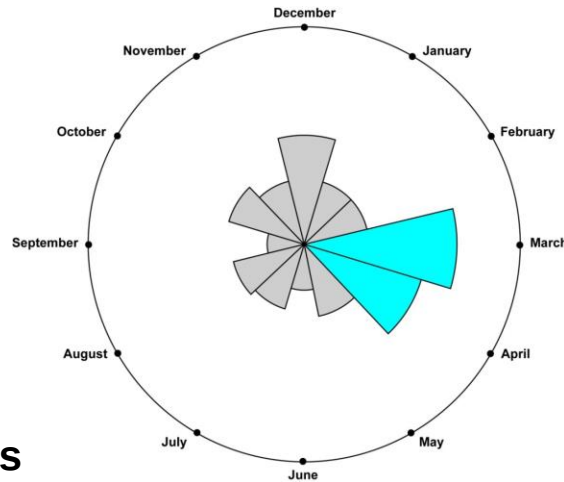
Spatial Distribution and Clustering of IIM in MYOVISION



Season of Disease Onset Varies Among Myositis Phenotypes

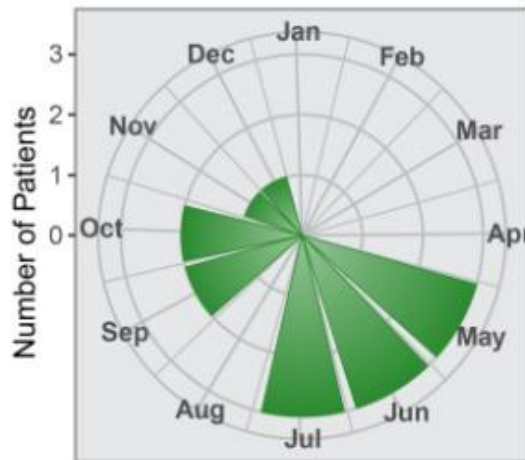
Anti-synthetase Autoantibodies

Adult



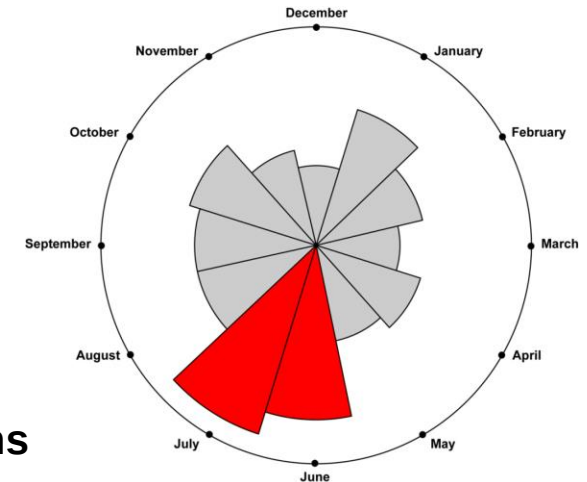
Onset peak in March-April;
Strongest associations in men with PM;
Onset peak in May to July in JIIM

Juvenile



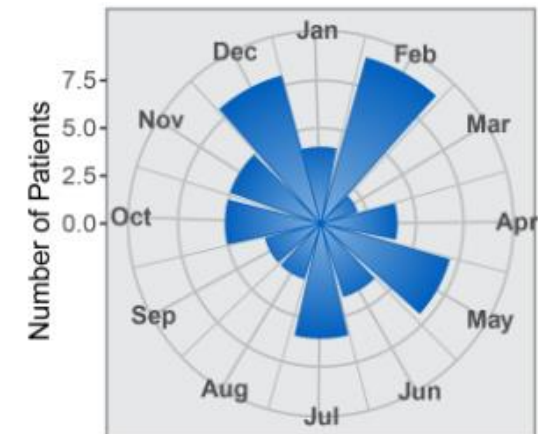
Myositis Autoantibody Negative

Adult



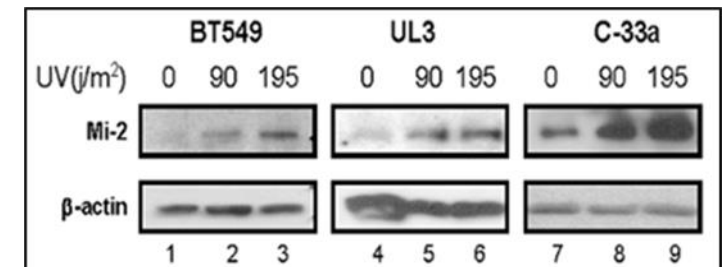
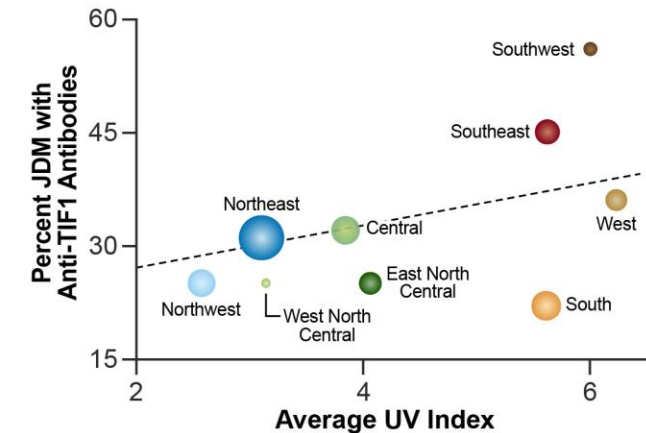
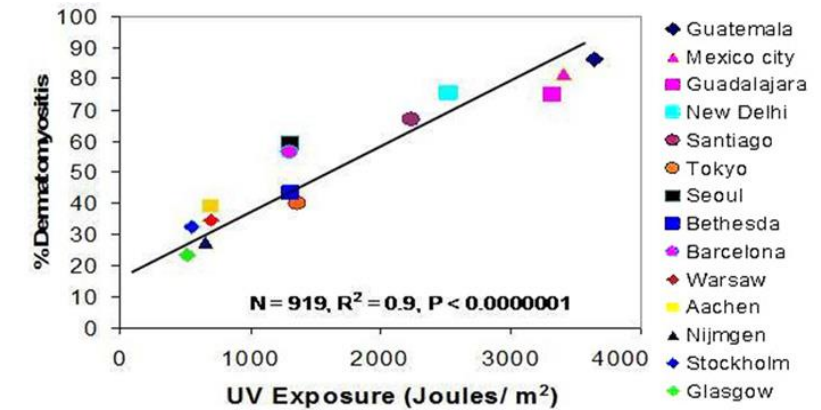
Onset peak in June-July;
Strongest associations in women with DM;
No seasonality in JIIM

Juvenile



Ultraviolet Radiation (UVR) May Play a Role in DM/JDM and Anti-Mi2, TIF1 Autoantibodies

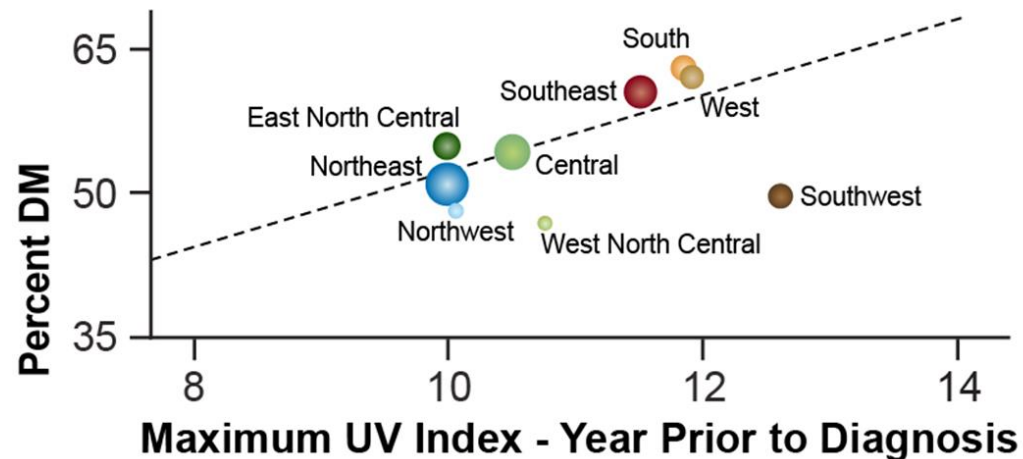
- Sunburn in year before diagnosis associated with DM vs. PM/IBM (MYOVISION)
 - ↑ sunburn in women, vs. healthy population (31% vs. 24%)
 - Sun exposure related to jobs in men, hobbies in women with DM
- Global UVR intensity correlates with proportion of DM and anti-Mi-2 autoantibodies, confirmed in US
 - Association in women, not men, and in whites, not in African-Americans
- Surface UVR intensity (residential) ≤ 1 month of illness onset associated with JDM, anti-TIF1γ and anti-MDA5 autoantibodies, not anti-NXP2 or autoAb-negative
 - Association stronger in girls for JDM/JPM
- UVR increases expression of DM autoantigen Mi-2 in cell lines via increased protein translation and message stability



MYOVISION National Myositis Patient Registry: Personal Sun Exposure Associated with DM



- Sunburn in year before diagnosis associated with DM, not PM/IBM
 - Frequency sunburns in women increased vs. healthy population (31% vs. 24%)
- Sun exposure related to jobs in men, hobbies in women with DM
- Residential UVB exposure in year prior to diagnosis associated with DM in women



Exposure	DM vs. PM/IBM OR (95% CI)*
Sunburn	
≥ 2 sunburns	
Females Only	1.5 (1.2, 1.8)
Males Only	1.0 (0.8, 1.2)
1 sunburn	
Females Only	1.5 (1.2, 1.8)
Males Only	1.0 (0.8, 1.2)
No sunburn	
Females Only	1.0 (0.8, 1.2)
Males Only	1.0 (0.8, 1.2)

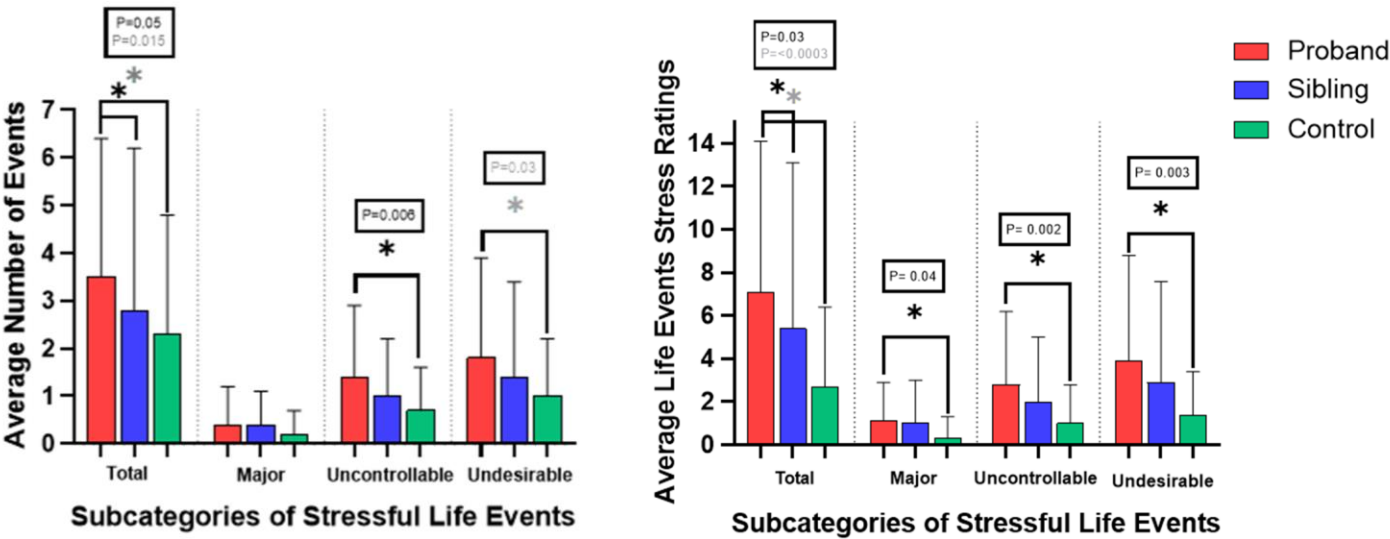
Exposure	DM vs. PM/IBM OR (95% CI)*
Job-related Sun Exposure	
High-Moderate	
Females Only	1.5 (1.2, 1.8)
Males Only	1.5 (1.2, 1.8)
Low/None	
Females Only	1.0 (0.8, 1.2)
Males Only	1.0 (0.8, 1.2)
Hobby-related Sun Exposure	
High-Moderate	
Females Only	1.5 (1.2, 1.8)
Males Only	1.5 (1.2, 1.8)
Low/None	
Females Only	1.0 (0.8, 1.2)
Males Only	1.0 (0.8, 1.2)



Stressful Life Events within Year of Diagnosis Associated with Systemic Autoimmune Diseases

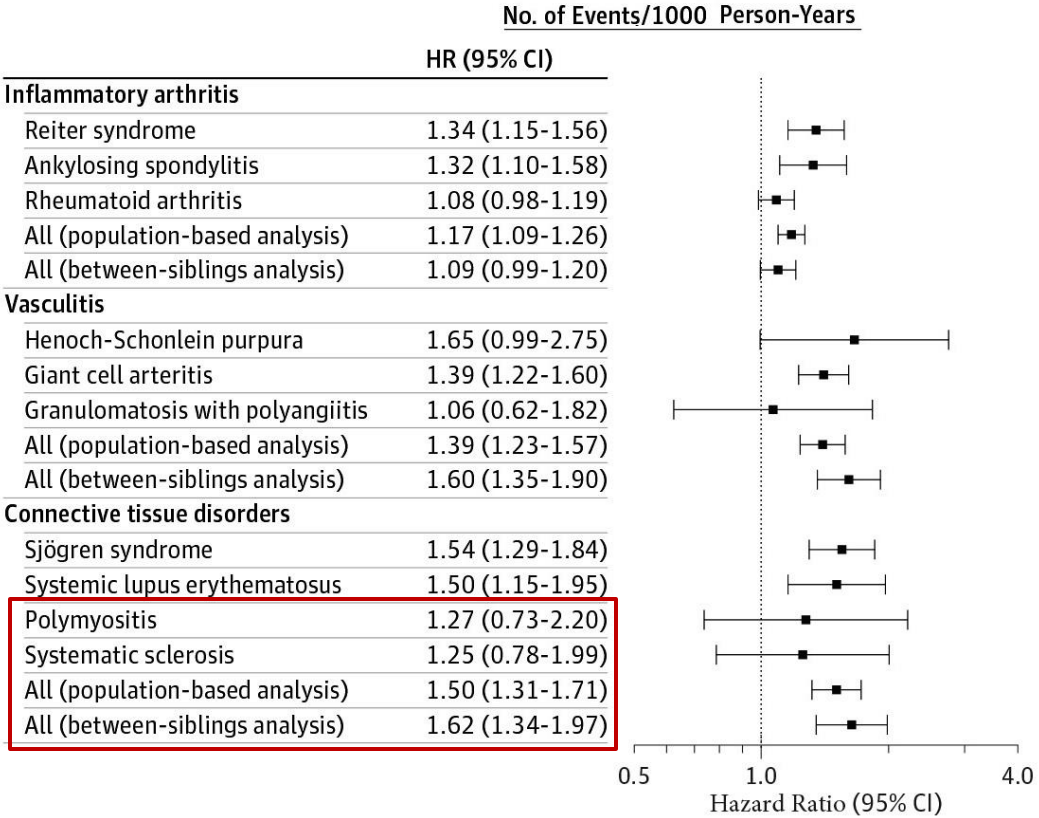


Adult SAiD Probands had More Frequent Stressful Life Events, Higher Stress Ratings in Year Prior to Diagnosis Compared Unaffected Siblings or Healthy Controls



Stressful Life Event Scores*	Events Score Odds Ratio	Weighted Event Score Odds Ratio
Total	1.31 (1.11 – 1.53)	1.23 (1.11 – 1.36)
Major	2.03 (1.00 – 4.11)	1.51 (1.08 – 2.11)
Uncontrollable	1.64 (1.14 – 2.35)	1.31 (1.09 – 1.57)
Undesirable	1.57 (1.17 – 2.11)	1.32 (1.12 – 1.55)

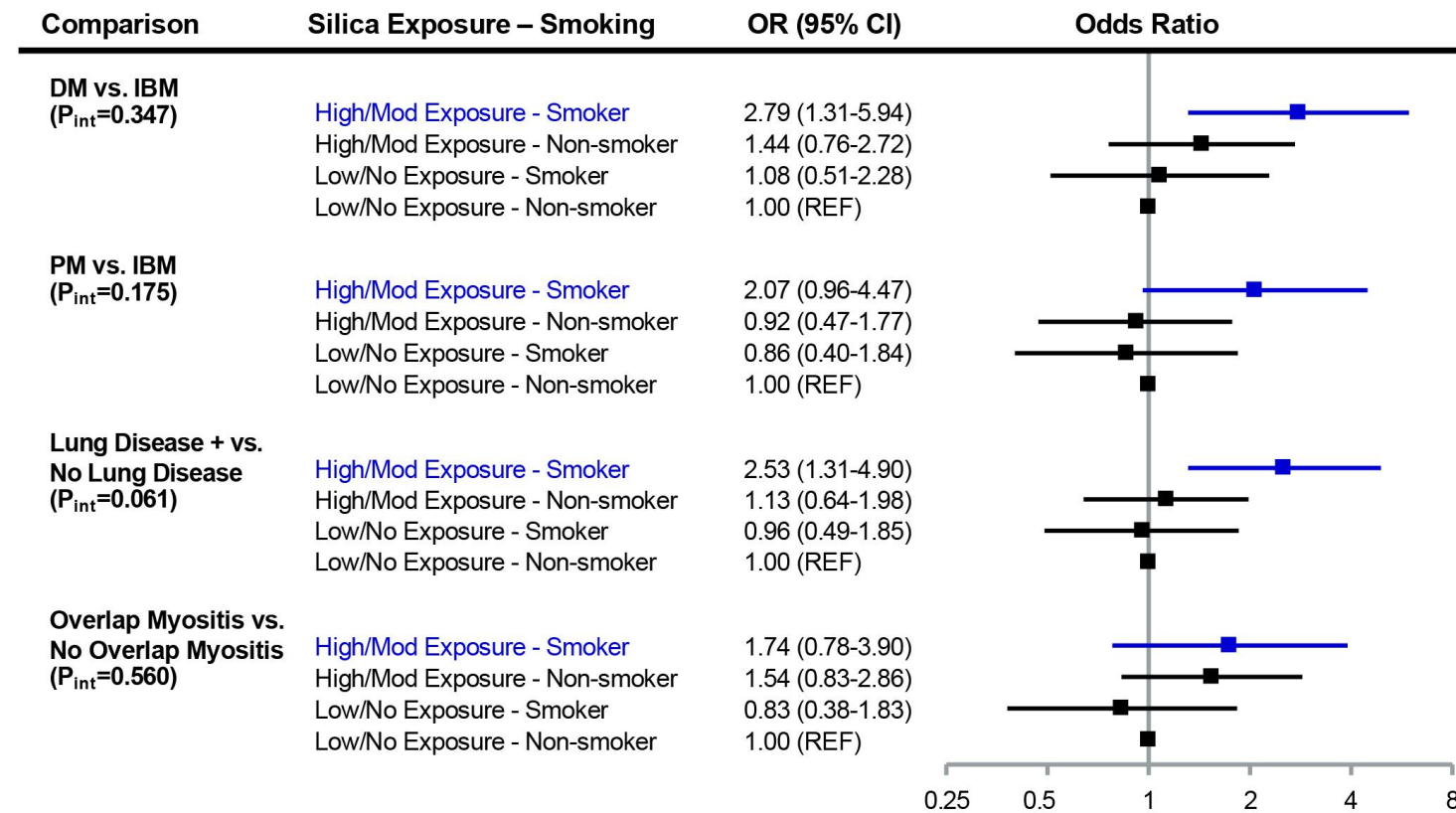
Models were adjusted for age, sex, race/ethnicity, education, smoking, diagnosis of anxiety and/or depression, and time from SAiD diagnosis to enrollment.



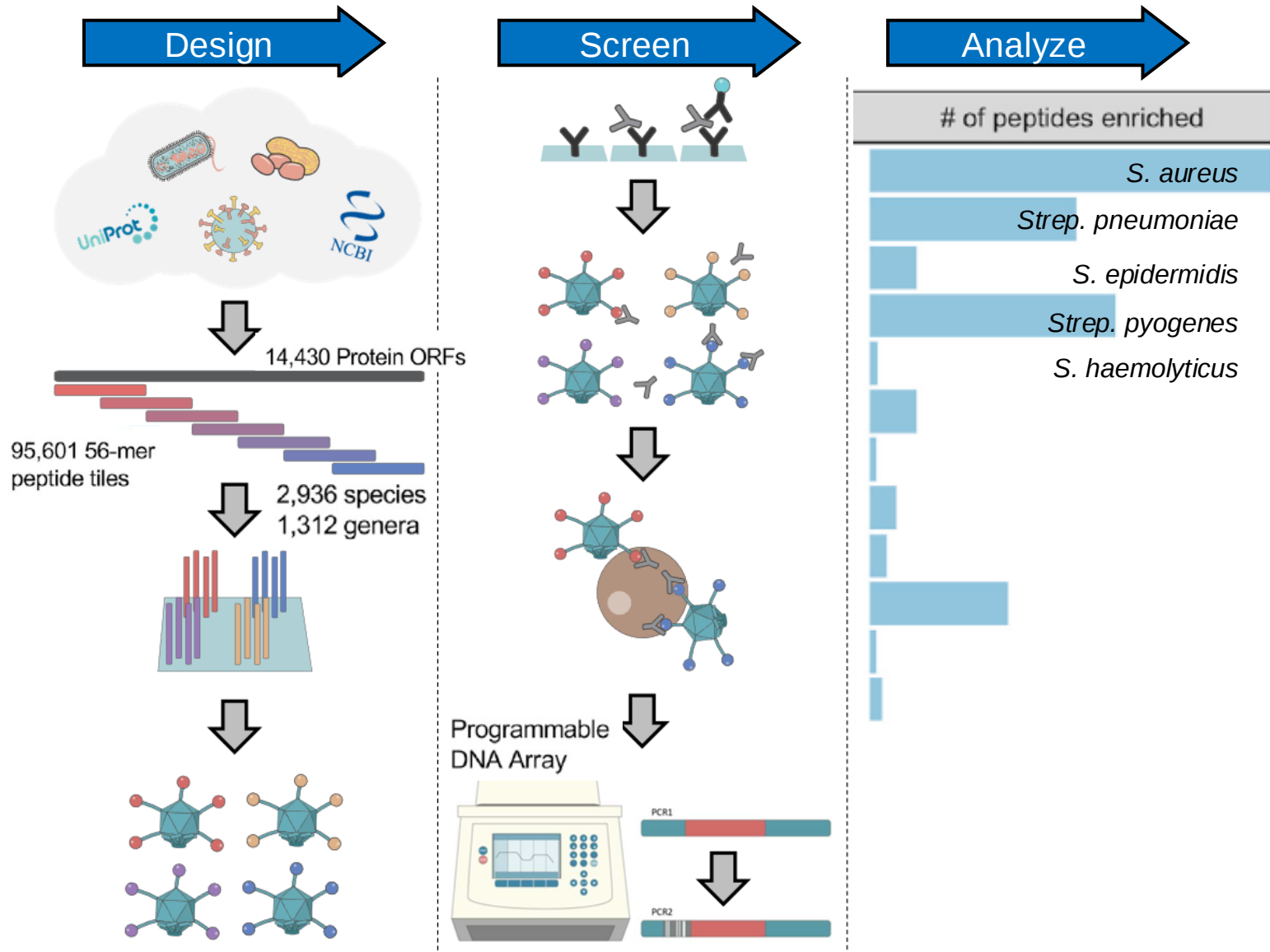
Occupational/Hobby Exposure to Silica, Heavy Metals, Solvents Associated with Myositis Subgroups



- Occupation/hobby exposures evaluated by occupation coding, epidemiologists assessed level of exposure and certainty
- High silica dust exposure associated with DM compared to IBM, with Lung Disease + (ASynS-like) and overlap myositis
- Moderate to high heavy metals and solvents also associated with Lung Disease + (ASynS-like) and overlap myositis
- Odds highest among smokers with these exposures and with greater number of exposures



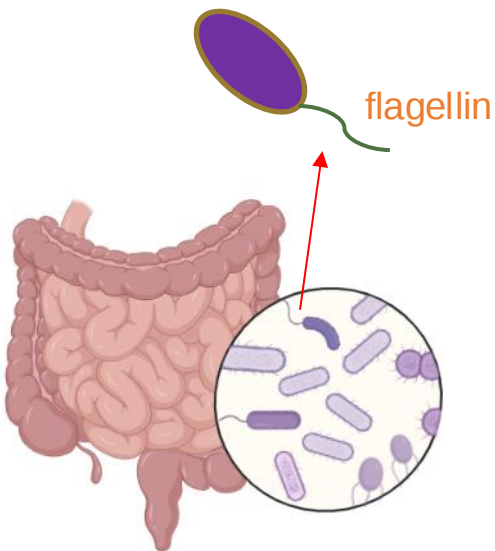
Phage-Immunoprecipitation Sequencing (PhIP-Seq) Assesses Immune Responses to External and Internal Antigens



- Unbiased tool for examining self and environmental antigen exposures, linking to human health/disease
- Protein libraries from:
 - **Human Proteome** (~250K 90-aa peptides)
 - **ToxScan** (~95K 56-aa peptides) – bacterial pathogens and human commensals
 - **Human Virome** (~100K 56-aa peptides)
 - **Allergome** (~19K 56-aa peptides)

Flagellin Reactivity with Conserved Peptide Motifs Enriched in JDM

Flagellin Protein, AA positions, Species	JDM Freq.	VRC (control) Freq.	P-value
Flagellin, 0-56, <i>B.burgdorferi</i>	0.3	0.02	3.0e-06
Flagellin B, 28-84, <i>H.pylori</i>	0.26	0.05	7.2e-06
Flagellin B, 0-84, <i>H.mustelae</i>	0.3	0.04	2.9e-05
Flagellin A, 0-56, <i>H.mustelae</i>	0.26	0.05	3.7e-05
Flagellin, 28-84, <i>B.pseudomallei</i>	0.26	0.05	1.2e-03



logo

FLAA HELMU mustelae 1....
 FLAB HELMU mustelae 1....
 FLAB HELPY pylori 2.....
 H7C7G3 pseudomallei 2....
 P11089 burgdorferi 1.....
 FLAB HELMU mustelae 2....
 consensus

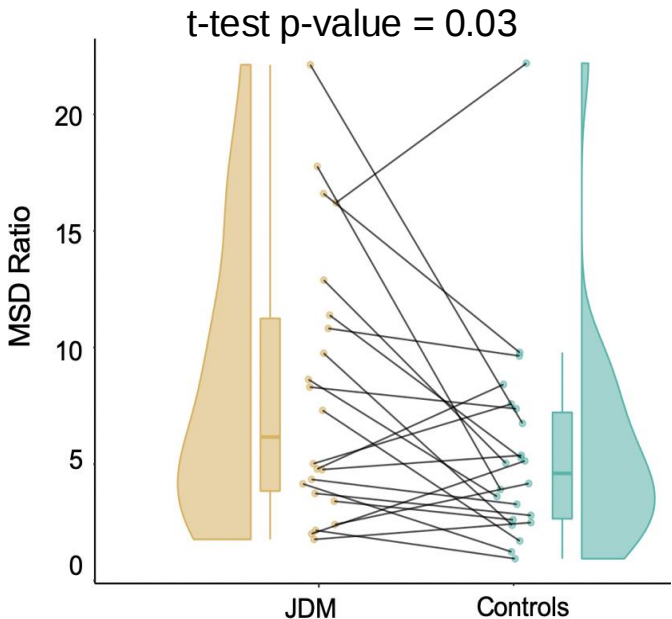


logo

FLAA HELMU mustelae 1....3
 FLAB HELMU mustelae 1....4
 P11089 burgdorferi 1.....2
 consensus



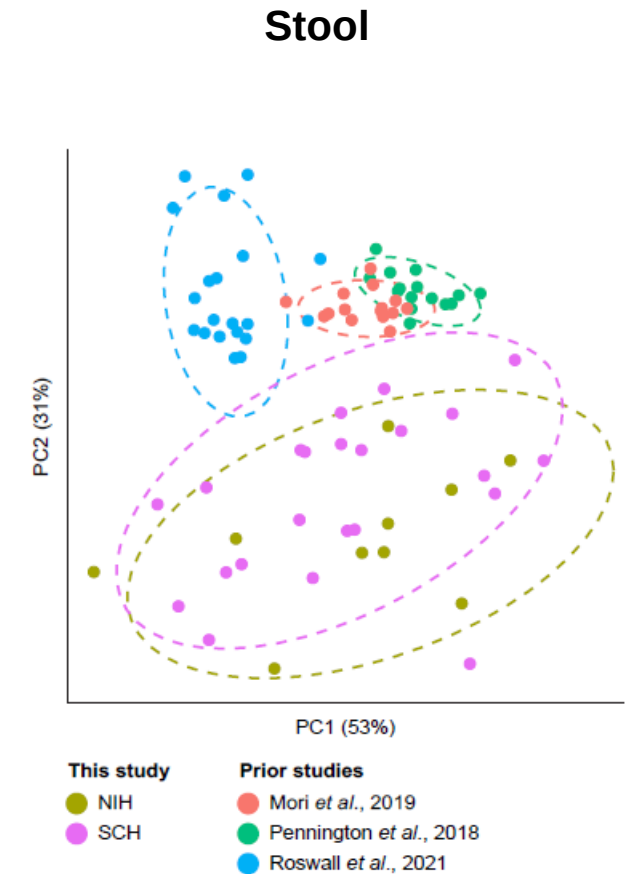
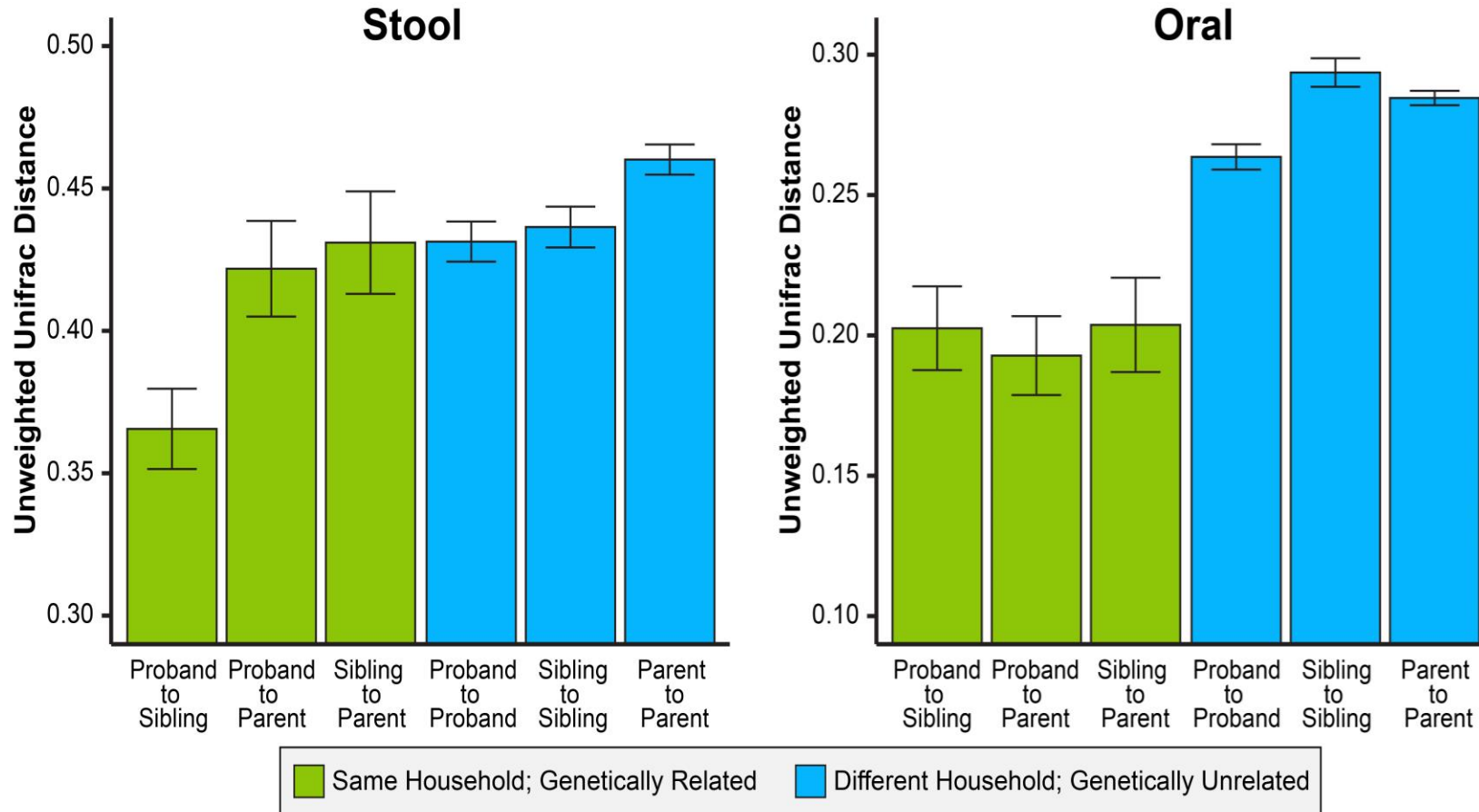
[X] non-conserved
 [X] ≥ 50% conserved
 [X] ≥ 75% conserved



11/23 JDM are Flagellin pos.
 → All children ≤10 years,
 9 + for anti-TIF1γ Abs;

 4/23 controls Flagellin pos.
 → All siblings/twins from
 same household

Familial Clustering of Dysbiotic Fecal and Oral Microbiomes in JDM

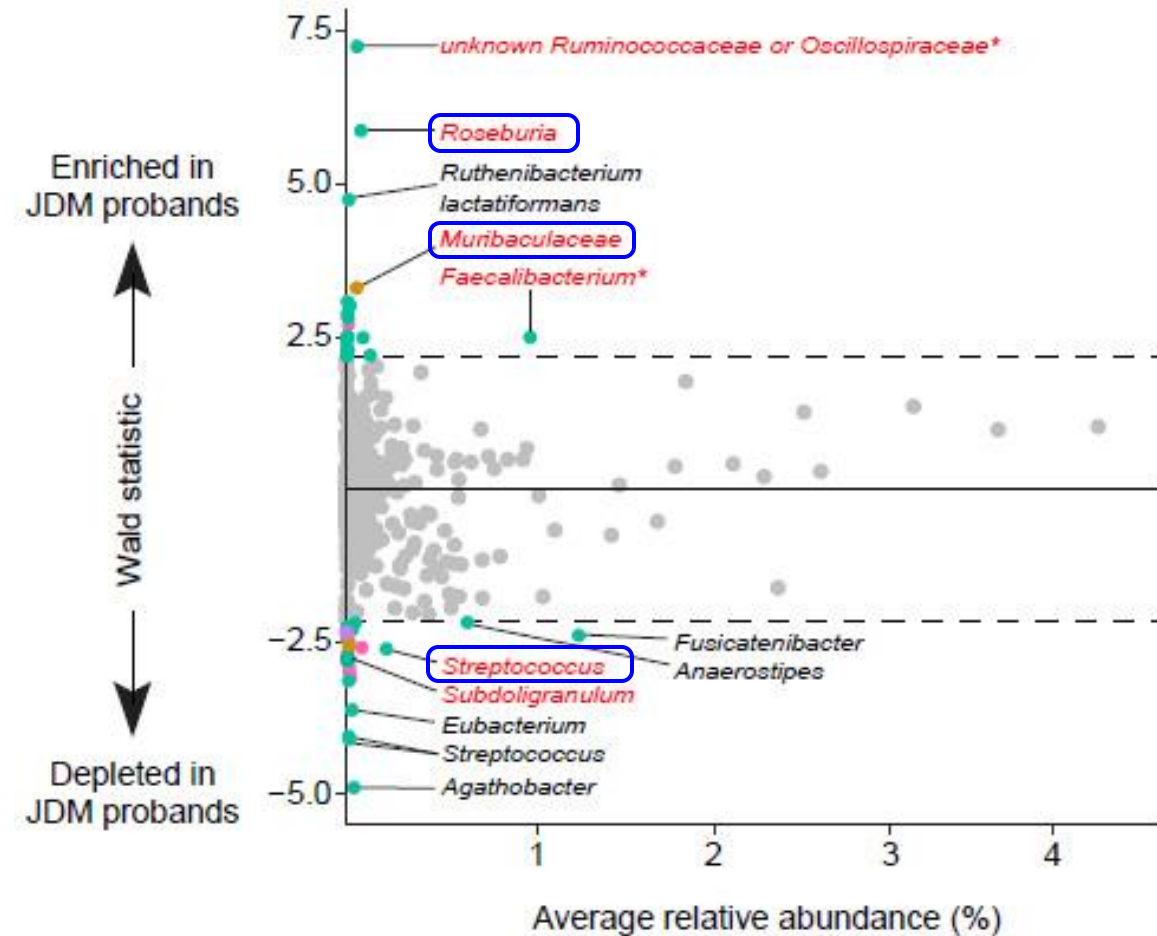


- Fecal microbiome of JDM probands more similar to healthy siblings than to other JDM probands or their parents; unrelated JDM probands more similar than among unrelated parents
- Oral microbiome shared within families, but unrelated JDM probands more similar than among unrelated siblings or parents

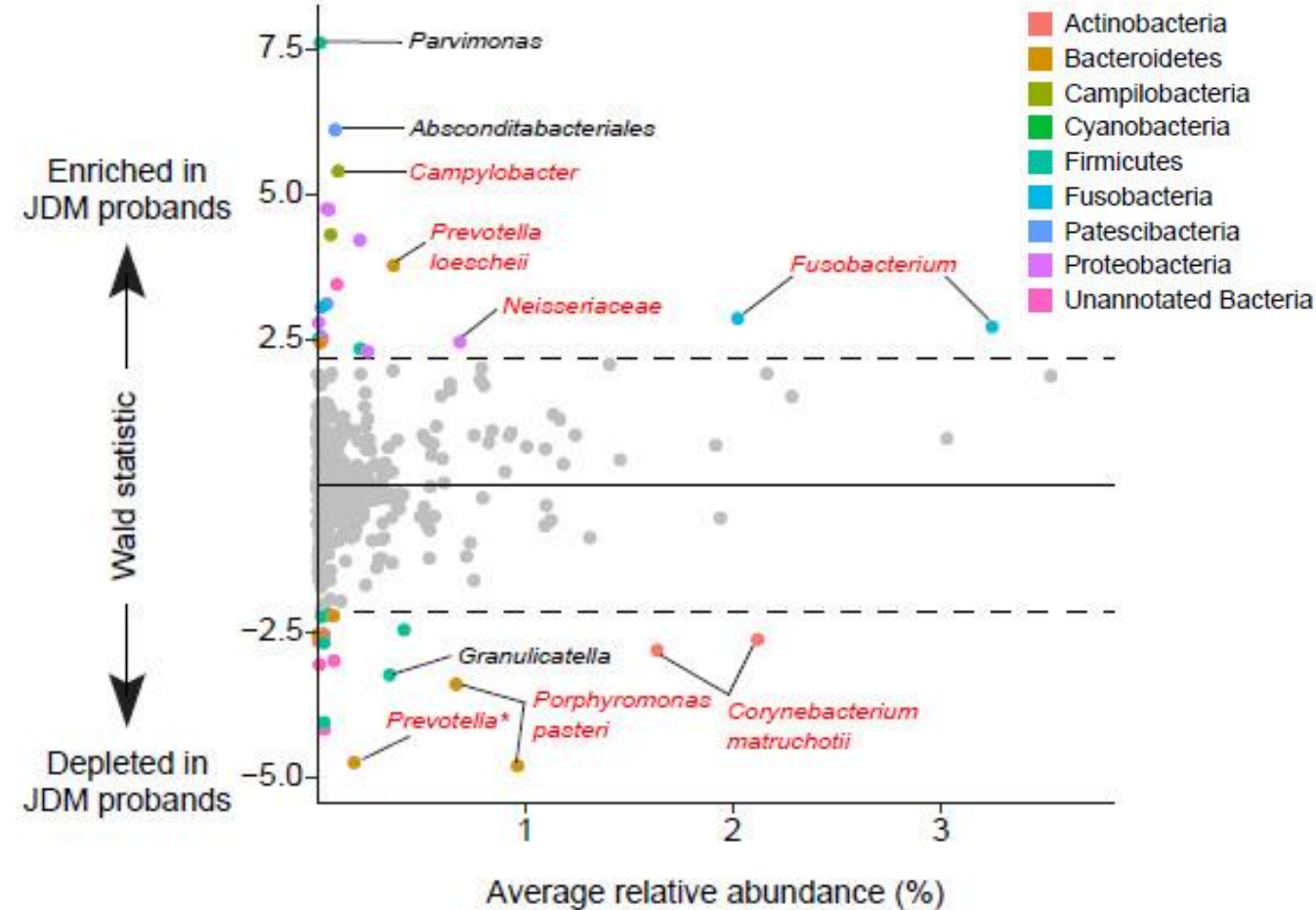
Gut microbiomes differ by center, but differ most from other healthy cohorts

Dysbiotic Fecal and Oral Microbiome Species in JDM are Pro-inflammatory, Shared with other AiDs

Stool



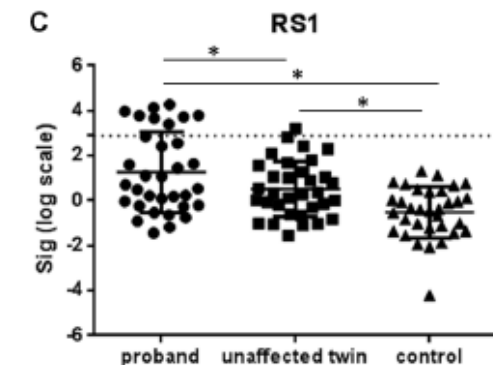
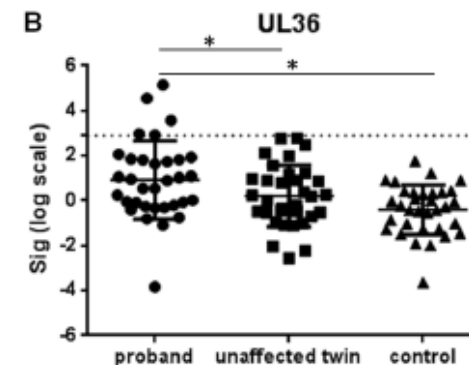
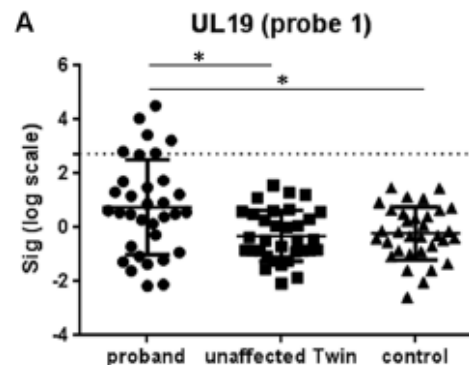
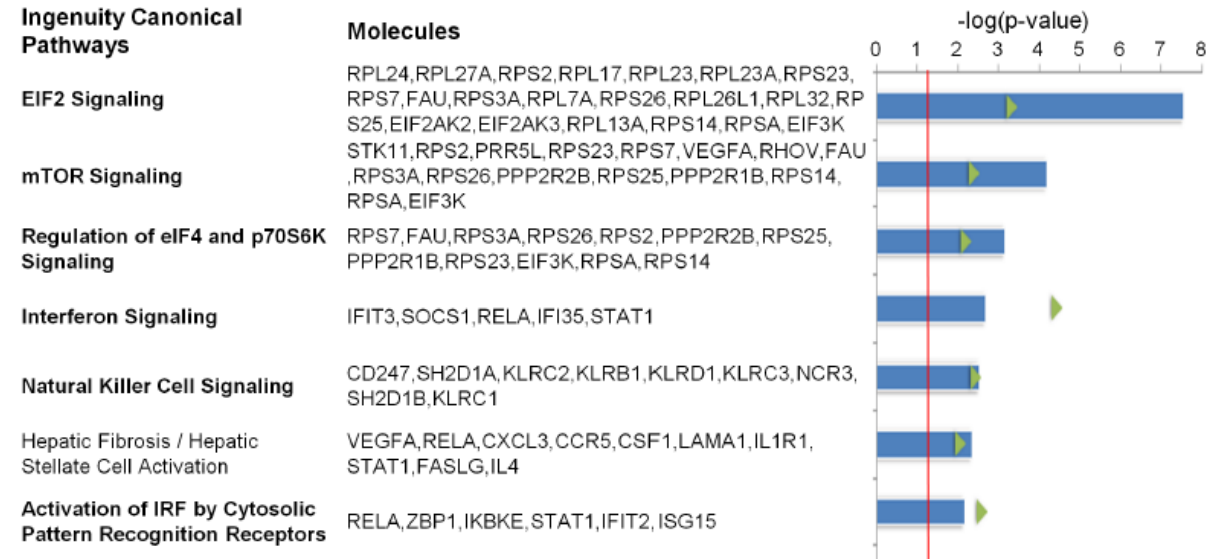
Oral – anterior subgingival dental plaque



4% ASVs in stool, 8% ASVs in oral differentially abundant between JDM probands and healthy siblings

Anti-Viral Pathways and HSV-2 Viral Exposure Shared Across SAiDs

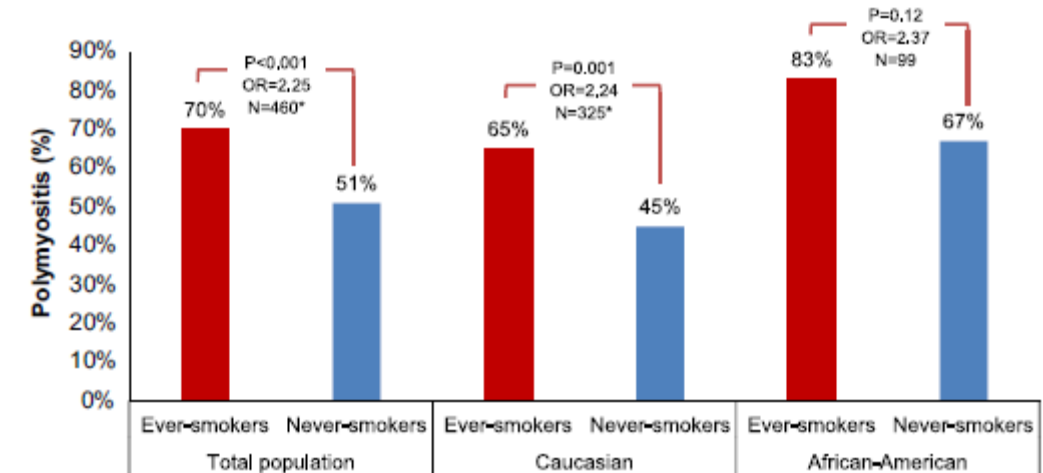
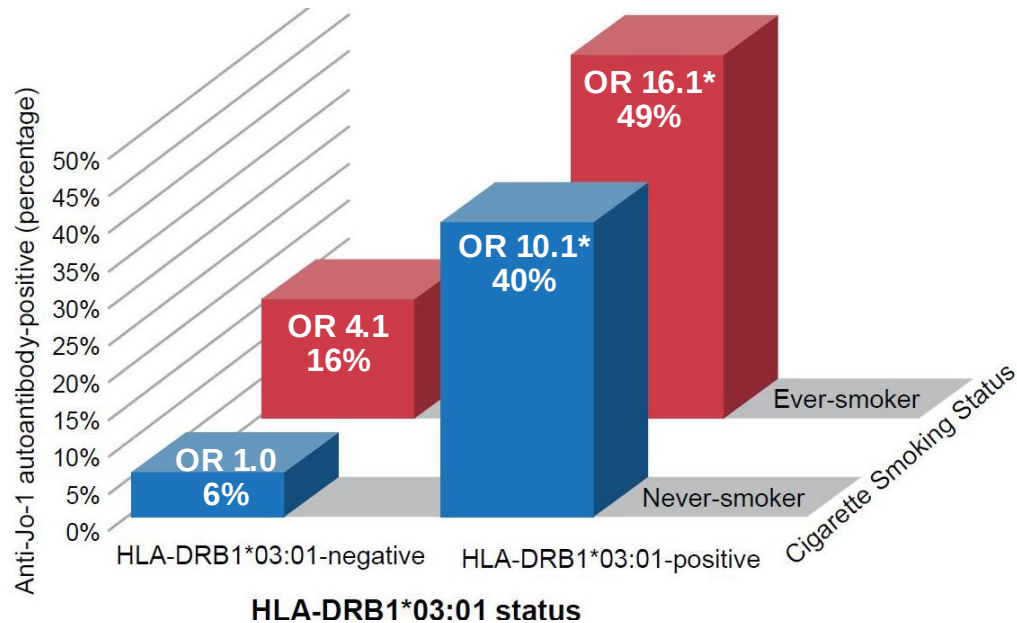
- 33 twins discordant for SAiD:
15 IIM, 12 RA, 5 SLE, 1 SSc,
75% juvenile
- Anti-viral response (IFN signaling)
and other inflammatory pathways
upregulated
- Viral gene expression for known
viral genes in 54 virus families:
64 viral probes from 40 viral families
distinguished SAiD probands from
unaffected sibs and healthy controls
 - Unaffected siblings intermediate
between probands and HCs
 - No difference in EBV, parvovirus,
varicella
- HSV-2 genes: 3 genes with different
stages viral infection, $\uparrow$ in probands



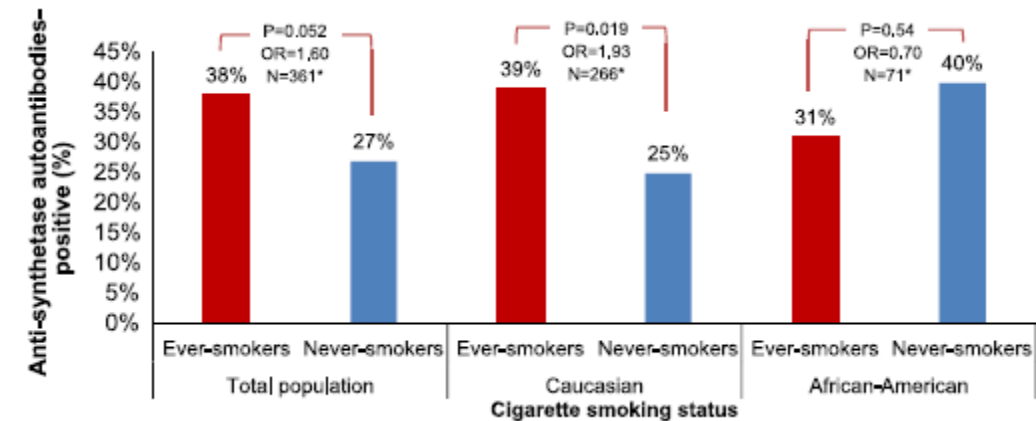
Tobacco Smoking Associated with Clinical Phenotypes and Anti-Synthetase Syndrome in DRB1*03:01+ Caucasian Patients



- Ever smokers more likely to have PM than DM and ILD, in Caucasian patients, not African American, associated with pack years of smoking
- Ever smokers more likely to have anti-synthetase and anti-Jo1 Abs, less likely to have anti-TIF1 γ Abs, in Caucasians
- Ever smokers with HLA-DRB1*03:01 had highest odds of PM, ILD, ASS, and anti-Jo1 Abs



Cigarette smoking status



Cigarette smoking status

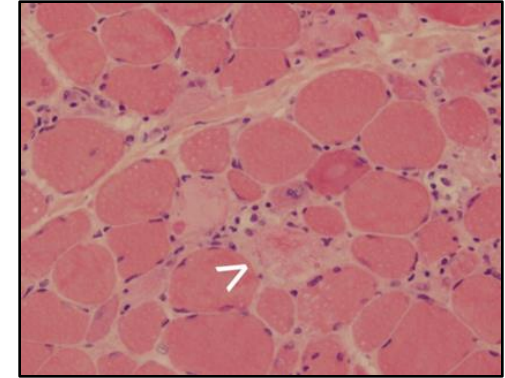
Anti-HMG CoA Reductase (HMGCR) Associated with IMNM, Statin Exposure and HLA DRB1*1101 in Adults

- 6-22% of adults with Immune-Mediated Necrotizing Myopathy (IMNM):

- Myofiber necrosis, minimal inflammation, regeneration, no DM rashes
- 2/3 with known statin exposure (drug or dietary), 80% > 50 years: symptoms persist/progress after statin withdrawal
- HLA DRB1*11:01 strong risk factor for anti-HMGCR Ab+ in adults with statin exposure (OR 4.92, 95% CI 2.52 – 9.97)

- In children, 1% with anti-HMGCR Ab+ IMNM

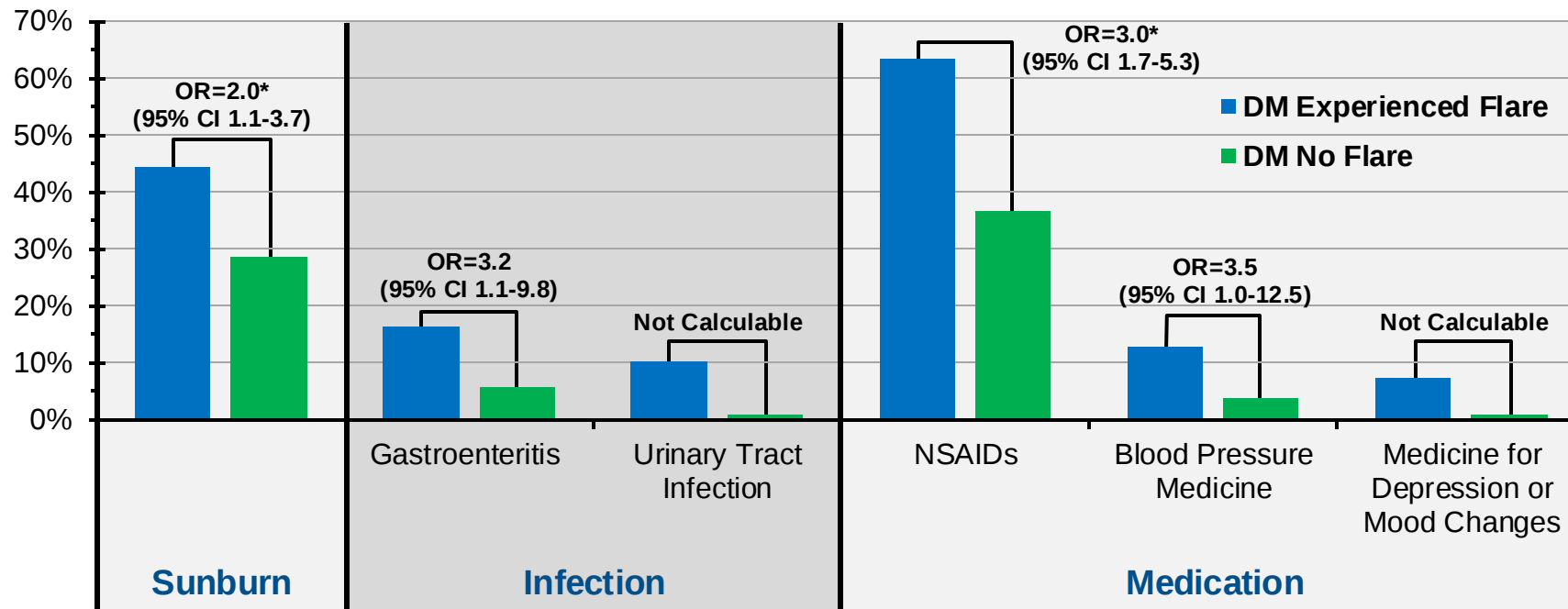
- Necrotizing myopathy on biopsy (IMNM)
- Clinical features similar to adults: myalgias, severe proximal and distal weakness, high CK, muscle atrophy, joint contractures, arthralgias
- No association with statin medications or dietary statins
- HLA DRB1*07:01-DQA1*07:01 haplotype strongly associated with anti-HMGCR Ab+ in juveniles



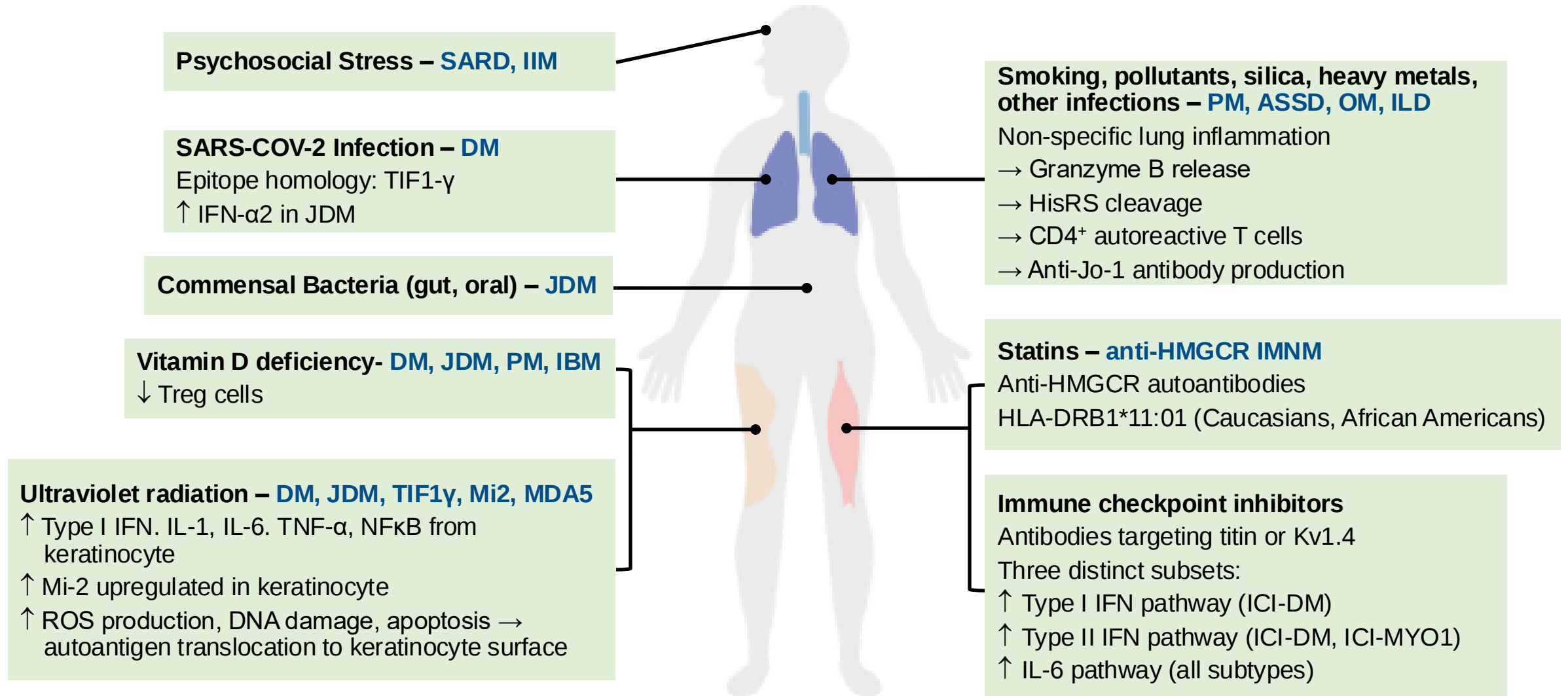
Environmental Factors Associated with JDM/DM

Initiation also Associated with Flares

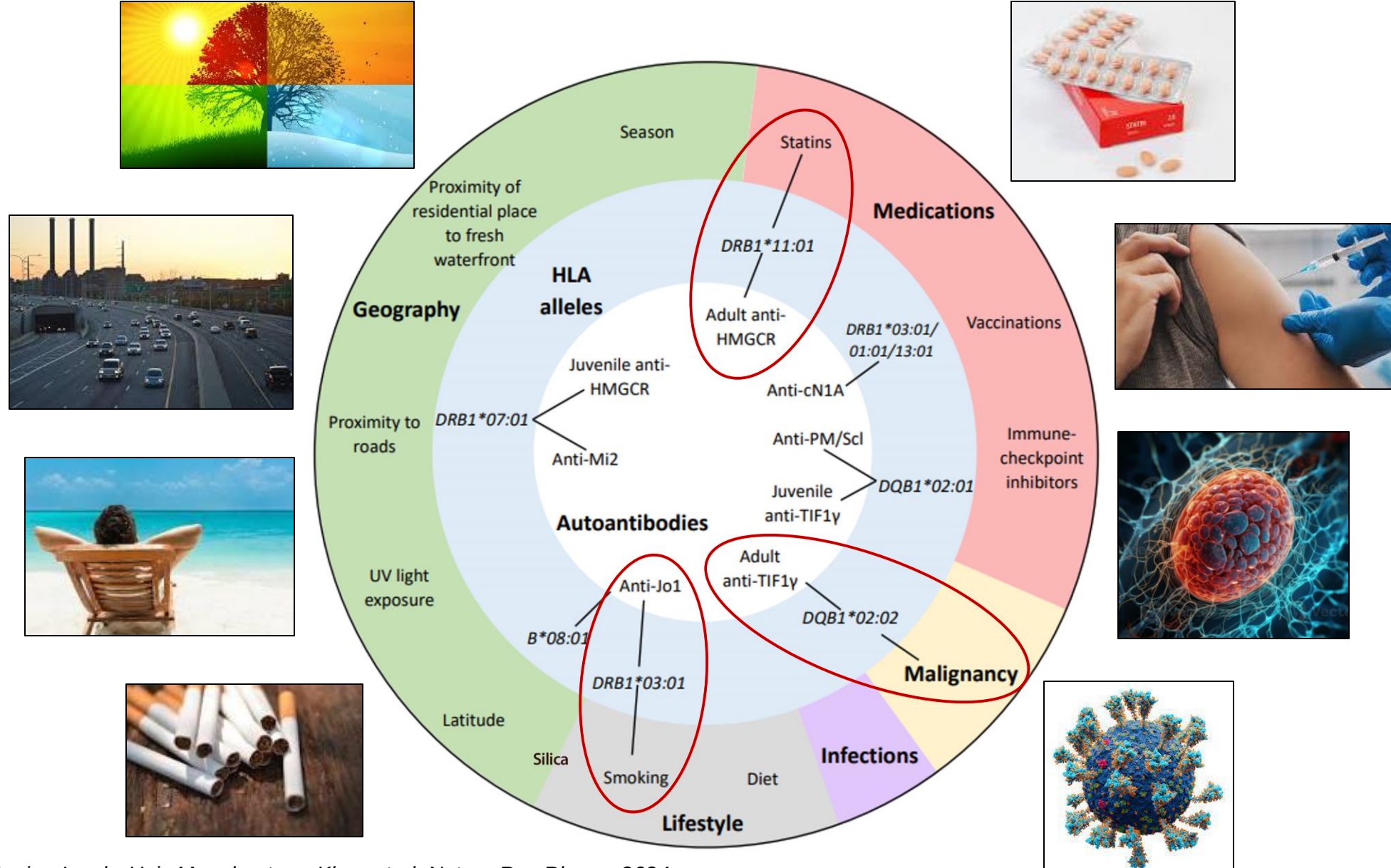
- Questionnaire study of 210 patients (164 JDM, 46 adult DM), 64% flared within 2 years
- Flare defined as increasing DM signs/symptoms (confirmed by physician), and required increase or addition of new medication
- Sunburn (red or painful, ≥ 1 day) and NSAID use within 6 months of flare were significant risk factors in multivariable analysis



Environmental Risks for Myositis – Key Findings



Gene – Environment Interactions in Myositis



Genetic and Environmental Risk Factors for Myositis

What Have We Learned?

- Genetic risk for autoimmune disease is important, but the rapid rise in autoimmunity/autoimmune diseases and other data implicate a strong role for environmental factors in myositis and other autoimmune diseases
- HLA 8.1 haplotype genes and alleles are major risk factors for many myositis clinical and autoantibody subgroups. GWAS suggest additional immune activation and cell signaling genetic risk factors and pathways to myositis that are shared with other AiD, and have implications for novel targeted therapies
- Environmental risk factors for myositis phenotypes include UV radiation, stress, certain infections/ commensal bacteria, medications, and in adults- tobacco use and occupational exposure to silica, heavy metals and solvents
 - Gene-environment- myositis autoantibody interactions beginning to be identified
- Certain exposures that are risk factors for development of myositis and specific phenotypes may also influence disease course and disease flares

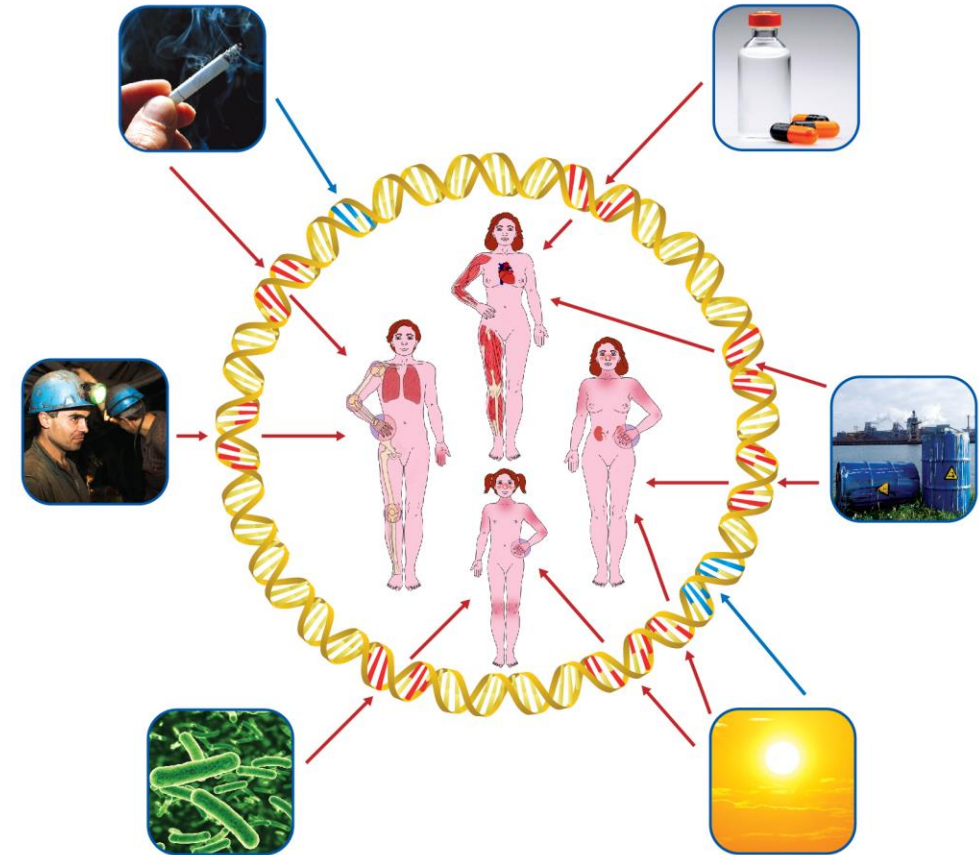
Future Directions: Risk Factors and Pathogenic Pathways

- **Genetic Risk Factors**

- WES/WGS of multiplex myositis families and singletons, including specific MSA groups

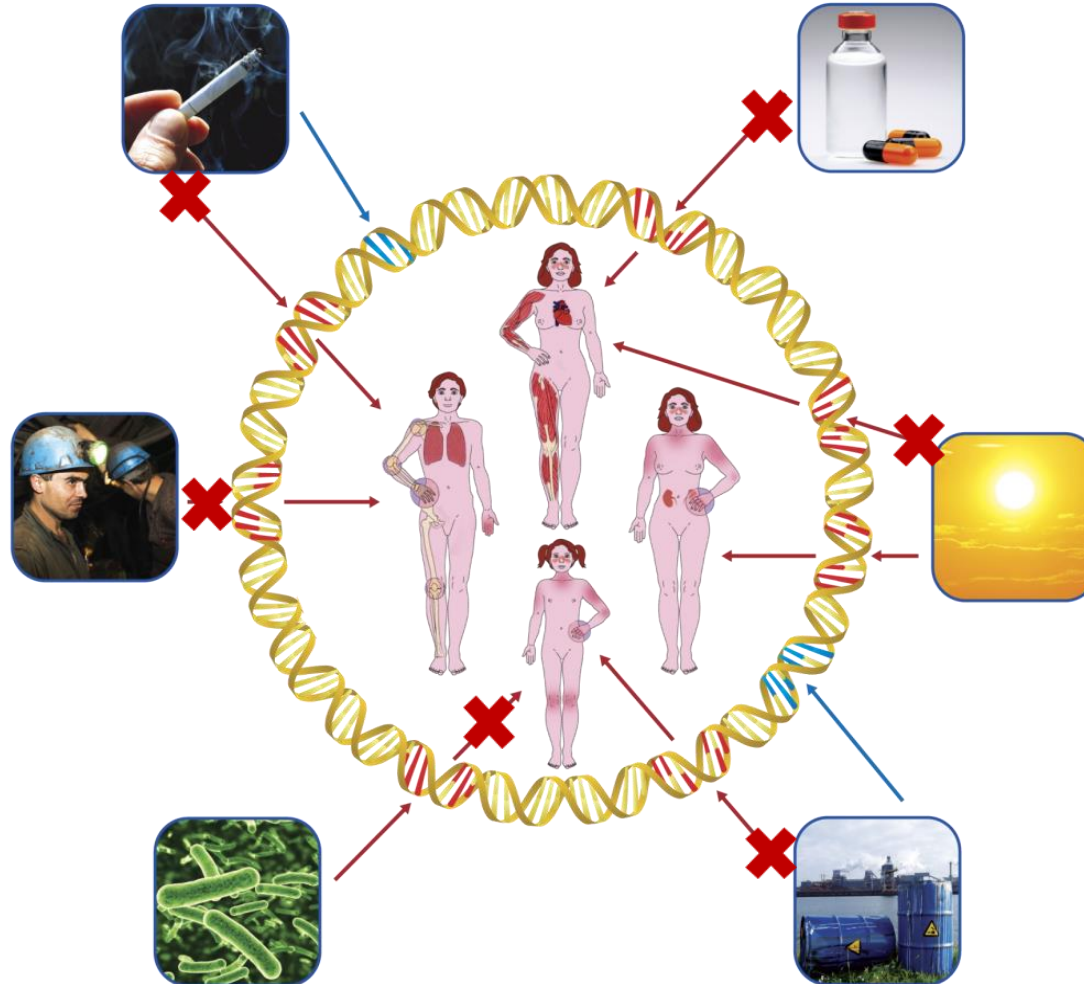
- **Environmental Risk Factors**

- Twin Sib, MYORISK, and MYOVISION exposure questionnaire data to identify risk factors
- Environmental, Genetic and GXE risk scores
- Exposomics and Metabolomics – Twin Sib study
- GIS analysis – MYOVISION
- Examine genome-wide epigenetic changes in immune response and metabolism genes in discordant twins – siblings
- New treatment trial focused on dietary intervention



Possible Outcomes of Enhanced Studies of Myositis and Autoimmune Disease

New approaches and technologies to carefully define myositis and autoimmune disease phenotypes and identify environmental and genetic risk/protective factors may lead to new disease pathways, better treatments, and possibly enable future prevention



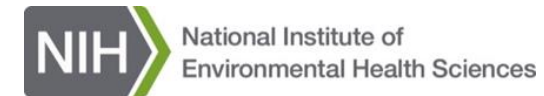
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