Autoantibodies in Idiopathic Inflammatory Myopathies

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Idiopathic Inflammatory Myopathies (IIM)

- **Heterogeneous group of systemic *autoimmune* syndromes characterized by *chronic muscle weakness* and striated *muscle inflammation***

- **Polymyositis (PM)**
- **Dermatomyositis (DM)**
- **Inclusion body myositis (IBM)**
- **Necrotising Autoimmune Myositis (NAM)**

Clinical features

- **Systemic conditions with predominant manifestations on skeletal muscle**
    - ***Muscle***
    - Joints
    - Lungs – interstitial lung disease
    - GIT
    - Cardiac
- **Patterns of muscle weakness**
    - PM/DM: symmetrical proximal upper and lower limbs, neck flexors
    - IBM: (asymmetrical) quadriceps weakness ≥ hip flexors, long finger flexors
    - dysphagia
    - DM: cutaneous features

Diagnosis of IIM

- **Clinical presentation**
- **Raised serum muscle enzymes – CK**
- **Electromyography: myopathic triad**
- **Muscle biopsy**
  - **definitive diagnostic test**
  - **Used to categorise disease**

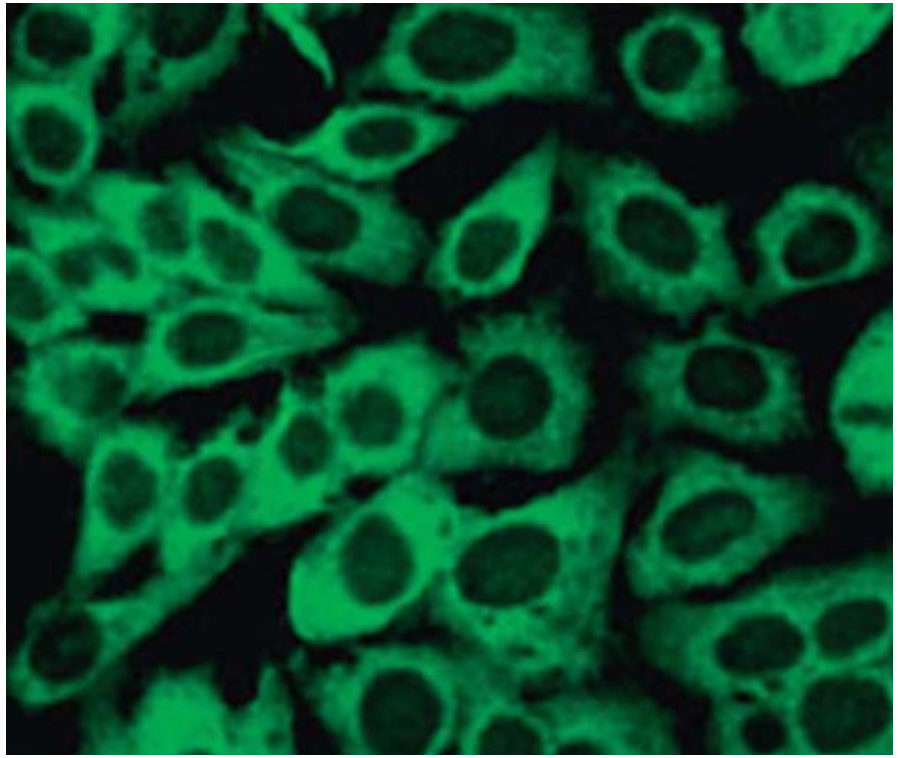
- ***Increasing interest in the role of autoantibodies in classification and prognostication***

Role of antibodies in IIM

- **Directed to nuclear and cytoplasmic antigens involved in protein synthesis**
- **Several strong associations between autoantibodies and clinical phenotypes**
- **Diagnostic markers for disease**
- **Divide patients into homogeneous subgroups**

- **Proposals for serological classification of IIM**

Negative ANA Does Not Imply Antibody Negativity



Homogeneous, diffuse
cytoplasmic staining

Dimitri, Muscle and Nerve, 2007

Myositis-Associated Autoantibodies

Autoantibody	Antigen	Clinical
PM-Scl	Unidentified	PM/ DM/ SSc overlap syndrome
U1-RNP	U1 small RNP	MCTD
Ro52	RNA protein TRIM21	IIM, pSS, SLE & ILD
Ku	DNA-binding proteins	DM/PM with SLE/SSc overlap

Myositis-Specific Autoantibodies

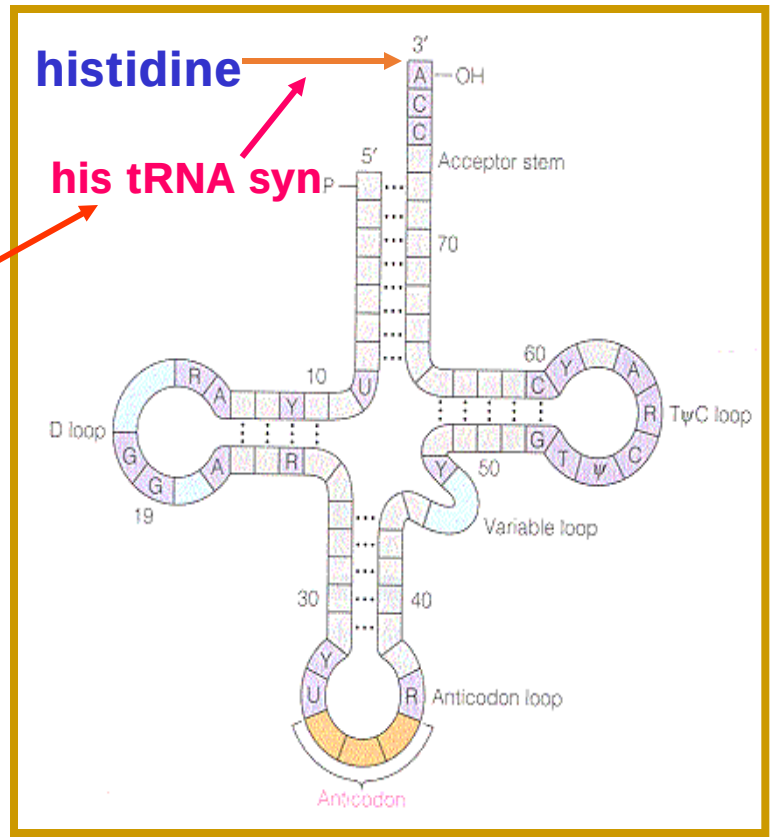
Antibody	Target	Subset	Phenotype
Synthetases	ARS	PM/DM	Anti-synthetase syndrome
Mi-2	NuRD	DM	Shawl, V-neck, Gottron's
SRP	SRP 72, 54 kDa	PM/NM	Severe/refractory NM
SAE	SUMO	DM	ILD, dysphagia
NXP2	NXP-2	JDM	Calcinosis, ulceration
TIF-1γ	TIF1γ (p155/140)	DM, JDM	Severe skin, malignancy
MDA-5	MDA-5	DM	Amyopathic, ILD
HMGCR	HMGCR	IMNM/NAM	Necrotizing myopathy

Anti-synthetase antibodies

Anti-synthetase Ab	tRNA synthetase	Clinical
Anti-Jo-1	Histidyl	PM, DM +ILD
Anti-PL-7	Threonyl	PM, DM +ILD
Anti-PL-12	Alanyl	ILD> myositis
Anti-EJ	Glycyl	PM>DM +ILD
Anti-OJ	Isoleucyl	ILD +PM/DM
Anti-KS	Asparaginyl	ILD> myositis
Anti-Zo	Phenylalanyl	ILD +PM/DM
Anti-Ha	tyrosyl	ILD +PM/DM

Anti-Jo-1 Autoantibody

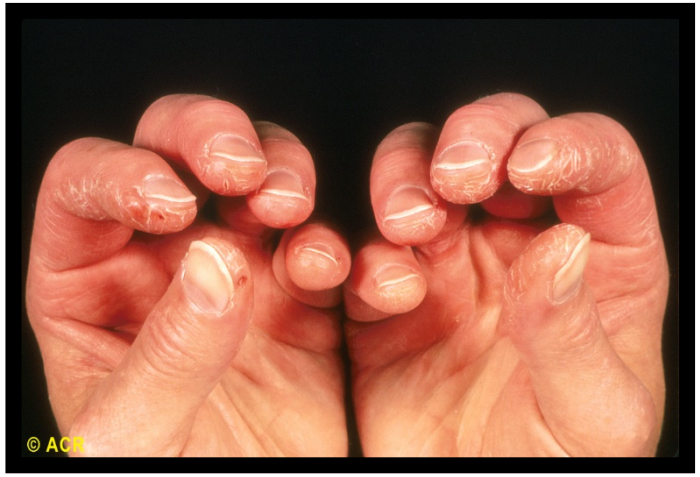
- **Directed against histidyl-tRNA synthetase**
- **Ag: enzyme that catalyzes binding of an amino acid to its tRNA in process of protein synthesis**

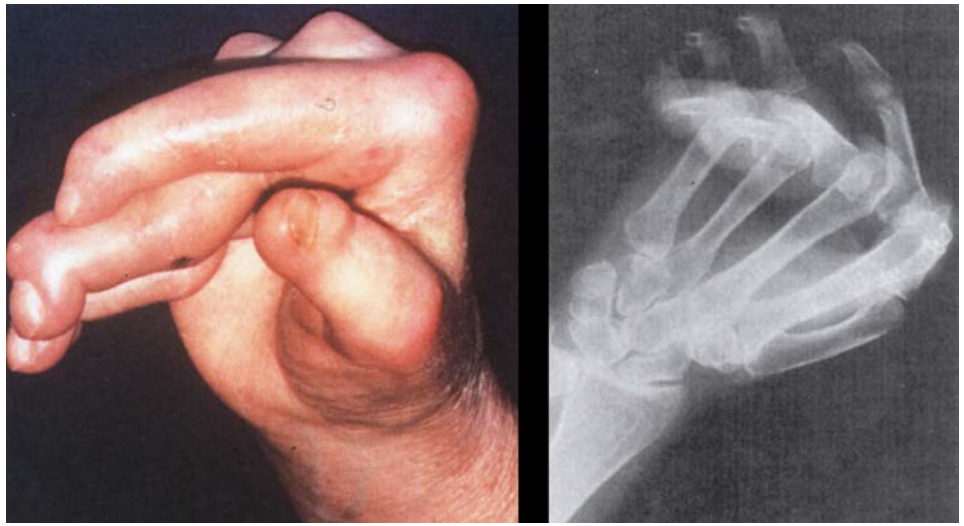


tRNA for histidine

Anti-synthetase syndrome

- **PM or DM**
- **Interstitial Lung Disease**
- **Fever**
- **Arthritis**
- **Raynauds phenomenon**
- **Mechanic’s hands**





Jo-1 versus non-Jo-1 antisynthetases

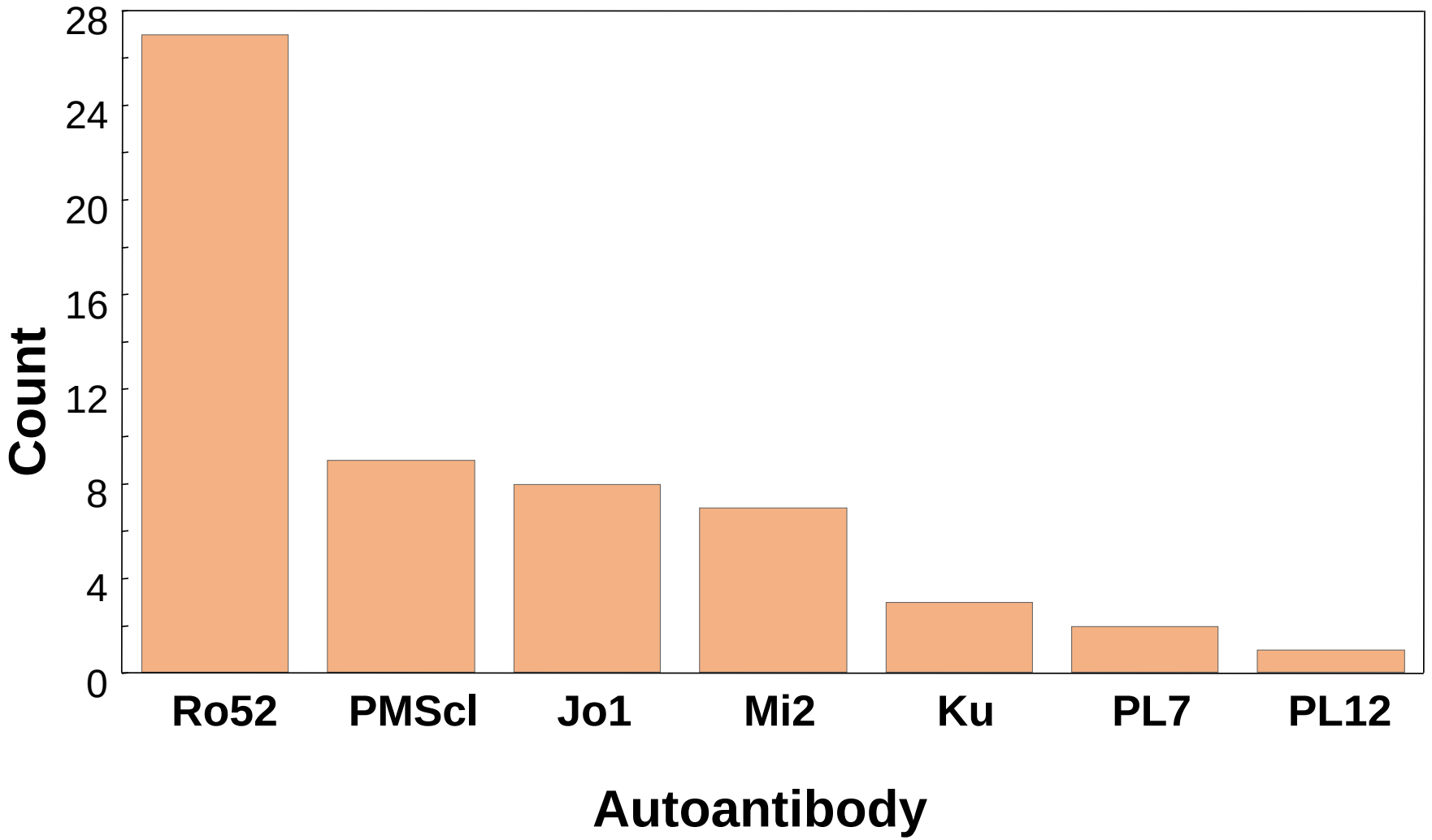
- **Jo-1**
  - **more likely muscle involvement**
  - **arthritis**
- **Non-Jo1**
  - **more likely ILD, CTD overlap**
  - **Raynaud's phenomenon more common**
- **Differences between each of the non Jo-1 antisynthetases**
  - **OJ – arthritis prominent, ILD then myositis**
  - **EJ: Heliotrope, Gottrons**
  - **KS: increase CK**

South Australian Myositis Database – Autoantibodies detected in 32%

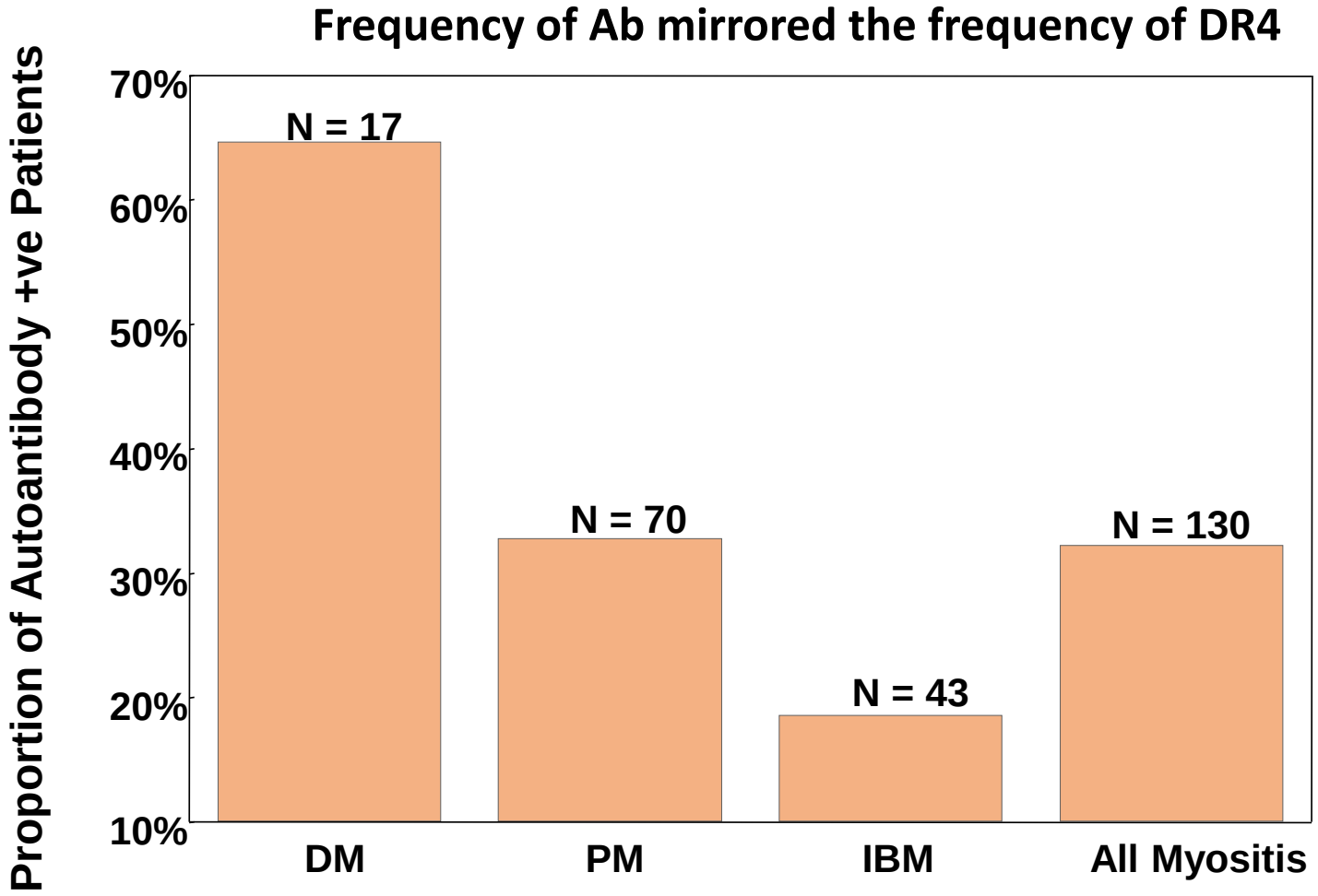
- **Biopsy-proven cases of IIM subsequent to 1980**
- **Central reporting of all adult muscle biopsies in SA in Neuropathology Lab**
- **DM, PM, IBM, necrotising myopathy**
- **DNA and serum stored**

- **Autoantibodies present in 42/130 (32%) myositis patients**

Antibodies to Ro52 were the commonest



Ab are more common in DM than PM or IBM



DM (11/17) cf PM (23/70), p = 0.033

DM cf IBM (8/43 =p = 0.002)

Myositis Ab are associated with both HLA DR3 and DR4

Previous reports - linked DR3 with MSA formation in IIM patients.

(Arnett FC, Arthritis Rheum 1996;39(9):1507-18)

DRB1	Autoantibody		Odds Ratio	P value
	Pos (n=37)	Neg (n=81)		
DRB1*03	23 (31%)	23 (14%)	3.3 (1.7, 6.6)	0.0005
DRB1*04	13 (18%)	12 (7%)	3.6 (1.5, 8.6)	0.004
Other	38	127	1	

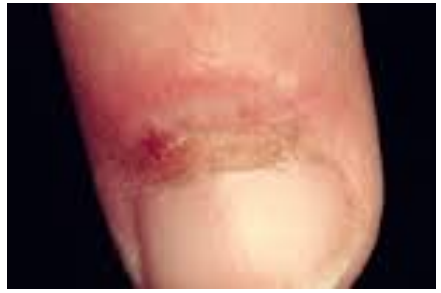
DR3 and DR4 are both systematically assoc with autoantibody production in IIM

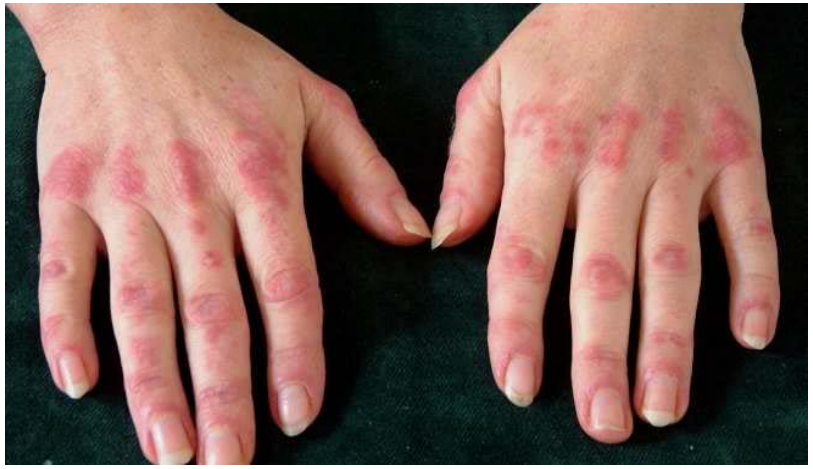
Limaye V et al, Rheumatol Int. 2012;32(3):611-9

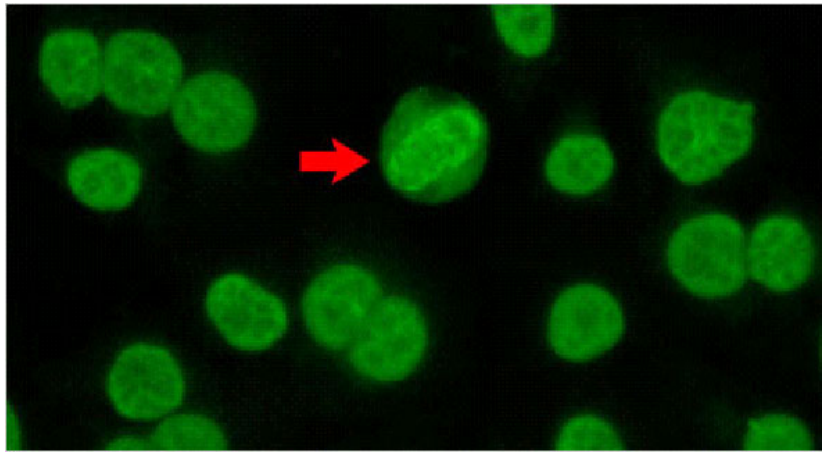
Antibodies in DM

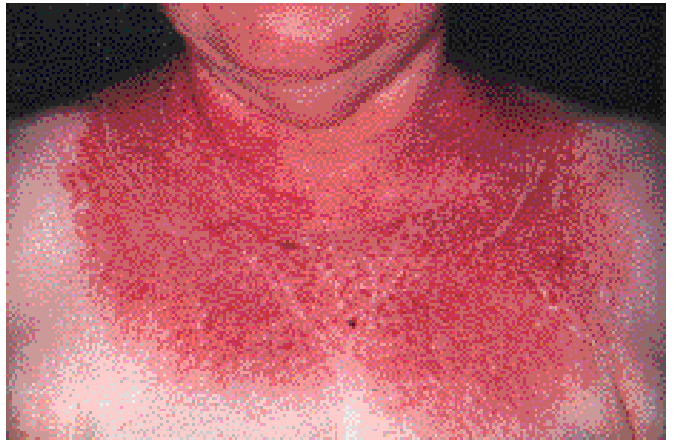
Anti-Mi2

- 11-59% prevalence in DM
- Skin manifestations
- relatively mild disease
- less internal organ involvement
- treatment response - fair
- latitudinal gradient (UV intensity)









Novel Autoantibodies in DM

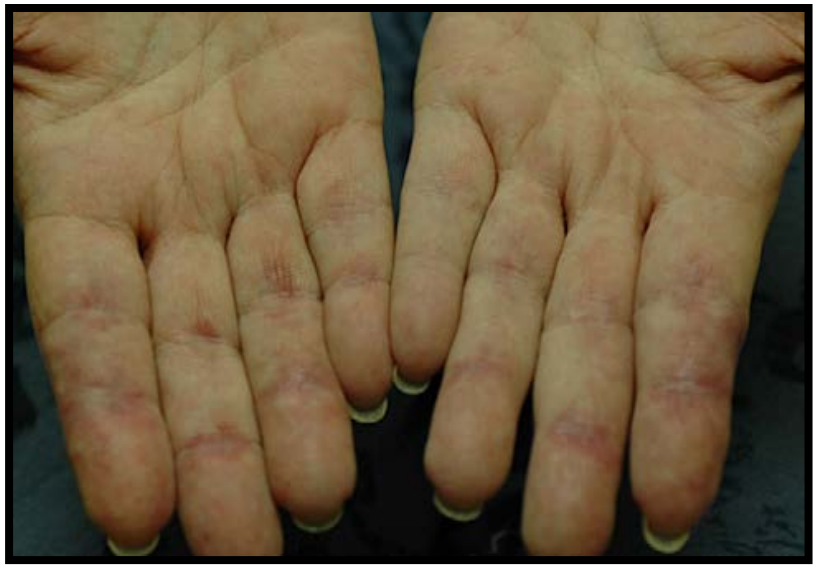
- **Ab in DM often assoc with distinct clinical phenotypes**
- **Tend to be mutually exclusive**
  - **specific immune responses may shape different phenotypes**

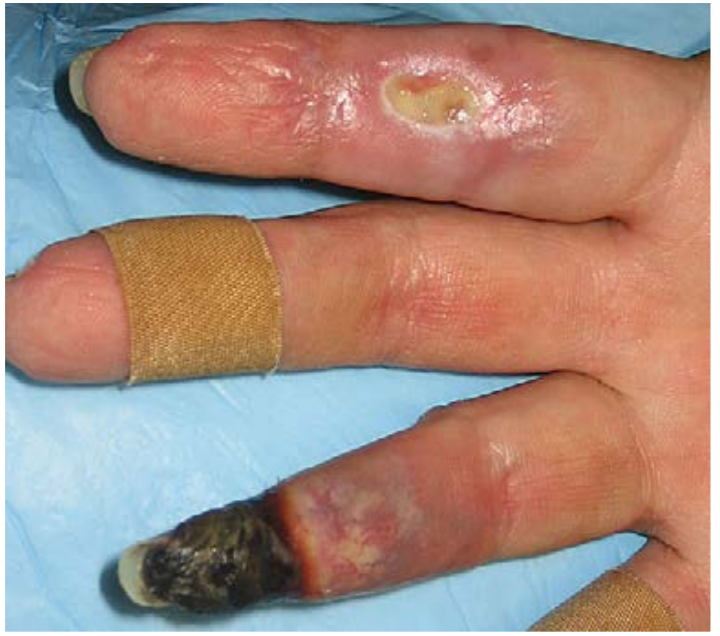
- **MDA5**
- **TIF1 ϒ**
- **NXP2**
- **SAE**

Antibodies to MDA5

- **Target antigen: melanoma differentiation-associated gene 5**
- **10-48% Asians, 0-13% Caucasians**
- **Clinically**
  - ***Rapidly progressive ILD***
    - **Sato, Arthritis Rheum 2005**
  - ***Novel cutaneous phenotype***
    - **palmar papules**
    - **cutaneous ulcerations**
    - **severe vasculopathy**
  - **Amyopathic DM**
- **HLA–DRB1*08**

Clinical phenotype of IIM with Anti-MDA5





Fiorentino, J Am Acad Derm, 2011

Antibodies to TIF1Ɣ

- **Target antigen: transcriptional intermediary factor 1-Ɣ**
- **Originally reported as anti-p155/p140**
- **13-31% DM**
- **Adults: Ca-associated DM**
  - **Sensitivity for Ca 78%**
  - **specificity for Ca 80%**
- **Less Raynauds, calcinosis and ILD**
- **Juvenile DM : no malig but skin ulceration**
- **DQA1*0301 association**

Antibodies to TIF1Ɣ: manifestations according to age

Children **young adults** **older adults**

Ulceration/ vasculitis **rash** **malignancy**

Amyopathic **myositis**

Frequency of Anti-MDA5 and Anti-TIF1ϒ

	Age	Mean (SD)	M:F	Total	Anti-MDA5 (%)	Anti-TIF1g (%)
DM	3-84	50 (19)	24:58	82	21 (26)*	12 (15)
CADM	3-84	48 (20)	7:24	31	20 (65)**	3 (10)
CA-assoc DM	48-80	66 (11)	5:7	12	0	7 (58)***
Classical DM	16-76	47 (17)	12:27	39	1 (3)	2 (5)
PM	32-70	57 (14)	0:6	6	0	0
SLE	15-76	50 (15)	5:16	21	0	0
SSc-ILD	30-75	58 (10)	3:23	26	1 (4)	0
Controls	46-72	54 (6)	4:16	20	0	0
Cancer	48-78	58 (7)	5:15	21	NA	0

*P<0.05 in DM vs SLE, SSc-ILD, healthy controls. **P<0.005 in CADM vs ca-assoc DM or classical DM without cancer by a chi-square test. ***P<0.005 in cancer-associated DM vs CADM or classical DM without cancer

Hoshino K et al, Rheumatology, 2010

Antibodies to NXP2

- **Ag: 140kDa nuclear matrix protein-2**
- **Frequency**
  - **<5% adult DM**
  - **JDM 23-25%**
- **Most frequent Ab in Italian cohort (17%)**
- **In JDM:**
  - **↑ risk of calcinosis**
  - **↑ disease severity**

Gunawardena H. Arthritis Rheum 2009

Ceribelli A. Arthritis Res Ther 2012

Antibodies to SAE

- **Ag: small ubiquitin like modifier activating enzyme (SAE)**
- **Frequency**
  - **<5% adult DM**
  - **<1% JDM**
- **Clinically**
  - **Often cutaneous features first**
  - **Mild muscle involvement**
  - **dysphagia**
- **Low freq malignancy and ILD**
- **HLA-DRB1*04-DQA1*03-DQB1*03**
  - **Betteridge ZE. Ann Rheum Dis 2009**

Low frequency of novel antibodies

Hungarian cohort IIM n=337

- **12 anti-TIF1g**
- **4 anti-NXP2**
- **4 anti-SAE**
- **0 anti-MDA5**

- **Bodoki L et al Autoimmune Rev 2014**

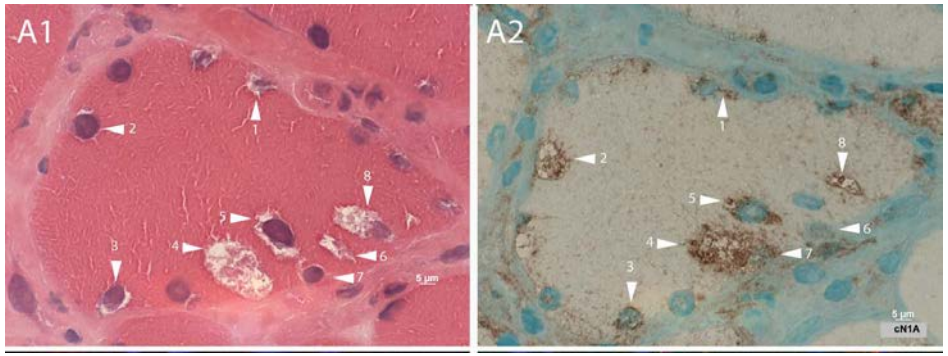
SA Myositis Registry n=193

- **3 TIF1g**
- **1 anti-NXP2**
- **0 anti-MDA5**

- **Together with Neil McHugh, Zoe Betteridge, Bath, UK**

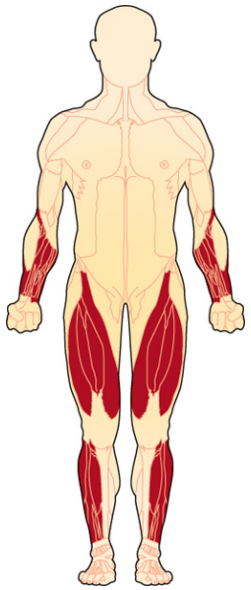
Antibodies in IBM

Anti-cN1A



- 43kDa muscle autoantigen: cytoplasmic 5'-nucleotidase 1A (cN1A)
- Strengthens role for B-cell mediated autoimmunity in IBM
- IgG anti-cN1A :>90% specificity and 34-70% sensitivity in IBM
    - Salajegheh M, PLoS One 2011
- Detection of multiple isotypes increased sensitivity to 76%
    - Greenberg SA Muscle Nerve 2014
- cN1A accumulates in perinuclear regions and rimmed vacuoles in IBM muscle and localises to areas of myonuclear degeneration
- ? Provide a link between dual processes of autoimmunity and myodegeneration
- ? Biomarker for IBM

Larmen B et al Ann Neurol 2013



Anti-cN1A: SA Myositis Database

- **Detected in 24/69 (35%) patients with IBM***
- **IgM isotype most frequent (n=17), IgG (n=13) and IgA (n=5)**
- **No gender difference: Ab+ve 15/24 female, Ab neg : 27/45 female**
- **No diff in frequency of malignancy in patients with anti-cN1A (3/20) compared to those without (10/39), p=0.51**
- **Antibodies to other MSA/MAA were present in a minority (8/56) of patients with IBM and were significantly less prevalent than anti-CN1A (p=0.01)**

Limaye V et al, Muscle Nerve. 2016 53(4):654-5

Necrotising autoimmune myositis

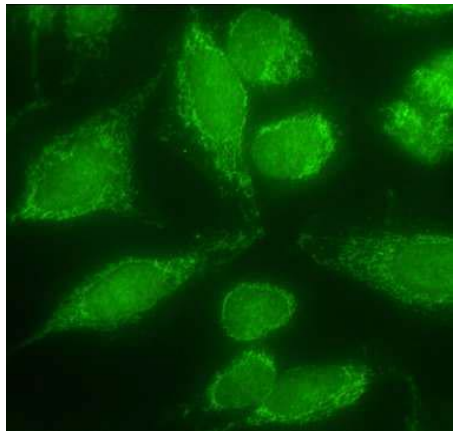
anti-SRP
anti-HMGCR

Antibodies to signal recognition particle (SRP)

- **Ribonucleoprotein –targets secretory proteins to endoplasmic reticulum**
- **Anti-SRP detected in 4-6% of patients with myositis**

- **Clinically**

  - **Rapidly progressive weakness**
  - **marked elevation of CK**
  - **Cardiac involvement**
  - **Muscle biopsy typically shows necrotizing myopathy**
  - **Traditionally - poor prognosis/ response to treatment**



Anti-HMGCR and necrotising autoimmune myositis

- **Statins can trigger an immune-mediated necrotizing myopathy which persists despite statin discontinuation**
  - **suggests immune mechanisms involved**

- **Statins up-regulate HMGCR**
- **Regenerating muscle fibres express high levels of HMGCR**
- **linked with anti-100kDa proteins –since identified as HMGCR**

Anti-HMGCR antibodies – what is already known?

- **Detected in 6% of 750 patients with suspected IIM (Johns Hopkins Centre)**
  - **Mammen AL, et al. Arthritis Rheum 2011**
- **Rarely detected in patients on statins with self- limited MSK symptoms**
- **Testing for anti-HMGCR by ELISA**
  - **high sensitivity (94%)**
  - **high specificity (99%)**
- **Levels of anti-HMGCR correlate with CK levels and proximal weakness**
- **Anti-HMGCR persists despite clinical improvement following immunosuppressive therapy.**
- **Testing for anti-HMGCR -proposed to be useful diagnostically in patients with suspected statin–mediated immune necrotizing myopathy**

SA Myositis Database: Anti-HMGCR detected 9% IIM/ NM

- **Detected in 19/207 (9.2%) sera from patients with IIM/NM***

- **Anti-HMGCR was not detected in any of 151 sera from a general reference Western Australian Busselton population.**

Anti-HMGCR is equally distributed among IIM subsets

IIM Subgroup	Anti-HMGCR +ve	Anti-HMGCR -ve
DM (n=26)	1 (4%)	25 (96%)
PM (n=74)	8 (11%)	66 (89%)
IBM (n=62)	6 (10%)	56 (90%)
IIM NOS (n=13)	1 (8%)	12 (92%)
Necrotizing (n=23)	2 (9%)	21 (91%)
Other (n=9)	1 (11%)	8 (89%)
Total (n=207)	19 (9%)	188 (91%)

prevalence of anti-HMGCR was comparable among subsets of IIM (p=0.95).

Associations of anti-HMGCR

Predictor	Anti-HMGCR +ve	Odds Ratio (95% CI)	p-value	PPV (95% CI)	NPV (95% CI)
Statin use	16/52 (31%)	39 (9, 361)	$<10^{-8}$	0.31 (0.19, 0.45)	0.99 (0.96, 1)
No statin use	1/130 (0.1%)				
DR11 positive	10/24 (42%)	50 (11, 486)	$<10^{-8}$	0.42 (0.22, 0.63)	0.99 (0.95, 1)
DR11 negative	1/105 (0.1%)				
DR11+ve & statin	9/10 (90%)	80 (10, 1108)	$<10^{-7}$	0.90 (0.55,1)	0.95 (0.75, 1)
DR11 –ve & statin	1/20 (5%)				
Males	11/77 (14%)	2.5 (1.0, 6.6)	0.079		
Females	8/130 (6%)				

Limaye V et al. Muscle Nerve 2014

Anti-HMGCR antibodies – role of statins?

- **Among anti-HMGCR positive pts, preceding statin exposure in**
  - **24/26 (92.3%) Mammen A et al 2011**
  - **20/45 (44.4%) Allenbach et al Medicine 2014**
  - **16/19 (84%) Limaye et al 2014**

- **Statins** → **↑ HMGCR expression** → **Anti-HMGCR immune response**

DRB1*1101

Statin-naïve patients – what triggers disease?

natural supplements which reduce cholesterol ? Trigger HMGCR expression

Statins found in food products and supplements

oyster mushrooms – lovastatin

red yeast – peking duck glaze

Other environmental triggers

Conclusions

- **Detection of autoantibodies in IIM : role for B-cell mediated autoimmunity**
- **A number of these antibodies are under genetic control**

- **Disease monitoring –Do levels of autoantibodies correlate with disease activity?**
- **Predictive value for development of disease**

- **Precise role in pathogenesis**

Conclusions

- **Antibodies are markers for distinct clinical phenotypes**
- **In clinical practice autoantibodies may help to establish a diagnosis**
- **May prompt**
  - **more intensive therapy**
  - **Screening for associated features eg ILD, malignancy**
- **May enable prognostication**
  - **autoantibodies may correlate with disease outcome**
  - **Differential risk for ILD, malignancy, cutaneous features**

ACKNOWLEDGEMENTS

Neuropathology Dept, SA Pathology
Peter Blumbergs
Sophia Otto
Caroline Smith
Barbara Koszkya

SA Clinicians
Peter Roberts Thomson
Les Cleland
Susanna Proudman
Sally Cox
Sajini Basnayake
SA neurologists

Boston
Steven Greenberg

Anti-cN1A

NXP2, TIF1g, SAE, MDA5

Bath, UK
Neil McHugh
Zoe Betteridge

PathWest, Perth Aust
Peter Hollingsworth
Chris Bundell

Anti-HMGCR

Statistical assistance
Sue Lester

Myositis patients SA