Treatment–resistant Ophthalmoplegia in Myasthenia Gravis

Jeannine Heckmann
Neurology Division
University of Cape Town
South Africa

Mathilda Karel Spak (1901 – 2005)

MYASTHENIA GRAVIS
foundation of california

Founder of MGFC

Mother had MG symptoms x 20 yrs

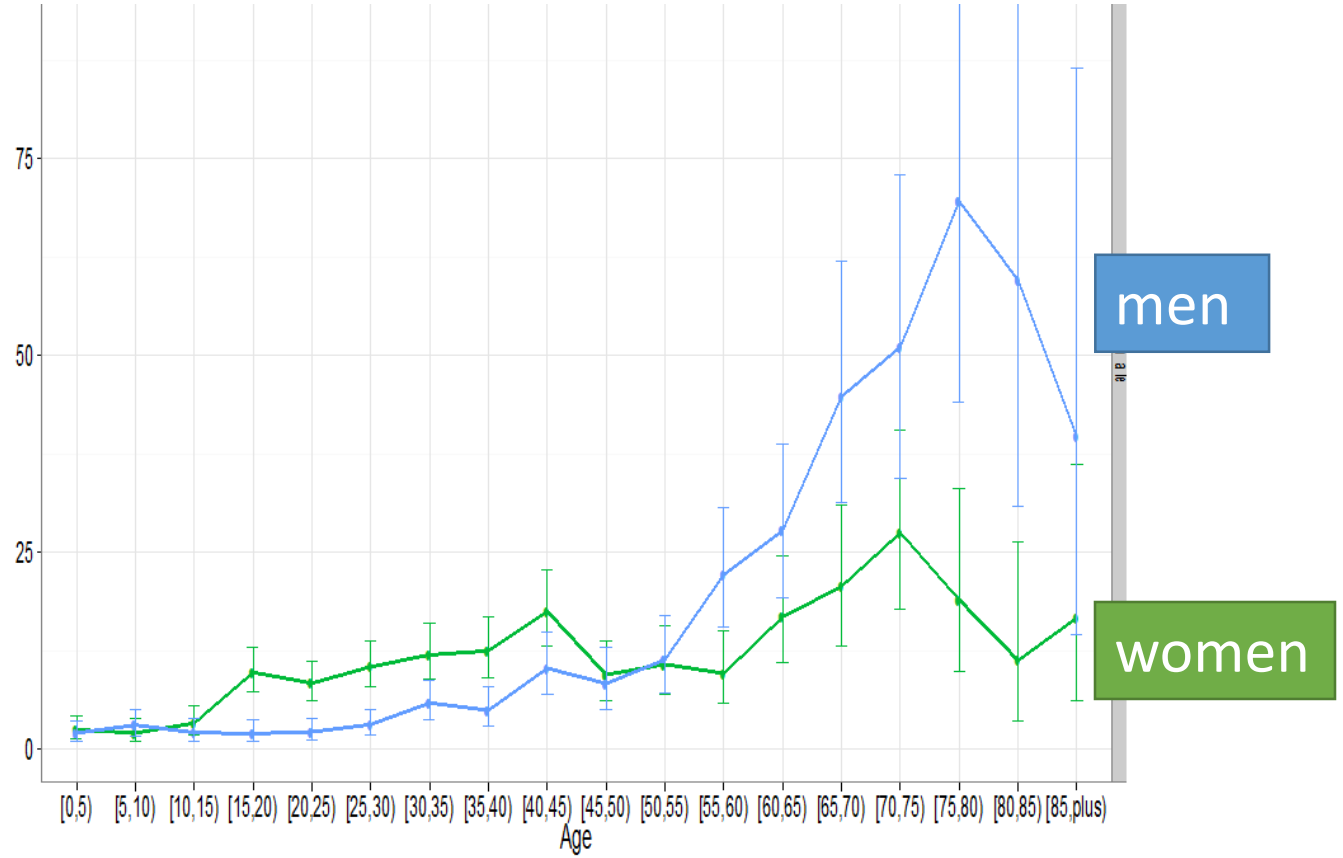
Treatment-resistant ophthalmoplegia in MG (OP-MG)

- Phenotype of OP-MG
- Histology
- "Genotype"
- Dynamic studies
- Hypothesis of OP-MG pathogenesis

INCIDENCE OF ACETYLCHOLINE RECEPTOR-ANTIBODY-POSITIVE MYASTHENIA GRAVIS IN SOUTH AFRICA

BUSISIWE MOMBAUR, MB, ChB,[1] MAIA R. LESOSKY, PhD,[2] LISA LIEBENBERG, BSc(Hons),[3]
HELENA VREEDE, MB, ChB,[4] and JEANNINE M. HECKMANN, PhD[1]

MUSCLE & NERVE April 2015



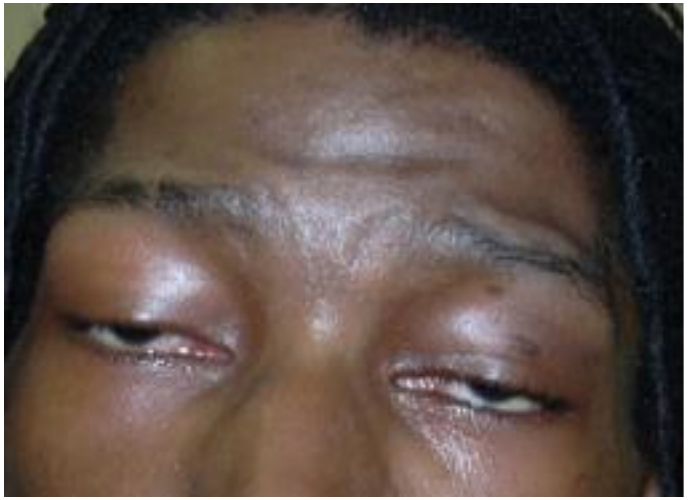
Ave annual incidence rate by age at symptom onset 2011-2012

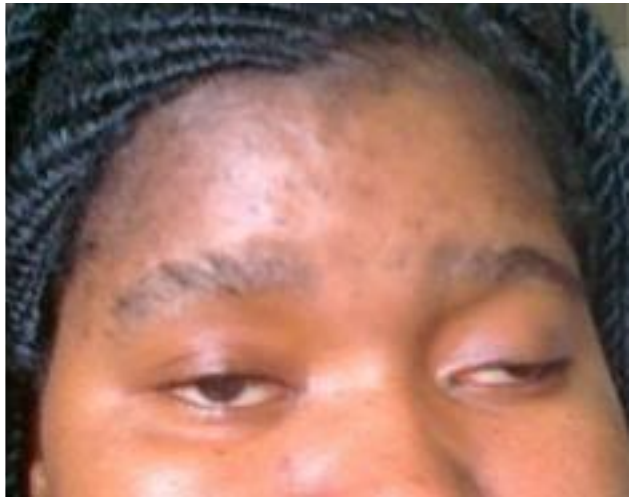
overall ~9 /million/year

Myasthenia gravis in South Africans:

Neuromuscular Disorders 17 (2007) 929–934

Subgroup of AChR ab+ MG patients





- Treatment resistant ophthalmoplegia
- Juvenile onset MG
- African genetic ancestry

Postulate:
Gene variant(s) have no consequence without MG –
however, MG triggers dysregulation in vulnerable EOMs

African Genetic ancestry in South Africa – racial categories in census

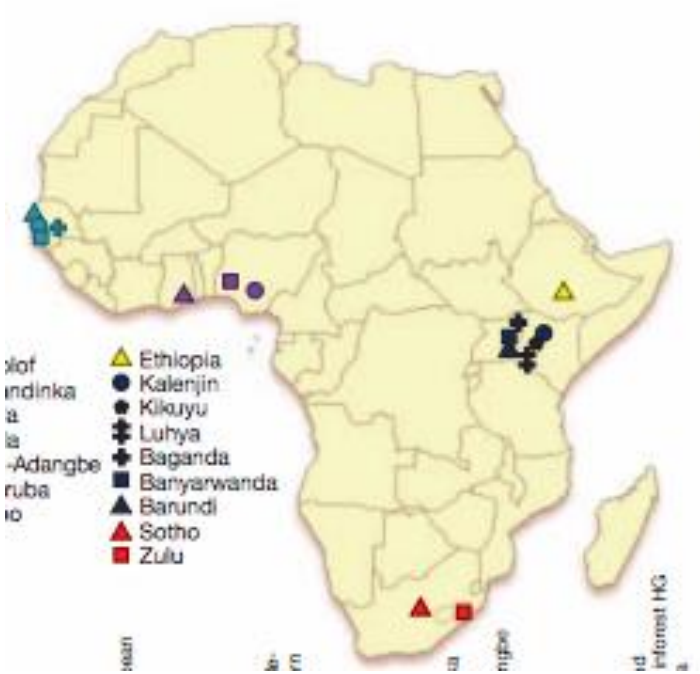
Indigenous black SA

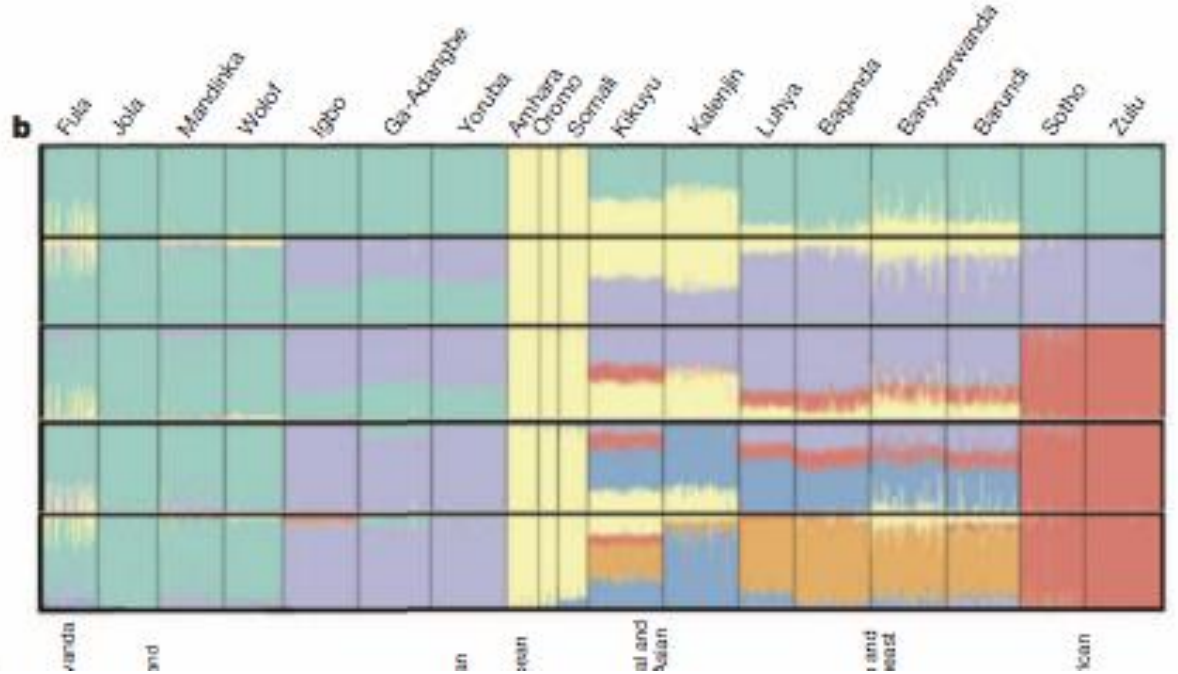
Cape mixed African

The African Genome Variation Project

Black Africans: colour-coded ancestral gene clusters

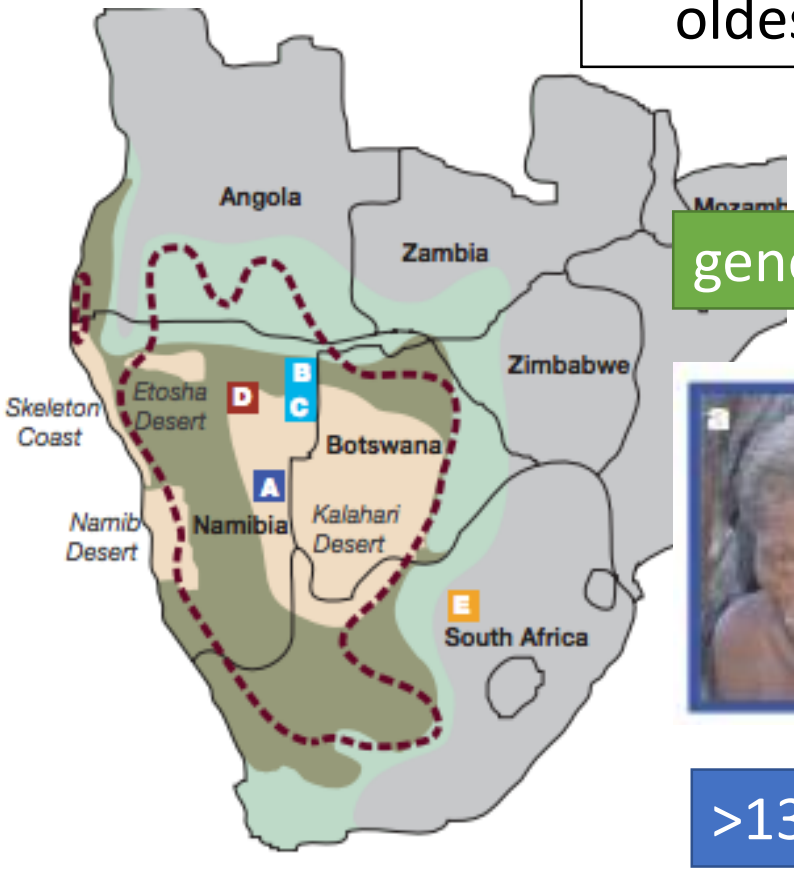
West African | East African | SA





Vol 463|18 February 2010|doi:10.1038/nature08795

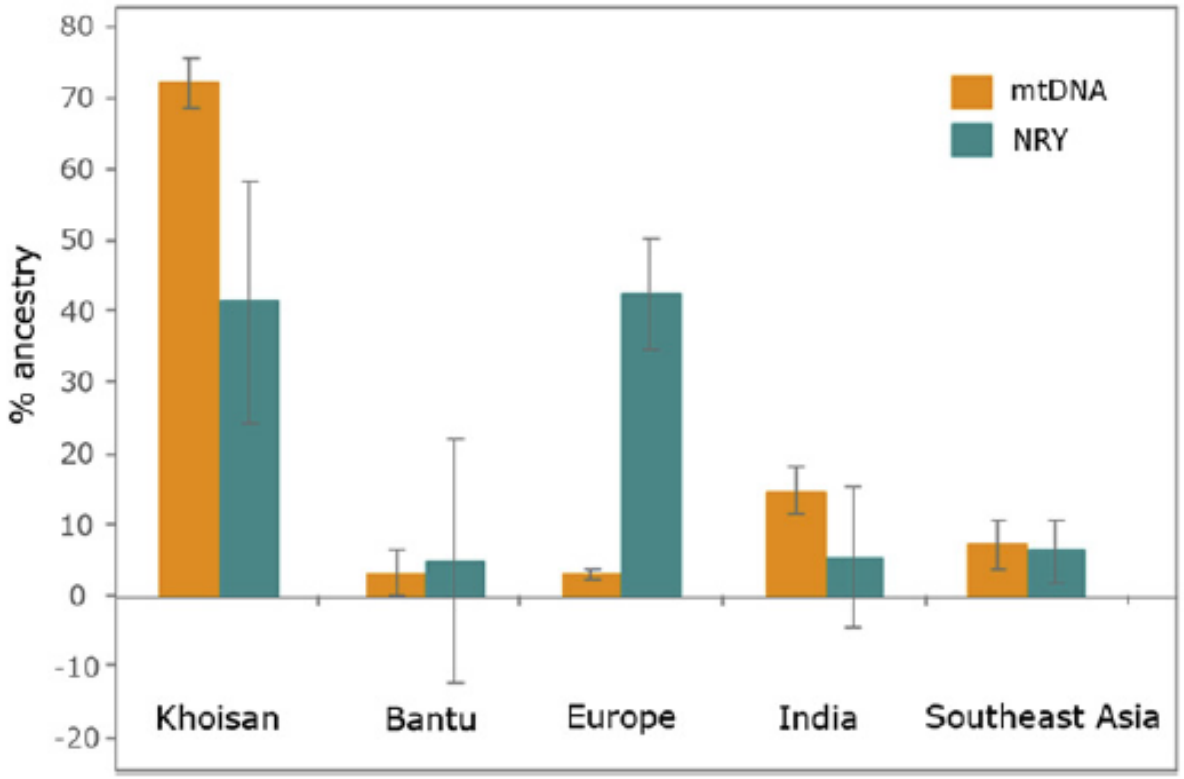
SA hunter gatherer genomes most diverse- oldest known lineage of modern humans



genome diversity - KhoiSan octogenarians

>13,000 novel amino acid variants

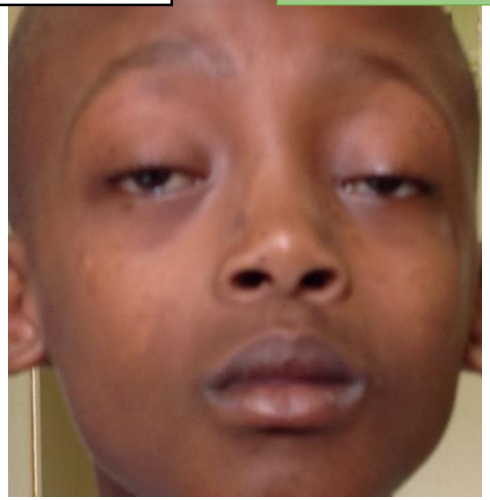
Cape mixed-African:
400 yrs – mainly Khoisan



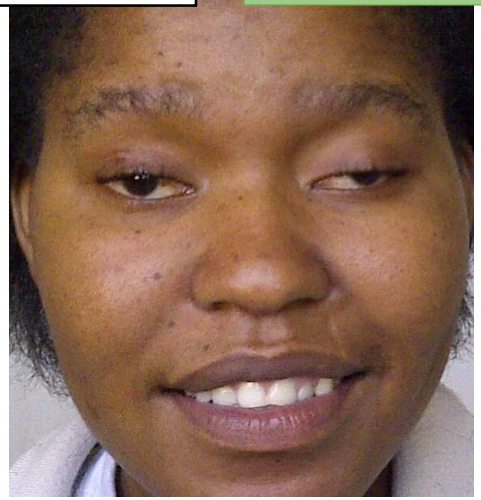
The American Journal of Human Genetics *86*, 611–620, April 9, 2010

Immediate onset: EOMs treatment-resistant from diagnosis while remaining muscles respond

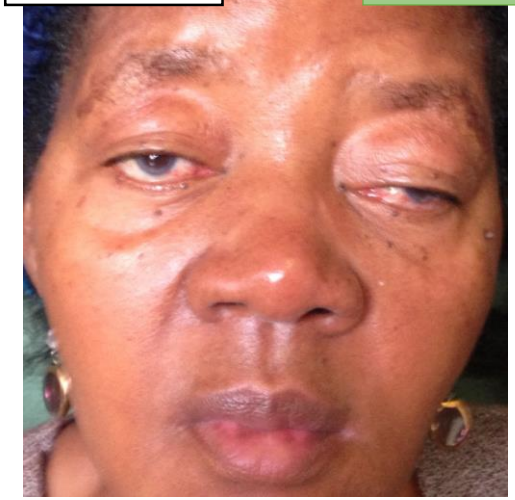
AAO-3 MGFA 2A



AAO-17 MGFA 4B

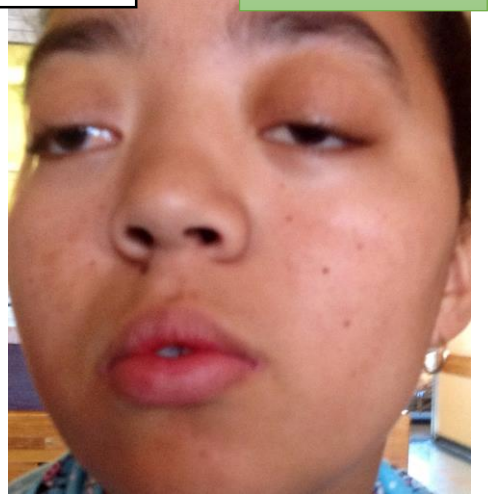


AAO-28 MGFA 3B

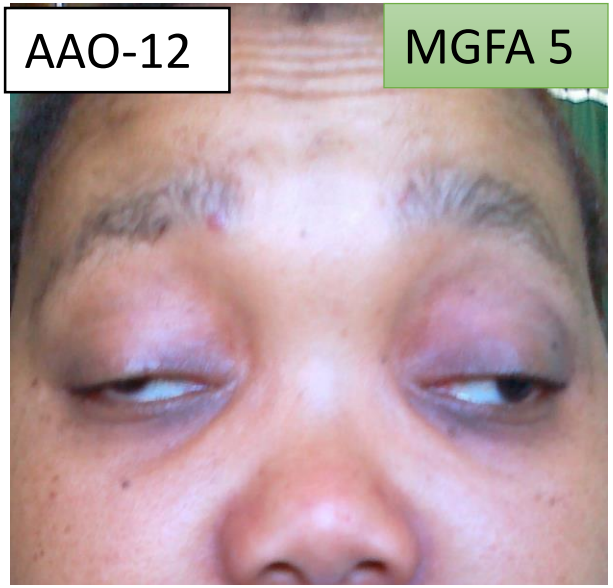


Delayed onset: EOMs initially responded to therapy and then a critical event triggered OP-MG

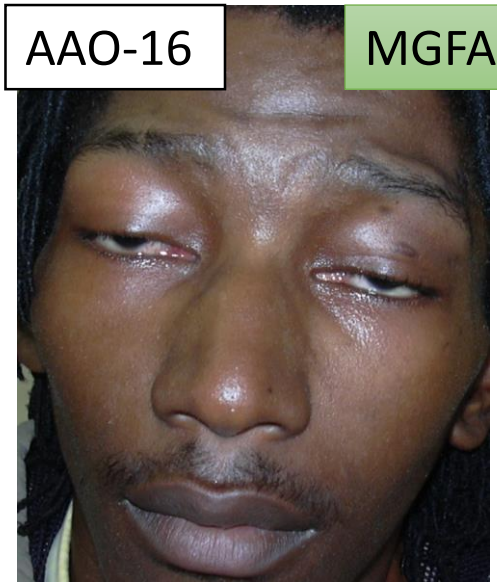
AAO-2 MGFA 3A



AAO-12 MGFA 5

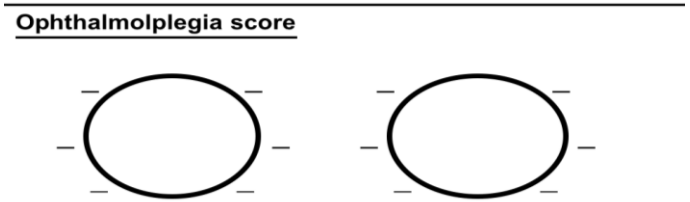


AAO-16 MGFA 5



Important to document the ophthalmoplegia objectively

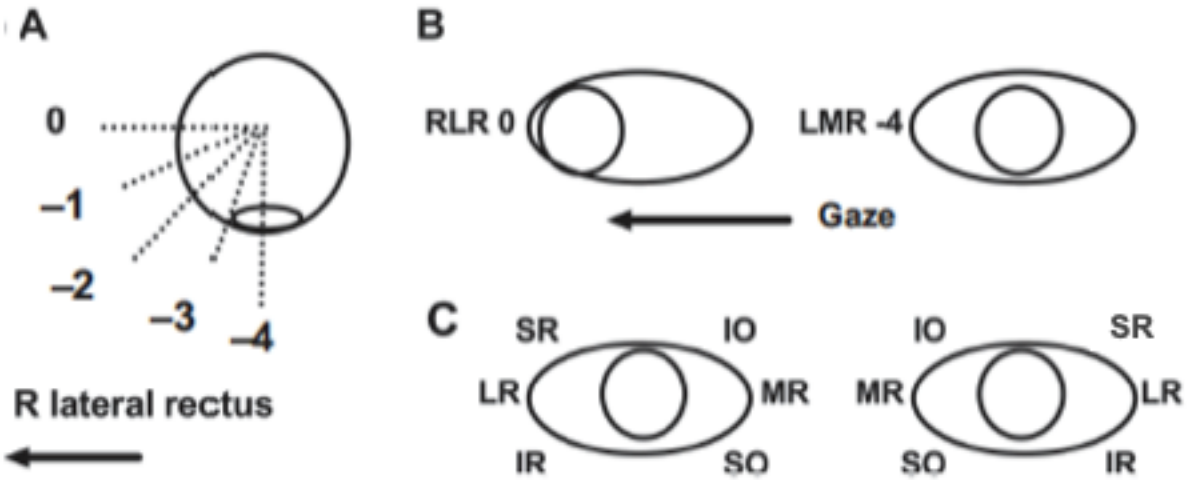
Every clinic visit: Ophthalmoplegia + MG composite score



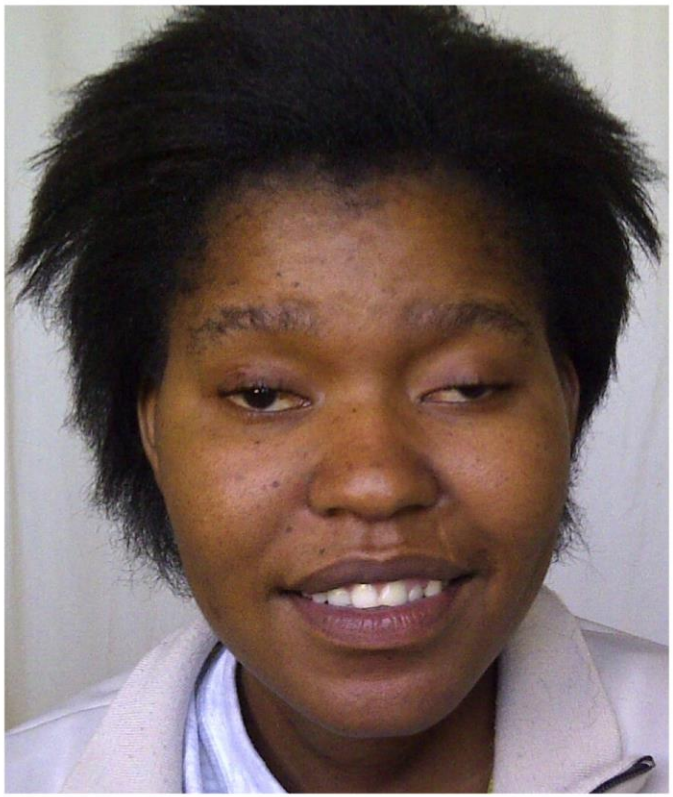
ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Issue: *Myasthenia Gravis and Related Disorders*
PERSPECTIVE

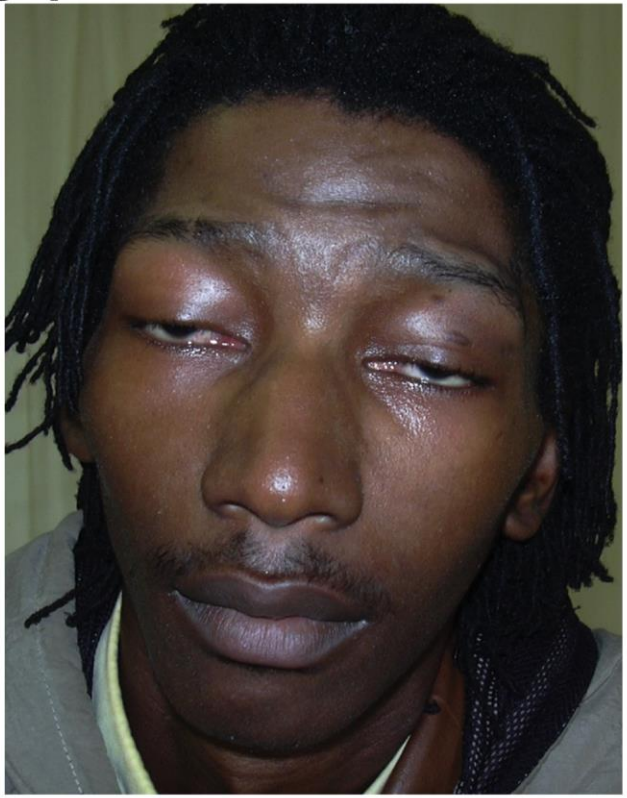
A unique subphenotype of myasthenia gravis

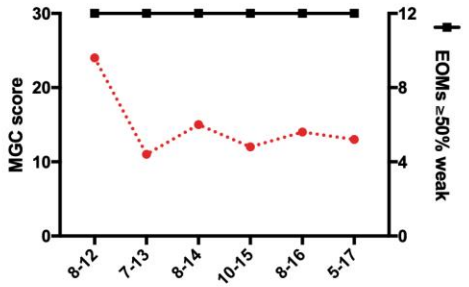
Jeannine M. Heckmann [1,2] and Melissa Nel[2]

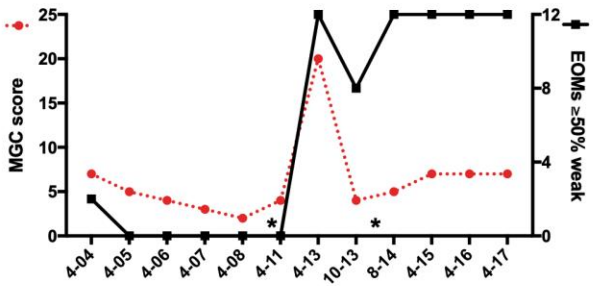


Complete ophthalmoplegia- no clinical observable movement +/- lagophthalmos





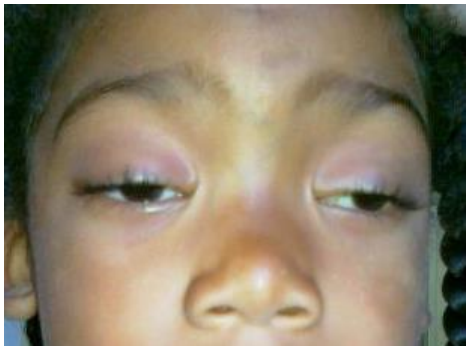


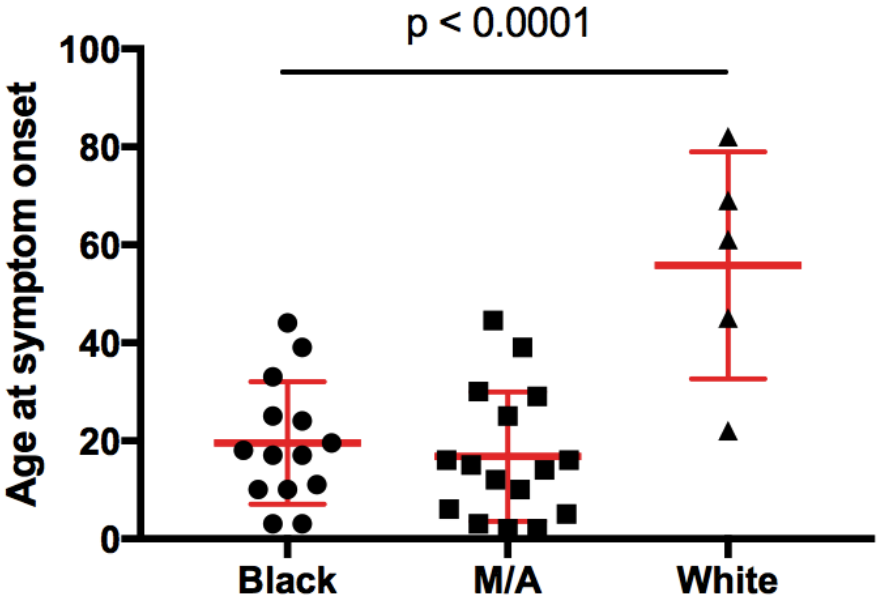


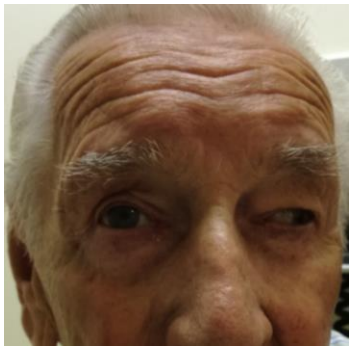
How frequently do we see OP-MG in the clinic?

- Defined as treatment-resistant ophthalmoplegia > 2 yrs
  - Self categorized by race

African genetic ancestry are younger







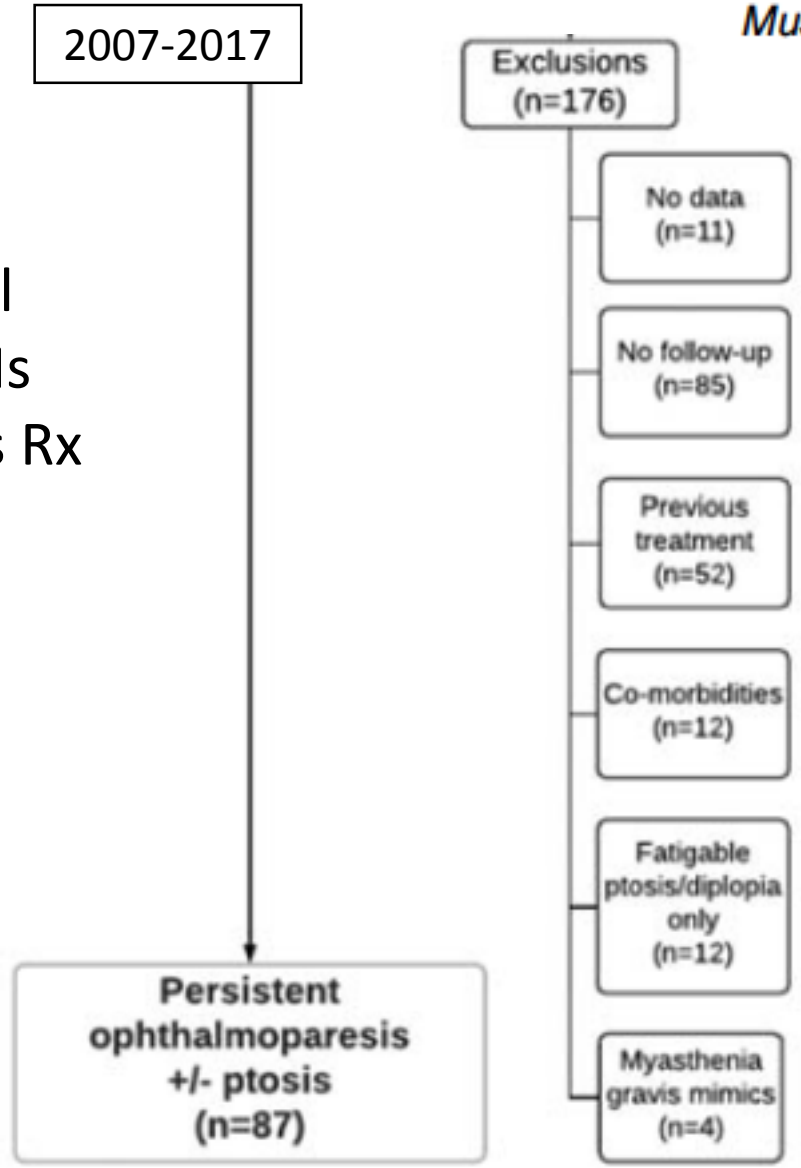
Total followed > 2 years	n=80	n=180	n=126
% OP-MG	17.5%	9.4%	3.1%

MYASTHENIC OPHTHALMOPARESIS: TIME TO RESOLUTION AFTER INITIATING IMMUNE THERAPIES

TARIN A. EUROPA, MBChB, MELISSA NEL, MBChB, PhD, and JEANNINE M. HECKMANN, MBBCh, PhD

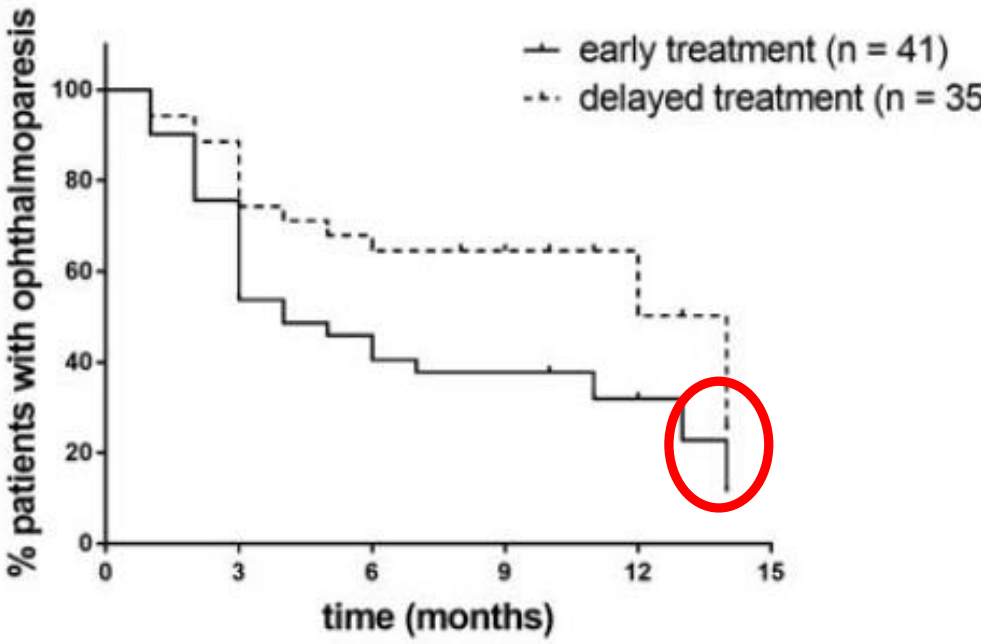
Muscle Nerve **58**:542–549, 2018

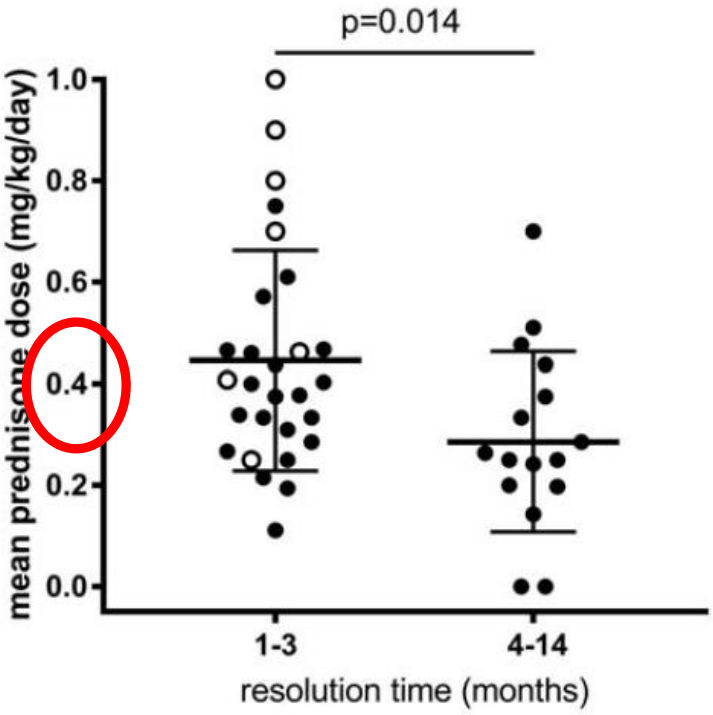
- Observational
- BL weak EOMs
- 1st 12 months Rx



13 OMG; 74 GMG

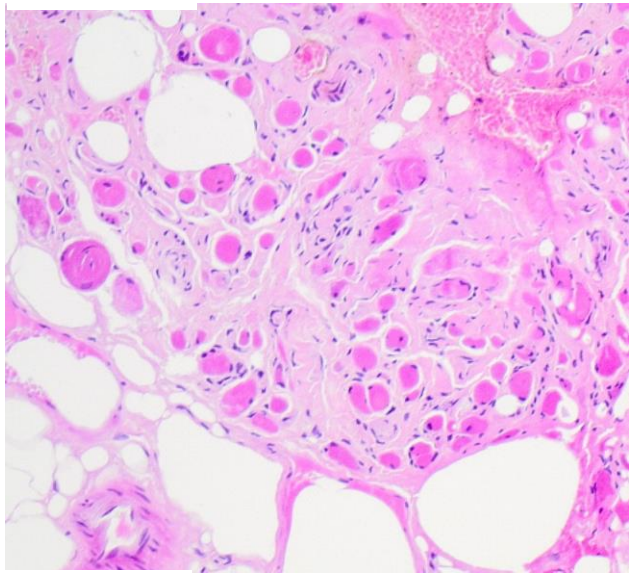
Earlier and “aggressive” immune therapies associated with resolution of ophthalmoparesis





<12 mo symptoms + immune therapy/pred. 2x > chance of resolution
Median resolution early rx group – 4 mo.

OP-MG



Extraocular Muscle Findings in Myasthenia Gravis Associated Treatment-Resistant Ophthalmoplegia: A Case Report

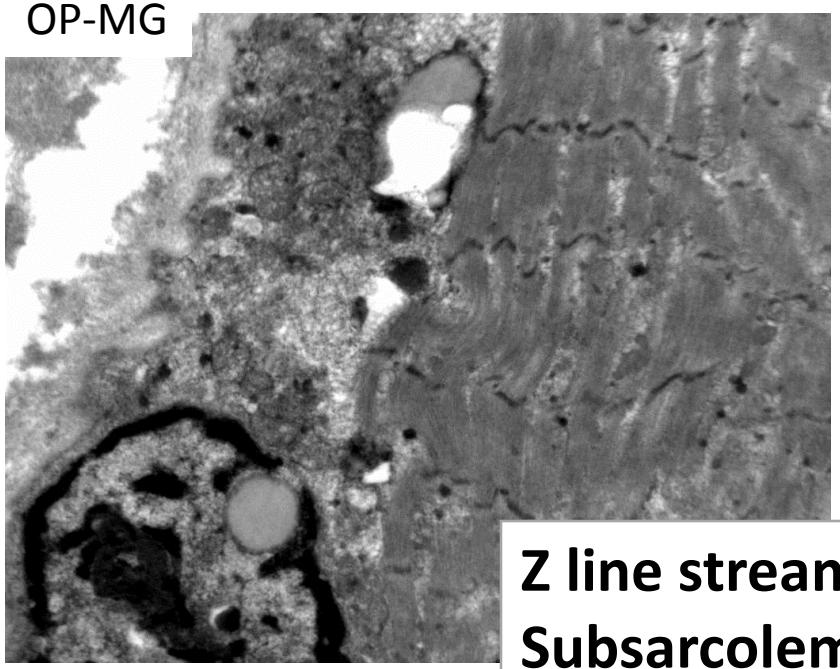
Rautenbach et al: *J Neuro-Ophthalmol 2017*; 0: 1-4

Paralysed medial rectus MG vs control

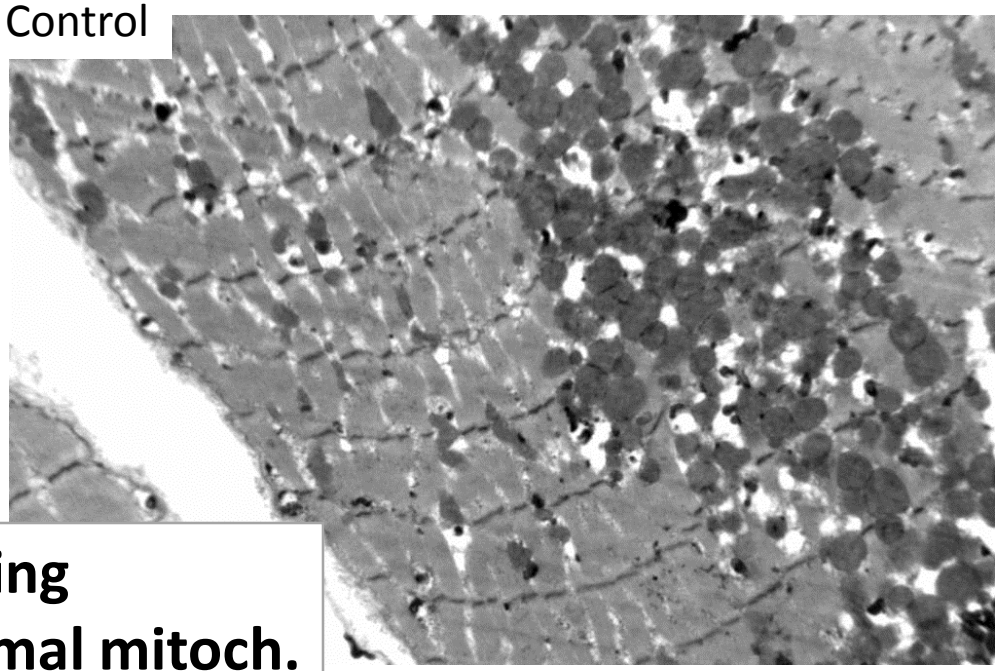
MGFA gr3b : Bilat. ophthalmoparesis x 3 yrs

Control: Stabbed & blind/sensory squint x 3yr

OP-MG



Control



Z line streaming
Subsarcolemmal mitoch.
Abnormal mitoch.

ELSEVIER

Neuromuscular Disorders xxx (xxxx) xxx

www.elsevier.com/locate/nmd

Review

A review of the histopathological findings in myasthenia gravis: Clues to the pathogenesis of treatment-resistance in extraocular muscles

Tarin A. Europa[a], Melissa Nel[a], Jeannine M. Heckmann[a,b,*]

Muscle type	Light microscopy (*n*)	Electron microsopy (EM)	
		EM general (*n*)	EM mitochondria (*n*)
MG limb before serotyping ($n=13$)*	AF type II >> type I (6), N-atrophy (6), MFD (4), LI (4), FCMR (2), lymphorrages (2), cores/targets (1), necrosis (2)	ZBS (2), IMCL (2)	Enlarged (2), SSA (2), abn. cristae (2)
MG limb AChR+ ($n=5$)**	N-atrophy (3), AF type II >> type I (5), MFD (1), rims (2), cores (2)	ZBS (1), IMCL (1)	Enlarged (1), SSA (2), abn. cristae (2)
MG limb MuSK+($n=5$)**	N-atrophy (2), AF (4), MFD (4), rims (2), cores (1)	ZBS (2), IMCL (2)	Enlarged (1), SSA (1), abn. cristae (1)
MG EOM ($n=6$)#	N-atrophy (2), AF (3), MFD (1), FCMR (3), lymphorrhages (1), LI (1), degenerative fibres (1)	ZBS (1), IMCL (1)	Enlarged (1), SSA (1)
Strabismus EOM ($n=9$)	AF (3), MFD (5), FCMR (3), rims (2), degenerative fibres (1), LI (1)	ZBS (5), IMCL (4)	Enlarged (3), SSA (3), abn. cristae (6), degenerated (1)

Neurogenic changes
Atrophy of type II
Mitochondrial stress

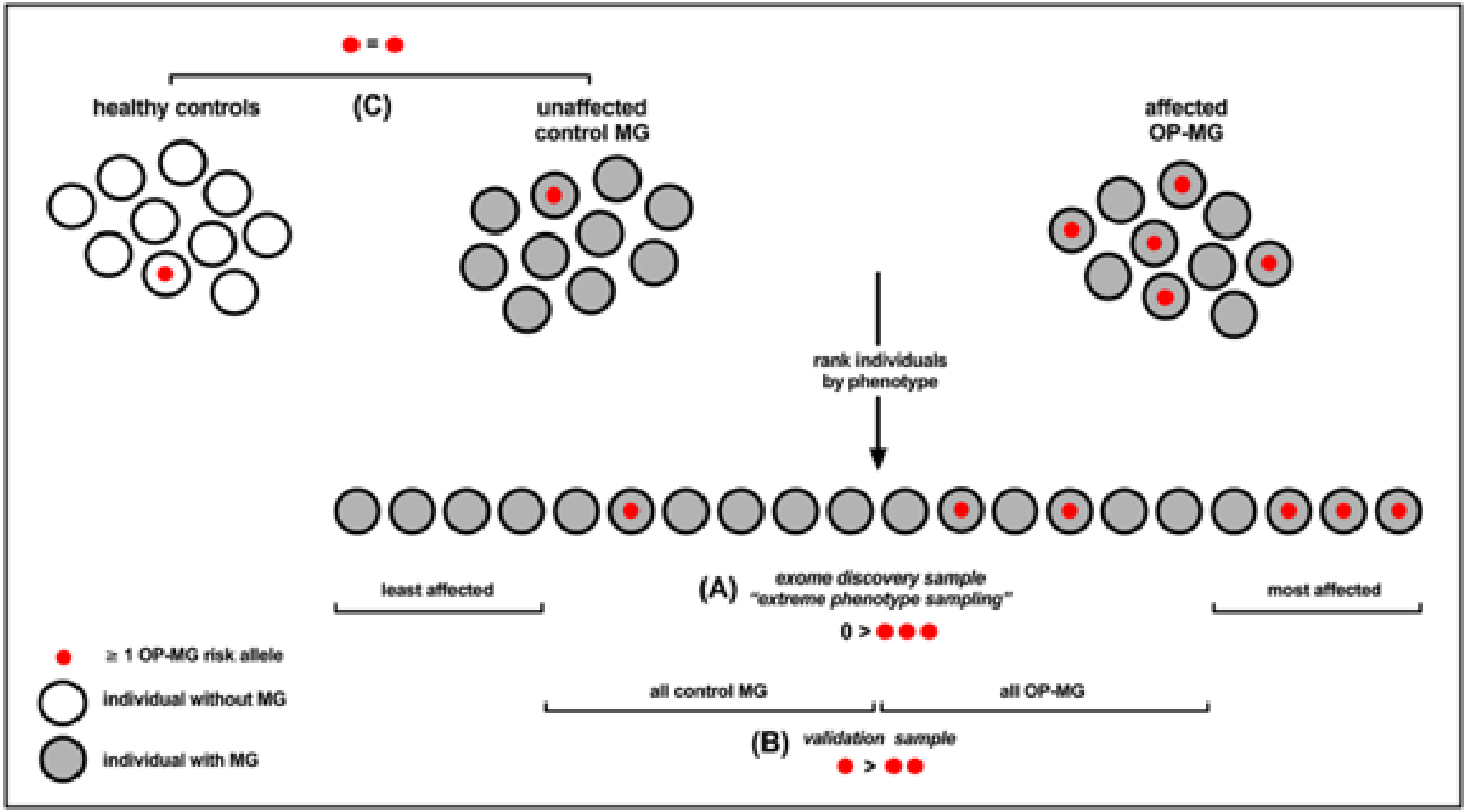
Poor muscle force & contractility

Neuromuscular Disorders 27 (2017) 816–825

Exome sequencing identifies targets in the treatment-resistant ophthalmoplegic subphenotype of myasthenia gravis

Melissa Nel [a], Mahjoubeh Jalali Sefid Dashti [b], Junaid Gamieldien [b], Jeannine M. Heckmann [a,*]

Extreme phenotyping approach to identify NOVEL genes/pathways

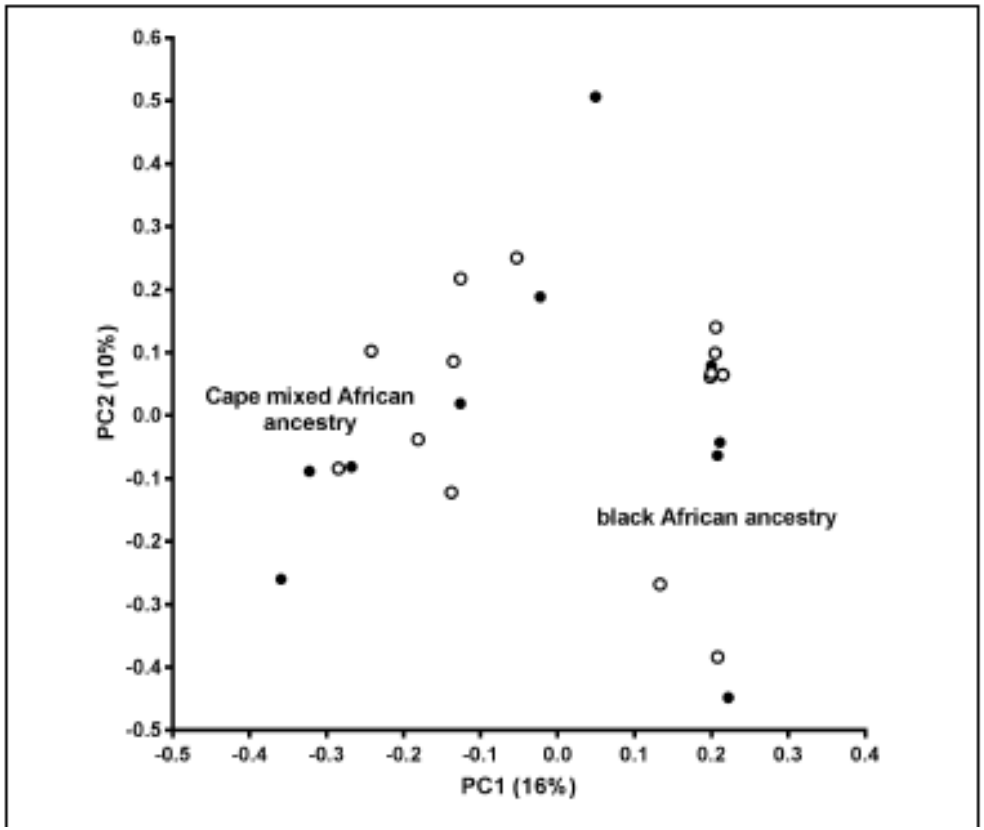


frontiers in Genetics

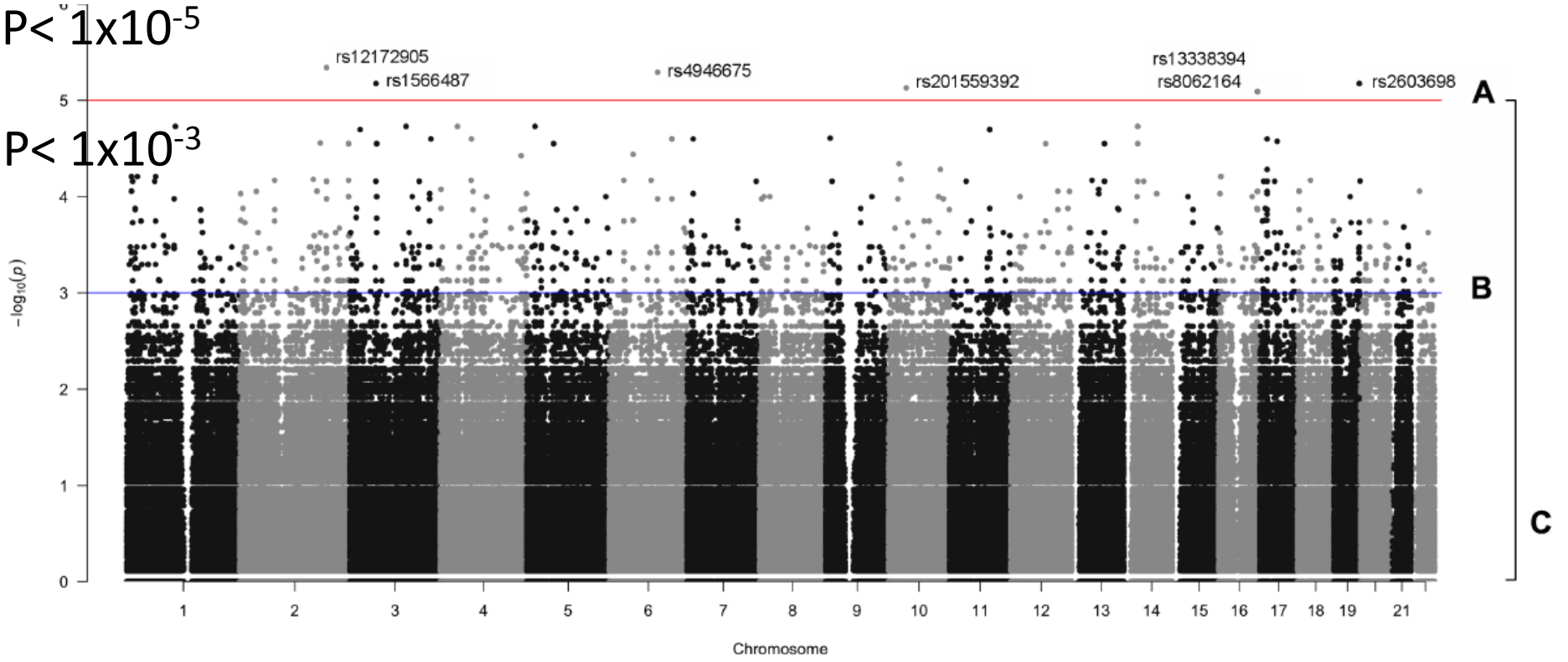
Using Whole Genome Sequencing in an African Subphenotype of Myasthenia Gravis to Generate a Pathogenetic Hypothesis

*Melissa Nel[1], Nicola Mulder[2], Tarin A. Europa[1] and Jeannine M. Heckmann[1]**

PCA plot –
2 subpopulations segregate
but not OP-MG vs control MG



Manhatten plot of GW single gene variant association OP-MG vs control MG



Subthreshold analysis: Top 2 ranked regulatory variants in gene promoters - expressed in muscle

3x more frequent in Africans > Europeans

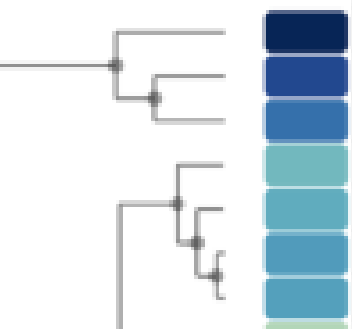
- Unbiased
- Gene-based association analysis of collective putative functional variant burden in genes in OP-MG vs cntrl MG p< 0.015
- Ranked according to GTEx expression level in skeletal muscle

Transcripts per million in skeletal muscle

+ Muscle atrophy

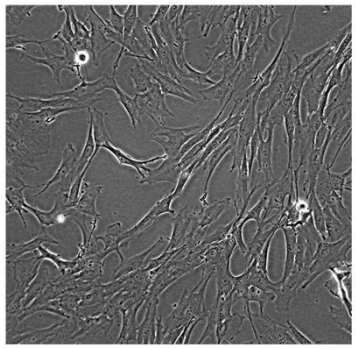
+ integrity Myosin HC

- Satellite cell act.



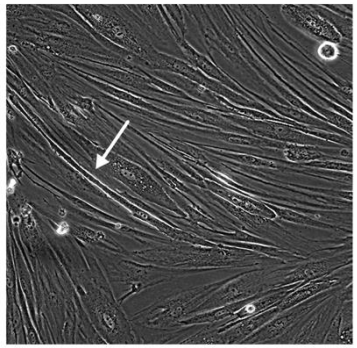
gene	p value	TPM	expression level
MKNK2	0,008	772	medium
SH3BGR	0,005	294	medium
MYL12B	0,001	116	medium
PPP1R12C	0,011	22	medium
AKT1S1	0,003	32	medium
PPP1R2	0,011	48	medium
ZFP36L2	0,007	43	medium

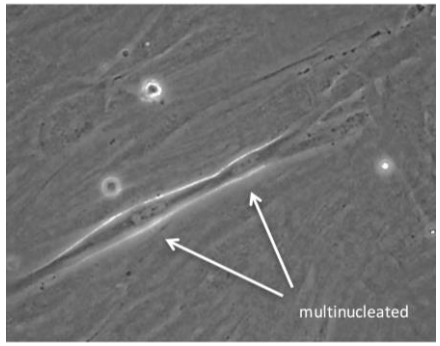
Gene expression? Patient-derived myoblast cultures



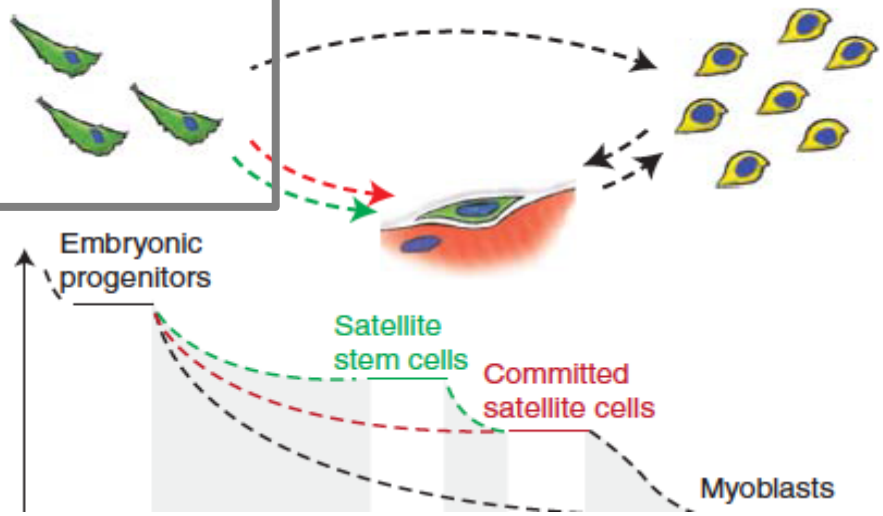
Primary dermal fibroblasts

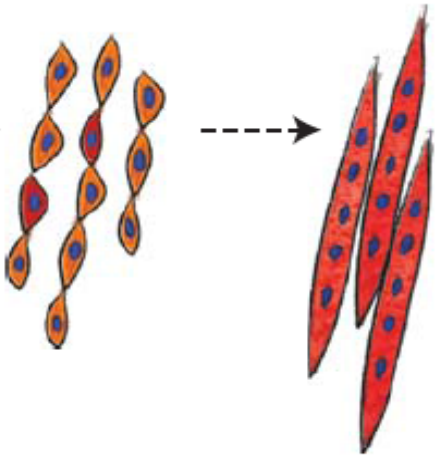
MyoD adenovirus





AdMyoD-myocytes





48 hrs

Figure adapted from C.F. Bentzinger et al. Cold Spring Harb Perspect Biol 2012

Nel et al. Orphanet Journal of Rare Diseases (2019) 14:24
https://doi.org/10.1186/s13023-019-1003-y

Orphanet Journal of Rare Diseases

RESEARCH Open Access

Profiling of patient-specific myocytes identifies altered gene expression in the ophthalmoplegic subphenotype of myasthenia gravis

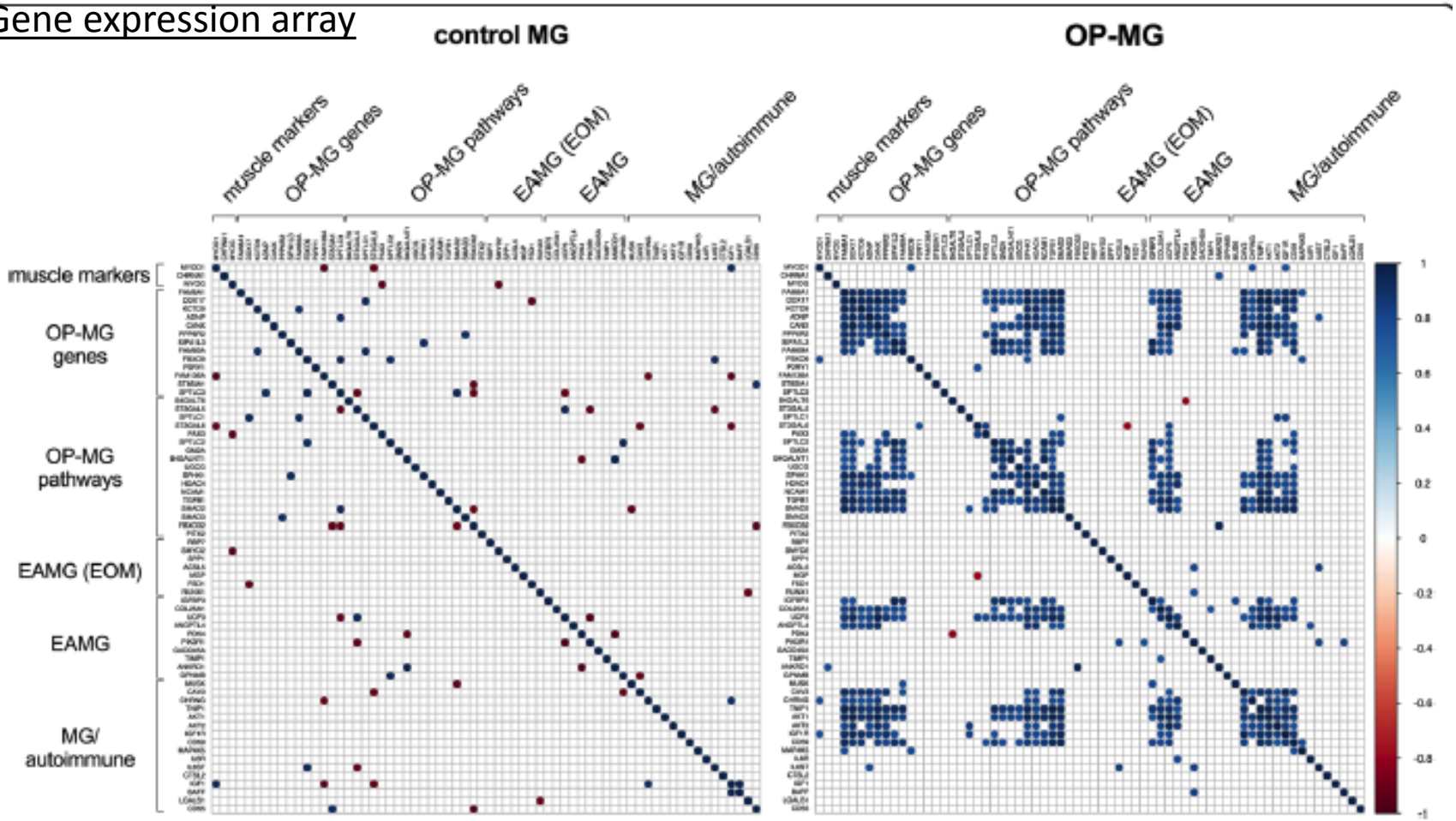
Melissa Nel[1], Sharon Prince[2] and Jeannine M. Heckmann[1,3*]

homologous 5% MG sera x 24 hrs

OP-MG (10) vs control MG (6)

Gene pair expression levels correlate in nodes (r>0.9; FDR <0.01)

Gene expression array



Surrogate OP-MG muscle model: dysregulated 'myocyte' gene expression suggests functional relationship

- 50% OP-MG genes correlated with 40% MG/EAMG genes
  - IGF1/AKT pathway –atrophy [EOMs]

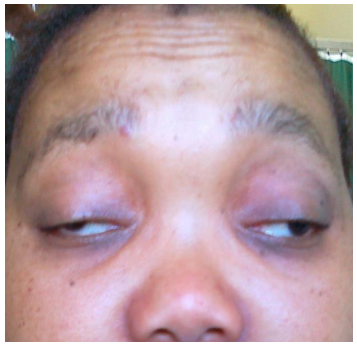
- pathway not previously considered relevant in MG correlate with MG/EAMG pathways
  - Myogenesis & satellite cell activation
  - Gangliosphingolipid & glycoprotein synthesis
    - Integrity of muscle endplate

Mitochondrial stress

EAMG muscles

Histopathology in MG

MGS induced mito. metab genes in muscle model

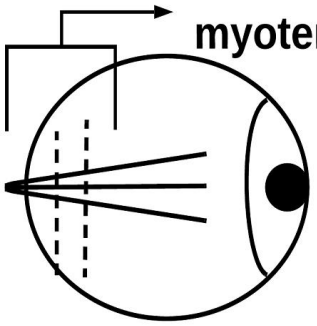


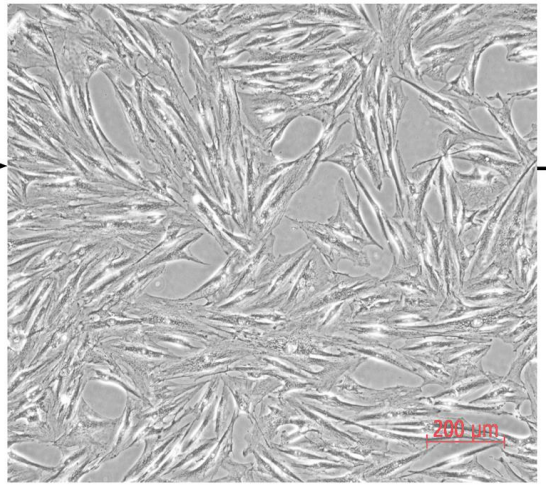
vs Non-paralytic strabismus

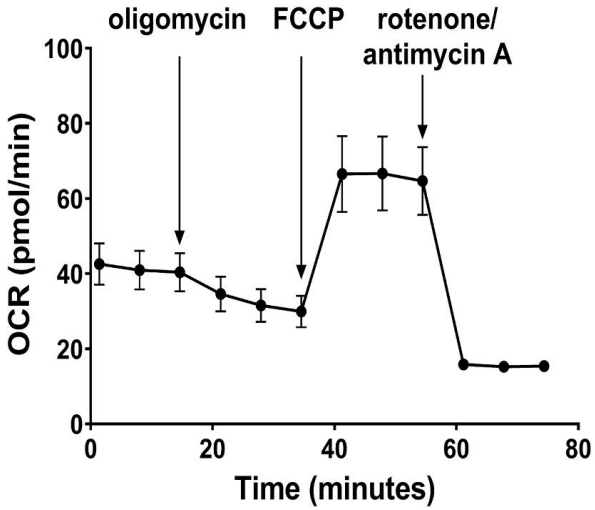
ocular re-alignment surgeries

perimysial ocular fibroblast cultures

Seahorse XF live-cell metabolic assays and qPCR (+/- MG sera)



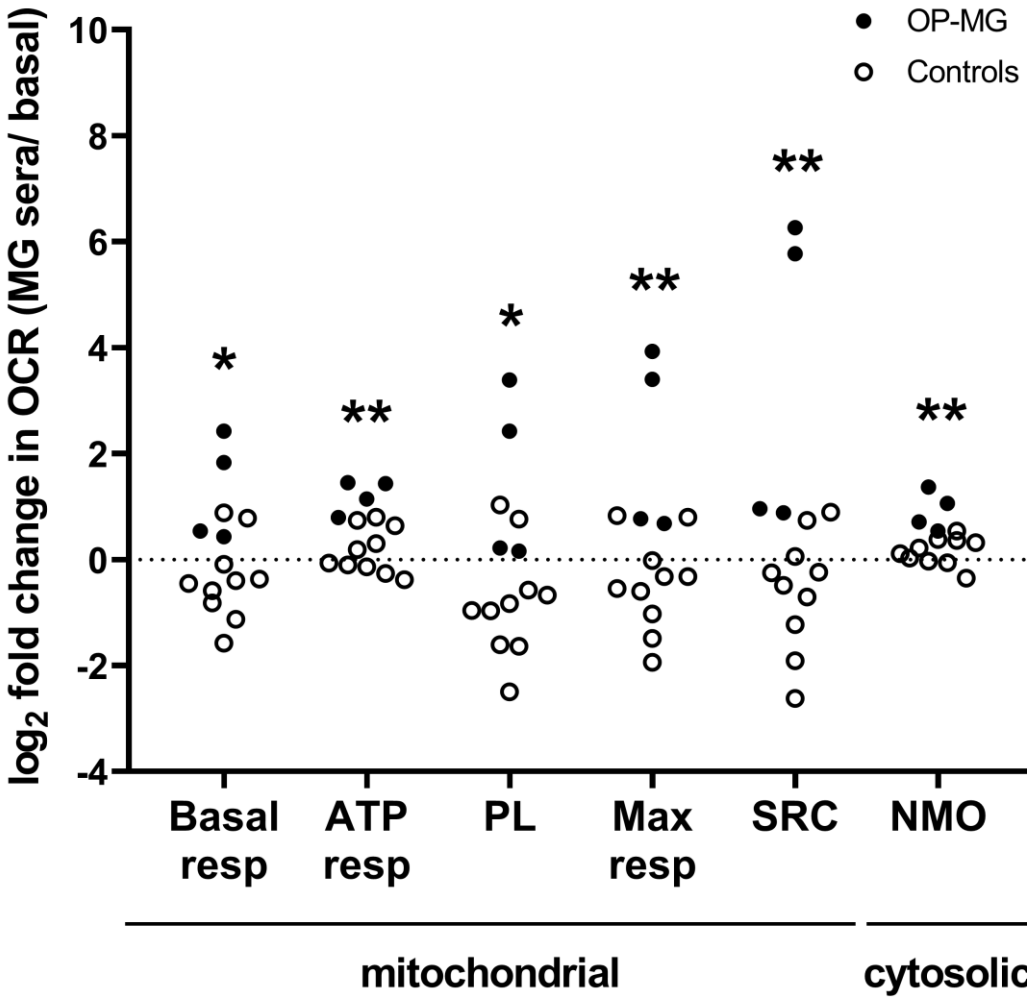




Metabolic assay: Oc-fibro cultures: 2 OP-MG vs 5 controls

Similar **basal OCR** and response to "**stressor mix**"

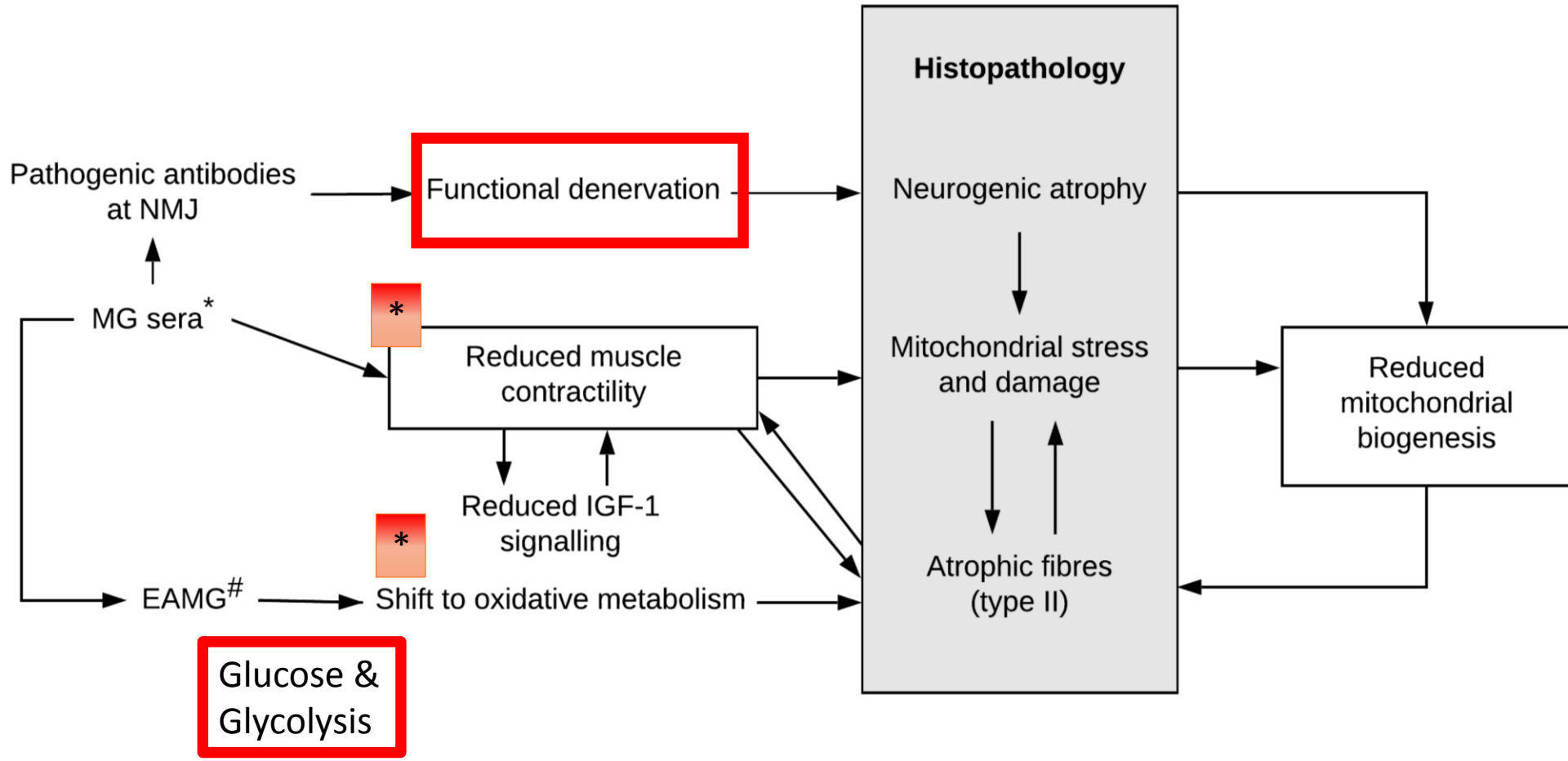
MG sera induced > **energetic** phenotype in OP-MG (3x)



* p<0.01 **p<0.001

Pathogenesis

Neuromuscular Disorders 29 (2019) 381–387



SUMMARY

Genetic studies OP-MG vs cntrl MG*		Dynamic studies MG sera		Gene expression
		MG muscle Model*	Ocular Fibro **	Orbic. Oc**
Gene	CD55, TGFB1	-/✓		?
WGS	Muscle atrophy	✓		?
	Mitochondrial metab	✓	✓	?
	Muscle regeneration	✓		?
	2 ‘muscle’ genes?			?

Histology suggests **poor contractility** critical in EOMs

** vs strabismus cntrl

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