Hereditary myasthenic syndromes: new genes and better treatment

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• Introduction and Diagnosis

• Eye movements

• Acute Crises

• Later onset progressive forms

• Treatment
Congenital myasthenic syndromes

- Genetic disorders of the NMJ
- Rare 1:100,000
- Mendelian inheritance
- Heterogeneous
- Treatable
- Additional cases genetically undiagnosed
Key proteins at the NMJ

The neuromuscular junction
NEUROMUSCULAR TRANSMISSION

NERVE

VGCC

AChR

VGNaC

MUSCLE

Membrane potential

---------- -40mV

------------- -50mV

Threshold

---------- -70mV
At least 26 different genes can be involved.
Features of CMS

• Genetically determined myasthenias
• Rare
• Muscle weakness:
  - ptosis, EOM, face, limbs, trunk, bulbar, respiratory
  - fatiguable
• Onset birth/infancy
• Stable throughout life
• Consanguinity / Family history
• EMG: decrement, jitter, blocking
• No antibodies
• Response to anti-cholinesterases & 3,4-DAP
CMS or what?

- hypermobility syndromes
- dyspraxias (Developmental Coordination Disorder)
- sero-negative MG
- chronic fatigue syndrome
- congenital myopathy
- SMA
- muscular dystrophy
Useful distinguishing features

From non-myasthenic conditions

- EMG – decrement > jitter/block, non-stim vs stim SFEMG
- fatiguable ptosis
- response to pyridostigmine
- fluctuation of weakness (better in morning)

From autoimmune MG

- early onset (< 3yrs) & Family History
- ankle dorsi-flexion weakness
- ophthalmoplegia static/lack diplopia
- non-acute onset
- ‘symmetrical’ ptosis
- no antibodies (common in childhood MG)
Ophthalmoparesis

**Pre-synaptic**
- CHAT
- COLQ

**Synaptic**
- AChR
  - Deficiency
    - AChR ε subunit
  - RAPSN
    - Glycosylation defects

**Post-synaptic**
- Kinetic Abn AChR
  - Slow channel
  - Fast channel

- Dispersion
  - DOK7
  - (MUSK)
  - (AGRIN)
AChR deficiency
(ε-subunit mutation)
24/24 severely restricted EOM
Restriction of eye movement develops over time

- Sister born 4 years later
- Slow feeder
- At 1 month full EOM
- 3 months later some restriction
- 6 months eye movements worse
- 10 months complete ophthalmoplegia
Variability in eye movements in CMS

**AChR mutations:**
- Deficiency: severe ophthalmoplegia
- Fast channel: 90% ophthalmoplegia (70% severe)
- SCS: 2/3 partial ophthalmoplegia

**Reduced clustering of AChR**
- RAPSN: ‘all’ full range EOM, maj. divergent squint
- DOK7: 93% normal EOM

**COLQ**: partial, normal, complete
**CHAT**: normal
**Glycosylation**: normal
Early life Crises

PRE
- CHAT

SYNAPTIC
- COLQ

POST
- AChR Deficiency
  - AChR ε subunit
  - RAPSN
    - Glycosylation defects
- Dispersion
  - DOK7
    - (MUSK)
  - (AGRIN)
- Kinetic Abn AChR
  - Slow channel
  - Fast channel
RAPSN CMS

Sudden life-threatening crises infancy & early childhood
Hospitalisation: 12/14, Ventilation: 10/14, Sibling deaths: 3/14

Induced by minor infections
Between attacks well
Significant morbidity and mortality
SYNAPTIC COLQ Dispersion DOK7 (MUSK) ε (AGRIN) (LRP4) AChR Deficiency AChR ε subunit RAPSN Glycosylation defects Kinetic Abn AChR Slow channel Fast channel worse with pyridostigmine
Cohort of patients with limb girdle pattern of weakness

- Sparing of face and ocular muscles
- Onset often childhood
- EMG decrement
- Pyridostigmine responsive
- Tubular aggregates
Data analysis from whole exome/genome sequencing

- Sequenced coding regions of the genome
- Identified all variants.
- Filtering out known SNPs
- All genes with two or more mutations in the same gene
- Genes shared between both patients

GFPT1/DPAGT1/ALG2/ALG14/GMPPB
N-linked glycosylation pathway
Glycosylation pathway mutations

- Clinical features
  - Limb-girdle muscle weakness
  - Usually no ptosis
  - No ophthalmoplegia
  - No facial involvement
  - No bulbar involvement
GMPPB - myasthenia

- Fatiguability of muscle weakness
- Myasthenic features are treatable
- Selective neurophysiological abnormalities

Dr Mathew Pitt. GOSH, London
Broadening of the phenotype

- Sparing of face and ocular muscles
- Onset can be in adulthood
- Can have raised CK
- Variable severity – overlapping myopathy
- Variable neurophysiology/muscle groups
- Pyridostigmine responsive, addition salbutamol/ephedrine beneficial
Aberrant glycosylation

- AChR subunits
- Other NMJ components
- Stability and structure of synapse
- Myofiber structure
- Dystrophin changes in muscle
- CNS involvement
- CNS and other tissues involvement
- Other components in CNS and other tissues

- signal transmission
- CMS
- LGMD
- CDG
Oxford CMS Cohort – genetic diagnosis 543 patients

25 Genes: >400 Mutations

- glycosylation defects
- CHAT + others
- COLQ (80% \(sp^{12}\))
- Fast channel (60% G153S)
- Slow channel
- DOK7 (60% 1124_1127dupTGCC)
- AChRε def (25% 1267delG)
- RAPSYN
- >90% N88K

Estimate <10% no known mutation
Better treatment?

(The DOK7 story)
Aneural postsynaptic specialisation

Yuji Yamanashi

NERVE

Clustered AChR

Agrin

Rapsyn

MuSK

Dok-7
DOK7 mutations

- 325G>T
- 415G>C
- 437delC
- IVS1+14del15
- 473G>A
- 481G>A
- 496G>A
- 539G>C
- 548_551delTCCT
- 596delT
- 601C>T
- 101_141del
- 1143insC
- 1185C>G
- 1263insC
- 1339_1342dupCTGG
- 1357_1370del14
- 1378insC
- 1487G>T
- 1504_1505insTA
- 1508insC
- 1522_1523delAC
- 1522C>T
- 1544_1547delTTCT
- 1558_1559delCT
- 1589_1592delAGAC
- 1604_1612del21
- 1618_1621del4
- 1630C>T
- 1634_1636delCTCT

Common mutation:

1124_1127dupTGCC
In vitro clustering assay

Myoblasts

Transfect with cDNA + Dok-7

Mutant Dok-7

Differentiate

Myotubes

C2C12

Myotubes

AChR

AChR clusters
C2C12 mouse myoblasts

Methods

Retroviral infection

Differentiation into myotubes

DOK7

AChR cluster induction
DOK7-induced AChR clusters

Myotubes – no DOK7

DOK7 WT

DOK7 common mutation

(Fewer and smaller clusters)
Type of AChR clusters formed following expression of Dok7 in C2C12 cells

Perforated  C-shaped  Branched  Endplate

Number

Complexity

(J.Cossins)
Clinical features DOK7 CMS

**Inheritance** - recessive

**Onset** - 1.5 – 4 years, sometimes respiratory problems at birth

**Symptoms** - limb girdle pattern of weakness, ptosis, but eye muscles unaffected

Unresponsive – pyridostigmine

Respond – ephedrine/salbutamol

(Palace et al., 2007)
DOK7 CMS patients respond to treatment with ephedrine

Disability score

QMG

DOK7 CMS patients respond to treatment with ephedrine

Lashley et al., 2010
Improvement over months

EPHEDRINE 4 months  15mg  

EPHEDRINE 1 yr  45mg

Originally non-ambulant
DOK7 CMS treated from an early age

Good response if treated from an early age

Age 3, severe weakness, unable to walk, in and out of ITU

Age 9, almost normal strength
How is the medication working?

Nerve terminal

ACh

Agrin

MuSK

Dok-7

Impaired signaling

Stabilise

Compensation 2\textsuperscript{nd} messenger

Salbutamol
Ephedrine

\(\beta_2\text{AR}\)
A group of patients with AChR deficiency who are severely disabled despite optimum therapy

Excellent initial response to anticholinesterase therapy but effectiveness tails off over time
AChR deficiency syndrome

Muscle

Nerve

Normal

Mutation

Bungarotoxin-labeled

Partial compensation

ε-subunit mutations (recessive)
Neurotransmission leads to disassembly of endplate AChR clusters

Focus on the factors that counteract disassembly
β2 agonists for patients on AChE inhibitors?

Nerve terminal

Excess

Destabilised postsynaptic structure

Pyridostigmine

Agrin

MuSK

Dok-7

Stabilise

Compensation 2nd messenger

ACh

Rapsyn

Salbutamol Ephedrine

β2AR

β2 agonists for patients on AChE inhibitors?
Adding salbutamol or ephedrine to severe AChR deficiency patients

(QMG scores)

(Rodriguez-Cruz et al., 2015)
Disorders affecting synaptic function

AChR deficiency

NERVE

Disorders affecting synaptic structure and stability

Agrin

LRP4

Muscle

Slow channel syndrome

Normal openings

Wild type

Prolonged openings

εL221F

Dok-7
Treatment summary

Destabilising effects of enhanced neurotransmission through anticholinesterase medication

Mestinon

Stabilisation through AGRN pathway

β2 ADR agonists

Tailored personalised therapy
Summary

- **CMS**
- Rare and heterogeneous
- Genetic disorders
- Neuromuscular transmission
- > 20 genes
- Fatigable muscle weakness
- Autoimmune MG

15% (NGS)
85% (Genetic dx)

Schematic representation of the NMJ
CMS due to COL13A1 mutation

Clinical features

- Onset at birth
- Breathing and feeding difficulties
- Early episodes of clinical deterioration
- Bilateral ptosis + normal eye movements
- Dysmorphic features, abnormal chest
- No response to pyridostigmine
CMS due to MuSK mutations

- 18 years old patient
- Onset at birth
- Severe muscle weakness
- Respiratory failure + tracheo
- Wheelchair bound
- Off-treatment
- Life-threatening deterioration with pyridostigmine
Response to treatment

Good response to salbutamol

Pre-treatment

Post-treatment
(salbutamol 4mg bd)
CMS ‘take home messages’

- distinct genotype / phenotype associations
- may not respond or get worse with pyridostigmine
- can present adulthood
- can look myopathic
- seronegative myasthenia:
  - check clustered AChR abs
  - weak ankle dorsiflexion consider CMS
- respond well to specific & different treatments
- important to obtain specific genetic diagnosis/mechanism
- perform RNS /SFEMG in weak muscles of limb girdle weakness
TREATMENT

SUSPECTED CMS

Features of DOK7, COLQ or SCS? (awaiting genetic diagnosis)

Yes

Avoid Pyridostigmine

DOK7

COLQ

SCS

Salbutamol

Fluoxetine

Quinidine

Add in 3,4-DAP*

No

Start Pyridostigmine

Rapsyn

Glycosylation

ChAT

AChR-def Fast Channel

Add in 3, 4-DAP*

Add in Salbutamol

First line treatment

Second line treatment

Additional treatment
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Working out how the AChR is affected

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Abnormalities of the AChR function

PROLONGED ACTIVATION

Excess Na$^+$, Ca$^{2+}$
Slow channel

SHORTENED ACTIVATION

Insufficient Na$^+$
Fast channel

REDUCED CONDUCTANCE

Insufficient Na$^+$
Conductance syndrome