Pharmacogenomics & Malignant Hyperthermia

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Learning Objectives

1. Describe the phenotypes of MH susceptibility
2. Discuss the what is known regarding the genetics of malignant hyperthermia susceptibility
3. Describe the molecular genetic testing used in malignant hyperthermia and genetic counseling regarding MH susceptibility.

Disclosures

None

Personalized Medicine

- Application of pharmacogenomics.
- Identify and characterize subpopulations with gene variants to predict
  - *Individual disease susceptibility*
  - *Individual risk for adverse responses*
  - *Individual with maximal drug Rx benefit*
  - *Individual risk for disease progression*

Pharmacogenomics & MH

- The application of genome science to the study of human variability in susceptibility to hypermetabolic responses to anesthetic drugs (MH reaction).
- Optimize outcome through knowledge of the genomic ***variability*** and its influence on MH susceptibility.
- Develop “personalized”strategies for individualizing anesthetic management to “predict and prevent” MH reactions.

Malignant Hyperthermia

Prevalence

1:50,000 anesthetics (adults)

1:15,000 anesthetics (children)

MH susceptibility ~ 1:2000

Triggering Agents

Volatile Anesthetic Agents

Depolarizing muscle relaxant-SDC

Diagnosis

Ex-vivo muscle biopsy

North America: CHCT (Caffeine halothane contracture test)

Europe: IVCT (In vitro contracture test)

DNA Testing?

Clinical Presentation of MH

1. Rigidity
2. Muscle Breakdown
3. Respiratory Acidosis
4. Temperature Increase
5. Cardiac Involvement
6. Others

Clinical Grading Score

Raw Scores	MH Rank	Description of Likelihood
0	1	Almost never
3-9	2	Unlikely
10-19	3	Somewhat less than likely
20-34	4	Somewhat greater than likely
35-49	***5***	***Very likely***
50+	***6***	***Almost certain***

MH Susceptibility (MHS)

Medical History

- History of suspected clinical MH reaction
- Central Core Disease
- Multiminicore Disease
- King Denborough Syndrome
- Severe masseter muscle rigidity
- ? Exertional rhabdomyolysis
- ? Exertional Heat Illness
- ? Elevated resting CK

Family History

- First degree or close relative of subject with MH reaction
- Close relative of individual diagnosed as MHS by CHCT or IVCT

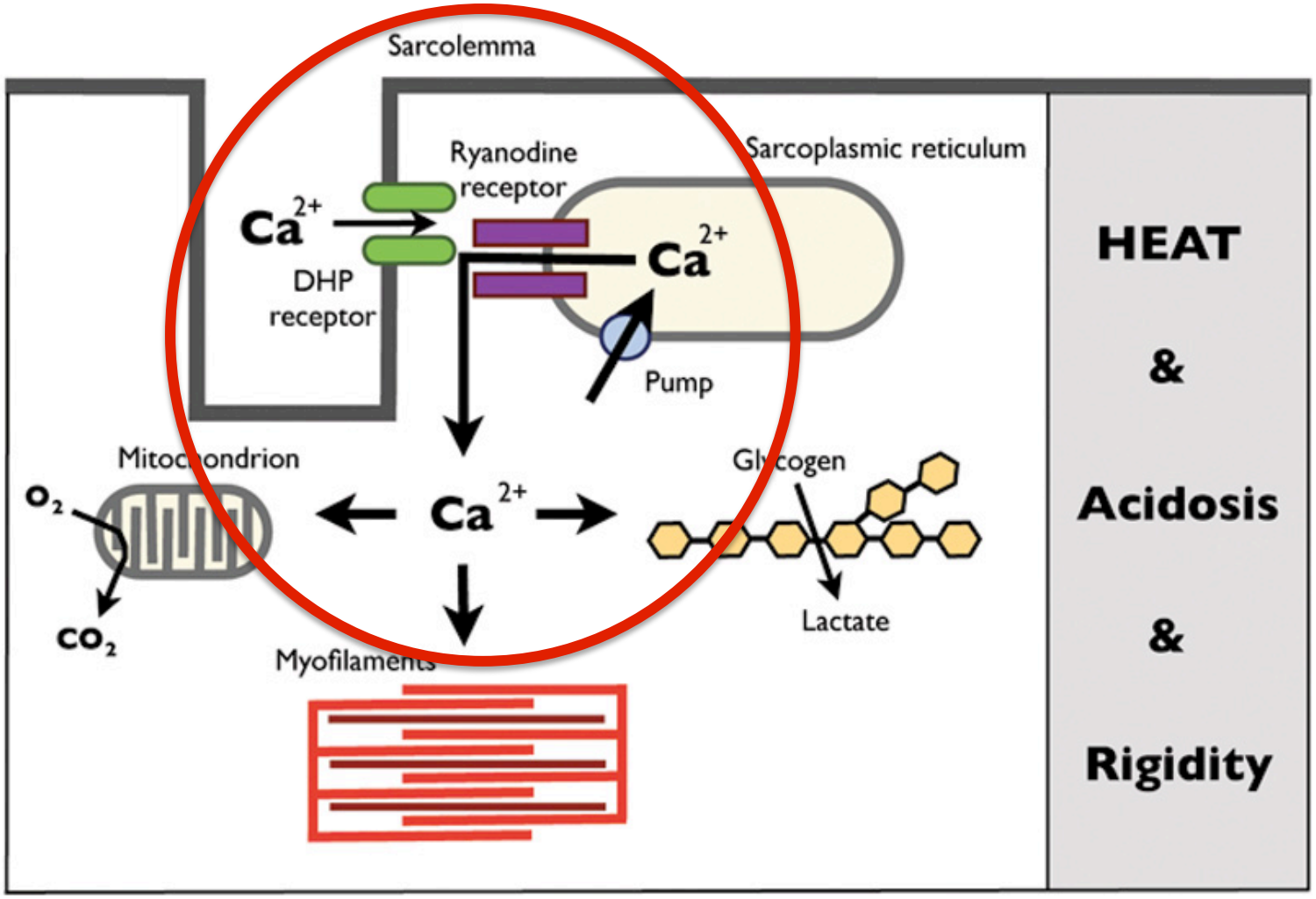
Muscle Biopsy Diagnosis

- CHCT (North America)
- IVCT (Europe)

In vitro Assays

- CICR (Japan)

MH & Calcium Dysregulation



Genetics of MH

- Genes identified as causative for MH
  - RYR 1 gene (Ryanodine receptor gene 1)
    - Located on chromosome 19
    - Up to 70% of MHS individuals have RYR1 mutations
    - 34 causative mutations identified by EMHG
  - CACNA1S gene (voltage-dependent, L type Ca channel, alpha 1S subunit)
    - Only 1% of MHS individuals have CACNA1S mutations
    - On chromosome 1
    - 2 causative mutations identified
  - STAC3 (native American myopathy)
    - On chromosome 12
- Four other loci mapped, genes **NOT YET** identified
  - Chromosome locus 17q11.2-q24
  - Chromosome locus 3q13
  - Chromosome locus 5p
  - Chromosome locus 7q21-q22

MH & Molecular Genetic Testing

- **Approaches**
  - Targeted gene screening
  - Targeted Exome sequencing (Hot Spots)
  - Whole Exome Sequencing
- **Cohort**
  - Unselected cohort
  - MHS individuals

Variants vs. Pathogenic Mutations

- Polymorphism
  - > 1% in overall population
- Rare Variants
  - ≤ 1% in overall population
  - May or may not be pathogenic
  - >450 rare RYR1 variants identified to date
  - >100 rare CACNA1S variants
- Pathogenic
  - 34 RYR1 causative mutations
  - 2 CACNA1S causative mutations

RYR 1 & MH

- RYR 1 gene
  - Located on chromosome 19
  - has 106 exons
  - encodes a protein with 5,038 amino acids
- 34 RYR1 causative mutations identified by EMHG
- Autosomal dominant transmission
- Predominantly missense mutations
- RYR1 mutations 70% MHS
- Males> females
- Phenotypes are more severe in
  - RYR1 associated with CCD
  - Males
  - Chronic elevated serum CK

Exertional Rhabdomyolysis & Exertional Heat Illnesses

- Tobin et al 2001 JAMA
  - Fatal heat stroke during football game in pt w/ MH episode successfully Rx w/ dantrolene
  - Temp=108˚F.
- Wappler et al 2001 Anesthesiology
  - 10/12 ER patient have abnormal IVCT and 3/12 with RYR1 mutations
- Sambuughin 2009 Clin Genetics
  - 6 AA males with history of ER
  - 5/6 showed RYR1 variants
  - 2/6 are known causal MH mutations
- Fizzer 2015 Anesthesiology

Exertional Rhabdomyolysis & Exertional Heat Illnesses

Fiszer 2015 Anesthesiology

- Study cohort
  - 57 samples (57 families): 29 MHS and 28 EHI
  - Additional 556 unrelated MHS & 211 MH normal
- Method
  - NGS sequence of entire RYR1 & CACNA1S coding regions in 57 samples
- Findings
  - 13/29 MHS confirmed or potential causative mutations identified
    - 2RYR1 & 1 CACNA1S
  - 7/28 EHI
    - 4 RYR1 and 2 CACNA1S
    - 5/7 abnormal IVCT

Genetic Studies of MH

from

Different Parts of the World

US Data

Brandom 2013 A&A

- 120 unrelated MHS
  - 108 positive CHCT
  - 12 no CHCT with hx of MH event confirmed by medical record review
- Screening Method
  - Tiered, targeted exome sequencing
  - 100 healthy Caucasian controls used to eval novel RYR1 variants frequency
  - CACNA1S screened if no RYR1 variants in >100 exons screened
- RYR1 mutations or variants
  - 2 CCD
  - More than ONE variant seen in 4 subjects
  - CHCT response greater in those with RYR1 mutation or variants

RYR1 mutation or other variants in MHS 52%

Data from Europe

Robinson 2003 Eur J Human Genetics

- IVCT centers from Europe
  - Belgium, Italy, France, Germany, Switzerland & UK
  - > 500 individuals
  - 15 mutations screened
- Identified three most prevalent RYR1 mutations
- **RYR1 mutations in MHS**
  - **12% Switzerland**
  - **27% UK**
  - **32% France**
  - **26% Italy**
  - **25% Belgium**

UK

Robinson Human Mutation 2002

- 297 MHS by IVCT
- Screened for 15 causal RYR mutations
- 85 identified with RYR1 missense mutations

RYR1 mutations in MHS 29%

(Specific 15 causal RYR mutations)

Data from Italy

Galli Human Mutation 2006

- 50 MHS (MH reaction, FH, chronic high CK)
- Entire RYR1 coding region screened
- 31 mutations in 43 individuals

RYR1 mutations in Italian MHS 86%

French Data

Monnier 2005 Human Mutation

- Study cohort
  - 129 IVCT-confirmed families
  - 189 MHS (Positive IVCT or MH episode)
- Genetic Screening
  - 25 causative RYR1 mutations
  - Additional screening for novel mutations

25 causative RYR1 mutations in MHS 44%

RYR1 variation & mutations 60%

Australian Data

Gillies Anaesth Intens Care 2008 and 2015

2008

- 38 IVCT- documented MHS
- Hot spot screening for RYR1 mutations
- 9/38 showed RYR1 mutations, 9/28 showed RYR1 variants

RYR1 variants and mutations in MHS 47%

2015

- 62 IVCT-documented MHS
- 6/62 with known causative RYR1 mutations
- Additional RYR1 or CACNA1S variants seen in 17/62
- Total 23/62 variants (one or more)

RYR1 or CACNA1S Variants and mutations in MHS 37%

- CGS and presence of variant or mutation
  - 80% of those with CGS 5 or 6 have a variant or mutation
  - CACNA1S variants with lower responses in IVCT

Combined rate of detection in Australian cohort 41%

Canada Data

Riazi 2014 A&A

Toronto General 1992-2011 CHCT patients

- 129 MHS & + CHCT & medical records available
  - 62% male
  - 95% Caucasian
  - 13% Prior unremarkable anesthetics
  - 56% CGS ranked 5 or 6
- RYR1 mutation analysis performed in 51/129

RYR1 mutations in MHS 47%

Canada Data

Kraeva Can J Anes 2011

Toronto General 2003-2008

- 36 MHS subjects
  - Positive CHCT
  - FH MH
  - Abnormal resting CK
  - CGS >35
- Genetic screening
  - Entire RYR1 transcript and selected regions of CACNA1S
- Findings
  - 7 known, 20 potential causal RYR1 and 15 novel RYR1 mutations

RYR1 mutations in MHS 86%

MH in Japan

Ibarra 2006 Anesthesiology

- Calcium-induced calcium release (CICR) Test for Dx
- 58 MHS or >1.5 SD for enhanced CICR
  - 41/58 have RYR1 variation
  - 33/58 with potentially pathogenic RYR1 mutations

RYR1 mutation in MHS ***57%***

- RYR1 Mutations
  - 48% have cores on muscle biopsy
  - All CCD have RYR1 mutation
    - **Prevalence of MHS 1:2,000 in Japan**

RYR1 Mutations or Variants

	Detection Rate	Screening Method
UK	29%	Specific for 15 causal RYR1 mutations
US	52%	entire coding regions of RYR1
France	60%	Combined elective and entire coding screening
Canada	86%	entire coding RYR1 & select regions of CACNA1S
Italy	86%	entire coding regions of RYR1
Australia	41%	entire coding regions of RYR1 & CACNA1S + hot spot screening
Japan	57 %	CICR

Genotype-Phenotype Correlation

- Clinical MH reaction & RYR 1 mutation correlation data limited
- Demonstrated for RYR1 mutations & IVCT responses.
  - Brandom et al 2013
  - Carpenter 2009
  - Monnier 2005
  - Robinson 2002
- RYR1 mutations causing CCD in MHS have worse IVCT
- RYR1 mutations have been identified in some individuals with exertional heat illnesses and exertional rhabdomyolysis

RYR1 Genotype & MH Phenotypes

Carpenter 2009 BJA

- MHS subjects
  - 504 individuals
  - 204 families
- RYR1 Screening and variant analysis
- Phenotypes examined
  - Onset of clinical reaction
  - Baseline CK
  - Pharmacological muscle contracture response

Different RYR1 variants

correlate with severity of IVCT and baseline CK

No sufficient data re clinical MH reaction

Clinical Scenarios

Questions

1. Why is this patient MHS?
2. Is muscle biopsy indicated?
3. Is muscle biopsy feasible?
4. Is genetic testing indicated
5. How would genetic testing inform the care of this patient?
6. Anesthetic plan for this patient.

Case #1

A 3 year old female child weighing 15 Kg is scheduled for femoral osteotomy. The patient's maternal uncle (mother's brother) had an MH episode and but did not have a muscle biopsy to confirm diagnosis of MH.

1. Is this patient MHS? *Yes*
2. Is muscle biopsy indicated? *In Uncle*
3. Is muscle biopsy feasible? *No. BW ≥ 20 kg*
4. Is genetic testing indicated? *Need to test child and family*
5. How would genetic testing inform the care of this patient? *Identify possible causative mutations.*
6. Anesthetic plan for this patient. *Treat patient as MHS*

Case #2

A 16 year old African American male patient with history of exercise-induced rhabdomyolysis is scheduled for emergency appendectomy. His resting CK is 600IU.

1. Is this patient MHS? *Potentially*
2. Is muscle biopsy indicated? ?
3. Is genetic testing indicated? *May be helpful*
4. How would genetic testing inform the care of this patient? *ER may have RYR1 mutations that are causative MH mutations.*
5. Anesthetic plan for this patient. *Should avoid using SDC*

Case #3

A 9 year old male patient reported to have a history of masseter spasm during GA for hernia repair at age 2 years. No anesthesia record is available for review. Normal CK. Patient's father & mother both had CHCT tests and the results were negative. No other significant family history. Patient is scheduled for T&A.

1. Why is this patient MHS? *Not likely.*
2. Is muscle biopsy indicated? *No*
3. Is muscle biopsy feasible? *If child >20 Kg, but not indicated*
4. Is genetic testing indicated? *No true clinical indication*
5. How would genetic testing inform the care of this patient?
6. Anesthetic plan for this patient. *Avoid SDC, no other concerns.*

CHCT vs Genetic Testing

CHCT

Gold standard

Sensitive

Expensive

Invasive muscle biopsy

At CHCT Testing centers only

Minimum bw $\geq$ 20 kg

(not possible in very young children)

Genetic Testing

Only detects ~30%

Specific

Cost varies

Non-invasive

A blood test

Can be performed in all ages

Should include genetic counseling

Genetic Testing Centers

Division of Molecular Diagnostics, Department of Pathology, UPMC

S701 Scaife Hall
3550 Terrace Street
Pittsburgh, PA 15213
412-648-8519
mdx@upmc.edu

Medical Neurogenetics

5424 Glenridge Drive NE
Atlanta, GA 30342
678-225-0222

Prevention Genetics, LLC

Eric W. Johnson, PhD
3700 Downwind Drive
Marshfield, WI 54449 USA
715-387-0484

Clinicaltesting@preventiongenetics.com

Pharmacogenomics of MH or What We Know About MH Susceptibility

- Prevalence estimated at 1: 2,000
- Ethnicity
  - Mostly reported from western world (may reflect anesthesia practice)
  - Caucasians, Japanese, Brazilians
- Males> Females
- Associated conditions
  - CCD
  - Other RYR1 myopathies
  - Exertional Heat Illness
  - Exertional Rhabdomyolysis