TRANSLATING SCIENCE TO BETTER HEALTH:
THE POWER OF DIVERSITY AND MULTICULTURAL ENGAGEMENT

CONCURRENT SCIENTIFIC SESSION 8
Advances in Heart Failure Etiology and Therapy

JIMMY T. EFIRD
University of Hawaii at Manoa
Gene Therapy for the Treatment of Heart Failure

Roger J. Hajjar, MD
Mount Sinai School of Medicine
New York, NY
Heart Failure - Continued Unmet Need

- Rising Patient Population
  - > 5 million people in US
  - Age related diagnosis, aging will double incidence
  - > 500,000 new cases per year

- Poor Prognosis
  - 4-fold increased mortality at any age
  - Average survival < four years
  - Five-year survival rate of 25% in men and 38% in women with NYHA classes II to IV

Projected incidence of heart failure

HF Mortality is Increasing
Despite Overall Decline in CV Deaths

National Center for Health Statistics

Racial Differences in Heart Failure
Treatment Trial

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>White</th>
<th>Black</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62 ± 10</td>
<td>57 ± 11</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic</td>
<td>70%*</td>
<td>46%</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>24%*</td>
<td>52%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>38%*</td>
<td>65%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>24%*</td>
<td>32%</td>
</tr>
<tr>
<td>Left-ventricular ejection fraction</td>
<td>25.0 ± 6.7</td>
<td>24.8 ± 7.0</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>1.22 ± 0.3*</td>
<td>1.31 ± 0.4</td>
</tr>
</tbody>
</table>

*P<0.01 vs black
Myocardium in Patients with Heart Failure

- Permanent loss of myocytes
- Dysfunctional myocytes
- Cardiac Fibroblasts
- Undiseased myocytes
- Hypertrophic Response
- Pathologic Stress

Calcium Handling in Failing Human Hearts

- Trabeculae Aequorin
- Isolated Cardiac Myocytes Fura-2

Control Dilated Cardiomyopathy


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SERCA2a Expression and Activity in Failing Hearts

Non-Failing   Failing

Phospho-

lamban

Non-Failing
Failing

ATPase Activity (nmol/mg.min)

Cell shortening (%)

Fura-2 Ratio

Non-Failing Myocyte + Ad.GFP
Failing Myocyte + Ad.GFP
Failing Myocyte + Ad.SERCA2a

Creating a Heart Failure Phenotype from iPS Derived Cardiomyocytes by Targeting SERCA2a

Fibroblasts

Shortening
calcium
siRNA
SERCA2a
Lentivirus

Normal Beating Myocyte

Human Cardiomyocytes

DCM + Ad.SERCA2a

SR ATPase Activity

Cytosol

Energy consumption/production

Mitochondria

Transcriptional regulation

ATP

Nucleus

Signalling pathways

Contraction

Cell growth

Cell death

Myofilaments
GENE THERAPY
• Unmet needs using current therapies
• Advances in the understanding of the molecular basis of heart failure
• Cardiomyocyte-specific targets have emerged that are difficult to manipulate pharmacologically

VECTORS
• Increasingly efficient gene transfer technology
• Safe vectors
• Homogeneous transduction of the cardiomyocytes
• Long-term expression
• Cell-specificity
• Effective and minimally invasive

Targeting by Gene Therapy

Choice of Vectors
Modes of Delivery
Immune Response
Clinical Tools

Viral Vectors for Gene Therapy
SIZE MATTERS!

<table>
<thead>
<tr>
<th>Solution (RI 788)</th>
<th>0.02 µm (Dark Red)</th>
<th>0.1 µm (Yellow Green)</th>
<th>10 µm (Red)</th>
</tr>
</thead>
</table>

Antegrade Injection  
Whole Heart

AAV Vectors
- Safe vectors
- Long-term expression
- Cardiac specificity
- Minimal immune reaction at low doses

AAV SEROTYPES

Muscle  Liver  Retina

Muscle  Liver  Retina  Like AAV1  Muscle  Liver  Heart
AAV1 Kinetics of Expression Dependent on Dose in vivo (AAV1/GFP) Injected into Mouse Hind-leg; Tumors quantified by optical imaging

AAV1.SERCA2a DNA: Vector Genome
- 4486 bp
- AAV2 ITRs flanking expression cassette
- <300 nucleotides of non-coding AAV DNA

Effects of SERCA2a Gene Transfer in Experimental Models of Heart Failure

<table>
<thead>
<tr>
<th>Pharmacological Inotropy (cAMP Dependent)</th>
<th>Targeting SERCA2a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energetics</td>
<td>↑</td>
</tr>
<tr>
<td>Arrhythmogencity</td>
<td>↑</td>
</tr>
<tr>
<td>cAMP</td>
<td>↑</td>
</tr>
<tr>
<td>Diastolic Calcium</td>
<td>↑</td>
</tr>
<tr>
<td>Survival</td>
<td>↑</td>
</tr>
</tbody>
</table>

Presented at the 13th RCMI International Symposium on Health Disparities | December 9-13, 2012 | San Juan, Puerto Rico
Does SERCA2a gene transfer induce an increase in SR Ca²⁺ Leak?

Effect of SERCA2a Gene Transfer on Survival in Rats with Pressure-Overload Hypertrophy in Transition to Heart Failure.
Targeting Calcium Cycling Abrogates Adverse Remodeling

Cardiac Injury
- Increased load
- Reduced systemic perfusion
- Activation of RAS, SNS, and cytokines
- Altered gene expression
- Growth and remodeling
- Contraction Abnormalities
- Apoptosis
- Cell death

Modes of Delivery

- Surgical Technique
  - Gene Transfer During Bypass
- Catheter Based Techniques
  - Extracorporeal Re-Circulating System (V-Focus™)
  - Retrograde Perfusion
  - Antegrade Epicardial Coronary Artery Infusion (AECAI)

Near-Infrared Surgical Imaging System
High Efficiency Transduction of Myocardium with Direct Intracoronary Infusion of AAV1 Based Vectors

- AAV1/LacZ dose: 2 x 10^{12} DRP
- Transduction efficiency: 60% ± 22 with a range of 16-98%

Preclinical Studies Demonstrate IC Infusion is Therapeutic at Low Doses

- AAV1.SERCA2a Detected by qPCR in Heart Sections After IC Infusion

Pre-Clinical Efficacy and Safety Studies

- Porcine Mitral Regurgitation Model of Heart Failure
  - Delivered into LCA through 10 minute infusion
    - 1 x 10^{12} DRP
- Porcine Myocardial Infarction Model + Shunt
  - Delivered into LCA through 10 minute infusion
    - 5 x 10^{12} DRP
- Ovine Pacing Model of Heart Failure
  - Delivered into LCA using either direct infusion or V-Kardia device over 10 minutes
    - 10^{10} - 2.5 x 10^{13} DRP
Study Design

MR creation (n = 26)

Gene Transfer (n = 16)

2 months

AAV1.SERCA2a (n = 9)

AAV1.βgal (n = 8)

2 months

Sacrifice SERCA2a (n = 7)

Sacrifice βgal (n = 6)

50% survival rate

SERCA2a Rescue

(A) SERCA2a level

(B) Western blot analysis

GAPDH (Cytoplasm)

S2a (Microsome)

CAPDH (Cytoplasm)

PKB (Microsome)

PKC (Mitochondria)

SERCA2a

NFAT

Cyt: cytoplasm

Mic: microsome

Mito: mitochondria

Nuc: nucleus

Pig Mitral Regurgitation Model of Heart Failure (Systolic)

Relative Median Change After 2 Months

Control

AAV1.SERCA2a

Fractional Shortening* -2% +55%

Ejection Fraction* -28% +16%

End Systolic Volume* +35% -14%

+dp/dt* -23% +33%

*p< 0.05

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Dose Response in Sheep Pacing Model of HF

Use of Nitroglycerin During Infusion
Vasodilators Increase Vector Uptake in the Heart

Minipigs, AECAS Delivery of MYDICAR

- Control
- IC Nitro Pre-infusion
- IV Nitro During Infusion

qPCR SERCA2a Expression (p<0.0001)

Intracoronary Bolus: 50 µg nitroglycerin
IV infusion: 1 µg/kg/min nitroglycerin

Changes in Regional, Myocardial Blood Flow by IC vs IV injection of NTG in Pigs

- Nitro IC
- Nitro IV
- Baseline

Immune Response following AAV Gene Transfer

- Neutralizing Antibodies to specific AAV serotype
- T cell (CD8+) response
NAB in Patients with Heart Failure

These Patients Would Need to be Excluded in Clinical Trials of Gene Therapy Especially at Low Doses of AAV

Concentration Dependent Recovery of AAV1/SERCA2a Infectivity After Incubation with NAbs In Vitro

Uptake of AAV1/SERCA2a In Vivo in the Heart in the Presence of Different Levels of NAbs
Potential for T-cell response
• Cells may transiently express AAV1 capsid protein on their cell surface.
• T-cell response could theoretically occur at any targeted tissue (MHC molecules, RES clearance) or heart (site of injection).
• In the clinical trials, to evaluate potential development of a T-cell response, a novel assay will be used: ELISPOT assay for anti-AAV1 capsid T-cell responses (IFN-γ release when patient's PBMCs are exposed to capsid peptide).

Adapted from K. High
**Eligibility**

### Main Inclusion Criteria
- Age 18-75 years old
- NYHA Class III/IV
- Ischemic or non-ischemic cardiomyopathy
- Maximal oxygen consumption ($\dot{V}_{O_2}$) of ≥ 15 ml/kg/min
- Left ventricular ejection fraction ≥ 30%
- KDA implanted
- If indicated, biventricular pacemaker implanted for ≥ 60 days
- Stable optimized HF regimen for 30 days, except for diuretics

### Main Exclusion Criteria
- Anti-AV1 neutralizing antibody titer (MRSA ≥ 1:2
- Clinically significant MI within 6 months
- 3 prior <14 kg/m² surgery with ≤ 6 months
- Expected survival <1 year
- Based on investigator’s clinical judgment of HF and co-morbid conditions

**Automatic Pericardial Artery Intracoronary Artery Infusion**

- Antegrade epicardial coronary artery infusion
- 60 mL divided into 1, 2, or 3 infusions depending on anatomy
- Delivered via commercially available angiographic injection system & guide catheters

**Time-to-Clinical Events Analysis: Multiple Events per Subject**

- Clinical events:
  - Worsening Heart Failure (WHF), MI and Silent MI, HF-related hospitalizations, All-Cause Death, LVAD, Transplant
- Observation period ends either at death or at Month 6
- Analysis pre-specified in statistical analysis plan (SAP)
### Baseline Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Subjects N=39</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean (SD)</td>
<td>60.5 (11.5)</td>
</tr>
<tr>
<td>Sex, n</td>
<td>34 Male</td>
</tr>
<tr>
<td>Race, n</td>
<td>34 White</td>
</tr>
<tr>
<td>HF Etiology, n (%)</td>
<td></td>
</tr>
<tr>
<td>Ischemic cardiomyopathy</td>
<td>19 (48.7)</td>
</tr>
<tr>
<td>Idiopathic cardiomyopathy</td>
<td>14 (35.9)</td>
</tr>
<tr>
<td>Hypertensive cardiomyopathy*</td>
<td>4 (10.3)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (7.7)</td>
</tr>
</tbody>
</table>

| Co-Morbidities, n (%) | | |
| Diabetes | 13 (33.3) |
| Hypertension | 25 (64.1) |
| Atrial Arrhythmia* | 17 (43.6) |
| Coronary Artery Disease | 24 (61.5) |
| Myocardial Infarction | 21 (53.8) |
| HF Regimen, n (%) | | |
| ACE Inhibitor / Angiotensin Antagonist | 36 (92.3%) |
| Aldosterone Antagonist | 17 (43.6) |
| Beta-Blocker | 35 (89.7) |
| Diuretic | 37 (94.9) |
| Other Concomitant Therapy, n (%) | | |
| Digoxin Therapy | 21 (53.9) |
| Antithrombotic Therapy** | 34 (87.1) |

* Includes atrial fibrillation, atrial flutter, atrial tachycardia, sick sinus syndrome and paroxysmal supraventricular tachycardia
** Warfarin, aspirin and/or clopidogrel

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Subjects N=39</th>
</tr>
</thead>
<tbody>
<tr>
<td>6MWT, m, mean (SD)</td>
<td>343 (124)</td>
</tr>
<tr>
<td>VO2max*, mL/kg/min, mean (SD)</td>
<td>13.9 (3.9)</td>
</tr>
<tr>
<td>LV EF, %, mean (SD)</td>
<td>25 (7)</td>
</tr>
<tr>
<td>LVESV, mL, mean (SD)</td>
<td>202 (91)</td>
</tr>
<tr>
<td>NYHA Class III, n (%)</td>
<td>39 (100)</td>
</tr>
<tr>
<td>MLWHFQ, mean (SD)</td>
<td>46 (22)</td>
</tr>
<tr>
<td>NT-proBNP, pg/mL, mean (SD)</td>
<td>2032 (3028)</td>
</tr>
<tr>
<td>Creatinine, mg/dL, mean (SD)</td>
<td>1.34 (0.53)</td>
</tr>
</tbody>
</table>
Clinical Events – Stage 2

MYDICAR Low

MYDICAR Mid

MYDICAR High

New Arrhythmias On-Study

<table>
<thead>
<tr>
<th>Type of Arrhythmia</th>
<th>Placebo</th>
<th>MYDICAR Low</th>
<th>MYDICAR Mid</th>
<th>MYDICAR High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-sustained VT, n (%)</td>
<td>3 (21)</td>
<td>1 (100)</td>
<td>2 (20)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>History of arrhythmia, n (%)</td>
<td>1 (13)</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td>1 (33)</td>
</tr>
<tr>
<td>Sustained VT, n (%)</td>
<td>1 (7)</td>
<td>0</td>
<td>2 (25)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>History of arrhythmia, n (%)</td>
<td>1 (7)</td>
<td>0</td>
<td>1 (100)</td>
<td>1 (33)</td>
</tr>
<tr>
<td>Atrial Fibrillation, n (%)</td>
<td>3 (20)</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td>1 (33)</td>
</tr>
<tr>
<td>History of arrhythmia, n (%)</td>
<td>1 (7)</td>
<td>0</td>
<td>1 (100)</td>
<td>1 (33)</td>
</tr>
</tbody>
</table>

*New* means baseline ICD interrogation had absence of any arrhythmia or baseline was not captured

*History of arrhythmia* means arrhythmia was noted as part of the past medical history at the time of screening

The Low & Middle Dose Groups

- Response seems to be limited to the first 6 months in these 2 dose groups
- From biopsy/explant data, little or no expression of exogenous SERCA2a in these two groups
### Patient Treatment Tissue qPCR

<table>
<thead>
<tr>
<th>PHASE 1</th>
<th>Patient</th>
<th>Treatment</th>
<th>Tissue</th>
<th>qPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>031001</td>
<td>AAV1.SERCA2a</td>
<td>Lowest Dose</td>
<td>Anterior/Posterior/Septal/Inferior</td>
<td>Not Detected</td>
</tr>
<tr>
<td>01002</td>
<td>AAV1.SERCA2a</td>
<td>Mid Dose</td>
<td>LV Apical Core</td>
<td>Not Detected</td>
</tr>
<tr>
<td>011002</td>
<td>AAV1.SERCA2a</td>
<td>Mid Dose</td>
<td>Anterior/Posterior/Septal/Inferior</td>
<td>Not Detected</td>
</tr>
<tr>
<td>091007</td>
<td>AAV1.SERCA2a</td>
<td>High Dose</td>
<td>LV Apical Core</td>
<td>200 ss</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PHASE 2</th>
<th>Patient</th>
<th>Treatment</th>
<th>Tissue</th>
<th>qPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>081011</td>
<td>Placebo</td>
<td>Apical Core</td>
<td>Not Detected</td>
<td></td>
</tr>
<tr>
<td>081011</td>
<td>Placebo</td>
<td>Anterior/Posterior/Septal/Inferior</td>
<td>Not Detected</td>
<td></td>
</tr>
<tr>
<td>051002</td>
<td>AAV1.SERCA2a</td>
<td>Mid Dose</td>
<td>LV Apical Core</td>
<td>Not Detected</td>
</tr>
<tr>
<td>081006</td>
<td>AAV1.SERCA2a</td>
<td>Mid Dose</td>
<td>Anterior/Posterior/Septal/Inferior</td>
<td>Not Detected</td>
</tr>
<tr>
<td>051002</td>
<td>AAV1.SERCA2a</td>
<td>Mid Dose</td>
<td>Anterior/Posterior/Septal/Inferior</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

### Coronary flow measurement

![Coronary flow measurement graph](image)

**P=0.01**

**P=0.02**

**SERCA2a Normal**

**MR-Heart Failure**

**AAV1.LPL ELISPOT+**

**No CPK elevation**

**High Dose of AAV1.SERCA2a in CUPID**

**AAV1.LPL ELISPOT+ + CPK elevation**

**Loss of Transgene**

**AAV2.FIX ELISPOT+ + LFTs elevation**

**Intrahepatic + LFTs Loss of Transgene**

### Should Higher Doses of AAV1.SERCA2a be Used?

- **AAV1.SERCA2a Dose of 10^12**
- **AAV1.SERCA2a Dose of 10^11**
- **AAV1.SERCA2a Dose of 10^10**
- **AAV1.SERCA2a Dose of 10^9**

**Should Higher Doses of AAV1.SERCA2a be Used?**

**Pre-Clinical Efficacy Dose AAV1.SERCA2a Cohort 1**

**Dose of CUPID Phase 1**
CUPID Pre-Screen Anti-AAV1 Antibody Results
≈50% Heart Failure Patients Qualify

Percent by NAb Titer
N=509

- <1:2 n=244
- 1:2 n=36
- 1:4 n=30
- 1:8 n=36

Plasmapheresis to Remove NAbs Prior to AAV1:SERCA2a Infusion

- Plasmapheresis for anti-AAV1 NAb
- Plasma is continuously prepared under appropriate anticoagulation and is pumped alternating through the adsorbers, where the antibodies are bound by the ligands.
- The plasma volume to be treated depends on the indication
- Potentially can reduce antibody titer 15-20 fold

Designer AAV Vectors

- Highly Tropic for Cardiac Muscle
- Highly Efficient in Primate & Human Cardiac Myocytes
- Has very little/no tropism to liver, lungs, spleen, kidneys.
- NO Antecedent Neutralizing Antibodies
CUPID SUMMARY

- In this phase 2 study of patients with advanced HF, AAV1.SERCA2a was found to be safe and associated with benefit in the following:
  - Clinical outcomes
  - Symptoms
  - Functional Status
  - Biomarkers
  - Cardiac Structure
- These encouraging results support phase 2b studies to advance AAV1.SERCA2a towards registration for advanced HF.
- November 2011, FDA gave AAV1.SERCA2a a “Fast Track Designation.”
- August 2012: Start of CUPID 2 which will enroll 200 patients in international centers 1:1 high dose AAV1.SERCA2a vs Placebo.
AAV1.SERCA2a Phase 3 Trial
N=200, 2-Arm

Sample Size/Power:
N=100 per treatment group with 180 recurrent events provides:
- 80% power, 0.05 two-sided significance level, to detect at least a 41% risk reduction (HR=0.59) based on time-to-recurrent HF-related hospitalizations in presence of terminal events (LV AD, MYDICAR 1x10^13 DRP, N=100 Placebo, N=100 PATIENT POPULATION

- 18-80 years of age, inclusive
- Chronic systolic HF
- Ischemic or dilated cardiomyopathy
- EF ≤ 35%
- NYHA Class III or IV
- Maximal, optimized HF

Long-Term Follow-Up
All subjects followed quarterly for clinical events until:
- Last enrolled subject completes 12 months of observation AND
- 180 adjudicated HF-related hospitalizations have occurred

CLINICAL TRIALS: AAV.SERCA2a

Enrolling:
- Patients undergoing LVAD insertion as destination-therapy or bridge to transplant receive AAV1.SERCA2a one month after VAD placement, 1x10^13 drp (15 patients) and saline (15 patients). (Harefield/Papworth, UK)
- Class III/IV heart failure patients receive AAV1.SERCA2a (22 patients) vs saline (22 patients) and LV function will be followed by multi-imaging modalities (Pitié-Salpetrière, Paris, France)

Other Trials being planned:
- Patients with normal Ejection Fraction HF
- Patients with primary pulmonary hypertension
- Patients with ventricular arrhythmias and ICD for secondary prevention
- Patients with muscular dystrophy and advanced cardiac disease

Patient-specific skin fibroblasts
Skin punch biopsy
Reprogramming factors
iPSC

Patient-specific cardiac myocytes in AAV.SERCA2a trials
Differentiation by cytokines
Differentiation by transcription factor activation

Single-cell Measurements
- Patch clamp
- 2D – models
- Micro-Electrode Array
- 3D-Cardiac tissue
- Force / contrility
- EKG / electric

SERCA2a Gene transfer efficacy
Functional assay to correlate with clinical improvements

DUPLICATION PROHIBITED
Integrated Proteomic Platform for SERCA2a Network

Display proteome
- Sub-organelles isolation (Cyt, Mito, Micro and Nuc)
- 2-D gel analysis (gel based)
- PF2D (gel free)
- Image analysis isolated spots
- Protein identification/Classification/Validation

Interactome
- Immunoprecipitation (IgG vs S2a)
- HPLC analysis
- Image analysis isolated bands/spots
- Protein identification/Classification/Validation

Phosphoproteome
- Phosphoprotein enrichment
- Phosphoprotein analysis
- Image analysis isolated spots
- Protein identification/Classification/Validation

Phosphorylated proteins (S2a phosphoproteome)
- ESI-MS/MS

SERCA2a Interactome Network

SUMO1 is identified as a SERCA2a Interacting protein

A. IP&2-DE
- Lysates (10% input)
- IP: Flag (SUMO1)
- WB: SERCA2a
- IP: myc (Ubc9)
- WB: SERCA2a

B. Validation
- Lysates (10% input)
- Image analysis isolated spots
- Protein identification/Classification/Validation

SUMO1 is Identified as a SERCA2a Interacting Protein
Small Ubiquitin-like Modifier Conjugation

SUMOylation can regulate protein-protein interactions, intracellular localization, enzymatic activity, and protect some modified targets from ubiquitin-dependent degradation.

SUMO1 Protein Level is Decreased in Failing Hearts

SERCA2a is Covalently Modified by SUMO1 at Lysine 480/lysine 585

SUMO consensus site: ψKXE
(ψ: hydrophobic residue)

Mouse Rat Rabbit Pig Human
IKQL MKKE FTLEFSRD - - LEDSANF
IKYE TNL

S-SERCA2a

IP: SUMO1
IB: SERCA2a

Input

IB: SERCA2a

kDa
150 250 100

SERCA2a
SUMO1 GAPDH

Protein/GAPDH (ratio to sham)

Mice

Pigs

P=0.001 P=0.03

P=0.004 P=0.03

n=5 n=3 n=8 n=5

n=8 n=5

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Protocol for rAAV9-Mediated SUMO1 Gene Therapy

- TAC operation
- Echo Tail-vein injection
- Echo Sacrifice

Mouse strain: B6C3F1
Injection dose:
- rAAV9.GFP (negative control)
- rAAV9.SUMO1: 5 x 10^11 VG
- rAAV9.SERCA2a (positive control)

rAAV9-mediated SUMO1 Treatment Improve LV Function in Heart Failure

SUMO1 Overexpression improves Cardiac Dysfunction by Regulating SERCA2a
Conclusion

SUMO1 may be an important therapeutic target to treat HF patients.

SUMO1

Simultaneous Gene Transfer of SUMO1 & SERCA2a in pre-clinical models

Concomitant Gene Transfer of SERCA2a and SUMO1

SUMO1 Activators

Proteinstability

ATPase activity

Calcium transient

Contractility

Cardiac dysfunction

SUMO1 may be an important therapeutic target to treat HF patients.
Concomitant Gene Transfer of SERCA2a and SUMO1 in a Porcine Model of Myocardial Infarction

Day 0 Day 28 Day 84
Myocardial Infarction

Change in EF

Saline
AAV.SUMO1
AAV.SERCA2a
AAV.SUMO1~SERCA2a

Euthanasia

1 2 3 4
Saline
AAV.SUMO1 AAV.SERCA2a AAV.SUMO1~SERCA2a

Effect of small molecules on SUMO1 accumulation

DUPLICATION AND DISTRIBUTION PROHIBITED
Effect of small molecule activators of SERCA-SUMO on myocyte contractility

Peak shortening

Maximal rate of contraction

Maximal rate of relaxation

Effect of small molecule activators of SERCA-SUMO on myocyte contractility

Calcium amplitude

Effect of small molecule activators of SERCA-SUMO on myocyte contractility

Calcium ($\mu$M)

Baseline

S-SERCA2a

Effect of small molecule #6 and its analogous compounds on SERCA2a SUMOylation

HEK-293 cells: in vivo sumoylation assay

10 $\mu$M, 24hr, Normal status

GAPDH

SERCA2a
Activators of SUMO1

Activators of SERCA2a

AAV1.SUMO1 Gene Therapy

AAV1.SERCA2a Gene Therapy Phase 3

BNP.11c Gene Therapy Phase 1

Acknowledgment

Thank you for your attention
GANGADARSHNI CHANDRAMOHAN

Charles R. Drew University of Medicine & Science

Vitamin D Deficiency and Cardiovascular and Renal Risk Factors among Children from Various Ethnic Groups in the United States

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Presented at the 13th RCMI International Symposium on Health Disparities | December 9-13, 2012 | San Juan, Puerto Rico

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What Does Vitamin D Do?

- Vitamin D is synthesized in the skin under the influence of sunlight and is also present in some foods.
- It plays a role in various biological processes, including bone health, immune function, and regulation of cell growth.

What is the Clinical Significance of Vit D?

- Prevalence of Vitamin D (25(OH)D) deficiency has been increasing over the past few years among children and adults.
- In the US, approximately 50% of children do not receive the recommended daily allowance of vitamin D from their diet or as a supplement.
- Lack of sun exposure in children due to increased indoor activities and increased use of sunscreen further aggravate this situation.
- Prevalence in Black and Hispanic children is known to be higher than in White children.

Vitamin D Deficiency by Age and Ethnicity:


- Data showing the prevalence of vitamin D deficiency by age and ethnicity.
Vitamin D Deficiency, CV Risk Factors and Ethnicity

- In adults, Vitamin D deficiency is known to be associated with CV risk factors (obesity, hypertension, diabetes and dyslipidemia).
- In children, Black and Hispanic children have a higher risk of developing these risk factors compared to White children.
- Therefore, we studied the risk association between Vitamin D deficiency and the cardiovascular risk factors among Black and Hispanic and White children.

Hypothesis

- There is a significant association between Vitamin D deficiency and cardiovascular risk factors:
  - Obesity
  - Hypertension
  - Dyslipidemia
  - Abnormal fasting blood sugar independently and in various combinations, among Black and Hispanic children compared to White children.

Specific Aims

- To determine the OR for having vitamin D deficiency in White, Black and Hispanic children who had cardiovascular risk when compared to their control groups.
- To determine its association to obesity and high BP in combination, in the same population.
Methodology

Retrospective, cross sectional analysis of data from the National Health and Nutrition Survey (NHANES) conducted between 2001-2004

Study Population:
- Nationally representative cross-sectional survey of non-institutionalized US population
- We studied 4849 children
- Inclusion criteria: Between the ages of 6-17 years
- Exclusion criteria:
  - Those with incomplete data
  - Those who were pregnant

Definitions

Vitamin D deficiency and insufficiency:
- Normal Vitamin D level: > 30 ng/ml
- Vitamin D insufficiency: 15-30 ng/ml
- Vitamin D deficiency <15 ng/ml

Cardio-Renal Risk Factors
- Hypertension: SBP or DBP ≥ 90th percentile or SBP > 120 mm.Hg or DBP > 80 mm.Hg
- Obesity: BMI ≥ 95th percentile
- High WC: ≥ 75th percentile
- Dyslipidemia: one or more of the lipid components is/are abnormal
- Abnormal Fasting Blood Glucose = >100 mg/dl

Statistical Analysis

All variables were stored and analyzed using SAS (SAS Institute Inc, 100 SAS Campus Drive, Cary, NC). Variables were then examined using SUDAAN statistical software 10.0 (Research Triangle Institute, Research Triangle Park, NC) and adjusted for the complex oversampling design.

Standard descriptive statistics, two-tailed student’s t-tests and chi square test will be used to compare categorical variables. Analysis of variance (ANOVA) were employed for continuous variables. Multiple regression analysis was performed where appropriate to identify the adjusted odds ratio for the 25(OH)D deficiency independent of other variables alone or in combination. A p-value less than 0.05 was considered statistically significant.
RESULTS

Demographic Distribution by Ethnicity

Distribution of High Waist Circumference and Obesity (BMI) by Ethnicity: NHANES 2001-2004

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Distribution of Cardiovascular Risk Factors by Ethnicity: NHANES 2001-2004

Distribution of Vitamin D Deficiency and Insufficiency by Ethnicity: NHANES 2001-2004

Mean of All Variables in White, Black and Hispanic Children

<table>
<thead>
<tr>
<th>Variables</th>
<th>White</th>
<th>Black</th>
<th>Hispanic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>11.8 (0.13)</td>
<td>11.57 (0.09)</td>
<td>11.50 (0.12)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.72 (0.19)</td>
<td>*21.48 (0.18)</td>
<td>*21.15 (0.18)</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>73.48 (0.51)</td>
<td>*71.61 (0.44)</td>
<td>73.91 (0.47)</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>105.33 (0.50)</td>
<td>105.79 (0.48)</td>
<td>105.79 (0.48)</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>58.03 (0.61)</td>
<td>56.78 (0.57)</td>
<td>57.87 (0.46)</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>50.76 (0.40)</td>
<td>*56.03 (0.46)</td>
<td>52.27 (0.40)</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>91.42 (1.54)</td>
<td>92.18 (1.15)</td>
<td>*88.54 (1.63)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>94.73 (2.70)</td>
<td>*70.31 (1.14)</td>
<td>92.13 (1.13)</td>
</tr>
<tr>
<td>FBS</td>
<td>93 (0.01)</td>
<td>94 (0.02)</td>
<td>95 (0.01)</td>
</tr>
<tr>
<td>25 OH VitD (ng/mL)</td>
<td>22.70 (0.14)</td>
<td>*18.33 (0.45)</td>
<td>*23.59 (0.35)</td>
</tr>
</tbody>
</table>
OR for Vitamin D Deficiency in White Children

OR for Vitamin D Deficiency in Black Children

OR for Vitamin D Deficiency in Hispanic Children
OR for Vit D Def. After Clustering Obesity and BP in **White Children**

- OR for Vit D Def. After Clustering Obesity and BP in **Black Children**

- OR for Vit D Def. After Clustering Obesity and BP in **Hispanic Children**

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Summary

- There was a significant association between vitamin D deficiency and female gender across all ethnic groups was observed.
- Vitamin D deficiency was independently associated only with age in Black children, but, with CV risk factors, high BP and abnormal FBS, in Hispanic children.
- When obesity and high BP were clustered, the association was 2.5 times higher in Black children and 6 fold higher in Hispanic children compared to their controls (non-obese normal BP children).

Conclusion

- Vitamin D deficiency is highly prevalent among Black and Hispanic children.
- Females demonstrated a higher risk of deficiency compared to males, overall.
- This study underscores the differential association between vitamin D deficiency and major cardiovascular risk factors in Black and Hispanic children compared to White children.

FUTURE DIRECTIONS

- Prospective studies should be conducted to determine the long term clinical effects of Vitamin D deficiency in children from each ethnic groups.
- Also, need to determine the causal effect of vitamin D and the underlying mechanism for its association with those CV risk factors in Black and Hispanic children.
Acknowledgement

- Magda Shaheen M.D, PhD, MPH
- Dulcie Kermah MSc
- Sheena Go M.D.
- Kam Kalanter M.D, PhD

EXaggerated EXercise
BLOOD Pressure AND
cEREBRAL BLOOD FLOW IN
BLACKS

Dr. Vernon Bond
Professor, Colleges of Arts & Sciences & Medicine
Howard University
Systolic Pressure Response To Exercise And Risk Of Cerebral Stroke

SBP > 19 mm Hg/min N = 1026 men


SBP < 16 mm Hg/min Stroke RR = 2.3
PURPOSE OF STUDY
To determine the cerebral blood flow during exercise in normotensive young adult African Americans with an exaggerated exercise blood pressure response.

Subjects
Exaggerated Exercise Pressor Response
18 – 25 yrs (N = 5)

Non-Exaggerated Exercise Pressor Response
(N = 5)
STUDY DESIGN

Laboratory Visit 1 Measures
- Peak oxygen uptake
- Exercise BP response
- Body composition

Laboratory Visit 2 Measures
- Cerebral blood flow
- Cardiac Output
- Heart rate variability
- ETCO2
- BP
<table>
<thead>
<tr>
<th>Variable</th>
<th>Exaggerated Exercise BP Response</th>
<th>Non-Exaggerated Exercise BP Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20.0 ± 0.6</td>
<td>20.0 ± 1.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.0 ± 5.2</td>
<td>168.3 ± 4.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.2 ± 3.9</td>
<td>60.3 ± 1.4 *</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>121.2 ± 5.3</td>
<td>119.6 ± 2.3</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>79.0 ± 1.4</td>
<td>79.0 ± 0.5</td>
</tr>
<tr>
<td>VO2peak (ml.kg⁻¹.min⁻¹)</td>
<td>27.9 ± 4.0</td>
<td>32.3 ± 3.4</td>
</tr>
</tbody>
</table>

* = P < .05

Baseline and submaximal exercise heart rate values in subjects with Non-EEBPR and EEBPR
Systolic blood pressure values at baseline and during submaximal exercise in subjects with Non-EEBPR and EEBPR

Baseline and submaximal exercise diastolic blood pressure in subjects with Non-EEBPR and EEBPR

Baseline and submaximal exercise cardiac output values in subjects with Non-EEBPR and EEBPR
Low frequency heart rate variability values for subjects with Non-EEBPR and EEBPR at baseline and during submaximal exercise

High-frequency heart rate variability values at baseline and during submaximal exercise in subjects with Non-EEBPR and EEBPR

End-tidal carbon dioxide values at baseline at and during submaximal exercise in subjects with Non-EEBPR and EEBPR
Cerebral blood flow at baseline and during submaximal exercise in subjects with Non-EEBPR and EEBPR

**CONCLUSION**

The findings of this study show that, compared to a control group, values of middle cerebral artery blood flow were not significantly different at rest but were lower during graded exercise in young adult African Americans exhibiting an exaggerated exercise blood pressure response. Future studies should determine whether the low cerebral arterial blood flow associated with an exaggerated exercise blood pressure response put these individuals at a higher than normal risk for development of cerebral perfusion dysregulation, cognitive deficits and/or stroke.

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