Second International Summit on Human Genome Editing November 27-29, 2018 Hong Kong

Genome Editing of Heritable Diseases:
Technical and Societal Considerations

Some Comments on Duchenne Muscular Dystrophy

R. Rodney Howell, M. D.
Miller School of Medicine
University of Miami
Miami, Florida USA

UHealth
UNIVERSITY OF MIAMI HEALTH SYSTEM

Duchenne Muscular Dystrophy (DMD)

Guillaume-Benjamin-Amand Duchenne, French physician born more than 200 years ago, studied medicine in Paris, and died in 1875. He provided accurate descriptions of many neuromuscular disorders, including pseudohypertrophic muscular dystrophy to which his name is attached today (Duchenne Muscular Dystrophy)

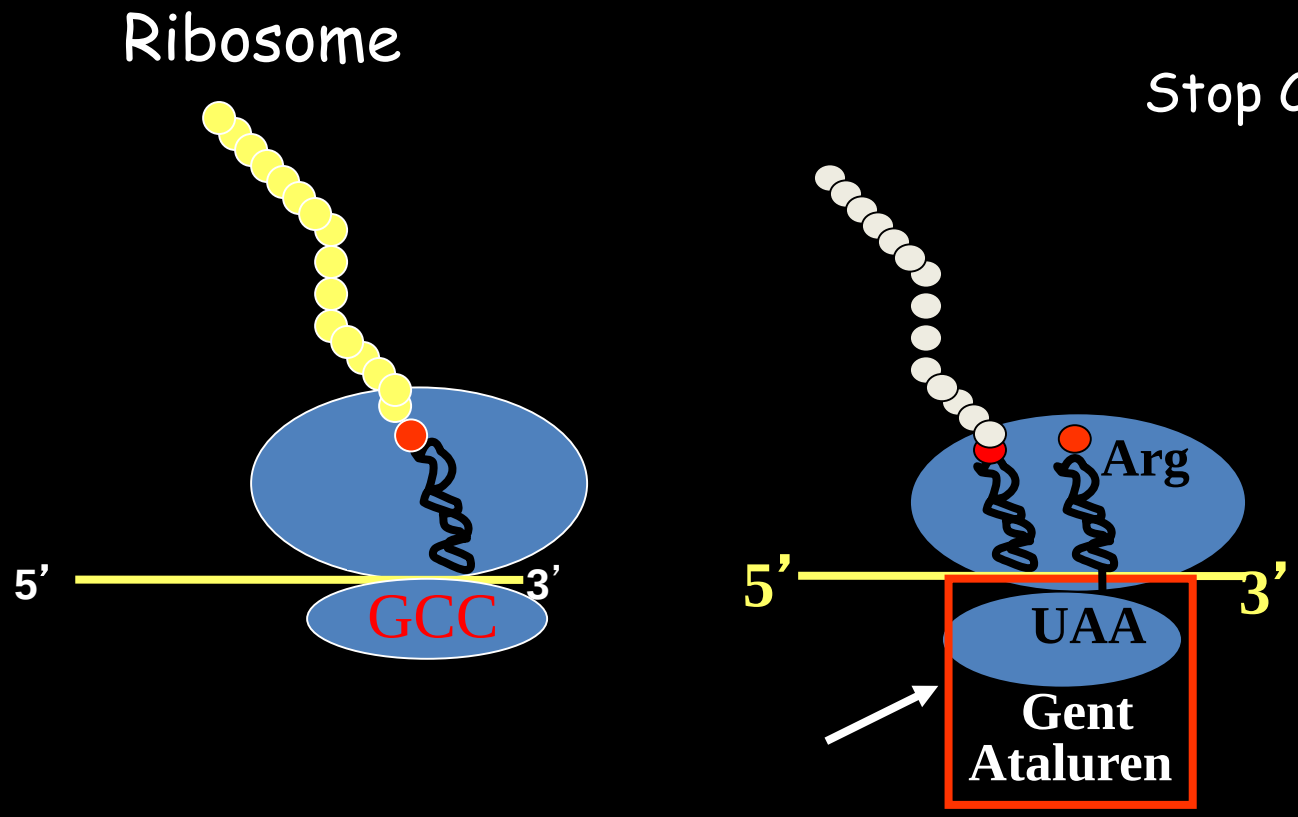
- One of the more common fatal genetic diseases in childhood
- This X-linked genetic disease has an incidence in most studies of between 1 in 4000 to 1:5000 in boys
- Mean age of death in the 1960s was 14.4 years, but this had increased and since the 1990s with ventilation, steroid use and other health-care improvements to 25.3 years of age
- Duchenne Muscular Dystrophy remains uniformly fatal
- The defect in DMD was discovered in the 1980s by Kunkel to be a deficiency of the protein he named dystrophin, the largest protein in the human body
- In spite of this ground-breaking discovery over 30 years ago, no specific treatment has been available for Duchenne muscular dystrophy until recently, and still no curative treatments are available.
- The disease has been an focus of extensive study over the past 50 years led by two very large voluntary health organizations, the Muscular Dystrophy Association in the United States, and the Association Francaise Contre Les Myopathies (AFM) in France, in addition to large numbers of smaller groups world wide in addition to the National Institutes of Health and other similar groups
- The widespread efforts of these organizations has made Duchenne Muscular Dystrophy one of the best known genetic disorders among the general public

Duchenne Muscular Dystrophy

- Corticosteroid treatment is the standard of care in Duchenne muscular dystrophy worldwide and has been shown to be clearly beneficial in both survival and function, although not specific in its action
- There has been extensive work delineating both the number and types of genetic mutations in Duchenne muscular dystrophy and there are current (2015) data in the TREAT-NMD DMD Global data base on more than 7,000 DMD mutations

  - A total of 5,682 large mutations have been observed, of which 4,894 were deletions (1 exon or larger) and 784 duplications (1 exon or larger)
  - There were 1,445 small mutations (smaller than 1 exon) and 358 were small deletions and 132 small insertions and 199 affected splice sites
  - Point mutations totaled 756 with 726 nonsense mutations and 30missense mutations; there were 22mid-intronic mutations
  - Mutations identified within the database that would potentially benefit from novel genetic therapies for DMD including stop codon read-through therapies (about 10% of mutations) and exon skipping therapy (eventually up to 55% of the total mutations)

Mutation Suppression



Gentamicin or Ataluren -Premature stop complex

Exon-Skipping Approach

SKIPPING EXON 51 ENABLES PRODUCTION OF FUNCTIONAL DYSTROPHIN PROTEIN (targets dystrophin region where skipping 51 corrects 13%

Duchenne Muscular Dystrophy

- Drug development has focused on the known mutations for DMD
- Currently, only one specific drug is FDA approved in the USA for DMD caused by deletions at exon 53 but that drug will be effective in about 13% of the DMD population; although very safe, the beneficial drug effects are not dramatic
- Gene therapy trials are underway, and very encouraging. Since the full-length gene for dystrophin is much too large to fit within the viral vectors this requires the use of a “mini-gene”, and the shortened dystrophin produced when effective will produce a Becker-like phenotype
- Specific, life-saving therapies for Duchenne Muscular Dystrophy are urgently needed

Screening for Duchenne Muscular Dystrophy

Jerry R. Mendell, MD,[1] Chris Shilling, MS,[1] Nancy D. Leslie, MD,[2] Kevin M. Flanigan, MD,[1] Roula al-Dahhak, MD,[1] Julie Gastier-Foster, PhD,[1,3] Kelley Kneile, BS,[3] Diane M. Dunn, BS,[4] Brett Duval, BS,[4] Alexander Aoyagi, BS,[4] Cindy Hamil, AS,[4] Maha Mahmoud, BS,[4] Kandice Roush, RN,[1] Lauren Bird, RN,[1] Chelsea Rankin, BS,[1] Heather Lilly, BS,[5] Natalie Street, MS, CGC,[6] Ram Chandrasekar, PhD,[5] and Robert B. Weiss, PhD[4]

Objective: Creatine kinase (CK) levels are increased on dried blood spots in newborns related to the birthing process. As a marker for newborn screening, CK in Duchenne muscular dystrophy (DMD) results in false-positive testing. In this report, we introduce a 2-tier system using the dried blood spot to first assess CK with follow-up *DMD* gene testing.
Methods: A fluorometric assay based upon the enzymatic transphosphorylation of adenosine diphosphate to adenosine triphosphate was used to measure CK activity. Preliminary studies established a population-based range of CK in newborns using 30,547 deidentified anonymous dried blood spot samples. Mutation analysis used genomic DNA extracted from the dried blood spot followed by whole genome amplification with assessment of single-/multiexon deletions/duplications in the *DMD* gene using multiplex ligation-dependent probe amplification.
Results: *DMD* gene mutations (all exonic deletions) were found in 6 of 37,649 newborn male subjects, all of whom had CK levels >2,000U/l. In 3 newborns with CK >2,000U/l in whom *DMD* gene abnormalities were not found, we identified limb-girdle muscular dystrophy gene mutations affecting *DYSF*, *SGCB*, and *FKRP*.
Interpretation: A 2-tier system of analysis for newborn screening for DMD has been established. This path for newborn screening fits our health care system, minimizes false-positive testing, and uses predetermined levels of CK on dried blood spots to predict *DMD* gene mutations.

ANN NEUROL 2012;71:304–313

Over the past 3 decades, creatine kinase (CK) testing on dried blood spots has been attempted as a method of newborn screening (NBS) for Duchenne muscular dystrophy (DMD),[1–9] because CK is elevated at birth in individuals with this condition.[10–12] CK elevation is then validated by retesting of venous blood at 4 to 6 weeks of age with subsequent *DMD* gene analysis employed to establish a definitive diagnosis. Presently, this approach survives only in Antwerp, Belgium[9] (NBS was stopped in Wales on November 30, 2011). It has been difficult for programs to justify NBS for DMD because of the lack of evidence that early treatment improves the outcome of affected newborns.[13,14] In addition, the Wales/Antwerp DMD NBS model, requiring extensive follow-up through retesting of venous blood several weeks after birth for CK with subsequent DNA testing, is impractical to implement in the United States.

Nevertheless, recent advances in diagnostic testing methods and promising molecular-based therapies for DMD have rekindled interest in establishing a pathway

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.23528

Received Nov 15, 2011, and in revised form Dec 31, 2011. Accepted for publication Jan 5, 2012.

Address correspondence to Dr Mendell, Center for Gene Therapy, Research Institute at Nationwide Children's Hospital, 700 Childrens Dr, Columbus, OH 43205. E-mail: Jerry.Mendell@nationwidechildrens.org or Dr Weiss, Department of Human Genetics, University of Utah, 15 North 2030 East, Salt Lake City, UT 84112. E-mail: bob.weiss@genetics.utah.edu

From the [1]Department of Pediatrics, Ohio State University and Nationwide Children's Hospital, Columbus, OH; [2]Department of Pediatrics, University of Cincinnati, Cincinnati, OH; [3]Department of Pathology, Ohio State University and Nationwide Children's Hospital, Columbus, OH; [4]Department of Human Genetics, University of Utah, Salt Lake City, UT; [5]Newborn Screening Laboratories of the Ohio Department of Health, Reynoldsburg, OH; [6]National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA.

Is Now the Time to Develop Newborn Screening for Duchenne Muscular Dystrophy?

BY RICHARD ROBINSON

Promising new therapies have begun to emerge for Duchenne muscular dystrophy (DMD), and that has researchers beginning to think about getting them to newborns at the earliest possible time. Now, a new study shows that newborn screening to identify at birth those carrying the gene for DMD can be done practically, accurately, and inexpensively. The results have experts wondering whether it is time to develop a comprehensive screening program for the disease, to ensure it is in place when any new therapy is approved.

For the study, whose findings appeared Jan. 12 in an advance online edition of the *Annals of Neurology*, Jerry Mendell, MD, director of the Center for Gene Therapy at the Research Institute of Nationwide Children's Hospital in Columbus, OH, and colleagues, used a two-stage screen, first to identify newborns with high creatine kinase and then to follow up with genetic testing for dystrophin mutations.

"The most powerful arguments for newborn screening," Dr. Mendell said, are the recent advances in exon skipping strategies, which he called "the most promising treatment." Within the past two years, two teams of European researchers have shown that oligonucleotides aimed at the most common mutation can restore dystrophin to the muscle membrane, and, according to one trial, may even provide clinically meaningful improvements in ambulation. Dr. Mendell is now conducting a blinded trial of this strategy. Mutation suppression and gene delivery strategies are also in development.

"We think there will be a treatment that can be introduced early in the disease, and we wanted to develop a means of screening boys early that could take advantage of potential early treatments," he said.

High creatine kinase (CK) in the blood is characteristic of newborns with DMD, but it is nonspecific, since it may be caused by multiple other conditions, including birth trauma. It remains high in DMD, while dropping in most other newborn conditions, but families often don't return for a second blood test. Direct genetic testing is highly specific, but current costs prevent it from being used as a first-pass screen.

Dr. Mendell tested for CK in blood spots collected for other disease screens,

DR. VALERIE CWIK: "This is an important study. It shows that population-based newborn screening is feasible for Duchenne muscular dystrophy, and it gives us some good information about how to conduct such a screening program."

and performed a genetic test on those with highly elevated CK. He conducted several screenings — first working with 30,000 samples from the Ohio Department of Health, then 7,000 newborns from Columbus and Cincinnati hospitals, 11,000 samples statewide (all boys), and 20,000 newborn boys, from a pooled samples of over 37,000. (See the table, "Screening Results: Duchenne Muscular Dystrophy" for the results in the different samples.)

Among the entire tested population, the researchers found a total of 308 samples with CK over 750 U/L. They also found 10 new samples with CK over 2000 U/L, and three new DMD mutations, for a total of six in the entire population.

The cost of CK testing was minimal, Dr. Mendell said, with no more than a dollar of materials, and a small marginal labor cost, since all newborns are screened for up to several dozen disorders using the same set of blood spots. Costs could come down even further with a higher CK threshold. "I think we could easily go to 1000 units per liter," based on the results from this study. DNA testing was an additional $150 in materials, although the cost is likely to continue to fall as technology improves, and may fall further if widespread screening is adopted.

A future publication from this study will look at the ethical issues involved in screening for DMD. "The critical question is how an early diagnosis impacts the family," Dr. Mendell said. Some concern has been raised whether receiving such a grave diagnosis at birth would lead parents to emotionally distance themselves from the newborn.

"I've been doing muscular dystrophy research for about 40 years, and I'm very involved in developing treatments," Dr. Mendell said. "But if I leave no other legacy in this field, I'd like to make sure we have a pathway for newborn screening so if we have a treatment, that we can really make a difference."

DR. JERRY MENDELL: "I've been doing muscular dystrophy research for about 40 years, and I'm very involved in developing treatments. But if I leave no other legacy in this field, I'd like to make sure we have a pathway for newborn screening so if we have a treatment, that we can really make a difference."

EXPERT COMMENTARY

"This is a valuable study that provides important information," said Thomas Bird, MD, professor of medicine, neurology, and

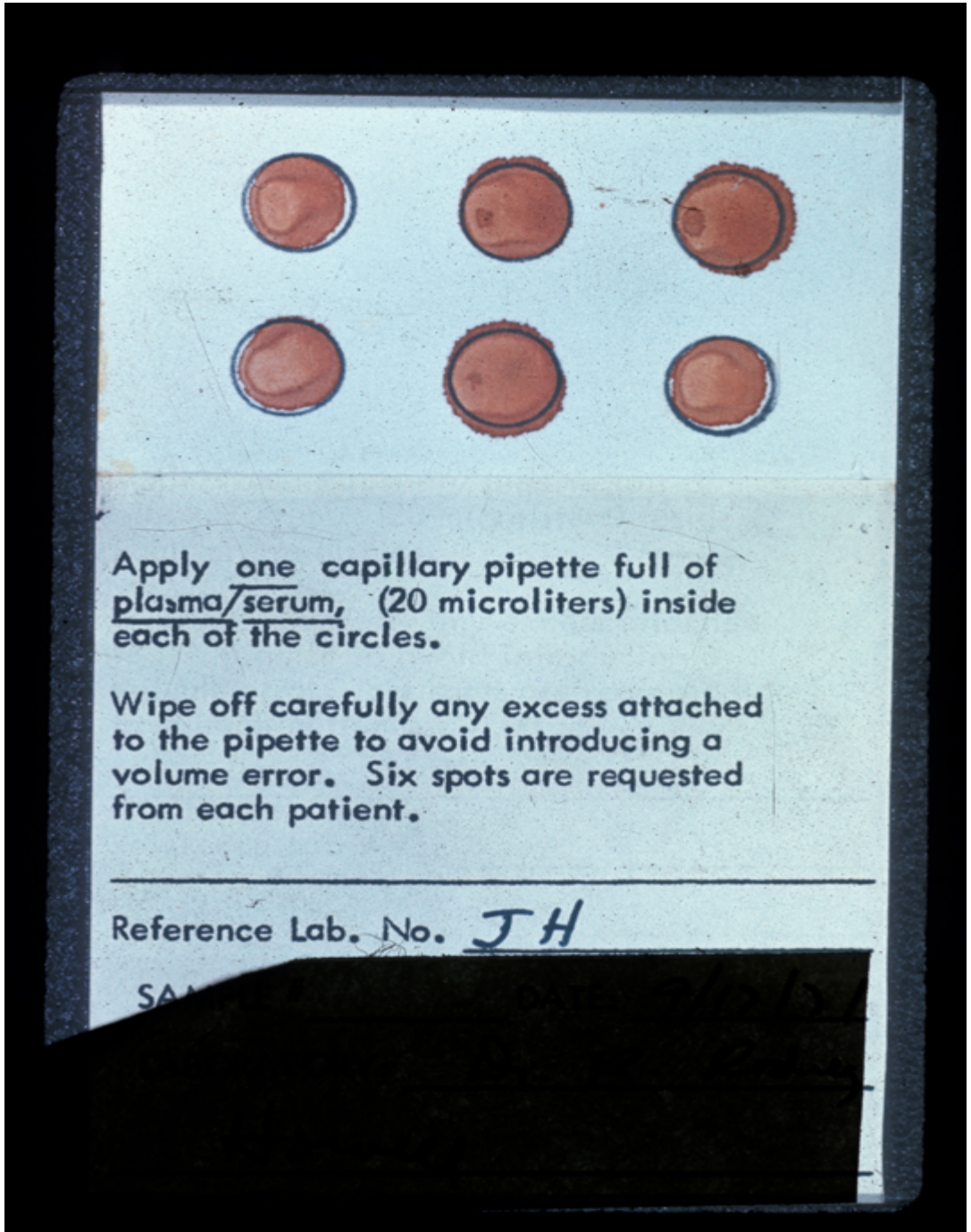
Continued on page 25

SCREENING RESULTS: DUCHENNE MUSCULAR DYSTROPHY

Phase	Total Number of Samples	#>600 U/L	#>750 U/L	#>2000 U/L	# of DMD cases	False- Positive Rate	Additional Notes
2: birthing hospitals in Columbus and Cincinnati	6,928	110		2	2	1.60%	478 declined testing
3: statewide screening, newborn boys	10,037		58	1	1	0.52%	
4: new samples, newborn boys	19,884			10	3		[n]=calculated; data not reported directly
4: total samples	37,740		308	13	6		

Newborn Screening for Genetic Disease in the United States

- **Routine newborn screening has been carried out in all 50 states since the 1970s; the programs have always been state sponsored public health programs, arguably one of the most successful ones**
- **Conditions such as phenylketonuria, with simple, reliable screening tests and proven treatment efficacy have been the targets of testing**
- **Over the years, congenital hypothyroidism and a handful of other diseases were added on a state by state basis**
- **As the programs grew and developed, there was extraordinary variation from state to state and there was little systematic evaluation of either the rationale for screening and/or the outcomes of such screening**
- **Over 4,100,000 infants are screened each year, making newborn screening among the most commonly performed genetic testing in the United States**



Apply one capillary pipette full of plasma/serum, (20 microliters) inside each of the circles.

Wipe off carefully any excess attached to the pipette to avoid introducing a volume error. Six spots are requested from each patient.

Reference Lab. No. JH

SA

Recommended Uniform Screening Panel For Newborn Screening in the United States By the Federal Advisory Panel Includes 35 Core Conditions (As of July 2018)

- Organic Acid Condition 9
- Fatty Acid Oxidation Disorder 5
- Amino Acid Disorder 6
- Endocrine Disorder 2
- Hemoglobin Disorder 3
- Other Disorders 10
  - Biotinidase Deficiency
  - Critical Congenital Heart Disease
  - Cystic Fibrosis
  - Classic Galactosemia
  - Glycogen Storage Disease Type II (Pompe Disease)
  - Hearing Loss
  - Severe Combined Immunodeficiencies
  - Mucopolysaccharidosis Type 1
  - X-linked Adrenoleukodystrophy
  - Spinal Muscular Atrophy

Newborn Screening for Duchenne Muscular Dystrophy (DMD)

- Screening will make use of the standard dried blood spots currently collected from every infant in the United States for newborn screening
- The initial test is for Creatine Kinase (CK) which has been shown to be substantially elevated in newborn infants affected with DMD.
  - It has been shown that CK-MM, one of the three isoenzyme forms of CK found predominately in skeletal muscle can be reliably measured in dried blood samples, and measuring this isoenzyme by a fluroimmunometric assay enhances the reliability of the CK assay for newborn screening
- Infants with elevations (usually large) of CK above an established normal for the newborn period will have the DNA from the same dried blood spots subjected to DNA sequencing and mutation analysis for whole genome amplification
- Since current treatments are mutation-specific, it is recommended that comprehensive sequencing be performed in order to be available for therapies currently under development

Duchenne Muscular Dystrophy(DMD) Moving Forward

- It is likely that we will soon have newborn screening for DMD which will identify virtually every person with Duchenne Muscular Dystrophy in the USA and many other countries
- Patients and their families know a great deal about DMD are very much aware of the developments that might lead to specific treatments
- A recent e-mail I received from a mother from India temporarily residing in Florida provides a family perspective which we hear:
  - “My son is suffering from DMD. I came to know at the age of 4—(describes how diagnosis was made). He is currently 8 years old and able to do almost all things on his own. I have read about the new technique crispr cas9 to cure human diseases. Is there any way using the above technique in DMD? We are ready to do anything for him. Currently he is in a very good condition, please give him one chance.”