

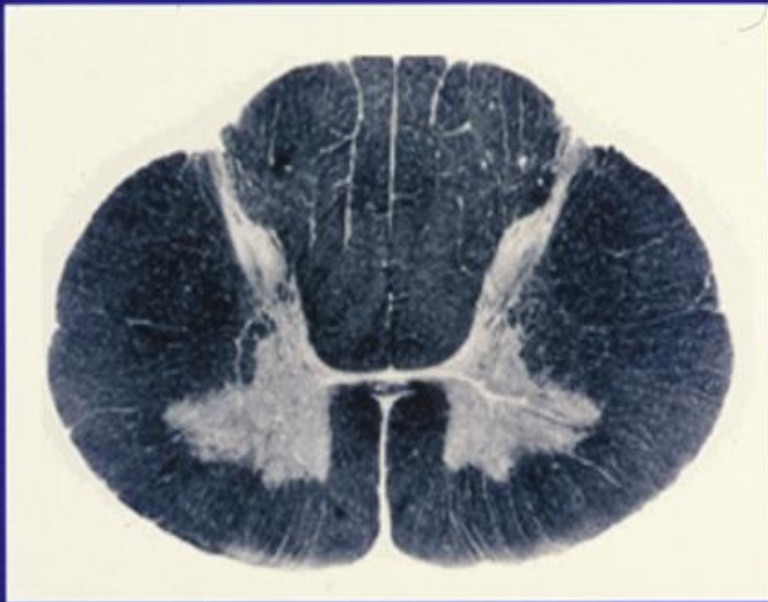
# **Genetic Screening**

Thomas W. Prior, Ph.D.  
Professor of Pathology and Neurology  
Ohio State University

# Spinal Muscular Atrophy

- Clinical
- Gene
- Molecular Testing
- Carrier Screening
- Genotype / Phenotype Associations
- Pathogenesis
- Newborn Screening

# Spinal Muscular Atrophy (SMA)



- Most common autosomal recessive genetic disorder lethal to infants
- Anterior horn cell degeneration
- Progressive weakness and muscle atrophy
- Only supportive treatment available



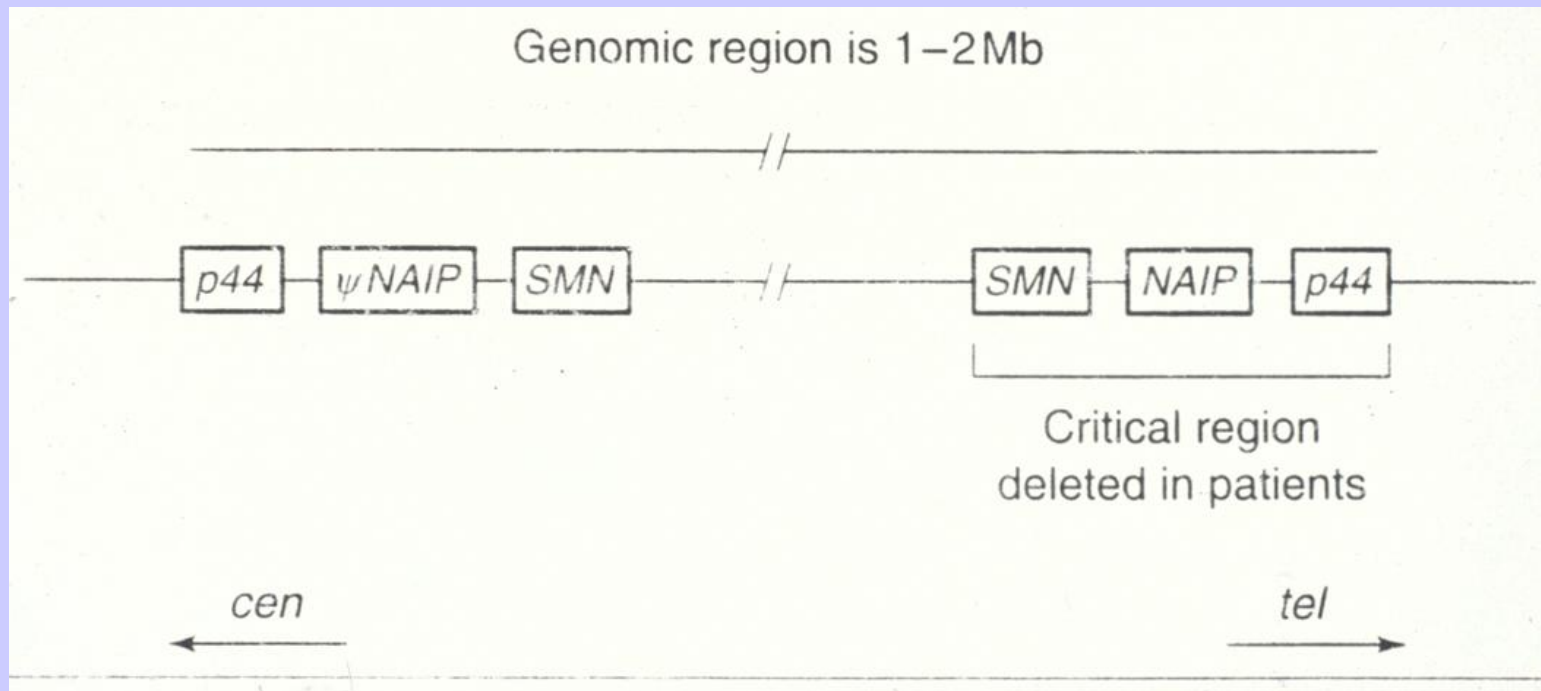
Picture archive Dr. K. Swoboda,  
Salt Lake Tribune

# Spinal Muscular Atrophy

- Male normal preg, labor, and birth
- 7 wks-decreased leg movement
- 10 wks– tongue fascics; no head control; slight hand movements
- Genetic testing + for SMA
- 4 mos - G-tube
- 6 mos – BiPap assistance
- 9 mos – expired in hospice care







Deletions were identified in the critical region in SMA patients

In 1995, there was evidence supporting 2 neighboring candidate SMA genes which were published in Cell.

# Survival Motor Neuron (SMN) Gene

Nearly 2 Identical Genes

Telomeric (SMN1)

Centromeric (SMN2)

About 95% of SMA Patients lack SMN1 Exon 7

SMN2 modifies the phenotype

294 amino / 38 KD Protein ubiquitously expressed

High Levels in Spinal Cord

Found in Cytoplasm and Nucleus

Functions in RNA metabolism



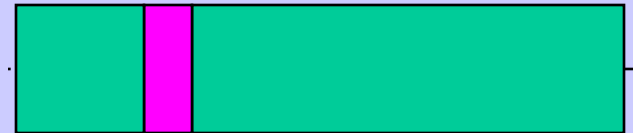
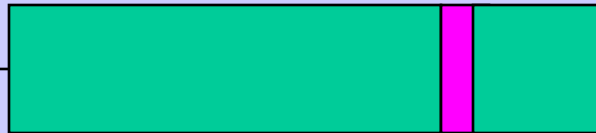
**Large inverted duplication in 5q13**

Telomeric

Centromeric

**SMN1 (SMNt)**

**SMN2(SMNc)**

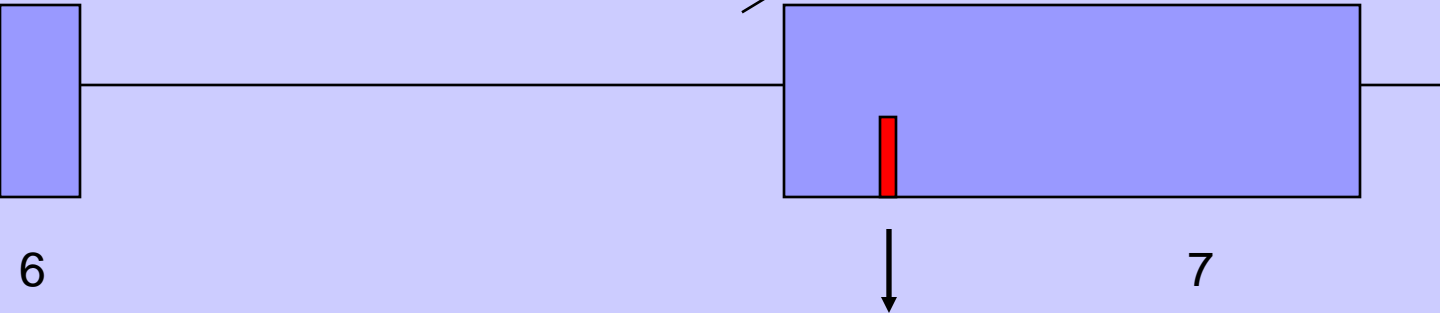


20kb

500kb

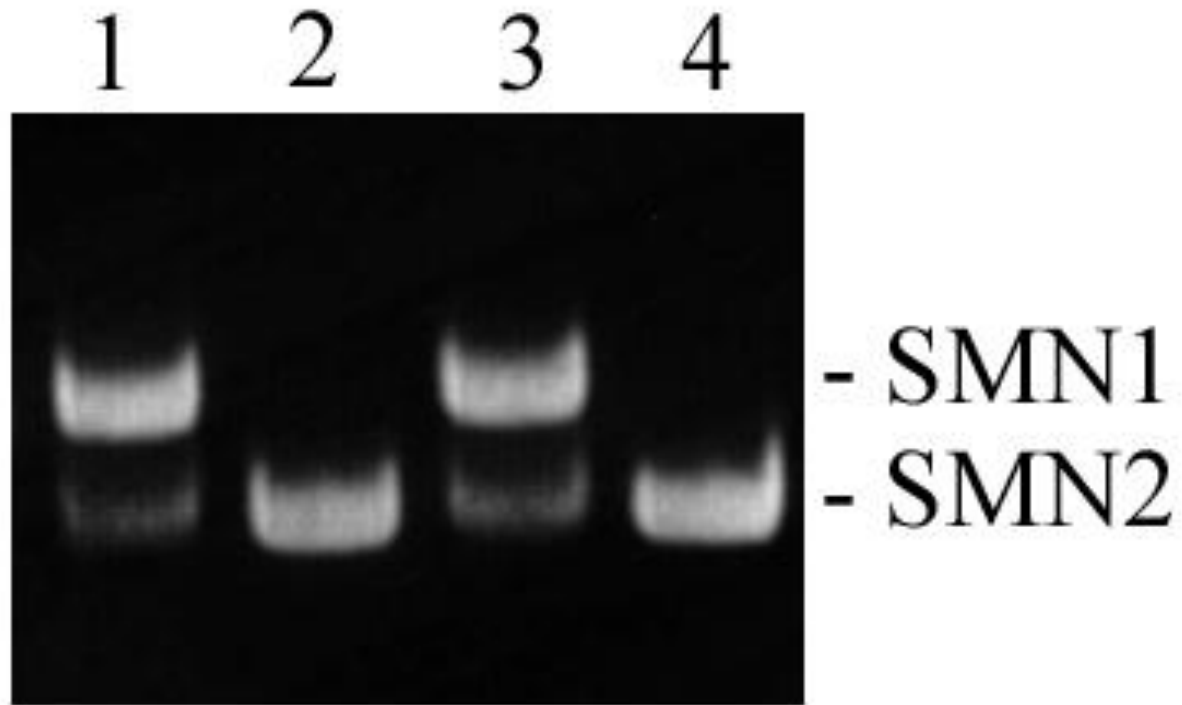


# SMN



**SMN1** → c.840 C  
**SMN2** → c.840 T

# Diagnostic Test for SMA



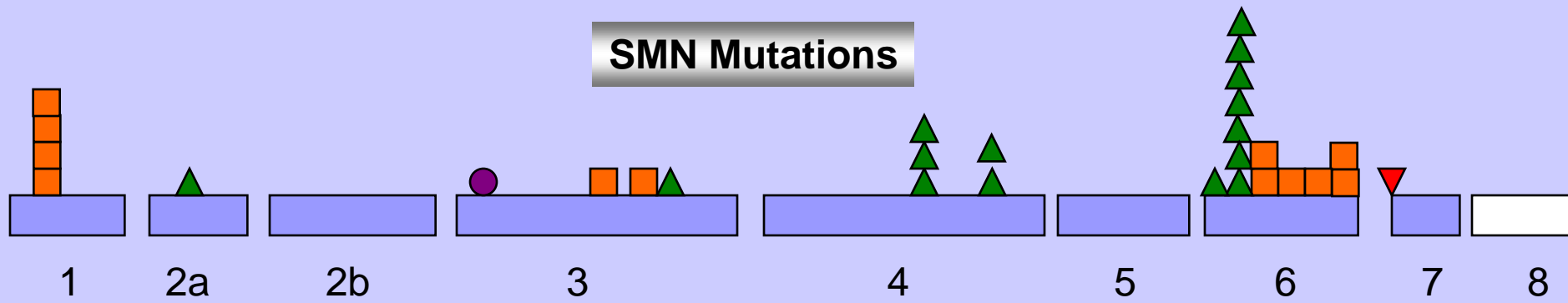
# The Others

## **Non-Deletion Mutations**

## **Nondeletion Patient study**

- Confirmation of the Primary Role of SMN1
- Identification of Important Functional Domains
- Improve Diagnostic Sensitivity

## SMN Mutations



- Nonsense Mutation
- Missense Mutation
- ▲ Frame Shift
- ▼ Splice Site

Exon	Mutation	Type	# of Patients
1	c.5 C>G; p.A2G	Missense	4
2a	c.109 ins A	Frameshift	1
3	c.305 G>A;p.W102X	Nonsense	1
3	c.389 A>G; p.Y130C	Missense	1
3	c.418-432 del 15 (GATCTACTTTCCCCA)	Frameshift	1
3	c.419 A>T; p. D140V	Missense	1
4	c.509-510 del GT	Frameshift	3
4	c.584 del C	Frameshift	2
6	c.735 ins C	Frameshift	1
6	c.770-780 dup 11 (CTGATGCTTTG)	Frameshift	7
6	c.785G>T;p.S262I	Missense	2
6	c.796T>C;p.S266P	Missense	1
6	c.818A>G;p.H273R	Missense	1
6	c.821C>T; p.T274I	Missense	2
intron 6	c.835-3 C>T	SpliceSite	1

**SMA Carrier Testing**

**Prevention**

## **Background**

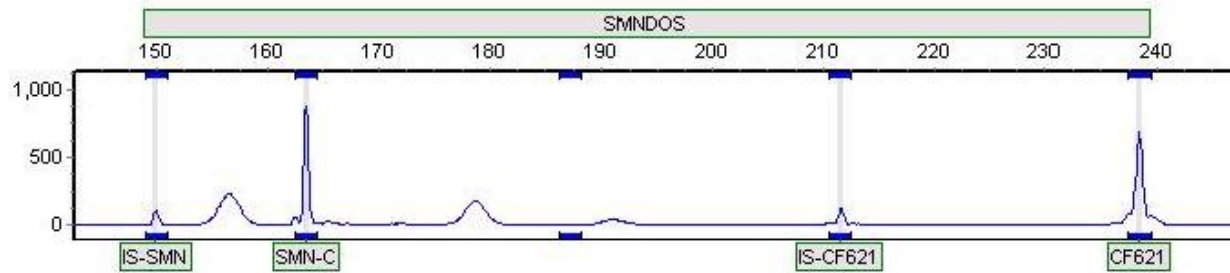
**In 1996 we started offering SMA carrier testing at Ohio State University and have currently performed > 2,500 carrier tests.**

**The majority of these tests have been performed on family members with previously affected SMA patients.**

**Why has this test not been performed as a more massive screen?**

# 200ng DNA Template; 20 Cycles

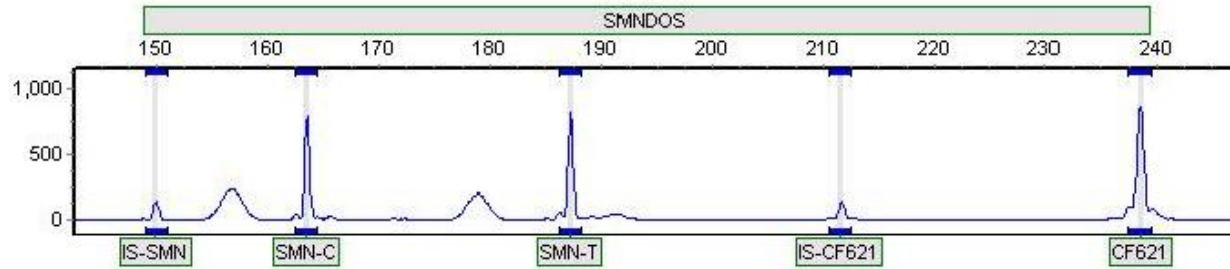
Sample 1:



smnT,smnC

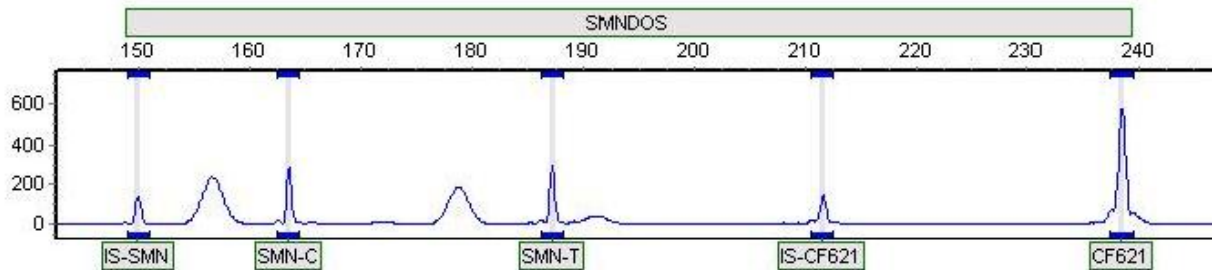
**0,3 (Affected)  
Ratio (0, 0.9)**

Sample 2:



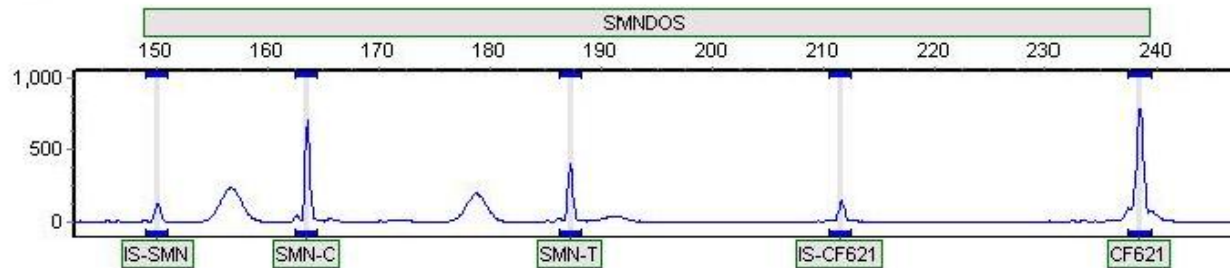
**2,2 (NON-Carrier)  
Ratio (0.68, 0.64)**

Sample 3:



**1,1 (Carrier)  
Ratio (0.36, 0.33)**

Sample 4:



**1,2 (Carrier)  
Ratio (0.36, 0.61)**

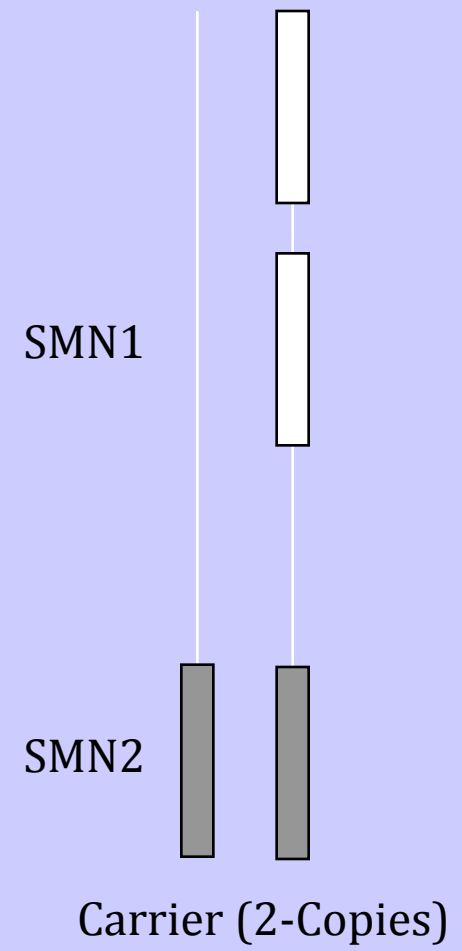
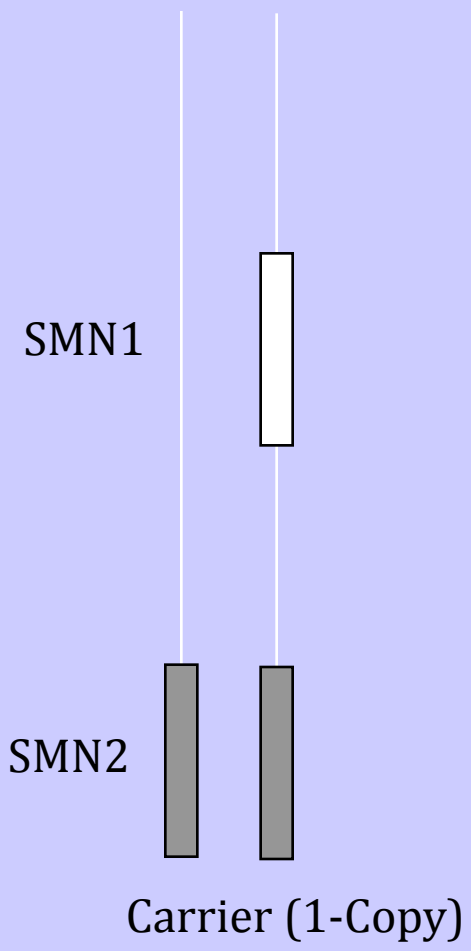
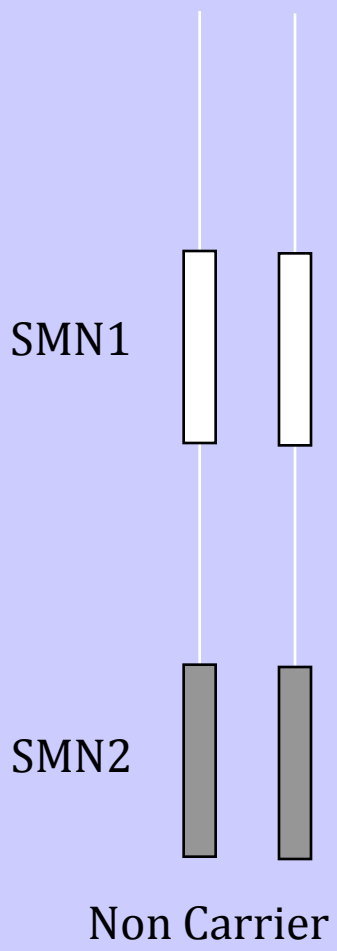
## *Limitations of SMA Carrier Testing*

Non Deletion Mutations

Lack of Phenotypic Prediction

De-novo Mutations

2 copy Cis *SMN1* Chromosomes



*Claire Altman Heine*

*1-29-04 to 11-12-04*



# *Criteria for Carrier Screening*

- Severe Disorder
- High Frequency of Carriers
- Reliable Test
- Availability of Prenatal Diagnosis
- Access to Genetic Counseling and Educational component

**Claire Altman Heine Foundation Pilot Program for  
Population SMA Carrier Screening Goals**

**Education**

**Estimate Carrier Frequencies**

**Determine Allele Frequencies**

**Collect Survey Data**

## **Method**

**Patients are recruited from 2 facilities in Columbus, Ohio: OSU Division of Maternal and Fetal medicine and Riverside Methodist Maternal-Fetal Medicine Clinic.**

**Facilities are staffed with 6 genetic counselors**

**Educational material is provided (Heine Foundation)**

**Demographic information is obtained**

**Post Survey is collected**

# SPINAL MUSCULAR ATROPHY

*What happens if  
both my partner and I  
are SMA carriers?*



CLAIRE ALTMAN HEINE FOUNDATION, INC.  
*dedicated to identifying carriers of Spinal Muscular Atrophy*

# SPINAL MUSCULAR ATROPHY

*Carrier Testing:  
The Decision is Yours*



CLAIRE ALTMAN HEINE FOUNDATION, INC.  
*dedicated to identifying carriers of Spinal Muscular Atrophy*

## Results

**500 carrier studies were performed**

**16 carriers (1/31)**

**46 individuals with >2 SMN1**

## SMN Genotypes

SMN1, SMN2	count
0,0	0
0,1	0
0,2	0
0,3	0
0,4	0
0,5	0
0,6	0

SMN1, SMN2	count
<b>1,0</b>	<b>1</b>
<b>1,1</b>	<b>3</b>
<b>1,2</b>	<b>7</b>
<b>1,3</b>	<b>4</b>
<b>1,4</b>	<b>1</b>
1,5	0
1,6	0

SMN1, SMN2	count
2,0	29
2,1	180
2,2	215
2,3	14
2,4	0
2,5	0
2,6	0

SMN1, SMN2	count
3,0	6
3,1	28
3,2	8
3,3	0
3,4	0
3,5	0
3,6	0

SMN1, SMN2	count
4,0	2
4,1	0
4,2	1
4,3	0
4,4	0
4,5	0
4,6	0

SMN1, SMN2	count
5,0	1
5,1	0
5,2	0
5,3	0
5,4	0
5,5	0
5,6	0

## Survey Results

76% of individuals: No knowledge of SMA

Majority (96%) of individuals found the Heine brochures “very helpful”

Reasons for Testing:

74% pursued testing because they were interested in their carrier status

57.3% worried about risk for pregnancy

98.7% were glad they were tested. Only 1 patient was not happy they underwent testing because of the added anxiety

## Survey Results

96.9% would have the test if it was covered by insurance

29% would have the test if it cost \$500 out of pocket

## **Recommendations for Population SMA Carrier Screening**

- 1. Since SMA is present in all populations, carrier testing should be offered to all couples regardless of race or ethnicity.**
- 2. Formal genetic counseling services must be made available to anyone requesting this testing.**
- 3. A negative screening test for one or both partners reduces but does not eliminate the possibility of an affected offspring, since the test sensitivity is less than 100% (~90% detection rate).**
- 4. As is true for all carrier screening programs, the testing is voluntary. Informed consent and the usual caveats must be addressed including: assurance of confidentiality, paternity issues, discrimination, self-esteem and cost.**

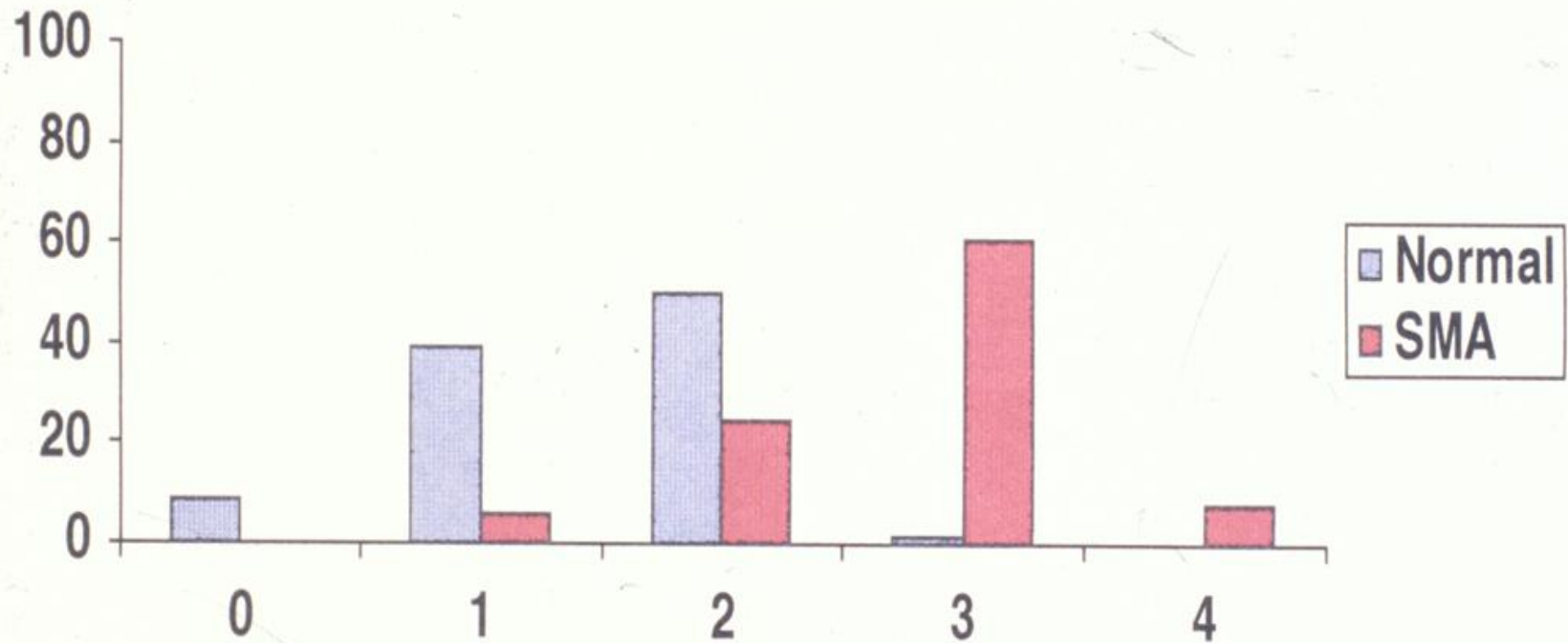
*Prior, Genetics in Medicine 2008:11:840-842*

*Accepted by American College of Medical Genetics*

## Genotype / Phenotype Association

- *SMN1* Deletion Frequency
- NAIP
- Gene Modifiers
- *SMN2*

# Total SMN<sup>C</sup> Copy Number



- Normal individuals have between 0-3 copies of SMN<sup>C</sup>; SMN<sup>C</sup> copy number in SMA patients ranges from 1-4.

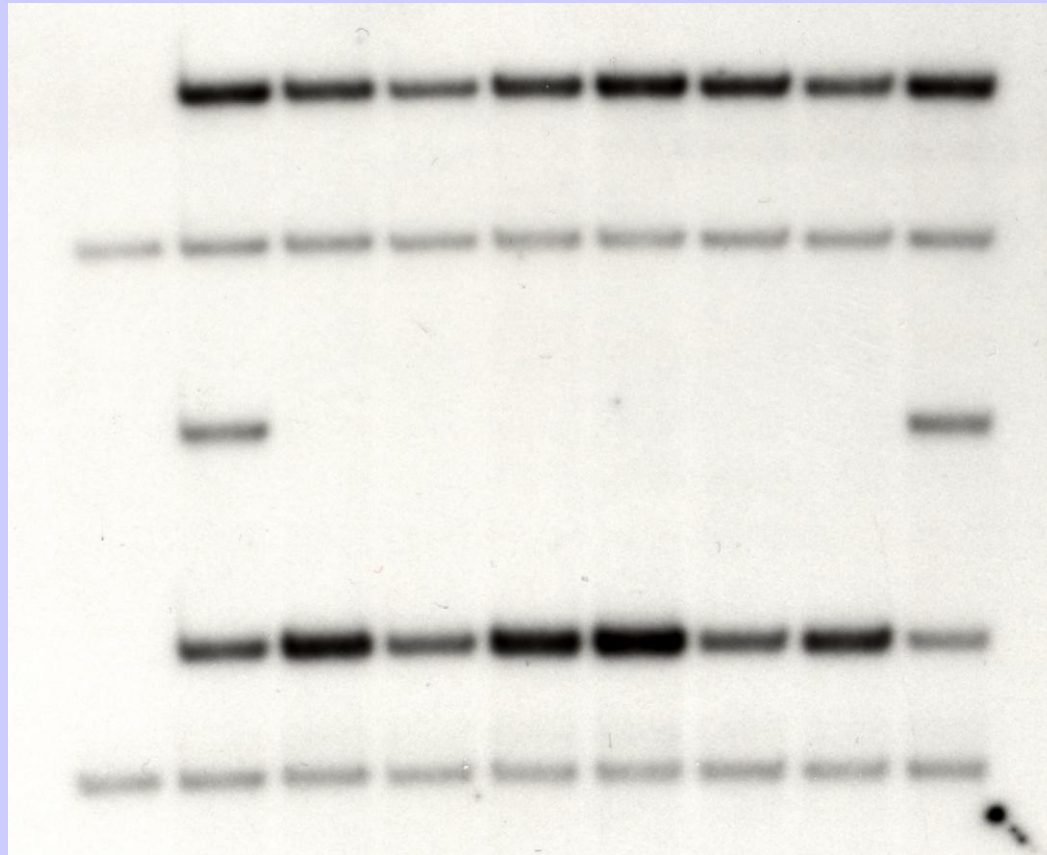
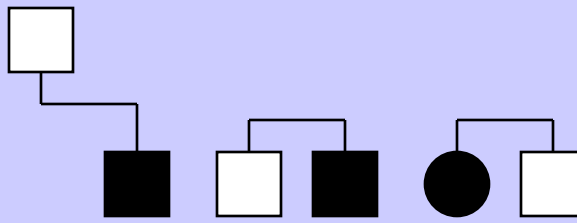
# Significant Difference in SMN2 Copy Number

	<u>Type I</u>	<u>Type III</u>	<u>Total</u>
1 Copy <i>SMN2</i>	7 (13.5%)	0 (0%)	7 (4.9%)
2 Copy <i>SMN2</i>	43 (82.7%)	0 (0%)	43 (30.3%)
3 Copy <i>SMN2</i>	2 (3.9%)	70 (77.8%)	72 (50.7%)
4 Copy <i>SMN2</i>	0 (0%)	20 (22.2%)	20 (14.1%)
<u>Total</u>	<u>52</u>	<u>90</u>	<u>142</u>

Statistically significant difference in copy number (**P<0.0001**)

96% of type I patients have 2 or fewer copies

100% of type III patients have 3 or greater copies



CFTR

CFTR-IS

SMN1

SMN2

SMN-IS

Lanes 1 2 3 4 5 6 7 8 9

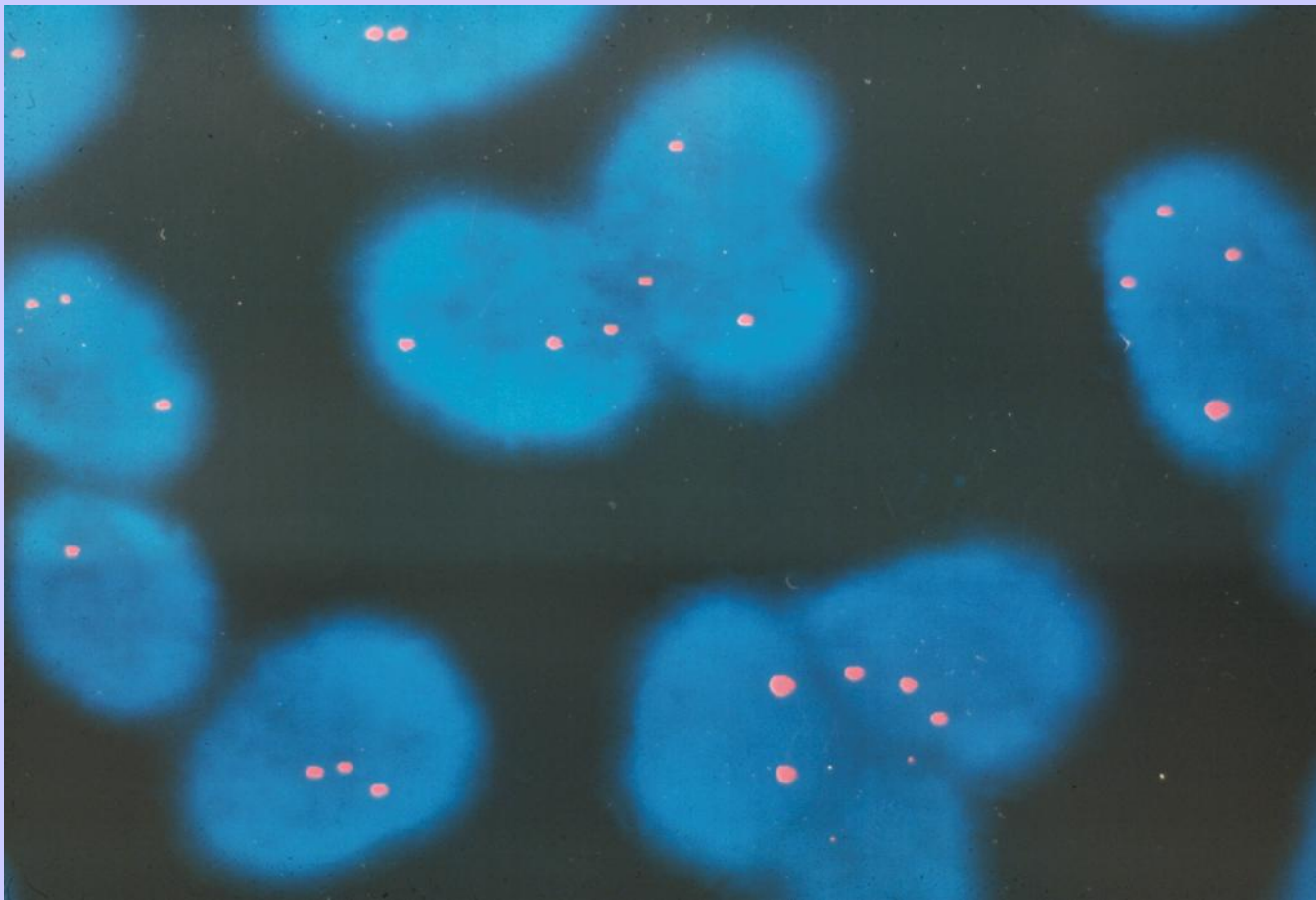
SMN2 # 0 2 5 3 5 5 2 5 1



In mice a severe phenotype results with the expression of 2 copies of SMN2. This phenotype can be rescued by expressing 8 copies of the SMN2 gene

# Questions

- Why does SMN protein deficiency cause a selective cellular death to motor neurons?
- Pathogenesis?
- Why does SMA exist?
- Therapy?
- When does the degeneration start?

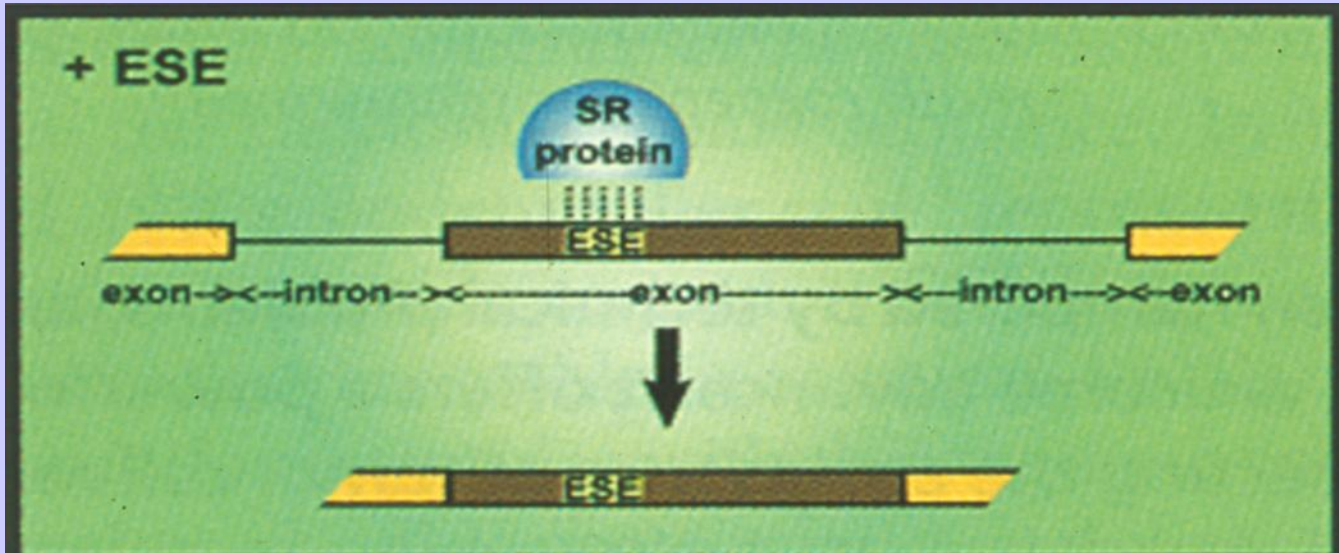


SMN is ubiquitously expressed and highly conserved through evolution.

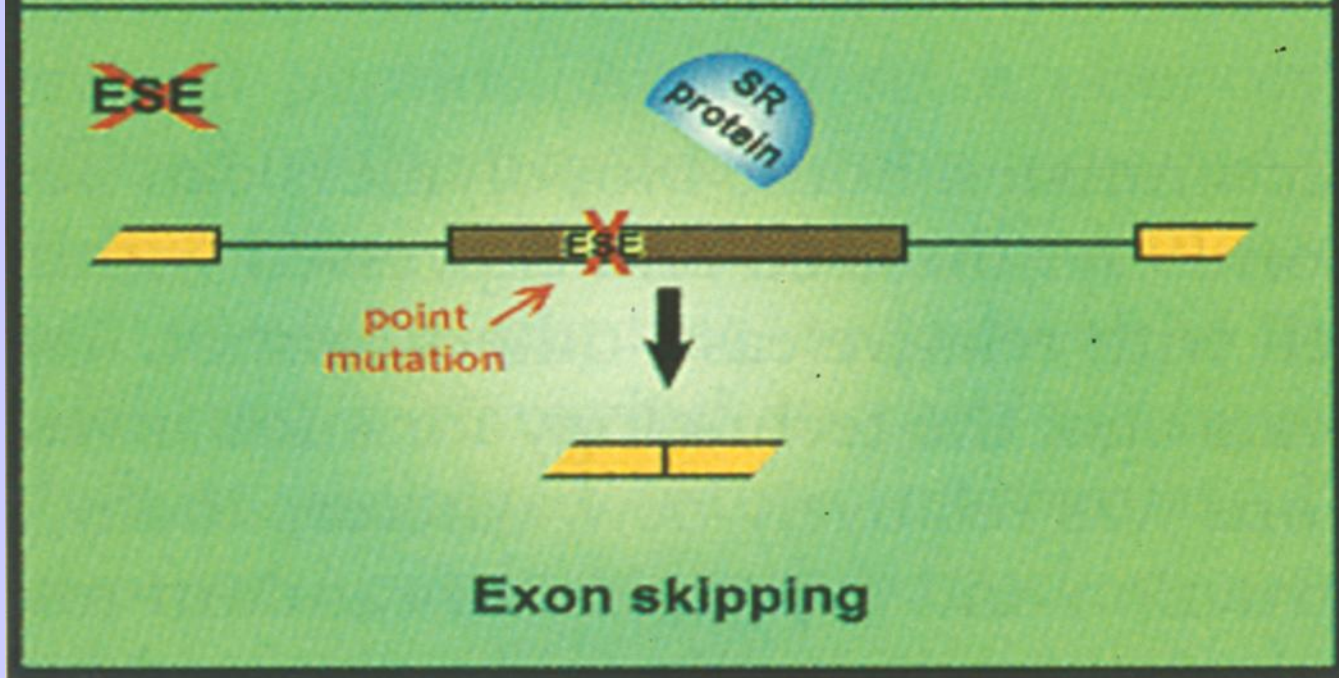
SMN is localized diffusely in the cytoplasm and concentrated in punctate structures in the nucleus, called gems. The gems are complexes rich in factors involved in RNA metabolism, snRNP biogenesis and pre-mRNA splicing.

SMN may also have a neuronal specific function, axonal mRNA trafficking?

SMN1



SMN2

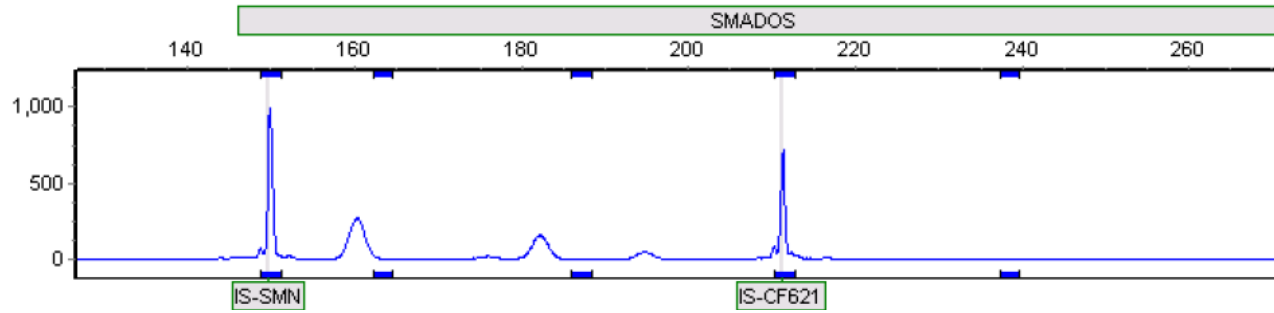


## Case I

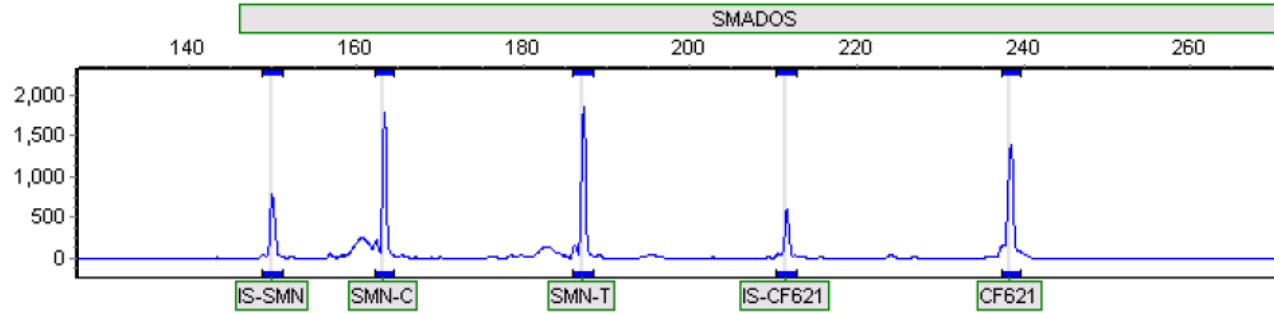
The affected in case 1 is a SMA 42-year-old type IIIb patient who had normal early motor milestones but walked on her toes for as long as she could remember. By high school she noticed slight weakness in her hips, but this was thought a normal variation by her physician. Her disease declared itself in her early 20s, when her husband witnessed her fall and became convinced that her leg strength was not normal. Her gait was stable with significant hyperlordosis. She was areflexic with an otherwise normal neurologic examination.

**\* DNA testing revealed a 0/0 SMN1:SMN2 genotype, which has never been reported and is considered lethal**

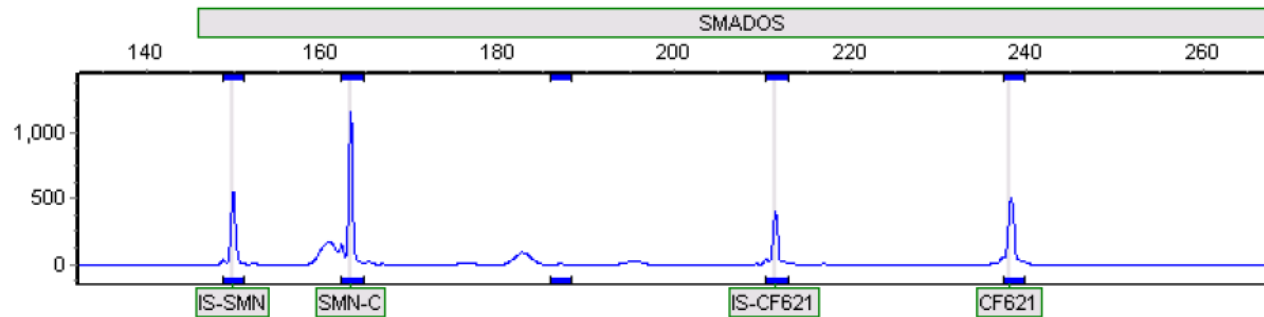
Blank w/  
Internal  
Standards



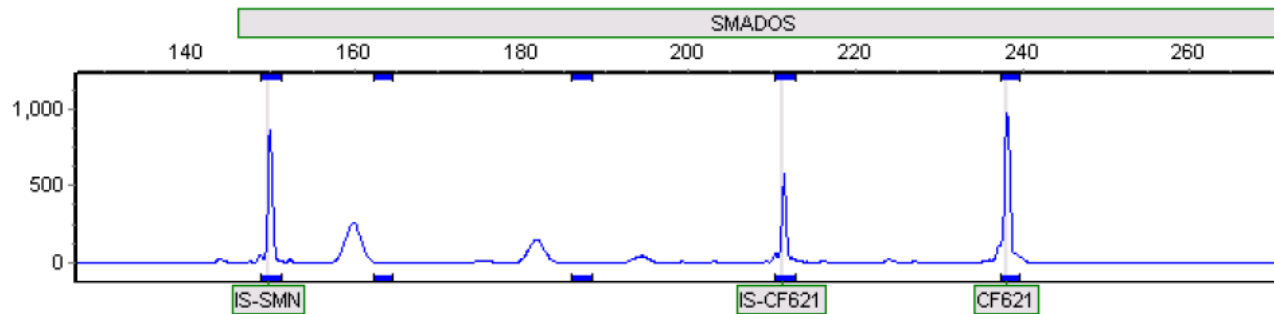
2,2



0,3



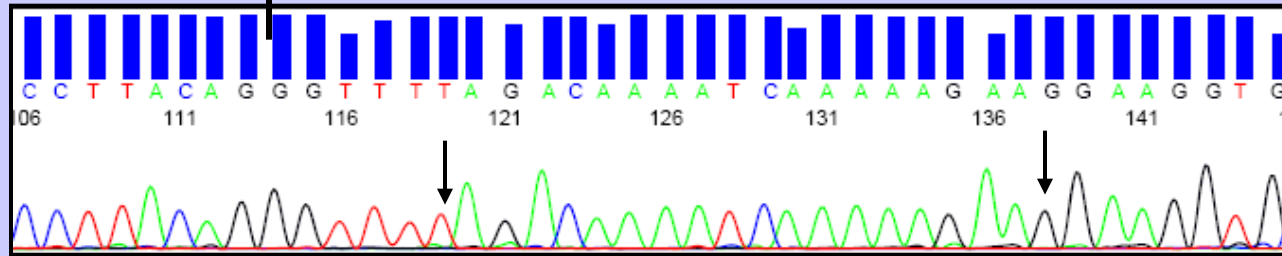
0,0



# SMN2

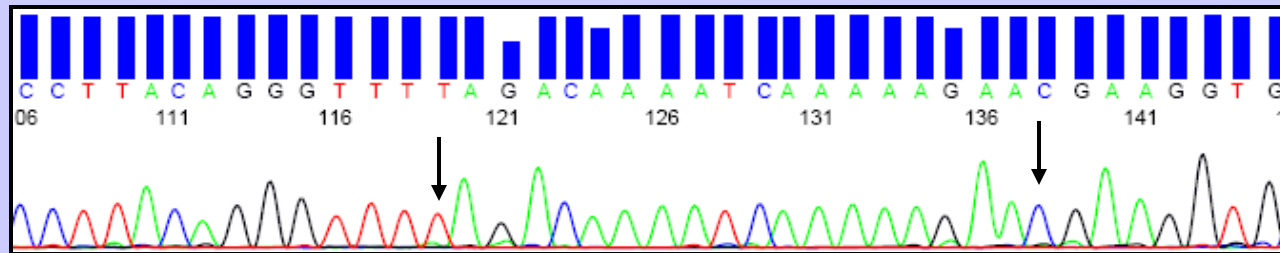
Intron 6      Exon 7

A



WT SMN2

B



Case I

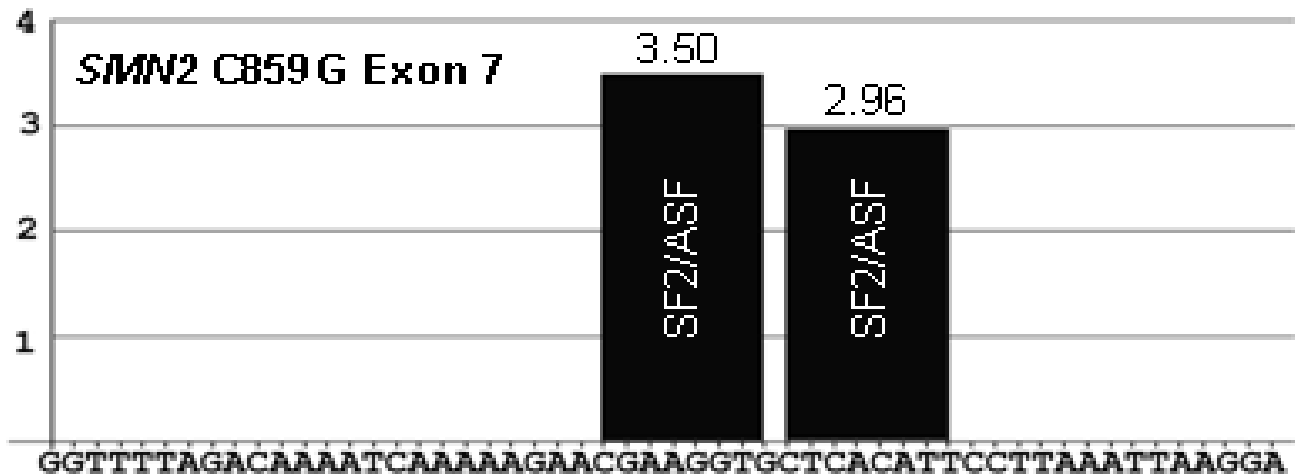
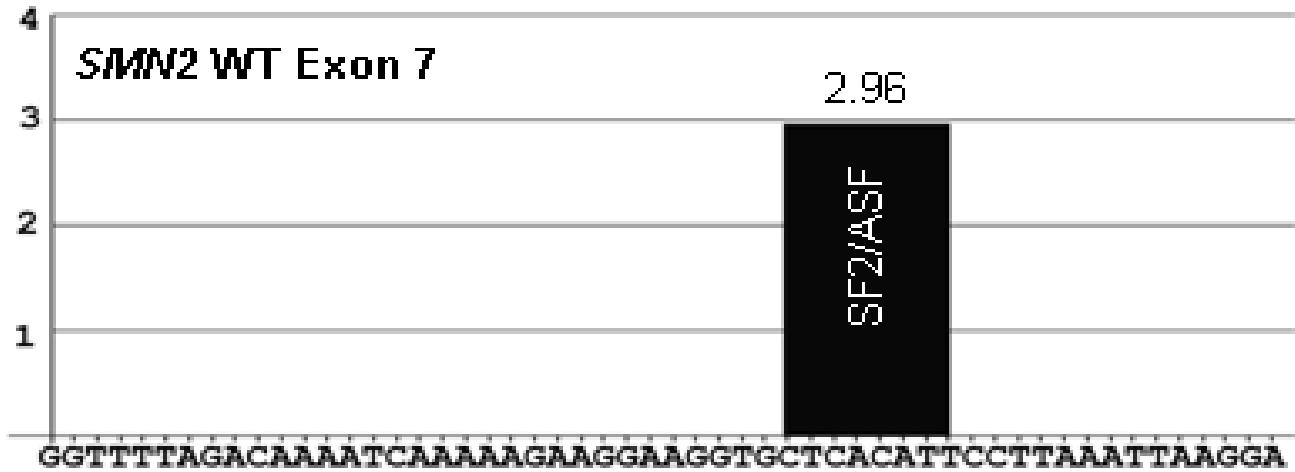
6

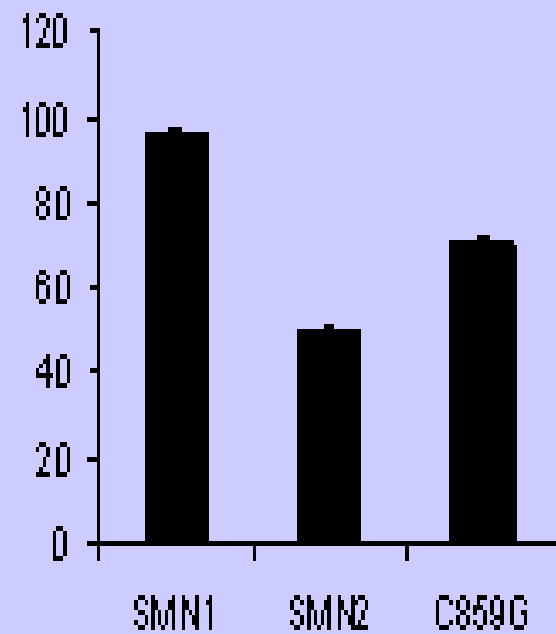
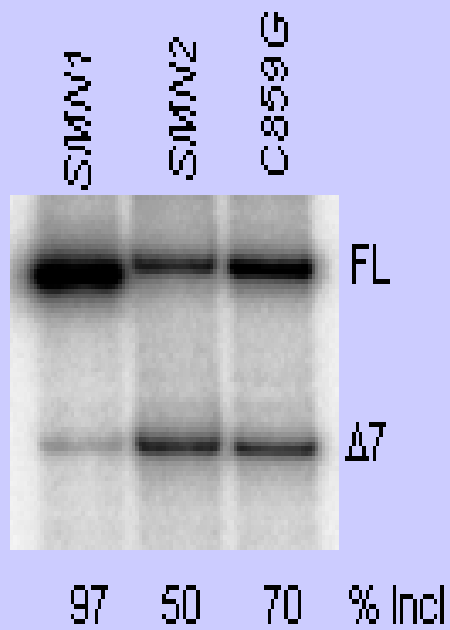
*SMN2* c.859G>C

p.Gly287Arg

Dosage testing reveals 2 copies both positive for the c.859G>C

**A**





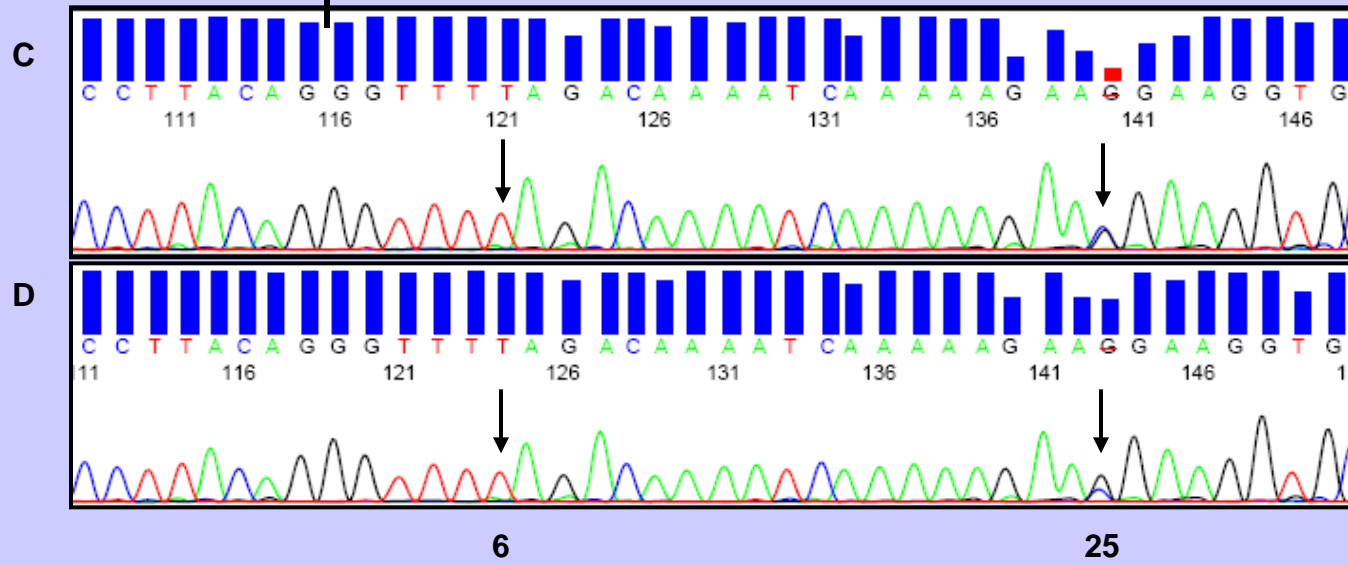
Since the c.859G>C substitution was initially identified in a type 3b patient with only two *SMN2* copies we decided to screen all of our discrepant cases and found c.859G>C in 2 out of a total 7 cases

Case II: 31 yo type IIIa female lost ambulation at age 12. DNA testing initially indicated that she had a 0 *SMN1*/1 *SMN2* genotype, which was not consistent with her milder type 3a presentation. Utilizing the redesigned *SMN2* primers, dosage testing revealed a second copy of *SMN2*, which was positive for the variant. Since we have observed the 0 *SMN1*/2 *SMN2* genotype in 90% of our type I patients, this finding in case 2 provided additional support for a positive modifying effect of the *SMN2* change.

Case III: 29 yo ambulant type IIIb male, He had difficulty running while in high school, but did not feel there was definitely something wrong until age of 22, when he realized he was not gaining strength despite an intensive weight lifting program. DNA testing initially indicated that he had a 0 *SMN1*/2 *SMN2* genotype. Since we had not observed this genotype in our type 3b population, the patient was re-tested with the new primer set and shown to have a third copy of *SMN2* with the c.859G>C.

# SMN2

Intron 6      Exon 7



Case II

Case III

## A New Positive Modifier in SMN2

The c.859G>C substitution is likely to be an important modifier accounting for some of the exception patients, we have identified the substitution in 3/8 unrelated discrepant cases and 0/41 type I cases.

It should not be assumed that all *SMN2* genes are equivalent and sequence changes found within the *SMN2* gene should be further investigated for potential positive or negative effects on *SMN2* transcription and post-transcriptional RNA processing.

\*\*These cases support the therapeutic benefit in increasing the SMN2 expression in order to decrease the severity of SMA.

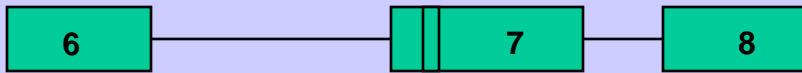
(Recently, we have identified an unaffected 26 year old through carrier screening who was 0/3 SMN1:SMN2. All three SMN2 copies were positive for the c.859G>C.)

# Molecular Basis of SMA

FL-FULL LENGTH

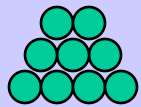
## Normal Individual

SMN1 (SMNt)



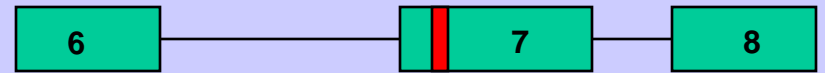
c.840 → c

Presence of ESE/  
Absence of ESS



90% FL-SMN Protein

SMN2 (SMNc)



c.840 → t

Disruption of ESE/  
Creation of ESS



10% FL-SMN Protein

SMN1 (SMNt)

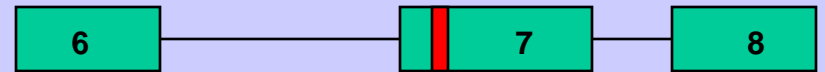
## SMA Patient

Mutation



LOSS OF 90% FL-SMN Protein

SMN2 (SMNc)



c.840 → t

Disruption of ESE/  
Creation of ESS



10% FL-SMN Protein

**If SMN2 did not have the 'T' nucleotide in exon 7, we would not be having this presentation.**

**SMA is not due to an absence of protein but a reduction.**

**Since all patients have at least one copy of SMN2, increasing the expression from SMN2 becomes a target in therapeutic strategies.**

**Newborn Screen**

**Treatment**

## Why newborn screen for SMA?

Significant disease progression occurs in the postnatal period, limited therapeutic window

Current ongoing clinical trials

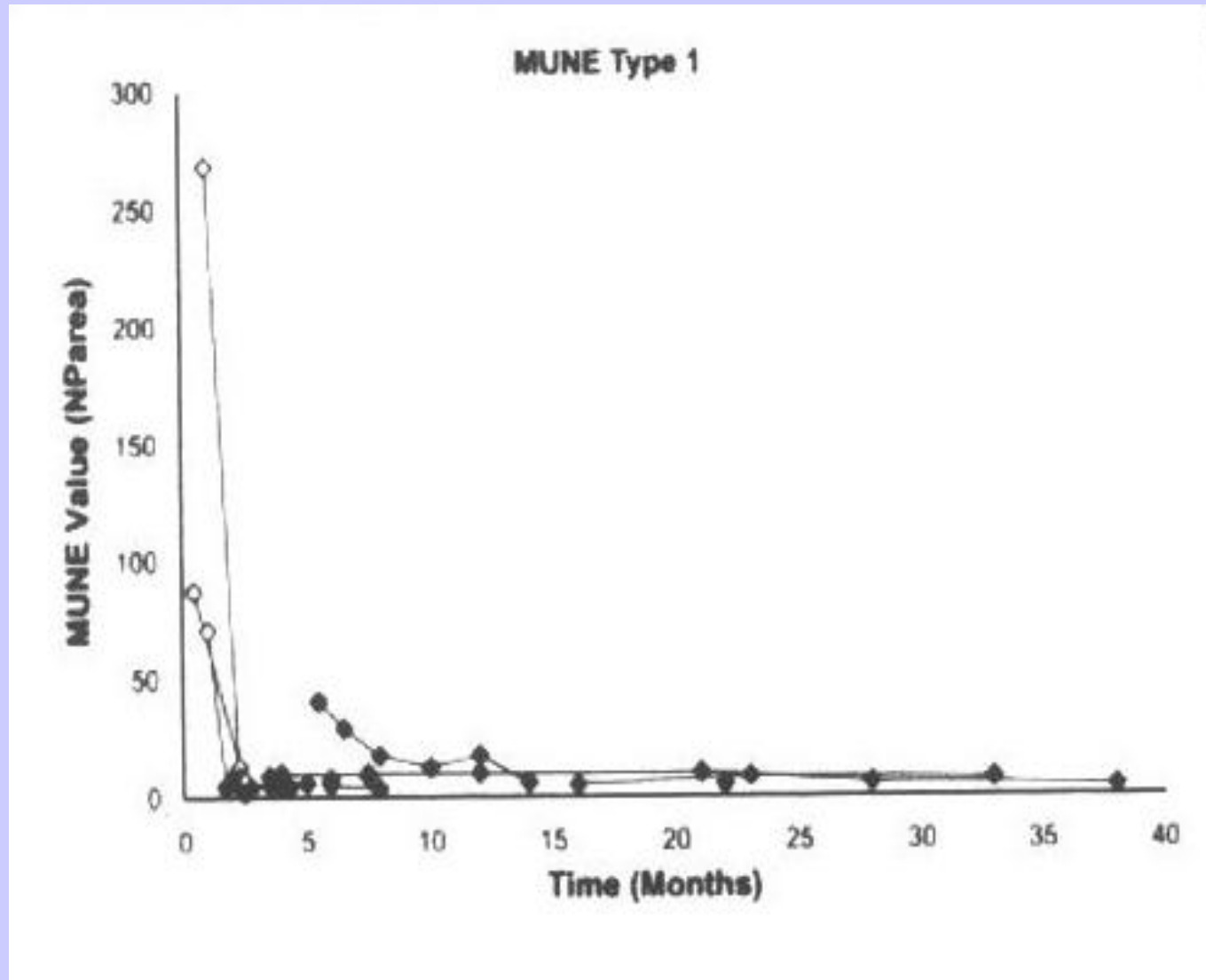
Timely diagnosis

Family Studies

Prognostic implications

Technologically Feasible

High incidence



Narrow Therapeutic Window

Pilot Screening Project #2

**Development of a Newborn Screen for SMA**

DNA



Blood Spot Extraction

# Luminex Workflow

4 Steps:

I

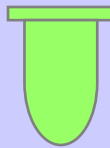
II

III

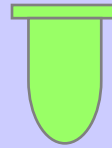
IV



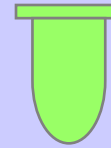
Genomic DNA



PCR



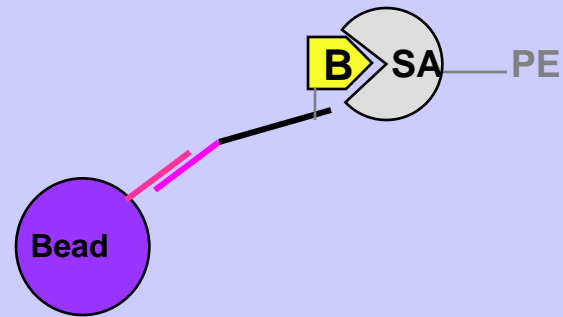
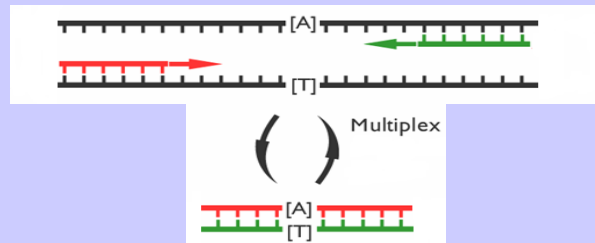
ASPE



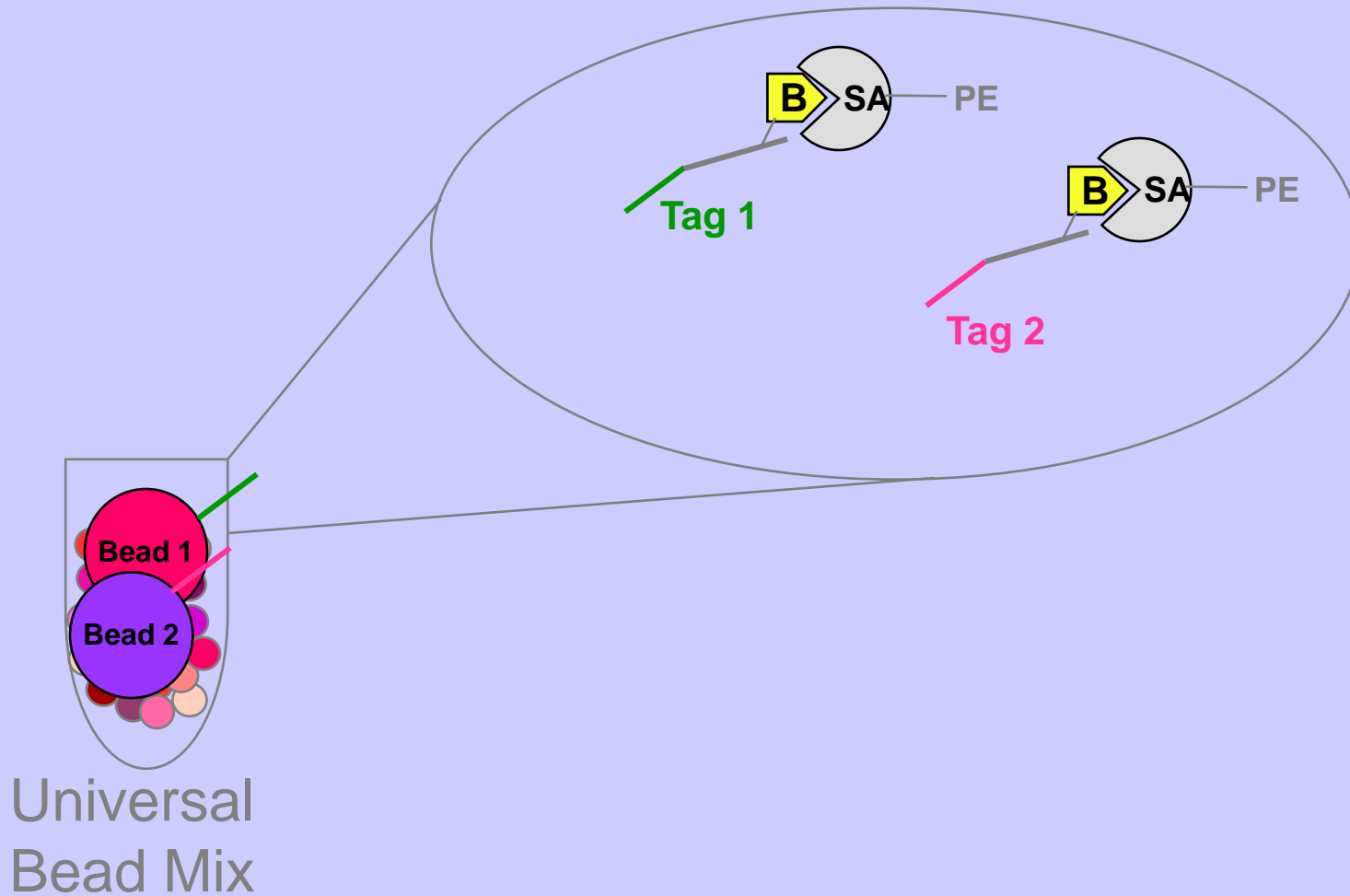
Sorting



Data Acquisition



# Universal Array Sorting



# Luminex xMAP System



## Multiplex PCR

*SMN1* & Amplification Control (*CFTR*)

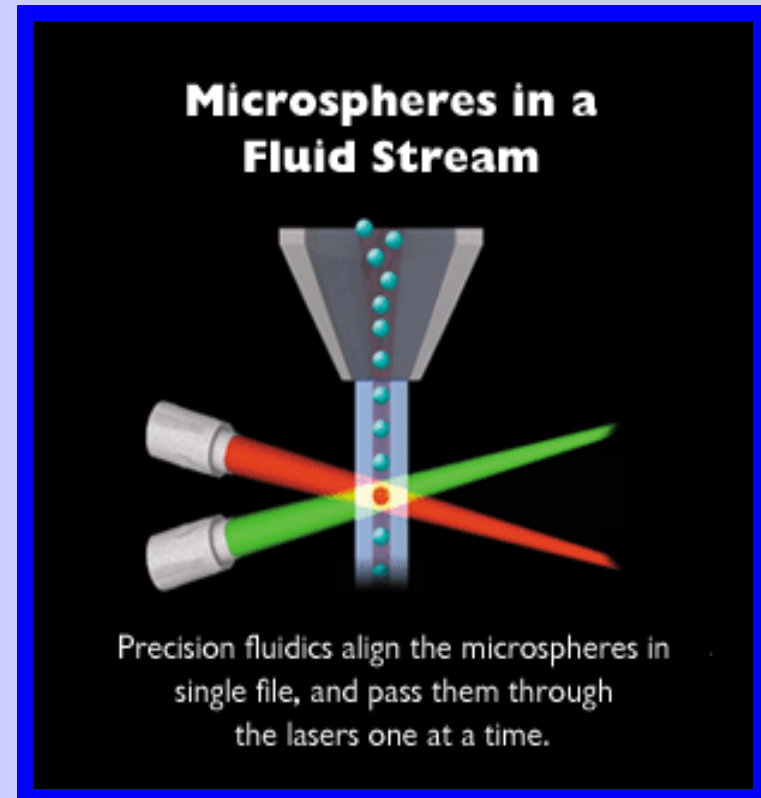


## Allele Specific PCR

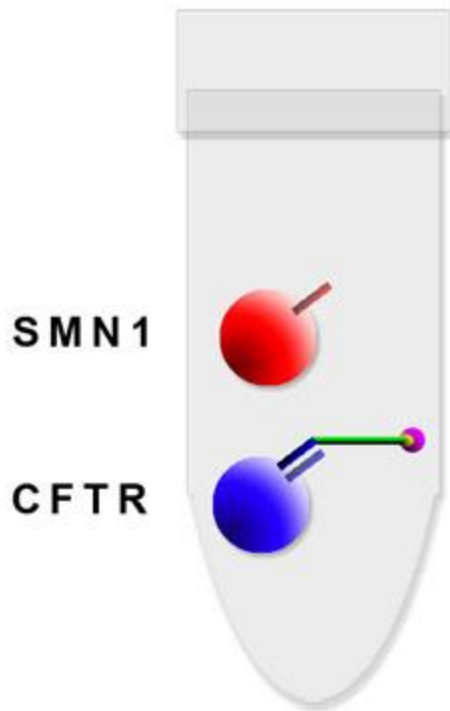
Primers specific for C allele in *SMN1* exon 7 & invariant base in *CFTR*



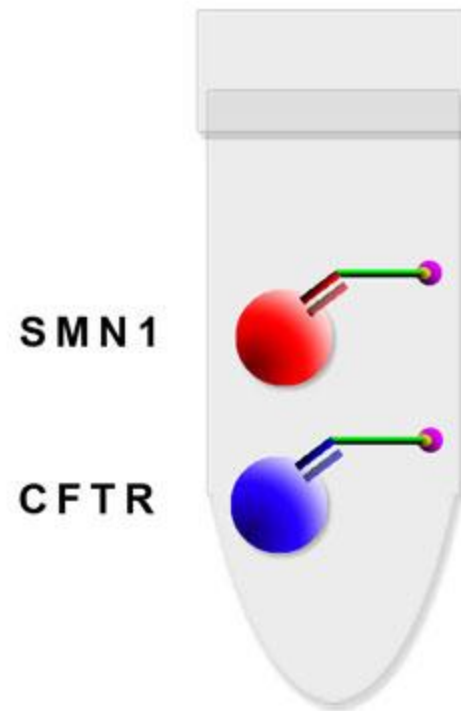
## Bead Capture



# Positive



# Negative



## Results

DNA, from 40,103 newborn blood spots were obtained from the newborn screening laboratory at the Ohio Department of Health, was extracted and tested

4 homozygous SMN1 deletions were identified

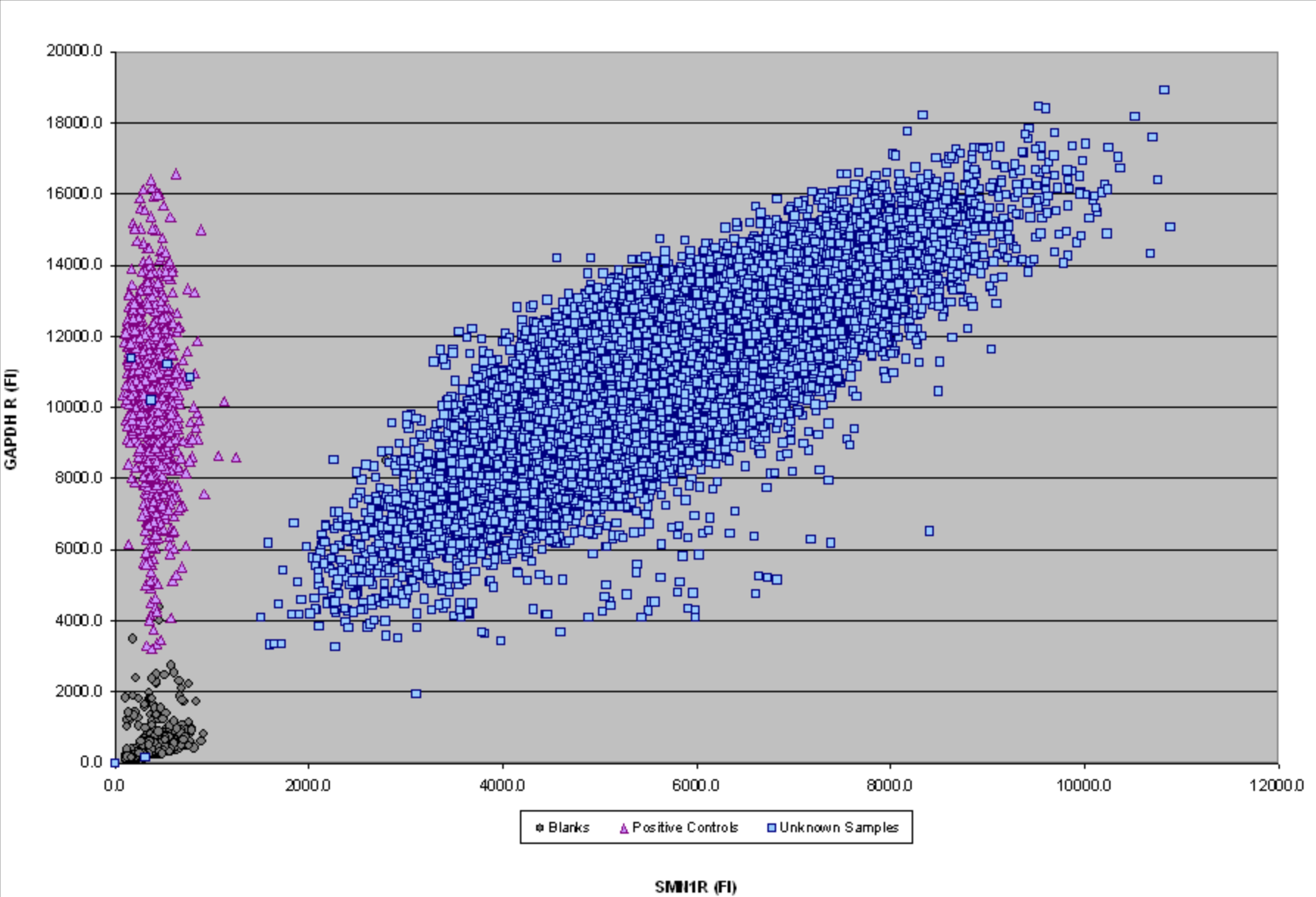
Deletions were confirmed and SMN2 copy numbers were determined:

2 had 2 SMN2

2 had 3 SMN2

< 1% sample Repeats

# GAPDH / SMN1R



## Final Thought

The need for the timely diagnosis of SMA to support an effective intervention will necessitate that the molecular diagnosis be included in the newborn screening program

Our pilot study has demonstrated that screening for SMA can be technically accomplished on a large scale newborn population.

# **Acknowledgments**

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