Genetics and Reproductive Options for SMA Families

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Louise R. Simard, PhD
University of Manitoba, Faculty of Medicine
Dept. Biochemistry & Medical Genetics
Winnipeg, MB, Canada R3E 0J9

Louise.Simard@umanitoba.ca

1987 - Ph.D. U Toronto – Medical Genetics.
Independent research program in SMA
DNA diagnostics Consultant, incl. SMA testing
2006-present – Professor and Department Head, U Manitoba

Questions are welcome!
SMA and Genetics: Outline

• SMA → lower Motor Neuron Disease → with a wide range of disease severity

• SMA is an Autosomal Recessive Genetic Disorder

• DNA and the SMA Gene, SMA Mutations & SMA severity

• SMA DNA Molecular Tests
  — Diagnosis
  — Carrier
  — Prenatal
Part 1: Genetics

SMA is a lower motor neuron disease that recurs in families (inherited).

**Type 0**
**Prenatal SMA**
- In utero
- Respiratory support
- Death before 1 month

**Type 1**
**Werdnig-Hoffman**
- < 6 months
- Never sit unaided
- Death before 2 years

**Type 2**
**Intermediate SMA**
- < 18 months
- Never stand unaided
- Decreased life expectancy

**Type 3**
**Kugelberg-Welander**
- 18 months
- Stand alone but loss of mobility
- Normal life expectancy

**Type 4**
**Adult SMA**
- ≥21 years of age
- Progressive muscle weakness
- Normal life expectancy

**Wide Clinical Presentation!**


Loss of MN cells leads to Muscle Atrophy

Genetics and Reproductive Options for SMA Families
Part 1: Genetics

SMA is inherited as an Autosomal Recessive Disorder

The fundamental unit of inheritance is DNA. Each cell has over 5 feet of DNA! It must be compacted to fit inside a cell → in the form of **chromosomes**.

We have 23 pairs of chromosomes; each set is inherited from our biological parents.

SMA → **autosomal recessive** mode of inheritance

There must be a mutation (error) in both copies of a gene (maternal and paternal) for the genetic disorder to be passed onto a child.

[Image of chromosomes]

[Gregor Mendel image]
SMA is inherited as an Autosomal Recessive Disorder

- Affected individuals have mutations in both copies of a recessive gene.
- Carriers have a mutation in only one copy of a recessive gene and is asymptomatic.
- Males and females can be carriers.
- Males and females can be affected.
- If both parents are carriers, the risk of having a child with the disease is 25%.

Having an affected child is a surprise because carrier parents are asymptomatic. From this point onwards – genetic counseling becomes possible.
Part 1: Genetics

Identifying the SMA gene:
1 gene 5 SMA types

Gene for chronic proximal spinal muscular atrophies maps to chromosome 5q
Nature (1990) 344:767-768

Genetic homogeneity between acute and chronic forms of spinal muscular atrophy
Nature (1990) 345:823-825

Genetic mapping of chronic childhood-onset spinal muscular atrophy to chromosome 5q11.2-13.3
Nature (1990) 540:540-541

Mapping of acute (type I) spinal muscular atrophy to chromosome 5q12-q14
JUDITH MELKI, PARAG SETH, SONIA ABDELHAK, PHILIPPE BURLET, MARIE-FRANCE BACHELOT, MARK G. LATHROP, JEAN FREZAL, ARNOLD MUNNICH, AND THE FRENCH SPINAL MUSCULAR ATROPHY INVESTIGATORS
### Genetic Code = Blueprint of life

~25,000 **genes** in the human genome

#### Part 1: Genetics

**DNA** = four nucleic acids (C, G, T, A)

- Different sequence of A-C-T-G = gene
- C (cytosine) pairs with G (guanine)
- T (thymine) pairs with A (adenine)

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**Part 1: Genetics**

**SMN1 vs. SMN2 gene:** Chromosome 5q13

Mutations in SMN1 = SMA

![Diagram showing SMN1 and SMN2 genes with promoter, transcription, and translation processes.](image)

- **DNA (a)**: SMN2 gene on chromosome 5q13
- **RNA (b)**: Transcription initiation at the promoter, followed by RNA synthesis up to the stop codon.
- **mRNA (c)**: Translation of mRNA into functional and nonfunctional proteins.

- ~10% Full-length transcripts
- ~90% Nonfunctional SMN protein
- 100% Full-length transcripts
- Functional Wild Type SMN protein

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**Genetics and Reproductive Options for SMA Families**
Mutations disrupt a gene’s function

Mistakes in copying the nucleic acids that make up a gene are called **mutations**

**Point Mutations**

* AAA to AAT    Lysine to Asparagine
** AAA to TAA    Lysine to STOP

* Missense mutation
** Nonsense mutation
Deletions (missing DNA) is a common mutation in SMA

Very large deletions are often associated with type I and type II SMA. Type I SMAs most often cannot make ANY SMN.

In Type II & III SMA, the mutation is often confined to SMN1 exon 7.

SMA locus is very variable

On average:

But...
also find:

No apparent
Clinical effect

SMA causing
Chromosomes
SMN2 is a major modifier gene

why Type 1 or Type 2 or Type 3?

% patients

# SMN2 copies

n=188
n=110
n=77

~94% patients = SMN1 exon 7 missing on both chromosomes
~3-4% patients = SMN1 exon 7 missing and a point mutation
~4-5% patients do not have 5q-SMA

<table>
<thead>
<tr>
<th>Genetics</th>
<th>Reproductive Options</th>
<th>SMA Families</th>
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<tr>
<td>deletion</td>
<td>T</td>
<td>C</td>
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<td>7</td>
<td>SMN2</td>
</tr>
<tr>
<td>point mutation</td>
<td>7</td>
<td>SMN2</td>
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</table>
Molecular Diagnostic Tests

- Detection of homozygous mutations
  - presence/absence of SMN1 Ex7 = diagnosis

- Detection of heterozygotes
  - quantification of exon 7 = carrier testing

- Analysis of point mutations = diagnosis
  - (research labs only)
Diagnostic Tests: SMN mutations

Diagnosis of SMA patients

Compared to EMG & muscle biopsy, the DNA test is:

- non-invasive
- sensitive, detects >95% of all SMAs
- accurate, all lacking SMN1 exon 7 have SMA
- ideal for newborn screening

Limitation: Some SMA individuals have a point mutation in the SMN1 gene. Such mutations are not detected by the deletion or quantitative PCR assay.

Diagnostic Tests: Carrier Detection
2 vs. 1 SMN1 gene test

• 95% of SMA patients are lacking SMN exon 7

• Because SMA is an autosomal recessive disorder, most SMA carriers should have only 1 copy of SMN1 exon 7

SMN quantitative test

McAndrew et al. (1997) Am J Hum Genet 60:1411-1422
Limitations with the SMA Carrier Test

Some individuals (2 to 5%) with 2 SMN1 copies are still carriers (2+0 genotype). The frequency of the 2+0 genotype varies between ethnic groups.

<table>
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<th>Population</th>
<th>Frequency</th>
<th>Risk</th>
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<tr>
<td>Caucasian</td>
<td>0.0009</td>
<td>1:632</td>
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<tr>
<td>Ashkenazi Jewish</td>
<td>0.0019</td>
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<tr>
<td>Asian</td>
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<td>1:628</td>
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<tr>
<td>African American</td>
<td>0.0041</td>
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<tr>
<td>Hispanic</td>
<td>0.0007</td>
<td>1:1061</td>
</tr>
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</table>

Therefore, we can never give a 100% Yes or No result.
Limitations with the SMA Carrier Test

This test does not detect the rare small mutations in the gene.

* Taken from Alías et al. (2009) Human Genetics 125:29
Prenatal SMA testing

• Prenatal diagnosis is offered when the SMA index case has a homozygous absence of SMN1 exon 7 or if the parents of an affected, deceased child have been shown to be carriers by quantitative PCR.

• Given the highly unstable nature of the 5q-SMA locus, surprises do occur.
should I have prenatal testing?
depends on your genotype! carrier status is VIP.

part 1: genetics

should I have prenatal testing?
depends on your genotype! carrier status is VIP.

25% risk of having SMA baby

50% risk of having SMA baby

Each baby = unique event = own combination of paternal/maternal genes.

Genetics and Reproductive Options for SMA Families
Should I have prenatal testing?

Depends on your genotype! Carrier status is VIP.

Part 1: Genetics

Normal non-carrier

<table>
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<th>Normal</th>
<th>Normal</th>
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<tbody>
<tr>
<td>N / N</td>
<td>N / N</td>
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Prenatal testing not necessary but … carrier risk = 50%.

Normal non-carrier

<table>
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<tr>
<td>N / m</td>
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</table>

Prenatal testing not necessary but … carrier risk = 100%.
Should I have prenatal testing?

**Depends on your genotype!**

Knowing carrier status can avoid unnecessary prenatal testing.

Knowing carrier status before pregnancy can facilitate choice of type of prenatal testing.

If carrier status is unknown – and you are expecting a child – the faster you get testing – the faster you will know if prenatal testing is necessary.
Part 2

Reproductive Options for Sma Families

Harvey J. Stern MD, PhD
Director, Reproductive Genetics
Genetics & IVF Institute
Workshop Goals

What are the genetic aspects of SMA especially as they relate to reproduction?

What are the reproductive choices for families at-risk for SMA?
RECESSIVE DISEASE

Unaffected
"Carrier"
Father

Unaffected
"Carrier"
Mother

Unaffected
1 in 4 chance

"CARRIER"
Unaffected
1 in 4 chance

"CARRIER"
Unaffected
1 in 4 chance

Affected
1 in 4 chance
Reproductive Options for SMA Families

Very difficult decisions about family building
Reproductive Options for SMA Families

• Decisions regarding childbearing are personal and reflect our own ethical, moral and religious views. This is not a “one size fits all” type of issue.

• Couples who are at-risk for a genetic disorder should be allowed to make up their own minds after considering all their reproductive options.
Reproductive Options for SMA Families

• Decisions are influenced by the couple’s experience with SMA. Those who have had a child or sib affected will react differently from a couple identified to be at-risk by genetic screening.

• Decisions are also influenced by couple’s attitudes towards prenatal diagnosis and assisted reproduction (“playing God”)
Most families do not wish to bring another child with SMA into the world.
Reproductive Options for SMA Families

- Have no or no more children. (Most Common Choice)
- Adoption.
- Use a gamete donor. (pre-tested for SMA)
Reproductive Options for SMA Families

- Natural conception with 1\textsuperscript{st} or 2\textsuperscript{nd} trimester prenatal testing.
- Preimplantation Genetic Diagnosis
What is PGD?

• Involves the use of assisted reproduction technologies (IVF) to provide a method of prenatal diagnosis.

• Offers an alternative to traditional methods of prenatal diagnosis including chorionic villous sampling and amniocentesis, with the option of termination of affected pregnancies.
Development of PGD

- This year represents the 27th anniversary of the first application of PGD in humans.
  -(Handyside et al. Nature 244 1990)
- Since 1990, approximately 400,000 PGD cycles have been performed worldwide.
- This has resulted in over 100,000 PGD babies born after the procedure.
PGD for Genetic Disease

Affected

Affected

Affected

Transfer unaffected embryos to the patient
In-Vitro Fertilization (IVF)
Questions About PGD

Why do I need to do IVF, I have no trouble getting pregnant?
Questions About PGD

- IVF is used to increase the odds of producing a healthy child.
- There is no change in the genetic material (egg or sperm).
- By creating multiple embryos, one increases the chance that good quality embryos which are not affected with SMA will be present and available to be returned to the uterus.
IVF Procedure
Components of IVF/PGD Cycle:

1. Down-regulation (preparation)
2. Ovarian Stimulation - FSH
3. Egg Retrieval
4. Fertilization (ICSI)
5. Embryo biopsy and testing
6. Embryo transfer to uterus
Parts Of An IVF Cycle

1. Cycle Preparation
2. Ovarian Stimulation
3. Egg Retrieval
4. Fertilization and Development
5. Embryo Transfer
6. Luteal Phase Support
7. Pregnancy Surveillance

IVF Timeline

Cycle Day

Days: 0, 7, 14, 21, 28, 35, 42, 49, 56
Normal Ovulation

Diagram showing the ovarian cycle, ovulation, LH surge, luteal phase, FSH, estradiol, and LH levels over the 28 days of the menstrual cycle.
Embryo Development

Adapted from Moore and Persaud, Saunders, 1993
**Natural fertilization**

1. **Egg maturation**
   - An egg matures in the ovary.

2. **Ovulation**
   - The egg is released and starts its migration to the uterus.

3. **Fertilization**
   - One sperm fertilizes the egg. A reaction in the egg blocks other sperm from entering.

4. **Cell division**
   - The fertilized egg starts dividing and is now called an embryo.

5. **Implantation**
   - The embryo attaches to the mucosa in the uterus, where it continues to develop.

**IVF**

1. **Egg isolation**
   - Through laparoscopy, near ultrasound and thin needle

2. **Fertilization**
   - Sperm is added to fertilize the egg.

3. **Implantation**
   - When the egg has divided a few times, it is transferred back to the uterus where the egg attaches to the mucosa.

IVF is used when sperm and egg cannot meet under normal conditions. Common causes include obstructed fallopian tubes, too few eggs or impaired production of sperm.
IVF: Monitoring Visit

- Estradiol levels
- Follicle measurement

Days of FSH Stimulation
Stimulated Ovary
Egg Retrieval
Egg Aspiration
Egg Ready For Fertilization
Fertilization by Intracytoplasmic Sperm Injection

ICSi
Embryo Development

Embryo Development

Morula stage embryos

Blastocyst stage embryos

Embryo Transfer
PGD for Single Gene Disorders

- Analysis involves whole genome amplification with multiplex PCR amplification of the mutation along with 3-4 linked polymorphic markers which generate a chromosomal haplotype.

- At the same time, the embryos are also tested by a chromosomal microarray for abnormalities (ie. Down syndrome) which can lead to failed implantation or pregnancy loss.
PGD for SMA

Embryo Biopsy

SMN gene

SMN 1 & 2 = unaffected

SMN 2 only = affected
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24 Chromosome Analysis by Microarray

Performed concurrently with SMA testing to identify the embryos with the best potential to make a baby as well as being free of SMA.
Accuracy of PGD

- Using the combination of mutational analysis and linked markers the accuracy of the test 98-99%.
- Contamination with external DNA is a major concern.
- Analyses done in surgical clothing in biohazard hood.
Specific Issues with IVF/PGD

- Patient Discomfort: Medications are given by injection (subQ).

- Abdominal distention and discomfort and some nausea are common.

- Egg retrieval done under anesthesia.

- Ovarian hyperstimulation syndrome 1-2%.
Questions about IVF/PGD

• Is IVF Safe?
• What will I feel?
• Does it increase cancer risk?
• Are the babies born normal?
• What are the costs?
• What about insurance?
Questions About IVF/PGD

What is the success rate of IVF/PGD?

• Varies, particularly with maternal age, but in large series 50-60% of patients became pregnant per IVF/PGD cycle.

• Improved with concurrent testing for chromosome abnormalities.
Thank you!!!