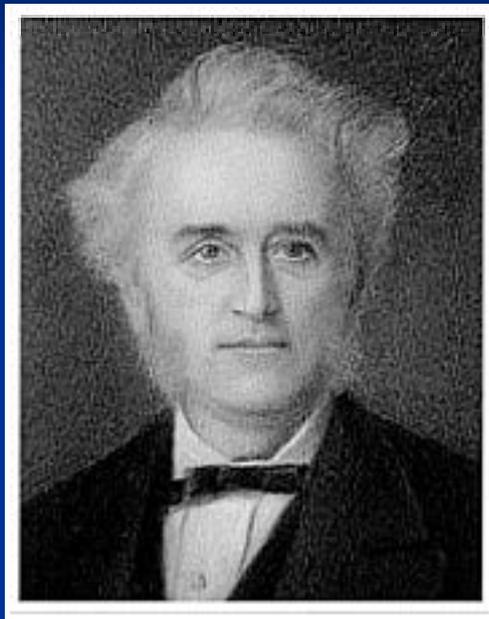


## Selected Clinical Genetic Disorders



Dr François LEUNG  
Paediatrician  
Burrenchobay Hall , 17.3.10

## Down Syndrome (1866)



**John Langdon Haydon Down**  
(1828-1896)

## Down syndrome / Trisomy 21

### Down's syndrome / Trisomy G / Syndrome de Down - Seguin

- Flemish painting of an angel: person with D.S. (1515).
- Esquirol J.E.D. (1772-1840).
- Séguin E: *Le traitement moral, l'hygiène et l'éducation des idiots* (1846) J B Baillière Paris
- Down, J.L.H., « Observations on an ethnic classification of idiots », dans *Clinical Lecture Reports, London Hospital*, vol. 3, 1866, p. 259–262.
- Séguin E: *Idiocy and its treatment by the physiological method* (1866).
- Lejeune J, Gautier M, Turpin R, « Etude des chromosomes somatiques de neuf enfants mongoliens », dans *Comptes Rendus Hebd Seances Acad Sci*, vol. 248, 1959, p. 1721–1722
- WHO officially dropped references to *mongolism* in 1965.

## Down Syndrome / Trisomy 21



- Most common chromosomal disorder.
- Most common cause of cognitive impairment.
- 1/800 live births.
- All races and economic groups.
- M:F ratio: 1.15:1

## Down syndrome (Trisomy 21)



Infant with Down syndrome. Note up-slanting palpebral fissures, bilateral epicanthal folds, flat nasal bridge, open mouth with tendency of tongue protrusion, and small ear with overfolded helix.



Child with Down syndrome. Note up-slanting palpebral fissures, bilateral epicanthal folds, a small nose with flat nasal bridge, open mouth with tendency for tongue protrusion, and small ears with overfolded helix.

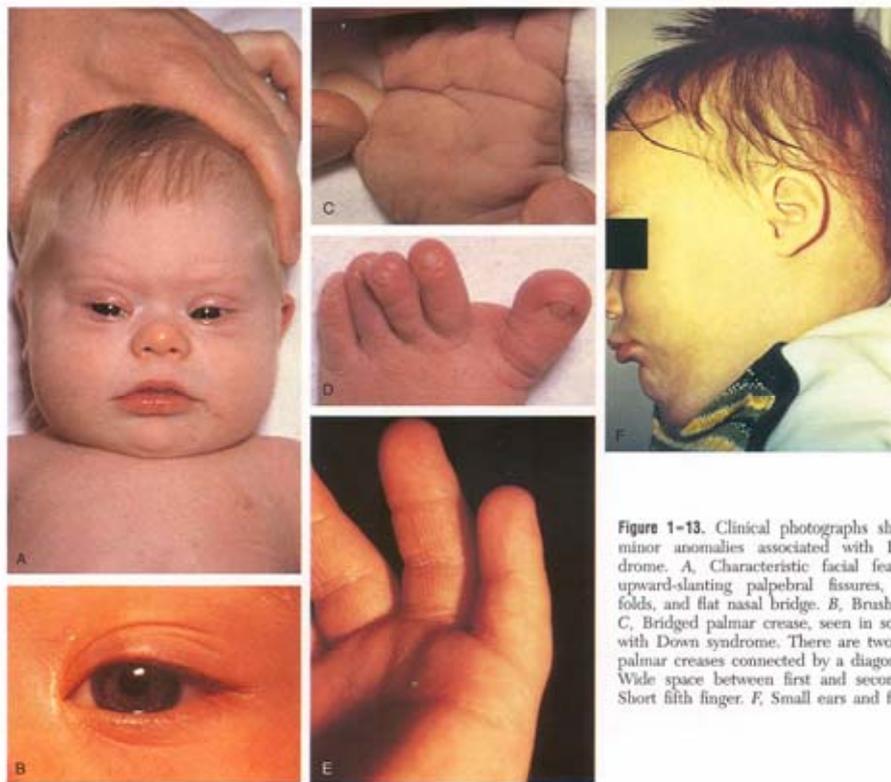
## Down syndrome (Trisomy 21)



Ear of an infant with Down syndrome. Note the characteristic small ear with overfolded helix.



Hand of an infant with Down syndrome. Note the transverse palmar crease and clinodactyly of the 5th finger.



**Figure 1-13.** Clinical photographs show several minor anomalies associated with Down syndrome. *A*, Characteristic facial features with upward-slanting palpebral fissures, epicanthal folds, and flat nasal bridge. *B*, Brushfield spots. *C*, Bridged palmar crease, seen in some infants with Down syndrome. There are two transverse palmar creases connected by a diagonal line. *D*, Wide space between first and second toes. *E*, Short fifth finger. *F*, Small ears and flat occiput.

## Brushfield spots



Brushfield spots consist of tiny areas of normal iris stroma that are surrounded by rings of mild iris hypoplasia. Brushfield spots give the iris a speckled appearance.

## Down Syndrome and Cognitive Impairment

- Leading cause for impaired cognition.
- Cognitive development is usually delayed.
- Learning difficulties persist throughout life.
- Average brain volume is small.
- Dysfunction of hippocampus and cerebellum.
- (Hippocampus: learning and memory).
- Which genes on the extra chromosome 21 affect cognition in Down syndrome?

## Medical Conditions Associated with Down Syndrome

- Heart defect (50%)
- Gastrointestinal abnormalities
- Acute leukemia and testicular cancer++ (most solid tumors--).
- Other medical conditions: infantile spasms, frequent ear infections, hearing loss, visual impairment, sleep apnea, hypothyroidism, cervical spine-instability, constipation, obesity, seizures, dementia, and early-onset Alzheimer's disease.
- Coexisting psychiatric and behavior disorders(18% to 38%): attention deficit hyperactivity disorder (ADHD), autism spectrum disorders, stereotypical movement disorders, obsessive compulsive disorder (OCD), and depression.

## Health Benefits of Down Syndrome

- Reduced incidence of many common malignancies except leukemia and testicular cancer.
  - role of tumor-suppressor genes on chromosome 21?
  - reduced exposure to environmental factors?
  - other unspecified factor?
- Low risk of atherosclerosis and diabetic retinopathy.

## Adults with Down Syndrome

- Premature aging.
- Increased risk for memory loss, dementia, late-onset seizures (tonic-clonic seizures in particular), and hypothyroidism.
- Dementia and develop early-onset Alzheimer's disease by age 40.
- By the age of 60: Alzheimer's disease in 50%-70%.

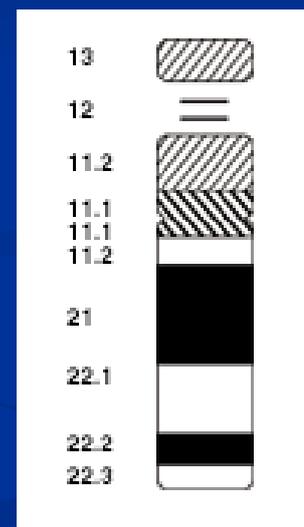
## Reproduction

- About 15-30% of females with trisomy 21 are fertile and have a 50% risk of having an affected child.
- 4 pregnancies fathered by 3 male patients with Down syndrome.
- Infertility in males has been attributed to defective spermatogenesis, but ignorance of the sexual act may be one of the contributing factors.

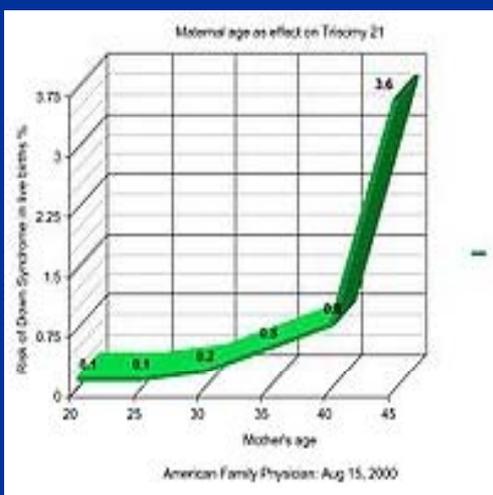
## Physiopathology

- The extra chromosome 21 affects almost every organ system and results in a wide spectrum of phenotypic consequences.
- **Down syndrome critical region (DSCR ):** 21q22.1-q22.3 region: gene(s) responsible for the congenital heart disease in DS.
- The extra copy of the proximal part of 21q22.3 appears to result in the typical physical phenotype.
- A new gene, *DSCR1*, identified in region 21q22.1-q22.2, is highly expressed in the brain and the heart.

Chromosome 21

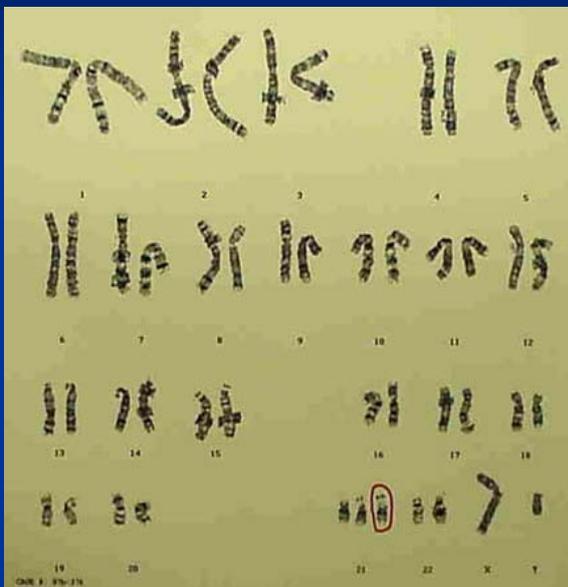


## Maternal Age-Specific Risk for Trisomy 21 at Live Birth

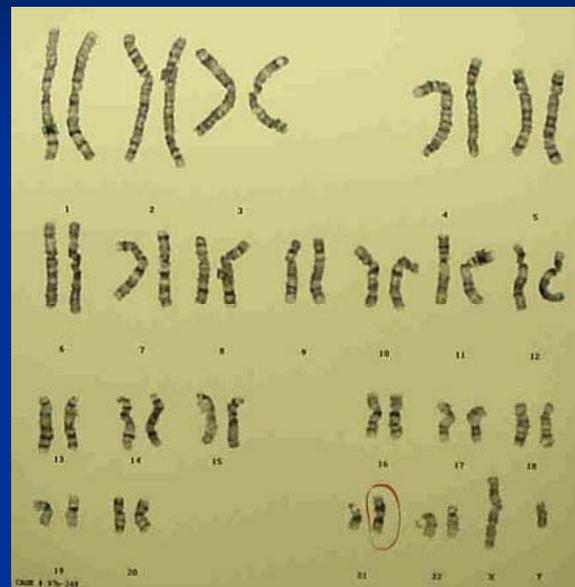


Maternal Age	Prevalence at Live Birth
20	1/1560
25	1/1350
30	1/900
35	1/400
36	1/300
37	1/230
38	1/180
39	1/135
40	1/105
42	1/60
44	1/35
46	1/20
48	1/16
49	1/12

## G-banded Karyotype



Trisomy 21 (47,XY,+21).



Trisomy 21 of isochromosome arm 21q type [46,XY,i(21)(q10)].

## Genetic counseling

- Trisomy 21
- Translocation Down syndrome (familial DS)
- Robertsonian translocation
- Mosaic Down syndrome

## Trisomy 21

- Previous history of trisomy can increase a woman's risk for a recurrence. If the couple has a child with trisomy 21, the risk of recurrence is about 1%. The risk does not appear to be increased in siblings of affected individuals

## Translocation Down syndrome or Familial Down syndrome

- If the child has a translocation, a balanced translocation must be excluded in the parents.



Karyotype of a normal male 14/21 centric fusion translocation carrier

## Robertsonian translocation

- Recurrence risk depends on type of translocation.
- de novo translocations: Recurrence risk is similar or slightly higher to that of the general population: 2-3%.
- Theoretic recurrence risk for a Robertsonian carrier parent to have a liveborn offspring with DS is 1 in 3. However, only 10-15% of the progeny of carrier mothers and only 2-3% of the progeny of carrier fathers have Down syndrome. The reason for this difference is not clear.
- Carrier parent with a 21q21q translocation or isochromosome, the recurrence risk is 100%.

## Mosaic Down syndrome

- Most patients with mosaic Down syndrome were once trisomy 21 zygotes.
- The phenotype varies and possibly reflects the variable proportion of trisomy 21 cells in the embryo during early development.
- In rare instances, low-level mosaicism in germinal tissue of a parent is postulated to be the cause of more than one trisomic child in the family.

# Prenatal Tests

## Screening Tests

- to estimate the risk that a fetus has DS.

## Diagnostic Tests

- 99% accurate in detecting Down syndrome and other chromosomal abnormalities. Associated with a risk of miscarriage and other complications.

# Prenatal Screening

## Nuchal translucency testing

- 11-14 w

## Triple screen or quadruple screen (Multiple marker test).

- 15-18 w
- (AFP, Estriol, HCG, INHA).

## Integrated screen

- uses results from first trimester screening tests (with or without nuchal translucency) and blood tests with second trimester quad screen.
- more accurate screening results.

## Genetic ultrasound

- 18-20 w
- Detailed ultrasound performed in conjunction with the blood tests.

## Prenatal Diagnostic Tests

### Chorionic villus sampling (CVS)

- 8-12 w
- Miscarriage 1-2%

### Amniocentesis

- 15 -20 w
- Preterm labour and miscarriage

### Percutaneous umbilical blood sampling (PUBS).

- >20 w
- Miscarriage++

### Fluorescent in situ hybridization (FISH analysis)

- extraction of fetal cells from the mother's blood

## New method of diagnosis

### Preimplantation diagnosis or blastomere analysis before implantation (BABI).

- Detect chromosome imbalances before an embryo is implanted during *in vitro* fertilization. (FISH method).
- This technique would primarily be used in couples who are at risk of passing on X-linked disorders, couples who have suffered repeated terminations of pregnancy, subfertile couples, or those at risk for single gene disorders.
- Technique successful so far for cystic fibrosis, Tay Sachs disease, and Lesch-Nyhan syndrome.

# Down Syndrome Treatment

No current treatment for Down syndrome.

Corrective surgery (soon after birth)

- Cardiac and gastrointestinal anomalies

Regular screening

- Vision problems, hearing loss, ear infections, hypothyroidism, and other medical conditions

Early intervention programs

- Physical therapy, occupational therapy, and speech therapy, are helpful. Special education and training for children with intellectual and developmental disabilities

Adolescents and young adults

- Proper education regarding sexual development and contraception.

Well-being of people with Down syndrome.

- Inclusion in family and community life

Current research to focus on understanding how cognition is impaired in Down syndrome.

- Finding therapies that might improve cognition in Down syndrome.

## Future Directions in Down Syndrome Research

- . Medical intervention including amino acid supplements and Piracetam.
- . Mouse model to analyze the developmental consequences of Down syndrome, and facilitate the development of effective interventions and treatment strategies.

(Mouse chromosome 16 has many genes in common with those on human chromosome 21).



## Outlook

- The overall outlook for individuals with Down syndrome has improved dramatically in recent years due to better medical treatment and social inclusion. However, life expectancy is still reduced compared to the normal population.
- Congenital heart disease is the major cause for early death.
- Many people with Down syndrome show signs of dementia and symptoms of Alzheimer's disease by age 40 years.

## Conclusion



## Notable individuals

- **Stephane Ginnsz**, actor (*Duo*) - In 1996 was first actor with Down syndrome in the lead part of a motion picture.
- **Pascal Duquenne**, Belgian film actor, co-starred with Daniel Auteil in the 1996 film *Le Huitième Jour* (The Eighth Day), both actors won the joint award for Best Actor at the Cannes Film Festival.
- **Joey Moss**, Edmonton Oilers locker room attendant.
- **Isabella Pujols**, adopted daughter of St. Louis Cardinals first baseman Albert Pujols and inspiration for the Pujols Family Foundation.
- **Paula Sage**, Scottish film actress and Special Olympics netball athlete. Her role in the 2003 film *AfterLife* brought her a BAFTA Scotland award for best first time performance and Best Actress in the Bratislava International Film Festival, 2004.
- **Chris Burke**, American actor who portrayed "Corky Thatcher" on the television series *Life Goes On* and "Taylor" on *Touched By An Angel*.
- **Edward Barbanell**, played Billy in 2005's *The Ringer*.
- **Danny Alsabbagh**, Australian actor who played Toby in the Australian mockumentary series *Summer Heights High*.
- **Tommy Jessop**, British actor who played Ben in *Coming Down the Mountain*, opposite Nicholas Hoult.
- **Rene Moreno**, subject of "Up Syndrome" - a documentary film about life with Down syndrome.
- **Nigel Hunt**, British author (*The World Of Nigel Hunt*; *The Diary Of A Mongoloid Youth* - his book was published in 1967, when "mongoloid" was still quite commonly used to refer to people with Down's Syndrome).
- **Hilly, Sam, Lucy and Megan**, 4 friends with Down's Syndrome who share a house in Brighton with their friend Lewis who has Williams Syndrome. Their lives are followed in the internet documentary series "The Specials".
- **Pablo Pineda**, Spanish actor who starred in the semi-autobiographical film *Yo También*.
- **Andrea Friedman**: actress who portrayed Ellen in the *Family Guy* episode "Extra Large Medium".
- **Nathalie Nechtschein**, French writer and poet, who composed the song « Si j'étais quelqu'un » in Céline Dion's album *D'Elles*.

## Paula Sage

Scottish film actress and Special Olympics netball athlete. Her role in the 2003 film *AfterLife* brought her a BAFTA Scotland award for best first time performance and Best Actress in the Bratislava International Film Festival, 2004.



Scottish award-winning film and TV actress Paula Sage receives her BAFTA award with Brian Cox.



Down Syndrome  
Duchenne Muscular Dystrophy

# Muscular Dystrophies



## Definition:

Heterogenous group of unrelated inherited disorders (different genetic trait, different clinical course and expression).

## 4 obligatory criteria:

- . Primary myopathies
- . Genetic basis
- . Progressive downhill course
- . Degeneration and death of muscle fibers at some stage.

## Muscular Dystrophies

- Duchenne Muscular Dystrophy (DMD) (Pseudohypertrophic )
- Becker Muscular Dystrophy (BMD)
- Emery-Dreifuss Muscular Dystrophy (EDMD)
- Limb Girdle Muscular Dystrophy (LGMD)
- Facioscapulohumeral Muscular Dystrophy (FSH or FSHD) (Landouzy-Dejerine)
- Myotonic Dystrophy (MMD or DM or Steinert Disease)
- Oropharyngeal Muscular Dystrophy (OPMD)
- Distal Muscular Dystrophy (DD) (Miyoshi)
- Congenital Muscular Dystrophy (CMD)

## Genetic traits

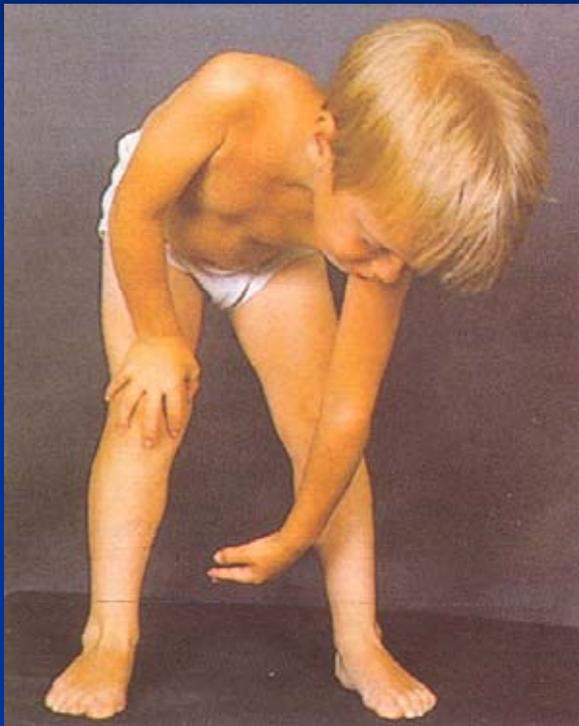
- Duchenne MD (DMD): X-linked Recessive.
- Becker MD (BMD): X-linked Recessive.
- Emery-Dreifuss MD (EDMD): X-linked Recessive; or Autosomal Dominant or Autosomal Recessive.
- Limb Girdle MD (LGMD) (Erb): Dominant, Recessive or X-linked.
- Facioscapulohumeral MD (FSH or FSHD) (Landouzy-Dejerine): Autosomal Dominant.
- Myotonic Dystrophy (MMD or DM or Steinert Disease): Autosomal Dominant.
- Oropharyngeal MD (OPMD): Autosomal Dominant or Autosomal Recessive.
- Distal MD (DD) (Miyoshi): Autosomal Dominant or Autosomal Recessive.
- Congenital MD (CMD): Autosomal Recessive or Autosomal Dominant.

## Duchenne Muscular Dystrophy (1868)



Guillaume Duchenne de Boulogne  
(1806-1875)

## Duchenne Muscular Dystrophy (DMD)



1868

Guillaume Duchenne  
Clinical description

1886

William Richard Gowers  
Transmission by mother

1891

Wilhelm Heinrich Erb  
Histological signs

## Duchenne Muscular Dystrophy (DMD)

- 1/3500 boys worldwide & 20 000 new cases each year.
- Mutations (mainly deletions) in the Dystrophin gene.
- DMD ; locus Xp 21.2
- Size of the gene: 2 500 000 base pairs.
- Largest locus, containing 79 exons. (1% of X).
- 2/3: Inherited; 1/3: Mutation.
- Girls: Mild form (Lyon hypothesis) (The normal X chromosome become inactivated, and the one with gene deletion become active).
- Turner Syndrome(45, XO): Full blown disease.

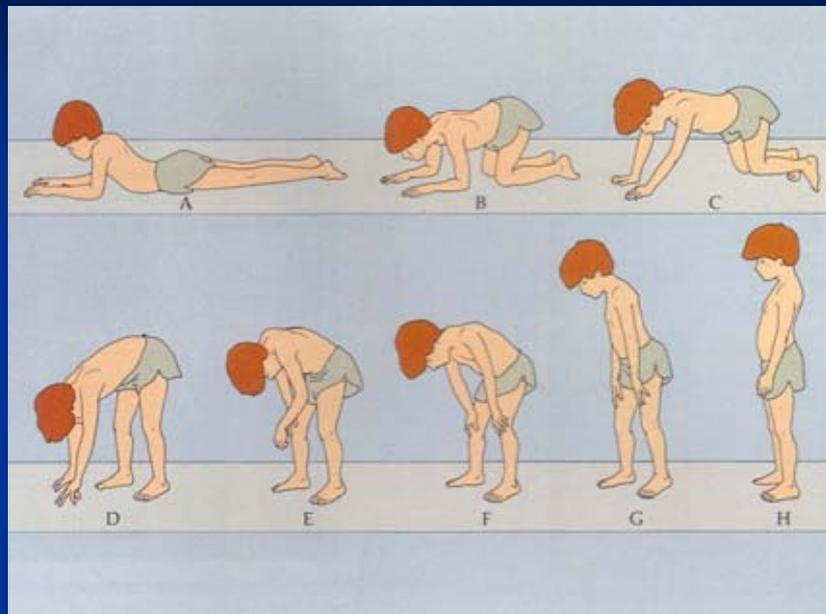
## Symptoms: Young Male

- Impairment of balance, resulting in frequent falls
- Large calf muscles (which try to compensate for weaknesses in other muscles)
- Abnormal difficulty getting up from a sitting or lying position
- Difficulty for walking, running and jumping, due to weak leg muscles
- Waddling gait (Trendelenburg gait)
- Lordosis, Scoliosis

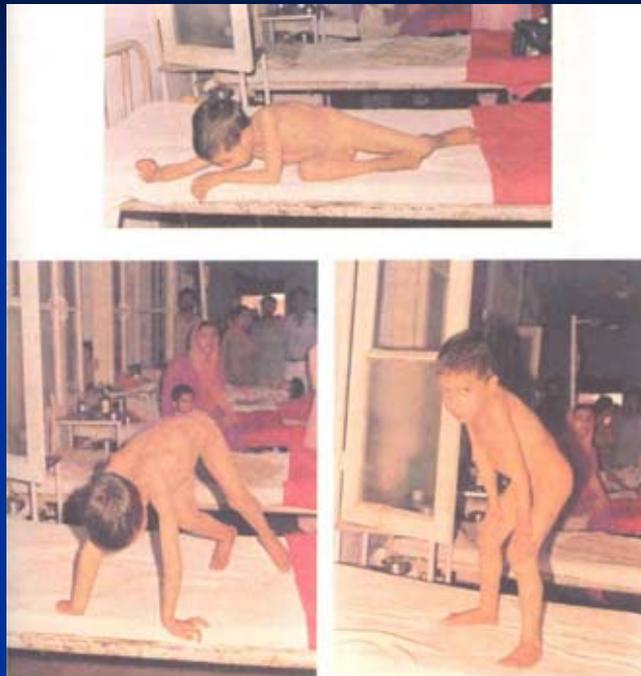
## Duchenne Muscular Dystrophy (DMD)

- **Age of onset:** 2 to 6 years
- **Symptoms:** General and severe muscle weakness and wasting, beginning from the upper arms, upper legs and pelvis. Weakness eventually progresses to all voluntary muscles. Calf muscles are generally hypertrophic . Mental retardation associated. (Dp7).
- **Rate of Progression:** Fast
- **Complications:** pneumonia, diaphragm muscle weakness, cardiomyopathy.
- **Expected Lifespan:** as much as 25 years, survival past the twenties is very rare.

## The Gowers' Manoeuvre (1879)



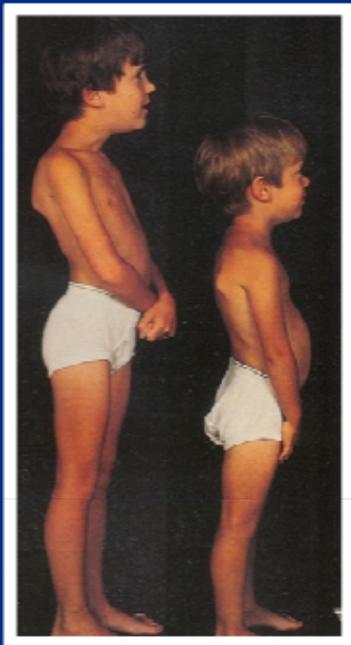
This series of diagrams illustrates the sequence of postures used in attaining the upright position. **A-C**, First, the legs are pulled up under the body, and the weight is shifted to rest on the hands and feet. **D**, The hips are then thrust in the air as the knees are straightened and the hands are brought close to the legs. **E-G**, Finally, the trunk is slowly extended by the hands walking up the thigh. **H**, The erect position is attained.



### Gowers' sign

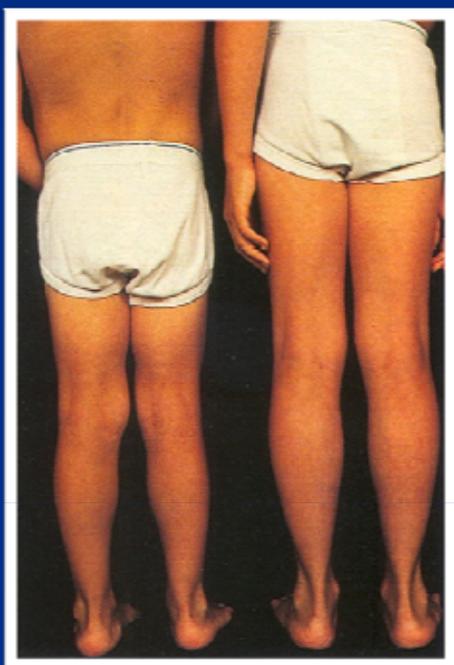
Succession of movements involved in arising from bed to an upright position.  
The child appears to be climbing his own thighs.

## Duchenne muscular dystrophy



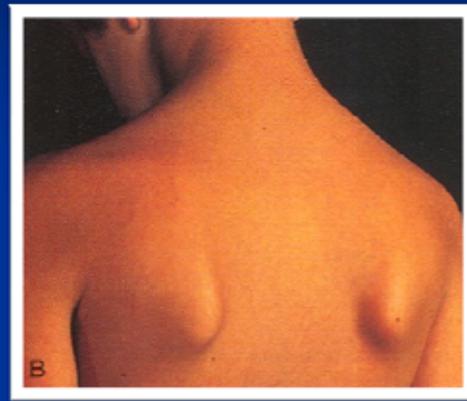
These brothers, ages 5 and 8, show progressive compensatory postural adjustments with broadening of stance, accentuated lumbar lordosis, and forward thrusting of the abdomen.

## Duchenne muscular dystrophy



Enlargement of the calves in brothers, ages 5 and 8.

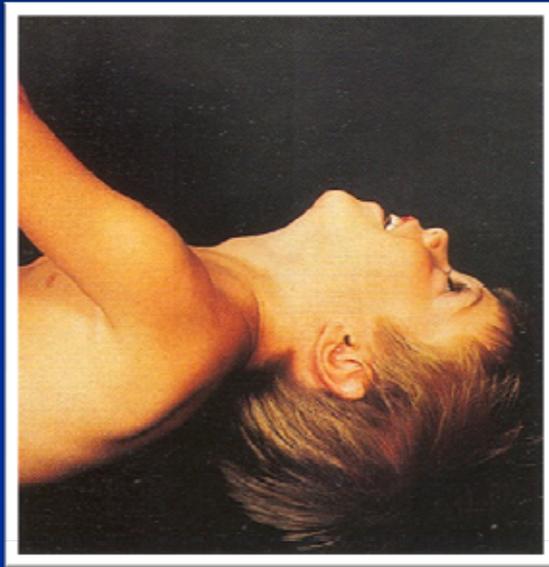
## Duchenne Muscular Dystrophy



*A*, This child, age 5, demonstrates weakness and hypotonia of the shoulder girdle musculature, Upward displacement of the shoulders and abnormal rotation of the scapulae are seen when the child is lifted with the examiner's hands under his arms.

*B*, Spontaneous winging of the scapulae can be noted in this 8-year-old child.

## Duchenne muscular dystrophy



This 5-year-old boy has neck flexor weakness. Note the marked head lag when the patient is pulled to sit from the supine position.

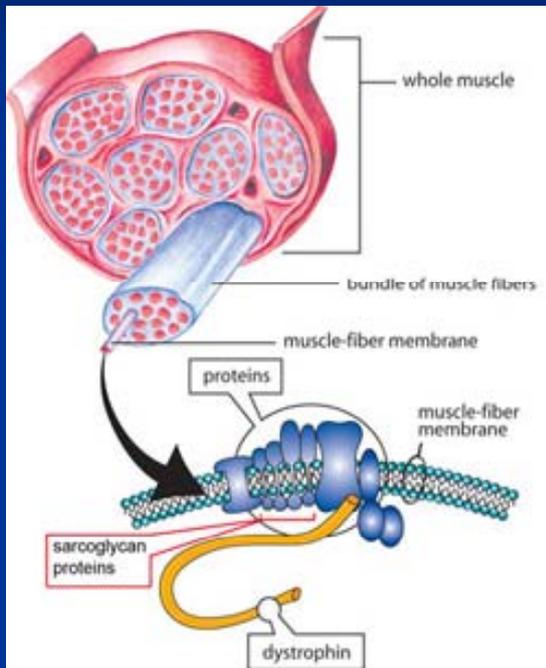
## Becker Muscular Dystrophy (BMD)

- **Age of onset:** 2 to 16 years
- **Symptoms:** Similar to Duchenne MD, but less severe. They include general muscle weakness, especially in the upper leg and arms, and pelvic muscles. Calf muscles are generally hypertrophic. Heart problems are also possible .
- **Rate of Progression:** Slower than Duchenne MD
- **Expected Lifespan:** Well into middle age .

## Clinical Features of the Muscular Dystrophies

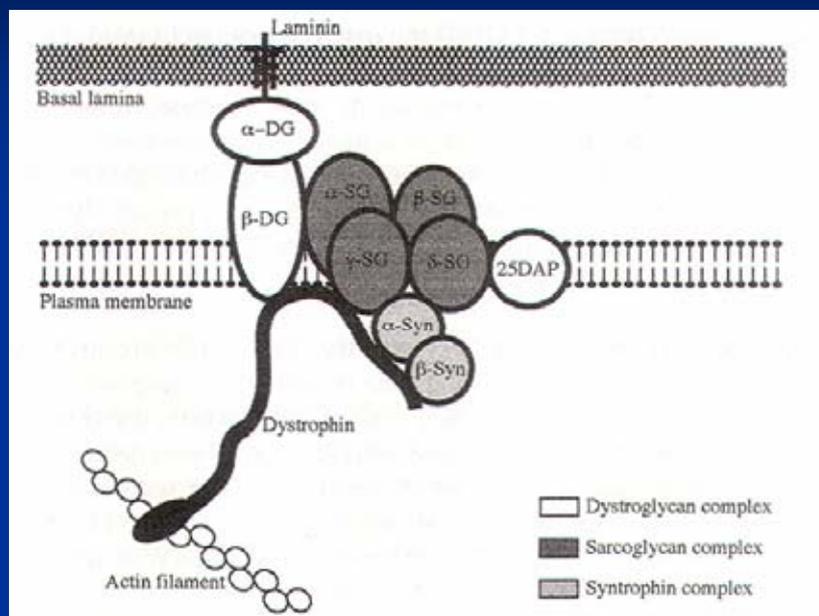
	Duchenne	Becker	Fascioscapulohumeral	Limb-Girdle	Myotonic
Inheritance	X-linked recessive	X-linked recessive	Autosomal dominant	Autosomal recessive	Autosomal dominant
Age at onset	Early childhood	Late childhood, adolescence	Variable: childhood through early adult life	Childhood to early adulthood	Highly variable
Pattern of weakness	Pelvic girdle, shoulder girdle	Pelvic girdle, shoulder girdle	Face, shoulder girdle	Pelvic girdle, shoulder girdle	Face, distal limbs
Rate of progression	Rapid	Slow	Very slow	Variable	Variable
Associated features	Pseudohypertrophy of calves	Pseudohypertrophy of calves	None	Pseudohypertrophy rare	Myotonia
Systemic features	Mental retardation, abnormal electrocardiogram, cardiomyopathy	Occasional mental retardation	None	None	Frequent mental retardation, heart block, cataracts, premature balding, testicular tubular atrophy, diabetes

## Physiopathology



Some forms of muscular dystrophy (i.e., DMD, BMD and several types of LGMD) are due to deficiencies of proteins associated with the membranes that surround each muscle fiber. These deficiencies lead to membrane damage.

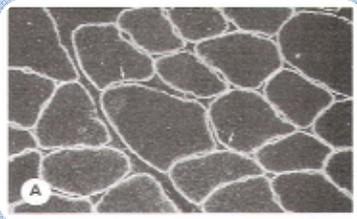
## The Dystrophin Complex



The molecular architecture of dystrophin and dystrophin-associated proteins at the muscle cell membrane.

DG, dystroglycan; SG, sarcoglycan; Syn, syntrophin.

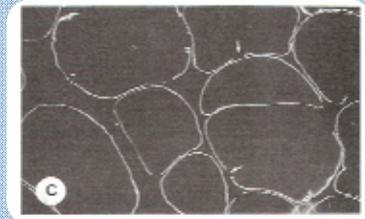
## Immunolocalisation of dystrophin



Normal human muscle shows reaction (*arrow*) at the sarcolemma of all fibers.



DMD sample shows lack of immunostain in all fibers.



BMD sample shows immunostain at the sarcolemma with regions of discontinuities (*arrows*).

## Genetics



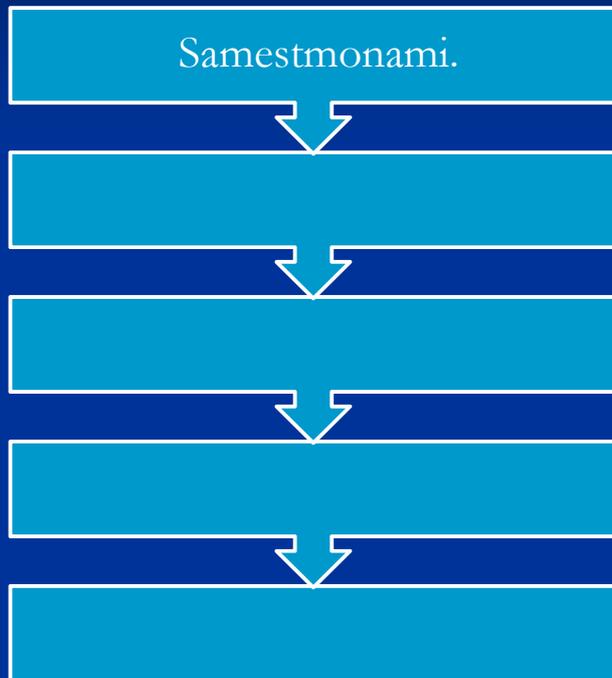
Gene: similar to a sentence in which all words consist of 3 letters (codons).

Start codon / Stop codon

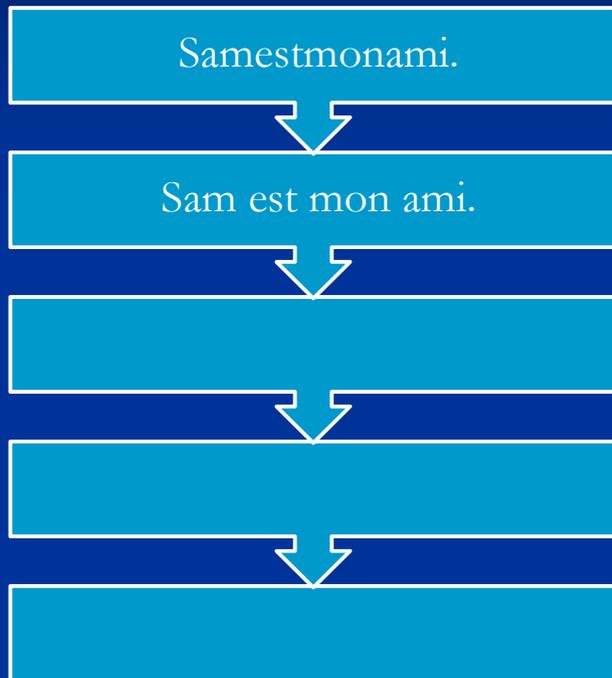
Synthesis of a functional protein.

« Samestmonami. »

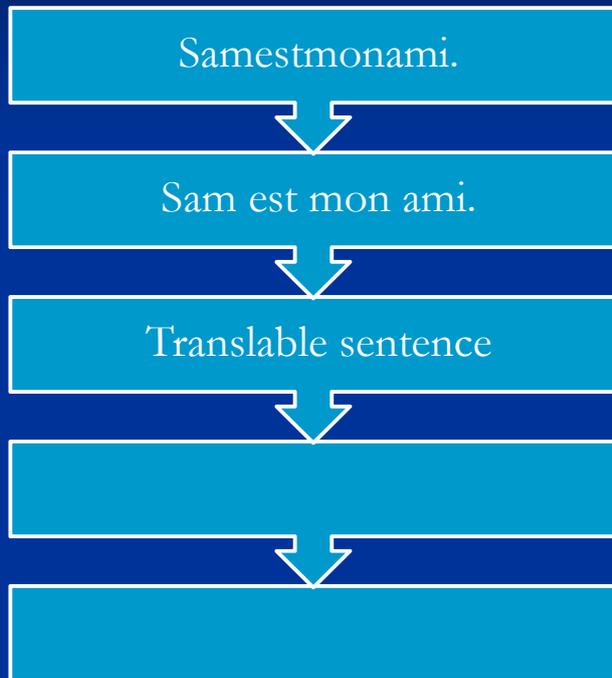
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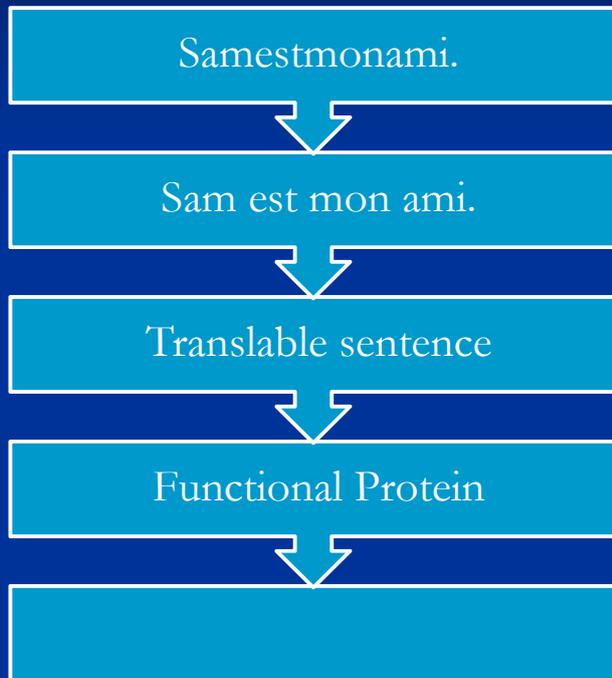
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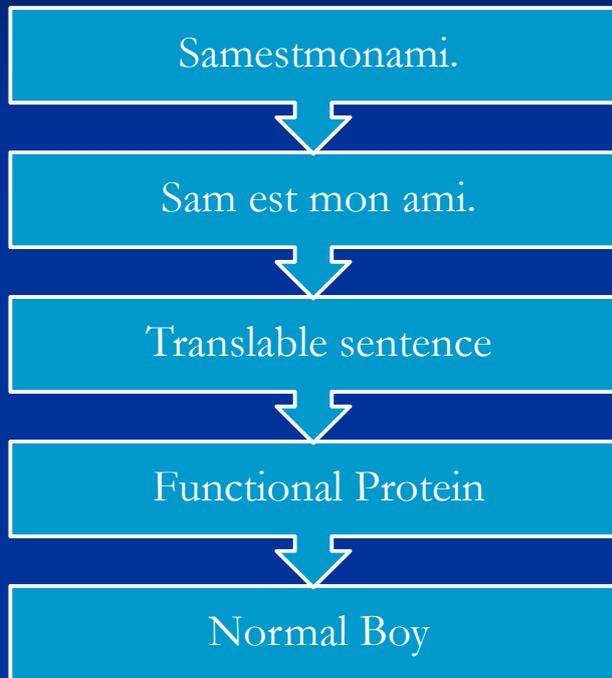
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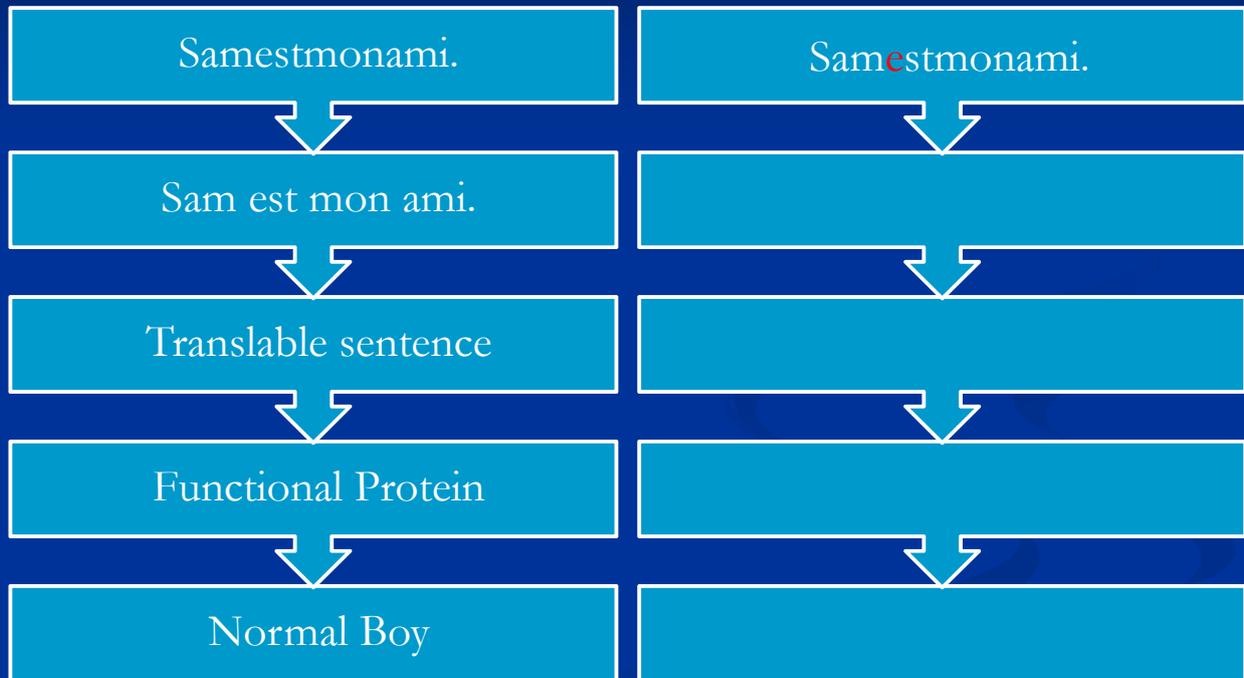
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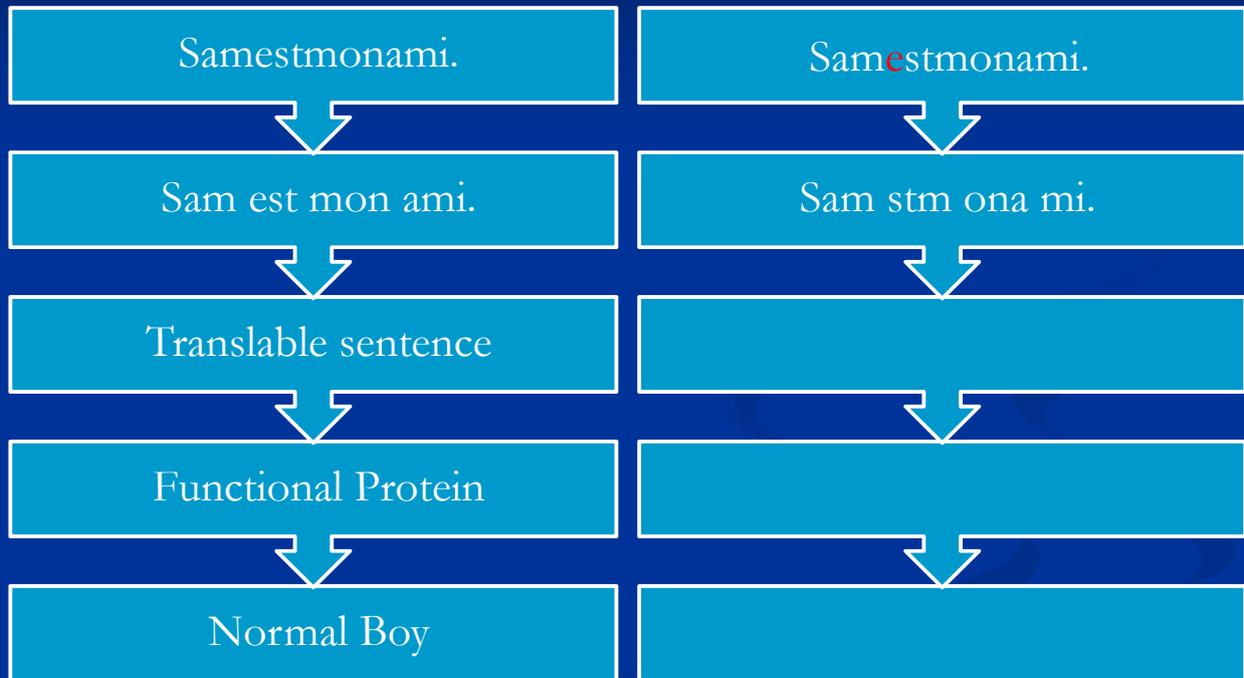
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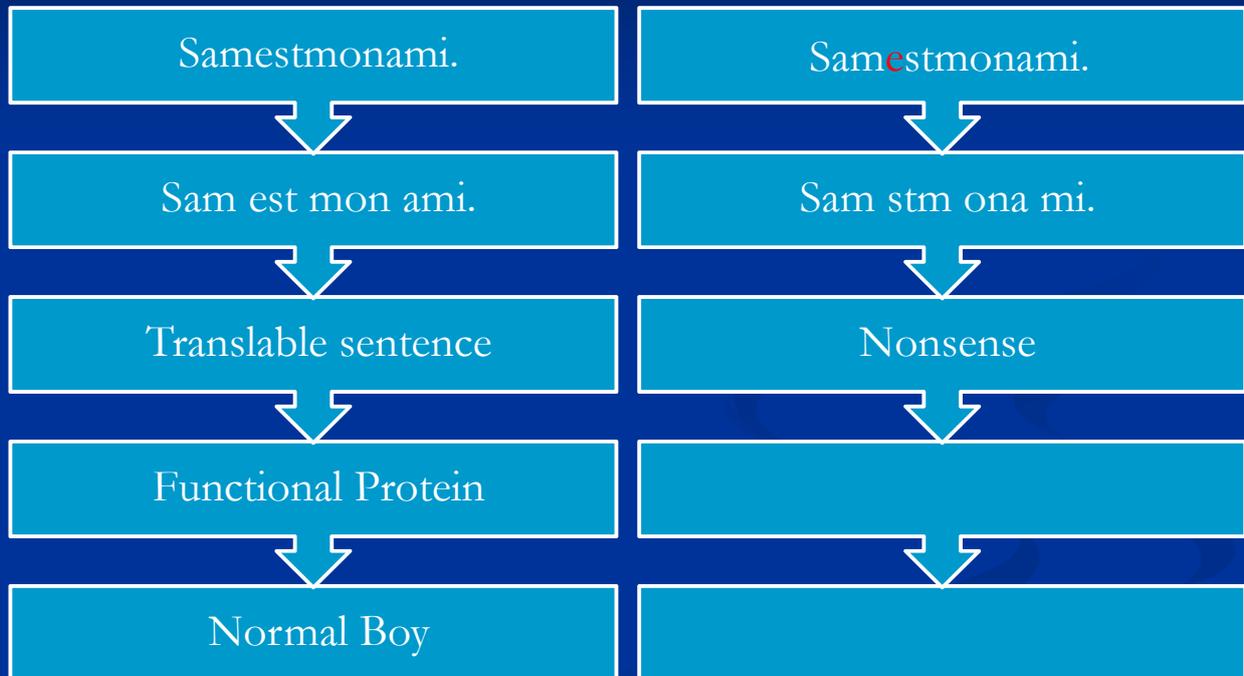
## « Samestmonami. »



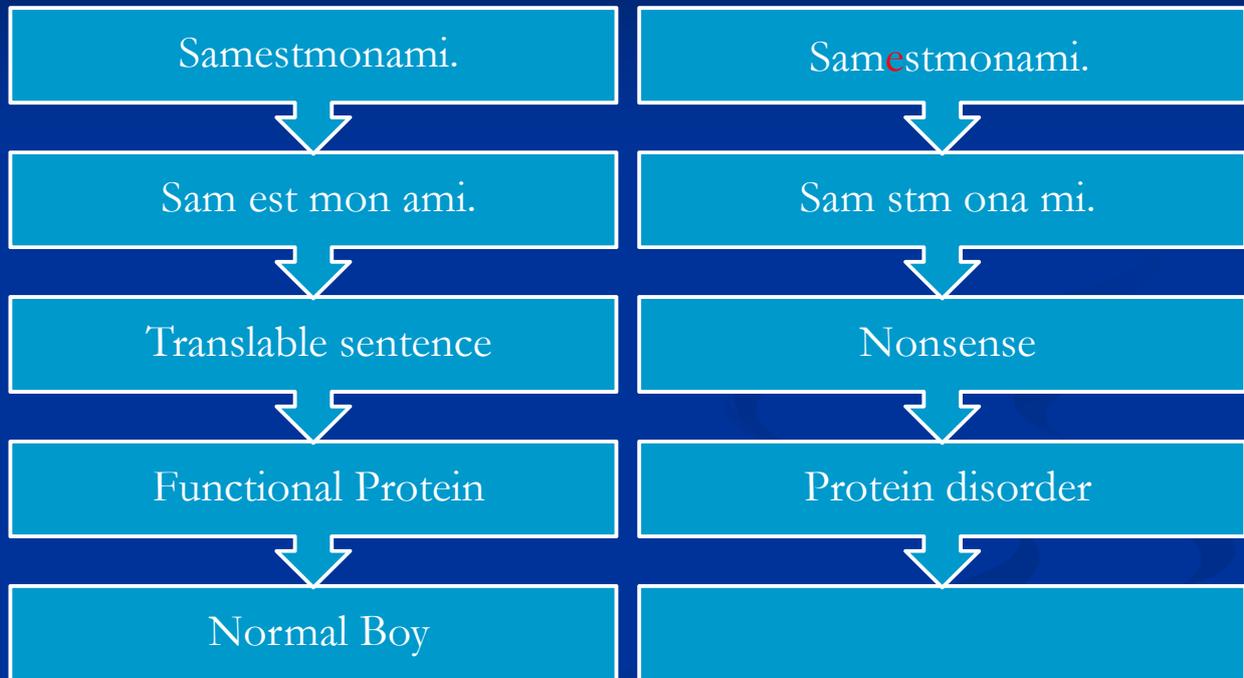
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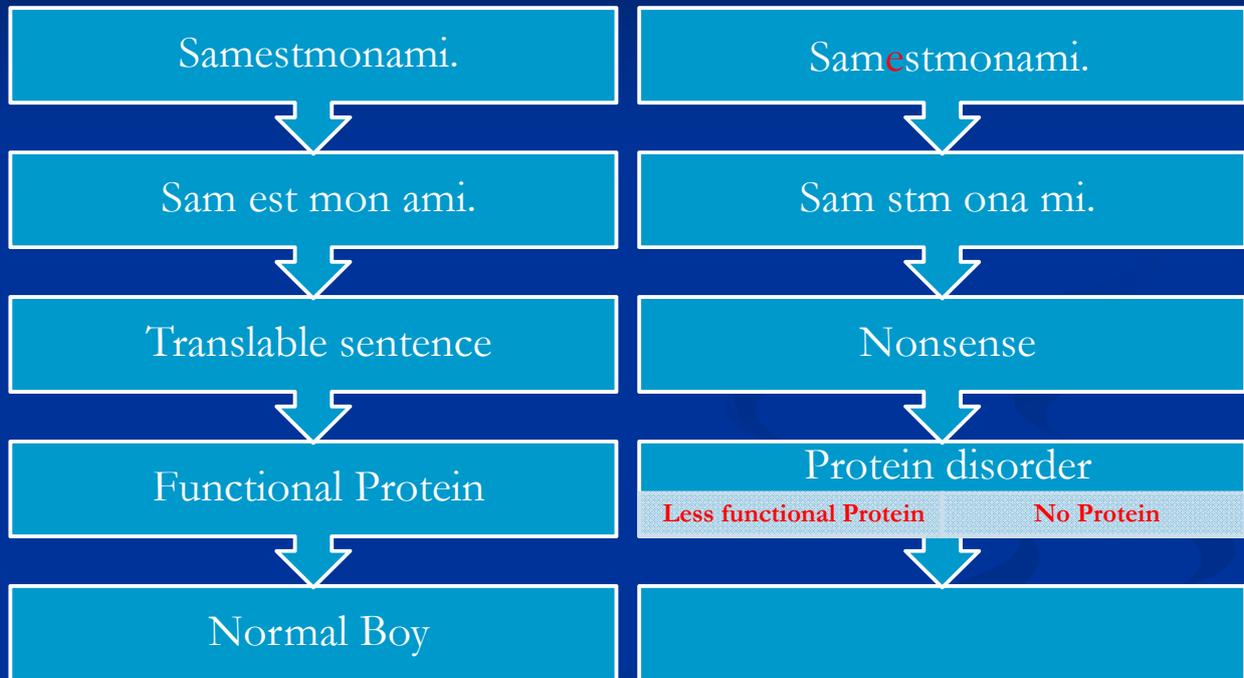
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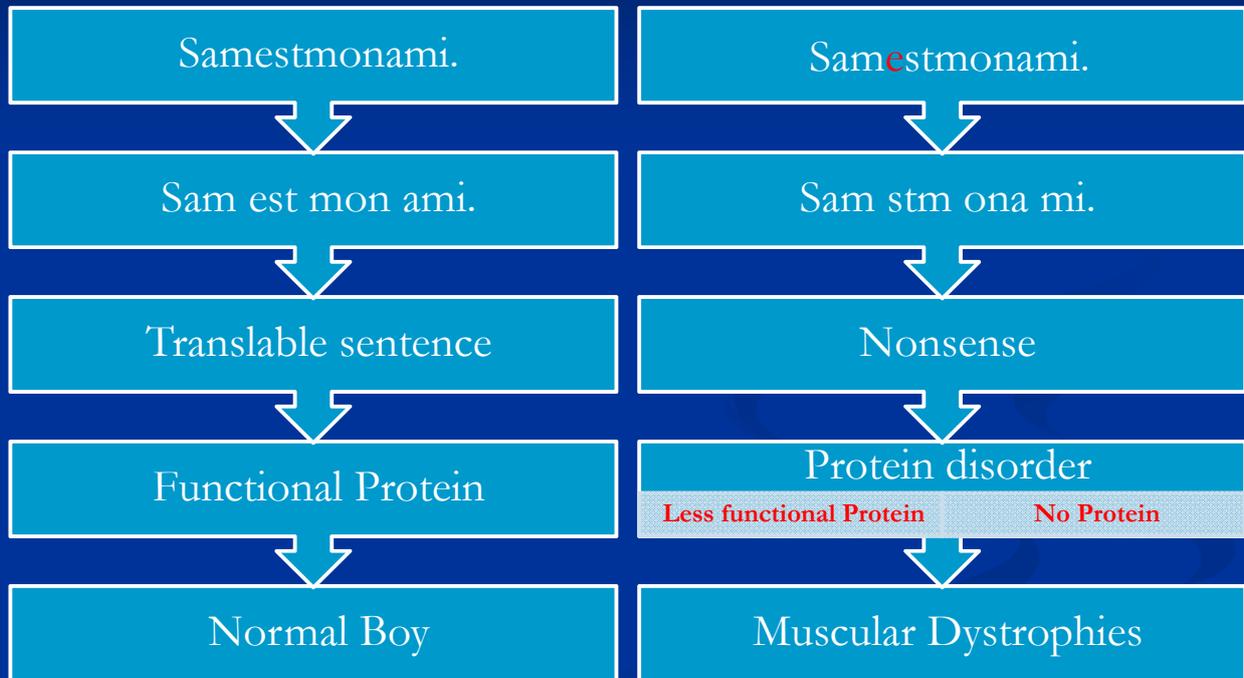
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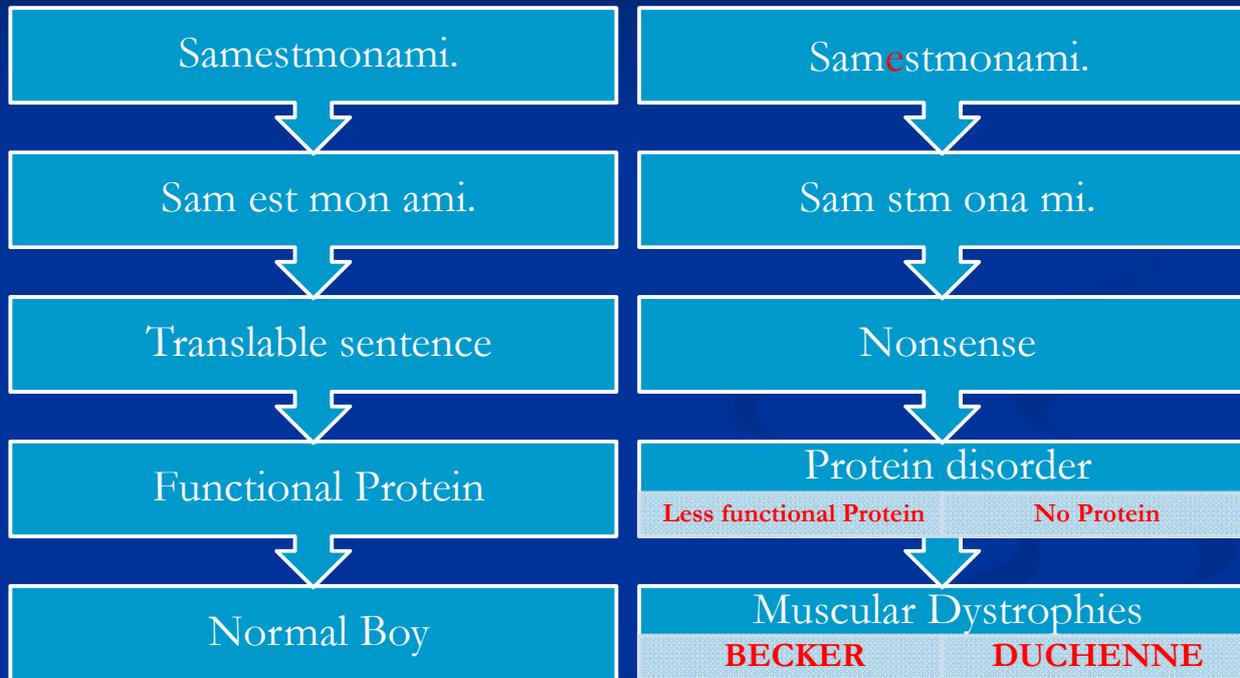
## « Samestmonami. »



## « Samestmonami. »



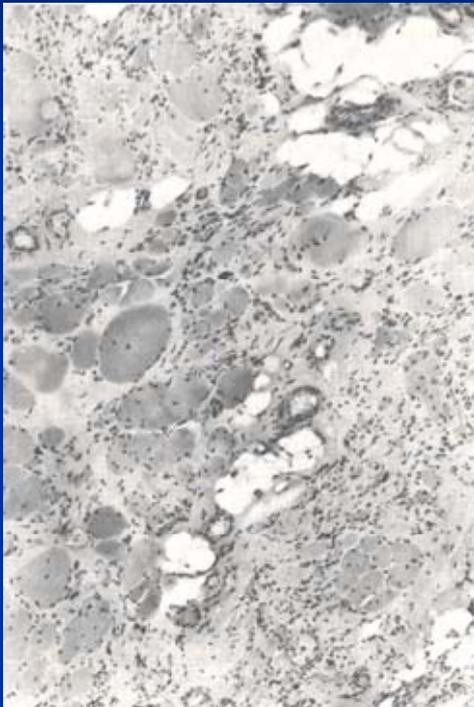
## « Samestmonami. »



## Paraclinical Tests

- CPK: x10 to x100 (Maximum level at 3 yrs)
- Aldolase
- LDH (M, PK)
- Transaminases (ALAT & ASAT)
- EMG: Reduced amplitude and duration of motor unit potentials.
- ECG: Tall right precordial R waves; deep Q waves in aVf and left precordial leads.
- Cardiac US: Hypokinesia of posterior and inferior part of LV.

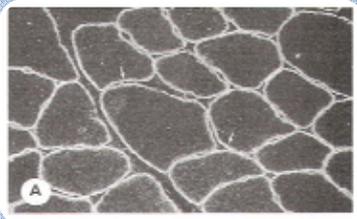
## Muscle Biopsy



DMD at an advanced stage:

Intense muscular lesions (connective and fat tissue infiltration, necrosis, fibers of unequal dimension, abundant central nuclei).

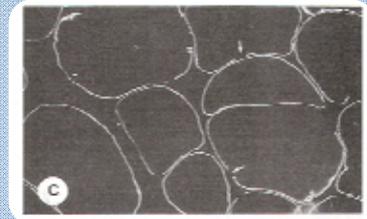
## Immunolocalisation of dystrophin



Normal human muscle shows reaction (*arrow*) at the sarcolemma of all fibers.



DMD sample shows lack of immunostain in all fibers.



BMD sample shows immunostain at the sarcolemma with regions of discontinuities (*arrows*).

- Golden Standard: DNA tests:
  - Specific mutation of the Dystrophin Gene,
  - Single condition amplification/internal primer (SCAIP) sequencing: Picture of the entire dystrophin gene. ...multiple variations.

## Genetic Counselling

- Recessive X-linked disease: males are affected, females are carriers of gene.
- Carriers: mother, sister, maternal (female) cousin.

## Treatment

- No specific treatments for DMD.
- Targeting of interventions to known manifestations and complications.
- Chronic steroid therapy gives gratifying initial results with improvement in muscle strength. Side-effects+++.
- **Prednisone: 0.75 mg/kg/day**  
(Max: 30 mg/day)
- **Deflazacort: 0.9 mg/kg/day.**  
(Max: 36 mg/day)

## Recommendations with regard to corticosteroid dosing schedules

- Daily dosing, typically given in the morning, is preferred over other methods.
- Physicians should reassess patients on low doses of corticosteroids after two to three months and increase if necessary.
- In cases where obesity is a concern, deflazacort is preferable to prednisone.
- Before abandoning corticosteroid treatment, efforts should be made to find an acceptable dosing regimen with minimum, manageable side effects.

## Recommendations for corticosteroid use

- Monitor for, prevent and/or manage corticosteroid-related side effects.
- Provide families with information on handling emergencies.
- Start steroid treatment at the “plateau” phase, when the child no longer is able to improve skills, but before skills have begun to decline.
- Long-term corticosteroid treatment: Side effects+++
- Long-term commitment to monitoring and managing the therapy is necessary.

## Other drugs and supplements (under investigation)

- **Albuterol:** . It is believed to work like prednisone - by stimulating muscle growth and suppressing the immune cells that remove debris and muscle cells.
- **Creatine:** a level of 5g/day seems sufficient to improve muscle strength in DMD patients.

## Other drugs and supplements (under investigation)

### ■ Gentamycine:

- . One of the most promising drugs for DMD.
- . Useful only for about 10% of DMD cases - those caused by "stop codons" that prevent the decoding of the full dystrophin gene.
- . When gentamycin is administered, the stop codons are ignored, and the full gene can be translated into a functional dystrophin protein.
- . Preliminary trial on men is currently being performed.

## Other drugs and supplements (under investigation)

- Use of the steroid **oxandrolone**, in conjunction with glucocorticoid therapy or alone, is neither necessary nor appropriate.
- The use of **botulinum toxin A** for prevention of contractures is not recommended.
- No recommendations were made for the use of **supplements, including coenzyme Q10, carnitine, amino acids, anti-inflammatories or antioxidants.**

## Other measures

- Muscle extensibility and joint contractures
- Skeletal management
- Respiratory management
- Cardiac management
- Nutrition, swallowing, speech
- Pain management
- Surgery and procedures/Emergency care

## Psychosocial well-being

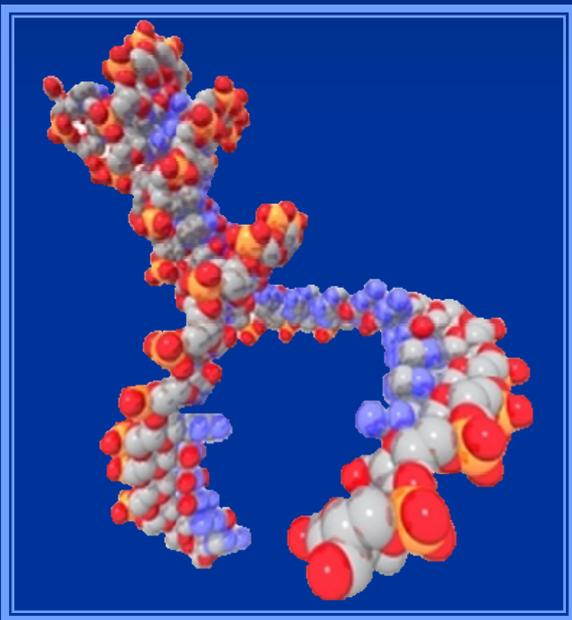
- Assessments should be made at the time of diagnosis, before the child begins school, and after a change in function occurs.
- Medication for psychiatric symptoms should be considered, with subsequent monitoring and follow-up with medical professionals.
- Palliative care should be offered, to prevent suffering and improve quality of life through such measures as pain management, emotional and spiritual support, and the making of medical decisions.

Molecular genetic engineering  
Gene Therapies



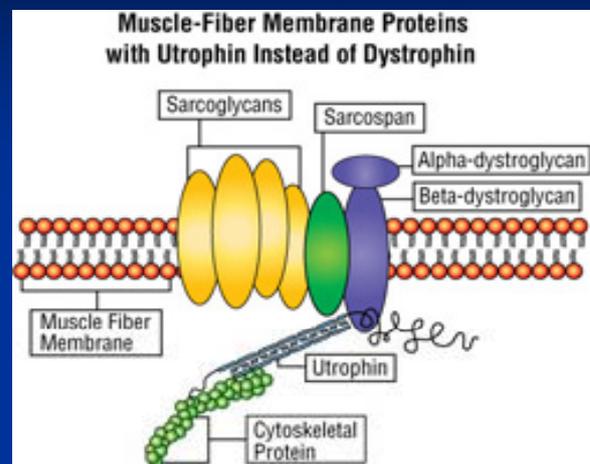
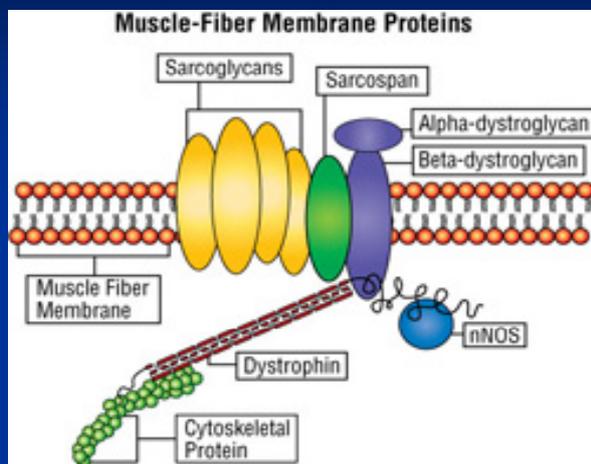
Hope of definitive treatments for  
Duchenne Muscular Dystrophy

## Miniaturised Dystrophin Gene



Miniaturization of the dystrophin gene has been necessary in order to fit the very large gene into small viral shells for delivery to muscle.

## Miniaturised Dystrophin Genes, including repeats 16 and 17



Neuronal nitric oxide synthase (nNOS) needs a specific section of the dystrophin protein (“spectrin-like repeats 16 and 17”) for its proper positioning. Adding repeats 16 and 17 to a dystrophin minigene provide it.

N.B: **Sildenafil (Viagra)**, has shown promise in dystrophin-deficient mice therapies to offset the loss of nNOS.

## Injection of full-length dystrophin genes (using modified AV)



Muscle fibers of the mice that received full-length dystrophin genes showed a more complete restoration of a cluster of proteins at the muscle-fiber membrane than can be achieved with AAV6-microdystrophin gene compounds.

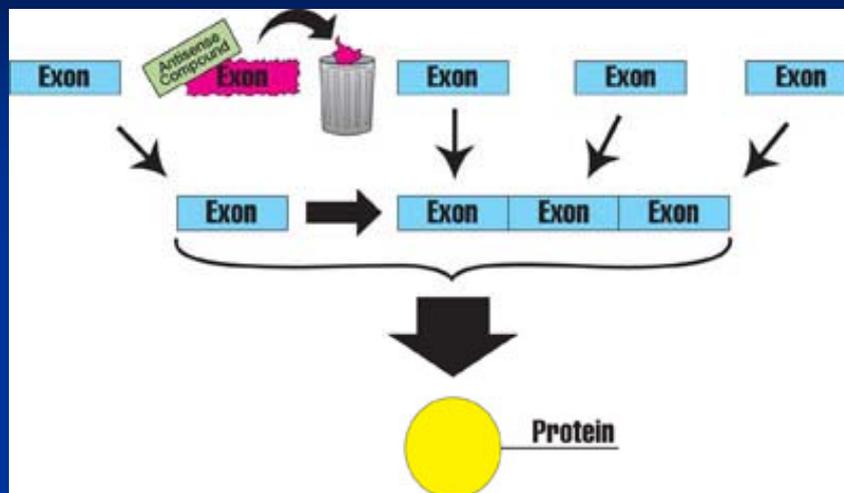
AVs caused a slight immune response but are unlikely to activate the immune system more than it's already activated in dystrophic muscles.

## Exon skipping Drug (Antisense Compound)



Exon skipping is among the most promising strategies for treatment of Duchenne Muscular Dystrophy.

## Exon Skipping



As a cell prepares the final version of instructions for making a protein, it removes excess material and leaves only the exons, the parts that will form the final protein recipe. Laboratory-designed antisense compounds can make a cell eliminate a specific exon along with the other unwanted material.

## AVI-4658

- AVI-4658 is designed to coax cells to skip (ignore) the part of the dystrophin gene called exon 51.
- (13 % of DMD: Mutation in the exon-51 area of the gene).
- Systemic Treatment with AVI-4658 Demonstrates RNA Exon Skipping and Dystrophin Protein Expression in Duchenne Muscular Dystrophy Patients.
- Any DMD drug based on exon skipping is expected to be administered regularly over the entire course of a patient's life.

## Modified morpholinos (AVI-5225)

- 5 to 6 weeks after IV treatment:  
**Reappearance of dystrophin in the heart. +++**
- Normalisation of histological and clinical cardiac parameters.
- 12 weeks after treatment:  
Natural elimination of the new dystrophin and no worsening of cardiac parameters for the following 7 months.

## Ataluren (PTC124)

- Nonsense mutation read-through.
- In some patients, the disease is caused by a mutation that prevents the full construction of dystrophin. (nonsense or premature stop codon mutation).
- Ataluren bypasses or "reads through" molecular signals in the gene for dystrophin and prevents the body from terminating prematurely the full synthesis of dystrophin.
- It has been proven to restore normal muscular function.

Ataluren is already in clinical trials: Ambulatory and nonambulatory DMD and BMD patients.

## T $\beta$ 4

- **T $\beta$ 4 Increases Skeletal Muscle**
- In exercised dystrophin deficient mice, chronic administration of T $\beta$ 4 increases the number of regenerating fibers in skeletal muscle.
- T $\beta$ 4 stimulates cell migration and anti-apoptosis pathways in skeletal muscle cultures: Role for T $\beta$ 4 in muscle degenerative diseases and injury.

## Follistatin / Myostatin

- Follistatin is a natural body protein that promotes muscle growth and strength by interfering with the actions of another natural protein, known as Myostatin, which limits these.

## Follistatin Gene



Injections of genes for the muscle-growth protein follistatin strengthened leg muscles in monkeys.

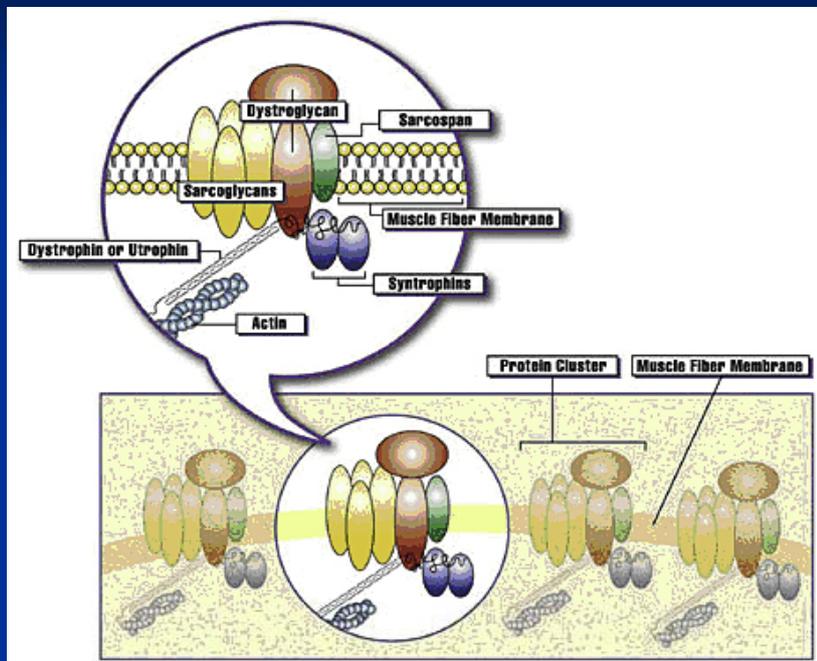
Potential for all nine forms of muscular dystrophy and possibly other types of muscle disease, such as inflammatory myopathies.

Since follistatin is a protein made by people with and without muscle disease, the immune system is likely to accept it without rejection.

## MYO-029

- Myostatin is a protein that inhibits the growth of muscle tissue.
- MYO-029 is a recombinant human antibody designed to bind and inhibit the activity of myostatin.
- **Myostatin blocking** is a strategy for maintaining muscle tissue in the face of degenerative disease.

## Dystrophin / Utrophin



Increase utrophin to sufficient levels by injecting utrophin genes or protein, or by increasing utrophin production from existing genes.

## Direct injection of Utrophin



Direct injections of utrophin protein into dystrophin-deficient mice strengthened their muscles.

Advantage: Less immunological reactions than with Dystrophin.

## BMN195



Compound designed to increase the body's production of utrophin, which may shelter fragile muscle cells in DMD & BMD.

## Zinc-finger protein 51 (ZFP51)

- Chemical switch that activates utrophin genes, thereby causing muscle fibers to make more utrophin protein and expanding utrophin's location from one small area to the whole fiber.
- The ZFP is a small protein and will probably not cause immunological intolerance.

## Protein therapy: TAT-micro-utrophin

- Different from gene therapy and gene upregulation.
- Injection of miniaturized utrophin protein molecules (micro-utrophin) into the abdomens of DMD mice.
- The **TAT-micro-utrophin** penetrated all the tissues examined. In addition, it aligned itself with the muscle-fiber membrane as part of a cluster of proteins the way dystrophin normally would.

## Sarcospan genes

- Sarcospan genes are given to dystrophin-deficient mice.
- Appearance of utrophin-containing protein clusters all around muscle fibers.
- Moreover, the sarcospan-enriched muscles looked healthier than those of the untreated dystrophin-deficient mice, with fewer degenerating fibers.
- Sarcospan does not increase utrophin production, but it stabilizes utrophin and allows it to expand its role and its territory and to substitute for dystrophin.

## Myoblasts

- Immature muscle cells called "myoblasts," taken from healthy relatives, were transplanted (injected) into the muscles of patients with DMD.
- It was hoped that these cells would repair damaged muscle fibers in the patients. However, very little such repair was seen.
- Most of the cells selected for transplantation were too far along in their maturation to join existing muscle tissue. These cells were not sufficiently flexible, or "stemlike," to perform the repair functions.

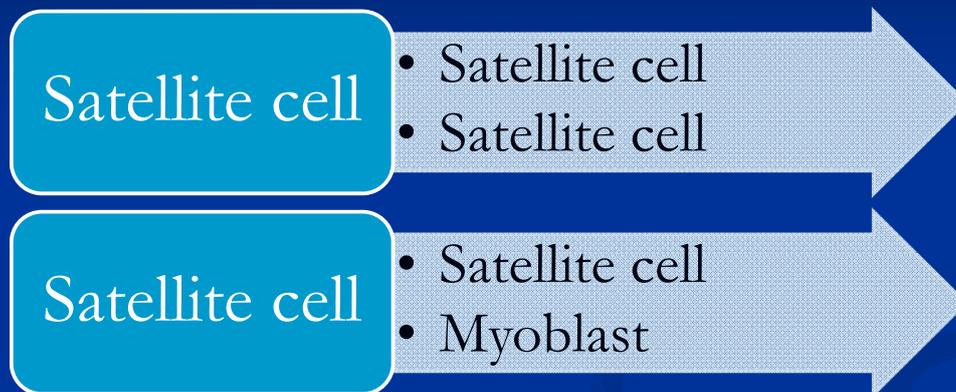
## Stem cells

- Stem cells - immature cells with the potential to develop into different tissue types - have been heralded as a major advance for developing treatments for a variety of diseases.
- Stem cells also can be developed from non-embryonic sources and can be « reprogrammed » by virus-free methods.

## Umbilical cord blood

- Stem cells which are isolated from the umbilical cord blood of healthy babies, then mixed in a lab dish with early-stage muscle cells (myoblasts) from people with **Duchenne muscular dystrophy (DMD)** or **Becker muscular dystrophy (BMD)**, can fuse.
- The resulting early-stage muscle fibers can produce dystrophin.
- These very immature (“undifferentiated”) umbilical cord stem cells, known as CD34-positive stem cells, are extremely flexible. These cells probably responded to chemical factors released by the dystrophic muscle cells that invited fusion and formation of muscle fibers.

## Satellite cells



Identifying the biochemical steps that underlie these processes should allow scientists to alter the balance between them and ultimately lead to better treatment of muscle degeneration.

## Muscle-Repair Booster (WNT7a protein )

- The WNT7a protein stimulates muscle repair by causing proliferation of "satellite stem cells."
- Injection of genes for the WNT7a protein into muscle fibers in mice: Increase in satellite stem cell numbers and enhanced muscle regeneration.

## Stem cells that renew repair cells

- A new type of skeletal-muscle stem cell in mice that appears to be particularly suited to repairing damaged muscle tissue.
- These cells are the **precursors of special muscle repair cells called “satellite cells.”** Satellite cells reside near muscle fibers and move into them to compensate for damage when necessary.
- The newly isolated cells are a subset of the muscle precursors known as muscle SP (“side population”) cells.

## Muscle SP (“side population”) cells.

- SP cells are “stemlike” in their ability to give rise to mature muscle fibers.
- Injection of satellite SP cells into leg muscles in mice lacking dystrophin: Extensive muscle regeneration and replenishment of dystrophin.
- These cells are presumably poised to conduct repair operations when needed and can replenish the satellite cells as well as repair muscle.

## New Muscle stem cell found in mice (PICs)



New type of muscle stem cell that appears capable of generating and repairing muscle and reproducing itself. PICs can become muscle, muscle progenitors or more PICs.

## PICs

- The PICs generated many additional PICs, prompting researchers to term this new cell type a "highly self-renewing stem cell. "
- If PICs also exist and play a similar role in humans, they could become the targets of small-molecule-based therapies or used for cell transplantation in the treatment of muscular dystrophies.



Thank you for your kind attention