PASSION ACADEMIC TEAM YU - MEDICINE

# MUSCULOSKELETAL SYSTEM

## Sheet#2 ( Part 2 ) - Biochemistry

## Lec. Title : Discuss the markers for bone formation and resorption

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If you come by any mistake , please kindly report it to shaghafbatch@gmail.com

Discuss the markers for bone formation and Resorption and their clinical use in diagnosis Describe the molecular basis of:

1. Duchene Muscular Dystrophy.

(It maybe diagnosed either by phenotype or markers.)

2. Glycogen storage diseases of muscle.

3. Muscle Mitochondrial diseases.

(mitochondrial diseases may relate to nervous disorders also)

4. Describe the molecular basis of:

Osteogenesis imperfecta and Ehlar Danlos syndromes

Ehlar: stretchy skin.

# Regarding to the previous slide

- When we say markers we mean that we use the clinical biochemistry to diagnose a disorder depending on detection of certain enzymes and molecules in serum and other specimen.

- First of all we make a clinical assessment then we have two ways for conformation of the disease :

1. Using the clinical biochemistry(markers).
2. Genetic investigation: extraction of DNA and analyze it.

Bone is a dynamic structure : we have mineralization and Resorption.
If there is an imbalance in the pathway an disorder will develop.

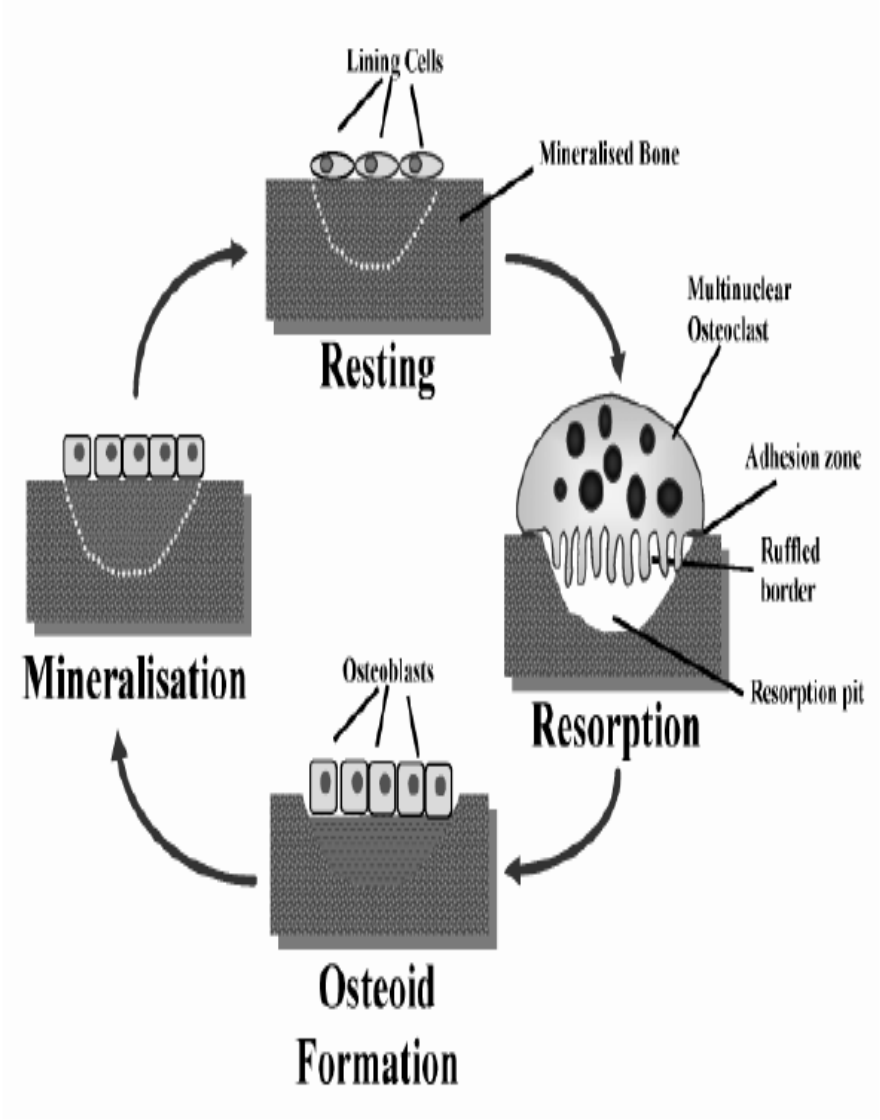



Figure 1. The bone remodelling cycle. Under normal conditions, the resorption (osteoclast) phase takes approximately 10 days, which is then followed by a formation

*Clin Biochem Rev Vol 26 November 2005*

# Biochemical Markers of Bone Turnover

The currently available markers of bone turnover include both enzymes and non-enzymatic peptides derived from cellular and non-cellular compartments of bone, they are usually classified according to the metabolic process they are considered to reflect.

**Markers of bone resorption are related to:**

- Collagen breakdown products such as hydroxyproline or the various collagen cross-links and telopeptides.

Other markers of bone resorption include non-collagenous matrix proteins such as **bone sialoprotein** (BSP), or osteoclast-specific enzymes like **tartrate-resistant acid phosphatase or cathepsin K.**

- also you can see high activity of certain enzymes like alkaline phosphates enzyme.
- BSP is acidic phosphoprotein that is responsible for mineralization.
- don't worry if you don't understand these two points , there is an explanation later on.

**Markers of bone formation are either by-products of collagen neosynthesis:**

(e.g. propeptides of type I collagen), or osteoblast-related proteins such as **osteocalcin** (OC) and **alkaline phosphatase (ALP).**

Bone acid phosphatase

Alpha chains that forms collagen

Fragments of collagen that located at the endings of the bone you will see them due to degradation (telo means at the end of something)

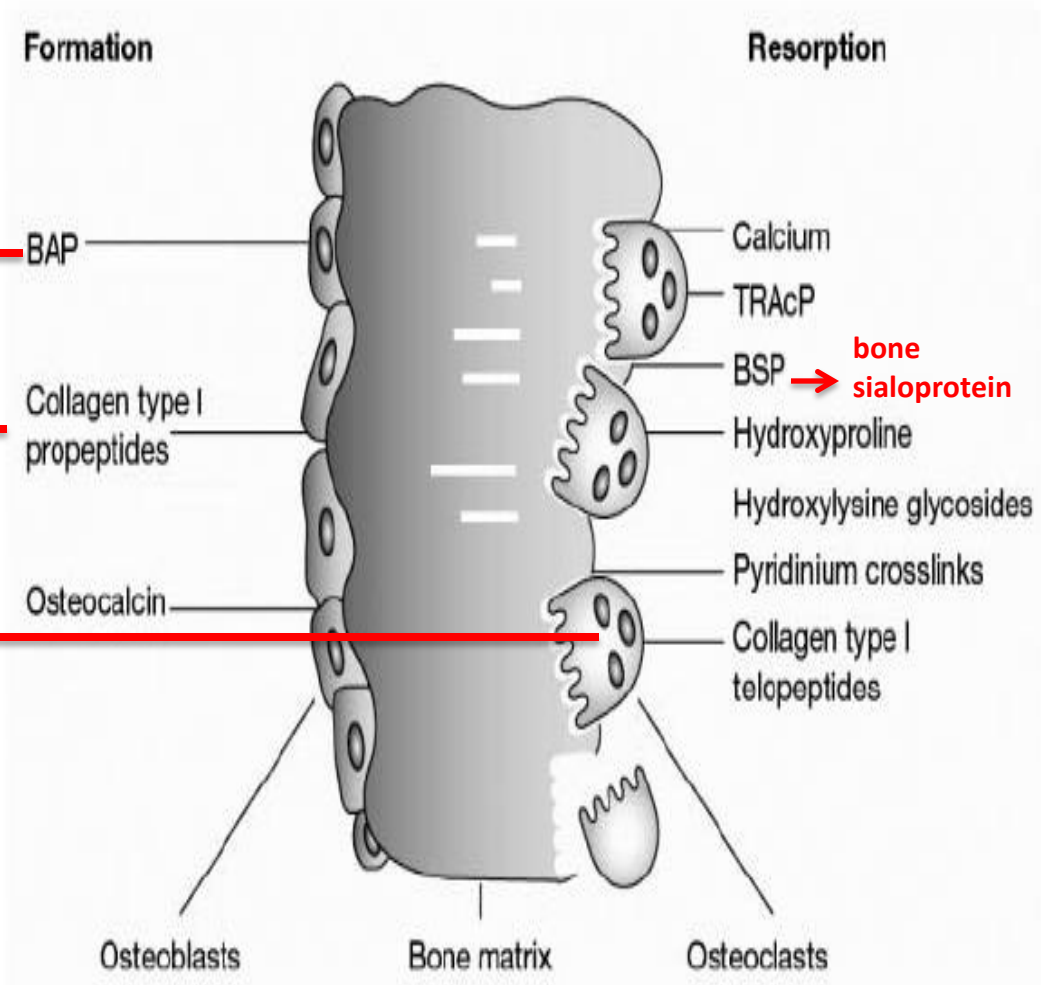



Figure 2. Biochemical markers of bone remodelling.

# Bone formation and bone resorption markers: (e.g. hydroxyproline, certain OC fragments).

- we could have the same markers for both processes because if you have resorption, it means that you need to compensate this problem by replacement of bone with new contents→ bone formation.

- In other word ,if you activate resorption ,sometime you could activate formation or deposition of the bone itself.

- so, we have some kind of overlapping markers.

- we can know if OC is related to resorption or formation by clinical assessment

Table 1. Markers of bone turnover.

المطلوب معرفته هون هو: أول عمود شو الأشياء الي specific for the bone and non specific ones العمود التاني انه كلهم من ال serum العمود التالت
المطلوب معرفته انه بنستخدم تقنية ال immune essay الهم كلهم خصوصًا ال ELISA... شرح طريقة ال immune essay بالسلايد الي بعد هاد

| Marker | Tissue of Origin | Specimen | Analytical Method | Remarks |
|---|---|---|---|---|
| **Markers of bone formation** | | | | الدكتور حكى انه هاد العمود مو مهم نعرفه هلا بس رح يكون مهم بمرحلة الكلينيكال |
| **Bone-specific alkaline phosphatase (BAP, bone ALP)** | Bone | Serum | Electrophoresis, Precipitation, IRMA, EIA | Specific product of osteoblasts. Some assays show up to 20% cross-reactivity with the liver isoenzyme (LAP) |
| **Osteocalcin (OC)** | Bone, platelets | Serum | RIA, IRMA, ELISA | Specific product of osteoblasts; many immunoreactive forms in blood; some may be derived from bone resorption. |
| **C-terminal propeptide of type I procollagen (PICP)** | Bone, soft tissue, skin | Serum | RIA, ELISA | Specific product of proliferating osteoblasts and fibroblasts. |
| **N-terminal propeptide of type I procollagen (PINP)** | Bone, soft tissue, skin | Serum | RIA, ELISA | Specific product of proliferating osteoblast and fibroblasts; partly incorporated into bone extracellular matrix. |

Bone acidic protein

Alkaline phosphatase protein

# ELISA technique (from recording)

- In this technique we will use an enzyme linked antibodies against the marker (the protein) which will be the antigen here. Also, we will add a substrate for the linked enzyme.

- When the antibody attaches to the antigen the enzyme will be activated and it will work on the substrate to change the color into different one, the intensity of the color indicates the amount of protein that present on the blood.

- Sometimes you can find a normally elevated amount due to physiological conditions as in children and pregnant women

**Markers of bone resorption**

ال3 سلايدات هدول حسب كلام الدكتور انه أول 3 أعمدة مطلوبة قرأ أسماء ال markers ع السريع و الأشياء الي ركز عليها بالشرح مشار عليها بالأحمر

| | | | | |
|---|---|---|---|---|
| **Collagen-related markers** | **Not highly specific for the bone** | **We use urine rather than serum (Some times serum)** | **We use HPLC and ELIZA techniques** | |
| **Hydroxyproline, total and dialysable** (Hyp) | Bone, cartilage, soft tissue, skin | Urine | Colorimetry HPLC | Present in all fibrillar collagens and partly collagenous proteins, including C1q and elastin. Present in newly synthesised and mature collagen, i.e. both collagen synthesis and tissue breakdown contribute to urinary hydroxyproline. |
| **Hydroxylysine-glycosides** | Bone, soft tissue, skin, serum complement | Urine (serum) | HPLC ELISA | Hydroxylysine in collagen is glycosylated to varying degrees, depending on tissue type. Glycosylgalactosyl-OHLys in high proportion in collagens of soft tissues, and C1q; Galyctosyl-OHLys in high proportion in skeletal collagens. |

| | | | | |
|---|---|---|---|---|
| **Pyridinoline** (PYD) | Bone, cartilage, tendon, blood vessels | Urine<br>Serum | HPLC<br>ELISA | Collagens, with highest concentrations in cartilage and bone; absent from skin; present in mature collagen only. |
| **Deoxypyridinoline** (DPD) | Bone,<br>Dentin | Urine<br>Serum | HPLC<br>ELISA | Collagens, with highest concentration in bone; absent from cartilage or skin; present in mature collagen only. |
| **Carboxyterminal cross-linked telopeptide of type I collagen** (ICTP, CTX-MMP) | Bone,<br>Skin | Serum | RIA | Collagen type I, with highest contribution probably from bone; may be derived from newly synthesised collagen. |
| **Carboxyterminal cross-linked telopeptide of type I collagen** (CTX-I) | All tissues containing type I collagen | Urine<br>(a-/ ß)<br>Serum<br>(ß only) | ELISA<br>RIA | Collagen type I, with highest contribution probably from bone. Isomerisation of aspartyl to ß-aspartyl occurs with ageing of collagen molecule. |
| **Aminoterminal cross-linked telopeptide of type I collagen** (NTX-I) | All tissues containing type I collagen | Urine<br>Serum | ELISA<br>CLIA<br>RIA | Collagen type I, with highest contribution from bone. |
| **Collagen I alpha 1 helicoidal peptide (HELP)** | All tissues containing type I collagen | Urine | ELISA | Degradation fragment derived from the helical part of type I collagen (alpha-1 chain, AA 620-633). Correlates highly with other markers of collagen degradation, no specific advantage or difference in regards to clinical outcomes. |

**- In the last 4 markers you have to know that the degradation will be for type 1 collagen (the most abundant collagen type in the bone)**
**- And that these markers are not related only to bone it maybe related to dentine for example.**

## Non-Collagenous Proteins

| | | | | |
|---|---|---|---|---|
| **Bone Sialoprotein** (BSP) | Bone, Dentin, hypertrophic cartilage | Serum | RIA ELISA | Acidic, phosphorylated glycoprotein, synthesised by osteoblasts and osteoclastic-like cells, laid down in bone extracellular matrix. Appears to be associated with osteoclast function. |
| **Osteocalcin fragments** (ufOC, U-Mid-OC, U-LongOC) | Bone **specific** | Urine | ELISA | Certain age-modified OC fragments are released during osteoclastic bone resorption and may be considered an index of bone resorption. |

## Osteoclast Enzymes

| | | | | |
|---|---|---|---|---|
| **Tartrate-resistant acid phosphatase** (TRAcP) | Bone<br>Blood | Plasma<br>Serum | Colorimetry<br>RIA<br>ELISA | Six isoenzymes found in human tissues (osteoclasts, platelets, erythrocytes). Band 5b predominant in bone (osteoclasts). |
| **Cathepsins** (e.g. K, L) | K: Primarily in osteoclasts<br><br>L: Macrophage, Osteoclasts | Plasma, Serum | ELISA | Cathepsin K, a cysteine protease, plays an essential role in osteoclast-mediated bone matrix degradation by cleaving helical and telopeptide regions of collagen type I. Cathepsin K and L cleave the loop domain of TRAP and activate the latent enzyme. Cathepsin L has a similar function in macrophages.<br>Tests for measurement of Cathepsins in blood are presently under evaluation. |

# Markers of Bone Formation (the most important markers)

**Serum Total Alkaline Phosphatase (AP)**: membrane-bound tetrameric enzyme attached to glycosyl-phosphatidylinositol moieties located on the outer cell surface

**Osteocalcin (OC):** OC is a 5.8 kDa, hydroxyapatite-binding, protein exclusively synthesised by osteoblast, odontoblasts and hypertrophic chondrocytes

**Procollagen Type I Propeptides:** The procollagen type I propeptides are derived from collagen type I, the most abundant form of collagen found in bone.

# Markers of Bone Resorption

**Hydroxyproline (OHP):**

Is formed intracellularly from the post-translational hydroxylation of proline and constitutes 12-14% of the total amino acid content of mature collagen. Ninety percent of the OHP liberated during the degradation of bone collagen is primarily metabolised in the liver.

**Hydroxylysine-Glycosides**: arise during the posttranslational phase of collagen synthesis and occur in two forms, glycosyl-galactosyl-hydroxylysine (GGHL) and galactosyl-hydroxylysine (GHL).
Both components are released into the circulation during collagen degradation

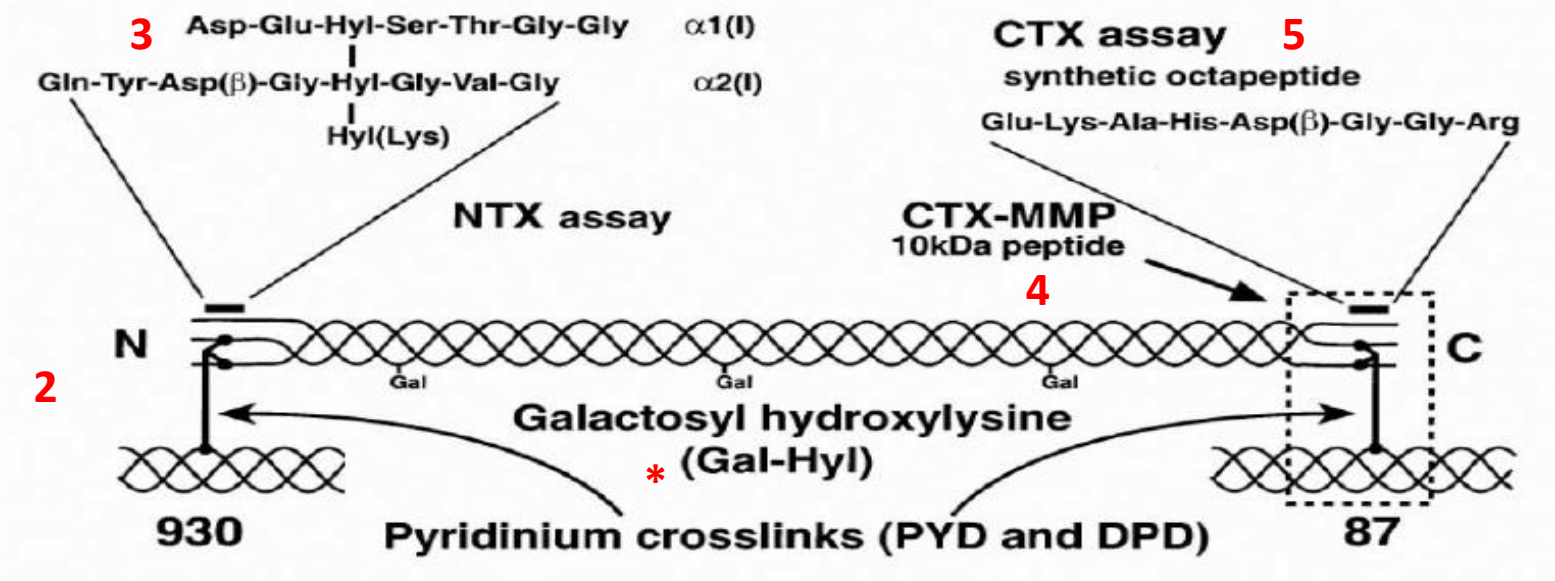



**Figure 5.** Molecular basis of currently used markers of type I collagen-related degradation. For more details, see text and Table 1. Figure courtesy Dr Simon Robins, Aberdeen.

This photo discuss that if collagen type I is degraded what are the fragments that will be produced for example:

1. c-terminal fragment
2. n-terminal fragment

*** Called pyridinium crosslinks markers**

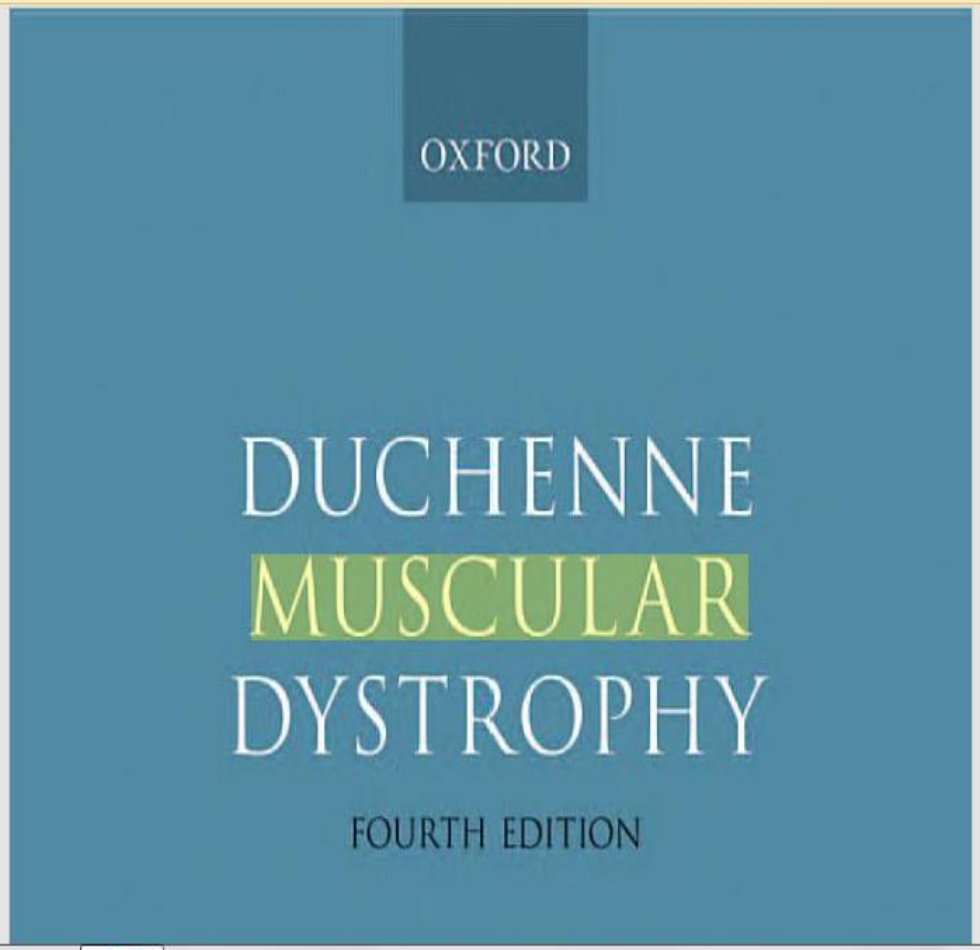
3. NTX fragment that will be detected by NTX assay.
4. CTX-MMP that was produced by metaloprotease enzyme.
5. synthetic octapeptide that will be detected by CTX assay

## 3-Hydroxypyridinium Crosslinks of Collagen Pyridinoline (PYD) and Deoxypyridinoline (DPD):

PYD and DPD are formed during the extracellular maturation of fibrillar collagens

**Crosslinked Telopeptides of Type I Collagen The:**

Crosslinked telopeptides of type I collagen are derived from specific regions of the collagen type I molecule, namely the amino terminal (NTP) and the carboxyterminal (CTP) telopeptide (Figure 5).



**Duchenne muscular dystrophy (DMD):**

Genetic disorder characterized by progressive muscle degeneration and weakness.

Emotionally, it's very bad disease.

There are nine types of *muscular dystrophy*.

Caused by an absence of ***dystrophin.***

Early childhood onset

X-linked

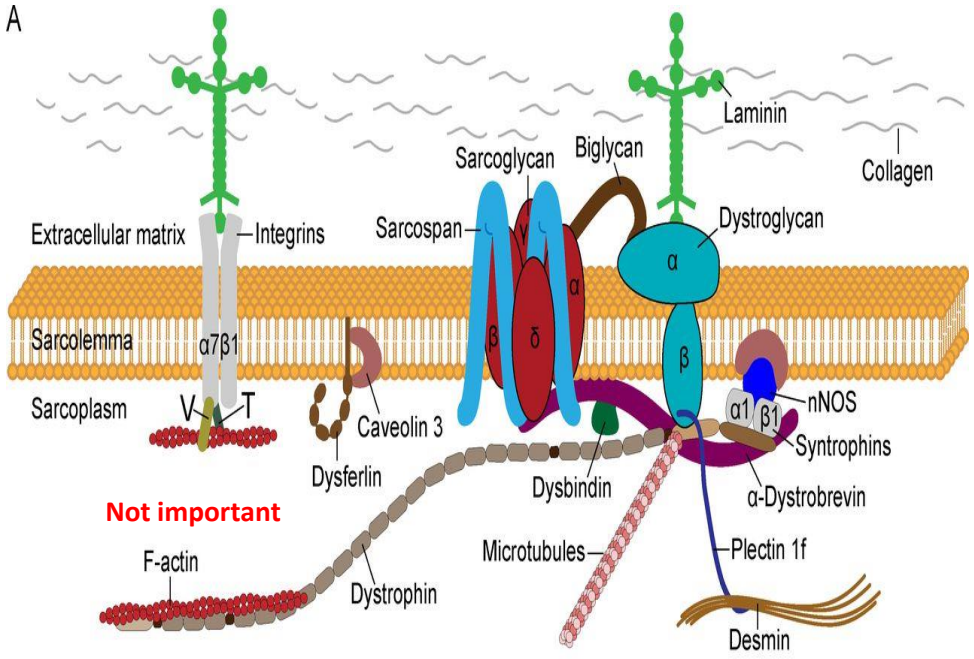
Prevalence 1:3,600 male infants.(very high)

## Sarcolemmal proteins and sarcomere structure.

- About slides 20,21,22:

❑ Dystrophin is an important protein for muscles integrity so the deficiency of it will affect the connection between the muscles.

❑ In the next two slides the doctor didn't discuss the cases but he said that you can see the phenotype of the patients that results from muscle destruction .




**Fedik Rahimov, and Louis M. Kunkel J Cell Biol 2013;201:499-510**



JCB

# Duchenne muscular dystrophy

Vineet Behera,[1] Manas Kumar Behera,[2] Rajeev Chauhan,[1] Velu Nair[1]

## DESCRIPTION

A 15-year-old boy presented with progressive proximal weakness of the lower limbs starting at 4 years of age followed by involvement of the upper limbs. He is the product of a consanguineous marriage; he had a family history of similar disease in a second-degree cousin and also had a history of delayed motor developmental milestones since birth. Clinically, he had flaccid quadriparesis with wasting and contractures without any sensory or neurological involvement. His weakness worsened leading to an inability to walk without support by the age of 9 and total wheelchair dependence by the age of 12. He was frequently admitted to hospital with chest infections.

The patient's creatine kinase was 2600 IU/L (normal 50–150 IU/L) and muscle biopsy from left quadriceps showed rounded small muscle fibres with evidence of degeneration and an absence of dystrophin protein. He was diagnosed as a case of duchenne muscular dystrophy. He is presently bed bound with weakness and contractures of all limbs and spinal deformities as shown in figure 1. He developed scoliosis at the age of 12 which has grad-

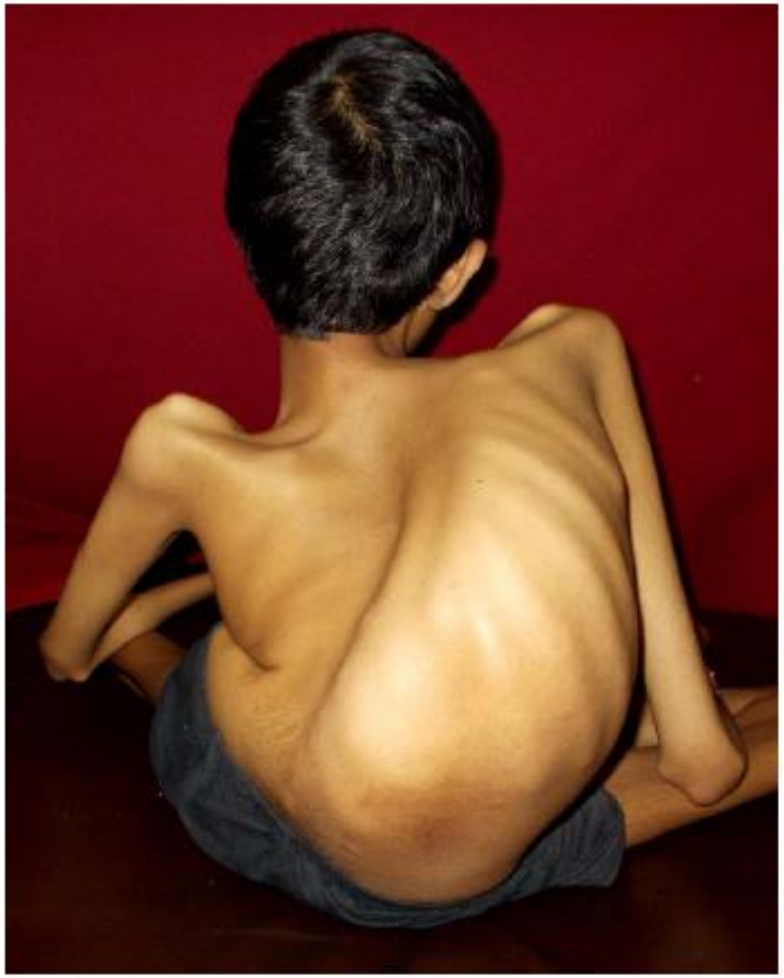


**Figure 3** Back view.

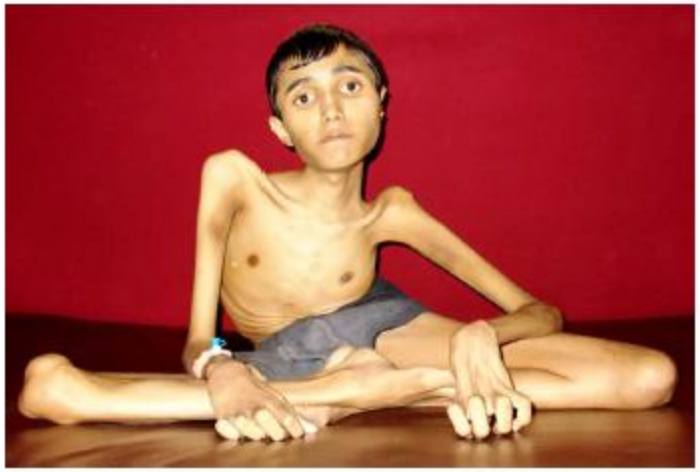


**Figure 1** Duchenne muscular dystrophy.

**Figure 2** Lateral view.

aggressive management of respiratory infections, periodical cardiac and respiratory follow-up, genetic counselling and other supportive therapies.

## Learning points

- Duchenne muscle dystrophy is a progressive inherited myopathy with an early onset in childhood.[1]
- It progresses to the bed-bound state in the second decade of life and patients usually succumb to respiratory or cardiac complications.
- Conservative management, active physiotherapy, genetic counselling and other supportive therapies hold the key to successful management of these cases.[2]

**Contributors** All authors have contributed to the manuscript.

**Competing interests** None.

**Patient consent** Obtained.

**Provenance and peer review** Not commissioned; externally peer reviewed.

## REFERENCES

1 Bushby K, Finkel R, Birnkrant DJ, *et al*. Diagnosis and management of duchenne muscular dystrophy: diagnosis, and pharmacological and psychosocial management. *Lancet Neurol* 2010;9:77–93.
2 Bushby K, Bourkeb J, Bullock R, *et al*. The multidisciplinary management of duchenne muscular dystrophy. *Curr Paediatr* 2005;15:292–300.

ORIGINAL ARTICLE

# Deletion mutations in Duchenne muscular dystrophy (DMD) in Western Saudi children

Mohammed T. Tayeb

**Table 1** Exon deletion frequencies in 15 unrelated DMD Saudi patients.

| Exon(s) deleted | No. of deletions | Deletion frequency (%) |
|---|---|---|
| Exon 19 | 1 | 6.7 |
| Exon 45 | 1 | 6.7 |
| Exon 48 | 1 | 6.7 |
| Exon 51 | 3 | 20.0 |
| Total deletions | 6 | 40 |

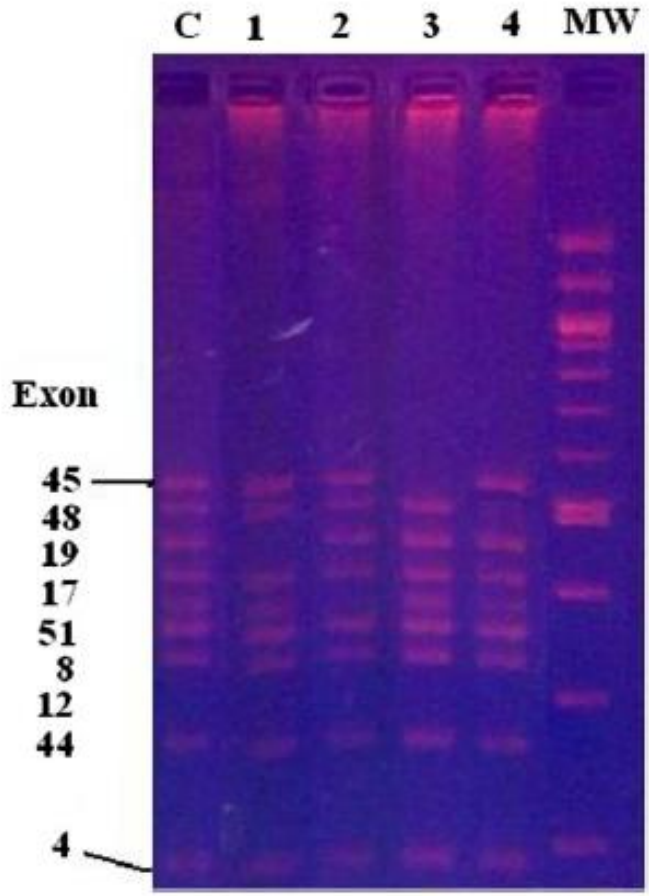



**Figure 1** Multiplex PCR of the human *DMD* gene electrophoresed on a 3% NuSieve gel-ethidium dye. 'C' represented 'normal control male' with no deletions, MW is size marker (100 bp ladder). Lanes 1, 2, 3, and 4 showed missing of exon 19, 51, 45 and 48, respectively.

A study on 15 patient with DMD with many deletions which means dystrophin gene is a big gene with many exons

# Regarding to the previous slide

Here we have a trial on 4 patients with DMD:

- C : the control sample.

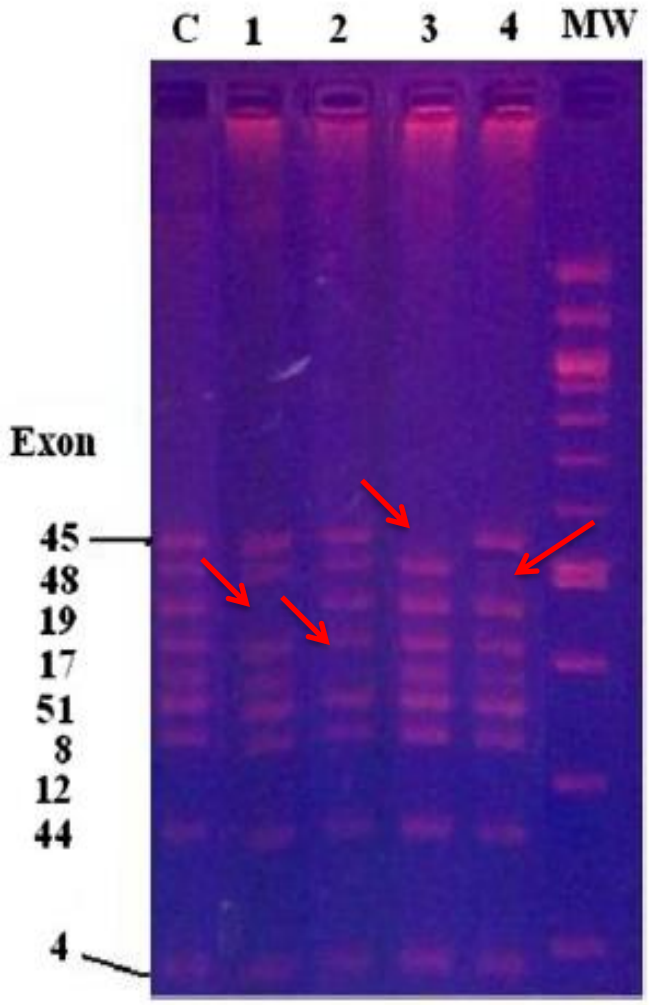
- MW : molecular marker(a fragments of DNA we put it in the gel in order to know the molecular weight (the size the DNA)).

-In patient 1 exon 19 is  absent.

-In patient 2 exon 51 is absent

- in Patient 3 exon 45 is absent

-In Patient 4 exon 48 is absent.

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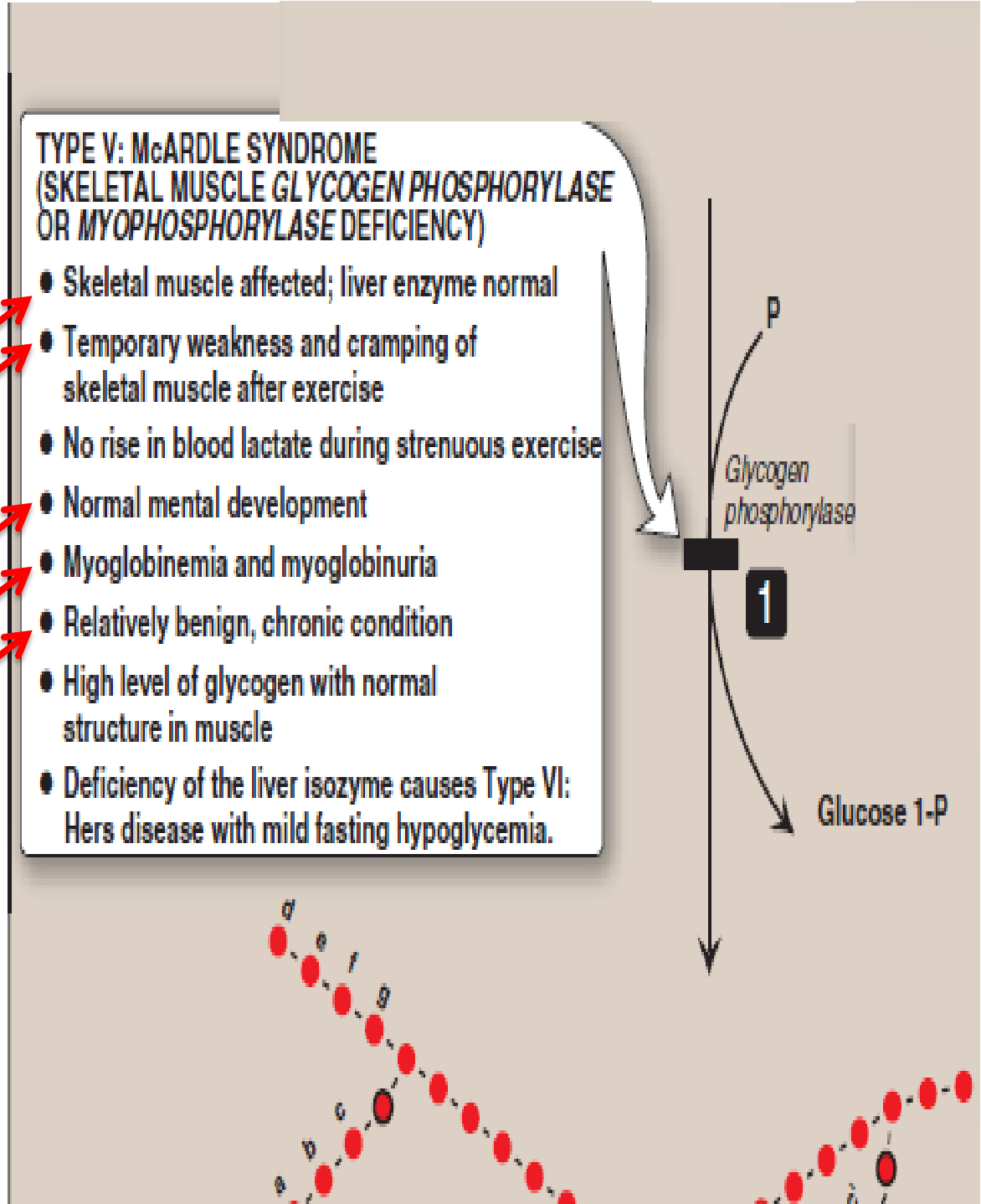
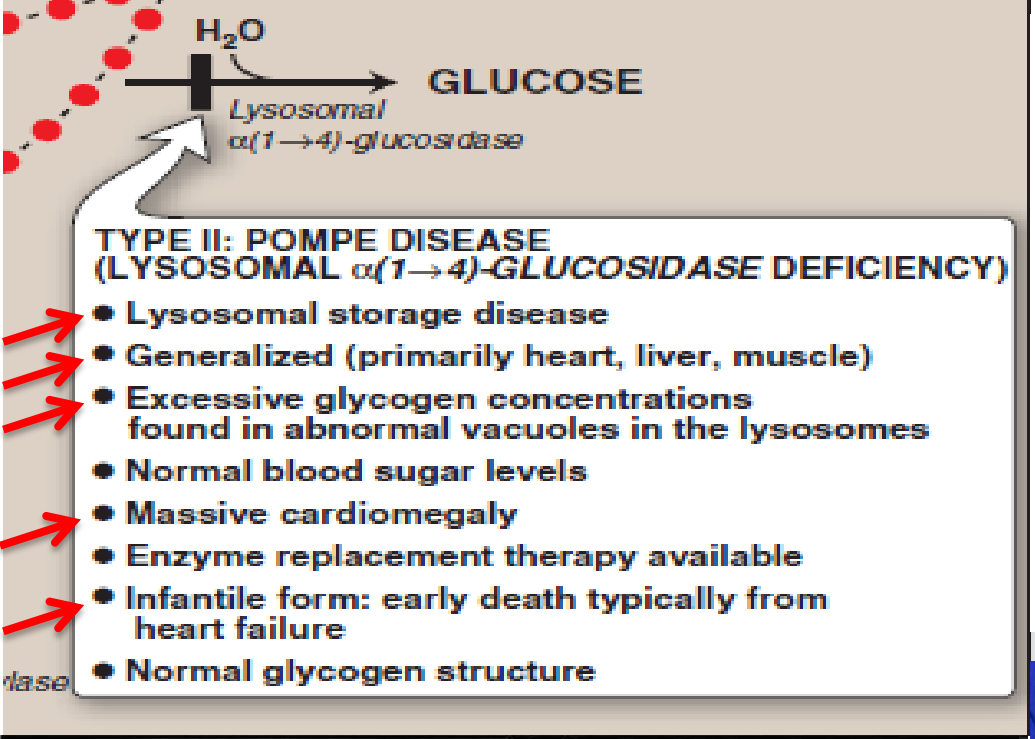
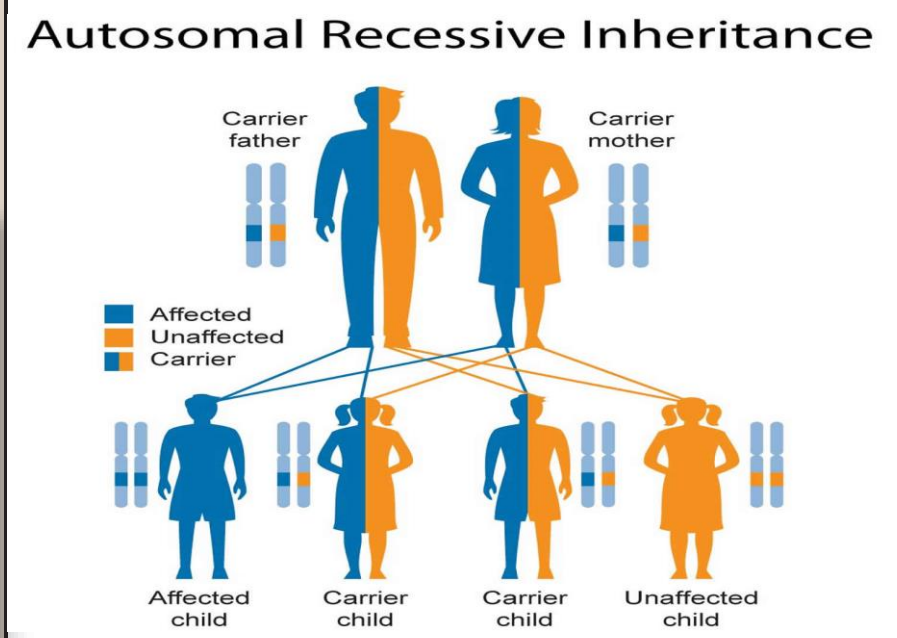
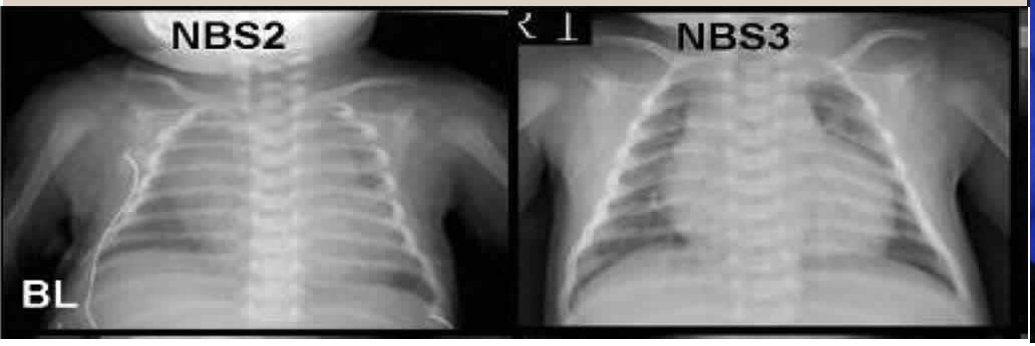
Types of glycogen disorders that causes muscle abnormality are:

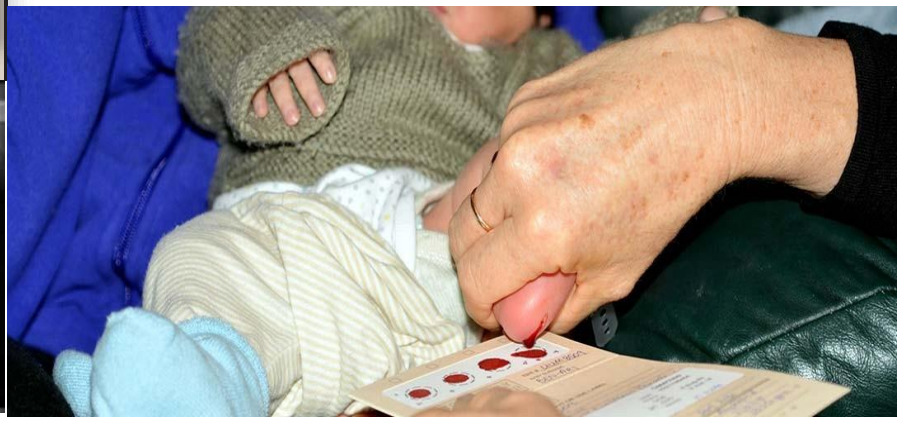
1. McARDLE syndrome: which caused by glycogen phosphorylase deficiency which is the enzyme that is responsible for glycogenolysis , this deficiency will store glycogen in muscles and prevent glucose production and may lead to hypoglycemia.



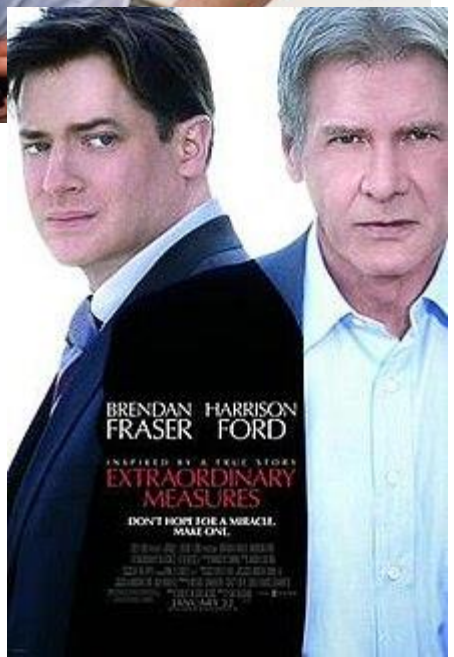


2. Pompe disease: type 2 glycogen storage disorder due to abnormality in lysosomal enzymes that don't break down glycogen in the muscles , this disease can be treated by enzymatic replacement.


BRENDAN FRASER
HARRISON FORD
INSPIRED BY A TRUE STORY
EXTRAORDINARY MEASURES
DON'T HOPE FOR A MIRACLE. MAKE ONE.

1. **Ehlers-Danlos syndrome (EDS):** This disorder is a heterogeneous group of generalized connective tissue disorders that result from inheritable defects in the metabolism of fibrillar collagen molecules. EDS can result from a deficiency of collagen-processing enzymes (for example, *lysyl hydroxylase* or *procollagen peptidase*), or from mutations in the amino acid sequences of collagen types I, III, or V. The most clinically important mutations are found in the gene for type III collagen. Collagen containing mutant chains is not secreted, and is either degraded or accumulated to high levels in intracellular compartments. Because collagen type III is an important component of the arteries, potentially lethal vascular problems occur. [Note: Although collagen type III is only a minor component of the collagen fibrils in the skin, patients with EDS also show, for unknown reasons, defects in collagen type I fibrils. This results in fragile, stretchy skin and loose joints (Figure 4.10).]

Deficiency with enzymes that are responsible for collagen processing or by mutations in collagen types.

**Figure 4.10**
Stretchy skin of Ehlers-Danlos syndrome.

***Very important

**2. Osteogenesis imperfecta (OI):** This disease, known as brittle bone syndrome, is also a heterogeneous group of inherited disorders distinguished by bones that easily bend and fracture (Figure 4.11). Retarded wound healing and a rotated and twisted spine leading to a "humped-back" (kyphotic) appearance are common features of the disease. Type I OI is called osteogenesis imperfecta tarda. The disease is the consequence of decreased production of α1 and α2 chains. It presents in early infancy with fractures secondary to minor trauma, and may be suspected if prenatal ultrasound detects bowing or fractures of long bones. Type II OI is called osteogenesis imperfecta congenita, and is the most severe. Patients die of pulmonary hypoplasia in utero or during the neonatal period. Most patients with severe OI have mutations in the gene for either the pro-α1 or pro-α2 chains of type I collagen. The most common mutations cause the replacement of glycine residues (in –Gly–X–Y–) by amino acids with bulky side chains. The resultant structurally abnormal pro-α chains prevent the formation of the required triple-helical conformation.

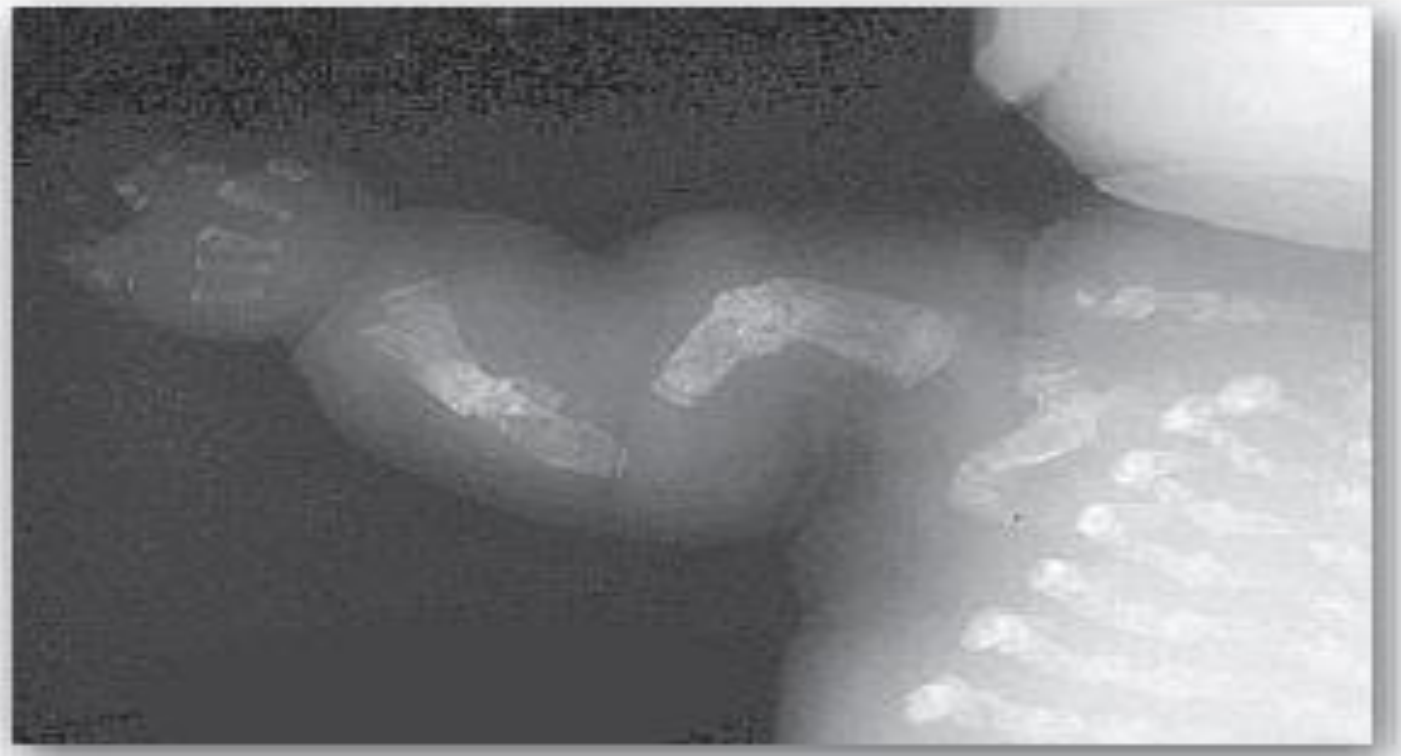


**Figure 4.11**
Lethal form (type II) of osteogenesis imperfecta in which the fractures appear in utero, as revealed by this radiograph of a stillborn fetus.

# Mitochondrial Myopathy

Group of neuromuscular diseases caused by damage to the mitochondria.

Most mitochondrial myopathies occur before the age of 20, and often begin with exercise intolerance or muscle weakness.

# Types of Mitochondrial Myopathies

- Kearns-Sayre syndrome (KSS)
- Leigh syndrome and maternally-inherited Leigh syndrome (MILS)
- Mitochondrial DNA depletion syndrome (MDS)
- Mitochondrial encephalomyopathy, lactic acidosis and strokelike episodes (MELAS)
- Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE)
- Myoclonus epilepsy with ragged red fibers (MERRF)
- Neuropathy, ataxia and retinitis pigmentosa (NARP)
- Pearson syndrome
- Progressive external ophthalmoplegia (PEO)