Limb-girdle muscular dysthrophies with special review on calpainopathies

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Limb-girdle muscular dystrophy is a term for a group of diseases that cause weakness and wasting of muscles of the upper and lower extremities. Most affected are muscles closer to the trunk (proximal muscles), especially muscles of the shoulder girdle, upper arms, pelvic area, and thighs.

- **1884 Erb**
- **in 1954 Walton i Nattrass proposed LGMD as a nosological entity**
- **Definition includes the folowing criteria:**

- **Expressed in both sexes**
- **Onset in first or second decade of life**
- **Usually autosomal recessive, less frequently autosomal dominant inheritance**
- **Involment of shoulder and pelvic girdle muscles with variable rates of progression**
- **Severe disability within 20-30 years of life**
- **Muscular pseudohyperthrophy and contracture are uncommon**

- **Genetically very heterogeneous diseases with relatively similar phenotypic characteristics**
- **1995 Bushby and Beckmann introduced the current classification based on clinical and molecular characteristics:**
- **Type 1 - autosomal dominant**
- **Type 2 - autosomal recessive**

Type	Gene location	Protein	Inheritance
LGMD 1A	5q22-q31	Myotilin	AD
LGMD 1B	1q11-q21	Lamin A/C	“
LGMD 1C	3p25	Caveolin-3	“
LGMD 1D	6q23	‘	“
LGMD 1E	7q	‘	“
LGMD 2A	15q15-q21	Calpain-3	AR
LGMD 2B	2p13	Dyspherlin	“
LGMD 2C	13q12	α-sarcoglycan	“
LGMD 2D	17q12-q21	β- “	“
LGMD 2E	4q12	γ-”	“
LGMD 2F	5q33-q34	δ-”	“
LGMD 2G	17q11-q12	Telethonin	“
LGMD 2H	9q31-q34	TRIM32	“
LGMD 2I	19q13.3	FKRP	“
LGMD 2J	2q24.3	Titin	“

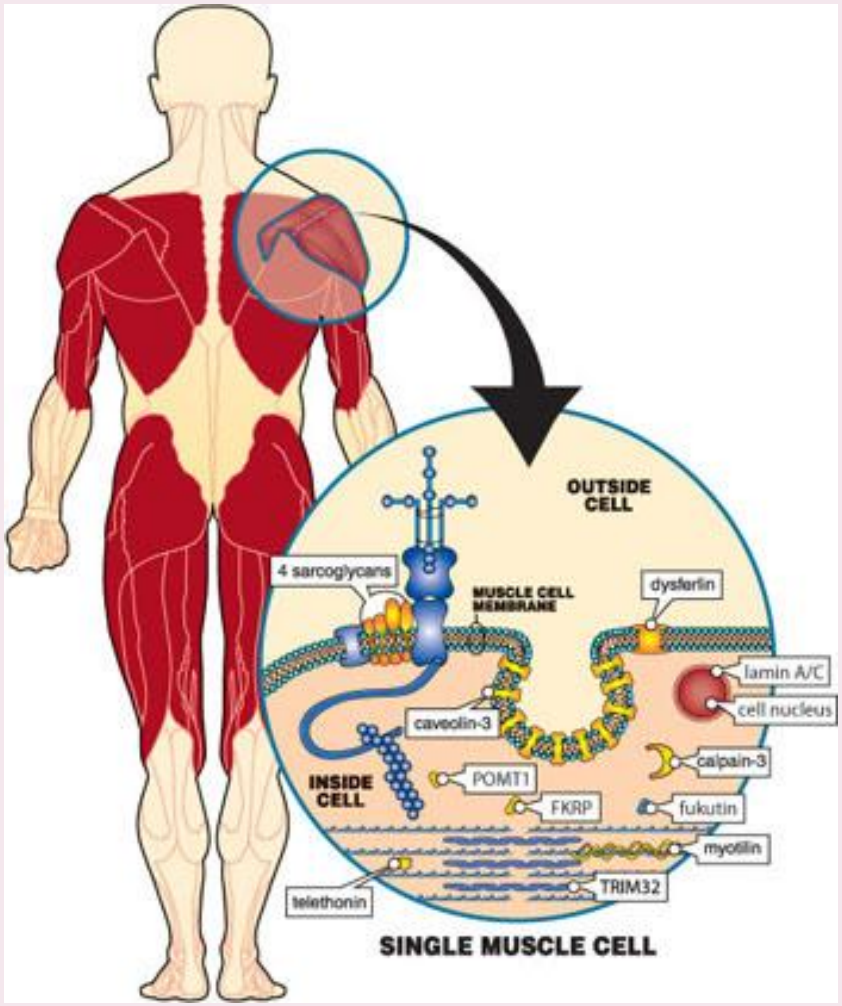
- **90% autosomal recessive**
- **10% autosomal dominat**
- **Some sporadic cases**
- **The frequency of certain types varies in different populations and ethnic groups.**
- **The widespread types are: LGMD 2A (calpainopathy), LGMD 2B (dysferlinopathy), LGMD 2C-F (sarcoglicanopathies)**

Autosomal dominant LGMD

- **Up to 10% of LGMDs**
- **Occur later in life**
- **Mild clinical picture and slow progression**
- **LGMD1A myotilinopathy**
  LGMD1B laminopathy A/C
  LGMD1A caveolinopathy-3

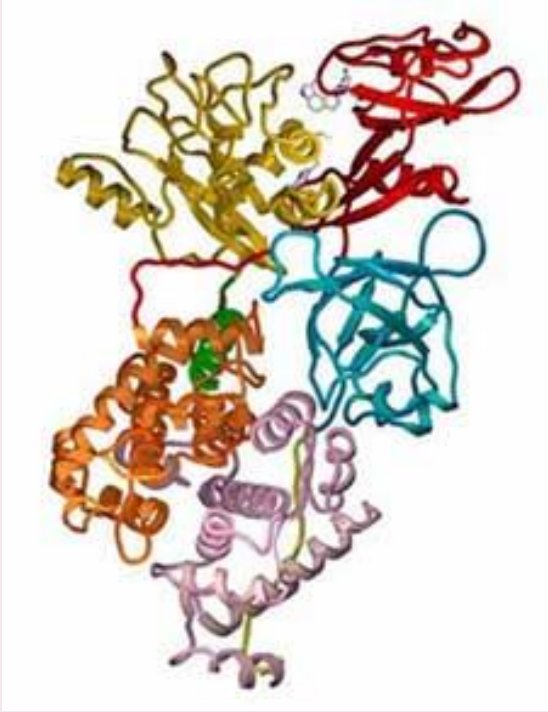
Pathophysiology

- **Gene mutation causes a defective protein coding with subsequent dysfunction of muscle cells**
- **Proteins are located in the sarcolemma associated with the contractile apparatus, or they are constituents of cellular enzymes of muscle cells**



LGMD 2A

- **Calpain 3 – is a calcium-dependent, neutral cysteine protease. Located almost exclusively in skeletal muscle. Calpain 3 consists of 821 amino acids of molecular weight 94K. It is associated with connectin (or titin), which is a basic component of the sarcomere.**



Clinical presentation LGMD 2A

- **It occurs between 3-30 years of age, usually between the age 10-20, slightly more common in men (3:1).**
- **The course is progressive.**
- **<u>I stage:</u> difficulties in running, clinical findings: weakness of m. gluteus max. and thigh muscles, while the m. quadriceps femoris is preserved for a long time.**
- **Mild hyperlordosis with weakness of the abdominal muscles**

Clinical presentation LGMD 2A

- **II stage: inability of climbing stairs, getting up from a squat, weakness of periscapular muscles**
- **III stage: weakness affects m. quadriceps femoris and distal muscle groups. Inability of walking at the age of 30.**
  Arm muscles, especially the hands, and facial muscles are spared for a long time.

Clinical presentation LGMD 2A

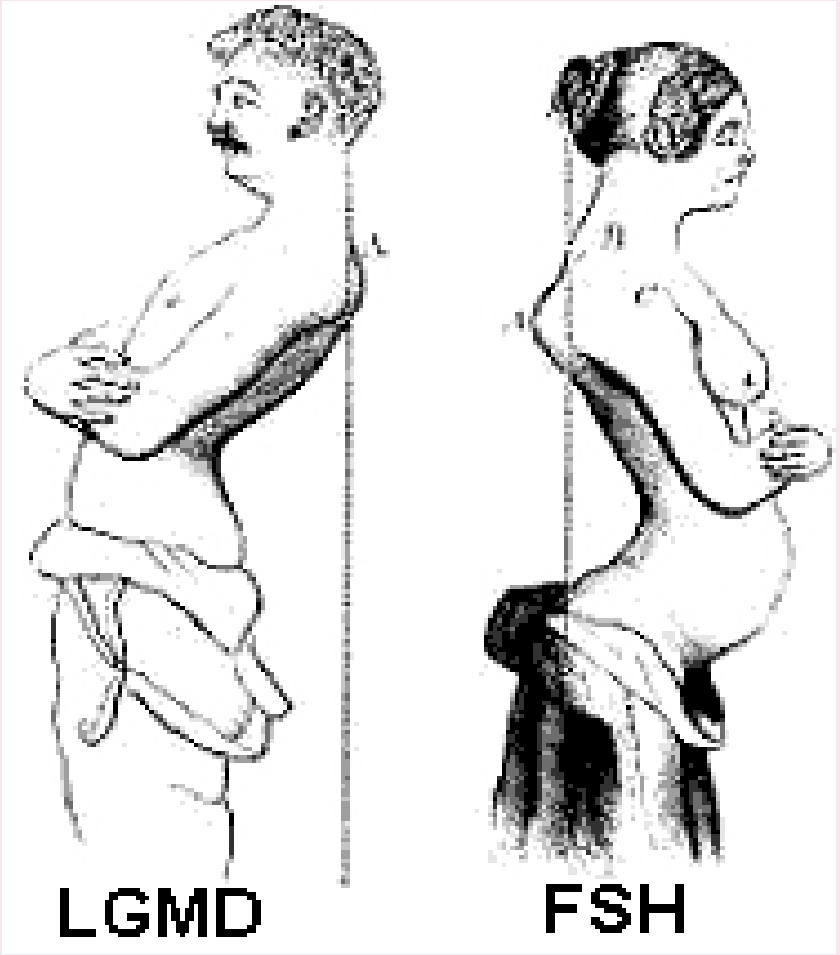
- **Frequent development of hip, knee and elbow contractures**
- **The heart muscle is preserved**
- **Intellectual development is preserved**

Differential Diagnosis

- **LGMD type 2C (sarcoglikanopathy) - similar to DMD, muscle pain and kramps**
- **LGMD type B (dysferlinopathy) – weaknes of distal muscle groups**

Differential Diagnosis

- **Facioscapulohumeral MD - autosomal dominant, pronounced weakness of facial muscles, asymmetric development of muscular weakness, slower progression**



Laboratory Finndigns LGMD 2A

- **CK increased up to ten times**
- **EMG: typical myopathic pattern in the affected muscles. Neurography: normal. ECG and ultrasound of the heart are regular.**
- **MRI of affected muscles**
- **Muscle biopsy: signs of chronic myopathy, not specific in relation to other types of dystrophy**
- **Immunohistochemical methods: deficiency of calpain 3**

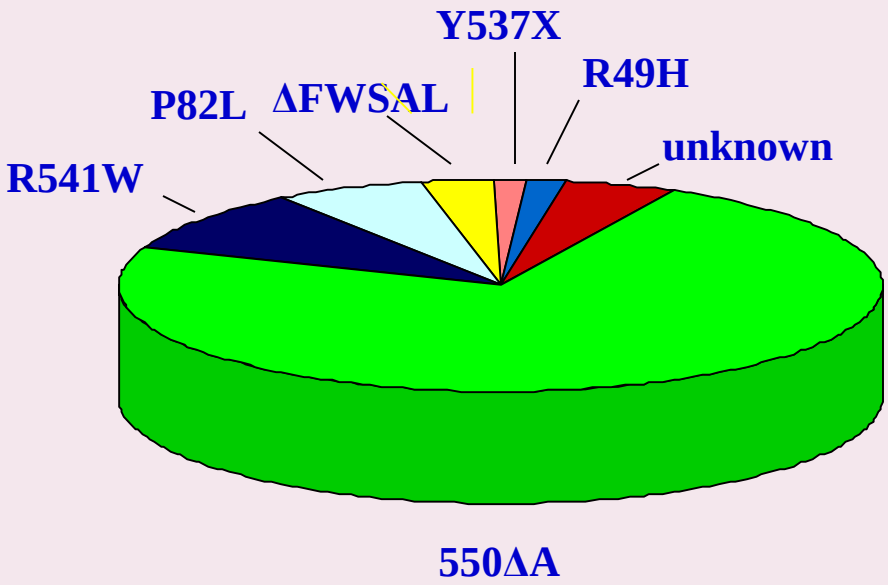
Molecular Analysis LGMD 2A

- **Analysis of CAPN3 gene**
- **Difficulties due to the large number of mutations (280)**
- **Determination of the type of mutations characteristic for a specific population would be useful**

CAPN3 gene analysis

CAPN3 genetic analysis of 32 families from Croatian LGMD2A resulted in the discovery of six CAPN3 mutations

MUTATION	Nmb. CAPN3 chromosome	Frequency
550ΔA	47/64	73,4%
R541W	6/64	9,4%
P82L	4/64	6,3%
ΔFWSAL	2/64	3,1%
Y537X	1/64	1,6%
R49H	1/64	1,6%
Unknown	3/64	4,7%

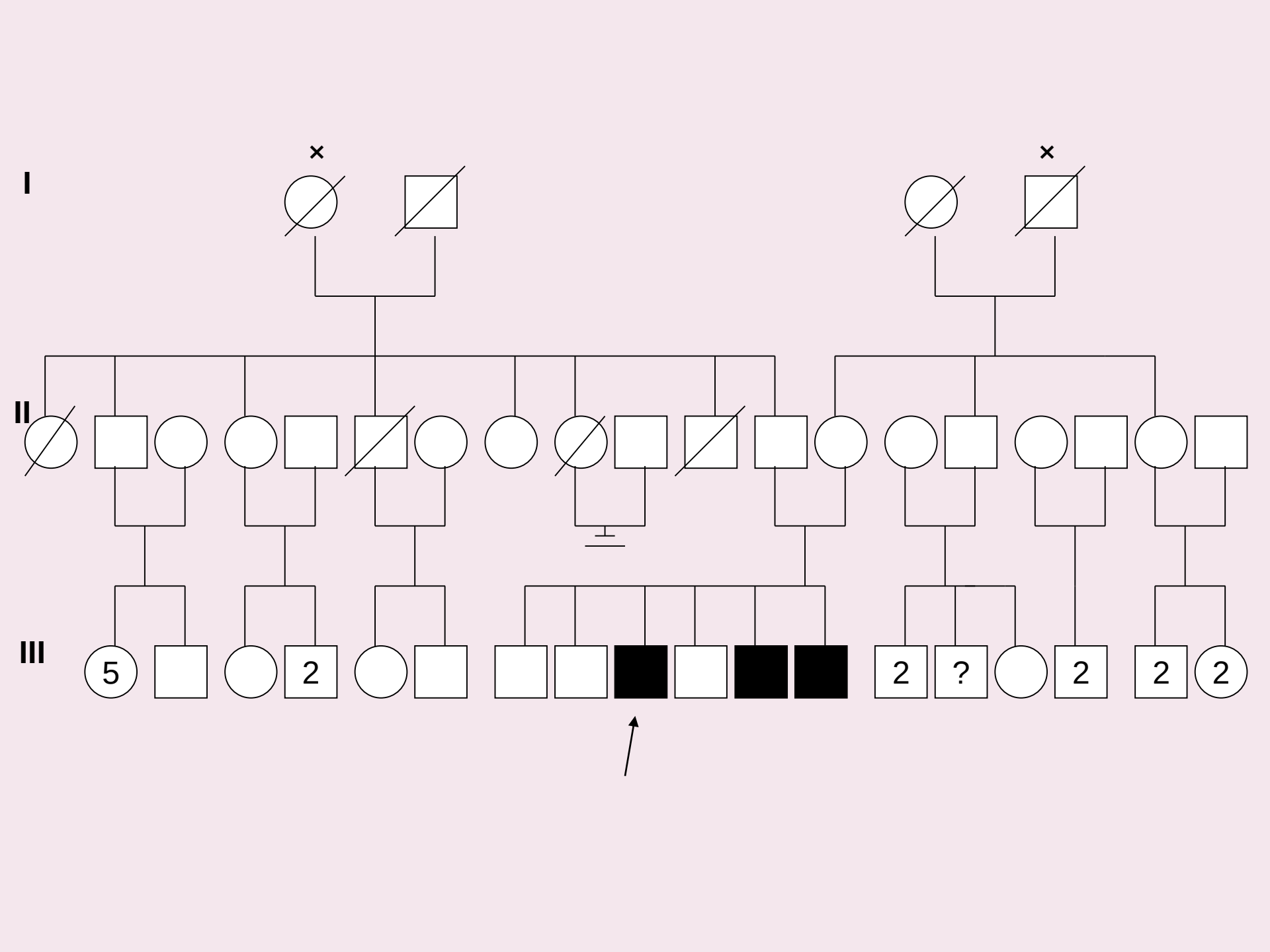


Mutation 550delA

- **Located in exon 4 CAPN3 gene**
- **Deletion of one adenine at nucleotide position 550. The mutation changes the reading frame, which leads to production of a defective protein with a different amino acid sequence. Such proteins are degraded soon after synthesis with consequent lack of the protein.**

Presentation of a family with LGMD 2A

- **Parents: father born in 1953, mother born in 1957, both are healthy**
- **Possible consanguinity: originate from two neighboring villages (maiden name of father's mother and the surname of mother's father are the same)**
- **Children: six sons, three of which are healthy**


I
II
III
5
2
2
?
2
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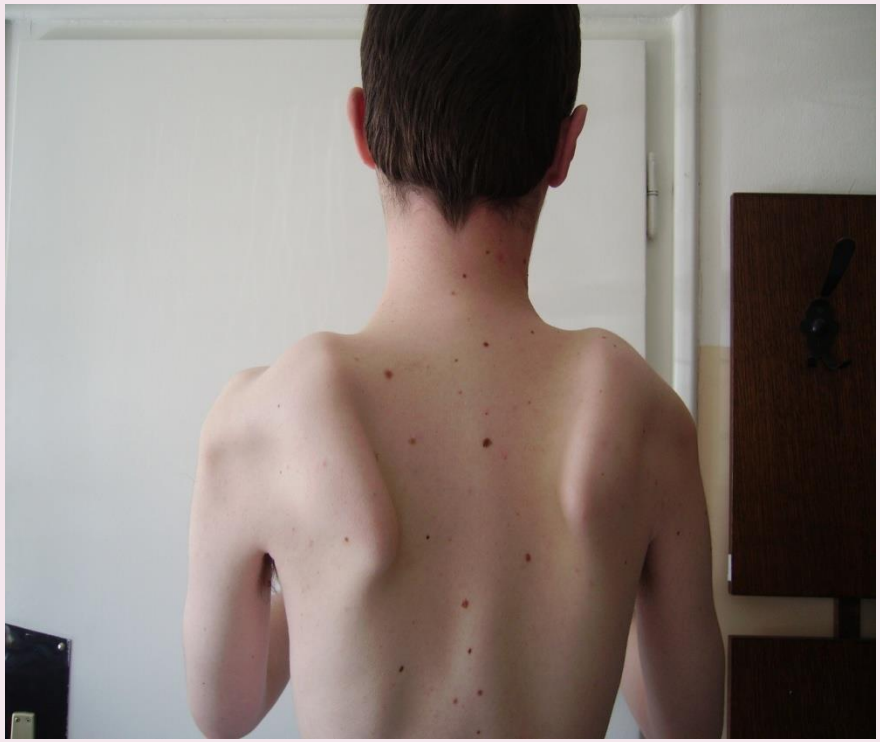
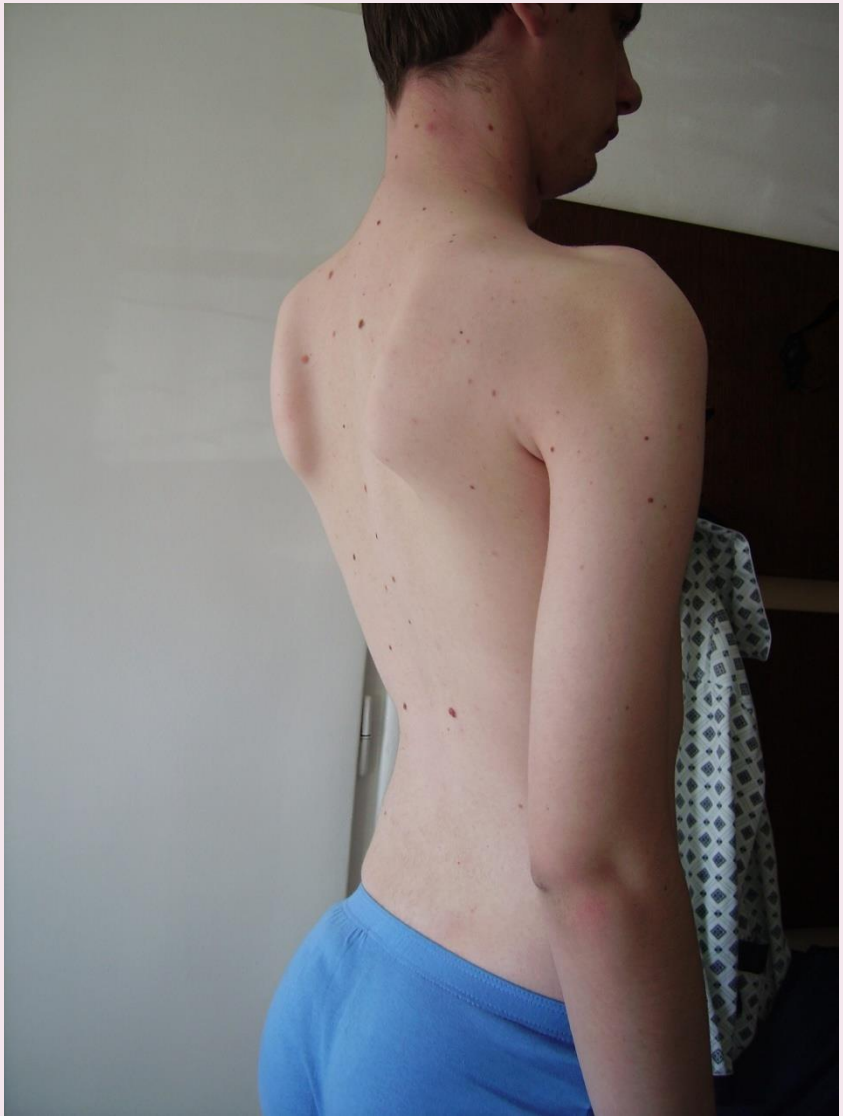
2

Presentation of a family with LGMD 2A

- **G. P., born in 1984**
- **Regular mother's pregnancy and early psyhomotor development**
- **Age 12-13: frequent falls**
- **Climbing stairs using handrail**
- **Age 16: education discontinued due to incapacity of walking to school (3 km).**
- **Weakness of shoulder girdle**

Presentation of a family with LGMD 2A





Clinical examination

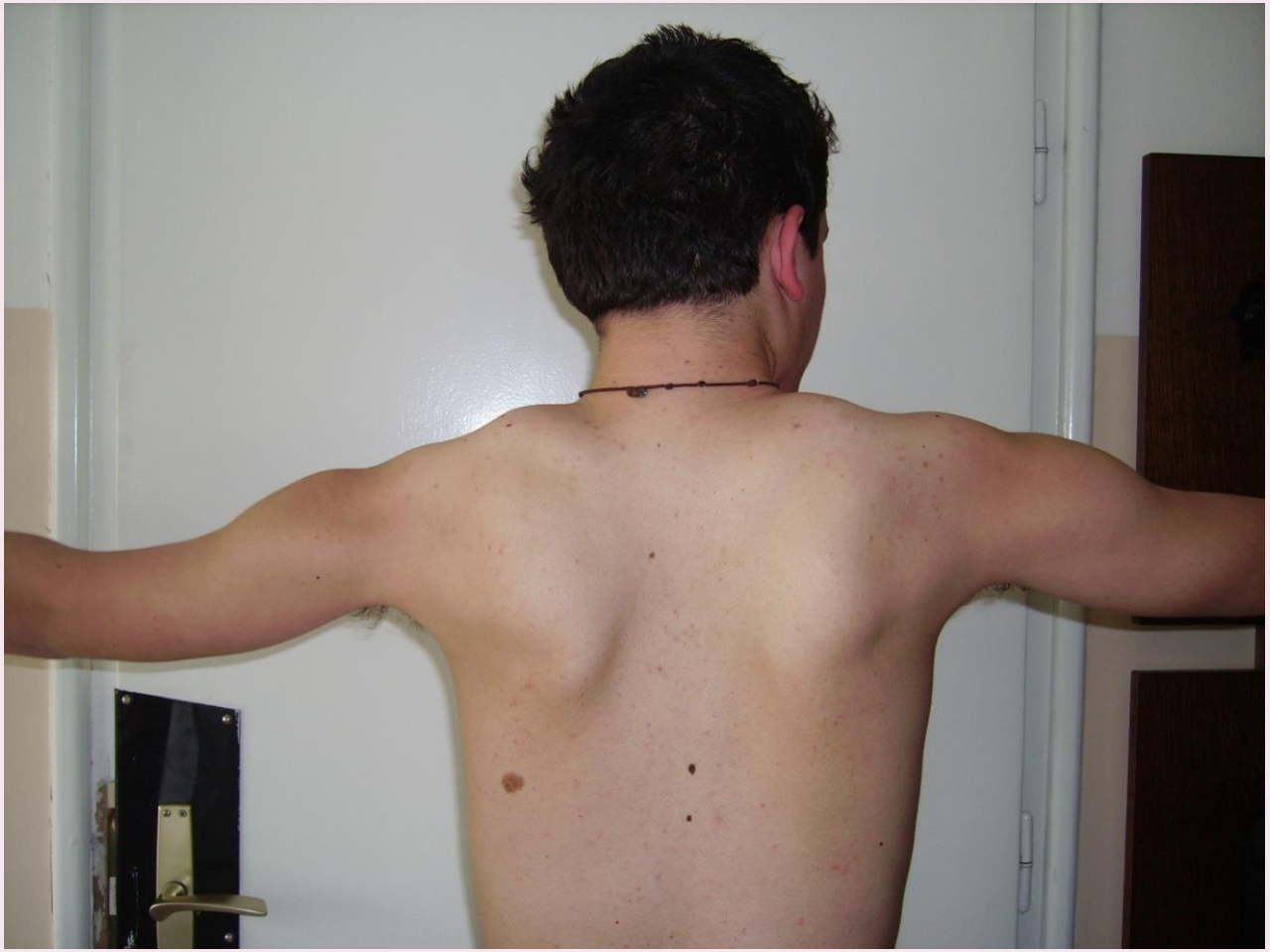
- **Slighty diminished pouting of lips, weakness of muscles of the scapular region, shoulder and pelvic girdle.**
- **Abduction of upper arms is possible up to 45 degrees.**
- **Elevated scapulas**
- **Waddling gait, hyperlordosis**
- **Inability of independently rising from sitting position**
- **Shortenig of Achilles tendons**

Laboratory findings

- **CK 1226 U/L**
- **EMG: Myopatic pattern in muscles of scapular region, m. biceps brachii, thigh muscles and lower leg musles.**
- **Neurography, ECG, ultrasound of the hart: normal**

Presentation of a family with LGMD 2A

- **P. F., born in 1989**
- **Early psyhomotor development regular**
- **Age 13: difficulties in walking**
- **Waddling gait with pronounced hyperlordosis**
- **Elevated scapulas**
- **Shoulder abduction possible up to 90 degrees. Walking on heels is not possible, walks several steps on his toes**



Laboratory findings

- **CK 1160 U/L**
- **EMG: signs of myopathy in shoulder muscles, upper arm, and more pronounced in the thighs**

Presentation of a family with LGMD 2A

- **B. P., born in 1993**
- **Age 12: difficulties in climbing stairs**
- **Neurologycal examination: waddling gait, elevateted scapulas bilaterally.**
- **Abduction of the the upper arms is preserved**
- **Positiv Gowers sign: climbs up his own legs when is tryng to rise from the floor.**

Laboratory findings

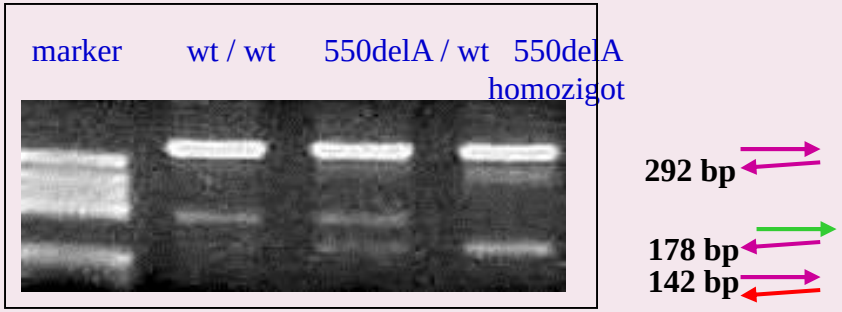
- **CK 986 U/L**
- **EMG: Signs of myopathy in the lower leg muscles, thighs, and relatively less pronounced in the upper arm and shoulder muscles.**
- **Neurography: normal.**

Features indicating the diagnosis of LGMD2a

- **Family history: healthy parents, three sick sons and three healthy sons – suggesting recessive mode of inheritance**
- **Onset of disease - at age of 12-14 years**
- **Clinical presentation: weakness of legs and pelvic girdle muscles et start, later involvement of shoulder girdle**
- **CK markedly elevated**

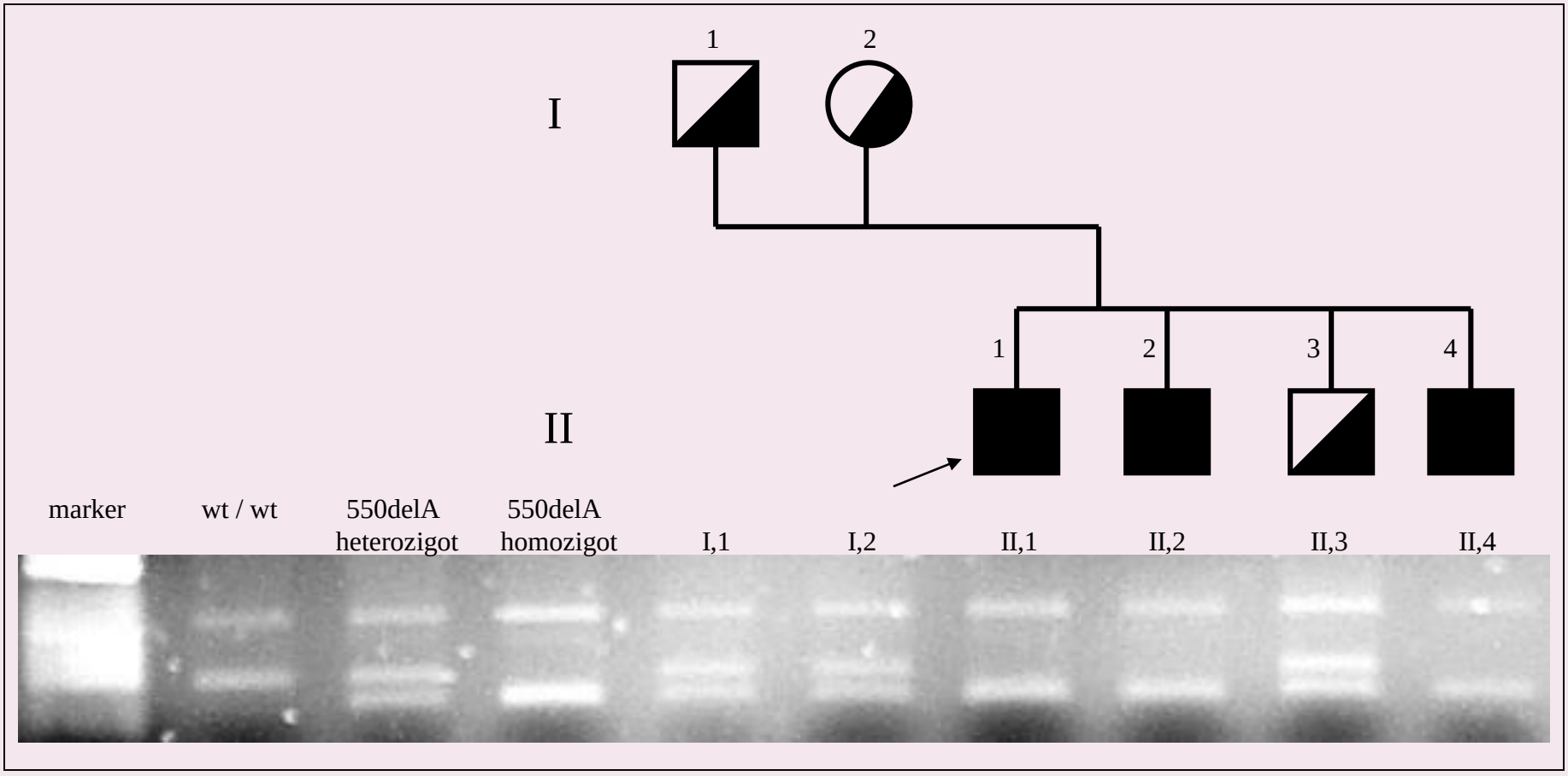
- **DNA analysis was performed at the Laboratory of Molecular Genetics and muscle disease at the Croatian Institute for Brain Research.**

- **M. P. born in 1984: homozygous carrier of two identical mutations on CAPN3 gene (550delA/550delA)**



CAPN3 gene analysis

Results



Conclusion

- **The knowledge of the most frequent CAPN3 mutations present in our population allows a quick, inexpensive and noninvasive diagnosis of LGMD2A by simple and rapid methods of molecular biology**