PS2 Group1 - 026 / #478

CALPAINOPATHIES IN CHILE

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Background and General Purpose

Calpainopathies in Chile

1. Limb girdle muscular dystrophy 2A (LGMD2A; MIM#253600) is an autosomal recessive disorder caused by mutations of the calpain gene (*CAPN3*) which encodes for calpain-3 (CAPN3)
2. Calpain-3 is a muscle specific calcium-activated neutral protease involved in remodelling of the sarcomere
3. No patients with calpainopathy have been reported hitherto from Chile, a country of 17 million inhabitants and a strong Basque ascendance
4. We describe five patients belonging to four unrelated Chilean families, harbouring *CAPN3* mutations
5. Two of the mutations found in Chilean patients are novel

Subjects and Methods

1. The local Ethics Committee of the Hospital Clínico Universidad de Chile and the Chilean National Commission of Scientific Research and Technology (CONICYT) approved the study protocol.
2. Patients were referred from all across the country
3. Diagnosis was based on clinical features, muscle biopsy by immunohistochemistry to exclude other limb girdle dystrophy or myopathy and clinical findings consistent with calpainopathy (Figure 1)
4. Work-up consisted of Functional Motor Measure (MFM), echocardiogram, baseline spirometry, standardized electrophysiological protocols and sequential entire body muscle MRI
5. The highest serum CK activity value was used for analysis.

Gene analysis.

Genetic screening for LGMD mutations was performed in Marseille, France in four patients 1-4 (Génétique Médicale et Génomique Fonctionnelle, INSERM UMR_S 910, Université d'Aix-Marseille, Faculté de Médecine de la Timone), on a NGS panel of 306 genes involved in neuromuscular diseases, using HaloPlex (Agilent TechnologiesTM) enrichment method and sequenced on the NextSeq500 (IlluminaTM) by HelixioTM (Biopôle Clermont-Limagne, France).

Families of patients 1 to 4 were analysed in Santiago, Chile (Programa de Genética Humana, ICBM, Facultad de Medicina, Universidad de Chile) or in Marseille, France (Patient 5) by Sanger sequencing using primers flanking each mutation. Primer sequences and the amplification conditions are available on request.

Magnetic Resonance Imaging.

Sequential entire body axial T1W and STIR MRI images were analysed and scored for muscle fatty replacement and oedema.

1. Standardized protocols were applied using a 1.5T system (Magnetom Symphony Maestro-Class, Siemens, Erlangen, Germany). Exploration was done in supine position, covering from the temporal regions to the ankle level. Axial T1-weighted spin-echo (T1W) sequence (TR: 610 - 640 ms, TE: 11 - 12 ms) and axial T2 Short – Time Inversion Recovery (STIR) sequence (TR: 3700 - 4500 ms, TE: 51 - 64 ms, TI: 150). Slice thickness was 8 mm, with a gap of 4 mm. The field of view was between 350-490 mm, with a matrix of approximately 384x240. DICOM OsiriX viewer was used for analysis. STIR sequences were used for edema
2. Score was adapted from Kornblum et al. (2006). **Stage 0,** normal (score 0); **stage 1**, discrete moth-eaten, with sporadic scattered T1 hyperintense areas (score 1); **stage 2a**, moderate moth-eaten, with numerous scattered T1 hyperintense areas (score 2); **stage 2b**, late moth-eaten, with numerous confluence areas of T1 hyperintensity (score 3); **stage 3**, complete fatty degeneration and fibrous replacement of muscle by connective tissue and fat (score 4)

Figure 1. Main Clinical Features of four patients. A. Patient 2, 44 yr old., note hyperl.ordosis, tip-toe standing is possible. B. Patient 3, 37 yr old, scapular winging is severe, note the marked hypotrophy of the lower limbs. Patient 1, at 18 yr old, tip-toe walking was obliged and hyperlordosis is also present. D. Patient 5, at 13 yr old, there is a generalized amyotrophy, symmetrical scapular winging, but is able to stand in tip-toe

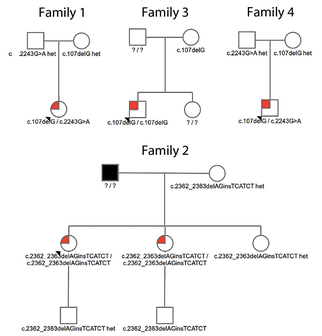

Figure 2. Pedigree of the four Chilean families harbouring CPN3 mutations. Note that the novel mutation c.107delG/p.Gly36Valfs*21 is present in three out of four families, thus suggesting a possible founder effect. See also Table 1.

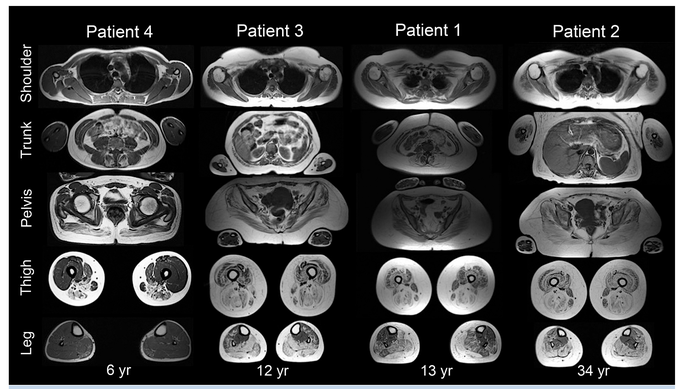

Figure 3. Muscle MRI features. At the bottom of each panel is indicated disease duration at the moment of MRI. Note the earlier involvement of the posterior compartment of the thighs, gluteal and paraspinal muscles. At later stages of disease, the posterior compartment of the legs, proximal paraspinal muscles and shoulder girdle become also involved. Patient 2, after 34 years of disease shows involvement of all muscle segments, including he arms and . Note in Patient 4, the relative sparing of the forearms at trunk level.

Table 1. Summary of main clinical features of the 5 Chilean patients with calpainopatthy.

		Clinical Features						Genetics	
Family	Patient	sex/age	Onset (yrs)	DD yrs (progress.)	Other features	CK level (UI/L)	EcoC/ Spir	Exon/cDNA change	Protein Change
1	1	W/26	<8	18 (C)	Tip-toe walking, hyperlordosis, asymmetric scapular winging.	x45	N/N	Ex1.HTZ c.107delG Ex 21. HTZ c.2243G>A	p.Gly36Valfs*21 p.Arg748Gln
2	2	W/44	7	37 (C)	Early onset with tip-toe waking, Achilles tenotomy, hyperlordosis	x1.5	N/N	Ex 22. HMZ c.2362_2363delAGinsTCATCT	p.Arg788Serfs*14
	3	W/40	25	15 (A)	Late onset, severe scapular winging	x5	N/N	Ex 22. HMZ c.2362_2363delAGinsTCATCT	p.Arg788Serfs*14
3	4	M/20	<12	9 (A)	Early onset, mild retractions	x32	N/N	Ex1.HMZ c.107delG	p.Gly36Valfs*21
4	5	M/25	10	11 (A)	Early onset, mild retractions	x32	N/N	Ex1.HTZ c.107delG Ex 22. HTZ c.2362_2363delAGinsTCATCT	p.Gly36Valfs*21 p.Arg788Serfs*14

Abbreviations: M male, W woman, DD: disease duration; progress.: progression (A), ambulant; (C) support with a cane. EcoC: echocardiography; Spir.: Basal spirometry. N: normal. CK [N]= 30-135 UI/L.

Summary of Results and Conclusions

1. Five patients, four affected Chilean families, (*an additional patient was recently identifyied*)
2. Variable onset, on average in the first decade
3. Typical clinical phenotype, non specific biopsy findings, need of WB analysis of CAPN3 in muscle
4. No cardiac involvement
5. Given the lack of specific clinical and laboratory features, diagnosis is frequently delayed
6. Two novel Mutations
7. Possible founder effect given the recurrence of novel mutations

Supported by Grants FONDECYT#1151383 to JABR