Mutations in amphiphysin 2 (BIN1) disrupt interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy

BIOLOGIE & SANTÉ 2011

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Acronyme: CENTROPATHIES ANR 06 MRAR 023 ; "Identification and characterization of new genes mutated in centronuclear myopathies : molecular links with the myotubularin-dynamin pathway implicated in several neuromuscular disorders"

Programme: Maladies Rares 2007-2008

CONTEXT

Centronuclear Myopathies (CNM) are a group of rare congenital disorders characterized by muscle weakness and typical skeletal muscle biopsies showing small rounded fibers with central nuclei without excessive regeneration. Several forms have been documented :

- X-linked neonatal form or myotubular myopathy (XLCNM, OMIM 310400): due to mutations in the phosphoinositides phosphatase **myotubularin** (MTM1)
- Autosomal dominant form (ADCNM, OMIM 160150): with mutations in the large GTPase **dynamin 2** (DNM2)
- Autosomal recessive forms (ARCNM, OMIM 255200): the onset of weakness typically occurs in infancy or early childhood, although cases were reported to present hypotonia at birth. **No genes** have been identified previously to this work.

OBJECTIVES

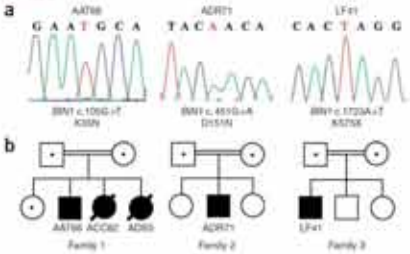
1. Identify novel gene(s) mutated in Centronuclear Myopathies
2. Establish genotype-phenotype correlations
3. Characterize the functional impact of disease-causing mutations
4. Decipher the molecular link betwwen different proteins implicated in Centronuclear Myopathies

RESULTS

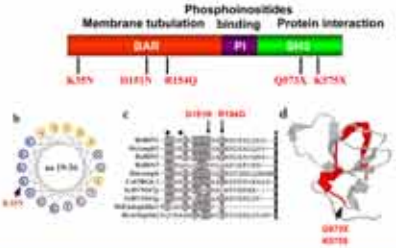
Strategy

1. Patients recruitment : **132 families with a CNM-like phenotype**
2. Exclusion of known genes : **MTM1** (Myotubularin) and **DNM2** (Dynamin 2) by Sanger sequencing, and RT-PCR and Western blot for MTM1
3. Selection of candidates genes (by data mining, comparative genomic, and yeast two hybrid) : **600 "functional" candidate** genes of which **the 5 best were sequenced** in our panel of patients
4. Homozygosity mapping with 11 consanguineous families, to prioritize families and candidate genes
5. Sanger sequencing of exons and intron-exon boundaries
6. Functional impact of the variants found

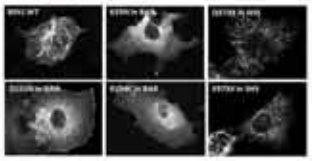
Amphiphysin 2 (BIN1) is mutated in Centronuclear Myopathies



Mutations of the BIN1 genes found in several consanguineous families with autosomal recessive Centronuclear Myopathies. Sequences and position of mutations are indicated.

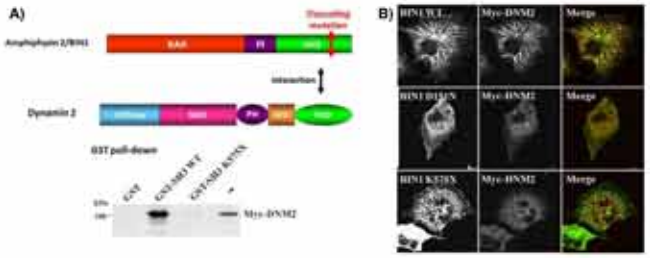


Mutations impair the membrane remodeling properties of Amphiphysin 2



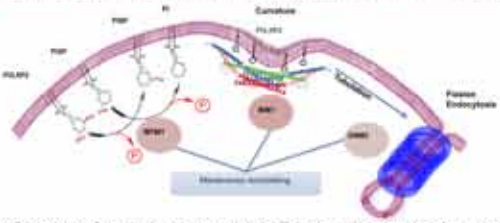
Mutations in the BAR domain impair the membrane remodeling properties of Amphiphysin 2 (BIN1). Exogenous expression of WT BIN1 and SH3 truncated mutants tubulates membranes, while BAR mutants do not tubulate membranes.

Mutations disrupt the complex Amphiphysin/Dynamin



BIN1 - Dynamin 2 uncoupling. A) BIN1 and DNM2 interact while truncation of the SH3 domain abrogates DNM2 binding by GST pull-down. **B)** Exogenous expression of WT BIN1 tubulates membranes and recruit exogenous DNM2. Mutations in the SH3 domain decrease DNM2 recruitment to the membrane tubules.

A novel membrane remodeling pathway in skeletal muscle ?



The M.A.D. pathway for membrane remodeling. Together with other proteins mutated in Centronuclear Myopathies, Amphiphysin 2 defines the M.A.D. pathway (Myotubularin, Amphiphysin, Dynamin) that may control membrane remodeling in skeletal muscle.

CONCLUSION – PERSPECTIVES - IMPACT

Through excellent collaborations with clinicians and histopathologists, we identified BIN1 (amphiphysin 2) as a novel gene mutated in Centronuclear Myopathies.

This work provides the knowledge for genetic diagnosis and counselling. The molecular diagnosis is now done in routine in several countries.

Moreover, it reveals the importance of Amphiphysin 2 in membrane remodeling and nuclei positioning in skeletal muscle and links this protein to Myotubularin and Dynamin superfamilies.

An animal model reproducing the disease will be envisaged to study its pathological mechanisms and further test therapeutic approaches.

PUBLICATIONS - VALORISATION

Routine genetic diagnosis for BIN1 mutations

Articles

•Nicot AS*, Toussaint A*, Tosch V, Kretz C, Wallgren-Pettersson C, Iwarsson I, Kingston H, Garnier JM, Biancalana V, Oldfors A, Mandel JL, Laporte J. Mutations in amphiphysin 2 (BIN1) disrupt interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy. **Nat Genet**. 2007 Sep;39(9):1134-9. *equal contributors

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Reviews

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•Jungbluth H, Wallgren-Pettersson C, Laporte J. Centronuclear (myotubular) myopathy. **Orphanet J Rare Dis**. 2008 Sep 25;3:26.

Didactic reviews

•Jungbluth H, Wallgren-Pettersson C, Laporte J. on behalf of the Centronuclear (Myotubular) Myopathy Consortium. 164th ENMC International Workshop: 6th workshop on Centronuclear (Myotubular) Myopathies, 16-18th January 2009, Naarden, The Netherlands. **Neuromuscul Disord**. 2009 Oct 19(10):721-8.

•Toussaint A, Nicot AS, Mandel JL, Laporte J. Mutations in amphiphysin 2 (BIN1) cause autosomal recessive centronuclear myopathy. **Med Sci** (Paris). 2007 Dec;23(12):1080-2

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