Autosomal Recessive Forms of Charcot-Marie-Tooth

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S. Girard[1], K. Poirier[1], H. Azzedine[1], N. Ravisé[1], A. Chojnowski[1], O. Dubourg[2], T. Stojkovic[2], S. Tardieu[3], E. Leguern[1,3] **(Coordinator)**

[1]Université Pierre & Marie Curie-Paris 6, UMR_S975, CNRS, UMR7225, CRICM, [2] APHP, Centre de référence des maladies neuromusculaires Paris-Est,[3] Département de Génétique et Cytogénétique, Hôpital de la Pitié-Salpêtrière, Paris, France

THE AR CMT WERE THE LESS KNOWN FORMS

The Charcot-Marie-Tooth disease (CMT) is an inherited neuropathy, which causes walking disability and difficulties in daily-life handling. The electrophygiological examination allows distinguishing demyelinating from neuronal forms (axonal or spinal). At the beginning of this project, very few was known about autosomal recessive (AR) forms, which were considered rarer than autosomal dominant CMT. Collaborations have been established with neurological departments in Maghreb in order to study larges siblings with related parents, which allow localizing and subsequently identifying the causative genes. Today, less than 30% of patients with ARCMT has mutations in the 12 already identified genes.

OBJECTIVES

1-Identification of new genes responsible for spinal ARCMT;
2-Collection of a large number of families with mutations in *SH3TC2* (CMT4C) to establish phenotype-genotype correlations;
3-Construction of cellular models to understand the functional consequences of the causative mutations in *SH3TC2*

RESULTS

A NEW GENE FOR SPINAL ARCMT

We previously incriminated the locus associated with the "Jerash" phenotype, a spinal ARCMT described in a large Palestinian pedigree (Christodoulou et al., 2000), in five families from Maghreb.
In 3 unrelated Algerian families (figure 1). we identified the same 7 bp frameshift mutation in a gene localized in the candidate interval. It consists of the TGGGCCG insertion in exon 1, leading to a premature Stop codon (p.Arg8Glyfs118).
Genotyping of affected members of these families is in favour of a founder effect of this mutation.

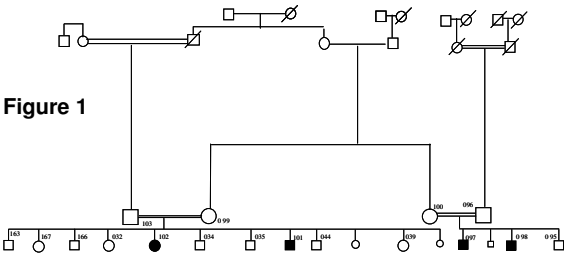


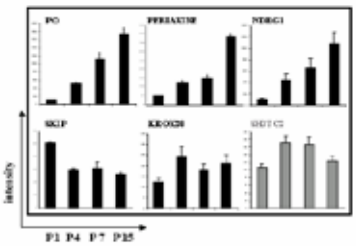
Figure 1

STUDY OF THE *SH3TC2* GENE

Mutations in SH3TC2 are frequent in France

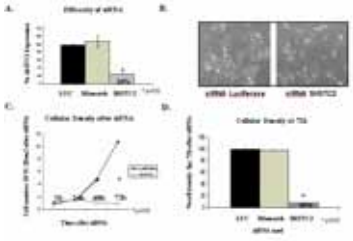
Since ARCMT were considered as rare in France, we showed that the *SH3TC2* gene is frequently mutated in French isolated cases with a demyelinating CMT: 15% of patients. Moreover, their electrophysiological profile, showing an heterogeneous demyelination, is characteristic and useful to direct the diagnosis of CMT (Azzedine et al., 2009, Ygier et al., Submitted).

SH3TC2 is expressed in early phase of myelination



SH3TC2 is expressed at early stages of myelination in the peripheral nervous system of the rat. Total RNA was extracted from rat sciatic nerves at P1, P4, P7 and P15. *SH3TC2*, *Krox20*, *P0*, *PRX* and *NDRG1* mRNA levels were quantified by real-time PCR and normalized to cyclophilin mRNA levels at each time. The mean ± SM of 3 independent experiments is shown.
The results were expressed, at each time point, as the percent of normalized mRNA levels in control siRNA-luciferase transfected cells

The knock-down of SH3TC2 decreases SC proliferation



SH3TC2 silencing by siRNAs decreases Schwann cell (SC) density in culture. (A) RNA interference reduced *SH3TC2* mRNA level to 26% of the control 2 h after transfection (B) *SH3TC2* silencing decreases Schwann cell density in culture compared to luciferase siRNA control (C) Quantification of Schwann cell number after 2, 24, 48 and 72 hours after RNAi.
(D) Representation of cell density 72 hours after si RNA. *Significantly different than the corresponding control ($p < 0.05$, **$p < 0.01$).
We also showed that the knock-down of *SH3TC2* increased the death iof Schwann cells n vitro .

CONCLUSION

Screening of SH3TC2 is crucial in isolated cases with a demyelinating CMT. Moreover, the electrophysiological profile is highly suggestive of CMT4C
RNA interference is useful to understand the cellular consequences of a loss of function in AR diseases and is complementary to the construction of the corresponding KO models. However, it requires time to test both the efficiency and specificity of the siRNAs.
Finally, the identification of a new gene, like the gene responsible for spinal ARCMT, is of great interest in terms of diagnosis and prognosis for the at-risk members of affected families and is crucial to determine new therapeutical approaches.

CONTACT :
Eric Le Guern:
Eric.le_guern@upmcfr