DU GÈNE AU TRAITEMENT : RECHERCHE TRANSLATIONNELLE ET NEUROPATHIES PÉRIPHÉRIQUES HÉRÉDITAIRES

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Team NEUROMYOLOGIE TRANSLATIONNELLE

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INHERITED PERIPHERAL NEUROPATHIES

Group of hereditay diseases affecting **the peripheral nervous system**

- **Charcot-Marie-Tooth disease** (motor and sensory)
- Pure motor forms (« spinal » CMT or dCMT)
- Pure sensitive forms (HSN, HSAN)

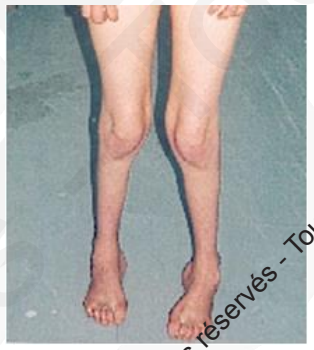
Charcot-Marie-Tooth disease

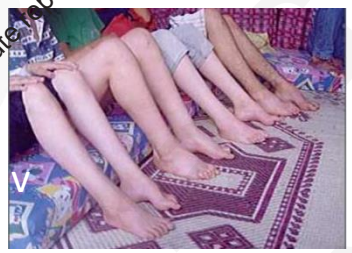
One of the most most common inherited neurological disorder ⇨ prevalence: **1/2500**

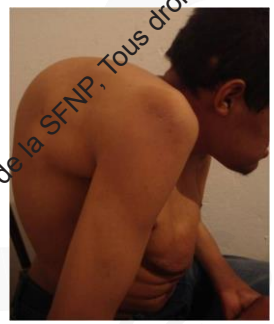
- Progressive distal muscular **weakness and atrophy** beginning at the lower limbs and slowly progressing to the upper limbs

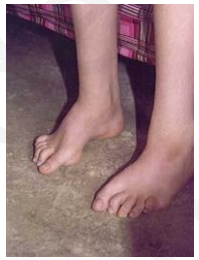
- Feet and hand **deformations**

- cramps
- scoliosis
- variable loss of sensitivity
- hypo- or areflexia



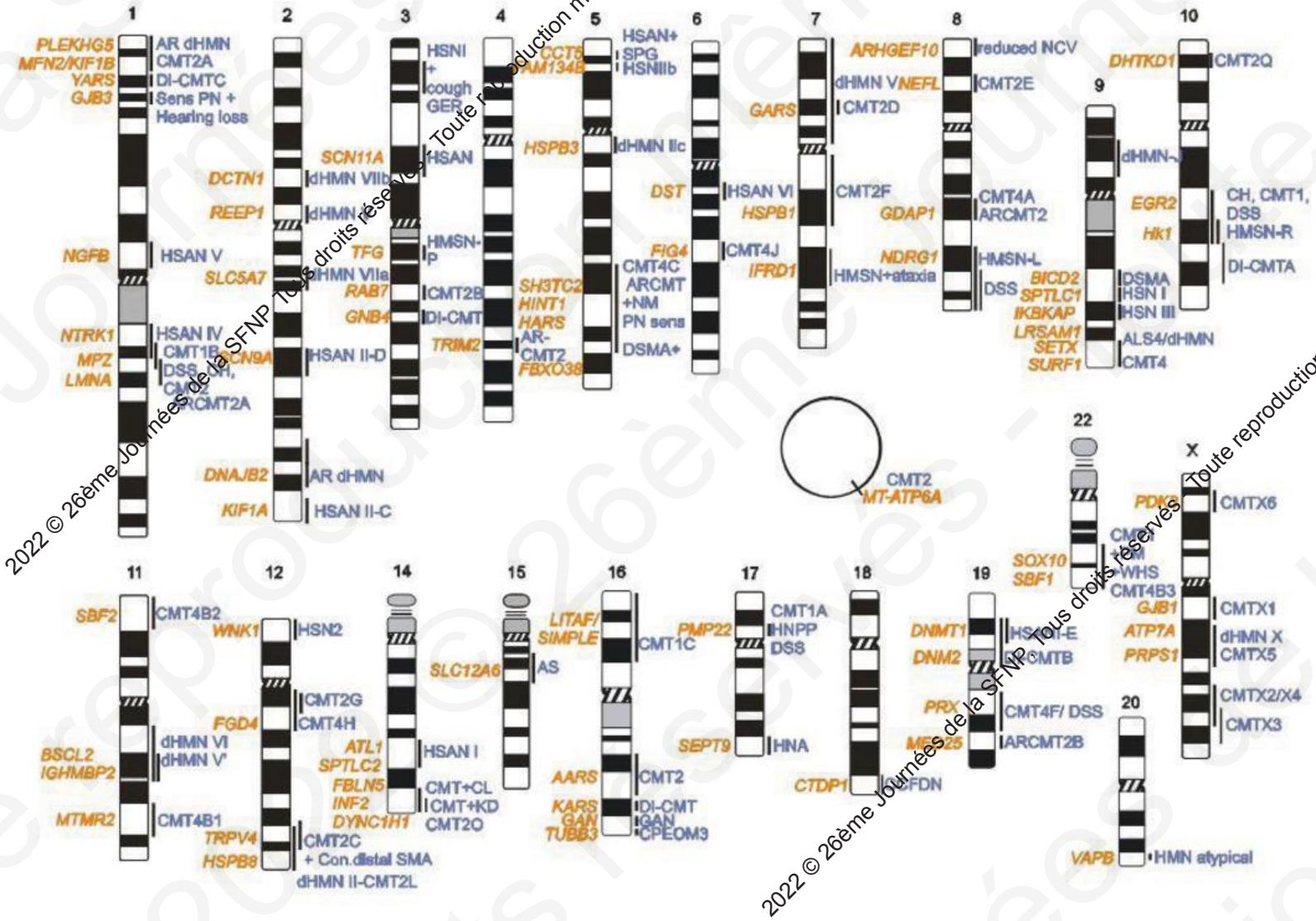






Charcot-Marie-Tooth disease

Genetic Heterogeneity: 100 genes described to date



D'après Timmerman et al., *Genes* **2014**, *5*, 13-32

MÉCANISMES PHYSIOPATHOLOGIQUES COMPLEXES

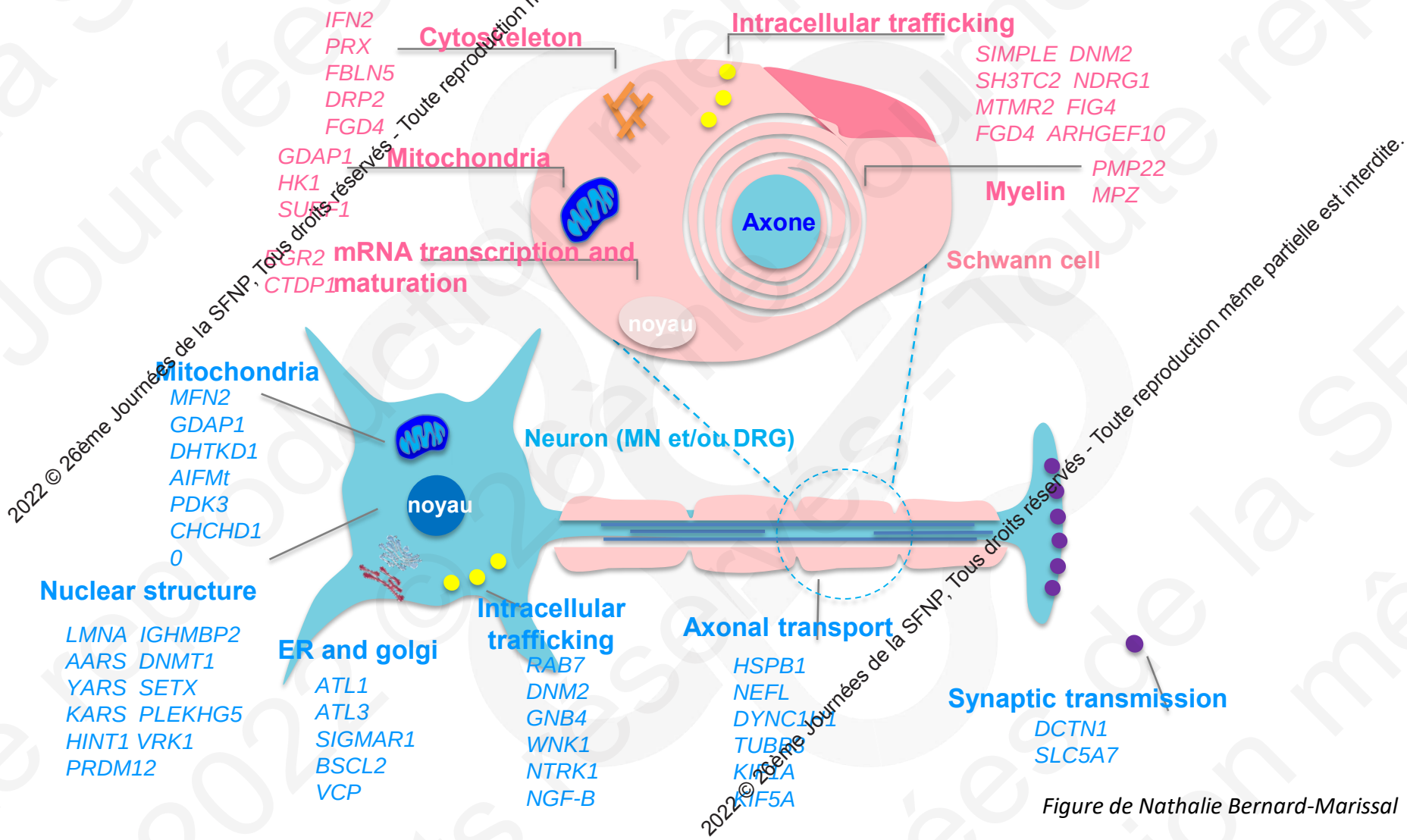
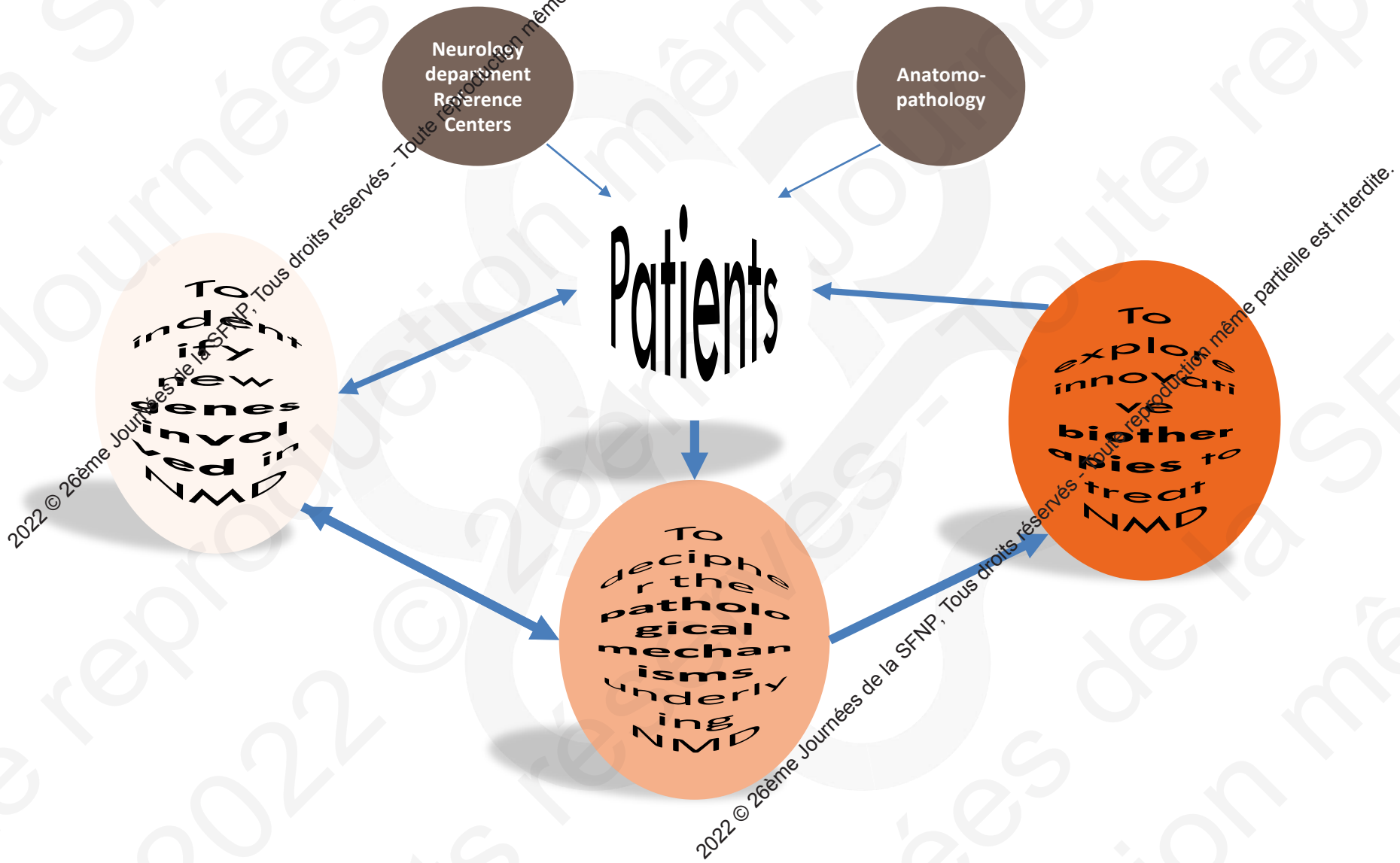


Figure de Nathalie Bernard-Marissal

RECHERCHE TRANSLATIONNELLE



OBJECTIFS

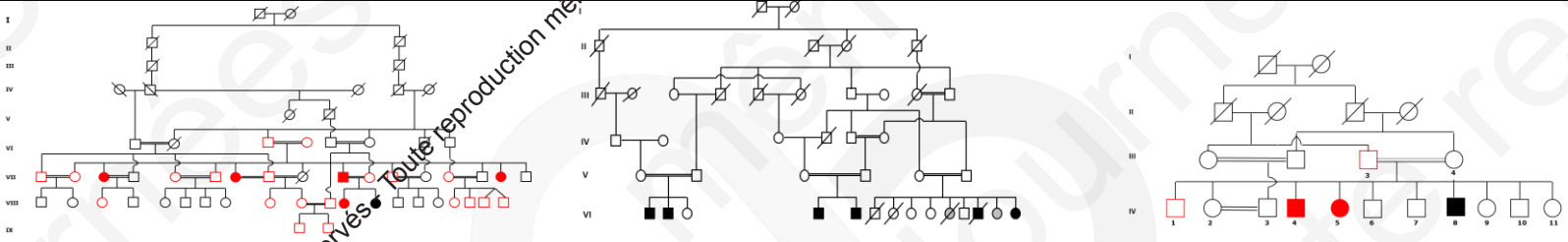
- Better understand the genetic bases of Inherited Peripheral Neuropathies to improve diagnosis

- Understand the pathomechanisms underlying those diseases by studying the role of the defective proteins in the Peripheral Nervous System and discover new actors of the PNS

- Propose and develop biotherapies to improve the treatments of these diseases

ETUDES GENETIQUES

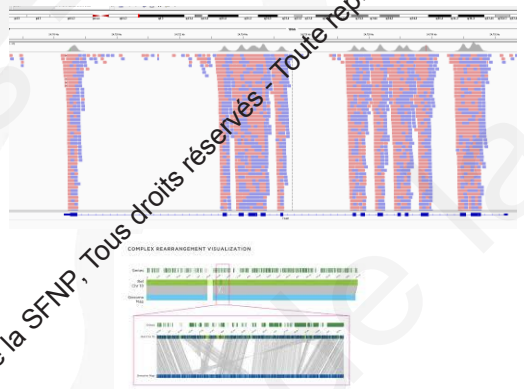
Identification de nouveaux gènes mutés dans les IPNs/CMT



- Large consanguineous families with peripheral nerve diseases
- Autosomal recessive transmission
- Collaboration network with geneticists from the Mediterranean area and the Middle East

Methodology

- Targeted sequencing
- WES, WGS sequencing of trios, quattors..
- Identity by Descent analysis of WES and WGS for SNV
- WGS-based linkage analysis combined with variant analysis of WGS data
- CNV detection on WES, WGS data or by using BioNano Genomics
- Study of the genetic "burden"



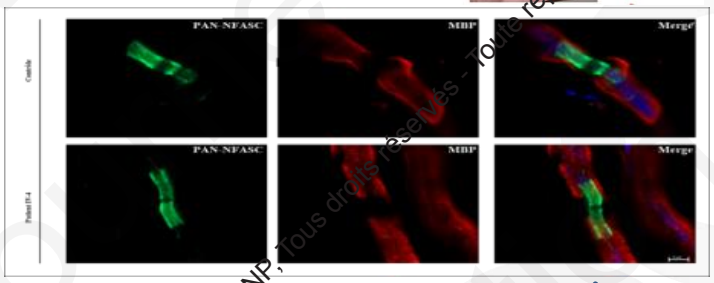
Objectives

- Contribute to improve diagnosis strategies
- Identify new players in muscle and peripheral nerves
- Study the underlying pathomechanisms

ANALYSES FONCTIONNELLES/ETUDE DES MECANISMES

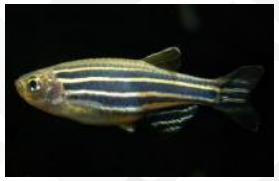
Quels modèles?

Tissus de patients



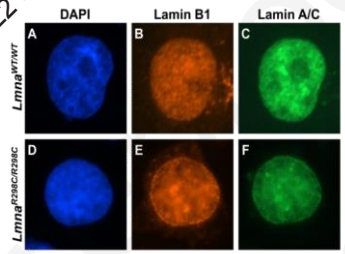
Fibres myélinisées de peau glabre

Modèles animaux

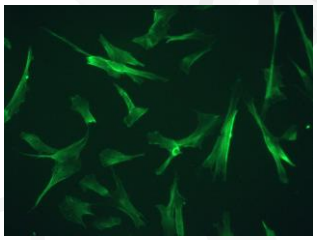


Modèles *in vitro*

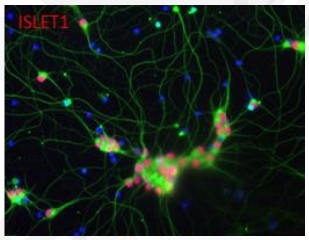
Cellules de patients



Lignées lymphoblastoides

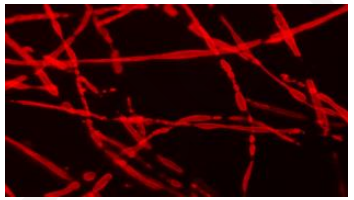


Fibroblastes



Neurones moteurs ou sensitifs dérivés d'hiPSC +/- coculture avec des myoblasts

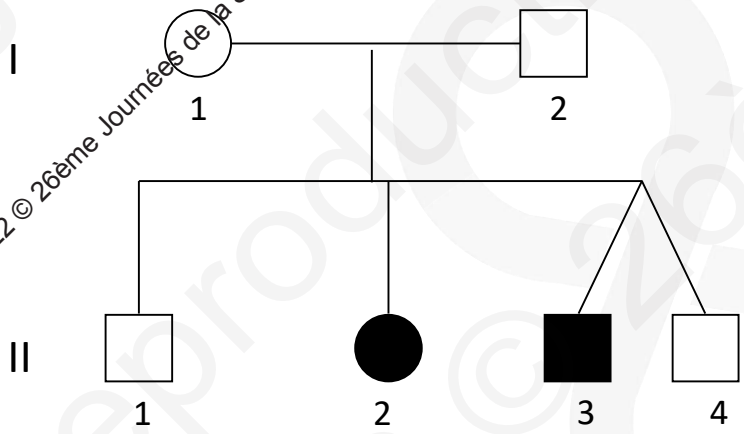
Modèles cellulaires dérivés de modèles animaux



SC/DRG cocultures

LEBANESE FAMILY

- Non consanguineous Lebanese family
- Two affected sibs
- Refered to me as affected with **dHMN+ Upper motor Neuron disease signs**
- Onset: second decade
- Slowly progressive



Clinical examination

- Motor deficit of the four limbs with distal predominance
- Muscle atrophy of hands and legs
- Proprioception and vibration sensation deficit distally bilaterally
- Hyperreflexia throughout
- Babinski signs bilaterally
- Normal cranial nerves

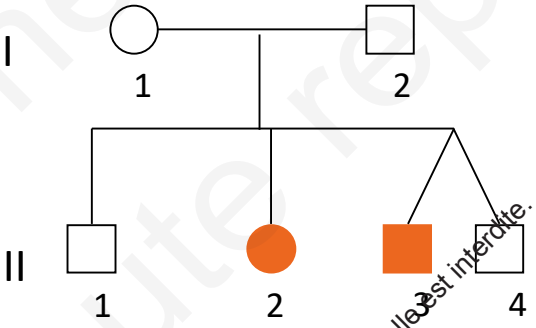
Electrophysiology

- EMG: axonal peripheral neuropathy, motor predominant

GENETIC ANALYSES

Variant segregation and filtering

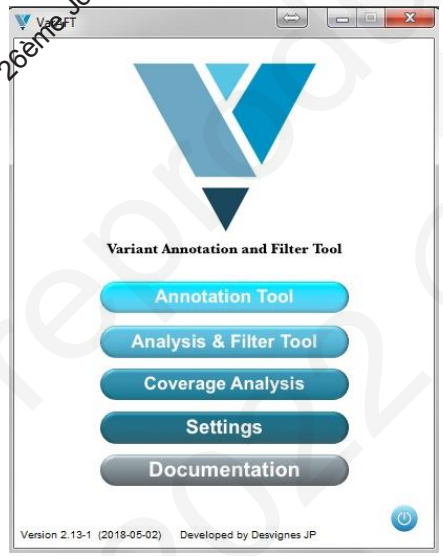
After alignment to the human genome (hg19) , we found:
20911 SNPs in patient II.2
21438 SNPs in her brother II.3



Autosomal recessive transmission

No Consanguinity

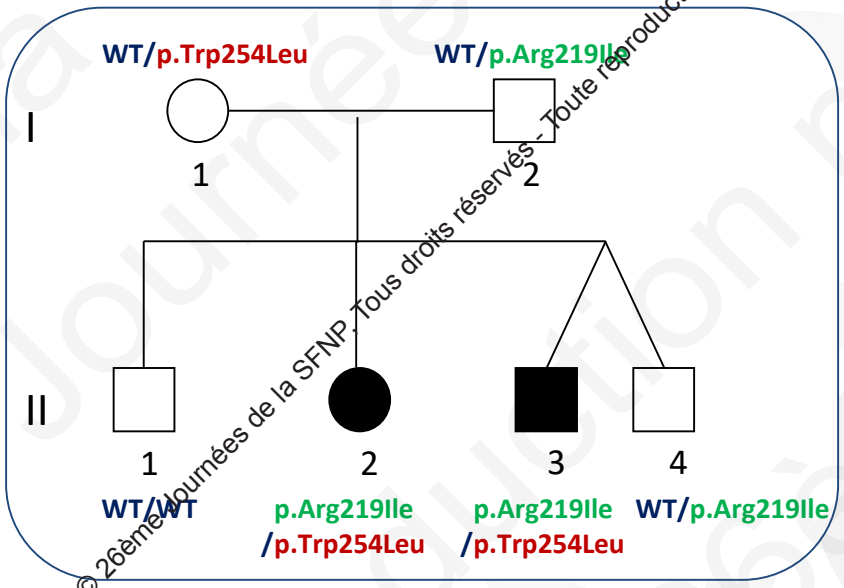
→ « Compound »heterozygous mutations **shared** by the 2 patients

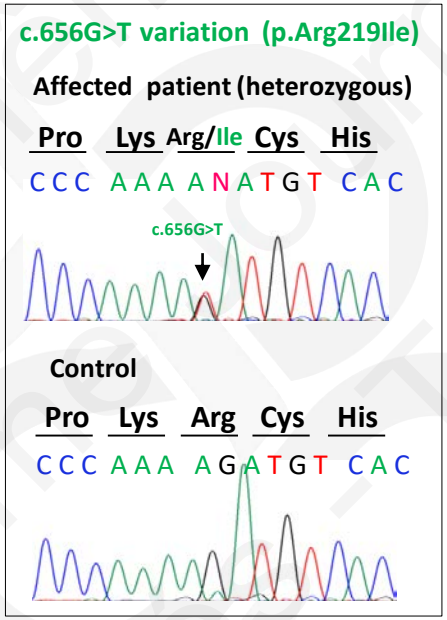


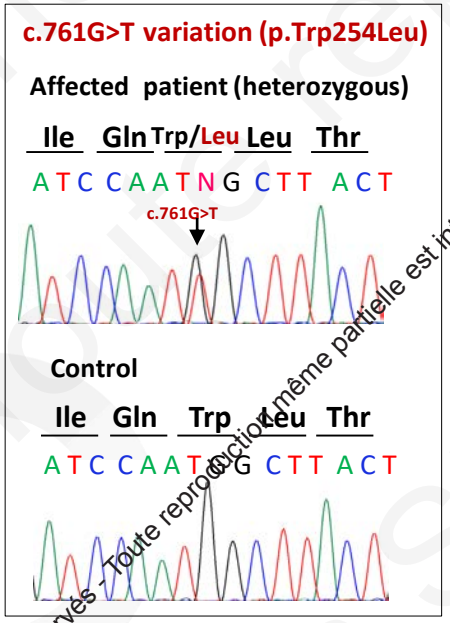
- VarAFT: Variant Annotation and Filter Tool. In house software tool développé by Christophe Beroud's team (U 1251, MMG)
- Desvignes et al. 2018. **VarAFT: a variant annotation and filtration system for human next generation sequencing data.** Nucleic Acids Res doi: 10.1093/nar/gky471 . [Epub ahead of print]
- https://varaft.eu/download.php

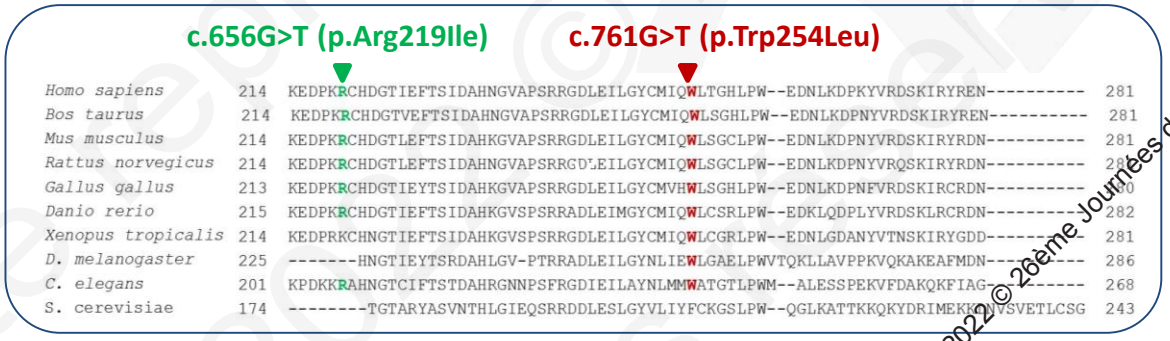
Identification of mutations in VRK1

VRK1 (NM_003384) : c.656G>T (p.Arg219Ile) et c.761G>T (p.Trp254Leu)









- ✓ Absent from frequency datasets (gnomAD, EXAC, base locale) and from a cohort of de 300 lebanese
- ✓ Predicted Pathogenic

VRK1: Vaccinia-related kinase 1

Fonctions cellulaires

Sérine-thréonine kinase

Prolifération cellulaire et tumorigénèse

Régulation trancsriptionnelle

Régulation du cycle cellulaire

Peu de données sur son rôle dans les neurones et le SN : Knock-down de vrk1 *in utero* dans le cerveau d'embryons de souris →

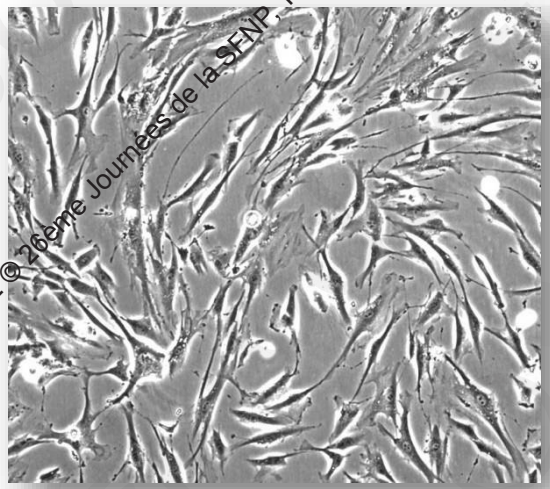
rôle dans la migration neuronale , dans prolifération des progéniteurs neuronaux et dans le guidage axonal (Vinograd *et al*; 2015 et 2018)

VRK1: Vaccinia-related kinase 1

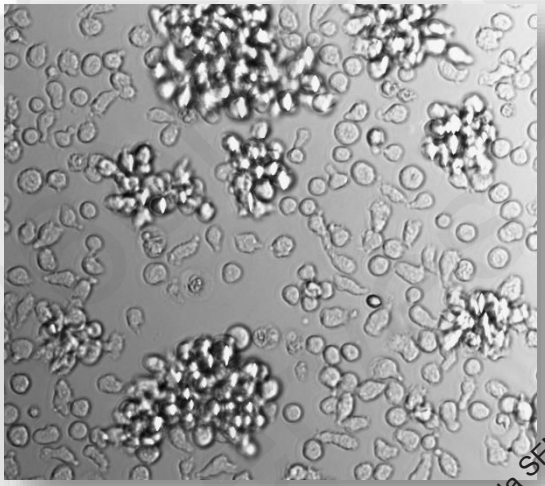
Etudes fonctionnelles

Modèles cellulaires issus de patients

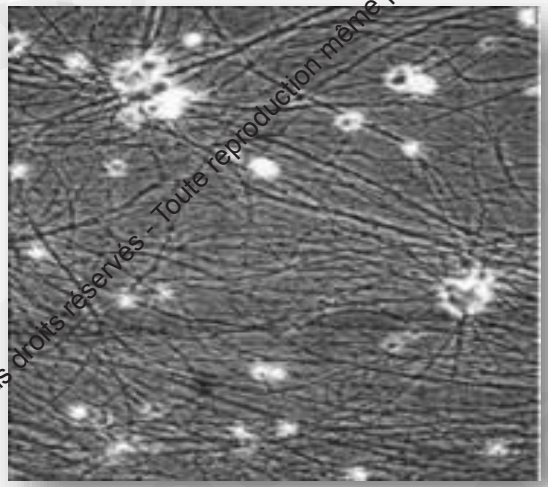
Fibroblastes



Lymphocytes



Motoneurones dérivés d'iPSC

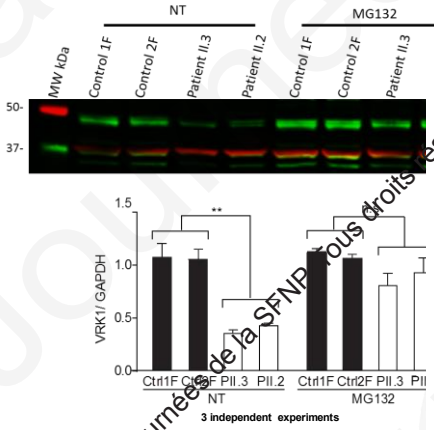


Protocole de différenciation développé par Khalil RIHAN

EXEMPLE 1

Novel mutations in VRK1 in a new form of CMT with upper motor neuron signs

1. In patient's fibroblasts: decrease of VRK1 levels in patients' cells is due to post-translational defects and VRK1 depletion in patients' fibroblasts leads to reduced coilin levels by facilitating its proteasomal degradation.



Human Molecular Genetics, 2019, Vol. 28, No. 14 2378–2394
doi: 10.1093/hmg/ddz060
Advance Access Publication Date: 2 April 2019
General Article

GENERAL ARTICLE

Loss of Cajal bodies in motor neurons from patients with novel mutations in VRK1

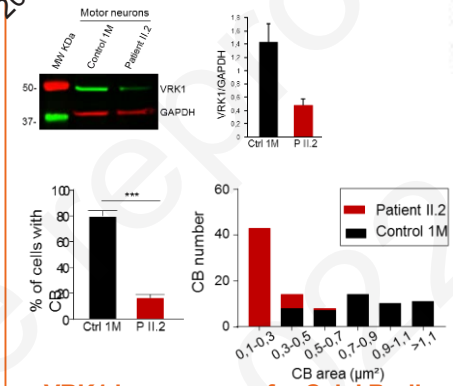
Lara El-Bazzal[1], Khalil Rihan[1], Nathalie Bernard-Marissal[1], Christel Castro[1], Eliane Chouery-Khoury[2], Jean-Pierre Desvignes[1], Alexandre Atlemson[1], Karine Bertaux[3], Salam Koussa[4], Nicolas Lévy[1,5], Marc Bartoli[1], André Mégarbané[6,7], Rosette Jabbour[8] and Valérie Delague[1,*,†]

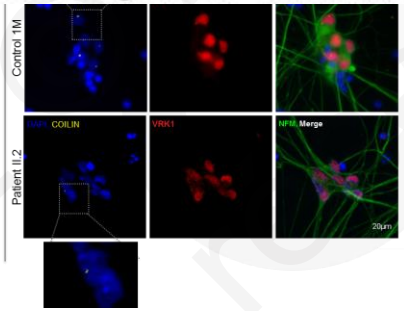
Blockade of the p

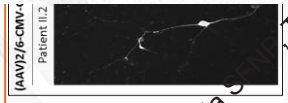
2. In patients' hiPSC-derived Motor in the nucleus lead to disassembly

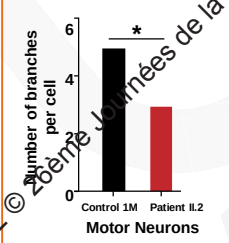
SC-derived MNs:

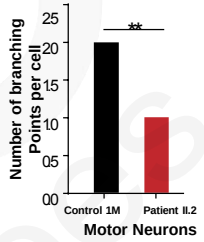
ent's motor signs. These ngth dependent axonopathy affecting both lower and upper motor neurons in patients.

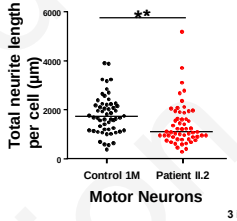












VRK1 is necessary for Cajal Bodies assembly in hiPSC-derived MNs

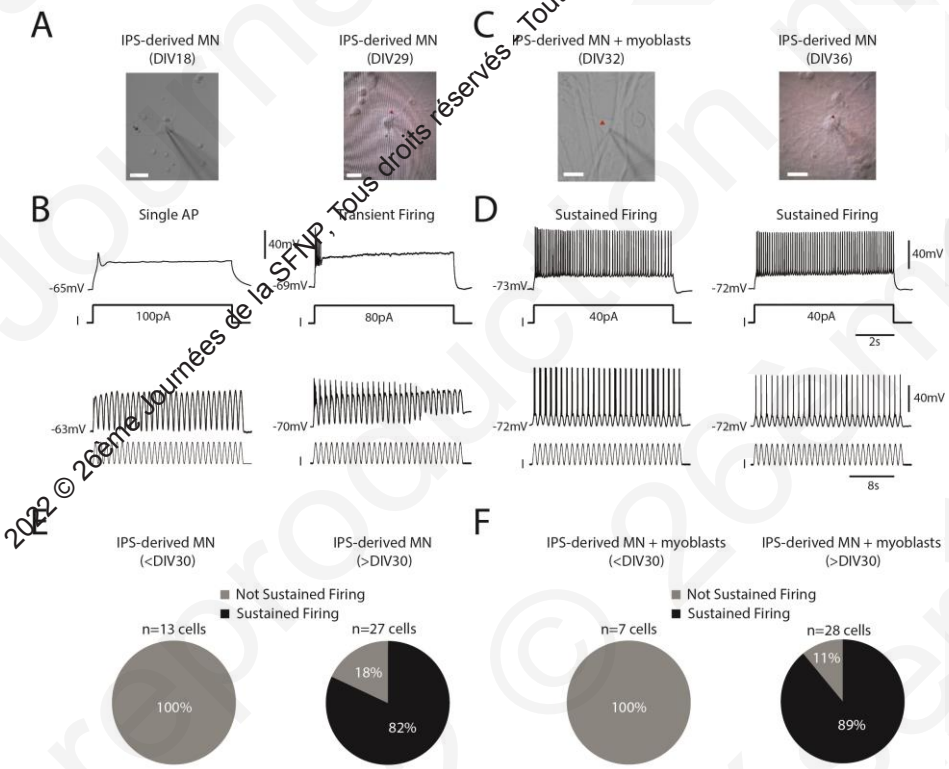
3 independent experiments

3 independent experiments

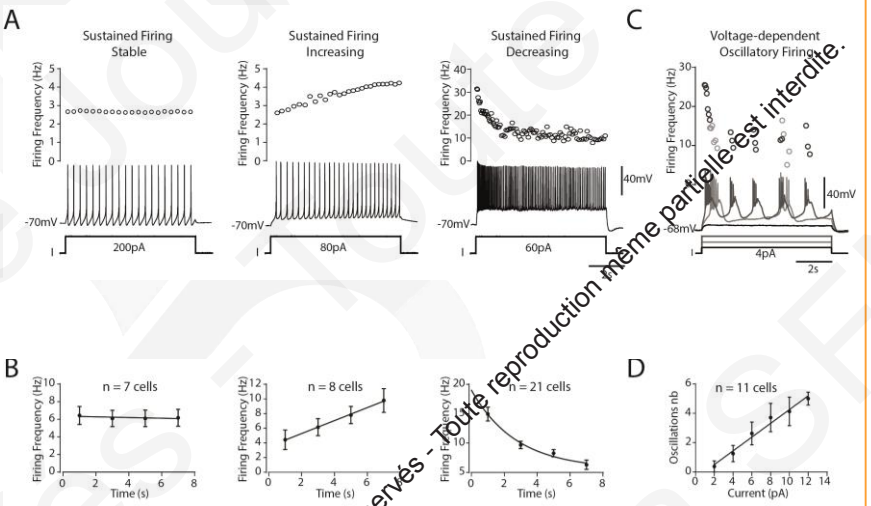
hiPSC-MNs are functional and sustain firing patterns, typical of spinal MNs

hiPSC-derived MNs are functional and sustain firing patterns, typical of spinal MNs

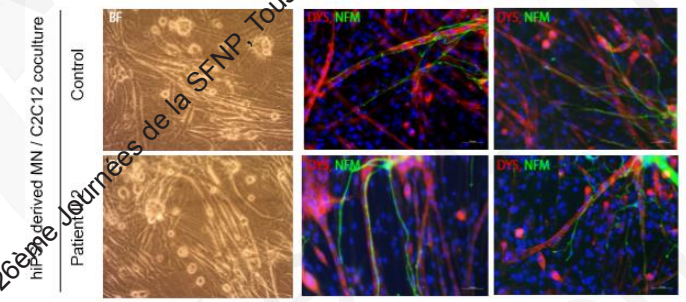
1. Co-culturing human hiPSC-MNs with myoblasts accelerates their functional maturation.



2. Control hiPSC-MNs co-cultured with myoblasts display four distinct electrophysiological signatures



3. Co-culture of control hiPSC-MNs co-cultured with mouse myoblasts



Bos, Rihan et al. **2022.** *Neurobiology of disease*

hiPSC-MNs from patients with VRK1 mutations have altered Action Potential waveform and shorter Axonal Initial Segment

1. hiPSC-MNs from patient II.2 display similar electrophysiological firing patterns than controls

Four distinct electrophysiological signatures obtained in Control hiPSC-MNs with myoblasts (>DIV30) and in patient II.2 hiPSC-MN

hiPSC-MNs from patient II.2 with VRK1 mutations (>DIV30)

Neurobiology of Disease 164 (2022) 105609

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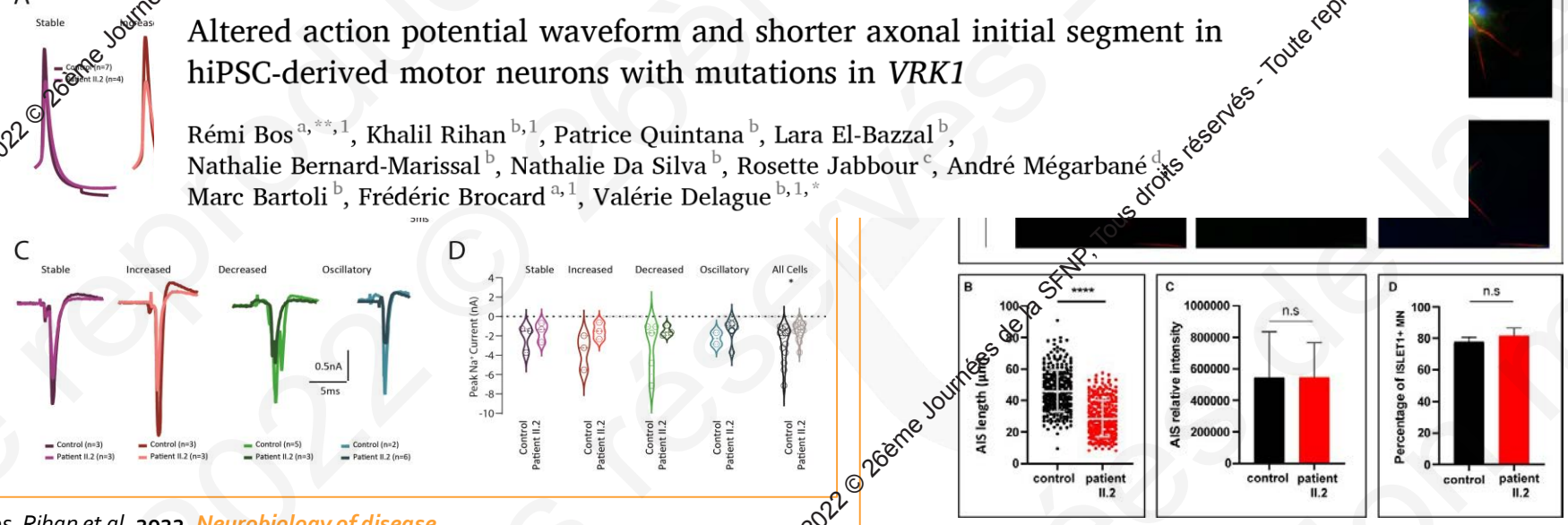
journal homepage: www.elsevier.com/locate/ynbdi

2. hiPSC-MNs f… decrease or pea… …ients with mutated



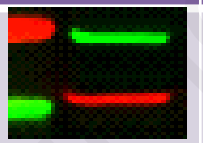
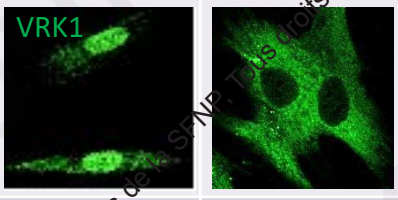
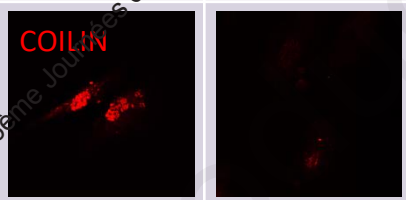
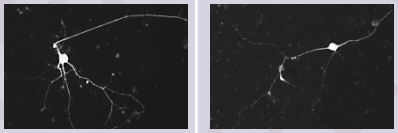
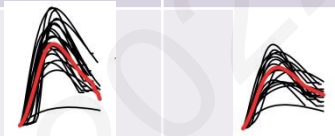
Altered action potential waveform and shorter axonal initial segment in hiPSC-derived motor neurons with mutations in *VRK1*

Rémi Bos [a,**,1], Khalil Rihan [b,1], Patrice Quintana [b], Lara El-Bazzal [b], Nathalie Bernard-Marissal [b], Nathalie Da Silva [b], Rosette Jabbour [c], André Mégarbané [d], Marc Bartoli [b], Frédéric Brocard [a,1], Valérie Delague [b,1,*]

Bos, Rihan et al. **2022.** *Neurobiology of disease*

Summary

Novel mutations in VRK1 in a new form of CMT with upper motor neuron signs

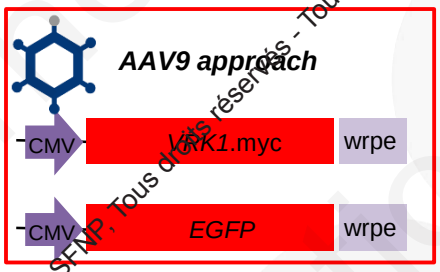
Control	Patient	
MNs		The levels of VRK1 are decreased in patients' cells including motor neurons
Fibroblasts		VRK1, a nuclear kinase is mislocalized to the cystoplasm
Fibroblasts		Coilin, a protein from the CB is not phosphorylated leading to ubiquitination and degradation by the proteasome, as well as CB disintegration
MNs		Role for VRK1 in the assembly of Cajal Bodies in motor neruons
MNs		Signs of axonal degeneration in iPS-MNs due to the absence of VRK1
MNs		AP of shorter amplitude and larger duration/ Shorter AIS

Perspectives: development of biotherapies

Gene replacement therapy by intrathecal injection of viral vectors in two mouse models of inherited peripheral neuropathies

1. Design of gene replacement therapy vectors

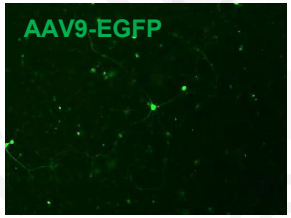
VRK1-HMN:
Delivery of VRK1 in MN



2. Evaluation of the therapeutic efficiency in vitro

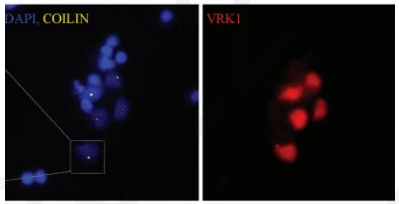
* Transduction efficiency of the cell type of interest

MN derived from hiPSC



* Efficiency capacity to improve the cellular defects

VRK1-HMN: restoration of Cajal bodies



El-bazzal et al., 2019

3. Evaluation of the therapeutic benefits in vivo

* Intrathecal injection at 6 week-old

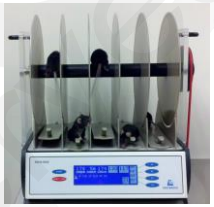
AAV9-CMV-VRK1 approach for VRK1-HMN

VRK1$^{GT3/GT3}$ mouse model

Vinogrand-Byk et al., 2018

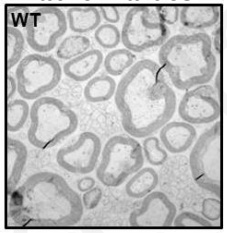
* Locomotor function assesment

At 6 and 12 months-old

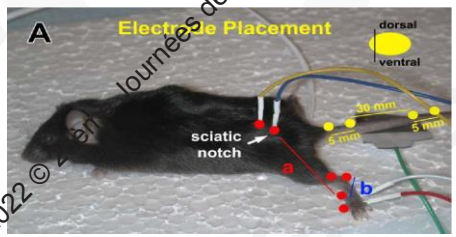


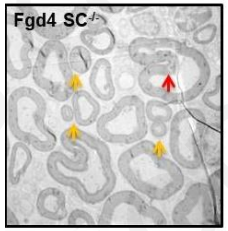
* Study of cellular defects

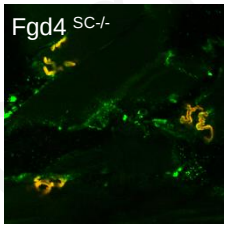
Myelin abnormalities



NMJ denervation

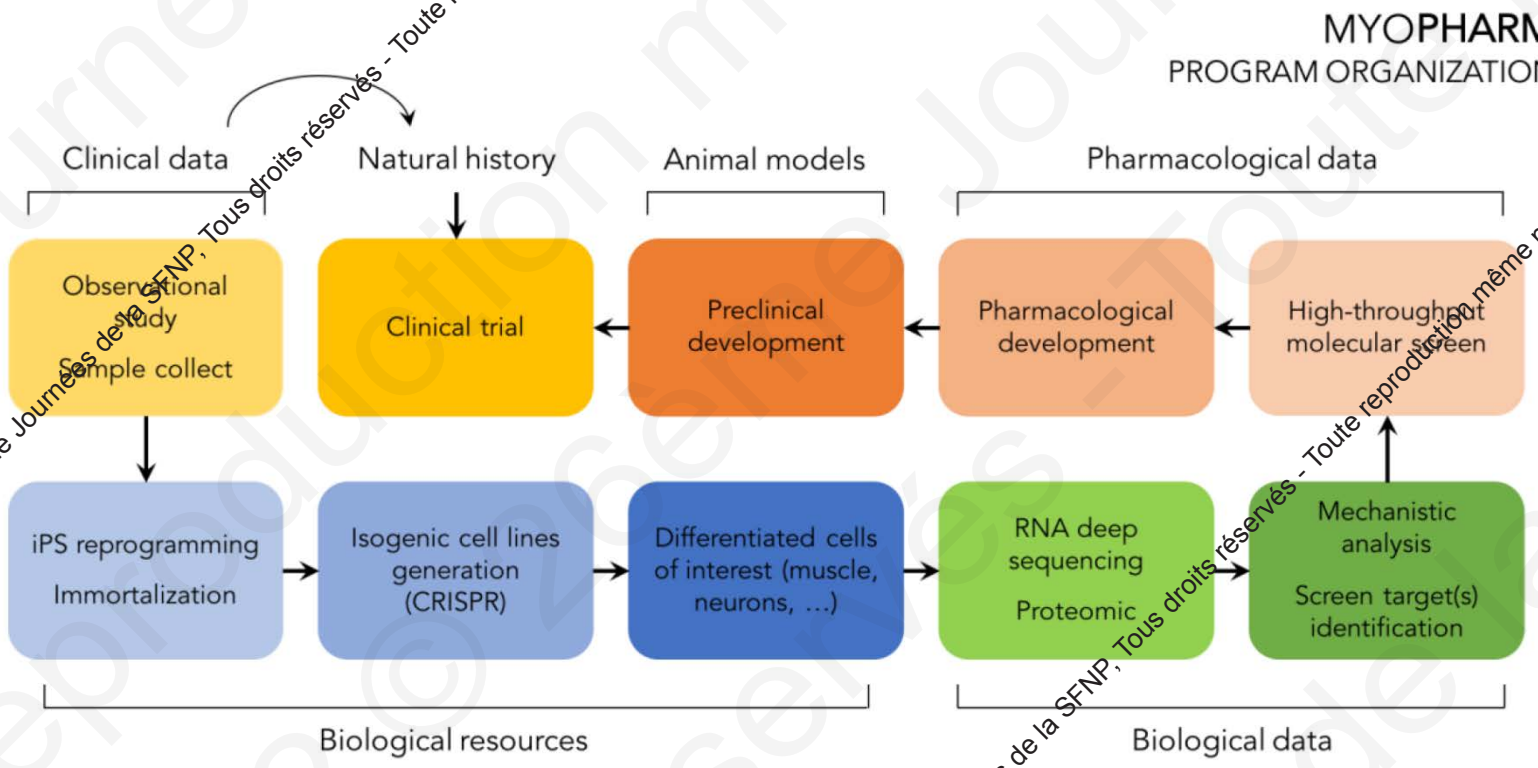






Perspectives

The MYOPHARM Project



The MYOPHARM Project

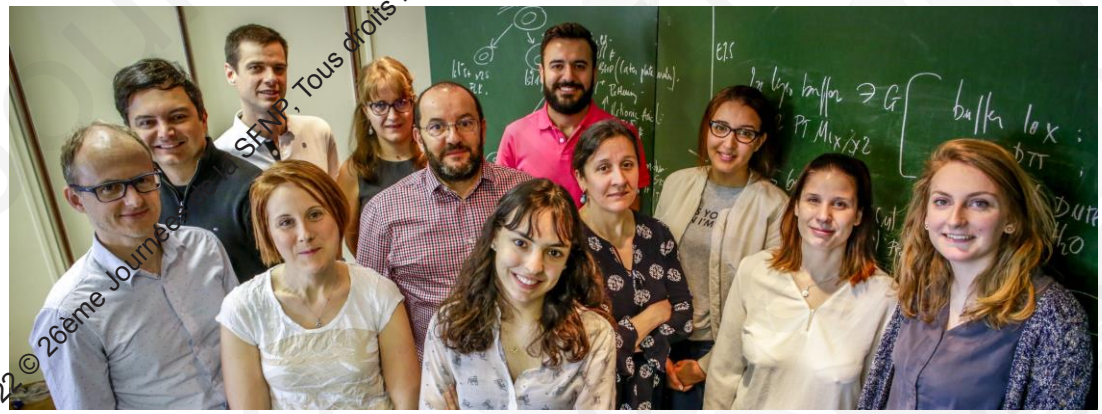
Novel mutations in VRK1 in a new form of CMT with upper motor neuron signs

- Use the molecular and electrophysiological defects already identified to define the readouts to use in the high throughput screen (HTS)
- Study these parameters in iPS-MN from the two other patients
- Define common readouts for the three different patients (with different mutations)
- Identify other readouts using transcriptomics and proteomics/phosphoproteomics study
- « Miniaturize » the processes to adapt to the conditions required for the HTS
- Use Multielectrode arrays to study electrphysiological properties of iPSC-MNs (also keeping in mind that we will need to adapt and « miniaturize » for HTS)

ACKNOWLEDGEMENT

- Patients and families

NEUROMYOLOGIE TRANSLATIONNELLE



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CONCLUSION

- We have identified novel mutations in VRK1 in a new form of CMT with upper motor neuron signs
- The levels of VRK1 are decreased in patients' cells including motor neurons
- Additionnaly, VRK1, a nuclear kinase is mislocalized to the cystoplasm
- Coilin, a protein from the CB is not phosphorylated leading to ubiquitination and degradation by the proteasome, as well as CB disintegration
- Absence of CB in motor neurons is likely to lead to altered transcription and splicing
- These findings suggest the existence of converging pathogenic mechanisms in our disease, SMA and ALS, with alteration/depletion of CBs as a hallmark