Pathophysiological mechanisms in a new form of Charcot-Marie-Tooth due to a mutation in *PDXK*

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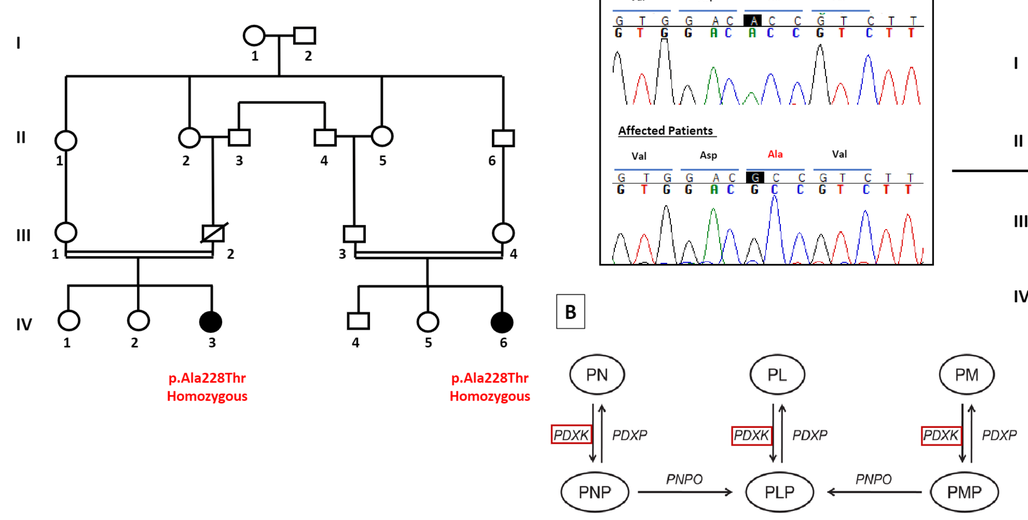
GENETIC ANALYSIS

Figure 1



Charcot-Marie-Tooth (CMT) disease is the **commonest inherited group of neuromuscular diseases**. This group of diseases, affecting the **peripheral** nervous system, is characterized by wide clinical and **genetic heterogeneity**, with around 100 genes identified to date[1]. Here, we present **4 new patients**, from two **consanguineous** Middle Eastern families, presenting with a rare subtype of CMT, for whom we identified, by Whole Exome Sequencing (WES), a **homozygous missense p.Ala228Thr mutation in the *PDXK* gene** (**A**). The same mutation was described previously by Chelban et al 2019, who first identified PDXK as responsible for CMT[2]. However, while the patients described by Chelban et al. are affected with axonal CMT associated with visual loss, our patients have **mixed axonal/demyelinating CMT** and no visual impairment. *PDXK* encodes for **Pirydoxal Kinase,** a cytoplasmic protein that converts the inactive B6 vitamers pyridoxine (PN), pyridoxal (PL), and pyridoxamine (PM) into catalytically active PLP (pyridoxal 5-phosphate) (**B**). PLP is an essential cofactor for more than 70 human enzymes implicated in diverse, essential, biological pathways, including amino acid and neurotransmitter metabolism.

FUNCTIONAL ANALYSIS

Figure 2 **Immortalized lymphoblastoid cells**

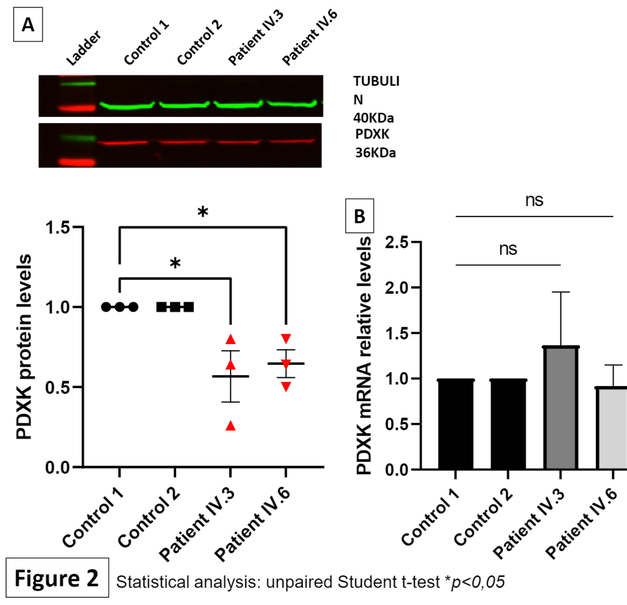
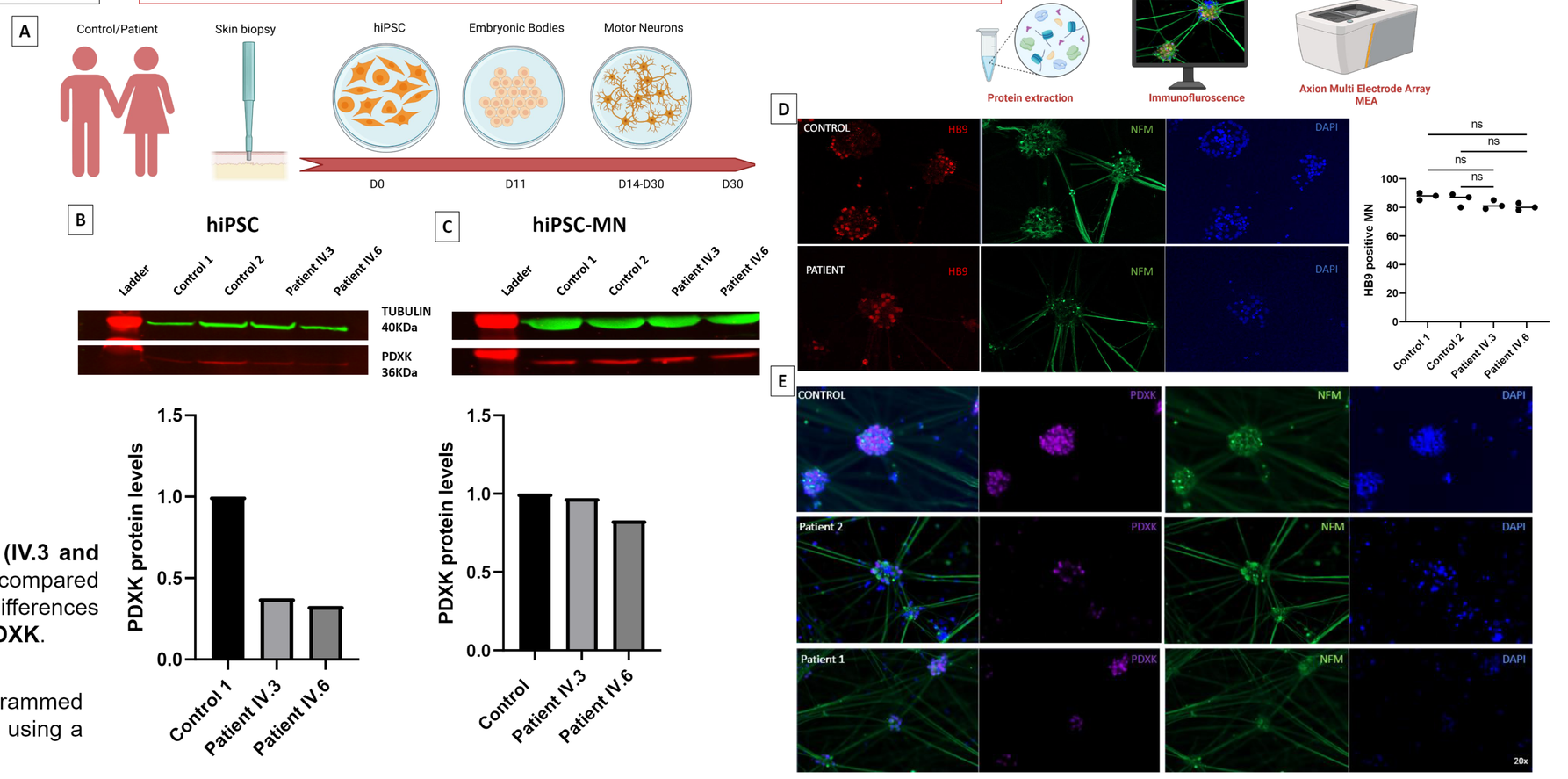


Figure 2 Statistical analysis: unpaired Student t-test *$p<0,05$

(**A**) In patients' immortalized lymphoblastoid cells from **Family 1 (IV.3 and IV.6)**, we have found a **40% decrease in PDXK protein levels** as compared to controls.(**B**) Interestingly, at the mRNA level, no significant differences were identified suggesting a **post-translational degradation of PDXK**.

Figure 3 **Human induced pluripotent stem cells derived Motor Neurons (hiPSC- MNs)**

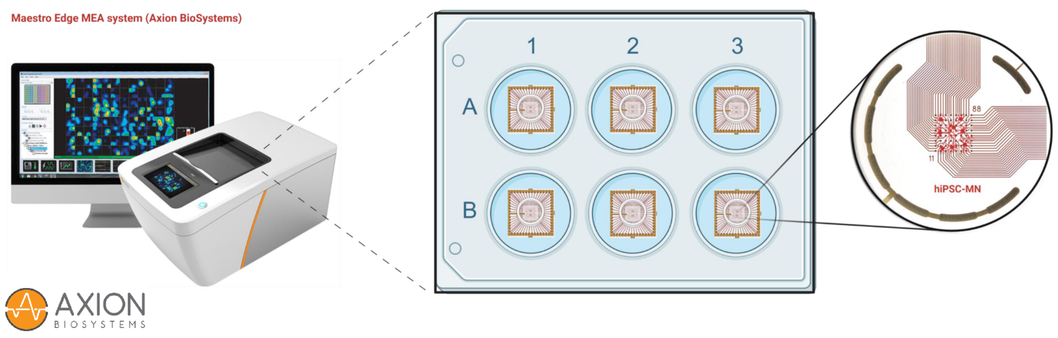


(**A**) **Human induced pluripotent stem cells (hiPSC)** were reprogrammed from skin fibroblasts. Mature spinal Motor Neurons were obtained using a 30-day differentiation protocol adapted from Maury et al.[3]

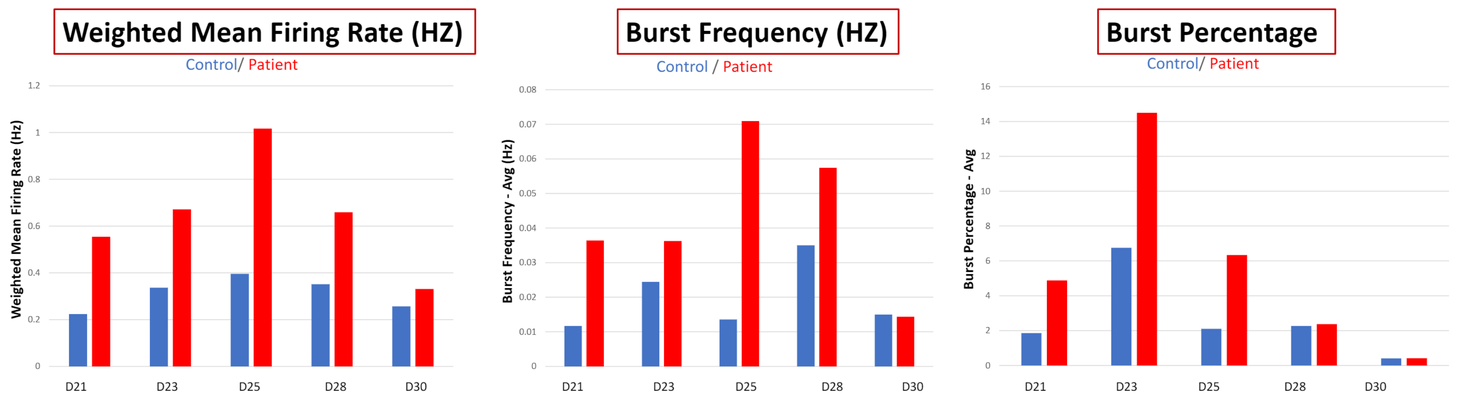
(**B**) We measured **PDXK protein levels in hiPSCs** and found the same **decrease** in PDXK protein levels than in lymphocytes. (**C**) Interestingly, in **Motor Neurons** differentiated from those hiPSCs (hiPSC-MNs), **this decrease was no further seen**. This finding suggests that, in dividing cells, the **degradation of mutant PDXK is more important than in "post-mitotic" cells, such as hiPSC-MNs.**(**D**) Immunolabeling of HB9 (red) and NF-M (green) at 30 days of differentiation showing no differences between conditions. (**E**) Immunolabeling of PDXK in hiPSC-MNs from patients and control show that **PDXK** (stained in purple) is found exclusively **in the cytoplasm of the nuclear body** and not the axons (labeled by **NFM, in green).**

ELECTRICAL ACTIVITY OF PATIENT'S hiPSC-MNs

Figure 4



DATA ANALYSIS



We have used Maestro Edge **MEA system** (Axion BioSystems) to measure the global electrical activity of our **hiPSC-MNs** at different differentiation stages (D21, D23, D25, D28 and D30). This his technology allows the recording of the action potentials of hiPSC-MNs plated in 6-well plates for 10 min inside a 37°C chamber at 5% CO2. We measured three different parameters, i.e. **weighted mean firing rate**, **burst frequency** and **burst percentages,** which characterize the general activity of the motor neurons. In this preliminary experiment (n=1), we found **an increase in all three parameters,** in both patients's hiPSC-MNs as compared to controls, at all differentiation time points. The increase seem to peak at differentiation day 25, but **the values come back to normal while MNs gets more mature (D25 to D30**). These results are preliminary and need to be completed, but they are in line with the differences observed by WB.

CONCLUSION

Here, we describe 4 new patients from 2 unrelated consanguineous families affected with a rare subtype of CMT, harboring **a homozygous p.Ala228Thr mutation in the *PDXK* gene.** Interestingly, our patients present a different phenotype than the ones described earlier with this mutation by Chelban et al, as they have mixed axonal/demyelinating CMT and no visual loss. Measurement of the global general activity using **MEA technology** suggest increased global electrical activity in patients' hiPSC-MNs before they reach maturity. These preliminary results will be completed by the assessment of the role for PDXK in myelination, by knock-down of the gene in an *in vitro* myelination model based on the coculture of mouse Dorsal Root Ganglion neurons and Schwann cells. This study is very encouraging toward using **hiPSC-derived motor neurons** to study the pathogenicity of mutations in this specific subtype of CMT, and in CMT in general.

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