ARHGAP39 variants cause microcephaly and cerebellar hypoplasia

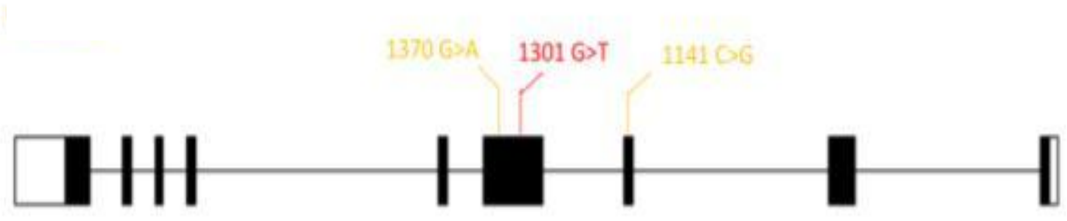
Carla Pagan - Angela Kaindl Laboratory

Introduction

Microcephaly is a neurodevelopmental disorder characterized by a significant reduction of the head circumference of affected children. The disturbance of various cellular processes such as cell proliferation/apoptosis and differentiation can lead to a deficit of mature neurons during development and thus to microcephaly. Rho GTPase activating proteins (RhoGAP) play a key role in several of these processes by catalysing the inactivation of Rho GTPases. We identified two non-related patients with novel mutations in *ARHGAP39*, a gene encoding a RhoGAP protein, harbouring microcephaly and cerebellar hypoplasia.

Aims

- Study of the second index patient (P2) fibroblasts to determine which cellular mechanism is altered by the mutations and how the mutations induce microcephaly.
- Creation of an *in vitro* model to study the effect of both patients mutations on cortical neurons.



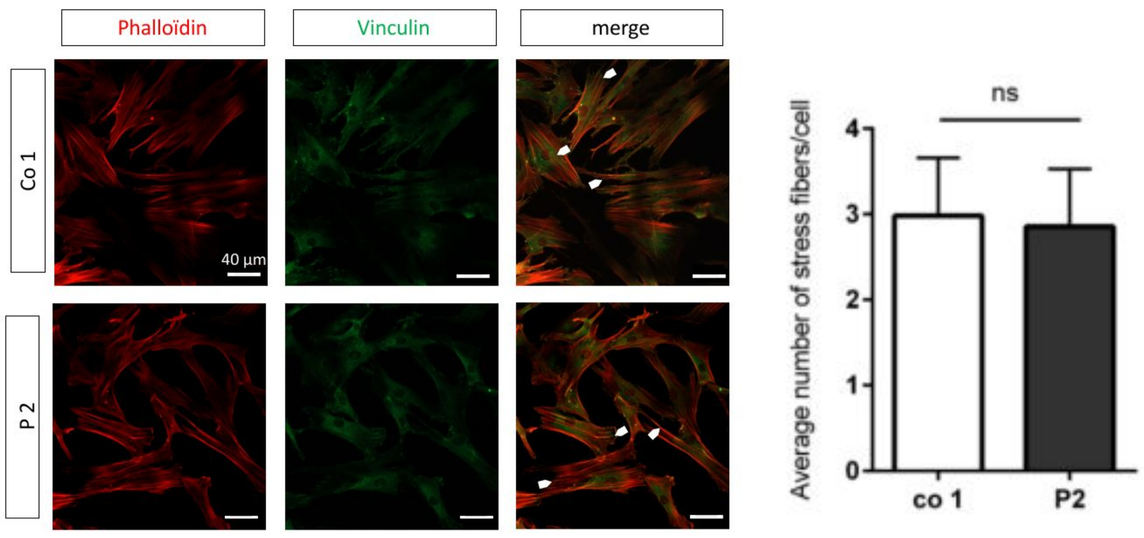
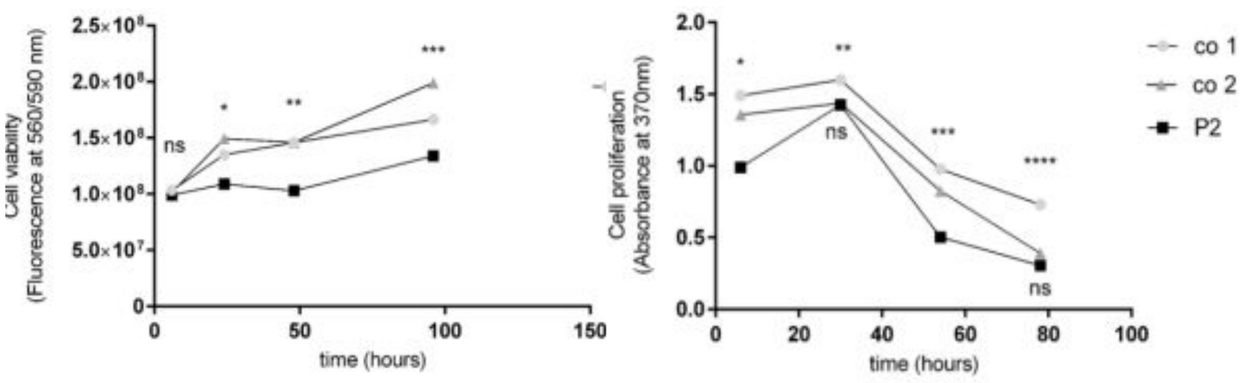


Fig. 3 : Actin and vinculin staining of patient (P2) and control fibroblasts. Arrows : stress fibers

76.64 Kb
Reverse strand

Sample	Variant	Mutation	Polyphen-2	SIFT	MutationTaster
Index patient 1	NM_025251	c.1301G>T; p.Cys434Phe	Possibly damaging (0.939)	Deleterious (0.02)	Disease causing
Index patient 2	NM_001308207	c.1141C>G, p.Arg381Gly	Possibly damaging (0.865)	Deleterious (0.01)	Disease causing
Index patient 2	NM_001308207	c.1370G>A, p.Arg457Gln	Benign (0.134)	Tolerated (0.07)	Polymorphism

Fig. 1 : ARHGAP39 genotype of the two patients



	24h	48h	72h
p-value co1/P2	0.0006	0.0379	0.0148
p-value co2/P2	0.0002	0.0019	0.0006

	6h	30h	54h	78h
p-value co1/P2	0.002	0.0499	0.0002	0.0002
p-value co2/P2	0.006	ns	0.0002	ns

Fig. 4 : Viability and proliferation assays of P2 fibroblasts

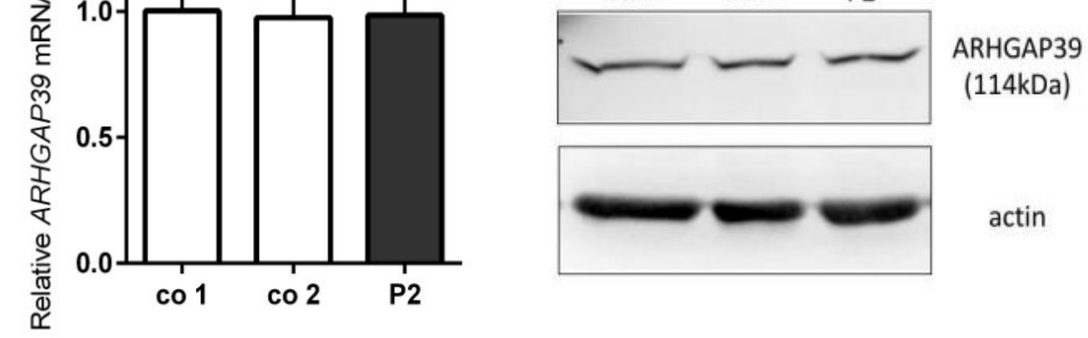


Fig. 2 : Analysis of ARHGAP39 expression in P2 fibroblasts. Quantification of mRNA and protein levels (RT PCR : n = 4, Western : n = 6)

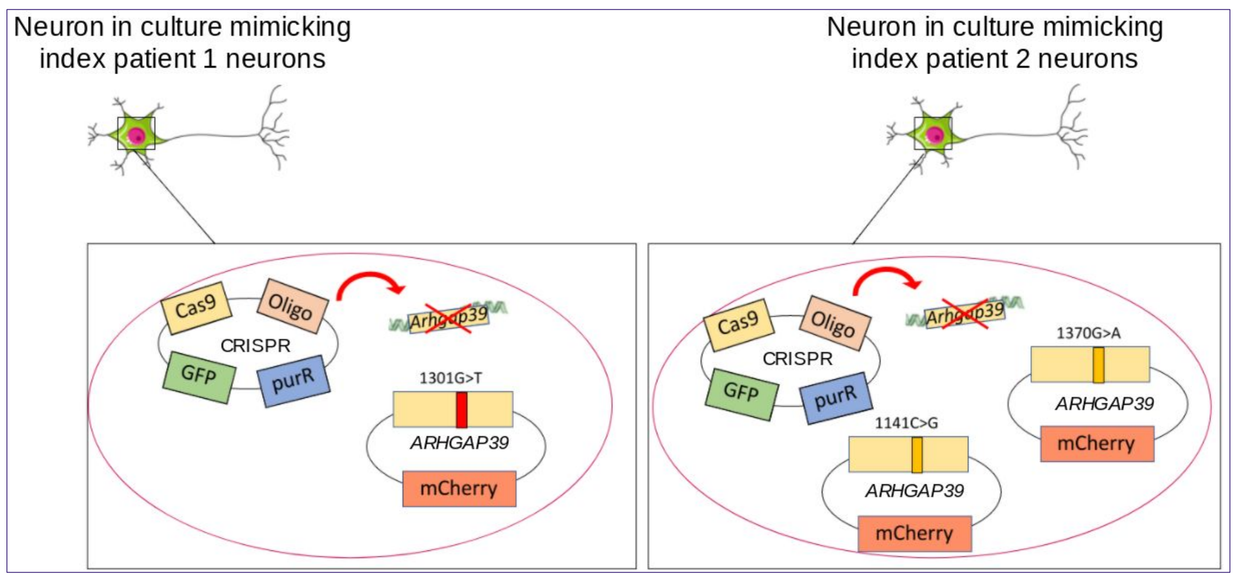


Fig. 5: In vitro model of patients' neurons

Experimental approach and results

Our study is divided in two parts :

- The analysis of the effect of the second patient (P2) mutations on his cellular phenotype (fibroblasts analysis)
- The use of an *in vitro* model to study the patients' mutations effect in cultured neurons

Fibroblasts analysis showed that P2 mutations do not induce changes in *ARHGAP39* expression or in the organization of fibroblasts cytoskeleton but seem to induce a defect of cell viability and proliferation. To build our *in vitro* model, we planned to generate neurons in which the endogenous *Arhgap39* has been inactivated by CRISPR-Cas9 and which are overexpressing the human *ARHGAP39* carrying one or two of the patients' mutations, induced by site-directed mutagenesis.

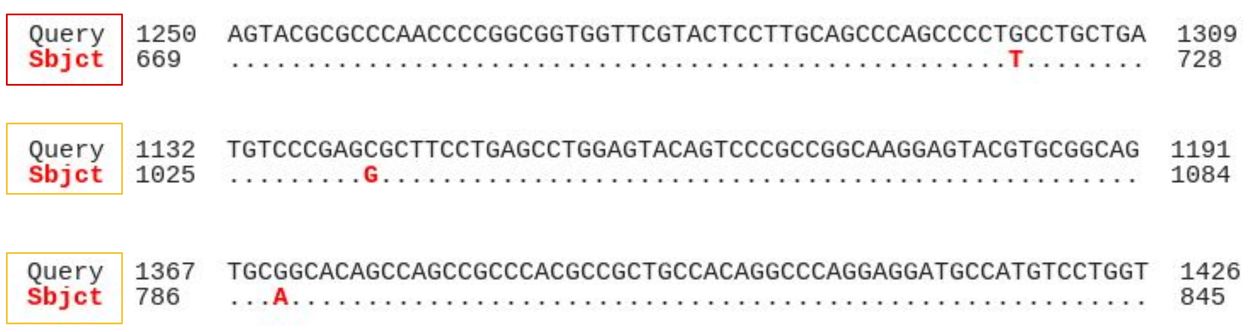


Fig. 6 : Sanger sequencing of the three plasmids containing mutated ARHGAP39 genes (obtained by site-directed mutagenesis)

Conclusion & perspectives

Once we have generated our plasmids containing mutated *ARHGAP39*, we will transfect cortical primary neurons with both the CRISPR and the mutated *ARHGAP39* plasmids. We will then analyse the proliferation and the dendritic arborization of the mutated neurons in order to determine if the patients mutations are also affecting neuronal proliferation and dendritic branching. In order to validate our model, we also planned to transfect our constructs in the deepest layers of mouse embryo cortex using electroporation in utero.

References :

1. von der Hagen, M. et al. (2014). Diagnostic approach to microcephaly in childhood: a two-center study and review of the literature. Developmental medicine and child neurology, 56(8), 732–741. https://doi.org/10.1111/dmcn.12425
2. Jean, F. et al. (2020). Dissecting the Genetic and Etiological Causes of Primary Microcephaly. Frontiers in neurology, 11, 570830. https://doi.org/10.3389/fneur.2020.570830
3. Lee, J. et al. (2017). Important roles of Vilse in dendritic architecture and synaptic plasticity. Scientific reports, 7, 45646. https://doi.org/10.1038/srep45646