Master de Sciences et Technologies
Mention Biologie Intégrative et Physiologie
Parcours : Neurosciences
Responsable : Professeur Régis Lambert

Internship Proposal
Academic Year 2018-2019

1. Host team :

Research Unit (e.g. Department or Institute) : Inserm UMRS-839, Institut du Fer à Moulin
Research Unit Director : Dr Jean-Antoine GIRAULT
Research Team Director : GOUTEBROZE Laurence / FRANCIS Fiona
Team name : Cortical Development and Pathology
Address : 17 rue du Fer à Moulin, 75005 Paris

Supervisor of the Research Intern for this project : GOUTEBROZE Laurence
Telephone : 33 1 45 87 61 44
E-mail : laurence.goutebroze@inserm.fr

2. Internship project title:

Contribution of autism-related Caspr2/*CNTNAP2* to mouse brain connectivity

3. Internship Description :

Autism Spectrum Disorders (ASD) represent a heterogeneous group of highly prevalent neurodevelopmental disorders (1/160 new-born in the world), which are characterized by social interaction and communication deficits, and repetitive/restricted behaviours. These conditions are considered to result from abnormal development and function of brain connectivity between cortical areas. Current consensus converges on the idea that the majority of ASD cases could be caused by a complex interaction of multiple genetic and environmental risk factors, with a strong heritable component. The gene *CNTNAP2*, encoding the protein Caspr2, is thought to be one of the major susceptibility genes for ASD, with a very large number of heterozygous missense variants identified in patients, in most cases inherited from one non-affected parent[1-3]. *Cntnap2* mutant mice display ASD-related behavioral deficits supporting human genetic data[4]. However, the clinical significance of the variants is still debated. **Our work** aims to provide a proof of principle that some *CNTNAP2* variants may be pathogenic and to evaluate their interactions with genetic background and environmental factors in ASD, by combining interrelated functional, phenotypical and genetic studies in mouse and human neurons, the latter derived from induced pluripotent stem cells (hiPSCs) from ASD families.

Caspr2 is a cell-adhesion glycoprotein of the neurexin family, which is well-known for its function in the juxtaparanodal domains of the nodes of Ranvier in mature myelinated neurons. We originally helped show that in these domains Caspr2 is involved in the organization of specific axoglial contacts, forming a cytoskeleton-associated complex *in cis* and *in trans* with the cell-adhesion molecule TAG-1, required for *Shaker*-type Kv1 channel clustering[5,6]. High expression of *Cntnap2* in the brains of mouse embryos suggested additional roles for Caspr2 during development. Performing cortical neuron cultures, we recently showed that Caspr2 plays a dose-dependent function in axon growth *in vitro*. A haplo-insufficiency reducing the expression of Caspr2 by half is sufficient to elicit axon growth defects, revealing a situation which may be relevant for *CNTNAP2* heterozygosity in ASD patients. Testing nucleotide variants identified in patients in a *Cntnap2* heterozygous genetic background, we found that they displayed either a dominant-negative effect or a loss-of-function during axon growth, through different

mechanisms. Thus, our data identify a new neurodevelopmental role for Caspr2, and provide evidence that *CNTNAP2* heterozygous missense variants may contribute to pathogenicity of ASD (Canali et al., in press).

During embryonic development, axon growth and guidance are early events essential for establishing brain connectivity. They control the development of cortico-cortical projections forming the corpus callosum (CC), which is a major myelinated interhemispheric tract allowing bilateral integration of lateralized sensory inputs and regulation of higher-order cognitive, social and emotional processing[7]. The cell-autonomous function of Caspr2 in axon growth supports the hypothesis that Caspr2 could contribute to the normal development and patterning of these projections *in vivo*. Performing preliminary morphological and electron microscopy analyses, we found that *Cntnap2* mutant mice present CC abnormalities at E17.5, as well as abnormalities of the anterior commissure (AC), which is another myelinated interhemispheric tract connecting mainly the piriform cortex. This suggests that Caspr2 may also play major roles in axon growth, fasciculation and/or guidance *in vivo*, the dysregulation of which may contribute to clinical manifestations of ASD. Axon defects may indeed influence network dynamics of cortical neurons and perturb long-distance functional connectivity, as previously described in ASD patients in whom long-range cortico-cortical functional and structural connectivity appears to be weaker than in controls[8,9]. On the basis of these observations, **we aim now to:**

(a) further characterize CC and AC abnormalities through development until adulthood in *Cntnap2* mutant mice, using immunostaining/epifluorescence microscopy, electron microscopy, and *in utero* electroporation coupled with light-sheet imaging to follow individual axons. This will help precise potential defects in axon diameter, fasciculation, guidance and myelination.

(b) investigate the molecular and cellular mechanisms underlying the axonal phenotypes, using mainly *in vitro* approaches (cultures of cortical neurons and explants, myelinating cultures; state-of-the-art imaging; biochemistry to evaluate protein-protein interactions).

(c) assess the degree of pathogenicity of a specific *CNTNAP2* variant *in vivo*. Collaborating with geneticists we identified a variant which leads to retention of Caspr2 in the endoplasmic reticulum (ER) and plays a dominant-negative effect on axon growth *in vitro*. We plan to evaluate to what extent this variant induces CC/AC development anomalies, the axonal mechanism dysregulation, and ASD-like behaviors (repetitive behaviors, social deficits), in a newly-derived knock-in (KI) mouse model.

(d) assess possible corrective strategies, by screening for drugs that overcome the dominant effect of the ER-retained variant. We will ask whether proteostasis regulators or chemical chaperones may improve folding and trafficking of the variant and rescue phenotypes in KI mouse cortical neurons *in vitro* and in KI mouse brain slices (CC/AC development). The efficacy of such drugs has been validated both *in vitro* and in mouse models[10,11].

The M2 student will contribute to projects a and b during its internship, and will be welcome to further develop projects b, c and d within the framework of a PhD.

References:

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5 Rasband MN & Peles E. 2015 *Cold Spring Harb Perspect Biol* 8, a020495.
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7 Leyva-Diaz E & Lopez-Bendito G. 2013 *Neuroscience*, **254**, 26.
8 Vissers ME et al. 2012 *Neurosci Biobehav Rev*, 36, 604.
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