APPEL A PROJETS DE THÈSE 2026
CONCOURS DE L'ED 658 – Sciences du vivant

Formulaire à compléter et déposer par les porteurs du projet de thèse HDR via l'adresse mail suivante : ed658@univ-amu.fr OU/ET romane.haddadi@univ-amu.fr
MERCI DE METTRE EN SUJET DU MAIL : Concours ED 658 2026 porteur de projet
Ces dossiers seront publiés pour que les candidats postulent.

au plus tard le 3 mars 2026.

INTITULÉ DU PROJET

Dysfonctionnement de KCC2 et altération de la transmission GABAergique dans un trouble du spectre autistique monogénique

KCC2 dysfunction and altered GABAergic signaling in a monogenic autism spectrum disorder

1- Directrice ou directeur de thèse HDR			
NOM	Porcher	Prénom	Christophe
Téléphone portable	0623148877	Courriel	Christophe.porcher@univ-amu.fr
Unité de recherche (UR) de rattachement			
Code (acronyme et n°) de l'UR à AMU :	UMR_S 1249		
Nom de l'UR :	INMED - Institut de Neurobiologie de la Méditerranée		
Directrice ou directeur de l'UR			
Nom	Cossart	Prénom	Rosa

2- Codirectrice ou codirecteur de thèse, le cas échéant			
NOM	Medyna	Prénom	Igor
Téléphone portable	0610118388	Courriel	Igor.medyna@inserm.fr
Unité de recherche (UR) de rattachement			
Code (acronyme et n°) de l'UR à AMU :	UMR_S 1249		
Nom de l'UR :	INMED - Institut de Neurobiologie de la Méditerranée		
Directrice ou directeur de l'UR			
Nom	Cossart	Prénom	Rosa

SPECIALITE : (Rayer les mentions inutiles)

~~Bio-informatique et Génomique / Biochimie Structurale / Biologie du Cancer / Biologie du Développement / Biologie Végétale / Biotechnologie / Immunologie / Microbiologie et interactions hôte-pathogènes~~ / Neurosciences

SOUS JURY POUR LE CONCOURS : (Rayer les mentions inutiles)
JURY 1 ~~: BIOLOGIE CELLULAIRE~~
JURY 2 ~~: MICROBIOLOGIE – GENOMIQUE~~
JURY 3 : NEURSOCIENCES

ED658 - Ecole Doctorale Sciences du Vivant T +33 (0)4 13 94 19 63 Site: https://ecole-doctorale- 658.univ-amu.fr/ Campus Luminy - 163 Avenue de Luminy - Marseille, 13009 Email: ed658@univ-amu.fr Bat TPR1 – RdC - bureau E.007

APPEL A PROJETS DE THÈSE 2026
CONCOURS DE L'ED 658 – Sciences du vivant

MERCI DE REMPLIR TOUTES LES INFRORMATIONS EN FRANÇAIS ET EN ANGLAIS

ED658 - Ecole Doctorale Sciences du Vivant T +33 (0)4 13 94 19 63 Site: https://ecole-doctorale- 658.univ-amu.fr/ Campus Luminy - 163 Avenue de Luminy - Marseille, 13009 Email: ed658@univ-amu.fr Bat TPR1 – RdC - bureau E.007

APPEL A PROJETS DE THÈSE 2026
CONCOURS DE L'ED 658 – Sciences du vivant

Description de la problématique de recherche - Project description

Ce projet vise à étudier une famille de patients (« Dylan ») présentant un trouble du spectre autistique (TSA) associé à deux mutations distinctes du gène *SLC12A5*, codant pour le cotransporteur potassium-chlorure KCC2. Spécifiquement exprimé dans les neurones, KCC2 joue un rôle central dans la régulation de la concentration intracellulaire des ions chlorure, conditionnant ainsi l'efficacité de la transmission synaptique inhibitrice médiée par le GABA.

De nombreuses données expérimentales suggèrent qu'une altération de l'homéostasie du chlorure et de l'équilibre excitation/inhibition constitue un mécanisme clé dans la physiopathologie des TSA. Toutefois, à ce jour, aucun lien de causalité direct entre un dysfonctionnement de KCC2, la perturbation de l'homéostasie du chlorure et l'émergence des TSA n'a pu être formellement établi chez l'homme. L'identification de mutations rares de *SLC12A5* dans cette famille représente une opportunité unique pour explorer, à l'aide de modèles cliniques et précliniques, les mécanismes neuronaux sous-jacents reliant la dysfonction de KCC2 aux altérations neurodéveloppementales observées dans les TSA.

This project aims to investigate a family of patients ("Dylan") affected by autism spectrum disorder (ASD) associated with two distinct mutations in the *SLC12A5* gene encoding the potassium-chloride cotransporter KCC2. KCC2 is specifically expressed in neurons and plays a pivotal role in regulating intracellular chloride concentration, thereby determining the efficacy of GABAergic inhibitory synaptic transmission.

Accumulating experimental evidence suggests that disruption of chloride homeostasis and excitation/inhibition balance represents a key mechanism in the pathophysiology of ASD. However, a direct causal relationship between KCC2 dysfunction, altered chloride homeostasis, and ASD has not yet been formally demonstrated in humans. The identification of rare *SLC12A5* mutations in this family provides a unique opportunity to investigate, using both clinical and preclinical models, the neuronal mechanisms linking KCC2 dysfunction to neurodevelopmental alterations associated with ASD.

ED658 - Ecole Doctorale Sciences du Vivant T +33 (0)4 13 94 19 63 Site: https://ecole-doctorale- 658.univ-amu.fr/ Campus Luminy - 163 Avenue de Luminy - Marseille, 13009 Email: ed658@univ-amu.fr Bat TPR1 – RdC - bureau E.007

Mots clés – Keywords

KCC2, autisme, GABA, épilepsie / KCC2, autism, GABA, epilepsy

Thématique / Domaine / Contexte - Theme / Field / Context

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder (NDD) characterized by impairments in social interaction and communication, as well as restricted and repetitive behaviors[1]. ASD arises from a combination of genetic and environmental factors and is frequently comorbid with epilepsy. Despite extensive research, the mechanisms underlying ASD remain poorly understood, and no curative treatment is currently available.

Although ASD is etiologically heterogeneous, converging evidence suggests that distinct genetic and environmental insults may converge on common neurobiological pathways. Several recent studies have proposed that impaired neuronal chloride (Cl^-) homeostasis, leading to altered inhibitory strength of GABA_A receptor–mediated neurotransmission, plays a critical role in ASD pathophysiology and may represent a shared mechanism across subsets of patients[2, 3, 4]. However, to date, no study has established a direct causal link between disrupted chloride homeostasis and ASD.

The neuron-specific potassium–chloride cotransporter KCC2 is a key regulator of intracellular chloride concentration ($[Cl^-]i$) during both neuronal development and adulthood[5]. Recently, Partner 2 identified and is following a family (hereafter referred to as "Dylan") in which ASD segregates with two previously unreported mutations in the *SLC12A5* gene encoding KCC2. We hypothesize that this extremely rare combination of functionally deleterious KCC2 mutations leads to dysregulation of neuronal chloride homeostasis, resulting in altered inhibitory neurotransmission and the emergence of ASD.

Adopting a translational approach spanning from the clinic to experimental models, this project aims to: (i) extend the clinical and genetic characterization of affected patients and their families; (ii) characterize the molecular, electrophysiological, and behavioral phenotypes of transgenic mice carrying Dylan-like KCC2

ED658 - Ecole Doctorale Sciences du Vivant T +33 (0)4 13 94 19 63 Site: https://ecole-doctorale- 658.univ-amu.fr/ Campus Luminy - 163 Avenue de Luminy - Marseille, 13009 Email: ed658@univ-amu.fr Bat TPR1 – RdC - bureau E.007

mutations generated for this project; and (iii) identify pharmacological compounds capable of restoring KCC2 function in vivo, with the long-term goal of therapeutic development.

Preliminary results

As a prerequisite for this project, we have generated compelling preliminary data supporting the central hypotheses. First, pathogenic mutations in *SLC12A5* were identified in the Dylan family. Second, functional analyses of the Dylan1 and Dylan2 KCC2 mutants, transiently expressed in murine N2a neuroblastoma cells, revealed a significant increase in resting intracellular chloride concentration and impaired KCC2-mediated ion transport. All mutant constructs also exhibited reduced surface expression. Third, Dylan-like transgenic mouse lines were generated using CRISPR-Cas9 technology through an international collaboration (University of Oldenburg, Germany; Weizmann Institute, Israel). Heterozygous KCC2D1/+ and KCC2D2/+ mice are viable and fertile, and their intercross produces offspring of all expected genotypes at Mendelian ratios. Homozygous KCC2D1/D2 mice, which genetically recapitulate the patient condition, display normal early postnatal development, making them suitable for in-depth phenotypic analyses, a major objective of the present project.

Finally, strong support for a causal relationship between KCC2 dysfunction and ASD-like phenotypes comes from previous studies using transgenic mouse models with impaired KCC2 regulation. In particular, KCC2E/+ mice carrying phosphomimetic loss-of-function mutations (T906E/T1007E) exhibit increased neuronal [Cl^-]i, reduced GABAergic inhibition, an altered glutamate/GABA synaptic ratio, abnormal ultrasonic vocalizations during early development, and persistent social deficits in adulthood[7]. Similar phenotypes have been reported in KCC2S940A mice with disrupted KCC2 phosphorylation[8]. Although these mutations are not associated with known human diseases, together these findings strongly support the hypothesis that KCC2 dysfunction is sufficient to drive ASD-like neurodevelopmental pathology. To date, however, no in vivo study has directly examined the causal impact of patient derived KCC2 mutations on neurodevelopmental disorders, a critical gap that this project aims to address.

ED658 - Ecole Doctorale Sciences du Vivant T +33 (0)4 13 94 19 63 Site: https://ecole-doctorale- 658.univ-amu.fr/ Campus Luminy - 163 Avenue de Luminy - Marseille, 13009 Email: ed658@univ-amu.fr Bat TPR1 – RdC - bureau E.007

Objective 1 – Phenotypic characterization of KCC2 mutant mouse models

A central objective of the PhD project is the comprehensive phenotypic analysis of transgenic mice carrying Dylan-like *Kcc2* mutations (KCC2D1/D2), alongside heterozygous and wild-type controls, across key developmental stages (P0, P7, P21, and P90). These analyses will focus on parameters known to be altered in neurodevelopmental disorders.

1.1 Behavioral and cognitive analyses

The PhD candidate will assess general health, social behavior, and cognitive performance using standardized behavioral paradigms relevant to autism-like phenotypes. Advanced behavioral monitoring tools, including long-term group analysis using artificial intelligence–based tracking systems, will be employed to quantify social interactions and activity patterns in enriched environments[7, 9, 10].

1.2 Anatomical, molecular, and biochemical analyses

The project will investigate brain architecture, KCC2 expression levels, and phosphorylation state in mutant mice, as these parameters critically regulate neuronal chloride homeostasis and inhibitory neurotransmission and are implicated in ASD and epilepsy.

1.3 Neuronal chloride homeostasis and excitatory/inhibitory balance ex vivo

Intracellular chloride regulation and the developmental shift of GABAergic signaling will be examined using electrophysiological recordings from CA3 pyramidal neurons in acute hippocampal slices. These experiments will target developmental windows during which altered chloride homeostasis has been implicated in neurodevelopmental disorders[3, 7, 9, 11].

1.4 Emergence of coordinated network activity in vivo

The impact of KCC2 mutations on the emergence of coordinated cortical and hippocampal network activity will be assessed using in vivo recordings in non-anesthetized mice. Network dynamics, including local field potential characteristics and neuronal recruitment, will be compared between mutant and control mice during critical developmental periods.

1.5 Seizure susceptibility

Given the strong association between neurodevelopmental disorders and epilepsy, seizure susceptibility will be assessed in KCC2 mutant mice using established pharmacological paradigms, providing functional links between KCC2 dysfunction, network excitability, and behavioral outcomes.

Objective 2 – Evaluation of therapeutic strategies targeting KCC2

ED658 - Ecole Doctorale Sciences du Vivant T +33 (0)4 13 94 19 63 Site: https://ecole-doctorale- 658.univ-amu.fr/ Campus Luminy - 163 Avenue de Luminy - Marseille, 13009 Email: ed658@univ-amu.fr Bat TPR1 – RdC - bureau E.007

To establish a functional link between the Dylan1 and Dylan2 mutations, altered KCC2 ion transport, and neurobehavioral and seizure phenotypes, this PhD project will evaluate the efficacy of pharmacological strategies aimed at restoring chloride homeostasis and GABAergic signaling in KCC2D1/D2 mice. The goal is to determine whether modulation of KCC2 activity can rescue cellular, network, and behavioral abnormalities associated with these mutations.

Several classes of KCC2- and chloride-modulating compounds will be investigated. These include:
(i) leptin receptor antagonists, previously shown to potentiate KCC2 function through inhibition of leptin signaling;
(ii) the antipsychotic drug prochlorperazine, recently identified as a pharmacological activator of KCC2 but not yet evaluated in mouse models of neurodevelopmental disorders[13];

(iii) the selective WNK kinase inhibitor WNK463, a potent activator of KCC2 shown to reduce seizure severity in experimental epilepsy models[14];

(iv) a set of putative KCC2-activating compounds developed by AstraZeneca, selected for their ability to enhance KCC2 function and authorized for evaluation in experimental models.

The effects of these compounds will be assessed in KCC2D1/D2 mice at early developmental stages (P15) by measuring neuronal intracellular chloride concentration, the excitatory/inhibitory action of GABA, the GABA/glutamate synaptic ratio, and susceptibility to flurothyl-induced seizures. At later stages (P60), drug efficacy will be evaluated through the analysis of social behavior. Together, these experiments will provide a mechanistic framework to evaluate the therapeutic potential of KCC2-targeting strategies in neurodevelopmental disorders associated with impaired chloride homeostasis.

Methodology

The PhD project will rely on a combination of well-established experimental approaches and state-of-the-art techniques commonly used in neuroscience research. These include patch-clamp electrophysiology[3, 7, 11], confocal imaging[15], biochemical analyses[11, 15], and live-cell immunolabeling[11, 15] to investigate KCC2 expression, localization, and function at the molecular and cellular levels.

In vivo and ex vivo experimental approaches will be complemented by behavioral analyses[3, 7, 9] using validated paradigms relevant to neurodevelopmental disorders. Advanced behavioral monitoring systems incorporating artificial intelligence–based analysis will be employed to quantify social interactions and activity patterns over extended periods.

All methodologies proposed in this project are already implemented within the host laboratory environment or through established collaborations and have been validated in previous peer-reviewed publications, ensuring technical feasibility within the timeframe of a PhD project.

ED658 - Ecole Doctorale Sciences du Vivant T +33 (0)4 13 94 19 63 Site: https://ecole-doctorale- 658.univ-amu.fr/ Campus Luminy - 163 Avenue de Luminy - Marseille, 13009 Email: ed658@univ-amu.fr Bat TPR1 – RdC - bureau E.007

Risk management

The experimental strategy integrates multiple complementary levels of analysis, from molecular and cellular investigations to network and behavioral readouts, thereby minimizing the risk associated with any single experimental approach. In the event that specific phenotypes are subtle or variable, alternative developmental stages, brain regions, or functional readouts will be explored to ensure robust conclusions.

Together, these approaches will allow a detailed assessment of how ASD-associated *SLC12A5* mutations affect KCC2 function and neuronal physiology in vitro and in vivo.

Ambition and expected outcomes

This PhD project is ambitious in that it aims to:

1. generate and characterize one of the first mouse models of autism spectrum disorder based on monogenic mutations in *KCC2*;
2. establish a direct causal link between KCC2 dysfunction, altered chloride homeostasis, and neurodevelopmental pathology;
3. describe KCC2-dependent alterations across molecular, cellular, and neuronal network levels;
4. provide a mechanistic framework for the development of therapeutic strategies targeting KCC2 and chloride homeostasis

Références bibliographiques

1. **Weintraub K.** The prevalence puzzle: Autism counts. *Nature* **479**, 22–24 (2011). https://doi.org/10.1038/479022a
2. **Cellot G., Cherubini E.** GABAergic signaling as therapeutic target for autism spectrum disorders. *Frontiers in Pediatrics* **2**, 70 (2014). https://doi.org/10.3389/fped.2014.00070
3. **Tyzio R., Nardou R., Ferrari D.C., Tsintsadze T., Shahrokhi A., Eftekhari S., Khalilov I., Tsintsadze V., Brouchoud C., Chazal G., Lemonnier E., Lozovaya N., Burnashev N., Ben-Ari Y.** Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring. *Science* **343**, 675–679 (2014). https://doi.org/10.1126/science.1247190
4. **Deidda G., Parrini M., Naskar S., Bozarth I.F., Contestabile A., Cancedda L.** Reversing excitatory GABA(_A) receptor signaling restores synaptic plasticity and memory in a mouse model of Down syndrome. *Nature Medicine* **21**, 318–326 (2015). https://doi.org/10.1038/nm.3827
5. **Medina I., Friedel P., Rivera C., Kahle K.T., Kourdougli N., Uvarov P., Pellegrino C.** Current view on the functional regulation of the neuronal K(^+)/Cl(^{-}) cotransporter KCC2. *Frontiers in Cellular Neuroscience* **8**, 27 (2014). https://doi.org/10.3389/fncel.2014.00027
6. **Moore Y.E., Kelley M.R., Brandon N.J., Deeb T.Z., Moss S.J.** Seizing control of KCC2: A new therapeutic target for epilepsy. *Trends in Neurosciences* **40**, 555–571 (2017). https://doi.org/10.1016/j.tins.2017.06.008
7. **Pisella L.I., Gaiarsa J.-L., Diabira D., Zhang J., Khalilov I., Duan J., Kahle K.T., Medina I.** Impaired regulation of KCC2 phosphorylation leads to neuronal network dysfunction and neurodevelopmental pathology. *Science Signaling* **12**, eaau0300 (2019). https://doi.org/10.1126/scisignal.aau0300
8. **Moore Y.E., Conway L.C., Wobst H.J., Brandon N.J., Deeb T.Z., Moss S.J.** Developmental regulation of KCC2 phosphorylation has long-term impacts on cognitive function. *Frontiers in Molecular Neuroscience* **12**, 173 (2019). https://doi.org/10.3389/fnmol.2019.00173

ED658 - Ecole Doctorale Sciences du Vivant T +33 (0)4 13 94 19 63 Site: https://ecole-doctorale- 658.univ-amu.fr/ Campus Luminy - 163 Avenue de Luminy - Marseille, 13009 Email: ed658@univ-amu.fr Bat TPR1 – RdC - bureau E.007

9. **Bertoni A., Schaller F., Tyzio R., Gaillard S., Santini F., Xolin M., Diabira D., Vaidyanathan R., Matarazzo V., Medina I., Hammock E., Zhang J., Chini B., Gaiarsa J.-L., Muscatelli F.** Oxytocin administration in neonates shapes hippocampal circuitry and restores social behavior in a mouse model of autism. *Molecular Psychiatry* **26**, 1–14 (2021). https://doi.org/10.1038/s41380-021-01227-6
10. **de Chaumont F., Torquet E., Lagache T., Dallongeville S., Imbert A., Legou T., Le Sourd A.M., Faure P., Bourgeron T., Olivo-Marin J.-C.** Live Mouse Tracker: Real-time behavioral analysis of groups of mice. *bioRxiv* (2018). https://doi.org/10.1101/345132
11. **Dumon C., Diabira D., Chudotvorova I., Bader F., Sahin S., Zhang J., Porcher C., Wayman G.A., Medina I., Gaiarsa J.-L.** The adipocyte hormone leptin sets the emergence of hippocampal inhibition in mice. *eLife* **7**, e36726 (2018). https://doi.org/10.7554/eLife.36726
12. **Valeeva G., Janackova S., Nasretdinov A., Rychkova V., Makarov R., Holmes G.L., Khazipov R., Lenck-Santini P.-P.** Emergence of coordinated activity in the developing entorhinal–hippocampal network. *Cerebral Cortex* **29**, 906–920 (2019). https://doi.org/10.1093/cercor/bhy309
13. **Liabeuf S., Stuhl-Gourmand L., Gackière F., Mancuso R., Sanchez-Brualla I., Marino P., Brocard F., Vinay L.** Prochlorperazine increases KCC2 function and reduces spasticity after spinal cord injury. *Journal of Neurotrauma* **34**, 1–10 (2017). https://doi.org/10.1089/neu.2017.5152
14. **Lee K.L., Abiraman K., Lucaj C., Ollerhead T.A., Brandon N.J., Deeb T.Z., Maguire J., Moss S.J.** Inhibiting with-no-lysine kinases enhances K(^+)/Cl(^{-}) cotransporter 2 activity and limits status epilepticus. *Brain* **144**, 100364 (2021). https://doi.org/10.1093/brain/awab343
15. **Friedel P., Ludwig A., Pellegrino C., Agez M., Jawhari A., Rivera C., Medina I.** A novel view on the role of intracellular tails in surface delivery of the potassium–chloride cotransporter KCC2. *eNeuro* **2**, ENEURO.0055-17.2017 (2017). https://doi.org/10.1523/ENEURO.0055-17.2017
16. **Mazzola L., Oliver K., Labalme A., Baykan B., Muona M., Joensuu T.H., Courage C., Chatron N., Borsani G., Alix E., Ramond F., Touraine R., Bahlo M., Bebek N., Berkovic S.F., Lehesjoki A.-E., Lesca G.** Progressive myoclonus epilepsy caused by a homozygous variant of *SLC7A6OS*. *Annals of Neurology* **89**, 402–407 (2021). https://doi.org/10.1002/ana.25941

Conditions scientifiques matérielles et financières du projet de recherche - Scientific, material, and financial conditions of the research project

Encadrement par le Dr. Igor Medyna (DR2 INSERM) et le Pr. Christophe Porcher (PRCE AMU).
La thèse se déroulera à l'INMED, qui possède l'ensemble des outils de recherche permettant la réalisation du projet de thèse dans des conditions optimales.
Publications dans des revues internationales de Rang A. Dépôt d'un brevet pour le traitement des patients.

ED658 - Ecole Doctorale Sciences du Vivant T +33 (0)4 13 94 19 63 Site: https://ecole-doctorale- 658.univ-amu.fr/ Campus Luminy - 163 Avenue de Luminy - Marseille, 13009 Email: ed658@univ-amu.fr Bat TPR1 – RdC - bureau E.007

Profil et compétences recherchées - Profile and skills required

Master 2 en biologie cellulaire ou neurosciences.
Graduate student in cellular biology and/or neurosciences.

ED658 - Ecole Doctorale Sciences du Vivant T +33 (0)4 13 94 19 63 Site: https://ecole-doctorale- 658.univ-amu.fr/ Campus Luminy - 163 Avenue de Luminy - Marseille, 13009 Email: ed658@univ-amu.fr Bat TPR1 – RdC - bureau E.007