Aix*Marseille université
Initiative d'excellence

Inmed
Institut de neurobiologie de la méditerranée
Mediterranean Institute of Neurobiology

Inserm
La science pour la santé
From science to health

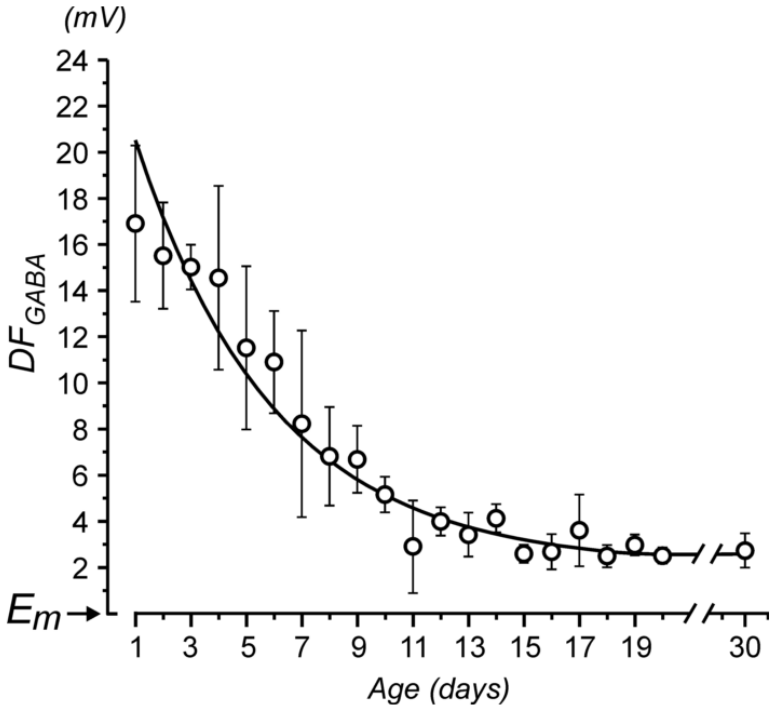
Виняткова роль йонів хлору в клітинному гомеостазі. Хлор-залежні патології

Ігор Медина

www.inmed.fr

Igor.medyna@inserm.fr

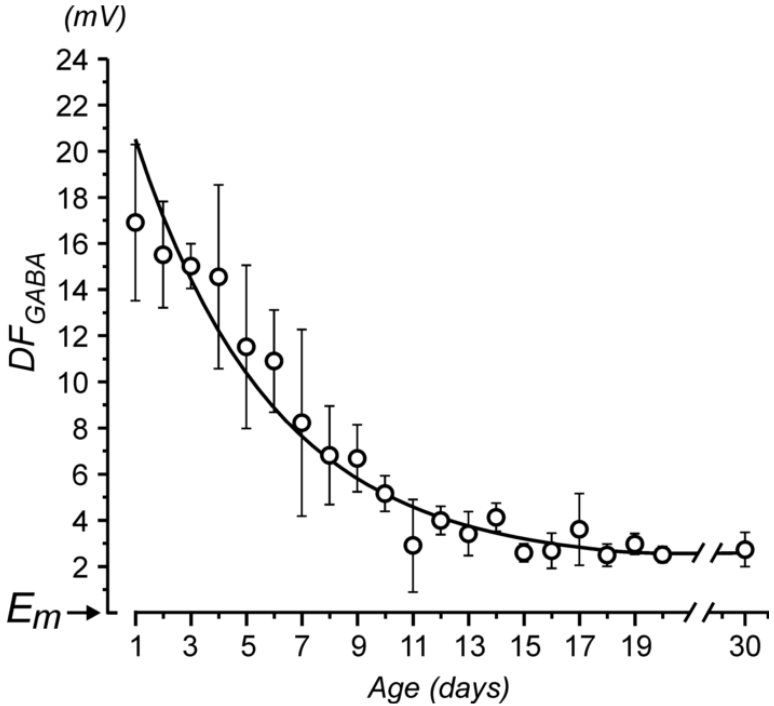
21/03/2024



series (in the bicarbonate-based ACSF), DF_{GABA} was 1.9 ± 0.6 mV (n = 9). In bicarbonate-free Hepes-buffered solution, DF_{GABA} was −3.7 ± 1.8 mV (n = 10; $P < 0.05$; Fig. 8A). These results indicate that bicarbonate conductance significantly contributes to DF_{GABA} in the soma of adult CA3 pyramidal cells. Subtraction of the bicarbonate component allows an estimation of the chloride equilibrium potential in the soma of adult CA3 pyramidal cells at around −82 mV. Assuming $[HCO_3^-]_I = 16$ mM and that the relative permeability $HCO_3^-/Cl^- = 0.2$, we further estimated $[Cl^-]_i$ according to the equation:

$$E_{GABA} = RT/F \ln[([Cl^-]_i + 0.2 \cdot [HCO_3^-]_i)/([Cl^-]_o + 0.2 \cdot [HCO_3^-]_o)]$$

We estimated $[Cl^-]_i$ in the soma of adult pyramidal cells as 4 mM, which corresponds to a $[Cl^-]_i$ equilibrium potential of −91 mV. This theoretical value is more negative than the value obtained in the bicarbonate-free Hepes-buffered ACSF (−82 mV). The difference is probably due to residual intracellular bicarbonate ions in the Hepes-buffered ACSF.

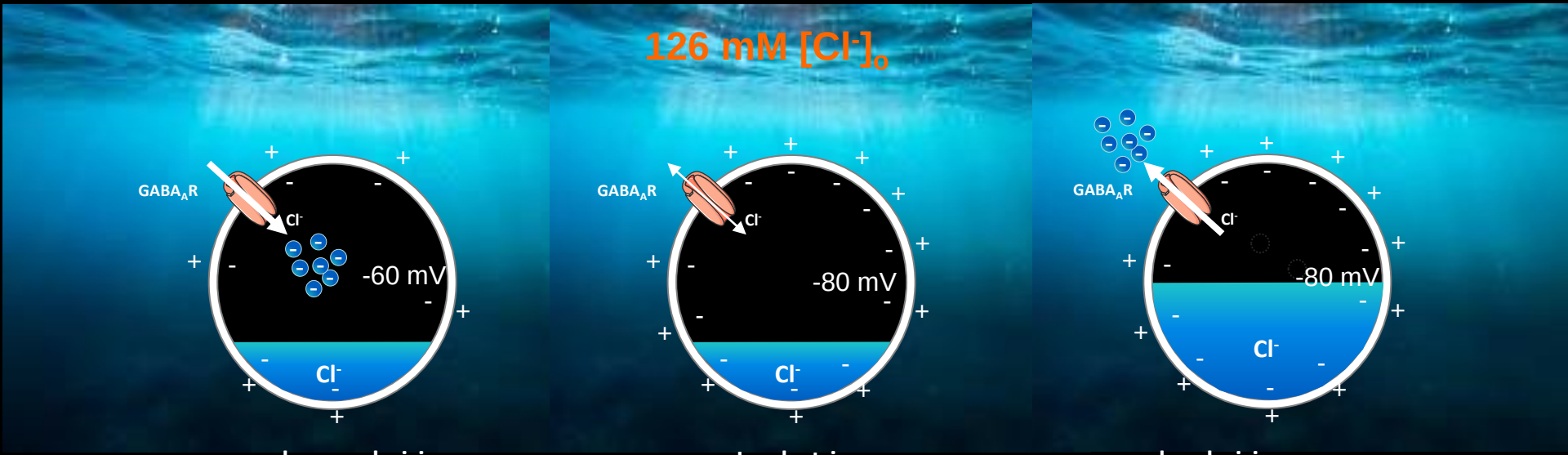


series (in the bicarbonate-based ACSF), DF_{GABA} was 1.9 ± 0.6 mV (n = 9). In bicarbonate-free Hepes-buffered solution, DF_{GABA} was −3.7 ± 1.8 mV (n = 10; $P < 0.05$; Fig. 8A). These results indicate that bicarbonate conductance significantly contributes to DF_{GABA} in the soma of adult CA3 pyramidal cells. Subtraction of the bicarbonate component allows an estimation of the chloride equilibrium potential in the soma of adult CA3 pyramidal cells at around −82 mV. Assuming $[HCO_3^-]_I = 16$ mM and that the relative permeability $HCO_3^-/Cl^- = 0.2$, we further estimated $[Cl^-]_i$ according to the Goldman equation:

$$E_{GABA} = RT/F \ln[([Cl^-]_i + 0.2 \cdot [HCO_3^-]_i)/([Cl^-]_o + 0.2 \cdot [HCO_3^-]_o)]$$

We estimated $[Cl^-]_i$ in the soma of adult pyramidal cells as 4 mM, which corresponds to a $[Cl^-]_i$ equilibrium potential of −91 mV. This theoretical value is more negative than the value obtained in the bicarbonate-free Hepes-buffered ACSF (−82 mV). The difference is probably due to residual intracellular bicarbonate ions in the Hepes-buffered ACSF.

$[Cl^-]_i$ effectively controls the neuron excitability



Розлади, пов'язані з $[Cl^-]_i$

Холера: цитолізин вібріона холери утворює аніонні канали та сприяє секреції Cl^- з інтактної слизової оболонки кишечника людини (Debellis, 2009)

Муковісцидоз: порушення функціонування хлоридних каналів CFTR, спричиняє збільшення в'язкості слизу та його накопичення в дихальних і травних шляхах. (Wikipedia)

Поділ ракових клітин потребує багато $[Cl^-]_i$. Зниження $[Cl^-]_i$ зупиняє їх поділ (Shiozaki, 2011)

Високий [Cl-]i є необхідною умовою запуску **апоптозу** (Heimlich & Cidlowski, 2006)

Неврологічні розлади, пов'язані з $[Cl^-]_i$

Розлади аутистичного спектру (Lemonnier та ін., 2013; Tyzio та ін., 2014)

Синдром Дауна (Deidda et al., 2015)

Синдром Ретта (Banerjee et al., 2016; Tang et al., 2016)

Шизофренія (Hyde et al., 2011; Merner et al., 2015; Tao et al., 2012)

Хвороба Альцгеймера (Chen et al., 2017)

Епілепсії (Huberfeld et al., 2007

Але

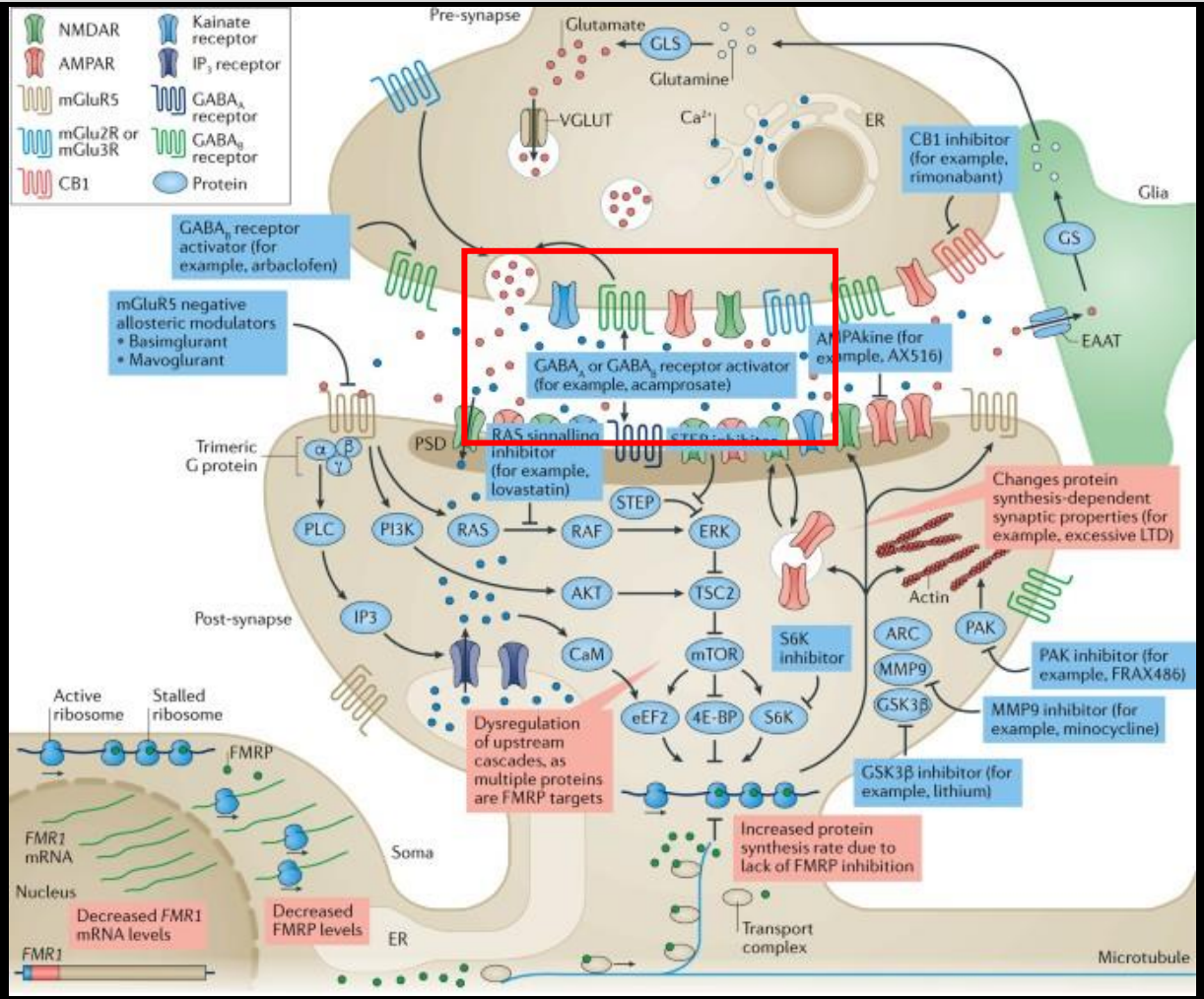
Невропатичні і фантомні болі (

Посттравматичні болі і відновлення спинного мозку (Boulenguez et al., 2010)

Посттравматичне відновлення головного мозку (Tazerart et al., 2020)

Всі перераховані розлади вважаються мультифакторними синдромами. Зміна Cl- може бути наслідком, а не причиною неврологічного розладу

lessons learned from fragile X syndrome



Berry-Kravis et al., *Nature Reviews*, 2018

2017. Секвенування геному пацієнтів з рідкісними захворюваннями.

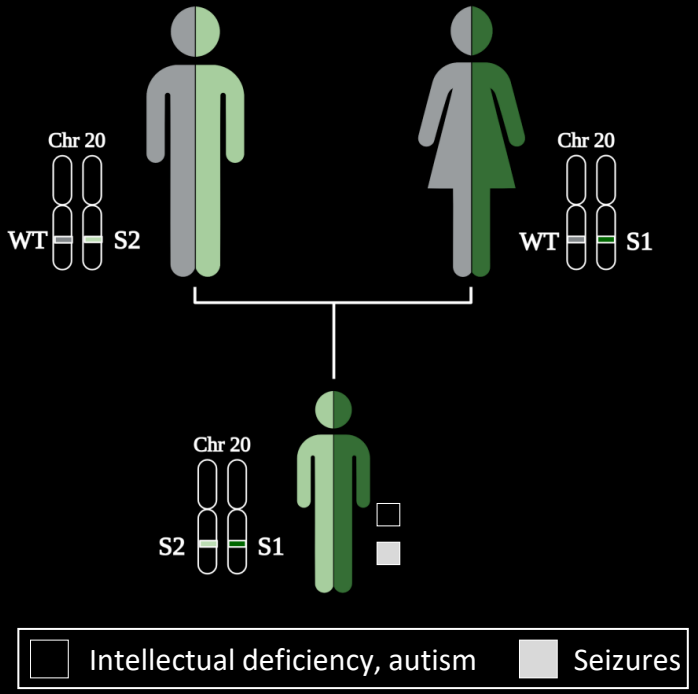
Dr Gaetan Lesca

Проект "Геном людини" зайняв 13 років і тисячі дослідників. Остаточна вартість в 2001: 2,7 мільярда доларів.

2023. Illumina: Досягнення в підготовці бібліотек, секвенуванні, біоінформатиці та аналізі варіантів дозволили перейти від зразка до звіту менш ніж за 30 годин. Вартість < $1000.

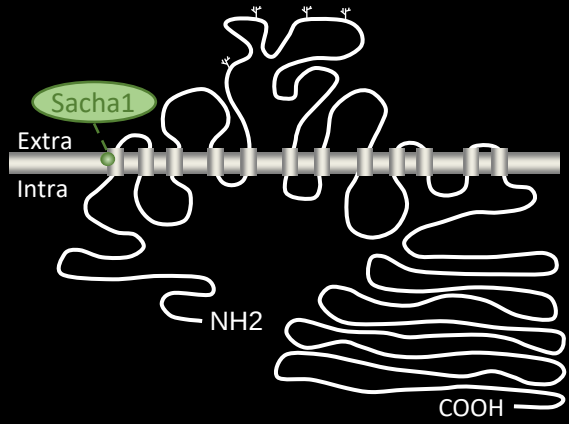
Whole-exome sequencing of patient with severe neurodevelopmental disorder* revealed missense variants of gene *SLC12A5* encoding neuronal K^+/Cl^- co-transporter KCC2.

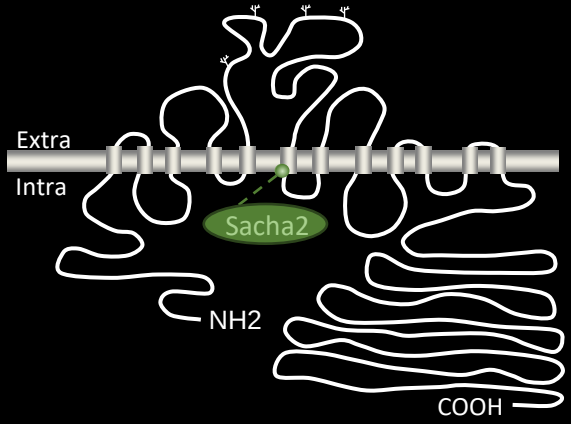
Dr Gaetan Lesca

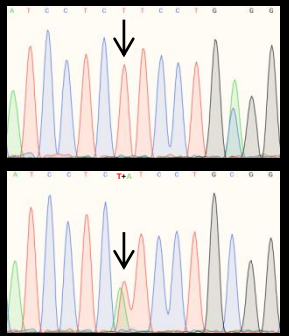


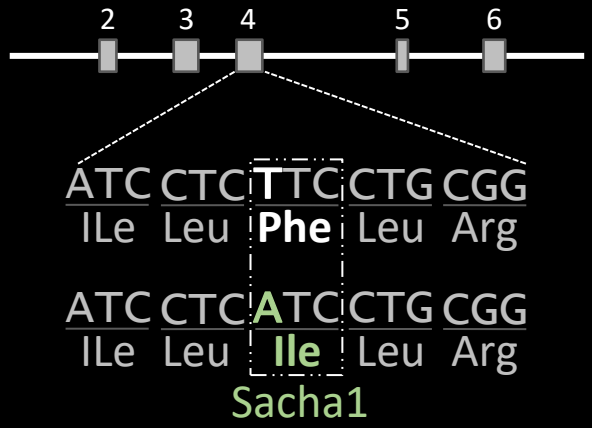
*developmental epileptic encephalopathy with drug-resistant focal seizures starting at three hours of life and occurring up to 100 times per day.

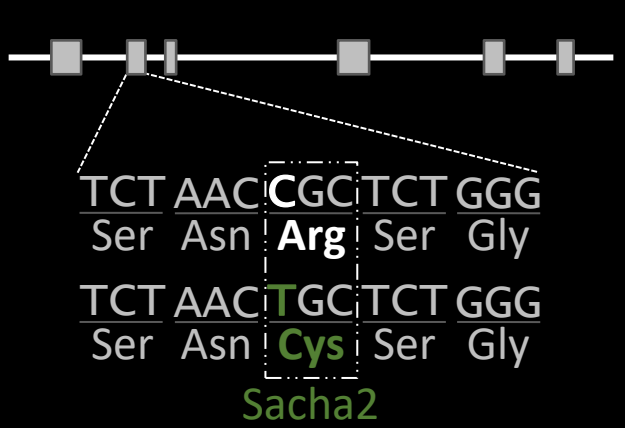
SLC12A5 Sacha variants

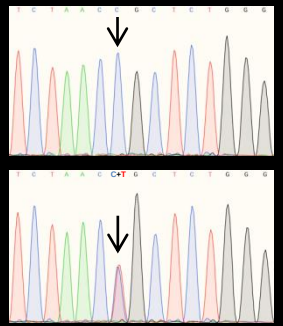












Multiplicity of molecules controlling $[Cl^-]_i$

Cation-chloride co-transporters

Anion exchangers

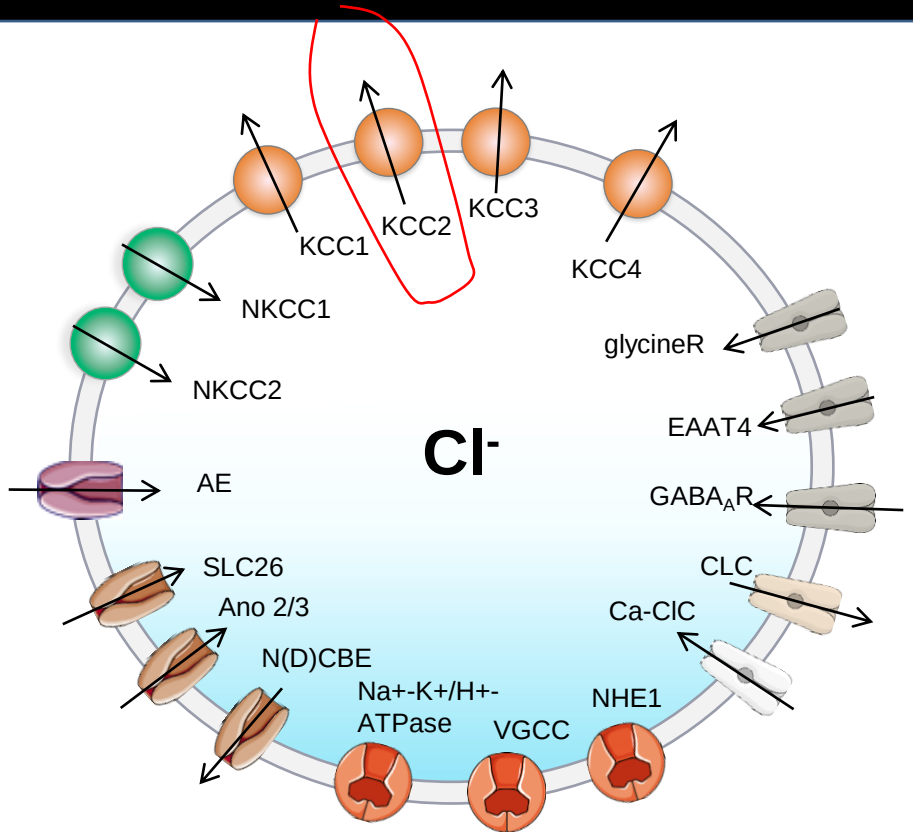
pH-sensitive transporters

Voltage dependent channels

Calcium dependent Cl- channels

Ligand gated channels/transporters

Secondary (primary ?) regulators

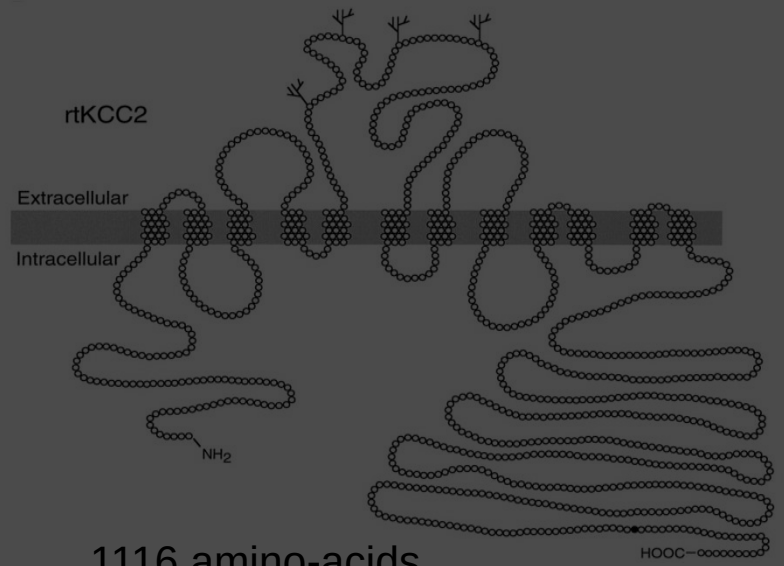


KCC2

1996

AchR (1982-1985) GABA$_A$ R (1983-1987)
Gly R (1987) Glutamate R (1989-1991)

Molecular rush / молекулярна лихоманка



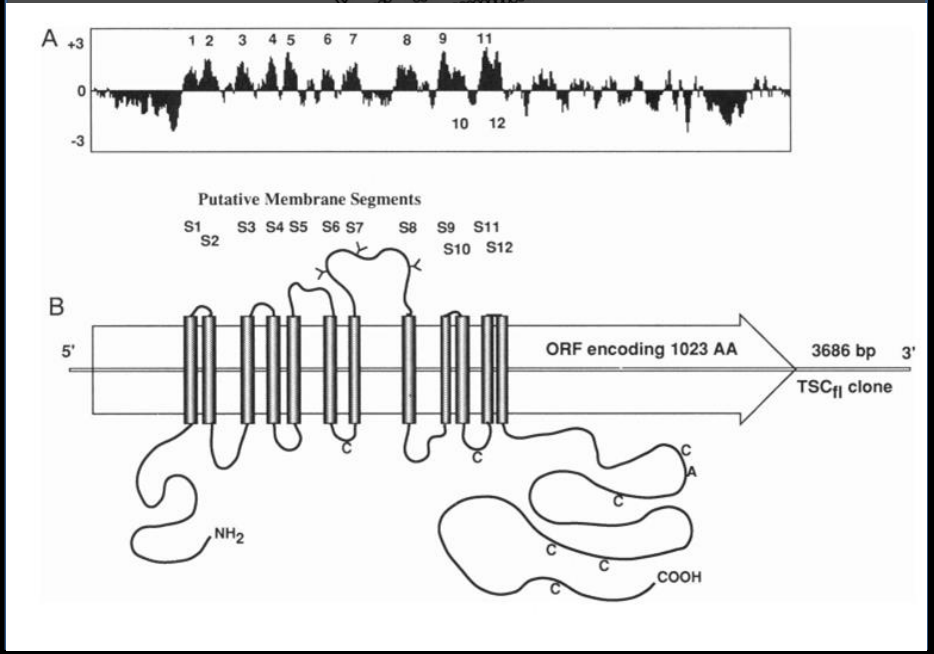
1116 amino-acids

Payne et al., *JBC*,1996

Cloning

1996

Molecular rush / молекулярна лихоманка



AchR (1982-1985) GABA$_A$ R (1983-1987)

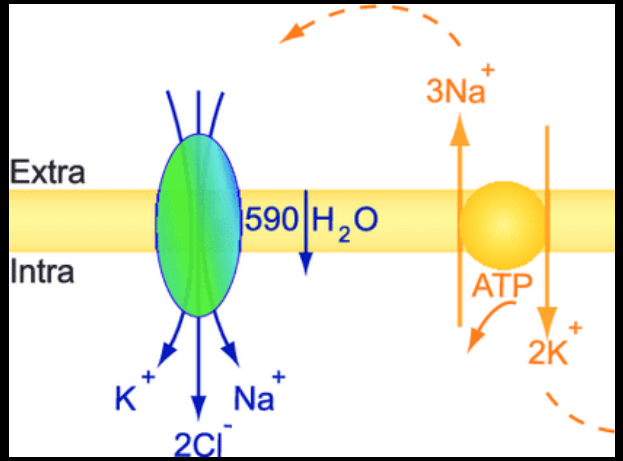
Gly R (1987) Glutamate R (1989-1991)

NKCC1 (1993; 1994), Transports Na^+, K^+, $2Cl^-$ from the blood into the cell. Maintains the cell volume.

NKCC2 (1994; 1994), Ascending limb of the loop of Henle; Transports Na^+, K^+, $2Cl^-$ from the urea to blood vessels.

KCC1 (1996)

KCC2 (1996)



Cloning

1996

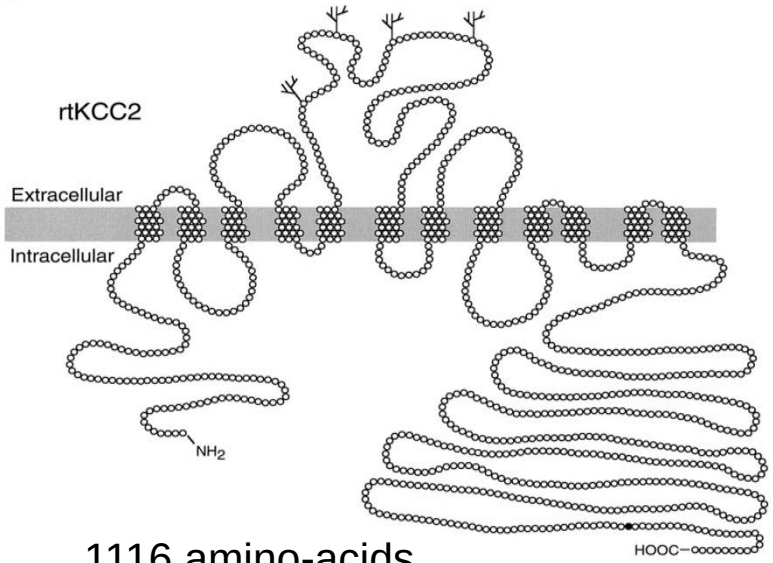
AchR (1982-1985) GABA$_A$ R (1983-1987)
Gly R (1987) Glutamate R (1989-1991)

NKCC1 (1993; 1994), Transports Na^+, K^+, $2Cl^-$ from the blood into the cell. Maintains the cell volume.

NKCC2 (1994; 1994), Ascending limb of the loop of Henle; Transports Na^+, K^+, $2Cl^-$ from the urea to blood vessels.

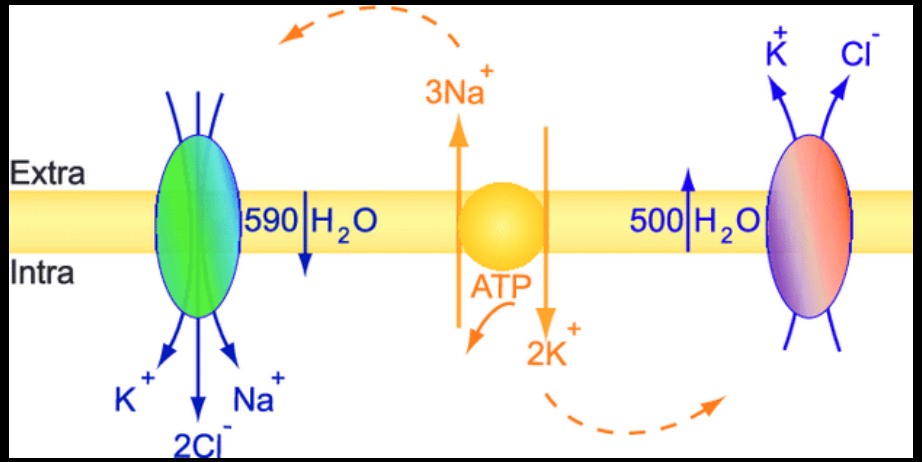
KCC1 (1996)

KCC2 (1996)



1116 amino-acids

Payne et al., *JBC*,1996



Cloning

1996

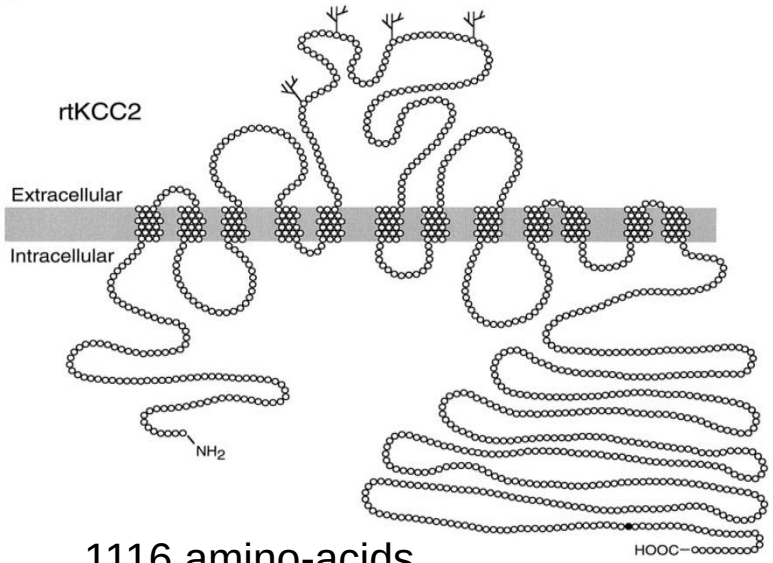
AchR (1982-1985) GABA$_A$ R (1983-1987)
Gly R (1987) Glutamate R (1989-1991)

NKCC1 (1993; 1994), Transports Na^+, K^+, $2Cl^-$ from the blood into the cell. Maintains the cell volume.

NKCC2 (1994; 1994), Ascending limb of the loop of Henle; Transports Na^+, K^+, $2Cl^-$ from the urea to blood vessels.

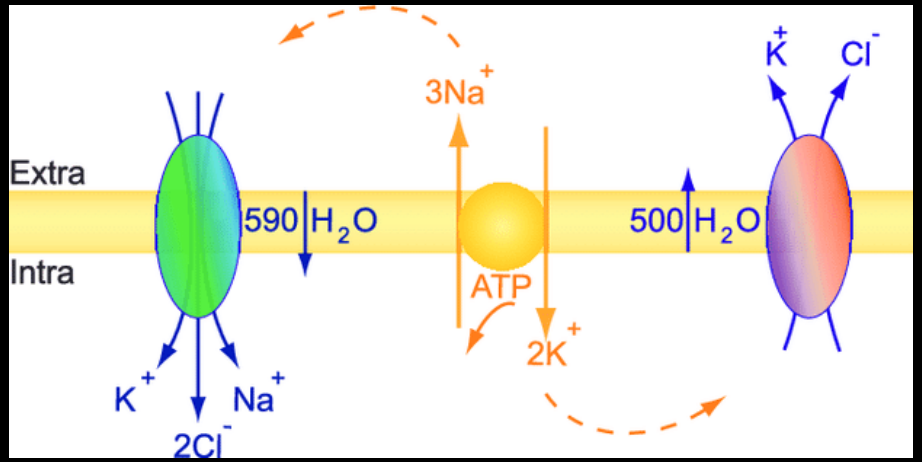
KCC1 (1996), ubiquitous, swelling activated, reduces cell volume during hypotonicity by extruding K^+ and Cl^-.

KCC2 (1996), neuron restricted, hypo- and hyper- tonicity resistant, constitutively active, **function unknown.**

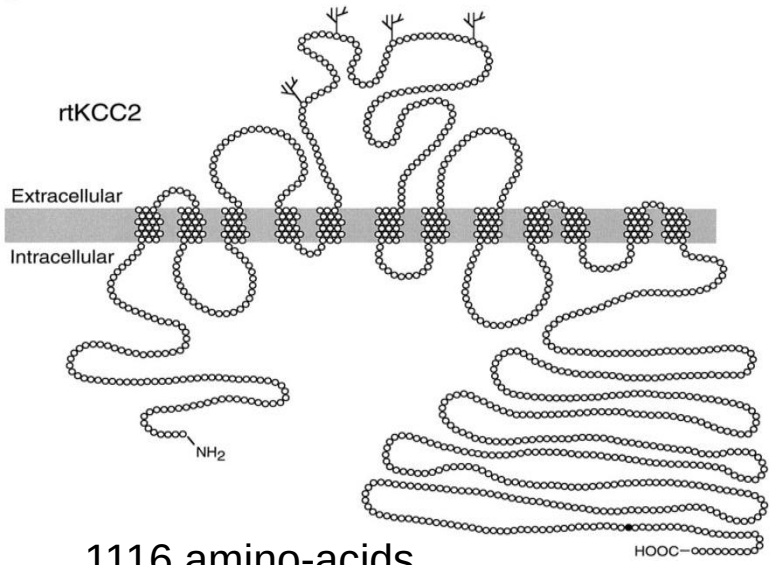


1116 amino-acids

Payne et al., *JBC*,1996

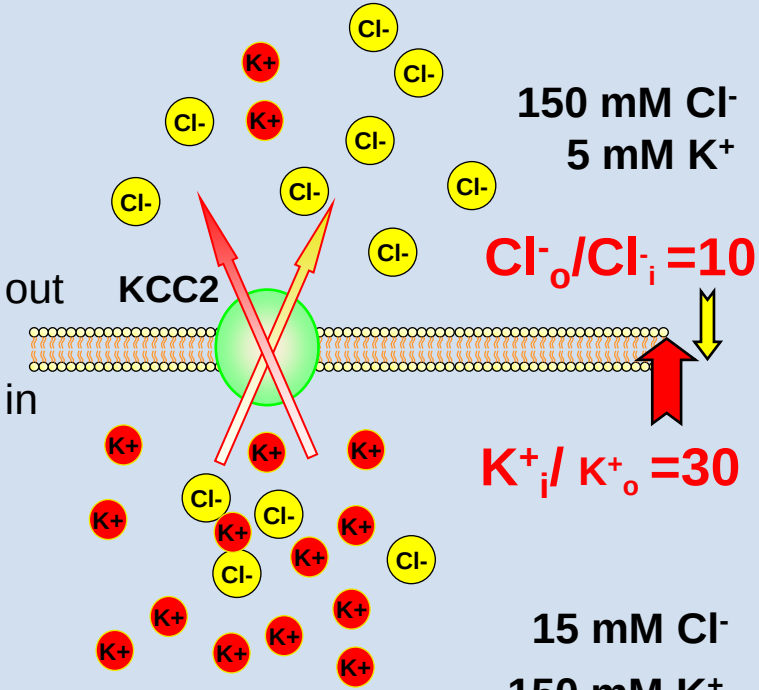


Challenges to study KCC2: 4 kb (140 kDa), electroneutrality

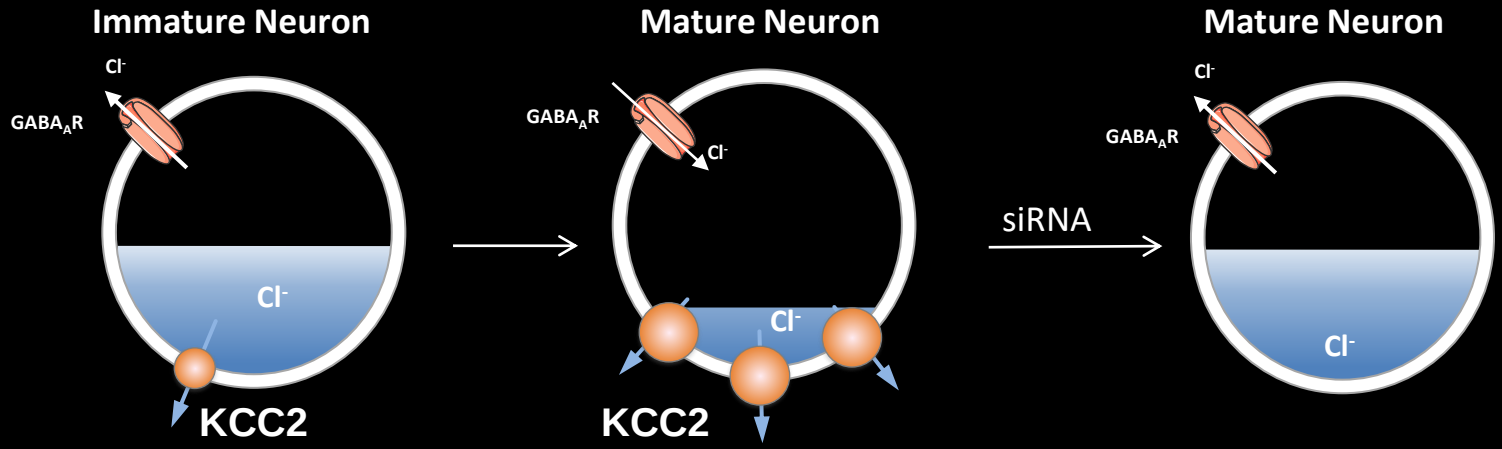


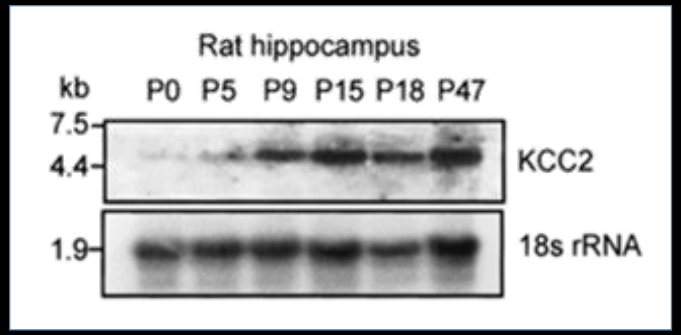
1116 amino-acids

Payne et al., 1996



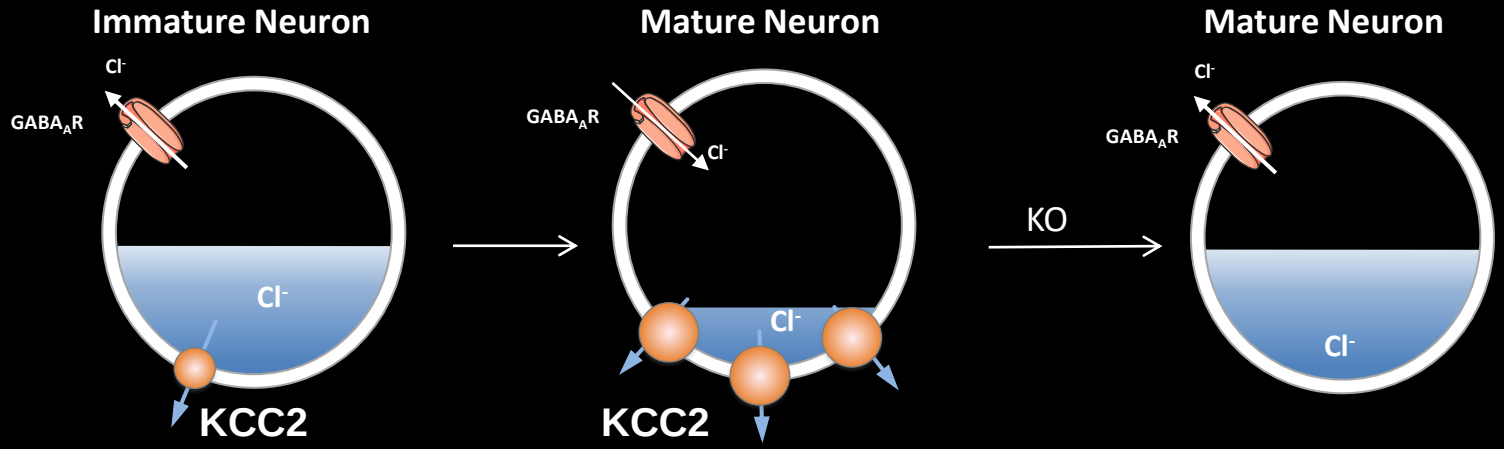
KCC2: Developmental upregulation, link to GABA functioning



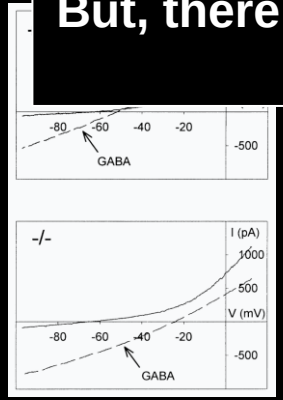


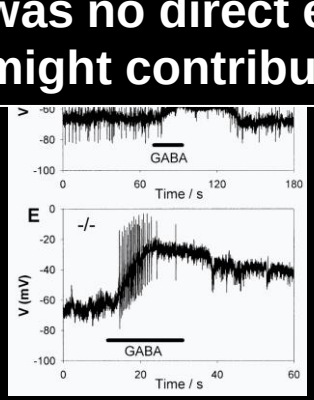
Rivera et al., **Nature** 1999

KO KCC2 -> lethal; KO KCC2b -> hyperactivity, dies at 14 DIV

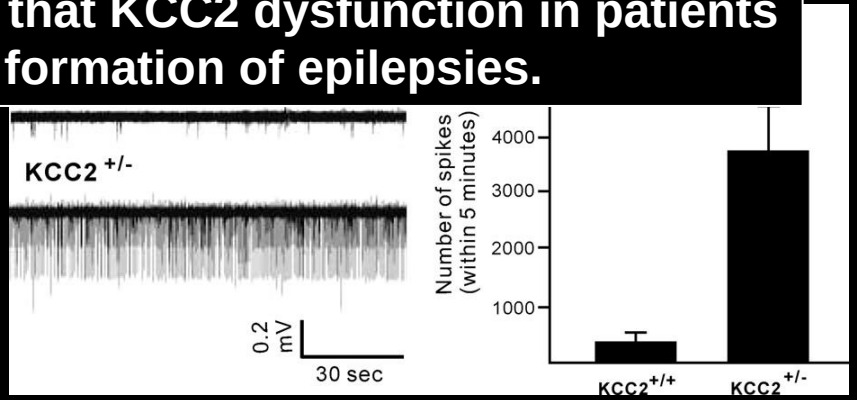


But, there was no direct evidence that KCC2 dysfunction in patients might contribute to the formation of epilepsies.





Hubner et al., 2001



Woo et al., 2002; Zhu et al., 2008

KCC2 mutations associated with epilepsy

Kahle et al., 2014

Scientific Report
TRANSPARENT PROCESS
EMBO reports

Genetically encoded impairment of neuronal KCC2 cotransporter function in human idiopathic generalized epilepsy

Kristopher T Kahle[1,2,†], Nancy D Merner[3,4,†], Perrine Friedel[5,6], Liliya Silayeva[7], Bo Liang[8], Arjun Khanna[2], Yuze Shang[1,2], Pamela Lachance-Touchette[9], Cynthia Bourassa[9], Annie Levert[4], Patrick A Dion[3,10], Brian Walcott[2], Dan Spiegelman[4], Alexandre Dionne-Laporte[4], Alan Hodgkinson[11], Philip Awadalla[11,12], Hamid Nikbakht[13], Jacek Majewski[13], Patrick Cossette[9], Tarek Z Deeb[7], Stephen J Moss[7], Igor Medina[5,6] & Guy A Rouleau[4,*]

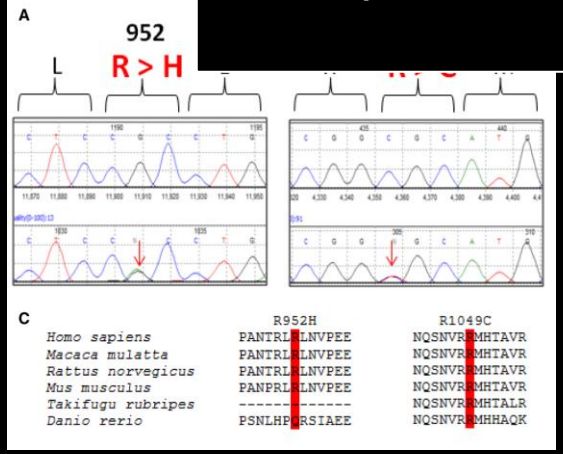
Puskarjov et al., 2014

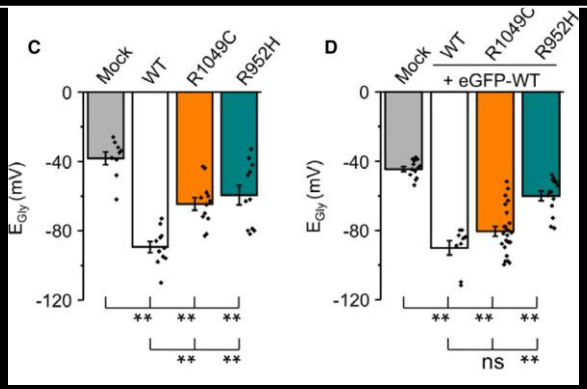
Scientific Report
DATA PROCESS ACCESS
EMBO reports

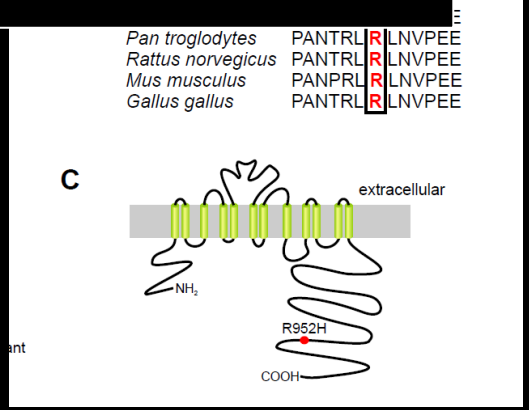
A variant of KCC2 from patients with febrile seizures impairs neuronal Cl^- extrusion and dendritic spine formation

Martin Puskarjov[1,2,†], Patricia Seja[1,2,†], Sarah E Heron[3,4,†], Tristiana C Williams[3], Faraz Ahmad[1,2], Xenia Iona[3], Karen L Oliver[5], Bronwyn E Grinton[6], Laszlo Vutskits[7], Ingrid E Scheffer[5,6], Steven Petrou[8], Peter Blaesse[1,9], Leanne M Dibbens[3,4], Samuel F Berkovic[5] & Kai Kaila[1,2,*]

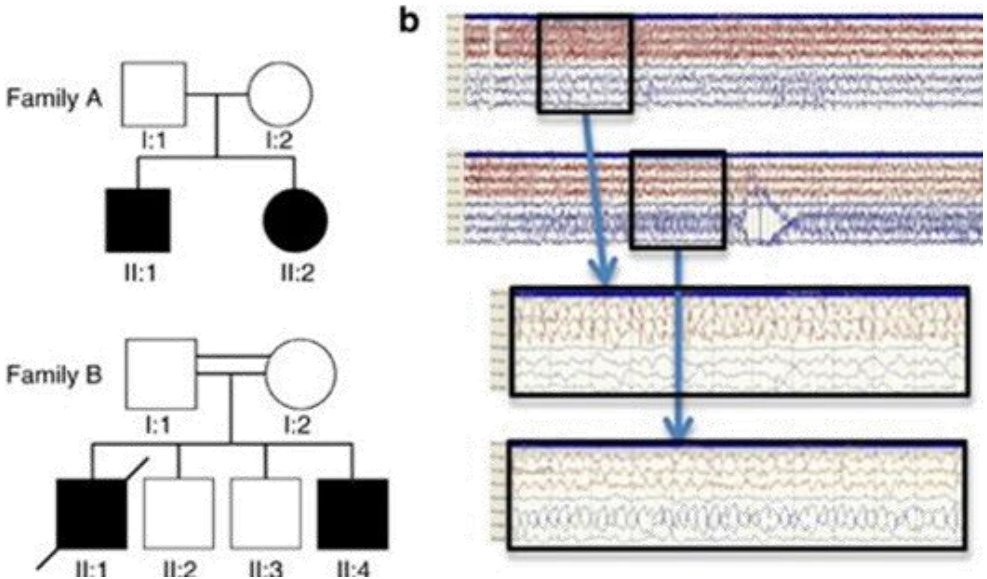
But, it remained unclear whether other mutations contribute to pathology.







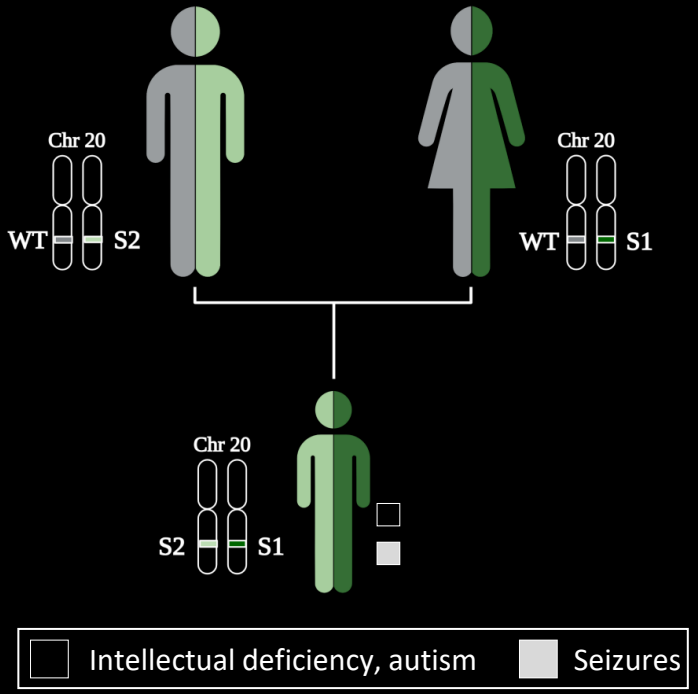
New cases revealed using whole exome sequencing



Stödberg et al., 2015; Saïtsu et al., 2016; Saito et al., 2017

Whole-exome sequencing of patient with severe neurodevelopmental disorder* revealed missense variants of *SLC12A5*, the gene encoding for KCC2

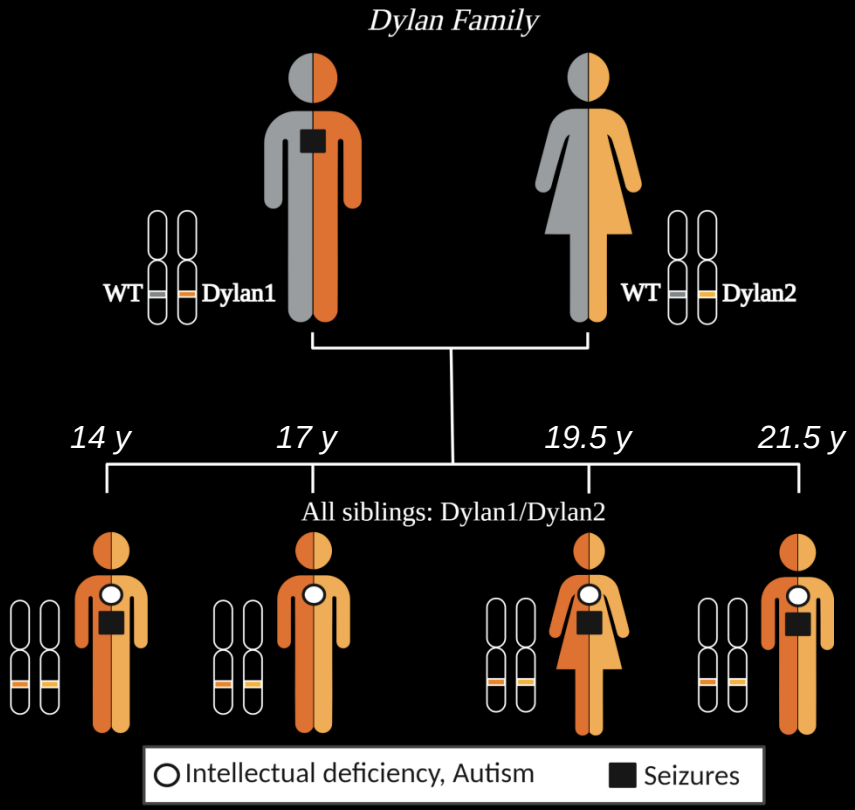
Dr Gaetan Lesca

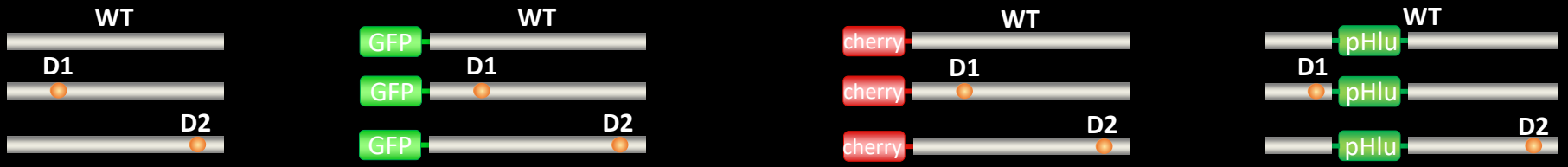


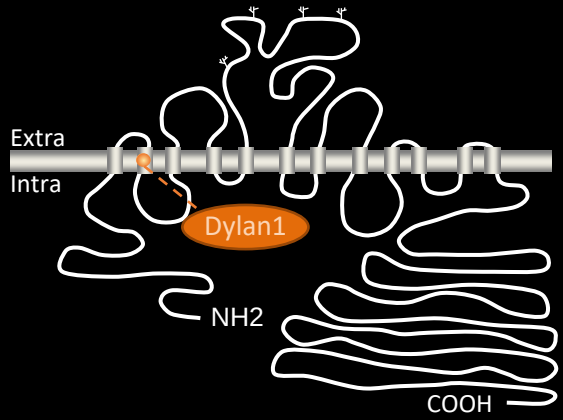
*developmental epileptic encephalopathy with drug-resistant focal seizures starting at three hours of life and occurring up to 100 times per day. Death at 9 y.

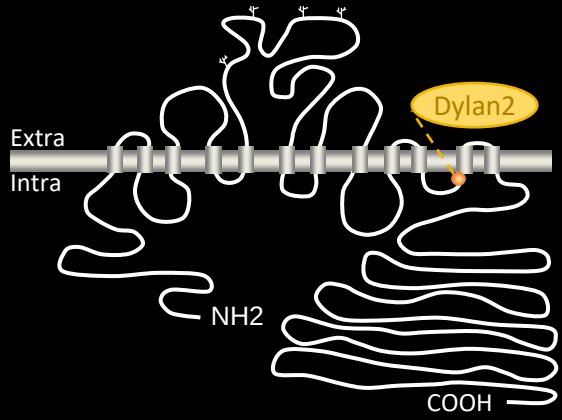
75 epilepsy-related gene sequencing in more than 300 patients with neurodevelopmental disorder revealed a family with missense variants of *SLC12A5,* the gene encoding for KCC2
No other mutations were detected (whole genome sequencing).

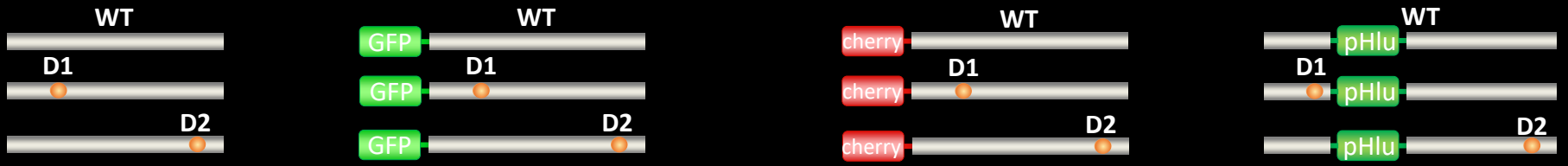
Dr Gaetan Lesca




WT
D1
D2
GFP
WT
GFP
D1
GFP
D2
cherry
WT
cherry
D1
cherry
D2
WT
pHlu
D1
pHlu
D2
pHlu


Extra
Intra
Dylan1
NH2
COOH


Extra
Intra
Dylan2
NH2
COOH



In-Vitro study

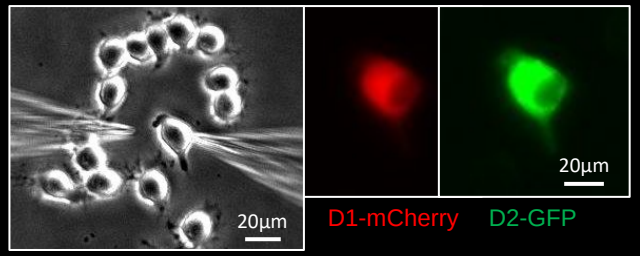
Transfection in heterologous cell line

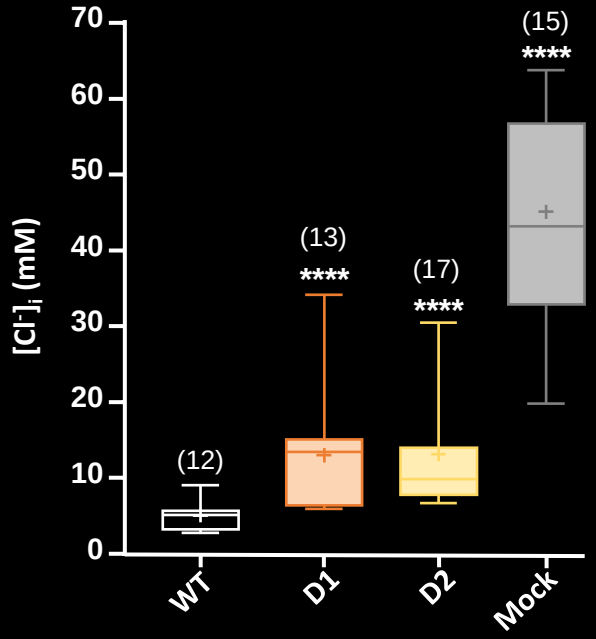
Neuroblastoma cells N2a
Mouse

HEK 293 cells (HEK)
Human

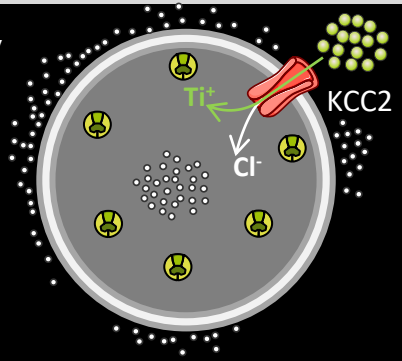
In-vitro study

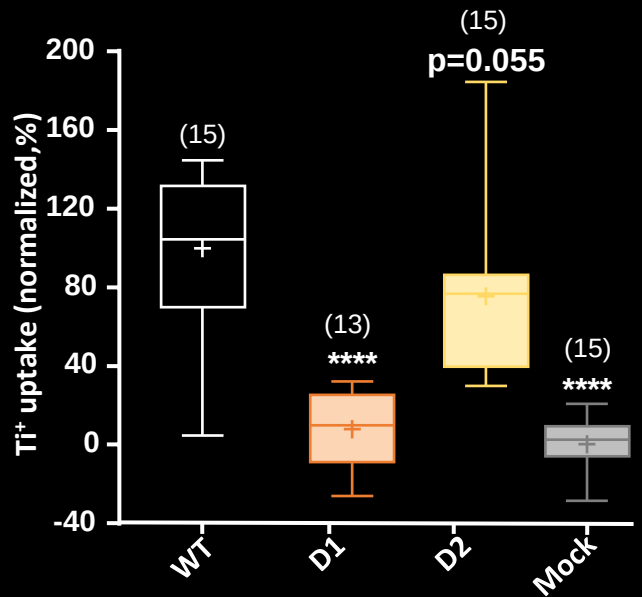
Gramicidin perforated patch:



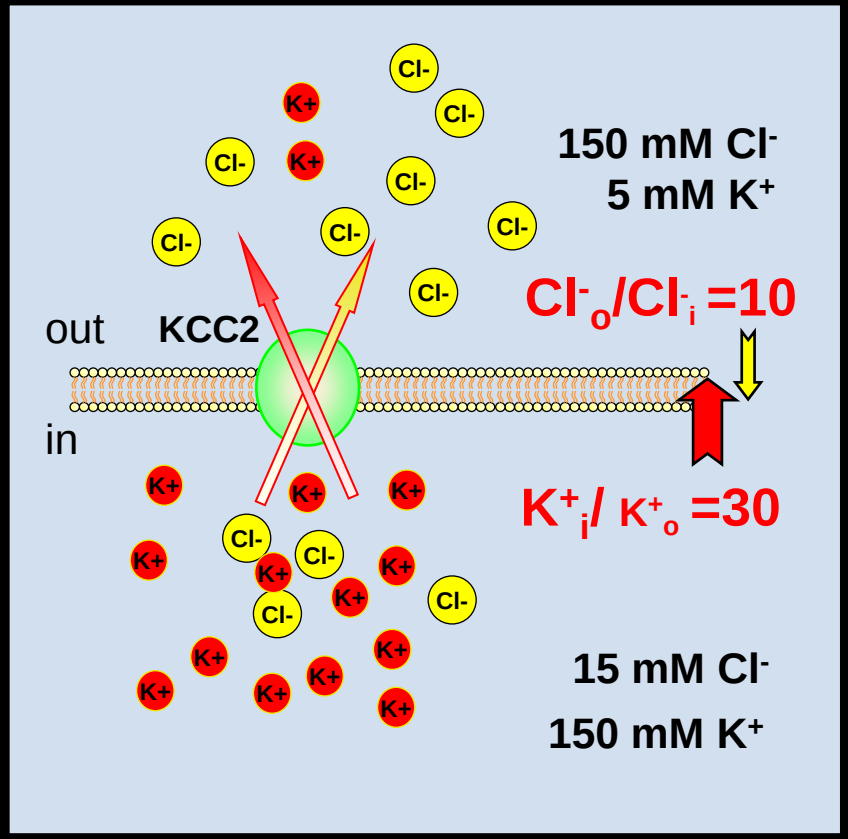


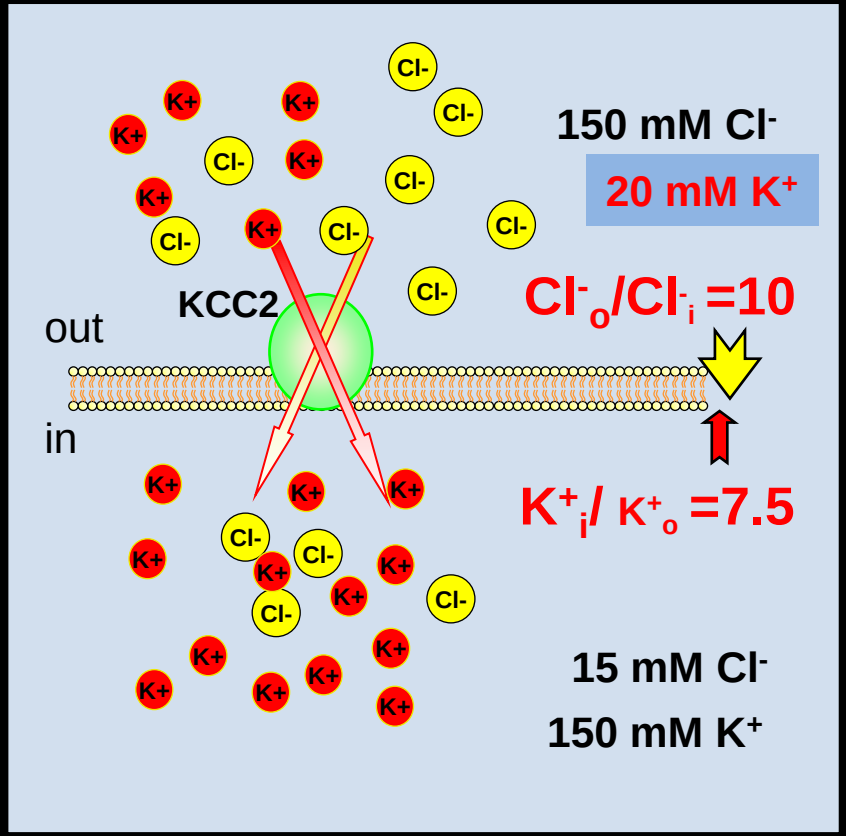
Ti⁺ flux assay





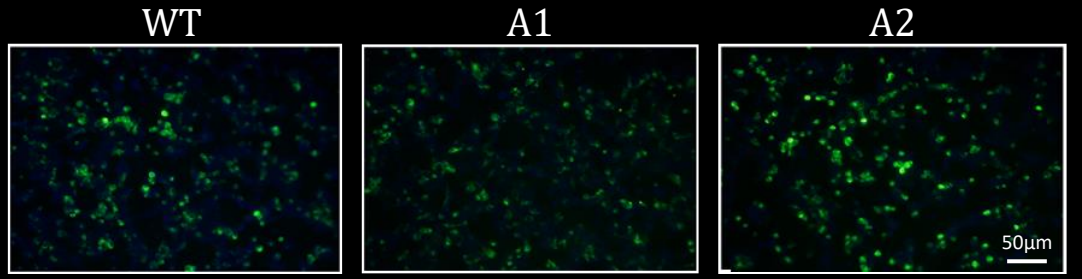
Challenges to study KCC2: 4 kb (140 kDa), electroneutrality

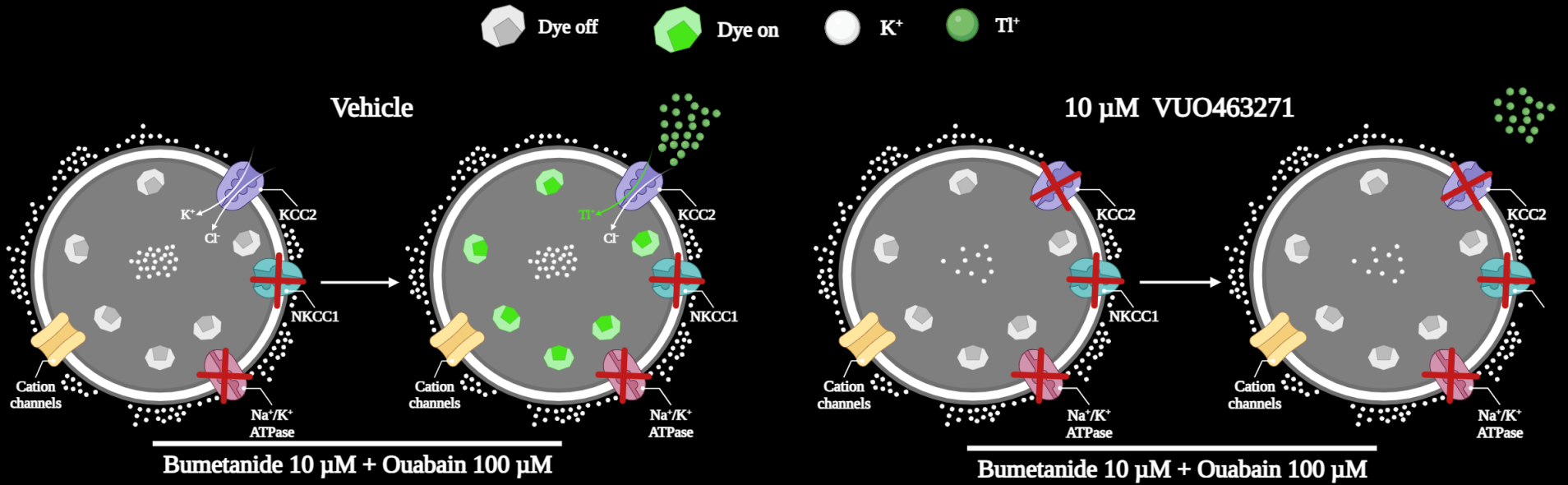




HEK293 cells: K^+ flux

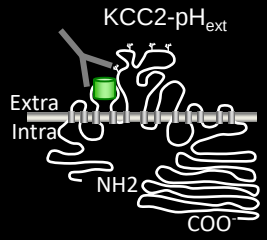
- Tl^+ flux assay

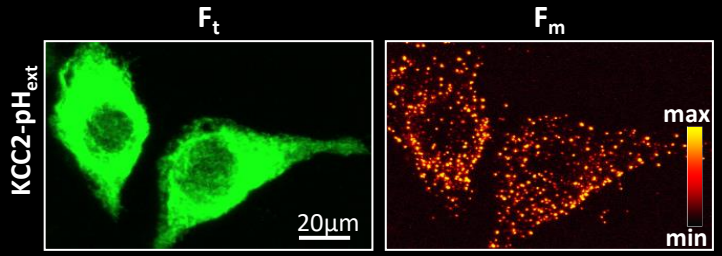


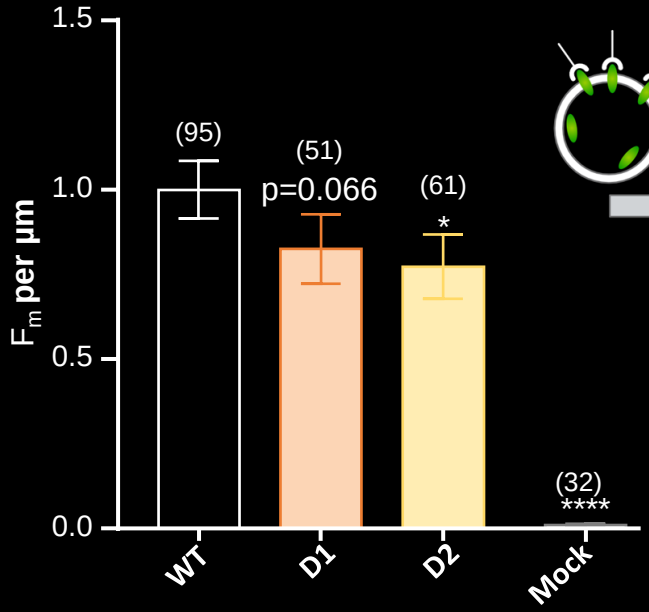


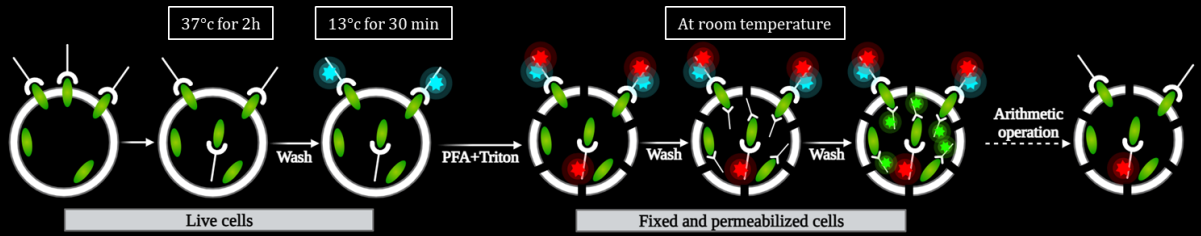
N2a: KCC2-pH$_{ext}$ surface expression (live staining)

Live staining









F_m: membrane pool

F_i: Internalized pool

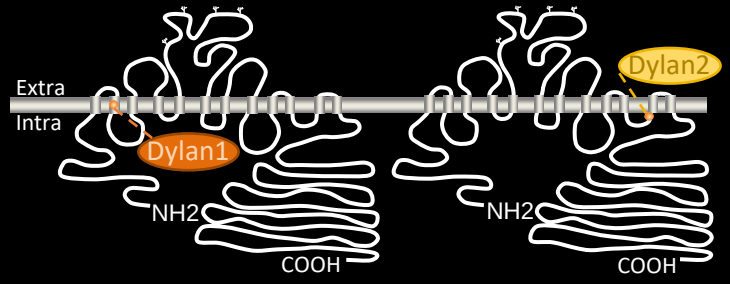
F_{all}: All clusters

F_t: Total expression

Dylan mutants

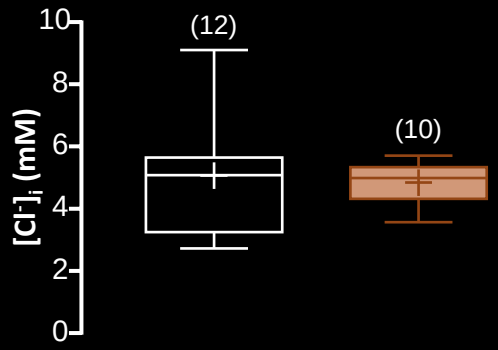
- Dylan 1 and Dylan 2 mutations alone decrease the ion transport activity and reduce the surface expression of the protein.

What happens in the patient like mixture?

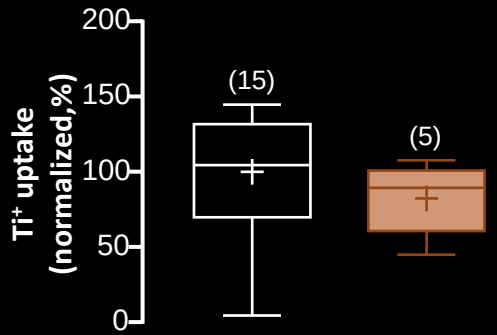


Dylan mutants

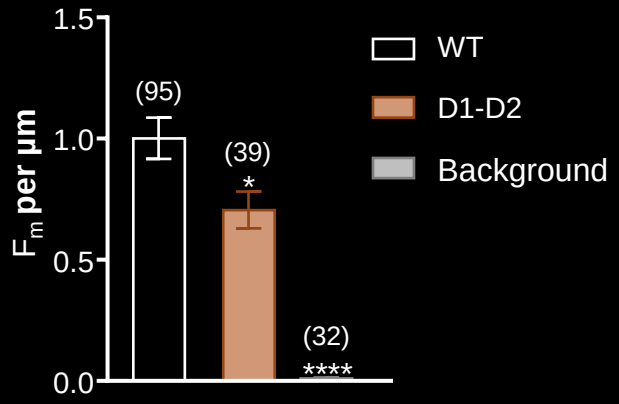
Gramicidin perforated patch:



Ti^+ flux assay:

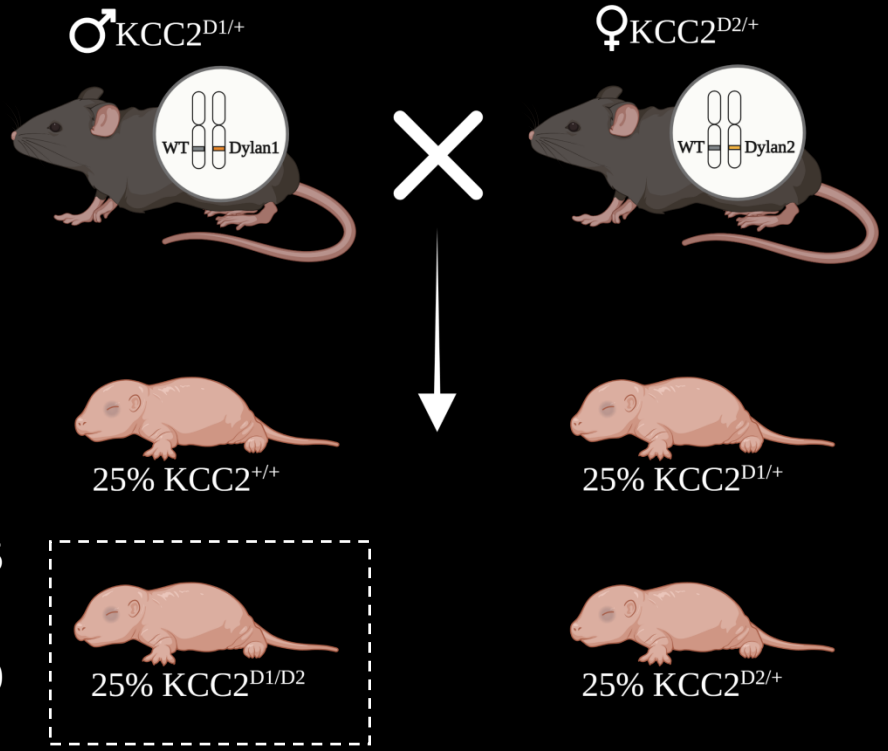


KCC2-pHext surface expression



➢ The patient like mixture of D1/D2 has no effect on the ion transport but decreases the surface expression of the protein.

Endogenous expression of human KCC2 mutants



Death at P60-P65
FMB

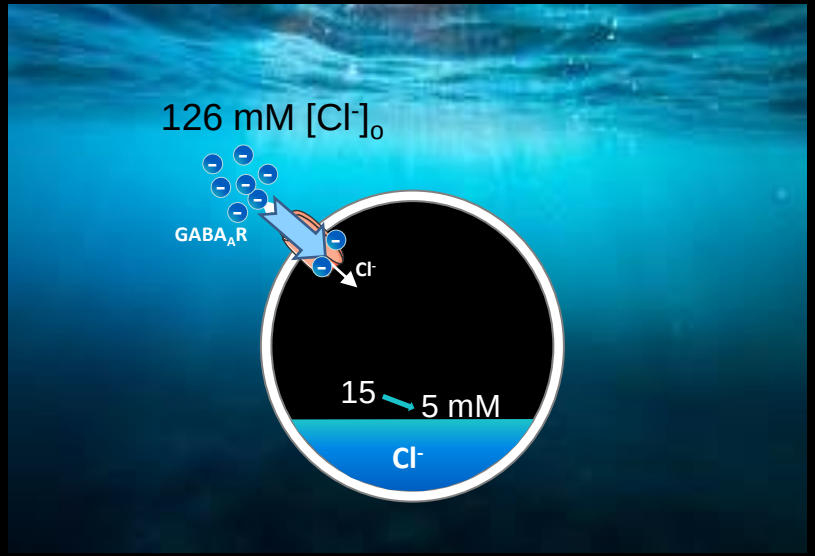
Death at P40-P50
in animal facility

Endogenous expression of human KCC2 mutants

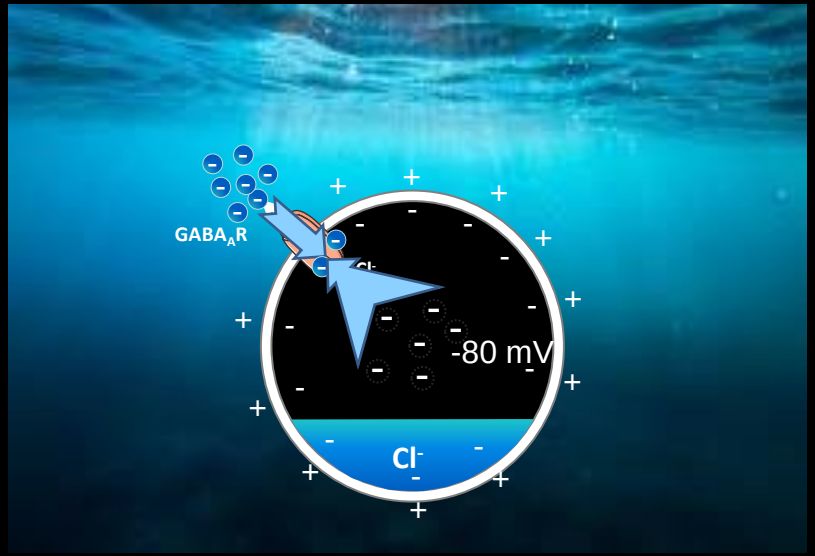
Video

Механізми дії

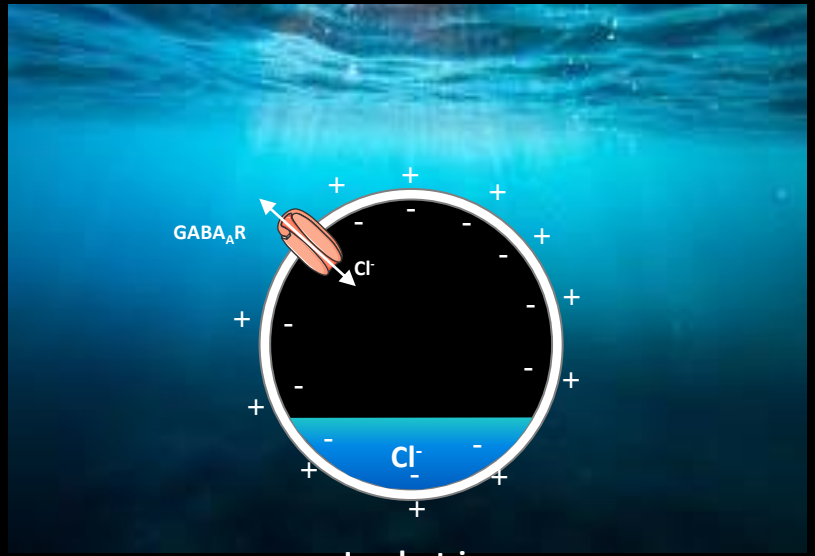
$[Cl^-]_i$ effectively controls the direction and strength of ion flux through $GABA_AR$



$[Cl^-]_i$ effectively controls the direction and strength of ion flux through $GABA_AR$

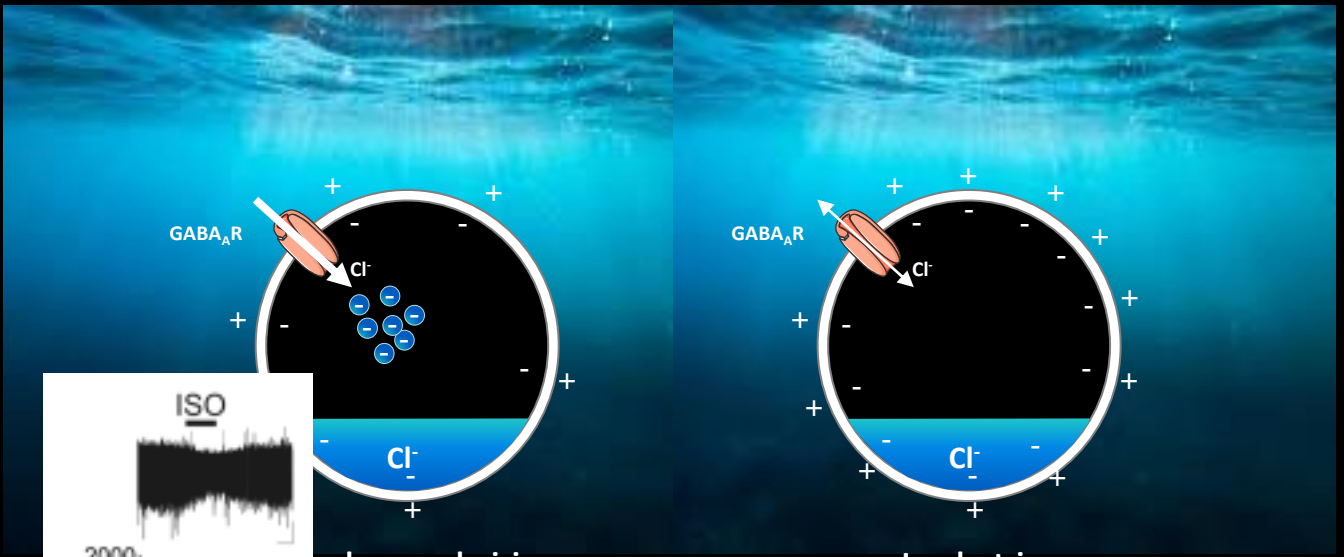


$[Cl^-]_i$ effectively controls the direction and strength of ion flux through $GABA_AR$



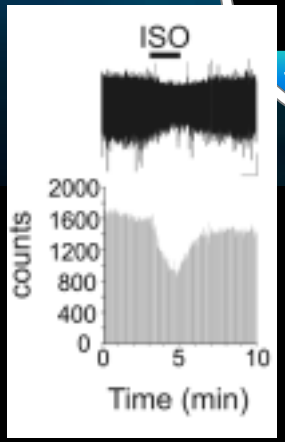
Isoelectric

$[Cl^-]_i$ effectively controls the direction and strength of ion flux through $GABA_AR$

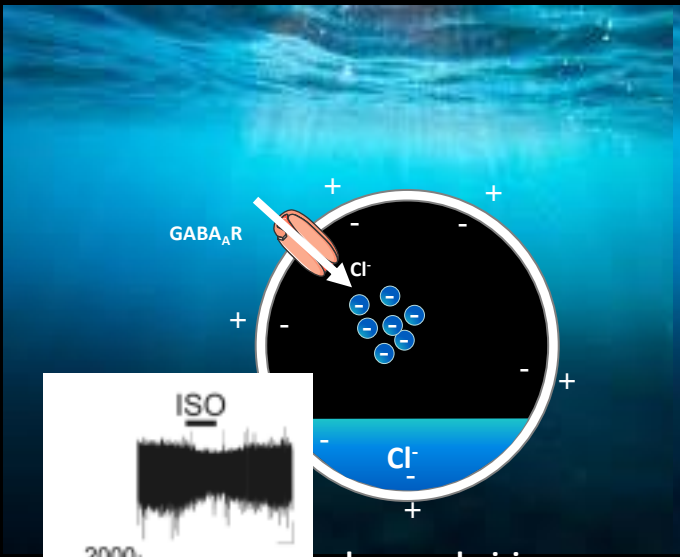


hyperpolarizing

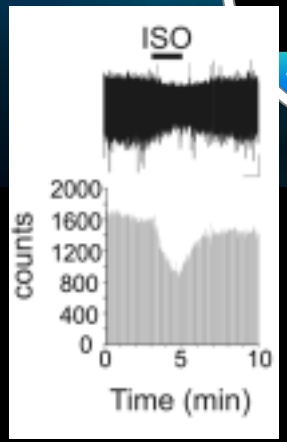
Isoelectric

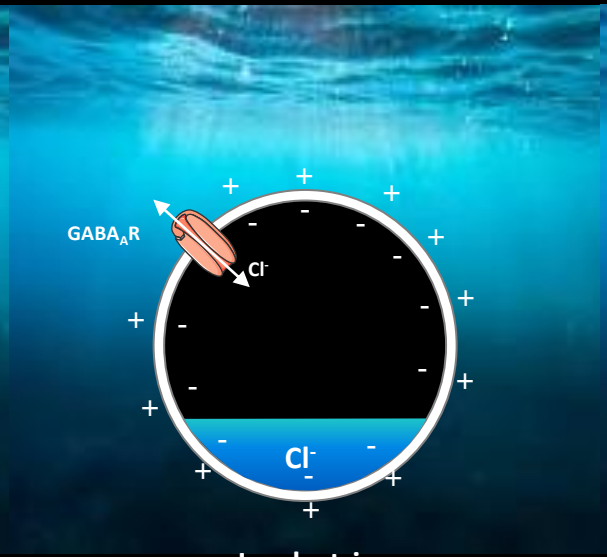


$[Cl^-]_i$ effectively controls the direction and strength of ion flux through $GABA_AR$

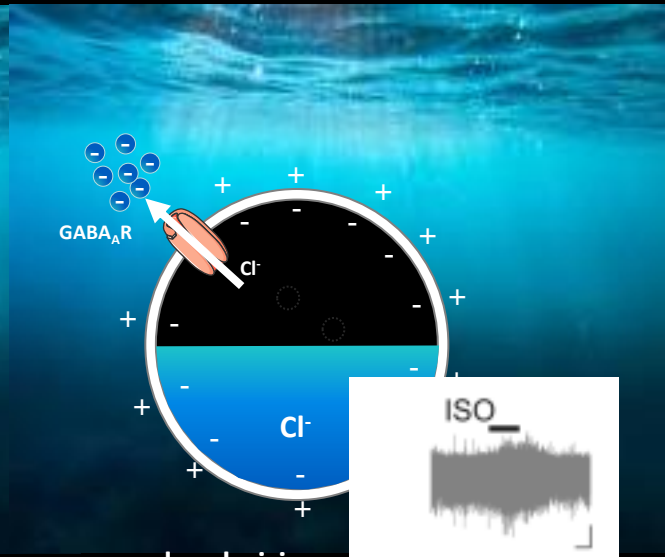


hyperpolarizing

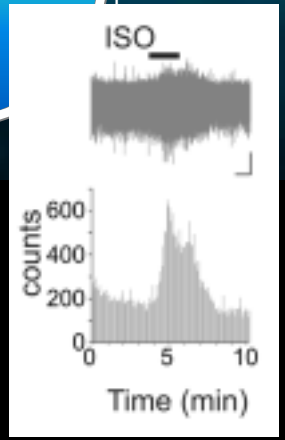




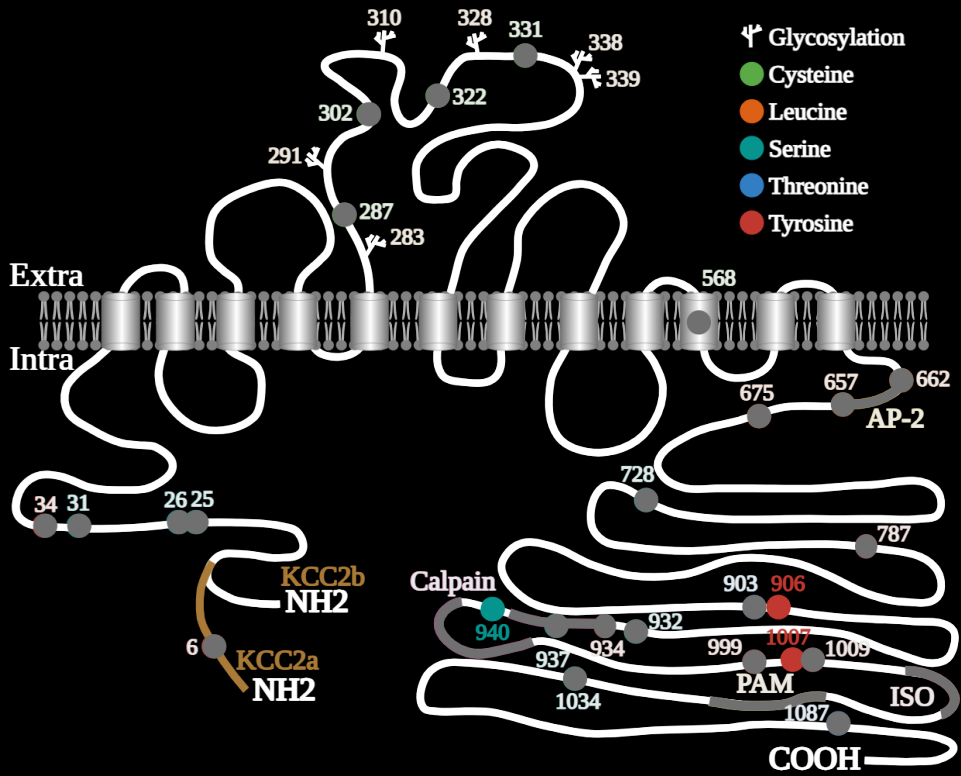
Isoelectric

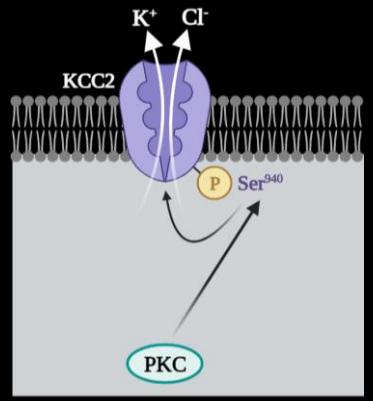


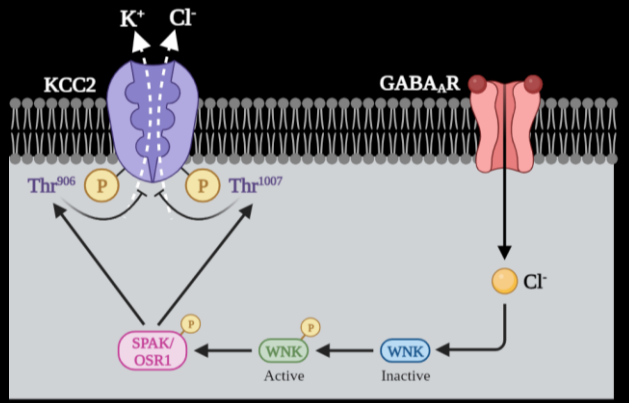
depolarizing



KCC2: Post-translational regulation





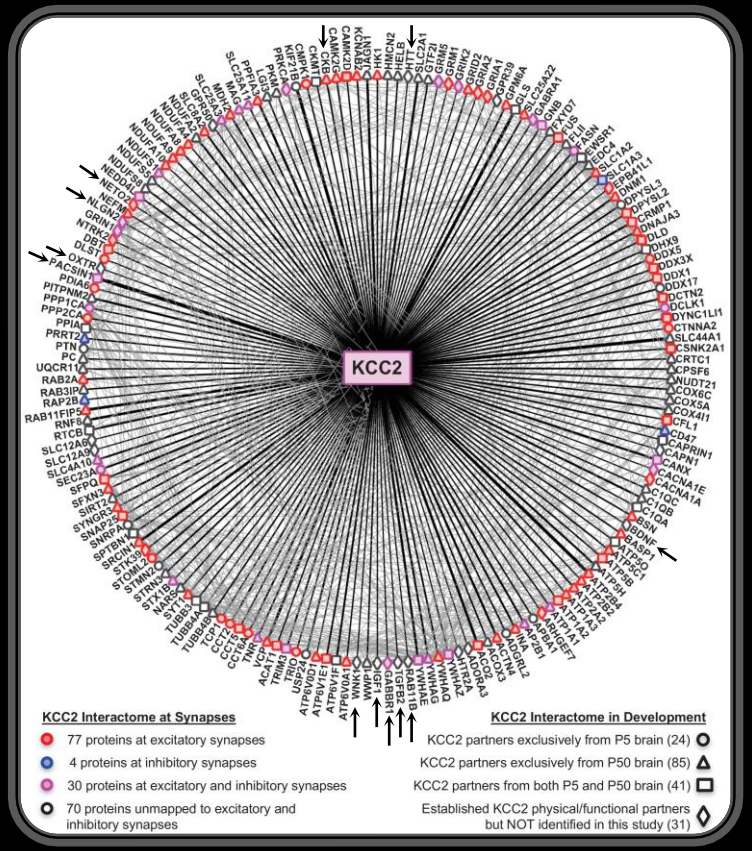


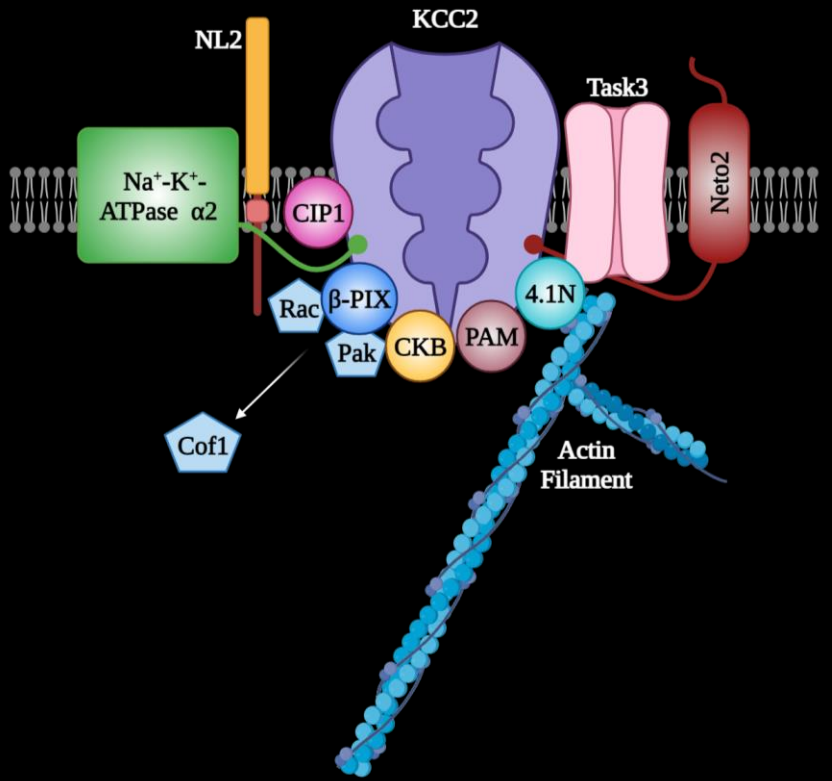
Hamze et al., in preparation

Others $[Cl^-]_i$ – independent mechanisms

Але

KCC: Protein-Protein interaction





Mahadevan et al., 2017; Hamze et al., in preparation

Jean-Luc Gaïarsa
Christophe Porcher
Diabé Diabira
Mira Hamze

Lucie Pisella
Perrine Friedel

Pierre-Pascal Lenck-Santini
Xavier Leinukugel

Facilities:

PBMC
Animal facility
InMAGIC platform
Histology platform

Collaborators:

Gaetan Lesca and Co, CHU Lyon, Lille
Sylvie Gory-Faure, Grenoble Institut des Neurosciences
Eric Denarier, Grenoble Institut des Neurosciences
Kristopher Kahle, Yale University, USA
Jinwei Zhang, University of Exeter, UK
Hans Gerd Nothwang, Oldenburg University, Germany
Anna-Maria Hartmann, Oldenburg University, Germany
Rebecca Haffner, Weizmann Institute of Science, Israel
Shifra Ben-Dor, Weizmann Institute of Science, Israel

IPSc facility, Marseille Medical Genetics