Neurobiology of Disease 80 (2015) 80–92

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

A recurrent *KCNQ2* pore mutation causing early onset epileptic encephalopathy has a moderate effect on M current but alters subcellular localization of Kv7 channels

Affef Abidi [a,b], Jérôme J. Devaux [c], Florence Molinari [d,e], Gisèle Alcaraz [a,b], François-Xavier Michon [d,e], Julie Sutera-Sardo [a,b,f], Hélène Becq [d,e], Caroline Lacoste [a,b,bb], Cécilia Altuzarra [g], Alexandra Afenjar [i,ii], Cyril Mignot [h,i], Diane Doummar [ii], Bertrand Isidor [j], Sylvie N. Guyen [k], Estelle Colin [l], Sabine De La Vaissière [m], Damien Haye [n], Adeline Trauffler [o], Catherine Badens [a,b,bb], Fabienne Prieur [p], Gaetan Lesca [q], Laurent Villard [a,b], Mathieu Milh [a,b,f,*], Laurent Anikstejn [d,e,**]

[a] *Aix-Marseille Université, GMGF, Marseille, France*
[b] *INSERM, UMR_S 910, Marseille, France*
[bb] *APHM, Hôpital d'enfants de la Timone, Département de génétique médicale et de biologie cellulaire, Marseille France*
[c] *Aix-Marseille Université, CNRS, CRN2M-UMR7286, Marseille, France*
[d] *Aix-Marseille Université, Institut de Neurobiologie de la Méditerranée (INMED), Marseille, France*
[e] *INSERM, UMR_S 901, Marseille, France*
[f] *APHM, Hôpital d'Enfants de la Timone, Service de neurologie pédiatrique, Marseille, France*
[g] *CHU Besançon, Service de génétique et neuropédiatrie, Besançon, France*
[h] *APHP, Service de Génétique Médicale et Centre de Références « Déficiences Intellectuelles de Causes Rares », Groupe Hospitalier Pitié-Salpêtrière, Paris, France*
[i] *Université Pierre et Marie Curie, Groupe de Recherche Clinique « Déficiences Intellectuelles de Causes Rares », Paris, France*
[ii] *APHP, service de neurologie pédiatrique, Hôpital Trousseau, Paris, France*
[j] *CHU de Nantes, Service de génétique médicale, Nantes, France*
[k] *CHU d'Angers, Service de neurologie pédiatrique, Angers, France*
[l] *CHU d'Angers, Département de Biochimie et Génétique, Angers, France*
[m] *CHU de Tours, Service de neurologie pédiatrique, Tours, France*
[n] *CHU de Tours, Service de génétique, Tours, France*
[o] *CHU de Lille, Service de neurologie pédiatrique, Lille, France*
[p] *CHU de Saint Etienne, Service de génétique médicale, France*
[q] *Hospices Civils de Lyon, Service de génétique, Lyon, France*

ARTICLE INFO

Article history:
Received 1 December 2014
Revised 28 March 2015
Accepted 15 April 2015
Available online 22 May 2015

Keywords:
Early epileptic encephalopathy
Kv7 channels
M-current
p.A294V mutation
p.A294G mutation
Subcellular channel expression

ABSTRACT

Mutations in the *KCNQ2* gene encoding the voltage-dependent potassium M channel Kv7.2 subunit cause either benign epilepsy or early onset epileptic encephalopathy (EOEE). It has been proposed that the disease severity rests on the inhibitory impact of mutations on M current density. Here, we have analyzed the phenotype of 7 patients carrying the p.A294V mutation located on the S6 segment of the Kv7.2 pore domain ($Kv7.2^{A294V}$). We investigated the functional and subcellular consequences of this mutation and compared it to another mutation ($Kv7.2^{A294G}$) associated with a benign epilepsy and affecting the same residue. We report that all the patients carrying the p.A294V mutation presented the clinical and EEG characteristics of EOEE. In CHO cells, the total expression of $Kv7.2^{A294V}$ alone, assessed by western blotting, was only 20% compared to wild-type. No measurable current was recorded in CHO cells expressing $Kv7.2^{A294V}$ channel alone. Although the total $Kv7.2^{A294V}$ expression was rescued to wild-type levels in cells co-expressing the Kv7.3 subunit, the global current density was still reduced by 83% compared to wild-type heteromeric channel. In a configuration mimicking the patients' heterozygous genotype i.e., $Kv7.2^{A294V}$/Kv7.2/Kv7.3, the global current density was reduced by 30%. In contrast to $Kv7.2^{A294V}$, the current density of homomeric $Kv7.2^{A294G}$ was not significantly changed compared to wild-type Kv7.2. However, the current density of $Kv7.2^{A294G}$/Kv7.2/Kv7.3 and $Kv7.2^{A294G}$/Kv7.3 channels were reduced by 30% and 50% respectively, compared to wild-type Kv7.2/Kv7.3. In neurons, the p.A294V mutation induced a mislocalization of heteromeric mutant channels to the somato-dendritic compartment, while the p.A294G mutation did not affect the localization of the heteromeric channels to the axon initial segment. We conclude that this

* Correspondence to: M. Milh, APHM, Hôpital d'Enfants de la Timone, Service de neurologie pédiatrique, rue St Pierre, 13005 Marseille, France.
** Correspondence to: L. Anikstejn, INMED-INSERM U901, parc Scientifique de Luminy, 13273 Marseille cedex 09, France.
E-mail addresses: mathieu.milh@ap-hm.fr (M. Milh), laurent.anikstejn@inserm.fr (L. Anikstejn).
Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2015.04.017

position is a hotspot of mutation that can give rise to a severe or a benign epilepsy. The p.A294V mutation does not exert a dominant-negative effect on wild-type subunits but alters the preferential axonal targeting of heteromeric Kv7 channels. Our data suggest that the disease severity is not necessarily a consequence of a strong inhibition of M current and that additional mechanisms such as abnormal subcellular distribution of Kv7 channels could be determinant.

Introduction

Kv7.2-5/*KCNQ2–5* channels are slow activating and non-inactivating voltage-gated potassium channels expressed in several neuronal populations (Jentsch, 2000; Brown and Passmore, 2009). Each subunit consists of intracellular N and C terminal domains and 6 transmembrane segments forming a voltage-sensing (S1–S4) and a pore (S5–P–S6) domains. Functional channels are formed by the homomeric or heteromeric assemblies of 4 subunits including Kv7.2 and Kv7.3, the two principal subunits underlying M current in many neurons (Wang et al., 1998; Battefeld et al., 2014; but see also Soh et al., 2014). Recent reports indicate that Kv7.2/Kv7.3 channels are selectively localized at the axonal initial segments (AIS) and at nodes of Ranvier in central and peripheral nervous systems where they are co-clustered with Nav channels (Devaux et al., 2004; Pan et al., 2006; Rasmussen et al., 2007; Battefeld et al., 2014). At these positions, Kv7 channels play a crucial role in controlling neuronal excitability (Yue and Yaari, 2006; Hu et al., 2007; Shah et al., 2008; Battefeld et al., 2014; Soh et al., 2014).

Previously, mutations in *KCNQ2* have only been reported in benign familial neonatal seizures (BFNS) (Biervert et al., 1998; Singh et al., 1998; Charlier et al., 1998). More recently, *de novo* mutations in *KCNQ2* were described in a severe form of neonatal epilepsy (early onset epileptic encephalopathy, EOEE), characterized by an early neonatal onset (first week) of stormy motor seizures, without any structural abnormality (but see Dalen Meurs-van der Schoor et al., 2014) and with variable outcome (Weckhuysen et al., 2012; Kato et al., 2013; Milh et al., 2013; Allen et al., 2014). Indeed, despite relatively similar early clinical features, the prognosis of *KCNQ2*-related epilepsies is highly variable. To date, there appears to be a broad separation between mutations of *KCNQ2* causing severe versus benign epilepsies but exceptions do exist (Borgatti et al., 2004; Steinlein et al., 2007). It was therefore proposed that the clinical variability of the *KCNQ2*-related epilepsy could be related to the functional consequence of mutations on M current and could thus be predictive of the neurological prognosis, a notion that was recently supported by two different studies (Miceli et al., 2013; Orhan et al., 2014).

Here, we investigated the early electro-clinical features and clinical evolution of 7 patients carrying a Kv7.2-p.A294V (Kv7.2^{A294V}) mutation located within the S6 pore domain and compared the functional consequences of this mutation to an inherited mutation associated with BFNS (Kv7.2^{A294G}, Steinlein et al., 2007). In those patients, the p.A294V mutation was always associated with severe forms of neonatal epilepsy. Both mutations had the same functional impact on M current, and modestly reduced the global current density by ~30% as measured in Chinese hamster ovary cells (CHO) in the configuration mimicking the situation found in patients. However, the p.A294V but not the p.A294G mutation induced a redistribution of Kv7 channels to the somato-dendritic compartment of neurons. Thus, our data indicate that the severity of the disease is not necessarily associated with strong inhibition of M current and we postulate that additional mechanisms such as abnormal subcellular distribution of Kv7 channels could be determinant.

Patients and methods

Patients

KCNQ2 screening was performed for diagnosis purpose at the "Département de Génétique Médicale" of the Timone Children's Hospital in Marseille and in the Hôpital Femme-Mère-Enfant in Lyon. We received a total of 237 patient's DNA having an early onset epileptic encephalopathy (EOEE, n = 208) or benign familial neonatal seizures (BFNS, n = 29). We found *KCNQ2* mutations in 46 patients; 23 presenting EOEE (see Fig. 1) and 23 presenting BFNS. Some of the EOEE and BFNS-related mutations have already been published (Milh et al., 2013; Soldovieri et al., 2014). We found 7 patients (5 in Marseille and 2 in Lyon) carrying the same mutation in the *KCNQ2* gene, c.881C>T/p.A294V (NM_172107.2), and presenting an EOEE (see the

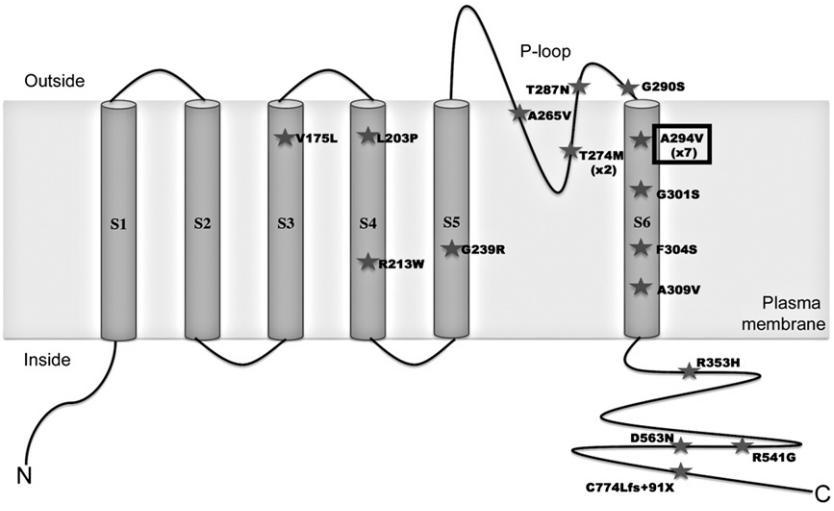

Fig. 1. Schematic presentation of the Kv7.2 subunit with the location of the different mutations associated with EOEE identified at the Timone Hospital in Marseille and in the Hôpital Femme-Mère-Enfant in Lyon. We analyzed the functional consequences and subcellular distribution of the Kv7.2 channel carrying the pore p.A294V mutation identified in 7 patients of our cohort.