Channels

ISSN: 1933-6950 (Print) 1933-6969 (Online) Journal homepage: http://www.tandfonline.com/loi/kchl20

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To cite this article: Maria Virginia Soldovieri, Francesco Miceli, Giulia Bellini, Giangennaro Coppola, Antonio Pascotto & Maurizio Taglialatela (2007) Correlating the Clinical and Genetic Features of Benign Familial Neonatal Seizures (BFNS) with the Functional Consequences of Underlying Mutations, Channels, 1:4, 228-233, DOI: 10.4161/chan.4823

To link to this article: http://dx.doi.org/10.4161/chan.4823

Published online: 31 Aug 2007.

[Channels 1:4, 228-233, July/August 2007]; ©2007 Landes Bioscience

Addendum

Correlating the Clinical and Genetic Features of Benign Familial Neonatal Seizures (BFNS) with the Functional Consequences of Underlying Mutations

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Original manuscript submitted: 08/02/07
Manuscript accepted: 08/02/07

Previously published online as a *Channels* E-publication:
http://www.landesbioscience.com/journals/channels/article/4823

KEY WORDS

epilepsy, Kv7.2 potassium channels, benign familial neonatal seizures, channel gating, mutations, genotype-phenotype correlations

ACKNOWLEDGEMENTS

The present study was supported by grants from: Telethon GP07125 and the European Commission STREP n. 503038 to M.T.

Addendum to:

Atypical Gating of M Type Potassium Channels Conferred by Mutations in Uncharged Residues in the S4 Region of KCNQ2 Causing Benign Familial Neonatal Convulsions

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J Neurosci 2007; 27:4919–28

ABSTRACT

Almost ten years have passed since the identification of *Kv7.2* and *Kv7.3*, the genes altered in Benign Familial Neonatal Seizures (BFNS), a familial autosomal dominant focal epilepsy of the newborn. Despite the rarity of the disease, clinical and genetic data have been gathered from more than 50 BFNS-affected families; these studies reveal that each family harbours a specific disease-causing mutation, and that the mutation-induced functional changes range from a subtle alteration in channel behaviour to a complete ablation of channel function. Prompted by the recent identification of peculiar gating changes in Kv7.2 subunits caused by novel mutations responsible for BFNS, in the present work we attempt to link, whenever possible, the specific genetic defect with the clinical evolution of the disease in the affected families on one side, and, on the other, with the functional defects revealed by expression studies. Such genotype-phenotype correlations may provide clues on the pathogenesis of the wide variety of neuropsychiatric manifestations often associated to BFNS, and should further our attempts to gain more detailed functional information which might help to elucidate the pathogenetic mechanisms of the disease.

Neonatal seizures with a strong familial component were first described in 1964,[1] revealing that genetic mechanisms, in addition to environmental perinatal factors, contributed to disease pathogenesis. The term benign was added few years later, in recognition of the fact that most of the patients display a normal psychomotor development.[2] Currently, Benign Familial Neonatal Seizures (BFNS; OMIM #121200) are classified within the group of familial autosomal dominant focal epilepsies;[3] in BFNS patients, convulsions begin around day 3 and disappear spontaneously around the third month of postnatal life.[4] More recent follow-up studies reveal that seizures or other neuropsychiatric abnormalities can occur in up to 15% of the patients, therefore questioning the benignity of the disease.[5]

In 1989, the first locus associated to BNFS was identified (20q13.3, EBN1); a second locus (8q24, EBN2) was subsequently discovered. Using positional cloning strategies, the genes altered in both EBN1[6,7] and EBN2[8] were identified; they encode for two highly homologous potassium (K^+) channel subunits named Kv7.2 and Kv7.3, respectively. The Kv7 K^+ channel subfamily also includes Kv7.1, which controls the repolarization phase of the cardiac action potential and is altered in the Long QT Syndrome-2;[9] Kv7.4, which is mutated in some forms of autosomal dominant deafness;[10] and Kv7.5.[11] Kv7 channel subunits are characterized by the presence of six transmembrane segments (S_1–S_6). At most neuronal sites, heteromeric tetramerization of Kv7.2 and Kv7.3 subunits is thought to underlie most of the M-current,[12] a widely-distributed K^+ current characterized by low activation threshold, slow activation and deactivation kinetics, absence of inactivation, and regulation by $G_{q/11}$-coupled receptors.

Since the first identification of the disease-causing genes, several families affected by BFNS have been described and genotyped for *Kv7.2* and *Kv7.3* mutations; these are found in approximately 70% of the cases, suggesting the existence of additional loci.[13] An updated list of the mutations identified in BFNS is given in Table 1. Collectively, these results reveal that *Kv7.2* is a major locus for BFNS, being affected in more than 90% of the cases, with a penetrance of about 85%. Missense, non-sense and frameshift mutations leading to truncated or extended protein sequences have been described, with most of the families showing a different genetic alteration; while most missense mutations alter the sequence of the transmembrane region of the protein, the largest number of mutations fall within the long C-terminus domain. This region, beside embedding the molecular

Table 1 **Overview of the available genetic, clinical and functional data from BFNS families**

Kv7.2

Amino Acid Change	Localization	Clinical Data (Beside BFNS)	Functional Effects	Reference
M1V	N-terminus	—	—	33
M1T		—	—	33
Frameshift at K69		—	—	33
Frameshift at Q78		LCS+GCS	—	34
In-frame deletion of S105	S1	—	—	34
S122L	S2	FS later in life; seizures until 7 years	Rightward shift in current voltage-dependence in the sub-threshold region; decrease in current activation kinetics	35
Splice site mutation at L129		—	—	17
Frameshift at S195	S2-S3 linker	—	—	36
A196V	S4	—	Rightward shift in current voltage-dependence; decrease in current activation kinetics; novel prepulse-dependence of current activation kinetics	26
A196V/L197P		—	Rightward shift in current voltage-dependence; decrease in current activation kinetics	26, 36
R207W		Myokymia	Marked rightward shift in current voltage-dependence; dramatic decrease in current activation kinetics	23
R207Q		Myokymia	Rightward shift in current voltage-dependence; decrease in current activation kinetics	24
M208V		GS between 4 and 7 years	Small decrease in maximal current; increased rate of channel deactivation	17
R214W		—	Slight rightward shift in current voltage-dependence; no effect on maximal current amplitude	25, 37
H228Q	S4-S5 linker	—	—	17
L243F	S5	—	—	17
S247W		Therapy-resistant seizures; epileptic encephalopathy	Markedly reduced maximal current amplitude (dominant-negative effect on Kv7.2 channels)	27
S247X		CS; FS at three years	—	35
V250G		—	—	36
E254X		mild mental retardation; West syndrome	Lack of functional homomeric channels; markedly reduced current amplitude in heteromeric channels	20
W269X	S5-S6 linker	1/7 late onset; 2/7 FS+GS in adulthood	—	17
G271V	Pore	BFIC	—	38
Frameshift at K283		5/19 GS between 21 and 45 years	—	6
Y284C		—	Markedly reduced current amplitude	6, 25
A306T	S6	11/69 FS; GS between 1 and 16 years	—	6
Q323X	C-terminus	2/6 BECTS at 2 and 4 years	Lack of functional homomeric channels; marked reduction in current amplitude in heteromeric channels	17
R333Q		—	Reduction in current amplitude; Faster rate of current deactivation	17
L339R		—	—	36
R353G		—	Reduced interaction with CaM	33
Splice site mutation at S373		LCS+GCS; 1 FS	—	34
S382X		late onset BFNS; 4/11 FS, GS until 10 years	—	6
Splice site mutation at N396		wide range of clinical manifestations, with partial seizures later in life	—	39
Splice site mutation at L397		—	—	40
Frameshift at K398		GTCS	—	41
Splice site mutation at R406		—	—	40

Continued

Table 1 **Overview of the available genetic, clinical and functional data from BFNS families (continued)**

R448X	—	30% reduction in maximal current amplitude in heteromeric channels	17, 33, 36
Splice site mutation at E509	—	—	33
Frameshift at Q494	—	—	40
Splice site mutation at D516	—	—	40
Frameshift at S522	late onset BFNS; 1/6 FS at 2 years	—	6
Frameshift at P534	—	Lack of functional homomeric channels; reduction maximal current amplitude of heteromeric channels	7
Splice site mutation at C544	late onset BFNS	—	6
R553Q	—	—	36
K554N	2/4 therapy-resistant seizures and mental retardation	Slight rightward shift in current voltage-dependence; no effect on maximal current amplitude	28
R581X	—	—	17
Splice site mutation at R588	—	—	33
R588X (intronic mutation)	1/11 seizures continued until 14 months of age; photosensitive myoclonic epilepsy at age 13 years; 1/11 mental retardation	—	42
L637R	—	Increased interaction with CaM	33
Frameshift at Y644 (+ 56aa)	In all patients, seizures persisted until 12–18 months	—	21, 43
Frameshift at T653	—	—	17
Frameshift at P709 (+ 56aa)	1/3 CTS at 3 years; 1/3 GS later in life	Lack of functional homomeric channels; reduction in current amplitude of heteromeric channels; decreased protein stability and enhanced degradation	15, 19, 44
Frameshift at G866 (+ 56aa)	—	Reduction of current amplitude in homomeric and heteromeric channels	22
Frameshift at W867 (+ 57aa)	3/12 seizures continued until age 2, 3, 7 years	Reduction in current amplitude; Slight shift in activation voltage-dependence; slight acceleration of current deactivation	17

Kv7.3

Amino Acid Change	Localization	Additional Clinical Data (Beside BFNS)	Functional Effects	Reference
D305G	Pore	—	Reduced current amplitude of heteromeric channels	17
W309R		—	—	45
G310V		—	slight (20%) reduction in heteromeric channels current	8
N468S	C-terminus	Three siblings affected by BFIC	No effect; possibly a polymorphism	17
N821S		Does not cosegregate with the disease	No difference versus wt; possibly a variant of unknown significance	20
nt c988t	?	—	—	46

LCS, lateral clonic seizures; GCS, generalized clonic seizures; FS, febrile seizures; GS, generalized seizures; CS, clonic seizures; BFIC, benign familial infantile convulsions; BECTS, benign epilepsy with centrotemporal spikes; GTCS, generalized tonic-clonic seizures; CTS, centrotemporal spikes. When necessary, amino acid numbering has been modified according to ref. 6, and is consistent with the longest variant "a" of Kv7.2 (872 amino acids; GenBank AF033348).

determinants required for subunit assembly, also provides the attachment site for a complex network of interacting molecules such as phosphatidylinositol 4,5-bisphosphate (PIP_2), calmodulin (CaM), A-kinase-anchoring proteins (AKAPs), Ankyrin-G (Ank-G), and, possibly, pK-A and pK-C, having crucial functional consequences on channel behaviour and sub-cellular distribution[14] (Fig. 1A). In heterologous expression systems, studies on the functional consequences of these mutations have been carried out for a significant fraction of the described *Kv7.2* and *Kv7.3* mutations (Table 1), with consequences ranging from slight changes in channel behaviour to a complete ablation of channel function. The available evidence converges on the hypothesis that a slight (≈ 25%) decrease of the M-current is sufficient to cause BFNS; thus, haploinsufficiency seems to be the primary pathogenetic mechanism for BFNS. However, genotype-phenotype correlations may provide clues on the pathogenesis of the wide variety of neuropsychiatric manifestations often associated with BFNS in different families or family members.

One paradigmatic example is possibly provided by the association between BFNS and an EEG trait characterized by centrotemporal spikes (CTS), or benign rolandic epilepsy (BRE), the most common idiopathic partial epilepsy.[15-17] Although the age-dependent CTS trait seems to segregate independently from Kv7.2,[18] in the families where

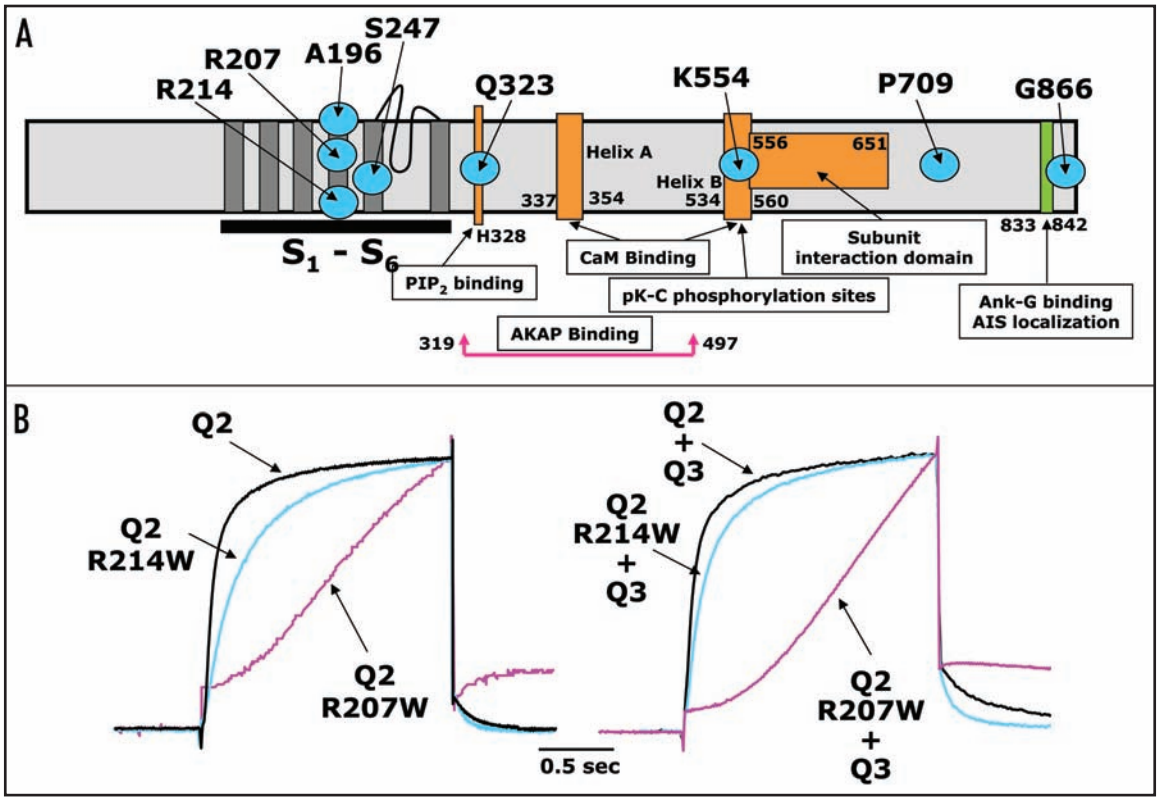

Figure 1. (A) Schematic representation of a single Kv7.2 subunit. With respect to the drawing, relevant sites for functional modulation are shown below; the amino acid positions affected by the mutations discussed in the text and indicated in Table 1 are represented by blue circles and identified above the drawing. AIS, Axon initial segment (B) Effect of the R207W and R214W mutations on homomeric Kv7.2 (left panel) and heteromeric Kv7.2 + Kv7.3 (right panel) channel function. Current traces were recorded upon expression in Xenopus oocytes of the indicated subunit(s) in response to membrane depolarization to +40 mV; holding potential: -90 mV. (For further experimental details, see ref. 25).

an association between BFNS and the CTS trait has been described, the CTS trait (or BRE) developed earlier than in classical rolandic epilepsy. Analysis of the functional consequences of Kv7.2 channels carrying the mutations found in families in which BFNS was followed by the appearance of the CTS trait, namely a complete truncation of the C-terminus (Q323X),[17] or the substitution of the last 163 amino acids within the same region (frameshift at P709),[15] revealed a complete loss of function of mutant subunits. A CTS trait has been also found in the original family described by Rett and Teubel;[1] interestingly, in this family, the same mutation found by Coppola etal.[15] was identified.[19] These results raise the intriguing possibility that specific *Kv7.2* gene defects may be responsible for the early appearance of BRE and, possibly, of various epilepsies or epilepsy-associated EEG traits. It is tempting to speculate that more dramatic functional consequences (such as a complete mutation-induced loss of channel function) maybe also responsible for more serious BFNS-associated clinical phenotypes, such as mild mental retardation and West syndrome,[20] or delayed age of remission;[21] this view seems to be supported by the observation that BFNS patients carrying more distal C-terminus mutations (frameshift at G866) which produced less dramatic consequences in functional expression studies, appear to be affected by classical BFNS with no atypical clinico-electrophysiological features after the BFNS symptoms have disappeared.[22]

Two families in which BFNS was associated with generalized myokymia, a disease characterized by skeletal muscle spontaneous twitching resulting from hyper-excitability of the lower motor neurons, have been described (see refs. 23 and 24). Surprisingly, both families carried missense mutations in the same amino acid, namely the third arginine residue in the voltage-sensing S_4 domain (R207W and R207Q, respectively). Functional studies revealed that the mutations did not lead to the loss of channel function, but rather to a dramatic rightward shift in the steady-state voltage-dependence of current activation, together with a marked decrease of current activation kinetics; as also shown in Figure 1B, the functional consequences on channel gating produced by mutations neutralizing the charge at R207 are considerably more dramatic than those prompted by a similar BFNS-causing mutation affecting another charged residues in the S_4 region (R214W),[25] both in homomeric and heteromeric configuration with Kv7.3 subunits, possibly suggesting a lesser involvement of the R214 residue in Kv7.2 voltage-sensing when compared to the R207 residue. Thus, subtle changes of Kv7.2 gating properties can cause BFNS because of a decrease in M-current repolarizing ability in cortical neurons during early developmental stages; on the other hand, when more dramatic changes in the gating properties of Kv7.2 channels are triggered by the specific underlying mutation, the intrinsic excitability of spinal motor neurons is also affected, thus leading, in addition to BFNS, to involuntary myokymia. Noticeably, mutations in uncharged residues in the S_4 region can also cause BFNS because of peculiar changes in the gating properties of Kv7.2 channel subunits.[26]

BFNS is among the best-recognized disease model for genetically-determined human epilepsies. The identification of

disease-causing mutations in *Kv7.2* and *Kv7.3* K^+ channel genes has allowed a clarification of the molecular pathogenesis of the disease and to explore the function of these genes in neuronal excitability. Nonetheless, several issues are yet to be resolved with regard to the evolution of the disease and its possible association with other convulsive manifestations or neuropsychological abnormalities, in order to bridge the existing gap between clinical characterization and functional studies. For example, clinical follow-up of BFNS family members often reveals more pronounced disease severity, with the occurrence of therapy-resistant epilepsies or epileptic encephalopathy.[27,28] Electrophysiological analysis of the functional consequences of the disease-causing mutations has not revealed a strong genotype-phenotype correlation: in one case,[27] the *Kv7.2* mutation (S247W) was able to reduce the channel currents by more than 50%, whereas in the other case[28] (K554N) only a slight reduction in channel function was observed. However, it should be underlined that most of the in-vitro functional experiments often do not cover the full spectrum of alterations in channel function, which might be prompted by the underlying mutation. In fact, the latter mutation falls within the calmodulin binding site along the Kv7.2 cytoplasmic C-terminus (Fig. 1A), next to a consensus sequence for pK-C-induced phosphorylation; whether the mutation impairs such regulation, which is crucial for channel function,[29,30] is yet to be investigated.

Given these limitations, and despite the rarity of the disease, a tighter link between clinical/genetic characterization and functional consequences is crucial to further our comprehension of BFNS and BFNS-associated phenomena. To this aim, investigators should pay particular attention to provide full descriptions of the phenotypic characteristics of the disease in each individual family, directing their efforts at discriminating the age of onset of symptoms, the characteristics and mode of onset of their convulsive episodes, response to available pharmacological therapy, and, in particular, the long-term evolution of the disease; moreover, functional studies should attempt to widen the range of techniques used to investigate the mutation-induced disease mechanism, possibly in experimental contexts closer to the in vivo situation, such as upon expression of mutant channels in a neuronal environment.[31] It can be anticipated that studies on the genotype-phenotype correlation in BFNS-affected patients may also boost research on Kv7 channels as pharmacological targets for hyper-excitability diseases.[32]

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