DOI 10.1007/s00702-002-0750-3
J Neural Transm (2002) 109: 1189–1194

Journal of Neural Transmission
Printed in Austria

Mutation analysis of the sodium/hydrogen exchanger gene (NHE5) in familial paroxysmal kinesigenic dyskinesia

Short Communication

S. D. Spacey[1,2], **B. I. Szczygielski**[2], **J. E. McRory**[2], **G. M. Wali**[3], **N. W. Wood**[4], and **T. P. Snutch**[2]

[1] Division of Neurology, Department of Medicine, and
[2] Biotechnology Laboratory, University of British Columbia, Vancouver, Canada
[3] Department of Neurology, Jawnharlal Nehru Medical Centre, KLE Society Hospital, Belguam, Karnataka, India
[4] Department of Clinical Neurology, Institute of Neurology, University College London, London, United Kingdom

Received March 16, 2002; accepted March 22, 2002
Published online June 28, 2002;

Summary. Familial Paroxysmal Kinesigenic Dyskinesia (PKD) is an autosomal dominant condition characterized by attacks of dystonia or chorea triggered by sudden movements. Recently two separate loci for PKD, Episodic Kinesigenic Dyskinesia 1 (EKD1) and Episodic Kinesigenic Dyskinesia 2 (EKD2), have been mapped to chromosome 16 but the causative genes have not been identified.

The Na^+/H^+ exchanger gene (NHE5) involved in regulating intracellular pH lies in the EKD2 region. The coding region of the NHE5 gene in familial PKD was sequenced. We did not identify any mutations in the exons, intron/exon boundaries or the 5′ and 3′UTR. This excludes mutations in the coding region of the NHE5 gene as a cause for familial PKD, but does not rule out a possible role of sequence variants in introns or regulatory regions.

Keywords: Na^+/H^+ exchanger gene (NHE5), paroxysmal kinesigenic dyskinesia, neurogenetics, movement disorders.

Introduction

The paroxysmal neurological conditions (migraine, epilepsy and the paroxysmal dyskinesias) are an intriguing collection of conditions characterized by episodes of neurological dysfunction with relative neurological recovery between attacks. Research into the underlying pathophysiology has revealed a dysfunction in ion channel activity in many of these disorders (Hanna et al.,

1998) and it is suspected that most of the paroxysmal neurological conditions are the result of ion channel dysfunction or a mechanism which affects neuronal stability.

Paroxysmal Kinesigenic Dyskinesia (PKD) is the most common of the paroxysmal dyskinesias (Houser et al., 1999). It is characterized by attacks of dystonia or chorea triggered by sudden movements. PKD patients are typically normal between attacks and laboratory and radiological studies are unremarkable (Houser et al., 1999; Fahn, 1994; Sadamatsu et al., 1999; Nagamitsu et al., 1999; Tan et al., 1998).

PKD is an autosomal dominant condition with a penetrance of approximately 80% (Valente et al., 2000). Recently two separate loci for PKD, EKD1 and EKD2 have been mapped to chromosome 16 but the causative genes have not been identified. EKD1 has been mapped to 16p11.2–q12.1 (Tomita et al., 1999), whereas EKD2 lies caudal to the EKD1 locus and has been mapped to 16q13–22.1 (Valente et al., 2000). Although many of the paroxysmal neurological conditions have proven to be defects in ion channel function (chanelopathies) (Hanna et al., 1998) no identified ion channels map to this region. However, given our understanding of paroxysmal neurological disorders, a candidate gene for PKD may not be limited to ion channel genes but could include any gene which codes for a protein that influences neuronal excitability. One such candidate gene is the Na^+/H^+ exchanger (NHE5) gene.

NHE5 is the fifth identified member of the Na^+/H^+ exchanger family (NHE1–6). The Na^+/H^+ exchanger (NHE5) gene regulates neuronal intracellular pH (Baird et al., 1999) and could potentially cause paroxysmal neurological disease from abnormal regulation. A mutation in this family of genes (NHE1) in mice results in an epilepsy phenotype (Cox et al., 1997). This demonstrates that mutations in this family of genes can cause paroxysmal neurological conditions. The NHE5 gene has been mapped to 16q22.1 (Klanke et al., 1995), the same region as the EKD2 gene and it is highly expressed in the brain, particularly in the basal ganglia (Baird et al., 1999). On this basis the NHE5 gene is an excellent candidate gene for PKD.

We performed mutational analysis of the NHE5 gene by direct sequencing of the exon, intron/exon boundaries and the 5′ and 3′UTR in a family with PKD linked to the EKD2 locus on chromosome 16q13–q22.1.

Material and methods

Subjects

The gene for PKD (EKD2) in the family studied here had previously been linked to chromosome 16q13–q22.1 (Valente et al., 2000). The family demonstrated autosomal dominant transmission over four generations (Fig. 1). Genomic DNA was isolated from peripheral blood in affected family members using standard techniques.

Sequence analysis of the NHE5 gene in a family with PKD

Based on the human genomic sequence of the NHE5 gene (Baird et al., 1999), we designed primers in the introns of the NHE5 gene for the sequencing of the exons, the

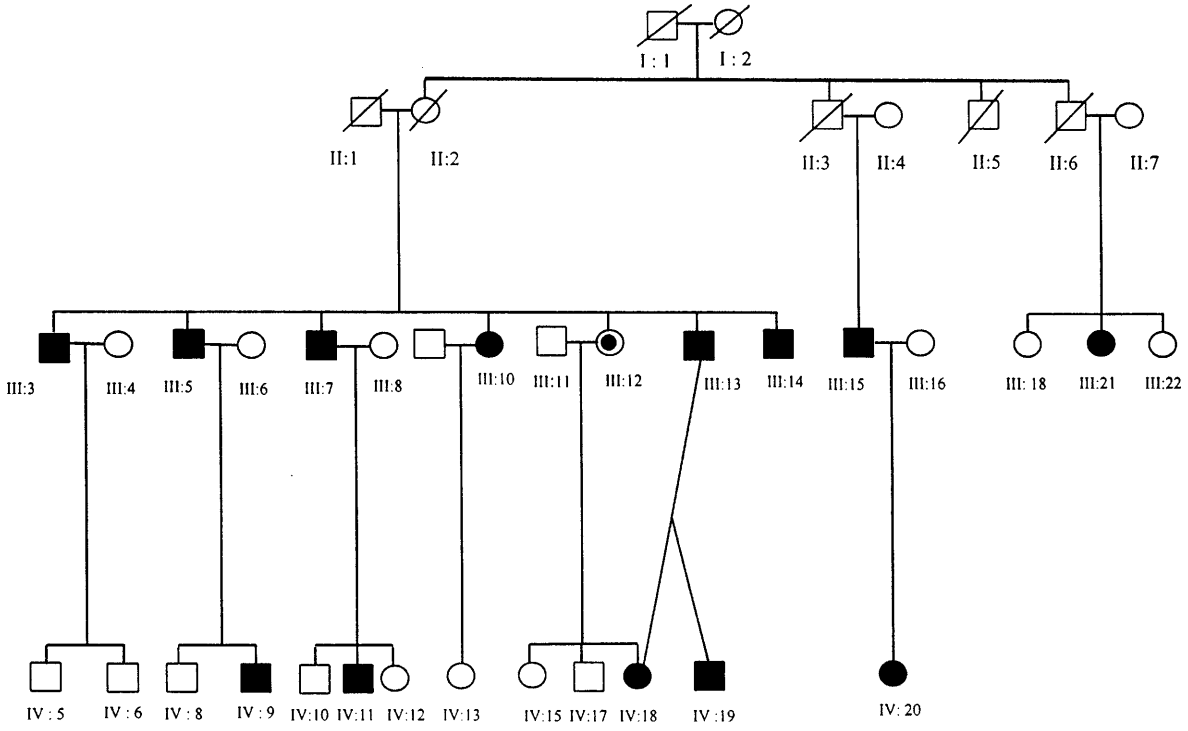

Fig. 1. Pedigree of PKD family. The circles represents females, the squares represent males. The black symbols denote individuals affected by PKD. The white symbol with a small black circle within it represents an obligate gene carrier. Deceased members are marked with a diagonal bar. Individual IV:18 married her uncle (III:13); individual IV:19 is their offspring

intron/exon boundaries and the 5′ and 3′UTR (Table 1). Each exon, its flanking intron/exon boundary and the 5′ and 3′UTR were PCR-amplified and subjected to automated sequencing using an ABI 377 automated DNA sequencer. For mutational analysis of the human NHE5 gene in familial PKD, samples of two affected individuals (III:7, III:15) in this PKD family were analyzed. The sequences obtained from the PKD affected individuals were compared to the human genomic sequence of the NHE5 gene in order to identify any potential mutations.

Results

We performed a mutation analysis of the NHE5 gene in familial PKD linking to the EKD2 locus. Direct sequencing of 16 exons, the intron/exon boundaries and the 5′ and 3′UTR revealed no disease causing heterozygous mutations.

Discussion

The Na^+/H^+ exchangers (NHE5) gene is an excellent candidate gene for PKD as it is involved in intracellular pH regulation and can influence general neuroexcitability (Baird et al., 1999). The NHE5 is the fifth of a family of six Na^+/H^+ exchanger genes, five are plasma membrane exchangers (NHE1–5) and one is mitochondrial (NHE6) (Baird et al., 1999). This family of Na^+/H^+

Table 1. Primer pairs used for sequencing of exons and intron/exon boundaries in the human NHE5 gene

Exon	Primer – forward	Primer – reverse	PCR conditions	Size of PCR fragment (bp)
5′UTR 1	TAT CGG AGC CGG GAT TGG	CCA ATC TGA GGA CCG AGG	35 cycles, 58°C AT	400
2	TAC TGC ACT CCA GCT TGG	TAG GAT GGA GCT TTC AGG	35 cycles, 58°C AT	540
3, 4	ATG CTG TCT TTG CCA TCC	GAA TAG CCT CTT CTG TCC	35 cycles, 56°C AT	660
5	ACA GAA GAG GCT ATT CGG	ATG GAC TCC TGA AGT TCC	35 cycles, 56°C AT	470
6, 7	AGT ATG CTC AAA CCC TGG	ACT GCA CTC AAG GTC TGG	35 cycles, 55°C AT	780
8, 9	AGA CCT TGA GTG CAG TGG	CCC TTC TCA GAA GTG TGG	35 cycles, 54°C AT	540
10	TGT CAG CCT TTC CCA TGA CA	AGG AAT TCC CAA CTC CCT GC	35 cycles, 58°C AT	430
11, 12	AAG TGC CTA GCA GAG TAC C	CTT A AG C TC TCC TCA G ACC	35 cycles, 55°C AT	540
13	AGA ACA CAG GGT CTC TGG	TGC TTG CTG TCA GCC TCC	35 cycles, 54°C AT	300
14	TGG AGG CTG ACA GCA AGC	GTC CTC CAG GAT TCT GGC	35 cycles, 55°C AT	600
15	CTG TGT AGT CCC AAC AGG C	AGG CTC TGC AAA TGC TGG C	35 cycles, 54°C AT	240
16, 3′UTR	AGA CAT TCA TCC GAT AAT CG	CCA TTC TCA ATC CCT GGG	35 cycles, 55°C AT	1,690

List of primers used for sequencing the exon, intron/exon boundaries and the 5′ & 3′UTR of the NHE5 gene. PCR conditions are documented concerning cycle annealing-temperature (AT,[°C])

exchangers are membrane proteins that mediate electro neutral exchange of extracellular Na^+ for intracellular H^+. In vitro data demonstrate that human NHE5 mediates H^+ dependent Na^+ influx (Baird et al., 1999). This is consistent with the observation that rat NHE5 mediates Na^+ dependent pH recovery from an acid load (Attaphitaya et al., 1999). Membrane voltage and ligand gated ion channels, transmitter uptake through transporters, intracellular signal transduction and intercellular communication via gap junctions are pH dependent (Takahashi et al., 1996). Dysfunction of NHE5 activity could result in decreased ability to regulate transient changes in pH and hence reduced threshold for propagation of an action potential. Triggers of PKD such as hyperventilation and movement, further suggest that pH may play a role in this condition. Considering its location in the genome, its known function and its high expression in the basal ganglia, the NHE5 gene is an excellent candidate gene for PKD.

This study excludes mutations in the coding sequences, the intron/exon boundaries and the 5′ and 3′UTR of the NHE5 gene as pathogenic in familial PKD linking to the EKD2 locus. Mutations in the intronic or regulatory regions of the gene can not be excluded and were not sequenced due to their large size. Further investigations will concentrate on other possible candidate genes in this region which include solute carriers SLC12A3, SLC12A4 and SLC7A6. The SLC12A3 gene codes for a sodium-chloride cotransporter which is expressed in the kidney and has been implicated in Gitelman's Syndrome, its expression has not been demonstrated in the brain and therefore is unlikely to be a candidate. However, the SLC12A4 and SLC7A6 genes have ubiquitous expression including expression in the brain and warrant further investigation.

Acknowledgement

This work was supported by a grant from the Canadian Institute of Health Research (CIHR) to TPS.

References

Attaphitaya S, Park K, Melvin JE (1999) Molecular cloning and functional expression of a rat Na^+/H^+ exchanger (NHE5) highly expressed in brain. J Biol Chem 274: 4383–4388

Baird NR, Orlowski J, Szabo EZ, Zaun HC, Schultheis PJ, Menon AG, Schull GE (1999) Molecular cloning, genomic organization and functional expression of Na^+/H^+ exchanger isoform 5 (NHE5) from human brain. J Biol Chem 274: 4377–4382

Cox GA, Lutz CM, Yang CL, Biemesderfer D, Bronson RT, Fu A, Aronson PS, Noebels JL, Frankel WN (1997) Sodium/hydrogen exchanger gene defect in slow – wave epilepsy mutant mice. Cell 9: 139–148

Fahn S (1994) The paroxysmal dyskinesias. In: Marsden CD, Fahn S (eds) Movement disorders, 1st ed. Butterworth-Heinemann, Oxford, pp 310–345

Hanna MG, Wood NW, Kullmann D (1998) Ion channel and neurological disease: DNA based diagnosis is now possible, and ion channels may be important in common paroxysmal disorders. J Neurol Neurosurg Psychiatry 65: 427–341

Houser MK, Soland VL, Bhatia KP, Quinn NP, Marsden CD (1999) Paroxysmal kinesigenic choreoathetosis: a report of 26 patients. J Neurol 246: 120–126

Klanke CA, Callen SYR, Wang Z, Meneton P, Baird N, Kandasamy RA, Orlowski J, Otterud BE, Leppert M, Schull GE, Menon AG (1995) Molecular cloning and physical mapping of a novel human Na^+/H^+ exchanger (NHE5/SLC9A5) to chromosome 16q22.1. Genomics 25: 615–622

Nagamitsu S, Matsuishi T, Hashimoto K, Yamashita Y, Aihara M, Shimizu K, Mizugauchi M, Iwamoto H, Saitoh S, Hirano Y, Kato H, Fukuyama Y, Shimada M (1999) Multicenter study of paroxysmal dyskinesias in Japan-clinical and pedigree analysis. Mov Disord 14: 658–663

Sadamatsu M, Masui A, Sakai T, Kunugi H, Nanko SI, Kato N (1999) Familial paroxysmal kinesigenic choreoathetosis: an electrophysiologic and genotypic analysis. Epilepsia 40: 942–949

Takahashi KI, Copenhagen DR (1996) Modulation of neuronal function by intracellular pH. Neurosci Res 24: 109–116

Tan LC, Tan AK, Tjia H (1998) Paroxysmal kinesigenic choreoathetosis in Singapore and its relationship to epilepsy. Clin Neurol Neurosurg 100: 187–1921

Tomita H, Yamada K, Niikawa, Nakane Y, Nagamitsu S, Matsuishi T, Wakui, Fukushima Y, Yamada K, Sadamatsu, Masui A, Konishi T, Matsuishi T, Aihara M, Shimizu K, Hashimoto K, Mineta M, Matsushima M, Tsujita T, Saito M, Tanaka H, Tsuji S, Takagi T, Nakamuar Y, Nanko S, Kato N, Nakane Y, Kiikawa N (1999) A gene for paroxysmal kinesigenic choreathetosis mapped to chromosome 16p11.2–q12.1. Am J Hum Genet 65: 1688–1697

Valente EM, Spacey SD, Wali GM, Bhatia KP, Dixon PH, Wood NW, Davis MB (2000) A second paroxysmal kinesigenic choreoathetosis locus (EKD2) mapping on 16q13–q22.1 indicates a family of genes which give rise to paroxysmal disorders on human chromosome 16. Brain 123: 2040–2045

Authors' address: Dr. S. Spacey, Rm. S-127, 2211 Wesbrook Mall, Division of Neurology, UBC Hospital Vancouver, B.C., Canada, V6T 2B5, e-mail: spacey@interchange.ubc.ca