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Novel \textit{PRRT2} mutation in an African-American family with paroxysmal kinesigenic dyskinesia

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Abstract

\textbf{Background:} Recently, heterozygous mutations in \textit{PRRT2} (Chr 16p11.2) have been identified in Han Chinese, Japanese and Caucasians with paroxysmal kinesigenic dyskinesia. In previous work, a paroxysmal kinesigenic dyskinesia locus was mapped to Chr 16p11.2 - q11.2 in a multiplex African-American family.

\textbf{Methods:} Sanger sequencing was used to analyze all four \textit{PRRT2} exons for sequence variants in 13 probands (9 Caucasian, 1 Caucasian-Thai, 1 Vietnamese and 2 African-American) with some form of paroxysmal dyskinesia.

\textbf{Results:} One patient of mixed Caucasian-Thai background and one African-American family harbored the previously described hotspot mutation in \textit{PRRT2} (c.649dupC, p.R217Pfs*8). Another African-American family was found to have a novel mutation (c.776dupG, p.E260*). Both of these variants are likely to cause loss-of-function via nonsense-mediated decay of mutant \textit{PRRT2} transcripts. All affected individuals had classic paroxysmal kinesigenic dyskinesia phenotypes.

\textbf{Conclusions:} Heterozygous \textit{PRRT2} gene mutations also cause paroxysmal kinesigenic dyskinesia in African-Americans. The c.649dupC hotspot mutation in \textit{PRRT2} is common across racial groups.

\textbf{Keywords:} PKD, PRRT2, African-American, ICCA, Hotspot mutation

Background

Paroxysmal kinesigenic dyskinesia (PKD, OMIM 128000), also known as episodic kinesigenic dyskinesia (EKD1) and paroxysmal kinesigenic choreoathetosis (PKC), is a rare autosomal dominant neurological disorder characterized by recurrent, brief attacks of involuntary movement usually triggered by sudden voluntary movement [1,2]. These attacks usually begin in childhood or early adulthood and may include various combinations of dystonia, chorea, and athetosis affecting the face, trunk, arms and legs. Oftentimes, PKD improves with age and most patients show a favorable response to anticonvulsant medications, particularly carbamazepine or phenytoin [1,2]. Recently, mutations in \textit{PRRT2} (Chr 16p11.2) have been causally associated with both familial and sporadic cases of PKD, infantile convulsions and choreoathetosis exercise-induced dyskinesia (PED), and paroxysmal non-kinesigenic dyskinesia-like (PNKD-like) syndromes in Han Chinese, Japanese and Caucasians [3-11].

PKD is clinically and genetically heterogeneous, and, in at least one British pedigree, does not map to Chr 16 [12]. Work to date suggests that fewer than 50% of patients with primary PKD harbor mutations in \textit{PRRT2} [6,8]. To expand the genotypic spectrum of \textit{PRRT2} mutations and examine the role of \textit{PRRT2} in other racial groups, we report the clinical and genetic data for 13 probands with paroxysmal dyskinesias including 1 Vietnamese, 1 mixed Caucasian-Thai and 2 African-Americans.

Methods

All human studies were performed in accordance with institutional review board guidelines at each participating institution, the Helsinki Declaration, and written informed consent for genetic studies and publication of clinical data was obtained from all subjects or, where participants were children, their parents. All genetic and phenotypic analyses and publication of the results were approved by the University of Tennessee Health Science...
Center Institutional Review Board (#01-07346-XP). Subjects were acquired from outpatient clinics at participating institutions. Clinical diagnoses were made by means of history and examination by one or more board-certified neurologists at each institution. Clinical and genetic details for 13 probands are presented Table 1.

DNA was extracted from peripheral blood leucocytes using Roche's DNA Isolation Kit for Mammalian Blood (Indianapolis, IN, USA). DNA quantity and quality were analyzed with a NanoDrop ND-1000 spectrophotometer (Wilmington, DE, USA) and agarose gel electrophoresis. With Primer3 (frodo.wi.mit.edu), four pairs of PCR primers were designed to encompass the four PRRT2 exons and flanking intronic regions (Additional file 1 Table S1). For Sanger sequencing, PCR was performed using 50 ng of template DNA, 1X PCR buffer, 2.5 mM MgCl₂ and 200 nM of each primer in a 20-µl reaction volume. The following cycling conditions were employed: 95°C for 15 min; 35 cycles at 95°C for 15 s, 60°C for 15 s, and 72°C for 45 s; and 72°C for 10 min. After agarose gel confirmation, 5 µl of the PCR products were cleaned using ExoSAP-IT® (United States Biochemical, Cleveland, OH, USA). Then, 1-2 µl of the purified PCR products were sequenced in the forward and reverse directions on the Applied Biosystems 3130XL Genetic Analyzer (Carlsbad, CA, USA). Control DNA samples (100 African-American and 100 Caucasian) were sequenced for detection of newly-identified PRRT2 mutations.

Results
Among 13 index cases with paroxysmal dyskinesias, two different mutations in three families were identified. A novel mutation was found in African-American Family A (Figure 1, c.776dupG, p.E260*). This mutation was not found in 100 African-American or 100 Caucasian normal controls. The proband was a 22-year-old female patient who noticed the first attack of chorei-

Discussion
Candidate regions for PKD and ICCA were mapped to Chr 16 over a decade ago. PKD was linked to a 15.8 cM region flanked by markers D16S685 and D16S503 on Chr 16q13-q22.1 with a maximum LOD score of 3.66 at D16S419 in a large Indian family [13]. This candidate region was telomeric to a locus identified in Japanese families with PKD [14], but showed overlap with a region identified in an African-American family with PKD [15]. A candidate region for ICCA had also been mapped to the pericentromeric region of Chr 16 in French [16] and Chinese [17] families. Just recently, several distinct loss-of-function frameshift mutations leading to protein truncation or nonsense-mediated decay in proline-rich transmembrane protein 2 (PRRT2) have been associated with PKD in numerous Han Chinese families [3-6]. A much smaller percentage of cases were associated with missense mutations (e.g., c.796C>T, p.R266W; c.913 G>A, p.G305R) [4,6]. In addition to classic carbamazepine-responsive PKD, the phenotypic spectrum of PRRT2 mutations includes cases of ICCA, BFIE, some “PNKD-like” syndromes, and PED [6-11]. PRRT2 is located on Chr 16p11.2, within the ICCA, Japanese PKD, and African-American candidate regions but outside the Indian PKD candidate region. The association of PRRT2 genotypes with specific neurological phenotypes may become apparent with the publication of additional well-characterized cases.

PRRT2 is a cell surface protein containing two predicted transmembrane domains and highly expressed in the developing nervous system, particularly the cerebellum [3]. Our study has shown that novel and hotspot
| Subject (Diagnosis) | PRRT2 Mutation | Age/Gender | Race                  | Age at onset(y) | Family history | Attack frequency | Attack duration | Triggers | Involuntary movements | Anatomical distribution | Response to anticonvulsants |
|---------------------|----------------|------------|-----------------------|-----------------|----------------|------------------|----------------|----------|----------------------|------------------------|----------------------------|
| Family A, II-1 (PKD) | NA             | NA/M       | African-American      | 10y             | Yes            | < 100/day        | 10-20 sec      | SM       | D, C                 | A, L                   | carbamazepine(+), phenytoin (+) |
| Family A, III-1 (PKD) | NA             | 28y/F      | African-American      | 12y             | Yes            | 20-30/day        | 10-40 sec      | SM       | D, C                 | F, A, L                 | phenytoin (+)               |
| Family A, III-2 (PKD) | NA             | 25y/F      | African-American      | 10y             | Yes            | 50-75/day        | 10-15 sec      | SM       | D, C                 | A, L                   | carbamazepine (+)            |
| Family A, III-3 (PKD) | c.776dupG      | 22y/F      | African-American      | 10y             | Yes            | 30-40/day        | 10-15 sec      | SM       | D, C                 | F, A, L                 | carbamazepine(+), phenytoin(+) |
| Family A, III-4 (PKD) | c.776dupG      | 18y/F      | African-American      | 13y             | Yes            | 20-30/day        | 10-60 sec      | SM       | D, C                 | F, A, L                 | carbamazepine(+)            |
| Family B (PKD)       | c.649dupC      | 30y/M      | African-American      | 12y             | Yes            | 50/day           | 10-60 sec      | SM       | D, C                 | F, A                    | phenytoin (+)               |
| Case 7 (PKD)         | c.649dupC      | 27y/M      | Caucasian-Thai        | 21y             | *Yes           | 3-6/day          | < 10 sec       | SM, S    | D                    | F, A, L                 | carbamazepine (+)           |
| Case 8 (PED)         | None           | 29y/F      | Caucasian             | < 28y           | No             | < 1/day          | 2-4 hrs        | Intense exercise | D                    | F, A, L                 | clonazepam (±)              |
| Case 9 (PKD)         | None           | 18y/M      | Caucasian             | 14y             | No             | 5-8/day          | < 15 sec       | SM       | D                    | F, A, L                 | carbamazepine (+)           |
| Case 10 (ICCA)       | None           | 19mo/M     | Caucasian             | 7 m             | No             | >100/day         | 40-50 sec      | SM       | D, C, A              | F, A, L                 | carbamazepine (+)           |
| Case 11 (PKD)        | None           | 41y/F      | Caucasian             | < 33y           | Yes            | 20-25/mo        | 2-30 min       | SM, S    | D                    | F, A, L                 | piracetam (±) clonazepam (±) |
| Case 12 (PKD)        | None           | 20y/M      | Caucasian             | 3y              | No             | 6-7/day          | 5-60 sec       | SM, S    | D, C, A              | F, A, L                 | carbamazepine (+)           |
| Case 13 (PKD)        | None           | 18y/M      | Caucasian             | 15y             | Yes            | 2-5/day          | 5-6 sec        | SM       | D                    | F, A, L                 | carbamazepine (+)           |
| Case 14 (PKD)        | None           | 18y/F      | Caucasian             | 15y             | No             | 3-4/day          | < 10 sec       | SM, S    | D, C, A              | F, A, L                 | carbamazepine (+)           |
| Case 15 (PKD)        | None           | 14y/M      | Vietnamese            | 12y             | No             | 10/day           | 15-60 sec      | S        | D                    | A, L                    | acetazolamide (+)           |
| Case 16 (PKD)        | None           | 26y/F      | Caucasian             | 16y             | No             | 30/day           | 20-30 sec      | SM       | D                    | A                      | phenytoin (+)               |
| Case 17 (PNKD)       | None           | 6y/F       | Caucasian             | 6 m             | Yes            | 2-10/mo         | 3-60 min       | Fatigue, sleep deprivation | D                    | A, L                    | NA                        |

Involuntary movements: D-dystonia, C-chorea, and A-athetosis. Anatomical distribution: F-face, A-arm, and L-leg. Triggers: SM-sudden movement and S-stress. NA, DNA or clinical detail not available. Response to anticonvulsants: +, good to excellent response and ±, partial response; *, early childhood seizures on maternal (Thai) side of the family.
mutations in PRRT2 are associated with classic PKD in African-Americans. The c.776dupG and c.649dupC mutations are heterozygous SNindels (single nucleotide insertions or deletions) predicted to cause nonsense-mediated decay of mutant transcripts rather than expression of a truncated protein [18,19]. SNindels occur at an estimated frequency of 0.887 per 10 kb of genomic DNA with more than half occurring in regions with mononucleotide repeats [19]. The novel c.776dupG mutation is located within a 6 nucleotide (nt) poly-G tract and the c.649dupC hot spot mutation is in a 9 nt poly-C tract. SNindels within regions of mononucleotide repeats may arise from replication slippage [19].

Conclusions
The novel c.776dupG mutation and c.649dupC hot spot mutation identified in our African-American families with classic PKD expands the molecular and racial spectrums of PRRT2 mutations. As evidenced from our patient of mixed Caucasian-Thai descent, the penetrance of PRRT2 mutations may depend on the origin of the normal or wild-type allele. Finally, a significant percentage of patients with PKD and ICCA do not harbor mutations in coding regions of PRRT2.

Additional file

Additional file 1: Table S1. PRRT2 Sequencing Primers.

Abbreviations
PKD, Paroxysmal kinesigenic dyskinesia; EKD1, Episodic kinesigenic dyskinesia; PNC, Paroxysmal kinesigenic choreoathetosis; ICCA, Infantile convulsions and choreoathetosis; BFIE, Benign familial infantile epilepsy; PED, Paroxysmal exercise-induced dyskinesia; PNKD, Paroxysmal non-kinesigenic dyskinesia.

Competing interests
The authors declare that they have no competing interests.
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References

1. Bruno MK, Hallett M, Gwinn-Handy K, Sorensen B, Considine E, Tucker S, Lynch DR, Mathews KD, Swooboda KJ, Harris J, Soong BW, Achzawa T, Jankovic J, Renner D, Fu HY, Paczek L. Clinical evaluation of idiopathic paroxysmal kinesiogenic dyskinesia: new diagnostic criteria. Neurology 2004, 63:2280–2287.

2. Bhatia KP. Paroxysmal dyskinesias. Mov Disord 2011, 26:1157–1165.

3. Chen WJ, Lin Y, Xiong QZ, Wei W, Ni W, Tan GH, Guo SL, He J, Chen YF, Zhang QJ, Li HF, Lin Y, Murong SX, Xu J, Wang H, Wu ZY. Exome sequencing identifies truncating mutations in PRRT2 that cause paroxysmal kinesiogenic dyskinesia. Nature Genet 2011, 43:1252–1255.

4. Wang JL, Cao L, Li XH, Hu ZM, Li JD, Zhang JG, Liang Y, San A, Li N, Chen SQ, Guo JF, Jiang H, Shen L, Zheng L, Mao X, Yan WQ, Zhou Y, Shi YT, Ai SK, Dai MZ, Zhang P, Xia K, Chen SD, Tang BS. Identification of PRRT2 as the causative gene of paroxysmal kinesiogenic dyskinesias. Brain 2011, 134:3493–3501.

5. Li J, Zhu X, Wang X, Sun W, Feng B, Du T, Sun B, Niu F, Wei H, Wu X, Dong L, Li C, Cai X, Wang Y, Liu Y. Targeted genomic sequencing identifies PRRT2 mutations as a cause of paroxysmal kinesiogenic choreoathetosis. J Med Genet 2012, 49:76–78.

6. Liu Q, Qi Z, Wan XH, Li JY, Shi L, Lu Q, Zhou XQ, Qiao L, Wu LW, Liu XQ, Yang W, Liu Y, Cui LY, Zhang X. Mutations in PRRT2 result in paroxysmal dyskinesias with marked variability in clinical expression. J Med Genet 2012, 49:79–82.

7. Heron SE, Grinton BE, Kvity S, Afawi Z, Zuberi SM, Hughes JN, Pridmore C, Hodgson BL, Iona X, Sadlie LG, Pelekanos J, Herlenius E, Goldberg-Stem H, Basset H, Haan E, Korczyn AD, Gardner AE, Corbett MA, Gécz J, Thomas PQ, Mulley JC, Berkovic SF, Scheffer IE, Dibbens LM. PRRT2 mutations cause benign familial infantile epilepsy and infantile convulsions with choreoathetosis syndrome. Am J Hum Genet 2012, 90:152–160.

8. Cao L, Huang XL, Zheng L, Xiao Q, Wang XJ, Chen SD. Identification of a novel PRRT2 mutation in patients with paroxysmal kinesiogenic dyskinesias and c.649dupC as a mutation hot-spot. Parkinson Relat Disord 2012, 18:704–705.

9. Ono S, Yoshiiura K, Kinoshita A, Kikuchi T, Nakane Y, Kato N, Sadamatsu M, Konishi T, Nagamitsu S, Matsushita M, Yasuda A, Komine M, Kanai K, Inoue T, Osamura T, Saito K, Hirose S, Koide H, Tomita H, Ozawa H, Niikawa N, Kurotaki N. Mutations in PRRT2 responsible for paroxysmal kinesiogenic dyskinesias also cause benign familial infantile convulsions. J Hum Genet 2012, 57:338–341.

10. Lee HY, Huang Y, Bruneau N, Roll P, Roberson ED, Hermann M, Quinn E, Maas J, Edwards R, Ashizawa T, Baykan B, Bhatia K, Bressman S, Bruno MK, Brunt ER, Caraballo R, Echenne B, Fejerman N, Frucht S, Gurnett CA, Hirsch E, Hoodlen H, Jankovic J, Lee WL, Lynch DR, Mohamed S, Müller U, Nespeca MP, Renner D, Rochette J, et al. Mutations in the novel protein PRRT2 cause paroxysmal kinesiogenic dyskinesia with infantile convulsions. Cell Rep 2012, 1:2–12.

11. Schubert J, Faravirdino R, Becker F, Berger A, Bebek N, Bianchi A, Brockmann K, Capovilla G, Dalla Bernardina B, Fukuyama Y, Hoffmann GF, Jukat-Rott K, Antonnen AK, Kurlemann G, Lehesjoki AE, Lehmahorn F, Mastrangelo M, Mause U, Müller S, Neubauer B, Pütt B, Rating D, Robbiano A, Ruf S, Schroeder C, Seidel A, Specchio N, Stephani U, Striano P, Teichler J, et al. PRRT2 mutations are the major cause of benign familial infantile seizures (BFI). Hum Mutat 2012, 33:1439–1443.

12. Spaczy S, Valente EM, Wall GM, Warner TT, Jammar PR, Schapira AH, Dixon PH, Davis MB, Bhatia KP. Wood NW. Genetic and clinical heterogeneity in paroxysmal kinesiogenic dyskinesia: evidence for a third EKD gene. Mov Disord 2002, 17:717–725.

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

14. Tomita H, Nagamitsu S, Wakai K, Fukushima Y, Yamada K, Sadamatsu M, 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, Nakamura Y, Nanko S, Kato N, Nakane Y, Niikawa N. Paroxysmal kinesiogenic choreoatethosisis locus maps to chromosome 16p11.2–q12.1. Am J Hum Genet 1999, 65:1688–1697.

15. Bennett LB, Roach ES, Bawcock AM. A locus for paroxysmal kinesiogenic dyskinesia maps to human chromosome 16. Neurology 2000, 54:125–130.

16. Szepetowski P, Rochette J, Berquin P, Piusan C, Latrhop GM, Monaco AP. Familial infantile convulsions and paroxysmal choreoathetosis: a new neurological syndrome linked to the pericentromeric region of human chromosome 16. Am J Hum Genet 1997, 61:889–898.

17. Lee WL, Tay A, Ong HT, Goh LM, Monaco AP, Szepetowski P. Association of infantile convulsions with paroxysmal dyskinesias (ICCA syndrome): confirmation of linkage to human chromosome 16p12-q12 in a Chinese family. Hum Genet 1998, 102:608–612.

18. Silva AI, Romão L. The mammalian nonsense-mediated mRNA decay pathway: to decay or not to decay! Which players make the decision? FEBS Lett 2009, 583:499–505.

19. Tan EC, Li H. Characterization of frequencies and distribution of single nucleotide insertions/ deletions in the human genome. Gene 2006, 376:268–280.

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