Identification of PRRT2 as the causative gene of paroxysmal kinesigenic dyskinesias

Jun-Ling Wang,1,* Li Cao,2,* Xun-Hua Li,3,* Zheng-Mao Hu,4 Jia-Da Li,4 Jian-Guo Zhang,5 Yu Liang,5 San-A,5 Nan Li,1 Su-Qin Chen,6 Ji-Feng Guo,1,7 Hong Jiang,1,7 Lu Shen,1,7 Lan Zheng,2 Xiao Mao,1 Wei-Qian Yan,1 Ying Zhou,1 Yu-Ting Shi,1 San-Xi Ai,1 Mei-Zhi Dai,5 Peng Zhang,5 Kun Xia,4 Sheng-Di Chen2 and Bei-Sha Tang1,4,7

1 Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan Province, 410008, China
2 Department of Neurology and Institute of Neurology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, 200025, China
3 Department of Neurology, the First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510089, China
4 State Key Laboratory of Medical Genetics, Changsha, Hunan Province, 410008, China
5 BGI-Shenzhen, Shenzhen, Guangdong Province, 518083, China
6 Department of Medical Genetics, Zhongshan Medical College, Sun Yat-sen University, Guangzhou 510089, China
7 Neurodegenerative Disorders Research Centre, Central South University, Changsha, Hunan Province, 410008, China

*These authors contributed equally to this work.

Correspondence to: Dr B.-S. Tang, Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan province, 410008, China E-mail: bstang7398@yahoo.com.cn

Correspondence may also be addressed to: Dr. Sheng-Di Chen. E-mail: chen_sd@medmail.com.cn
or Dr. Kun-Xia. E-mail: xiakun@sklmg.edu.cn

Paroxysmal kinesigenic dyskinesias is a paroxysmal movement disorder characterized by recurrent, brief attacks of abnormal involuntary movements induced by sudden voluntary movements. Although several loci, including the pericentromeric region of chromosome 16, have been linked to paroxysmal kinesigenic dyskinesias, the causative gene has not yet been identified. Here, we identified proline-rich transmembrane protein 2 (PRRT2) as a causative gene of paroxysmal kinesigenic dyskinesias by using a combination of exome sequencing and linkage analysis. Genetic linkage mapping with 11 markers that encompassed the pericentromeric of chromosome 16 was performed in 27 members of two families with autosomal dominant paroxysmal kinesigenic dyskinesias. Then, the whole-exome sequencing was performed in three patients from these two families. By combining the defined linkage region (16p12.1–q12.1) and the results of exome sequencing, we identified an insertion mutation c.649_650InsC (p.P217fsX7) in one family and a nonsense mutation c.487C>T (p.Q163X) in another family. To confirm our findings, we sequenced the exons and flanking introns of PRRT2 in another three families with paroxysmal kinesigenic dyskinesias. The c.649_650InsC (p.P217fsX7) mutation was identified in two of these families, whereas a missense mutation, c.796C>T (R266W), was identified in another family with paroxysmal kinesigenic dyskinesias. All of these mutations completely co-segregated with the phenotype in each family. None of these mutations was identified in 500 normal unaffected individuals of matched geographical ancestry. Thus, we have identified PRRT2 as the first causative gene of paroxysmal kinesigenic dyskinesias, warranting further investigations to understand the pathogenesis of this disorder.
Introduction

Paroxysmal kinesigenic dyskinesias (PKD) is a relatively rare neurological disorder of unknown cause. The characteristic of PKD is recurrent and brief attacks of involuntary movements that are triggered by sudden voluntary movements (Kertesz, 1967; Goodenough et al., 1978; Demirkiran and Jankovic, 1995; Bruno et al., 2004). The episodes usually present with dystonia, chorea, athetosis, ballism, or their combination. The attacks usually last from a few seconds to a few minutes. During the attacks, there is absolutely no loss or alteration of consciousness. However, the prognosis of PKD is favourable and the patients usually show excellent responses to antiepileptic drugs (Kertesz, 1967; Goodenough et al., 1978; Houser et al., 1999; Lotze and Jankovic, 2003; Bruno et al., 2004). It is noteworthy that in some patients or their first- and second-degree relatives, PKD is often combined with other intermittent neurological disorders, like infantile convulsions or infantile convulsion and choreoathetosis (Tomita et al., 1999; Swoboda et al., 2000; Bruno et al., 2004). PKD can be classified as primary or secondary according to its aetiology (Goodenough et al., 1978; Demirkiran and Jankovic, 1995; Blakeley and Jankovic, 2002). Most of the cases with secondary PKD are caused by multiple sclerosis, head injury, metabolic derangements or cerebral perfusion insufficiency (Blakeley and Jankovic, 2002; Perona-Moratalla et al., 2009). Primary PKD can be further classified as idiopathic or familial PKD according to the hereditary nature. Familial PKD cases are the most common and are usually inherited in an autosomal dominant manner (Goodenough et al., 1978; Demirkiran and Jankovic, 1995; Marsden, 1996; Perona-Moratalla et al., 2009). Tomita et al. (1999) performed a genome-wide linkage and haplotype analysis in eight Japanese families and assigned the first PKD-critical region to a region between D16S3093 and D16S476. They named this 12.4cM region at 16p11.2–q12.1 as EKD1 (episodic kinesigenic dyskinesias 1). In the following years, a number of other linkage studies on a variety of ethnicities confirmed EKD1, which encompassed the pericentromeric of chromosome 16 and shared a minimum overlap region between D16S3093 and D16S3396 (Bennett et al., 2000; Swoboda et al., 2000; Cuenca-Leon et al., 2002; Kikuchi et al., 2007; Wang X et al., 2010). Moreover, two other regions that did not overlap with EKD1 were also identified and defined as EKD2 (16q13–q22.1) and EKD3, respectively (Valente et al., 2000; Spacey et al., 2002). Nevertheless, no causative gene of PKD has been identified thus far.

A recently developed technology using whole-exome capture and high-throughput sequencing provides a powerful and affordable means to identify disease-causing genes. Recent studies have demonstrated the sensitivity and accuracy of the exome-sequencing approach in the identification of the causal genes of several rare monogenic diseases such as Miller syndrome (Ng et al., 2010) and Bartter syndrome (Choi et al., 2009). In 2010, we identified transglutaminase 6 as a novel causative gene of spinocerebellar ataxias using the combined strategy of exome sequencing and linkage analysis (Wang J et al., 2010). Here, we applied a similar strategy to screen the causative gene of PKD.

Materials and methods

Families and patients

This study was approved by the Expert Committee of Xiangya Hospital of the Central South University in China (equivalent to an Institutional Review Board). Written informed consent was obtained from all subjects.

Five Chinese Han PKD families presenting as autosomal dominant inheritance were included. A total of 75 members including 24 patients with PKD were enrolled. Families A and B were PKD pedigrees associated with other intermittent neurological disorders, in which both Patients III: 2 and III: 5 in Family A as well as Patients III: 3 and III: 5 in Family B were diagnosed with infantile convulsion and choreoathetosis syndrome. Families C–E were PKD pedigrees without any other paroxysmal conditions such as epilepsy, migraine or episodic ataxia. Among the 24 patients, the mean age at onset of the disease was 9.6 years (range 4–18 years). Attacks were usually precipitated by sudden movements, intention to move, startles, stress and anxiety. The symptoms of attacks consisted of dystonia, chorea and athetoid movements. The duration of the entire attack lasted from a few seconds to 1 min. Neither loss nor impairment of consciousness was observed in the attacks. Neurological examinations and imaging findings between attacks were entirely normal.

All the available affected individuals were subjected to a thorough neurological examination by two or more experienced neurologists. Clinical data were collected by interviews and clinical questionnaires, and the diagnosis of PKD was determined according to the criteria designed by Bruno et al. (2004). Individuals were diagnosed with infantile convulsions if they experienced non-febrile convulsions at the age of 3–12 months (Rochette et al., 2008). Infantile convulsion and choreoathetosis syndrome diagnoses were made according to Szepestowski et al. (1997). Unaffected individuals (n = 500) of matched geographic ancestry were also included as healthy controls. Genomic DNA was prepared from venous blood by standard procedures.

The first set of analyses was performed in Families A and B (Fig. 1). A total of 11 subjects (Patients I: 1–2; II: 1, 3–6; III: 1–2; 4–5) in Family A and 16 subjects (Patients I: 1–2; II: 1–7; III: 1–7) in Family B were included in the linkage study. Three individuals (Patient II: 4 in Family A, Patients II: 2 and II: 4 in Family B) were studied with exome sequencing. Three additional unrelated autosomal dominant PKD pedigrees (Families C–E) were used for subsequent mutational screening.

Genotyping for linkage analysis

Genotyping was performed using 11 microsatellite markers (D16S3041, D16S403, D16S401, D16S3136, D16S2623, D16S419, D16S415, D16S3057, D16S514, D16S503) in chromosome 16 with primers tagged with fluorescence (HEX, FAM). The markers...
were amplified by polymerase chain reaction using the method as described (Tang et al., 2004). Relative positions were derived from the human genome draft sequence and the Marshfield map (www.ncbi.nlm.nih.gov). Two-point logarithm of odds scores were calculated using Linkage 5.2 package, with the assumptions that, in the two families, PKD is inherited in an autosomal dominant mode with 95% penetrance, that the frequency of the mutant allele is 0.0001, and that the allele frequency of each marker locus is equal (Lathrop and Lalouel, 1984; Dib et al., 1996). The haplotype in the two pedigrees was reconstructed manually using the Cyrillic program.

Exome sequencing

Library preparation and exome sequencing

Qualified genomic DNA extracted from the three patients (Patient II: 4 from Family A, Patients II: 2 and II: 4 from Family B) was sheared by sonication and then hybridized to the SureSelect Biotin lated RNA Library (BAITS) for enrichment, according to the manufacturer’s instructions. The enriched library targeting the exome was sequenced on the HiSeq 2000 platform to get paired-end reads with read length of 90 bp.

Alignment, single-nucleotide polymorphism and insertions or deletions calling

The sequenced reads were aligned to the human genome reference (UCSC hg 18 version) using SOAPaligner (Li et al., 2008). Those reads were then aligned in the designed target regions and were collected for single-nucleotide polymorphism (SNP) calling and subsequent analysis. We estimated the consensus genotype and quality by SOAPsnp (v 1.03) (Li et al., 2010). The low-quality variations were filtered out by the following criteria: (i) consensus quality score \( \geq 20 \) (Q20); (ii) average copy number at the allele site \( \leq 2 \); (iii) distance of two adjacent SNPs \( \geq 5 \) bp; and (iv) sequencing depth \( \leq 400 \). For insertions or deletions (indels) in the targeted exome regions, we aligned the reads to the reference genome using bwa. The alignment result was used to identify the breakpoints by gatk. Finally, we annotated the genotypes of insertions and deletions (Li and Durbin, 2010).

Analysis protocol for exome-sequencing results

Based on the hypothesis that the mutation underlying families with PKD should not be present in the general population, non-synonymous/splice acceptor and donor site/insertions or deletions (NS/SS/Indel) variants reported in the dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/, Build 129), eight previously exome-sequenced HapMap samples (‘HapMap 8’) and 1000 Genome Project (http://www.ncbi.nlm.nih.gov/Ftp/) were removed. Synonymous changes were identified and filtered from the variant list using SIFT software (version 4.0, http://sift.jcvi.org/).

Moreover, we only focused on the genes with NS/SS/Indel variants occurring in the linkage region. We further assumed that all three exome-sequenced patients should show NS/SS/Indel variants in the same gene, although the NS/SS/Indel variants might not be identical in all patients. Sanger sequencing using customized primers was
performed to determine the presence of the variants in all the clinically affected subjects and to screen the unaffected members in Families A and B for co-segregation analysis. We then sequenced all the exons and flanking introns of the PRRT2 gene (NM_145239) in patients of three additional families (Families C–E) to detect other mutation sites using the traditional method. As an additional step, the detected variants were sequenced in 500 neurologically normal control subjects.

**Results**

**The clinical characteristics of families with paroxysmal kinesigenic dyskinesias**

Detailed clinical characteristics are summarized in Table 1. The five families in our study shared some common features of PKD such as trigger and duration of attack, no loss of consciousness during attack and good response to treatment with anticonvulsants. Besides these features, the five families also presented with a wide spectrum of clinical heterogeneity. The onset age of the patients in Families A and C–E was ~10 years old, while some patients in Family B (Patients III: 3 and III: 5) were younger (4 and 5 years old). The characteristics of the attacks varied not only between families but also among individuals in the same family.

For example, there were two members separately diagnosed with infantile convulsion and choreoathetosis syndrome in infancy in Family A (Patients III: 2 and III: 5) and Family B (Patients III: 3 and III: 5). The main manifestation in Family A was dystonia, choreoathetosis or athetosis, while dystonia were predominant in Family B. We noted that dystonia of the upper limbs was more obvious in Family D, while in Family C lower limbs were more likely to be involved and the patients tended to fall down when attacks occurred. Although sudden movements induced attacks in all cases, there may have been some other triggers such as anxiety, startle and intention to move in a relatively smaller number of patients.

**Linkage mapping of the syndrome to chromosome 16p12.1-q12.1**

Two-point logarithm of odds scores are shown in Table 2. A maximum two-point logarithm of odds score of 3.77 (I = 0) was obtained at mark D16S409, when penetrance was assumed to be 0.95. The highest probability haplotype in Families A and B was reconstructed manually using the Cyrillic program (Fig. 1) and some key recombinant events were identified. In Family A, recombination was present between D16S403 and D16S401 in the short arm as well as between D16S2623 and D16S419 in the long arm.
Exome sequencing identified PRRT2 as the candidate gene

To identify the causative gene of PKD, exome sequencing was performed on DNA samples obtained from three affected members of Families A and B (Fig. 1: Patient II: 4 in Family A, Patients II: 2 and II: 4 in Family B). We generated an average of 6.82 Gb of sequence from each affected individual as paired-end, 90-bp reads; 6.15 Gb (90.18%) passed the quality assessment and were aligned to the human reference sequence; and an average of 3.62 Gb of sequence data was mapped to the target region. After SNPs and Indel calling, we identified a mean of 96,645 variants from the reference sequence per subject, in which an average of 10,756 NS/SS/Indel variants were detected in each of the PKD patients sequenced. Given that this is a rare disorder, it is unlikely that causative variants will be present in the general population. We therefore removed the NS/SS/Indel variants reported in the dbSNP129, ‘HapMap 8’ and the SNP release of the 1000 Genome Project. After this initial filter, we generated an average of 612 NS/SS/Indel variants from each patient. We then focused on the genes with NS/SS/Indel variants occurring in the linkage region (16p12.1-q12.1). During this step, we identified five, three and five NS/SS/Indel variants from Patient II: 2 in Family A, and Patients II: 2 and II: 4 in Family B, respectively. Finally, we assumed that all three patients should show NS/SS/Indel variants in the same gene. As a result, PRRT2 was found to be the sole gene with mutations occurring in all three patients (Fig. 3F). The mutations included an insertion mutation c.649_650InsC (p.P217fsX7) in Family A, and a nonsense mutation c.487C>T (p.Q163X) in Family B. The mutations were confirmed by Sanger sequencing (Fig. 3B and C).

Mutation in PRRT2 gene

As shown in Fig. 3E, the PRRT2 gene consists of four exons and its protein has two domains: two low complexity segments followed by two transmembrane segments located at amino acids 268–290 and 315–337, respectively. The c.494_650insC (p.P217fsX7) mutation is a frameshift mutation, which generates a different sequence starting at position 217 and introduces a premature stop codon at position 224, generating a truncated protein with only 223 amino acids. The c.487C>T (p.Q163X) mutation is a nonsense mutation found in the second exon of PRRT2, which substitutes the codon for Q163 (CAG) with a stop codon (TAG) and generates a truncated protein with only 162 amino acids. As a result, both mutant proteins lack the transmembrane segments.

To confirm PRRT2 as the causative gene of PKD, we used Sanger sequencing to screen all members of Families A and B with respective variants (c.649_650insC in Family A and c.487C>T in Family B). As shown in Fig. 1, all five clinically affected subjects, but none of those who were unaffected in Family A, carried the heterozygous c.649_650insC mutation. Similarly, all seven patients, but none of the unaffected individuals in Family B, were heterozygous for the c.487C>T mutation. Thus, the mutations in PRRT2 completely co-segregated with the PKD phenotype within these two families.
To further study the contribution of PRRT2 to PKD, we sequenced all the exons and flanking introns of the PRRT2 gene of patients and unaffected members in another three unrelated PKD pedigrees. As shown in Fig. 2, the c.649_650InsC (p.P217fsX7) insertion was identified in Families C and D. We also identified a missense mutation c.796C>T (p.R266W) in Family E (Fig. 3D). The c.796C>T (p.R266W) is a missense mutation found in the second exon, causing an arginine to tryptophan substitution at codon 266 (Fig. 3D and E). As shown in Fig. 2, these two variants also co-segregated with the PKD phenotype in Families C–E. Additionally, these three mutations were not detected in 500 unaffected control individuals of matched geographical ancestry, as examined by Sanger sequencing.

Discussion

PKD is a neurological disorder with dystonic posturing, chorea, athetosis, ballism or a combination of these hyperkinetic symptoms (Kertesz, 1967; Goodenough et al., 1978; Demirkiran and Jankovic, 1995; Bruno et al., 2004). We have identified PRRT2 as the causative gene of PKD. PRRT2 had three different mutations, c.487C>T (p.Q163X), c.649_650InsC (p.P217fsX7) and c.796C>T (p.R266W), in five different autosomal dominant PKD families. Several lines of evidence supported the causal role of PRRT2 in PKD: (i) the linkage analysis in two families mapped locus of our PKD families to a pericentromeric region (16p12.1–q12.1) on chromosome 16; (ii) PRRT2 was the only gene with mutations shared by all three patients in the linkage region; (iii) two additional PKD families (Families C and D) carried the insertion mutation (c.649_650InsC), and a third PKD family (Family E) carried a different missense mutation (c.796C>T); (iv) all the mutations co-segregated with the phenotype; and (v) absence of such mutations in 500 normal unaffected individuals of matched geographical ancestry.

Although all of the patients with PKD in our study had mutations in PRRT2, their clinical features, except the core syndrome, varied among families. For instance, the insertion mutation (c.649_650InsC) was found in Families A, C and D, but the clinical manifestation of these three families varied from each other. Family A had two patients (Patients III: 2, III: 5) who presented with infantile convulsion and choreoathetosis, whereas the patients in Families C and D did not report any other history of intermittent neurological disorder. Families C and D also had different presentations. Family C presented with dystonia in lower limbs; however, Family D presented mainly with dystonia in upper limbs. It is possible that other unappreciated gene mutations may contribute to the heterogeneity of PKD phenotypes. Indeed,
Figure 3 Mutations of the PRRT2 gene. (A) Sanger sequencing of codons 214–220 of PRRT2 in a wild-type (WT) subject and (B) the c.649_650insC (p.P217fsX7) mutation. The mutation results in a frame-shift, which generates different sequences starting at position 217 and introduces a premature stop codon at position 224. (C) Sanger sequencing of codons 162–164 of PRRT2 in a wild-type subject (left) and an affected subject (right) with c.487C>T (p.Q163X) mutation. (D) Sanger sequencing of codons 265–267 of PRRT2 in a wild-type subject (left) and an affected subject (right) with c.796C>T p.R266W mutation. The mutant sites are indicated by red triangles in B–D. (E) Schematic diagram of PRRT2 gene and wild-type or mutant PRRT2 protein. The three mutations identified in this study are indicated with arrows. PRRT2 gene consists of four exons. PRRT2 protein has two domains: two low complexity segments followed by two transmembrane segments. All three mutations are located upstream of the coding region of the first transmembrane segment of PRRT2 protein. (F) The diagram of chromosome 16 indicating the relative position of PKD locus. The minimal linkage interval of PKD is 19.16 cM between D16S401 and D16S2623 on chromosome 16p12.1-q12.1. The PRRT2 position is indicated with a red triangle.
Table 3 Identification of the causative gene for PKD from three patients by exome sequencing

<table>
<thead>
<tr>
<th>Filter</th>
<th>Sample II: 4 (Family A)</th>
<th>Sample II: 2 (Family B)</th>
<th>Sample II: 4 (Family B)</th>
<th>Shared genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of variants</td>
<td>98581</td>
<td>90317</td>
<td>101037</td>
<td>9083</td>
</tr>
<tr>
<td>Number of NS/SS/Indel</td>
<td>10811</td>
<td>10530</td>
<td>10927</td>
<td>2840</td>
</tr>
<tr>
<td>Number of NS/SS/Indel after Filter 1</td>
<td>1603</td>
<td>1572</td>
<td>1607</td>
<td>265</td>
</tr>
<tr>
<td>Number of NS/SS/Indel after Filter 2</td>
<td>1122</td>
<td>1098</td>
<td>1162</td>
<td>187</td>
</tr>
<tr>
<td>Number of NS/SS/Indel after Filter 3</td>
<td>562</td>
<td>539</td>
<td>736</td>
<td>135</td>
</tr>
<tr>
<td>NS/SS/indel in 16p12.1-q12.1</td>
<td>SULT1A2(K258N)</td>
<td>CD19(V217M)</td>
<td>AC138894.2-1(N44S)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FAM57B(P27A)</td>
<td>LAT(V163M)</td>
<td>PRRT2(Q163*)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ITGAX(Q856H)</td>
<td>PRRT2(Q163*)</td>
<td>CORO1A(V229L)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PRRT2 (P217fsX7)</td>
<td>ZNF646(G1793E)</td>
<td>ZNF646(G1793E)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ZNF720 (Indel)</td>
<td>PRRT2</td>
<td>AC136932.2 (Indel)</td>
<td></td>
</tr>
</tbody>
</table>

*Shared genes* indicates the gene mutations occurred in all three samples. In the step of Filter 1, we first removed the NS/SS/Indel variants reported in the dbSNP129. Then, the NS/SS/Indel variants reported in the eight previously exome-sequenced HapMap samples (‘HapMap 8’) were further removed in Filter 2. Consequently, the NS/SS/Indel variants reported in the 1000 Genome Project were removed in Filter 3; * = stop codon.

we also found abundant novel mutations and SNPs in other genes by exome sequencing in every patient with PKD (Table 3).

PRRT2 is a member of the transmembrane protein family, including PRRT1-4. Interestingly, two of the mutations identified in this study (p.Q163X and p.P217fsX7) resulted in truncated PRRT2 proteins lacking the transmembrane domain. These truncated proteins cannot anchor to the membrane and may be loss-of-function. However, similar to some soluble membrane receptors, the soluble PRRT2 may still preserve binding ability to the interactive proteins or ligands and attenuate their binding to the receptors, the soluble PRRT2 may still preserve binding ability to the wild-type PRRT2, i.e. dominant negative effect (Celli et al., 1998). Nevertheless, the exact underlying molecular mechanism as to how these mutations caused PKD, either haploinsufficiency or dominant negative effect, requires further investigation.

There is a limited amount of research regarding the function of PRRT2; however, some indirect evidence suggested its role in the pathogenesis of PKD. First, PRRT2 was found to be mainly expressed in the basal ganglia (http://human.brain-map.org), a brain area possibly involved in the PKD pathogenesis (Hamano et al., 1995; Hayashi et al., 1997; Ko et al., 2001; Shirane et al., 2001; Volonte et al., 2001; Iwase et al., 2004). Secondly, SNAP25, an interactive protein of PRRT2, is also expressed in the brain, especially the basal ganglia (Stelzl et al., 2005). Third, SNAP25 participates in the regulation of neurotransmitter release (Graham et al., 2002). Finally, recent research studies have revealed that SNAP25 might be involved in the aetiology of the attention-deficit hyperactivity disorder (Gizer et al., 2009; Banaschewski et al., 2010), whereas the core features of PKD are also a paroxysmal hyperkinetic (Demirkiran and Jankovic, 1995).

Since the first description by Kertesz (1967), PKD has been widely studied. However, the mechanism is still unclear. Here, we have identified PRRT2 as the first causative gene for PKD, warranting future research to understand the pathogenesis of PKD.

**Acknowledgements**

We are indebted to all the patients and family members for their generous participation in this work.

**Funding**

The Major State Basic Research Development Program of China (973 Program) (grant number 2011CB501000) and the State Key Program of National Natural Science Foundation of China (grant number 81130021) was provided by Department of Neurology, Xiangya Hospital, Central South University. (They mainly completed the work about Family A, Family E, and exome analysis). The Major State Basic Research Development Program of China (973 Program) (grant number 2011CB504104) and Key Discipline program of Shanghai Municipality (grant number ZS2002) were provided by Department of Neurology & Institute of Neurology, Ruijin Hospital. (They mainly completed the work about Family B, and linkage analysis). National Natural Science Foundation of China (grant number 30671154) was provided by Department of Neurology, the First Affiliated Hospital, Sun Yat-sen University. (They mainly completed the work about Family C, Family D, and sequencing analysis).

**References**


Marsden CD. Paroxysmal choreoathetosis. Adv Neurol 1996; 70: 467–70.


