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RESEARCH ARTICLE

Penetrance estimation of *PRRT2* variants in paroxysmal kinesigenic dyskinesia and infantile convulsions

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Abstract **Proline-rich transmembrane protein 2 (*PRRT2*) is the leading cause of paroxysmal kinesigenic dyskinesia (PKD), benign familial infantile epilepsy (BFIE), and infantile convulsions with choreoathetosis (ICCA). Reduced penetrance of *PRRT2* has been observed in previous studies, whereas the exact penetrance has not been evaluated well. The objective of this study was to estimate the penetrance of *PRRT2* and determine its influencing factors. We screened 222 PKD index patients and their available relatives, identified 39 families with pathogenic or likely pathogenic (P/LP) *PRRT2* variants via Sanger sequencing, and obtained 184 PKD/BFIE/ICCA families with P/LP *PRRT2* variants from the literature. Penetrance was estimated as the proportion of affected variant carriers. *PRRT2* penetrance estimate was 77.6% (95% confidence interval (CI) 74.5%–80.7%) in relatives and 74.5% (95% CI 70.2%–78.8%) in obligate carriers. In addition, we first observed that penetrance was higher in truncated than in non-truncated variants (75.8% versus 50.0%, *P* = 0.01), higher in Asian than in Caucasian carriers (81.5% versus 68.5%, *P* = 0.004), and exhibited no difference in gender or parental transmission. Our results are meaningful for genetic counseling, implying that approximately three-quarters of *PRRT2* variant carriers will develop *PRRT2*-related disorders, with patients from Asia or carrying truncated variants at a higher risk.**

Keywords penetrance; *PRRT2*; paroxysmal kinesigenic dyskinesia; infantile convulsions

Introduction

Paroxysmal kinesigenic dyskinesia (PKD; OMIM #128200) is the most common type of paroxysmal movement disorder; it is characterized by recurrent and transient attacks of dystonia, choreoathetosis, or their combination, triggered by sudden voluntary movement. PKD frequently begins in childhood, and the average age at onset is 8–13 years old and seldom later than 18 years old [1,2]. The number of male patients is more than that of female patients, with a ratio of 2–4:1 [1,2]. Familial PKD is typically inherited in an autosomal dominant pattern; in 2011, variants of proline-rich transmembrane protein 2 (*PRRT2*) were identified to cause PKD in our eight Chinese families [3]. At present, *PRRT2* variants are the major cause of PKD, accounting for 11%–45% of sporadic cases and 69%–100% of familial cases in different cohorts [4–6]. Later studies have revealed other *PRRT2*-related disorders, including benign familial infantile epilepsy (BFIE), infantile convulsions with choreoathetosis (ICCA) (an overlap of BFIE and PKD), hemiplegic migraine, episodic ataxia, and febrile seizures [7–11]. Moreover, approximately 95% of reported patients with *PRRT2* variants presented PKD/BFIE/ICCA [11].

The reduced penetrance of *PRRT2* variants has been widely observed [12–15]. However, exact penetrance remains unclear, and only a few papers have reported *PRRT2* penetrance estimates. Three previous estimates were 90% [13], 82% [16], and 94% [17], which were evaluated independently in 3 ICCA, 39 BFIE, and 8 ICCA families, by calculating the proportion of affected variant carriers among index patients and relatives. The same family-based method was used to evaluate the penetrance of other genes in earlier studies, such as *GCH1* causing dopamine-responsive dystonia [18] and *OPA1* causing

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autosomal dominant optic atrophy [19]. Notably, researchers have argued that family-based studies may lead to an inflation of penetrance estimation, particularly in complex diseases [20,21]. Multiple-case families are more likely to be selected (ascertainment bias) for study, and other genetic and/or environmental factors in these families may also contribute to a disease apart from the culprit gene.

Several methods have been developed to reduce ascertainment bias in later studies. The first method calculates penetrance in relatives after the exclusion of index patients, or in obligate carriers. An obligate carrier is defined as an individual who may be clinically unaffected but definitely carries a variant and passes it to offspring on the basis of family history. The penetrance of *LGI1* causing autosomal dominant partial epilepsy with auditory features and *DCC* causing isolated agenesis of the corpus callosum was evaluated using this method [22–24]. The second method is based on maximum likelihood estimates and survival analysis. It requires an exact onset age of symptomatic variant carriers. And the penetrance represents the cumulative risk of developing a specific disease by a given age. Such method has been applied to estimate the penetrance of genes for some relatively late-onset diseases, such as *TTR* causing transthyretin amyloid neuropathies [25] and *SDHB* causing paraganglioma and pheochromocytoma [26]. A Bayesian approach was developed recently to estimate penetrance on the basis of disease prevalence and the allelic frequencies of variants in patients and control populations, such as the Exome Aggregation Consortium (ExAC). The penetrance of *PRNP* causing prion disease was evaluated using this Bayesian algorithm [27].

PKD typically occurs in childhood and remits in adulthood, and thus, the exact onset age of PKD is relatively clear in adolescent patients, but their older relatives may have forgotten the onset age. In this regard, obtaining accurate penetrance estimation by using the second method is difficult. Moreover, the application of the Bayesian approach is hindered without an exact prevalence of PKD. Therefore, we adopted the first method to evaluate the penetrance of *PRRT2* and adjust for ascertainment bias. We screened 222 PKD index patients and their available relatives and identified 39 families with pathogenic or likely pathogenic (P/LP) *PRRT2* variants for penetrance evaluation. We also reviewed the published pedigree figures and found 184 qualified PKD/BFIE/ICCA families. Penetrance was evaluated in the relatives of index patients, and in obligate carriers. The effects of variant type, gender, parental transmission, and ethnicity on penetrance were evaluated.

Materials and methods

Study design

PRRT2 variants were screened in 222 unrelated Han Chinese PKD patients. Among them, 146 PKD patients (30 familial and 116 sporadic) were newly recruited between September 2013 and August 2020 from the Second Affiliated Hospital of Zhejiang University School of Medicine and Huashan Hospital of Fudan University, and 76 patients (20 familial and 56 sporadic) had been described in our previous reports [3,5,28]. Direct sequencing of *PRRT2* exons and exon–intron boundaries was performed on the index patients, and single-site testing was conducted on 250 available relatives from 118 families. Families with P/LP variants were selected for penetrance analysis. The clinical diagnosis of PKD was based on Bruno's criteria, which include identified kinesigenic trigger, short duration of attacks (< 1 min), no loss of consciousness or pain during attacks, exclusion of other organic diseases, and age at onset between 1 and 20 years if no family history [2]. The study was approved by the local ethics committees. All participants or their guardians provided written informed consent.

We also reviewed the literature reported by other researchers published between November 2011 and May 2020 in English to search for PKD/BFIE/ICCA pedigrees by using PubMed. Families with P/LP *PRRT2* variants and obligate carriers were selected. After excluding families with unclear disease statuses of some variant carriers, we found 184 qualified families (173 familial and 11 sporadic). Relevant references are listed in Table S1. We extracted the following information from each family: *PRRT2* variant, ethnic origin, family history, total number of variant carriers and affected ones, and total number of obligate carriers and affected ones. For each obligate carrier, we recorded the gender, the phenotype, and the origin of the variant (parental transmission), if available. The complete lists of families and obligate carriers are provided in Tables S2 and S3.

Genetic analysis

Genomic DNA was extracted from peripheral blood by using the QIAamp® DNA Blood Mini Kit (QIAGEN, Germany). The primer sequences and annealing temperatures used for polymerase chain reaction amplification were the same as those in our previous report [3]. Direct sequencing was performed on ABI 3500xL Dx Genetic Analyzer (Applied Biosystems, USA). The frequencies of identified variants in the general population were evaluated via the 1000 Genomes Project, ExAC, and the Genome Aggregation Database (gnomAD). Five computational programs, namely, SIFT, Polyphen-2, Mutation Taster, REVEL, and MetaLR, were used to predict the functional effect of variants. The prediction results of the latter two programs were obtained from an integrated database, i.e., VarCards. The splicing effect of the splice site variant was evaluated using three prediction tools: NNSplice, Human Splicing Finder, and NatGene2. The pathogenicity of a

variant was evaluated in accordance with the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG) [29].

Penetrance analysis

The penetrance of *PRRT2* was calculated as the proportion of affected individuals with P/LP *PRRT2* variants. We evaluated penetrance in our families and the published families. Penetrance was calculated in relatives and obligate carriers. The advantages of obligate carriers were that they were over the age period of risk, and they were included in the pedigrees regardless of their disease status. Non-obligate carriers were more likely to be included in the pedigrees or be referred for genetic testing if they were affected. Thus, including all family members in the penetrance analysis would result in selection bias [23].

Statistical analysis

The binomial 95% confidence interval (CI) of *PRRT2* penetrance was calculated using the normal approximation method when sample size was more than 50. Otherwise, exact CIs were computed through the following website: JavaStat–Binomial and Poisson Confidence Intervals. Chi-squared test was used for categorical variables. The Fisher exact test was adopted if the expected frequency in one or more cells was less than five. All the tests were two-tailed, and the level of statistical significance was set as $P \leqslant 0.05$. Analyses were performed in SPSS version 22.0 (IBM, USA).

Results

Clinical and genetic features of our cohort and the published families

In our cohort, we found 22 *PRRT2* variants (Fig. 1A) in 78 of the 222 index patients, including 10 novel variants (Fig. 1B). The detailed features of the novel variants are provided in Table 1. After pathogenicity classification, 12 variants, carried by 67 index patients, were classified as P/LP variants in accordance with ACMG guidelines. In addition, 61 relatives of the 39 index patients carried these P/LP variants. Thus, 100 variant carriers from 39 families were identified for penetrance analysis; among which, 43 were obligate carriers. The study design and related results are presented in Fig. 2. The most prevalent variant in our 39 families was the hotspot variant c.649dupC (30/39, 76.9%). Males accounted for 82.1% (32/39) of the index patients and 45.9% (28/61) of the relatives. Most families had two generations (33/39, 84.6%), and 14 families (14/39, 35.9%) had no family history. A total of 61 relatives consisted of 3 grandparents, 33 parents, 3 aunts, 2 uncles, 16 siblings, and 4 children. The phenotypes of 100 variant carriers can be categorized into three types: PKD (71), ICCA (11), and BFIE (3). No *PRRT2*-related phenotypes were reported in 14 parents and 1 sibling. All these asymptomatic relatives were over 20 years old and had passed the period of onset. The clinical information of 100 variant carriers is provided in Table S4.

In the 184 published families, 816 germline variant carriers (184 index patients and 632 relatives) were identified. Among which, 353 were obligate carriers carrying 27 disparate P/LP *PRRT2* variants. The hotspot variant c.649dupC was carried by 143 families (143/184, 77.7%). Most families were familial, and only 11 had no family history (11/184, 6.0%). With regard to ethnic origin, 78 of the 184 (42.4%) families were Asian, 88 (47.8%) were Caucasian, 2 (1.0%) were African American, and 16 were unknown. Males comprised 49.0% (173/353) of obligate carriers. Moreover, 6 phenotypes were observed in 266 of the 353 obligate carriers, namely, PKD (91), BFIE (140), ICCA (29), febrile seizures (3), epilepsy (2), and migraine (1).

Penetrance estimation

The penetrance evaluated in relatives was marked as "penetrance 1," and the penetrance evaluated in obligate carriers was marked as "penetrance 2." We calculated penetrance 1 and 2 for each *PRRT2* variant and for all *PRRT2* variants combined. A total of 28 P/LP *PRRT2* variants were found in our and the published families. Penetrance estimation varied from 0 to 1 for different variants (Table 2). In our 39 families, 46 of the 61 relatives were affected, yielding a penetrance 1 estimate of 75.4%, which is similar to the penetrance 1 estimate in the 184 published families (492/632, 77.8%, $P = 0.663$), as indicated in Table 3. Penetrance 2 evaluated in our obligate carriers (29/43, 67.4%) was lower than that in the published obligate carriers (266/353, 75.4%). However, the trend was not statistically significant ($P = 0.261$). No statistical difference was found between penetrance 1 and 2 in our families (75.4% versus 67.4%, $P = 0.372$, chi-squared test) and the published families (77.8% versus 75.4%, $P = 0.373$, chi-squared test). After combining our and the published families, the *PRRT2* penetrance estimate calculated in relatives was 77.6% and that in obligate carriers was 74.5% (Table 3).

Factors that affect penetrance

The correlations between penetrance and variant type, gender, parental transmission, and ethnic origin were then assessed (Table 4). Penetrance was evaluated in obligate carriers. After combining our and the published families, 396 obligate carriers were included for the succeeding

Table 1 Pathogenicity classification of 10 novel *PRRT2* variants

Variants	Amino acid changes	Frequency of population			*In silico* prediction					ACMG classification
		1000 g	ExAC	gnomAD	SIFT	Polyphen-2 (HumVar)	Mutation Taster	REVEL	MetaLR	
c.5C>T	p.A2V	Absent	Absent	Absent	D	D	D	T	D	US (PM2, PP3)
c.680G>A	p.R227Q	Absent	9.79E–06	1.36E–05	T	P	N	T	T	US (PM2, BP4)
c.766delG	p.V256Wfs*57	Absent	Absent	Absent	D	NA	D	NA	NA	Pathogenic (PVS1, PM2, PP3)
c.881C>T	p.S294F	Absent	Absent	Absent	D	D	D	D	D	US (PM2, PP3)
c.902G>C	p.G301A	Absent	Absent	Absent	D	D	D	D	D	US (PM2, PP3)
c.952_953dupAT	p.V319Sfs*19	Absent	Absent	Absent	D	NA	D	NA	NA	Pathogenic (PVS1, PM2, PP3)
c.962T>C	p.L321P	Absent	Absent	Absent	D	D	D	D	D	US (PM1, PM2, PP3)
c.976_978delCTC	p.L326del	Absent	8.52E–06	1.23E–05	D	NA	D	NA	NA	LP (PM1, PM2, PM4, PP3)
c.1012G>A	p.V338M	Absent	8.58E–06	4.17E–06	T	B	D	T	T	US (PM1, PM2)
						NatGene2		HSF	NNSplice	
c.879 + 4A>G	NA	Absent	Absent	Absent		Donor site lost		Donor site lost	Donor site lost	US (PM2, PP3)

Reference sequence, NM_145239.2; 1000 g, 1000 Genomes Project; ExAC, Exome Aggregation Consortium; gnomAD, Genome Aggregation Database; SIFT, sorting tolerant from intolerant; Polyphen-2, polymorphism phenotyping v2; REVEL, rare exome variant ensemble learner; HSF, Human Splicing Finder; D, damaging, disease-causing or deleterious; T, tolerated; P, possibly damaging; N, polymorphism; B, benign; US, uncertain significance; LP, likely pathogenic; NA, not available.

analysis. The variant type was categorized into two groups: truncated variants (frameshift, nonsense, and splicing) and non-truncated variants (missense and non-frameshift deletion). Penetrance was higher in carriers with truncated variants than those with non-truncated variants (75.8% versus 50.0%, $P = 0.01$). Among the 396 obligate carriers involved, 48.7% were male and 51.3% were female, with no difference in penetrance between genders (72.0% versus 76.8%, $P = 0.271$). The parental transmission was *de novo* in 1 obligate carrier and unknown in 255 obligate carriers because either the information was missing in the pedigrees or it was difficult to determine whether the variant was inherited from the father or the mother. Among the remaining 140 individuals, 73 maternally transmitted offspring showed a higher penetrance than 67 paternally transmitted ones. However, this difference was not statistically significant (90.4% versus 85.1%, $P = 0.334$). In terms of ethnicity, we identified 178 Asian obligate carriers and 197 Caucasian obligate carriers and found higher penetrance in Asian carriers (81.5% versus 68.5%, $P = 0.004$).

Discussion

With the widespread application of whole-exome/genome sequencing, an increasing number of genetic variants are being discovered. Appropriate explanations and predictions for the risk of a genetic variant have become a pressing issue in genetic counseling. In the current study, we evaluated the penetrance of *PRRT2* variants and investigated the factors that affect penetrance. On the basis of our 39 families and the 184 published families, the analysis yielded a penetrance estimate of 77.6% (95% CI 74.5%–80.7%) in relatives and 74.5% (95% CI 70.2%–78.8%) in obligate carriers.

The penetrance estimated in this study was lower than previous estimates (90% [13], 82% [16], and 94% [17]). The reason for such result may be as follows: we excluded the index patients in our calculations to reduce ascertainment bias. If both the index patients and relatives were included, then the estimated penetrance was 83.1% (761/916) in combined 223 pedigrees; this value was similar to previous estimates [16]. This result reflected that including index patients would affect penetrance estimate, and ascertainment bias should not be disregarded in family-based penetrance analysis; otherwise, such neglect would lead to the inflation of penetrance estimates as described in the other literature [23,26,30]. Considering this condition, we believed that our penetrance was closer to the true penetrance than previous estimates. However, neither the exclusion of index patients nor restricting the analysis to obligate carriers can eliminate other genetic and/or environmental factors that may interact with known genes to cause disease in multiple-case families. In this regard, our penetrance estimation may exceed true penetrance.

Frameshift and nonsense *PRRT2* variants are more

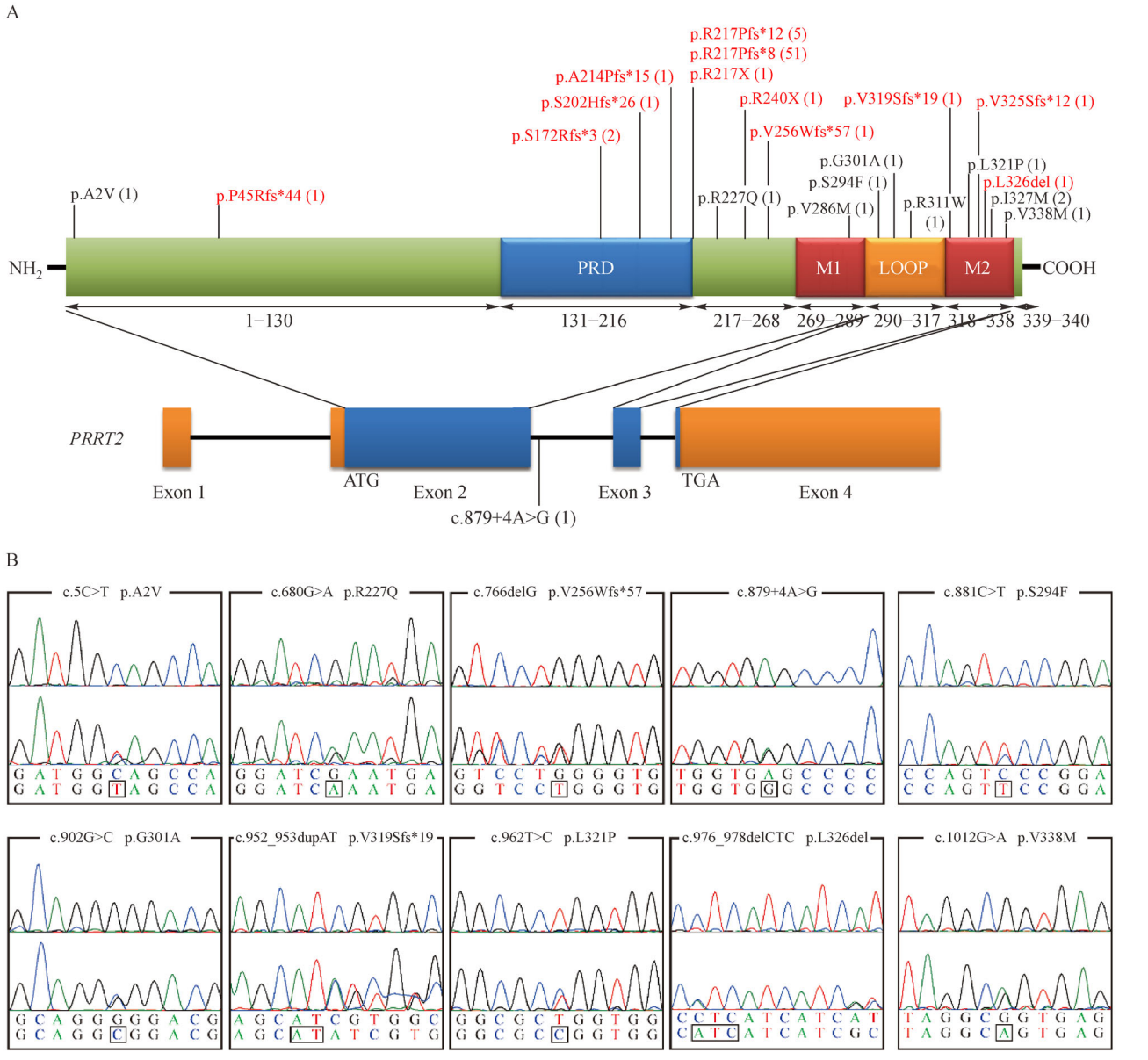

Fig. 1 *PRRT2* variants identified in our 222 PKD index patients. (A) Diagram of *PRRT2* variants superimposed to the domain structure of PRRT2 protein. *PRRT2* contains 4 exons that encode a 340 amino acid protein. The "pathogenic/likely pathogenic" variants are labeled in red, and variants of "uncertain significance" are depicted in black. The number in the bracket represents the number of index patients carrying that variant. PRD, proline-rich domain; M1, transmembrane domain 1; M2, transmembrane domain 2; LOOP, domain between M1 and M2. (B) Chromatograms of 10 novel *PRRT2* variants. The upper chromatogram in each frame represents the reference sequence, while the lower one represents the variant.

common than missense variants according to the Human Gene Mutation Database (65 versus 40) accessed in September 2020. Thus, we investigated whether penetrance varies by variant type and found a higher penetrance estimate of truncated variants (primarily frameshift and nonsense) than that of non-truncated ones (primarily missense). Truncated variants typically cause reduced protein levels and subcellular mislocalization (from the plasma membrane to the cytoplasm) [8,31,32]. This phenomenon indicates that truncated variants cause diseases via a loss-of-function mechanism (haploinsufficiency). However, the functional effect of missense variants differs. Some missense variants may alter both protein levels and localization, while some only impair protein levels or only disturb localization and others may have no effect on both functions [33,34]. Thus, missense variants may lead to a partial loss of function and exhibit lower penetrance than truncated variants. In addition,

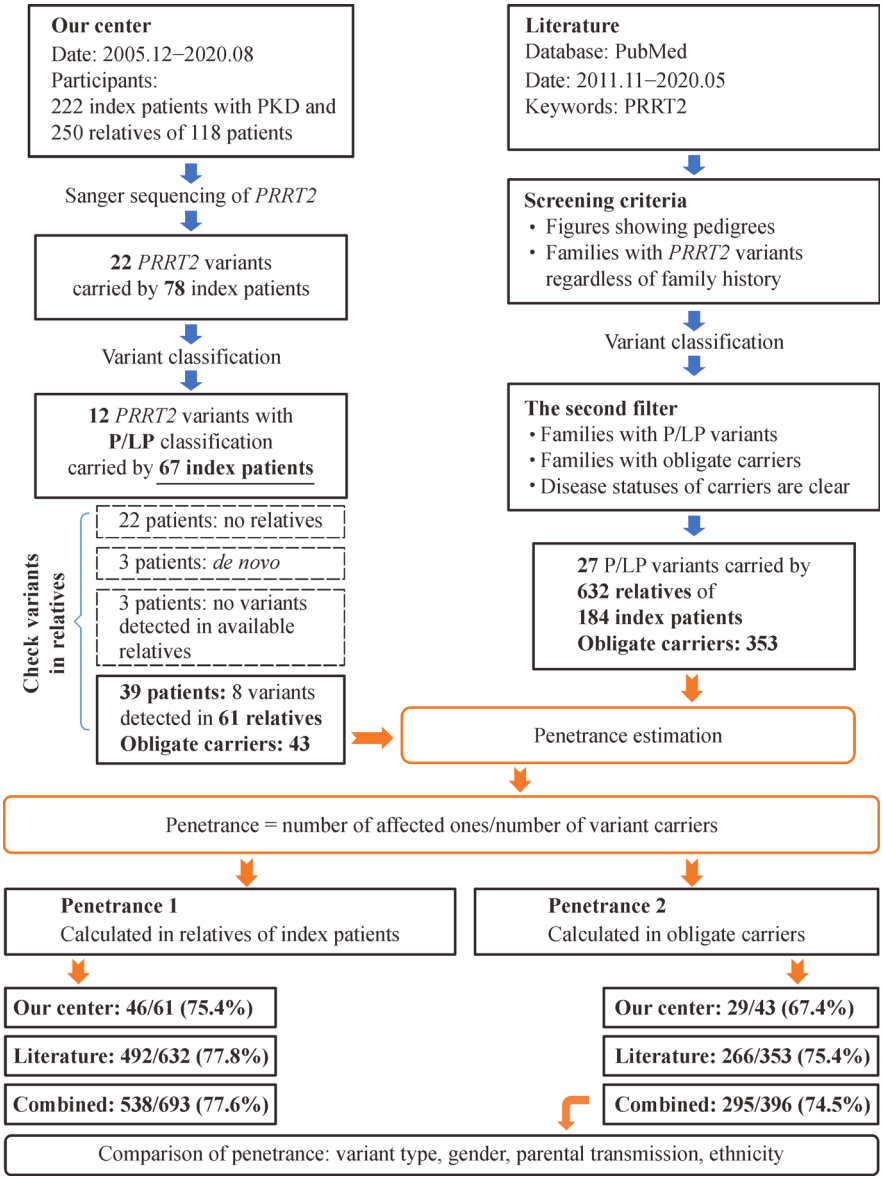

Fig. 2 Flowchart of the study design and penetrance estimation.

missense variants with normal protein expression levels and subcellular localization may have a dominant negative effect. As postulated by other researchers, these mutant proteins may preserve and interfere with the interaction between wild-type PRRT2 and its interacting proteins, such as SNAP25 [11]. Further experiments are necessary to test this effect.

Interestingly, the estimated *PRRT2* penetrance was higher in Asian variant carriers than in Caucasian carriers (81.5% versus 68.5%). Considering that penetrance differs by variant types, we examined whether penetrance difference in ethnicity was caused by the different compositions of variant types. A higher proportion of individuals carried non-truncated variants in Caucasian obligate carriers (12/197, 6.1%) than in Asian obligate carriers (6/178, 3.4%). However, the difference was not statistically significant (P = 0.218, chi-squared test). Furthermore, whether calculated only in truncated variant carriers (Asian: 140/172, 81.4% versus Caucasian: 132/185, 71.4%, P = 0.026, chi-squared test) or non-truncated variant carriers (Asian: 5/6, 83.3% versus Caucasian: 3/12, 25%, P = 0.043, Fisher's exact test), penetrance was still higher in Asian subjects than in Caucasian subjects. Thus, the difference cannot be fully explained by variant types. Other genetic differences, such as single nucleotide polymorphism (SNP), epigenetic modification and modifier genes, and/or environmental differences among different ethnic groups may also affect

Table 2 Penetrance estimation of 28 *PRRT2* variants identified in our and the published families

Nucleotide change	Protein change	Number of families (n = 223)	Number of affected/ variant carriers[b]	Penetrance 1	Number of affected/ obligate carriers	Penetrance 2
c.121_122delGT[23]	p.V41Tfs*92	1	4/5	0.8	1/2	0.5
c.133_136delCCAG[25,a]	p.P45Rfs*44	2	0/2	0	0/2	0
c.186_187delGC[22]	p.P63Qfs*70	1	1/1	1	1/1	1
c.291delC[4]	p.N98Tfs*17	1	2/2	1	1/1	1
c.388delG[12]	p.A130Pfs*46	1	1/1	1	1/1	1
c.433delC[6]	p.R145Gfs*31	1	1/2	0.5	1/2	0.5
c.487C>T[1]	p.Q163X	1	5/7	0.71	3/3	1
c.514_517delTCTG[19,a]	p.S172Rfs*3	2	3/4	0.75	2/3	0.67
c.604_607delTCAC[24,a]	p.S202Hfs*26	2	4/5	0.8	2/2	1
c.621dupA[27]	p.S208Ifs*17	1	10/12	0.83	5/6	0.83
c.629dupC[1]	p.A211Sfs*14	1	3/3	1	2/2	1
c.629delC[4]	p.P210Qfs*19	1	4/7	0.57	1/3	0.33
c.649dupC[1–9,11,14-20,23,25,26,28,29,a]	p.R217Pfs*8	173	427/536	0.80	237/307	0.77
c.649delC[19–21,23,a]	p.R217Pfs*12	12	22/38	0.58	11/20	0.55
c.649C>T[26,a]	p.R217X	2	2/2	1	2/2	1
c.697_698delAG[20]	p.S233Wfs*9	1	2/2	1	1/1	1
c.718C>T[3,6,16,28,a]	p.R240X	6	18/19	0.95	9/10	0.9
c.741delC[6]	p.S248Afs*65	1	1/1	1	1/1	1
c.776dupG[10,26]	p.E260X	2	4/5	0.8	2/2	1
c.879 + 1G>T[2,20]	NA	2	3/5	0.6	1/2	0.5
c.880-2A>T[5]	NA	1	1/1	1	1/1	1
c.904_905insG[19]	p.D302Gfs*39	1	0/1	0	0/1	0
c.916G>A[5]	p.A306T	1	1/1	1	1/1	1
c.917C>A[13]	p.A306D	1	5/5	1	4/4	1
c.919C>T[12]	p.Q307X	1	0/1	0	0/1	0
c.950G>A[2,28]	p.S317N	2	11/22	0.5	3/13	0.23
c.971G>A[5]	p.G324E	1	2/2	1	1/1	1
c.976_978delCTC[a]	p.L326del	1	1/1	1	1/1	1

Reference sequence, NM_145239.2. The superscript number represents the reference listed in Table S1. [a]The variant was also detected in our 39 families. [b]Variant carriers did not include the index patients. Penetrance 1 was evaluated in relatives. Penetrance 2 was evaluated in obligate carriers.

Table 3 *PRRT2* penetrance estimation in our and the published families

	Combined	Families		P value[a]
		Our	Published	
All families, n	223	39	184	
Total variant carriers (excluding index patients), n	693	61	632	
Affected, n	538	46	492	
Penetrance 1, % (95% CI)	77.6 (74.5–80.7)	75.4 (64.3–86.5)	77.8 (74.6–81.1)	0.663[b]
Total obligate carriers, n	396	43	353	
Affected, n	295	29	266	
Penetrance 2, % (95% CI)	74.5 (70.2–78.8)	67.4 (51.5–80.9)	75.4 (70.8–79.9)	0.261[b]

[a]Statistical difference was compared between our and the published families. [b]No statistical difference was detected with the chi-squared test.

penetrance [35]. According to a recently proposed Bayesian approach to evaluating penetrance that correlates penetrance with allelic frequencies and disease prevalence [27,36], a higher penetrance may reflect the difference in disease prevalence or allelic frequencies of *PRRT2* variants between the Asian and Caucasian groups. We checked the

Table 4 *PRRT2* penetrance by variant type, gender, parental transmission, and ethnic origin

	Obligate carriers, *n*	Affected, *n*	Penetrance, % (95% CI)	*P* value
Variant type				0.01[a]
Truncated variants	376	285	75.8 (71.4–80.1)	
Frameshift	355	267	75.2 (70.7–79.7)	
Nonsense	18	16	88.9 (65.3–98.6)	
Splice site	3	2	/	
Non-truncated variants	20	10	50.0 (27.2–72.8)	
Missense	19	9	47.4 (24.5–71.1)	
Non-frameshift deletion	1	1	/	
Gender				0.271[b]
Male	193	139	72.0 (65.6–78.4)	
Female	203	156	76.8 (71.0–82.7)	
Parental transmission				0.334[b]
Maternal	73	66	90.4 (83.5–97.3)	
Paternal	67	57	85.1 (76.3–93.8)	
Unknown	255	/	/	
De novo	1	/	/	
Ethnic origin				
Asian	178	145	81.5 (75.7–87.2)	0.004[c]
Caucasian	197	135	68.5 (62.0–75.1)	
African American	2	2	/	
Unknown	19	/	/	

[a]Significant difference ($P \leqslant 0.01$) compared between truncated and non-truncated variants, detected using the chi-squared test. [b]No statistical difference was detected with the chi-squared test. [c]Significant difference ($P \leqslant 0.01$) compared between Asian and Caucasian carriers, detected using the chi-squared test.

ExAC database and found that more *PRRT2* variants were detected in the Asian group than in the European group regardless of the pathogenicity of variants. In particular, 139 *PRRT2* variants (0.4%) were detected in approximately 35 000 Europeans (Finnish and non-Finnish), while 92 *PRRT2* variants were detected in the East Asian and South Asian groups that consisted of 11 300 individuals (92/11 300, 0.8%). Moreover, the allele frequency of SNP c.412C>G was also higher in the Asian group (1222/25 116, 4.9%) than in the European group (65/72 690, 0.1%). Currently, PKD prevalence is considered as 1:150 000, but detailed evidence is not available [11]. Whether the prevalence of PKD differs among various ethnic groups has yet to be determined.

A preponderance of males among PKD patients was widely observed, whereas gender bias in *PRRT2* penetrance has never been assessed. Here, we found similar penetrance in males and females (72.0% versus 76.8%), which may imply that gender bias in prevalence was not due to penetrance difference. Moreover, penetrance difference between maternally and paternally transmitted offspring was not statistically significant, probably suggesting the lack of genetic imprinting effect in *PRRT2*.

In conclusion, we comprehensively evaluated *PRRT2* penetrance and investigated influencing factors for the first time. Our analysis was based on clinically ascertained and published families regardless of family history, and it yielded an estimated penetrance of 74.5%–77.6%. In addition, we observed for the first time that *PRRT2* penetrance is affected by variant types and ethnicity. Our findings provide useful information for genetic counseling. Approximately three-quarters of *PRRT2* variant carriers will develop *PRRT2*-related disorders, and the risk appears to be higher in Asians or patients with truncated variants. When penetrance is considered, a patient with a heterozygous *PRRT2* variant has a 37.3%–38.8% chance of having a child with *PRRT2*-related disorders.

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Compliance with ethics guidelines

Yulan Chen, Dianfu Chen, Shaoyun Zhao, Gonglu Liu, Hongfu Li, and Zhi-Ying Wu declare that they have no conflict of interest. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional

and national) and with the *Helsinki Declaration* of 1975, as revised in 2000 (5). Informed consent was obtained from all subjects for being included in the study.

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