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Original Article

A newly discovered mutation in *PICK1* in a human with globozoospermia

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Abstract

Globozoospermia is a human infertility syndrome caused by spermatogenesis defects (OMIM 102530). Acrosome plays an important role at the site of sperm-zonapellucida binding during the fertilization process. Thus, malformation of the acrosome is the most prominent feature seen in globozoospermia. Disruption of several mouse genes, including *Gopc* (Golgi-associated PDZ and coiled-coil motif containing protein), *Hrb* (HIV-1 Rev binding protein), *Csnk2a2* (casein kinase 2, α prime polypeptide) and *Pick1* (protein interacting with C kinase 1), results in a phenotype similar to globozoospermia in humans, which suggests their potential role in the disease. However, no mutations with a clear link to globozoospermia have been identified in these genes in humans. In this study, we screened the candidate genes mentioned above in three globozoospermia type I patients and discovered a homozygous missense mutation (G198A) in exon 13 of the *PICK1* gene in a Chinese family. The family member affected by this homozygous missense mutation showed a complete lack of acrosome. Using the candidate gene screening strategy, our study is the first to identify an autosomal recessive genetic mutation in *PICK1* that was responsible for globozoospermia in humans.

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1 Introduction

Acrosome is a unique structure of the mature spermatozoon and plays an important role at the site of sperm–zonapellucida binding during the fertilization process. Globozoospermia (also called round-headed spermatozoa) is a rare (incidence < 0.1% among male infertile patients) but severe disorder causing male infertility [1, 2]. The most prominent feature of globozoospermia is the malformation of the acrosome, which is totally absent in the most severe cases. Intracytoplasmic sperm injection is a treatment option for these patients, but the fertilization rates are low because of a reduced ability to activate the oocyte [3]. In humans, several case reports of globozoospermia have demonstrated that two or more siblings were affected in each family, which suggested a genetic component to this disease [4–8]. In mice, genetic studies have provided direct evidence that disruption of genes, including *Gopc* (Golgi-associated PDZ and coiled-coil motif containing protein), *Hrb* (HIV-1 Rev binding protein), *Csnk2a2* (casein kinase 2, α prime polypeptide) and *Pick1* (protein interacting with C kinase 1), results in a globozoospermia phenotype with decreased fertility. However, there has not been a clear link between homozygous mutations in these genes and globozoospermia in humans [9]. One study using a genome-wide scan and sequencing analysis suggested that a homozygous mutation in *SPATA16* (spermatogenesis-associated 16) was associated with

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male infertility in human globozoospermia [4]. Here we reported a homozygous missense mutation (G198A) in exon 13 of the *PICK1* gene in a Chinese family. The family member affected by this homozygous missense mutation showed a complete lack of acrosome. Therefore, our study is the first to identify a mutation in *PICK1* responsible for globozoospermia in humans using the candidate gene screening strategy.

2 Materials and methods

2.1 Clinical information

A total of 100 unrelated, anonymous, fertile men were genotyped by sequencing exon 13 of the *PICK1* gene. All subjects were natives of China and had normal somatic karyotypes. No testicular biopsies were performed, and all sperm analyses were performed at least twice in accordance with the recommendations of the World Health Organization (WHO) [10]. The subject whose parents were cousins showed typical round-headed spermatozoa (Table 1). The study was approved by the local ethics committee of CITIC-Xiangya Hospital, Changsha, China and informed consent was obtained from all participants.

2.2 Blood sampling and DNA extraction

Blood samples (5 mL taken into EDTA by venipuncture) were obtained from all subjects. Immediately after collection, whole blood was stored at –80ºC until use. Genomic DNA for polymerase chain reaction (PCR) analysis was isolated from thawed whole blood using a phenol/chloroform extraction procedure.

2.3 Mutation analysis

Primers were designed according to the *PICK1* sequence (Table 2) and used in a PCR assay with Advantage 2 DNA polymerase (Clontech, Mountain View, CA, USA). PCR was performed as follows: (1) initial denaturation at 95ºC for 1.5 min, (2) 35 cycles of 94ºC for 10 s, anneal for 30 s, and 72ºC for 1 min, (3) 72ºC for 5 min, and (4) hold at 4ºC. The PCR fragments were separated by size in a 2% agarose gel in 1 × TAE buffer (40 mmol L^{-1} Tris, 20 mmol L^{-1} glacial acetic acid, 1 mmol L^{-1} EDTA, pH 8.0) and purified using an Agarose Gel DNA Fragment Recovery Kit (Takara, Tokyo, Japan) if necessary. Primers for other genes, including *GOPC*, *HRB* and *CSNK2A2*, were synthesized according to a previously published method [9].

Table 1. Sperm parameters of the affected individual.

Sperm characteristics	Parameter	Reference range
Round-headed spz (%)	100	
Sperm volume (mL)	3	≥ 2
Number spz × 10^6 mL^{-1}	30	≥ 20
Motility (%)	20	(A + B) ≥ 50%
Grade A spz	0	
Grade B spz	16.7	
Grade C spz	3.3	
Grade D spz	80	
Acrosome enzyme (μIU per 10^6 spz)	25.1	48.2–218.7

Abbreviation: spz, spermatozoa.

Table 2. Summary of PCR primers of *Pick1*.

Gene–exon	Annealing T	PCR primers (5′–3′)		Product sides (bp)
		Forward	Reverse	
Pick1–Ex1	60	GGGAAGGGAAGGATTGTCTGC	CAAGTGCCTAAATGCCAACGCC	395
Pick1–Ex2	60	GAGGGGTGGCGTTGGCATTTAG	AAGGGTCTGTGGGACTGGGAAG	252
Pick1–Ex3	58	CAGTGGAGCCCCTCAGGATGGTTAG	CAGGTGGTCAGAAAGCCCCTCTG	341
Pick1–Ex4	63	GAGCAGAGGGTAGAGTGGAAGACAG	ACAAGGAAGGGGGCGGTGAG	358
Pick1–Ex5	62	AGGAGTCTCAGTCCAGAACAGTCTTG	TTGGTCAGAGGTCAGAGCCCAC	301
Pick1–Ex6	61	CCACAGACTCACCAGGTCCTTTG	TGGTGACTTCTCAGTTCCACGG	268
Pick1–Ex7	58	TGTTAGAAGTGTGGGAGCTTTGC	TGCTGGGATTACAGGCGTGAG	508
Pick1–Ex8	61	TCGGACTGAGCTTTTACCCCTG	CATCGCAAATCCCAGCACCTGG	373
Pick1–Ex9	61	GCTTCTCCCCAACAAACCCCTG	CTCCAGCATACGACCTTCTCTGC	295
Pick1–Ex10	61	AGTCCACCAACAAGGGTGACGC	AGCATGGCTGACTGAAGTGGGG	263
Pick1–Ex11	61	GCCCTGTCATCCCTCAGCAC	GGAACGAGAGTCCAGCCAAGTC	320
Pick1–Ex12	60	AGGTCTCAGGAATGAAGAACAGCC	TTTCCCACCTCTGAAATGGAGAG	288
Pick1–Ex13	61	CTCCTGCGTTCCCTGAACTG	CCTCGTGTATCCCTGGACGG	430

Abbreviations: PCR, polymerase chain reaction; T, temperature.

Sequencing reactions were carried out using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem, Foster City, CA, USA).

2.4 Tracking PICK1 mutation with restriction digest

We prepared Pvu II digests of PCR products of the subjects by individually adding the required components to a clean microtube in the following order: 5.6 μL distilled water, 1 μL Buffer G (10 mmol L^{-1} Tris-HCl, pH 7.5, 10 mmol L^{-1} $MgCl_2$, 50 mmol L^{-1} NaCl and 0.1 mg mL^{-1} BSA), 3 μL of PCR product and 0.4 μL of Pvu II restriction enzyme. The tube was mixed by flicking and spun for 5 s in the microfuge to bring all the components to the bottom. This digest mixture was then incubated at 37ºC for 2 h (in a 37ºC water bath). DNA fragments were separated by size through an 8% PAGE gel in 0.5 × TBE buffer (45 mmol L^{-1} Tris, 45 mmol L^{-1} boric acid, 1 mmol L^{-1} EDTA, pH 8.0).

3 Results

3.1 Identification of homozygous mutation in PICK1 in a patient with globozoospermia

We identified three globozoospermia type I patients in our clinical study. To assess the potential genetic basis for globozoospermia in humans, we performed PCR and DNA sequencing analysis on four candidate genes (*GOPC*, *HRB*, *CSNK2A2* and *PICK1*) in these patients. We chose these four genes because disruption of these genes results in a globozoospermia phenotype in mice [9]. The subjects were screened for these genes, and we found a homozygous missense mutation in the *PICK1* gene in one of these patients. The affected individual showed only round-headed spermatozoa (Figure 1) with decreased motility and acrosomal enzyme activity (Table 1). We sequenced exons 1–13 of *PICK1* and the flanking sequences (primers shown in Table 2) in the affected individual and found a homozygous G→A transition at nucleotide 1567 according to the Genebank entry NM_012407 (Figure 2A). The transition generated a missense substitution (G393R) located in the C-terminal acidic domain. Thus, we have identified a homozygous mutation in the *PICK1* gene that is associated with globozoospermia in humans.

3.2 Tracking PICK1 mutations across generations of the patient's family members

To determine the mode of inheritance of this mutated gene, we tracked *PICK1* mutations across multiple generations of the patient's family members. As shown in Figure 2, we also found a heterozygous mutation at the same location in his parents, a sister and a niece. These findings demonstrated that the mutation was transmitted in an autosomal recessive mode.

Finally, this mutation created G198A in exon 13 and disrupted a Pvu II site, which was subsequently used to confirm and identify the mutation in the pedigree of this patient and other individuals. Figure 3 shows that the homozygous mutation was not present in 100 control chromosomes.

4 Discussion

Screens for mutations of candidate genes in the pedigree of patients and other individuals are a common method to investigate the function of a gene [11, 12]. In this study, we screened four candidate genes in three globozoospermia type I patients. We did not observe any mutations in *GOPC*, *HRB* or *CSNK2A2*, which may be due to limited samples or the controversial mechanism of *CSNK2A2* [13]. Fortunately, we discovered a homozygous missense mutation (G198A) in exon 13 of

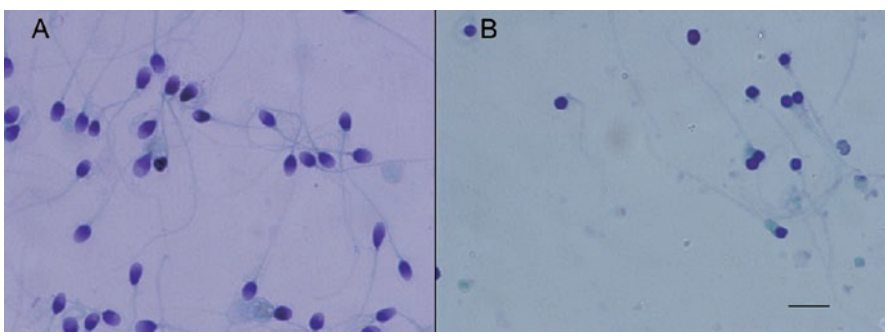

Figure 1. Morphology of sperm by Papanicolaou staining. (A) Normal human sperm and (B) globozoospermia. Globozoospermia results in the loss of the typical acrosome of normal sperm; instead, they have round ball-like heads. Bar = 10 μm.

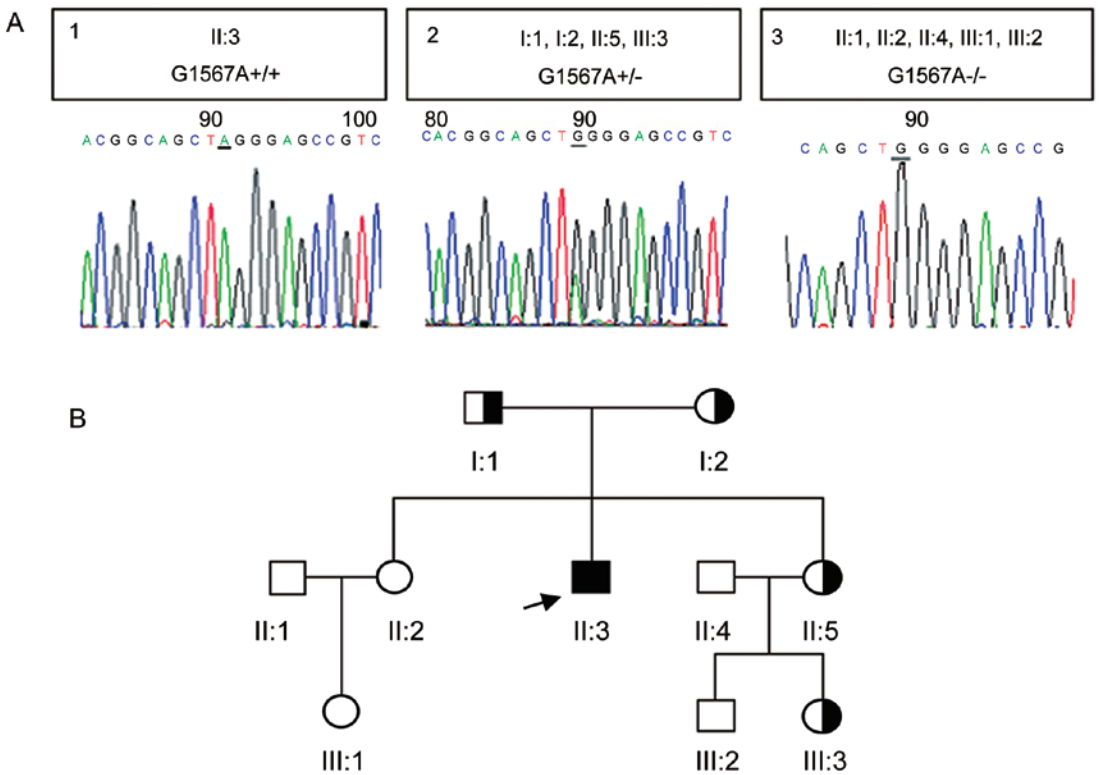

Figure 2. Sequence analysis and pedigree. (A1)–(A3) *PICK1* sequence analysis in the proband and his family. The mutation position of the sequence is underlined. The transition generates a missense substitution (G393R) located in the C-terminal acidic domain of the PICK1 protein. (A1): In the proband (II:3), automated sequencing revealed a homozygous G→A transition at the base pair 1 567 of the *PICK1* gene. (A2): The heterozygous G→A transition at the same position of the *PICK1* gene was observed in the subject's parents (I:1 and I:2), a sister (II:5) and a niece (III:3). (A3): Brothers in law (II:1 and II:4), sister (II:2), niece (III:1) and nephew (III:2) were homozygous for the wild-type allele. (B): The pedigree of the family.

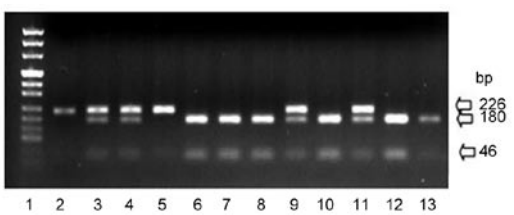

Figure 3. The agarose gel of the product from a Pvu II enzyme cut (Lane 1: Puc Mix Marker 8, Lane 2: PCR product without an enzyme cut, Lane 3: I:1, Lane 4: I:2, Lane 5: II:3, Lane 6: II:1, Lane 7: II:2, Lane 8: III:1, Lane 9: II:5, Lane 10: II:4, Lane 11: III:3, Lane 12: III:2 and Lane 13: normal man). The G198A mutation in exon 13 disrupted a Pvu II site. Thus, the homozygous individual (Lane 5) and the PCR product without an enzyme cut (Lane 2) showed one band (226 bp); heterozygous individuals (Lanes 3, 4, 9 and 11) showed three bands (226, 180 and 46 bp); and individuals homozygous for the wild-type allele (Lanes 6, 7, 8, 10, 12 and 13) showed two bands (180 and 46 bp). The positions of the molecular mass markers and enzyme cut product are indicated (bp).

the *PICK1* gene, which resulted in the complete lack of acrosome in one patient. Therefore, we have provided evidence for the first time that a *PICK1* mutation transmitted by an autosomal recessive mode was responsible for globozoospermia in humans.

The human *PICK1* gene contains 13 exons encoding a 415 amino-acid protein. The cytosolic protein interacts with many membrane proteins via its postsynaptic density-95/Discs large/zona occludens-1 (PDZ) domain. Its interactions with membrane proteins regulate their subcellular targeting and surface expression. PICK1 has been repeatedly reported to be involved in the neuronal system [14]. In neurons, synaptic targeting and surface expression of AMPA (α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid) glutamate receptors were found to be critical for synaptic plasticity. PICK1 is a cytosolic protein that interacts with many membrane proteins, including AMPA receptors, via its PDZ domain. Lipid binding of the PICK1 BAR (Bin/

amphiphysin/Rvs) domain was positively regulated by its PDZ domain and negatively regulated by its C-terminal acidic domain. Recently, Xiao *et al.* [15] reported that male mice deficient in PICK1 were infertile and had a phenotype resembling the human disease globozoospermia. The primary defect in the testes of *Pick1*-knockout mice was fragmentation of acrosomes in the early stages of spermiogenesis. This fragmentation was followed by defects in nuclear elongation and mitochondrial sheath formation, which led to round-headed sperm, reduced sperm count and severely impaired sperm motility. However, the genetic mutations in *Pick1* genes were not identified, and the correlation with globozoospermia remains unknown.

Here, we found a homozygous mutation in the C-terminal domain of *PICK1* in a globozoospermia patient, and observed heterozygous mutations in family members. This is the first reported mutation in the *PICK1* gene that has resulted in globozoospermia in humans.

In the present study, the G198A homozygous patient did not show any obvious physiological or anatomical defects other than the infertility, which suggested that the altered amino acid G393R in the C-terminal domain did not result in alterations of somatic functions. The heterozygous parents, who were cousins, did not have reduced fecundity. In addition, his heterozygous sister bore a heterozygous son. Similar to humans, homozygous mutant mice had no apparent somatic defects, and both male and female heterozygous mice showed normal fertility. Only homozygous males failed to produce pups. The *PICK1* mutant phenotype in the patient in this study was consistent with the phenotype observed in *Pick1*-deficient mice. In our opinion, the homozygosity for an autosomal gene defect underlies this phenotype. The altered amino acid G393R in the C-terminal domain may influence the negative regulation with the BAR domain and affect the formation of the acrosome because of the perturbation of lipid-BAR domain binding. We conclude that the homozygous mutation of *PICK1* results in globozoospermia.

Specifications

All DNA and amino acid numeration in this manuscript referred to *PICK1* isoform 1, which is the longest isoform (references NM_012407 and NP_036539.1, respectively). All three variants encoded the same protein. Thus, the described mutation (G198A) affects all three isoforms in the same manner.

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