3 Biotech (2021) 11:208
https://doi.org/10.1007/s13205-021-02761-4

ORIGINAL ARTICLE

Novel compound heterozygous missense variants (c.G955A and c.A1822C) of *CACNA2D4* likely causing autosomal recessive retinitis pigmentosa in a Chinese patient

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Received: 23 December 2020 / Accepted: 29 March 2021 / Published online: 10 April 2021

Abstract

Retinitis pigmentosa (RP) is a rare and heterogeneous group of inherited ocular diseases. However, the relationship between *CACNA2D4* mutations and RP is not well understood. In this study, a Chinese autosomal recessive retinitis pigmentosa (arRP) pedigree was enrolled and targeted next-generation sequencing was employed for identifying the causative gene in the proband. These steps were followed by confirmatory Sanger sequencing and segregation analysis. RNA-sequencing (RNA-seq) data and semi-quantitative reverse transcription polymerase chain reaction analysis were then applied to examine the expressions in the human and mouse tissues. Novel compound heterozygous, deleterious missense variants of the *CACNA2D4* gene, NM_172364.4: c.G955A (p.D319N) and c.A1822C (p.I608L), were identified in the arRP pedigree, co-segregating with the clinical phenotype in the patient. The CACNA2D4 protein is highly conserved among species. The *CACNA2D4* mRNA expression showed the highest expression in the retina of humans and in the later four developmental stages/times of retinal tissues in mice, indicating its role in retina/eye functions and developments. This study is the first to identify novel compound heterozygous mutations c.G955A (p.D319N) and c.A1822C (p.I608L) in the *CACNA2D4* gene. These might be disease-causing mutations, thereby extending the mutational spectra. The identification of pathogenic *CACNA2D4* variants is expected to enhance our understanding of the genotype–phenotype correlations of arRP for disease diagnosis and genetic counseling. The relationship between the *CACNA2D4* variants and diseases/phenotypes other than RP has also been reviewed and discussed in this paper.

Keywords Retinitis pigmentosa · *CACNA2D4* gene · Compound heterozygous mutations · Next-generation sequencing · *CACNA2D4* expression

Abbreviations

RP	Retinitis pigmentosa
arRP	Autosomal recessive retinitis pigmentosa
CACNA2D4	Homo sapiens calcium voltage-gated channel auxiliary subunit alpha2delta 4 gene
NGS	Next-generation sequencing
TGS	Targeted next-generation sequencing
RNA-seq	RNA-sequencing
RT-PCR	Revere transcriptional-polymerase chain reaction

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ExAC	The Exome Aggregation Consortium database
HGMD	The Human Gene Mutation Database
RPKM	Reads Per Kilobase of transcript per Million
OMIM	Online Mendelian Inheritance in Man
NX	Consensus normalized expression
VGCC	Voltage-gated calcium channels
BWA	Burrow–Wheeler Aligner
ERG	Electroretinogram

Introduction

Retinitis pigmentosa (RP, OMIM: 268000) is a rare, heterogeneous group of inherited ocular diseases. RP leads to progressive retinal degeneration, eventually culminating in severe visual impairment or blindness (Fu et al. 2019). The condition affects roughly 1 in 4,000 people in the United States (Ali et al. 2017; Fu et al. 2018). RP is both clinically and genetically heterogeneous and is inherited according to Mendelian inheritance patterns. The most common inheritance pattern of RP is autosomal recessive (50–60%), followed by autosomal dominant (30–40%) and rarely X-linked (5–15%) (Hamel 2014; Hartong et al. 2006). To date, mutations in over 80 genes have been identified to cause RP and result in progressive photoreceptor loss.

The calcium voltage-gated channel auxiliary subunit alpha2delta4 (*CACNA2D4*) gene (NM_172364.4) (OMIM: 608171), also known as *RCD4*, is located on chromosome 12p13.33. The gene has 38 exons spanning 128,909 bases in the human genome (GRCh38/hg38) and encodes a putative 1137-amino acid protein with a predicted molecular mass of 127,938 Da (NP_758952.4) (Qin et al. 2002). Mutations in the *CACNA2D4* gene have been linked to autosomal recessive cone dystrophy 4 (RCD4, OMIM 610478) or autosomal recessive retinitis pigmentosa (arRP) (Wycisk et al. 2006b; Ba-Abbad et al. 2016). However, the *CACNA2D4* gene mutations and diseases/phenotypes, including RP, are not well understood.

The present study aims to decipher the causative gene and its mutations accounting for the occurrence of arRP in a Chinese family. Targeted next-generation sequencing (NGS) (Wang et al. 2014; Zhang et al. 2016; Valencia et al. 2015) was used to identify the possible genetic cause for arRP in this family. The relationship between the *CACNA2D4* variants and diseases/phenotypes other than RP has also been reviewed and discussed.

Materials and methods

Pedigree, proband, clinical assessment, and sample collection

Detailed ophthalmic examinations were performed on the recruited pedigree with a proband (M338). Blood samples were collected and gDNA was extracted from the patient (proband) and the parents as well as healthy control volunteers using a standard phenol/chloroform extraction method (Fu et al. 2002, 2020a). Prior approval was obtained from the Ethics Committee of Southwest Medical University. Informed written consent was acquired from all the participating subjects.

Targeted-NGS sequencing

Targeted-NGS (TGS) analysis was conducted using family M338 according to the instructions for Illumina (Wang et al. 2014; Zhang et al. 2016; Fu et al. 2018; Salvo et al. 2015). The reads were aligned with the hg19 reference genome of humans using Burrow–Wheeler Aligner (BWA) and the public online database (Li and Durbin 2009). Variations in single nucleotide polymorphisms (SNPs) and insertions/deletions were refined using Atlas-SNP2 and Atlas-Indel2 (Challis et al. 2012). Variant frequency data were applied to the CHARGE consortium (Psaty et al. 2009), Human 1000 Genomes (Genomes Project et al. 2010), ANNOVAR (Wang et al. 2010), ESP-6500 (Tennessen et al. 2012), and Exome Aggregation Consortium (ExAC) databases to identify all pathogenic variants in the candidate genes and were assessed by PolyPhen-2, SIFT (Sorting Intolerant From Tolerant), MutationTaster, I-Mutant2.0, and HGMD (Human Gene Mutation Database).

Sanger DNA sequencing and co-segregation analysis

Polymerase chain reaction (PCR) amplification and Sanger DNA sequencing of the variants were applied to the gDNA of the participants for mutation verification and pedigree segregation analysis (Imani et al. 2018a). Locus-specific primer pairs, CACNA2D4-955 and CACNA2D4-1822, were designed using the online Primer3 Program for NM_172364.4: c.G955A or c.A1822C mutations in the *CACNA2D4* gene (Table 1).

For sequencing, PCR amplification was performed using a total volume of 20 µL of reaction mixture containing the specific primers CACNA2D4-955L and CACNA2D4-955R for the c.G955A variant or CACNA2D4-1822L and CACNA2D4-1822R for the c.A1822C variant (Table 1), 50 ng of

Table 1 The primer sequences for PCR and the sizes of PCR products

Primer name	Forward primer	Sequence (5′–3′)	Reverse primer	Sequence (5′–3′)	Size	°C
CACNA2D4-955	CACNA2D4-955L	aacctggcatagatgggttg	CACNA2D4-955R	accgtggacactcacctctc	563	60
CACNA2D4-1822	CACNA2D4-1822L	agtgggatcttggtgaatgc	CACNA2D4-1822R	tgcagcaagcactcaactct	339	60
RT-cacna2d4	RT-cacna2d4L	catgctgtcctctagccaca	RT-cacna2d4R	caacagcctcgactttcctc	347	60
RT-b-actin-m	RT-b-actin-mL	tgttaccaactgggacgaca	RT-b-actin-mR	tctcagctgtggtggtgaag	392	60

gDNA template, and 10 µL of 2×PCR TaqMaster mix (Tiangen Biotech, Beijing, China) in a Veriti™ 96-Well Thermal Cycler from Applied Biosystems, USA. The amplification conditions were as follows: initial denaturation at 95 ºC for 90 s, followed by 32 cycles at 94 ºC for 40 s, 60 ºC for 60 s, 72 ºC for 40 s, and final extension at 72 ºC for 5 min.

The PCR products were then resolved by electrophoresis on 1.2% agarose gels in 0.5×TAE buffer. The gels were visualized by staining with 0.5 µg/mL ethidium bromide, and specific bands of 563 bp for the c.G955A variant or 339 bp for the c.A1822C variant were gel-extracted and purified. The purified PCR products were then sequenced using the Sanger DNA sequencing method with specific primers, CACNA2D4-955L or CACNA2D4-1822L, as presented in Table 1. All ethnically matched normal controls were amplified and sequenced using the aforementioned primers. Co-segregation analyses for the pedigree were conducted based on Sanger DNA sequencing results.

Protein structure analysis and bioinformatics

A search of the conserved domains of CACNA2D4 was performed using the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) by inputting the amino acid sequences of CACNA2D4 (NP_758952.4) (Marchler-Bauer et al. 2017; Imani et al. 2018b). Homologs of *CACNA2D4* were revealed by the NCBI HomoloGene program (https://www.ncbi.nlm.nih.gov/homologene?Db=homologene&Cmd=Retrieve&list_uids=26544).

RNA-seq data analysis for *CACNA2D4* expression

To determine the tissue-specificity, the mRNA expression profiles of *CACNA2D4* were examined using the RNA-seq data from 27 different tissues (https://www.ncbi.nlm.nih.gov/gene/93589). This project was called HPA RNA-seq normal tissues (Zhou et al. 2019; Cheng et al. 2020; Fu et al. 2020b). The mRNA expression profiles of *CACNA2D4* in different human tissues, including the retina, were obtained via the HPA (Human Protein Atlas) (https://www.proteinatlas.org/ENSG00000151062-CACNA2D4/tissue) (Uhlen et al. 2010; Wei et al. 2020; Fu et al. 2020b).

RNA extraction and semi-quantitative reverse transcription (RT)-PCR

Total RNA was isolated from mice according to our previously reported protocol (Fu et al. 2018). The sequences of the RT-primers, product sizes, and PCR conditions, including the annealing temperature, are listed in Table 1. RT-PCR was conducted using the primer pair RT-cacna2d4 (RT-cacna2d4L and RT-cacna2d4R) targeting the mouse *Cacna2d4* gene (NM_001033382.2). The mouse *β-actin* gene was used as an internal control and was amplified using the primer pair described previously (Cheng et al. 2019) (Table 1). RT-PCR amplification was performed in a total volume of 10 µL using cDNA as a template on a Veriti™ 96-Well Thermal Cycler. The amplification conditions for the mouse *Cacna2d4* gene were as follows: initial denaturation at 95 ºC for 90 s, followed by 30 cycles at 94 °C for 40 s, 60 ºC for 60 s, 72 ºC for 40 s, and final extension at 72 ºC for 5 min. The amplified PCR products were then separated on 1.2% agarose gel in 1 × TAE buffer (Liu et al. 2020). The gels were then visualized, and the images were documented as reported previously (Zhou et al. 2019; Cheng et al. 2019).

Results

Pedigree and proband clinical characteristics

The proband (Fig. 1a, II: 1) was a 62-year-old man belonging to a Chinese family. The patient claimed a reduction in visual acuity and peripheral field loss at the age of 35. His clinical features are summarized in Table 2. Fundus examination revealed pale fundus, optic nerve atrophy, vessel attenuation, osteocyte-like pigmentation, and retinal pigment epithelial degeneration (Fig. 1b). Electroretinogram

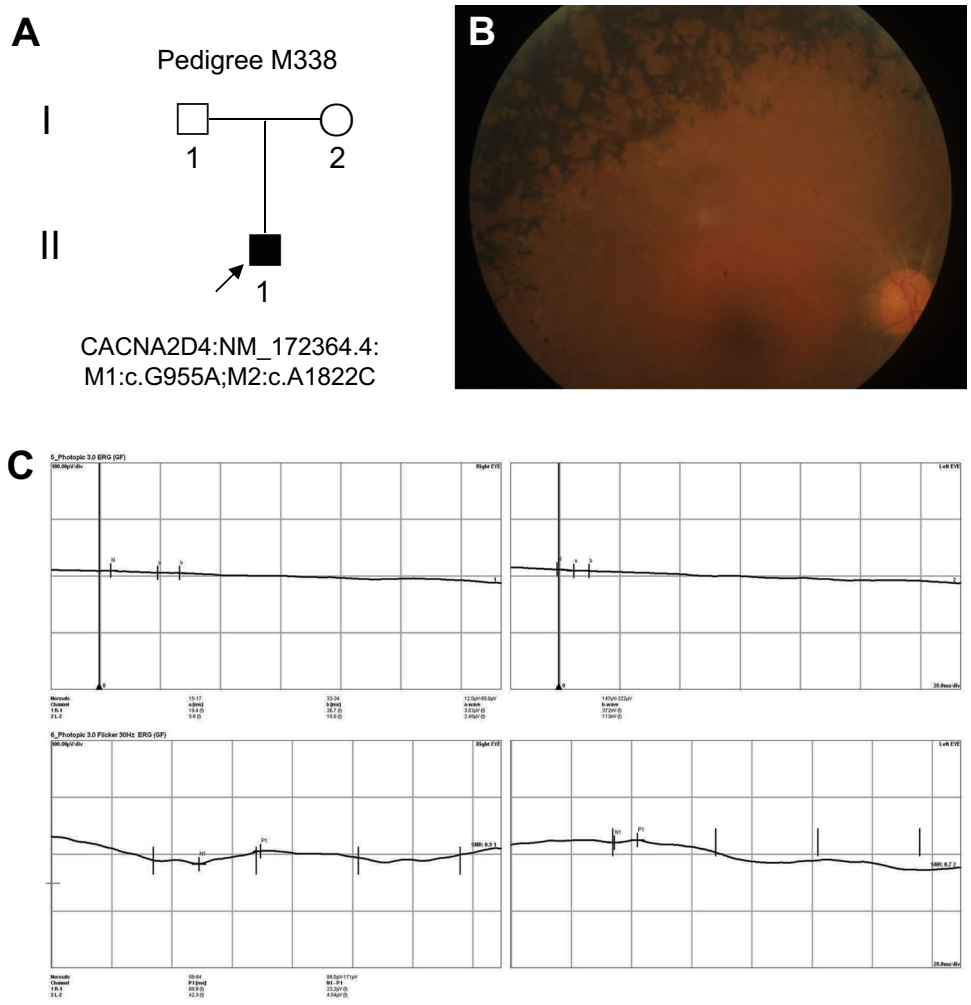

Fig. 1 A M338 pedigree of retinitis pigmentosa. **a** A pedigree of the M338 family. Normal individual is indicated as a clear circle (female), whereas the affected male patient is indicated as a filled square symbol with an arrow (II: 1) for the compound heterozygous variants of *CACNA2D4*: NM_172364.4:M1:c.G955A; M2:c.A1822C. **b** Fundus photography of the right eye of the proband. **c** Electroretinogram (ERG) of the proband. Upper panels, photopic of 3.0 ERG (right and left eyes); Bottom panels, photopic of 3.0 flicker 30 Hz ERG (right and left eyes). M338 is the molecular number for the proband

Table 2 Clinical features of the RP patient

Proband	II:1
Gender	M
Current age (yrs)	62
Age at onset (yrs)	35
VA (OD/OS)	LP/HM
Initial symptoms	Decreased vision, needs for more light
Ocular features	Nystagmus, oculomotor apraxia
Fundus features	A pale fundus, optic nerve atrophy, vessel attenuation, osteocyte-like pigmentation outside retinal vascular arch, and retinal pigment epithelial degeneration

yrs years, *M* male, *HM* hand movement, *LP* light perception, *OD* right eye, *OS* left eye, *VA* visual acuity

revealed that the amplitudes of the α and β waves were significantly decreased in the apparent dark vision reaction, and the reaction was extinguished. (Fig. 1c). The proband's parents had normal eye examinations and showed no retinal disease. Thus, the M338 proband was characterized as arRP.

NGS analyses and putative pathogenic mutation identification

To assess the disease-causing gene and its mutations, a targeted NGS panel of retinal disease-causing genes was conducted successfully using the gDNA of the M338 patient (Fig. 1, pedigree II: 1). Compound heterozygous, missense variants c.G955A and c.A1822C at exons 8 and 18, respectively, in the *CACNA2D4* gene (NM_172364.4) were identified, which led to amino acid (aa) changes (p.D319N and p.I608L) in the CACNA2D4 protein (NP_758952.4) (Fig. 1, II: 1). P olyPhen-2 analysis suggested probable damage (PD) for c.G955A: p.D319N change (score 1) and benign for c.A1822C: p.I608L change (score 0.268); MutationTaster revealed disease-causing (DC) (scores of 1 for c.G955A: p.D319N change and 0.9998 for c.A1822C: p.I608L change); SIFT alluded damaging (scores of 0.02 for c.G955A: p.D319N change and 0.04 for c.A1822C: p.I608L change); and I-Mutant2.0 of the free-energy change values indicated decreased stability (DDG = − 1.00 kcal/mol, < 0) for c.G955A: p.D319N change and increased stability (DDG = 0.75 kcal/mol, > 0) for c.A1822C: p.I608L change. The deleterious and pathogenic aspects of the *CACNA2D4* gene: c.G955A and c.A1822C mutations are presented in Table 2. Hence, the compound heterozygous, missense mutations (c.G955A and c.A1822C) in the *CACNA2D4* gene are likely to have induced damaged protein function in the patient. These variants were determined to be novel mutations through

Table 3 Characteristics of CACNA2D4 variants and analyses of disease-causing effects

Gene	Exon	Variation				Polyphen-2	MutationTaster	I-Mutant2.0 (DDG)	SIFT	ExAC
		Nucleotide	Amino acid	Type	Status					
CACNA2D4	8	c.G955A	p.D319N	Mis	Com het	PD (1)	DC (1)	–1.00 kcal/mol	D (0.02)	Novel
CACNA2D4	18	c.A1822C	p.I608L	Mis	Com het	B (0.268)	DC (0.9998)	1.34 kcal/mol	D (0.04)	Novel

CACNA2D4 Homo sapiens calcium voltage-gated channel auxiliary subunit alpha2delta 4, *c* variation at the cDNA level, *p* variation at the protein level, *Mis* missense, *Com het* compound heterozygote, *PD* probably damaging, *B* benign, *DC* disease-causing, *DDG* free-energy change value, *DDG > 0* increased stability, *DDG < 0* decreased stability, *D* damaging

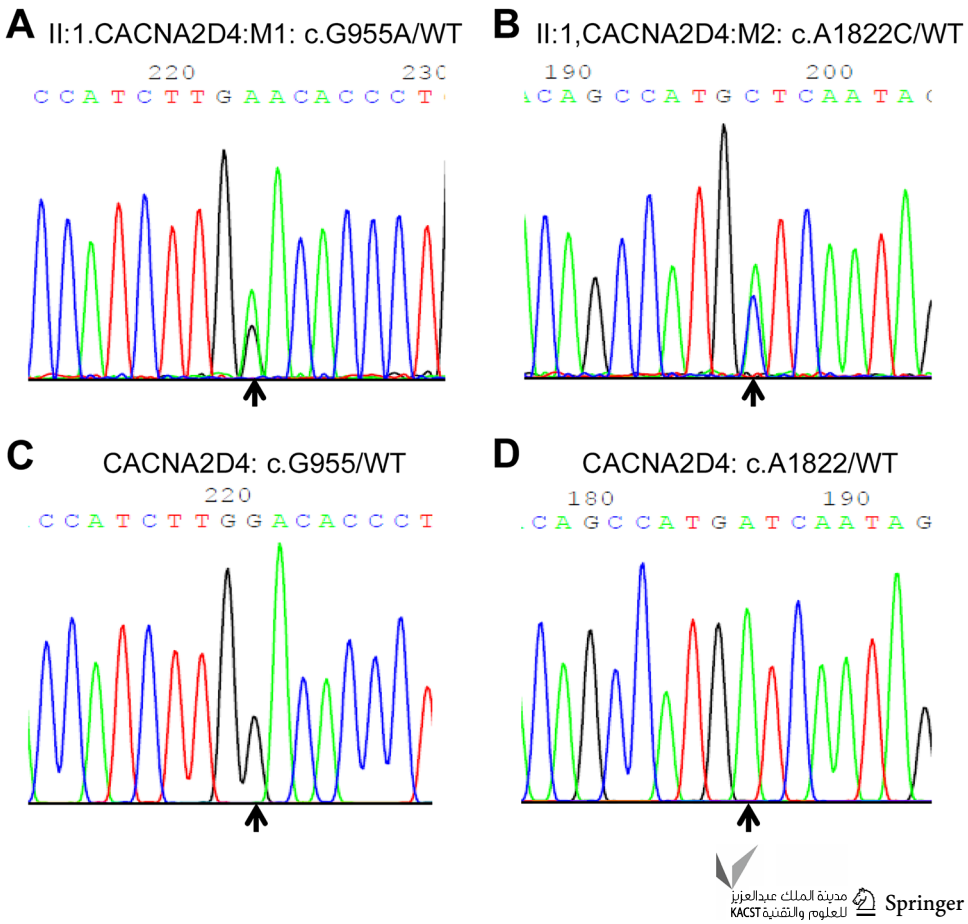

Fig. 2 Pyrogram profiles for variant verification by Sanger DNA sequencing. **a**, **b** The sequencing results in II: 1 for *CACNA2D4* compound heterozygous mutants for c.G955A and c.A1822C, and **c**, **d** The sequencing results in a normal male for wild-type in both the sites c.G955 and c.A1822, respectively. The arrows indicate the mutations at the nucleotide position NM_172364.4: M1:c.G955A; M2: c.A1822 in *CACNA2D4*

ExAC or HGMD (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=CACNA2D4) database searches (Table 3).

Verification of candidate mutations and segregation analysis

Confirmation of the mutations (c.G955A and c.A1822C) and segregation analysis were done by DNA sequencing (Fig. 2). The variants c.G955A and c.A1822C of the *CACNA2D4* gene was verified to be compound heterozygous in patient M338 (pedigree II:1; Fig. 2a, b). A heterozygous variant c.G955A in the M338 father and a heterozygous variant c.A1822C in his mother (pedigree I:1, I:2; data not shown) were identified, while the normal males had wild-type forms of the gene (Fig. 2c, d). Thus, we were able to establish that the c.G955A and c.A1822C variants in the *CACNA2D4* gene co-segregated with the RP phenotype of the family members. Both variants were absent in the 100 normal controls. These findings indicate the co-segregation of the mutations in the pedigree of the RP family and define their disease-causing roles in pathogenesis.

Functional effects of the variants c.G955A (p.D319N) and c.A1822C (p.I608L) in *CACNA2D4*

Search for conserved domains within the CACNA2D4 protein in the NCBI database revealed that CACNA2D4 had four conserved domains, namely VWA_N (pfam08399) (aa interval: 154–264), vWA_VGCC_like (cd01463) (aa interval: 277–460), dCache_1 (pfam02743) (aa interval: 454–576), and VGCC_alpha2 super family (cl07190) (aa interval: 687–1070) (Fig. 3a). The p.D319N mutation was located in the vWA_VGCC_like domain (Fig. 3c). The *CACNA2D4* gene was found to be conserved in the chimpanzee, rhesus monkey, dog, cow, rat, mouse, chicken, zebrafish, mosquito, fruit fly, and frog upon searching for homologs. Orthologous comparisons of the CACNA2D4 protein in *Homo sapiens* with the abovementioned 12 species indicated that p.D319 and p.I608L of the CACNA2D4 protein were highly conserved (Fig. 3b, c, respectively). In essence, our study has asserted that the pathogenic variants c.G955A (p.D319N) and c.A1822C (p.I608L) of the *CACNA2D4* gene with compound heterozygous, missense mutations are responsible for the patient's arRP disease.

Results of *CACNA2D4* and *Cacna2d4* mRNA expression profiles

When RNA-seq was performed, *CACNA2D4* gene expression in the different human tissues showed that the Reads Per Kilobase of transcript per Million (RPKM) values in the testis and the appendix were the highest, with an approximate score of 1.0 (Fig. 4a), demonstrating that the levels were not quite high in any of the tested tissues. Unfortunately, no eye tissues, including the retina, were available. Thus, the mRNA expression profiles of the *CACNA2D4* gene in 61 different tissues and cells, including the retina, were obtained. The results revealed that the *CACNA2D4* gene expression was the highest in the human retina, with a consensus normalized expression (NX) value of 47.0. The second highest expression was in the T-cells, but with an NX value of only 4.7 (Fig. 4b). Hence, this finding strongly indicates that

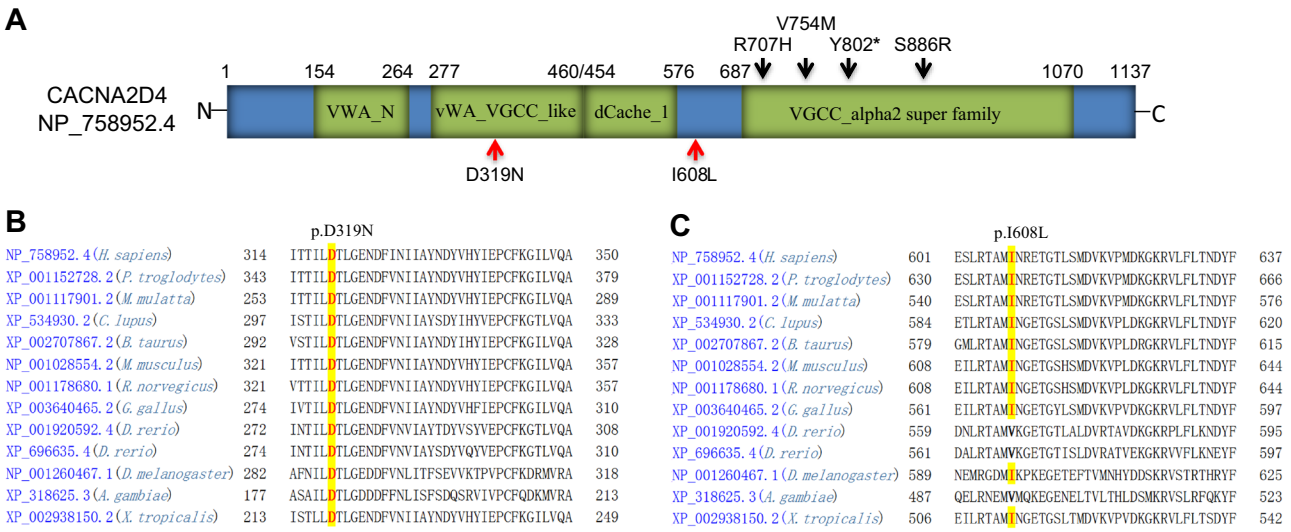

Fig. 3 CACNA2D4 protein structure and comparison. **a** CACNA2D4 domains. Arrows indicate the mutated amino acid positions. Red arrows indicate the missense mutation positions identified in this study, whereas the black arrows indicate the missense/nonsense mutation positions reported earlier. "*" indicates the stop codon. **b** Orthologous conservation analysis in the CACNA2D4 p.D319 variant in the indicated species. **c** Orthologous conservation analyses in the CACNA2D4 p.I608 variant in the indicated species

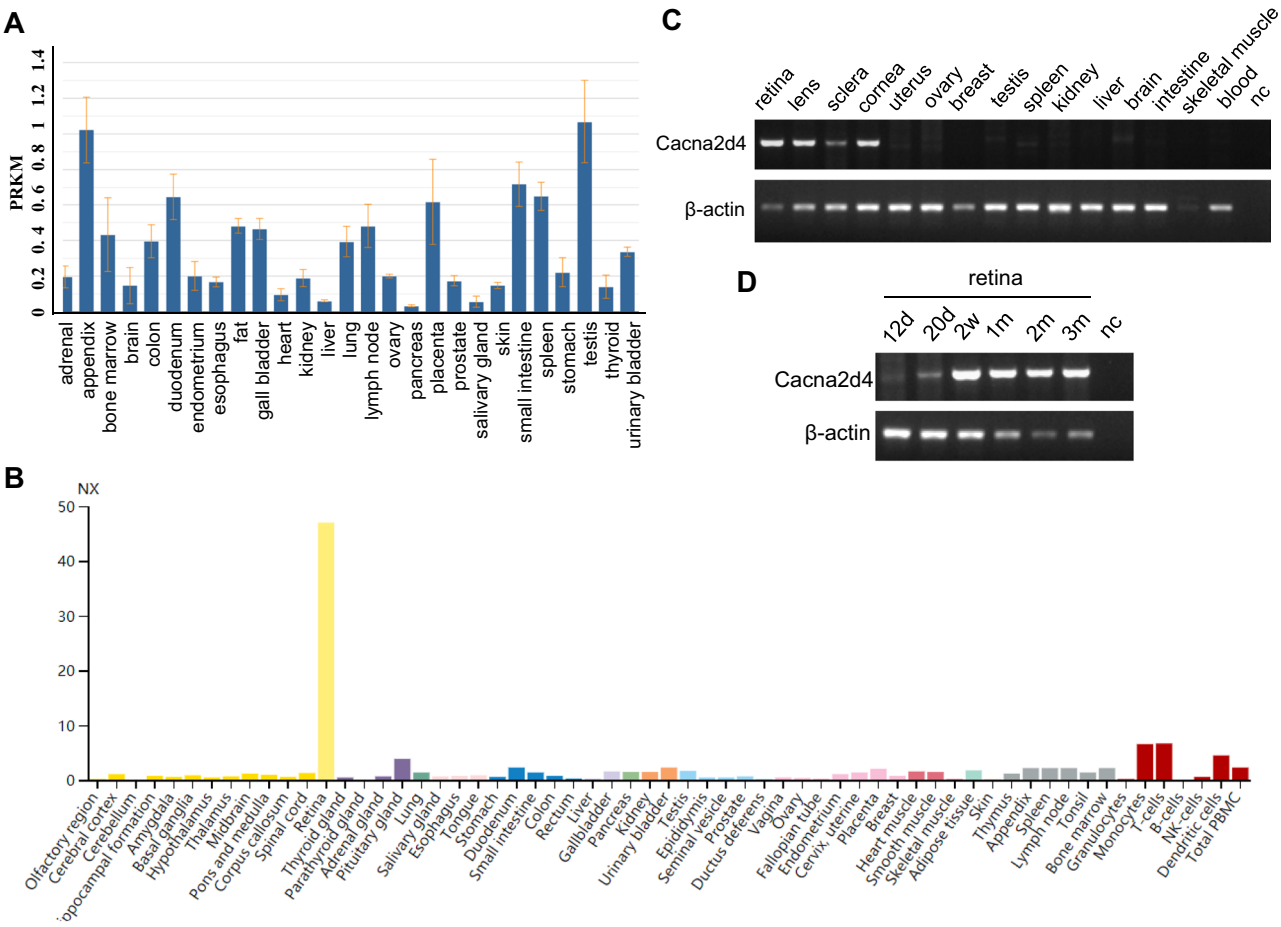

Fig. 4 The mRNA expression profiles of the genes of human *CACNA2D4* and mouse *Cacna2d4*. The *CACNA2D4* expressions in the indicated human tissues (**a**). The expression for *CACNA2D4* in 55 indicated tissues and 6 blood cells (**b**). The expression profiles of *Cacna2d4* in the indicated tissues in mice (**c**) and the indicated development stages of retina in mice (**d**). NX, consensus normalized expression. RPKM, Reads Per Kilobase of transcript per Million mapped reads. d indicate day(s); w, week(s); m, month(s); nc, negative control

CACNA2D4 is highly expressed only in the retinal tissue and plays an important role there.

Meanwhile, the expressions of *Cacna2d4* mRNA in the different tissues and developmental stages of the retina were analyzed by quantitative RT-PCR, and the results are shown in Fig. 4c, d. From Fig. 4c, it is evident that the levels of *Cacna2d4* mRNA are the highest in the retina, followed by the lens, cornea, and sclera of the eyes. However, no expression was detectable in the uterus, breast, ovary, testis, kidney, spleen, liver, intestine, brain, skeletal muscle, and blood (Fig. 4c). Furthermore, *Cacna2d4* mRNA of the retinal tissue was highly expressed in the later four stages of (Fig. 4d). Whole eyeballs of the embryos at 12.5 days and 20.5 days (Fig. 4d) were taken because of technical difficulties in sample collections. The higher expression of *Cacna2d4* gene in the retina and other tissues of the eyes further supports the notion that CACNA2D4 plays important roles in the functioning of the retina and the eye as a whole.

Discussion

The *CACNA2D4* gene encodes a member of the alpha-2/delta subunit family, a protein with a molecular mass of 127,938 Da in the complex of the voltage-dependent calcium channel. Calcium channels can mediate the influx of calcium ions into the cells when the membranes polarize and consist of a complex in a 1:1:1:1 ratio with alpha-1, alpha-2/delta, beta, and gamma subunits. The abundance of the presynaptic calcium channel and the probability for release are determined by alpha-2-delta expression since the release of the synaptic neurotransmitter is driven by Ca^{2+} influx through the active zone voltage-gated calcium channels (VGCC) (Hoppa et al. 2012). As a regulatory subunit, CACNA2D4 can alter the properties of the pore-forming alpha-1 subunits of VGCC. CACNA2D4 can also be processed into two peptides (subunits of alpha-2 or delta) and be held together via a disulfide bond. Mutations in the *CACNA2D4* gene cause

Table 4 The CACNA2D4 variants and diseases/phenotypes

Disease/phenotype	Mutation numbers	CACNA2D4 variants	Refs.
Takotsubo (stress) cardiomyopathy?	2	Missense (p.V754M) Small insertion ACCCC^(1017)GTGacaTTCGTGTA	(Goodloe et al. 2014)
Atrial fibrillation?	1	Missense (p.S886R)	(Weeke et al. 2014)
Bipolar disorder, late-onset?	1	Gross deletion (35,740 bp incl ex. 17–26)	(Van Den Bossche et al. 2012)
Cone dystrophy	1	Missense (p.Y802*)	(Wycisk et al. 2006b)
Retinal dystrophy	1	Missense (p.R707H)	(Huang et al. 2015)
Schizophrenia?	1	Small deletion ACACC^(1116)TCAgCCTCGCCG	(Purcell et al. 2014)

Small deletions/insertions are shown in terms of the deleted bases in lower case plus, in upper case, 8-bp DNA sequence flanking both the sides of the lesion. The numbered codon is indicated by a sequence by the caret character (^); p, variation at the protein level; *, stop codon; incl, including; ex., exon; ?, low confidence; Ref., references

autosomal recessive cone dystrophy, retinal dystrophy, or arRP (Wycisk et al. 2006b; Ba-Abbad et al. 2016; Gustafson et al. 2017; Huang et al. 2015). Interestingly, a homozygous mutation has also been identified in the *Cacna2d4* gene of mice, which leads to autosomal recessive cone–rod dysfunction in the visual system (Wycisk et al. 2006a). However, the *CACNA2D4* gene mutations and their involvement in RP have not been well studied in the Chinese population (Huang et al. 2015). In this study, we have successfully identified novel compound heterozygous, pathogenic missense variants of the *CACNA2D4* gene, namely c.G955A and c.A1822C, by NGS-based genetic diagnosis in a Chinese family, which led to RP. Upon searching the HGMD (access date: December 1, 2020), we found that only seven pathogenic variants of the *CACNA2D4* gene have so far been reported, including four missense/nonsense mutations (Fig. 3a), one small deletion, one small insertion, and one gross deletion. The relationship between the *CACNA2D4* variants and diseases/phenotypes other than RP have also been discussed, and the relevant literature has been reviewed. Table 4 lists the *CACNA2D4* variants and the associated diseases/phenotypes. From the above identified variants, we found that the mutational spectra and disease phenotypes were variable (Goodloe et al. 2014), for example, familial atrial fibrillation was associated with the CACNA2D4 variant: p.S886R (Table 4) (Weeke et al. 2014). To the best of our knowledge, the variants c.G955A and c.A1822C of the *CACNA2D4* gene are novel and might cause arRP, thereby extending its mutational spectra.

Orthologous comparison of the CACNA2D4 protein *of H. sapiens* with 12 other species indicated that these proteins are higher conserved. The CACNA2D protein has four conserved domains, namely VWA_N (pfam08399), vWA_VGCC_like (cd01463), dCache_1 (pfam02743), and VGCC_alpha2 super family (cl07190). The p.D319N mutation is located in the vWA_VGCC_like domain. The exact biochemical function of this domain is not clear; however, the alpha 2 delta complex has been reported to regulate various functional properties of the channel complex, suggesting its role in complex stability. Four other studies have documented that missense/nonsense variants are located in the VGCC_alpha2 super family domain of the CACNA2D protein (Fig. 3), but the clinical phenotypes of the patients are entirely different (Table 4).

Human *CACNA2D4* gene expression based on the RNA-seq of 61 different tissues and cells revealed highest expression in the retina and only very low expression in others. Our results from mouse tissues showed that *Cacna2d4* mRNA is expressed only in the retina, lens, sclera, and cornea of the eye, suggesting that the Cacna2d4 protein plays important roles in the development and functioning of the retina/eye. Using KO-mice model, Kerov et al. established that Cacna2d4 is essential for maintaining the structural and functional integrity of the rod and cone synapses. Thus, their disruption by *CACNA2D4* gene mutations might contribute to visual impairment (Kerov et al. 2018). To sum up the findings, our study has revealed that the *CACNA2D4* compound heterozygous, missense mutations c.G955A and c.A1822C are likely to lead to vision impairment and arRP. However, further studies are needed to determine whether these variants are truly the causative factors for different clinically variable diseases.

Conclusion

In conclusion, our study is the first to identify two novel compound heterozygous missense variants, c.G955A and c.A1822C, in *CACNA2D4*, which may be the disease-causing mutations for RP in the present Chinese pedigree, thereby expanding its mutation spectrums. Targeted-NGS thus provides an effective approach for genetic diagnosis (Fu et al. 2018; Adams and Eng 2018; Rahbaran et al. 2019). The identification of pathogenic CACNA2D4 variants also enhances our understanding of the genotype/phenotype correlations in RP for gene diagnosis as well as for genetic counseling.

Acknowledgements This work was supported by the National Natural Science Foundation of China (31701087), the Special Training Program for Young Science and Technology Talents from Southwest Medical University (00031726), the Joint Research Foundation of Luzhou City and Southwest Medical University (2018LZXNYD-YL01).

Authors' contributions JF was in charge of the idea. J.F. and S.A. supervised the project. JC, JF, LZ, and CW performed DNA extraction, PCR, sequencing and data analysis. HL and QZ recruited the clinical patients and were in charge of the clinical assessment. JF and SK wrote the manuscript, and JF, SA and JC revised the manuscript. All authors read and approved the final manuscript.

Declarations

Ethics approval and consent to participate The study has the Ethical Committees approval granted by the Southwest Medical University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent for publication Written informed consent was obtained from all participants.

Competing interests The authors declare no conflict of interests.

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