New mutation in PTEN identified in patient with rare bilateral choroidal ganglioneuroma

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Abstract

Background

Choroidal ganglioneuroma is an extremely rare tumor, with little known regarding its pathogenesis. The present study aimed to investigate the phenotype and genetic alterations in one sporadic patient with rare bilateral choroidal ganglioneuroma.

Methods

A 6-year-old boy with histological diagnosis of bilateral ganglioneuroma was recruited in this study. Comprehensive ophthalmic examinations were performed in the patient. Genomic DNA was extracted from peripheral blood collected from the patient, the patient's unaffected family members, and from 200 unrelated control subjects from the same population. Whole exome sequencing was carried out and raw reads were aligned to the human genome reference (hg19) using the Burrows Wheeler Aligner. All available family members were subjected to Sanger sequencing for segregation analysis.

Results

Bilateral and extensive retinal detachments were observed in OCT. The diffuse thickening of choroid was identified in B scan and MRI. Genetic analysis revealed the presence of a novel heterozygous *PTEN* frameshift mutation, c.498delA (p.Thr167LeufsTer16), in exon 6. It was identified in the affected individual, but not in any of the unaffected family members. Genetic analysis showed that there was no mutant in neurofibromatosis-related genes. There were no obvious abnormalities in other organs in comprehensive systemic examinations.

Conclusions

A novel de novo PTEN mutation was identified in a bilateral choroidal ganglioneuroma case. Although PTEN mutation has been considered to induces multiple abnormalities, choroidal ganglioneuroma can be the first manifestation without abnormalities in other organs. Further studies are needed to confirm this new association between choroidal ganglioneuroma and the PTEN mutation.

Background

Choroidal ganglioneuroma is an extremely rare disease, that only 13 cases have been reported in literatures [1–13]. Ganglioneuroma is benign neurogenic tumor that occurs with an approximate incidence of 1 case per million in the United States for children [14,15]. It is arising from neural crest [16], and considered as a subset of neuroblastomas that histopathologically consist of mature ganglion-like cells

with scarce immature cells [17, 18]. The underlying mechanism for development of ganglioneuroma is still unclear.

According to the previous reports, blind painful eye was the common feature, and 12 cases have established neurofibromatosis type 1 (NF-1), which lead to the suggestion that choroidal ganglioneuromas could be recognized as a rare manifestation within the clinicopathologic spectrum of NF-1 syndrome [19]. Recently, we confronted with the first case of bilateral choroidal ganglioneuromas, and reported the multimodal imaging features in the early stage[20].

Aiming to unravel the pathogenesis of this rare disease, whole exome sequencing was carried out in this patient. As a phosphatase and tensin homolog (PTEN) mutation was identified, comprehensive systemic examinations were performed to screen the presence of concomitant abnormalities.

Methods

Study participants

This study was carried out according to the guidelines approved by the Ethics Committee of Zhongshan Ophthalmic Center (ZOC), Sun Yat-sen University and in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects.

A 6-year-old boy complaining of gradual vision loss bilaterally for 2 years presented in Pediatric Ophthalmology Department, ZOC. Comprehensive ophthalmic examinations were performed including visual acuity, intraocular pressure (IOP), slit lamp microscopy, fundus photography (Heidelberg Engineering, Inc., Heidelberg, Germany), optical coherence tomography (OCT; Carl Zeiss Meditec, Inc., Dublin, CA, USA), ultrasound B scan (Aitomu Machinery Co. Ltd, Shanghai, China), and magnetic resonance imaging (MRI; General Electric, Milwaukee, WI). As choroidal ganglioneuroma was diagnosed based on previous choroidal biopsy, extensive examinations were performed to screen any systemic involvements. Whole exome sequencing was carried out to unravel the genetic pathogenesis of this rare disease.

DNA sample collection

Blood samples were collected from this proband, his unaffected family members, and 200 unrelated control subjects from the same population. Genomic DNA was extracted using the TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China) as instructed by the manufacturer. The quantity and quality of DNA was verified by NanoDrop (2000c Model, Thermo Fisher, US).

Library preparation and targeted sequencing

According to the manufacturer's protocol, Illumina paired-end libraries were prepared using Kapa LTP library prep kit (Roche, Basel, Switzerland). Briefly, genomic DNA was sheared into fragments of approximately 300–500 bp in length. The DNA fragments were end-repaired and an extra 'adenine' base

was added to the 3' end. Illumina adapters were ligated to the ends of the DNA fragments and four cycles of PCR amplification were applied to each sample after ligation. The DNA libraries were quantified by the Qubit 3.0. Pre-capture libraries were pooled together for one capture reaction. Agilent SSELXT Human All Exon V6 was used for whole exome sequencing (Agilent, Santa Clara, CA, USA). The enriched DNA library was sequenced on Illumina Xten Analyzers for 150 cycles per read to generate paired-end reads following the manufacturer's standard sequencing protocols.

Bioinformatics analysis of sequencing results

Raw reads were aligned to the human genome reference (hg19) using the Burrows Wheeler Aligner. Single-nucleotide variants (SNVs) and insertions and deletions (InDels)were called by GATK4.0 HC. The frequency of all SNVs and InDels were annotated using the ExAC, gnomAD, HGVD, CHARGE, 1000 Genome, UK10K databases, and the internal database of Clinbytes Inc. to filter the common variants, with an allele frequency cutoff of 0.5% and 0.1% for recessive and dominant variants, respectively.

Genetic validation

After any pathologic variants were confirmed in the proband, samples from all available family members were subjected to Sanger sequencing for segregation analysis. PCR primer sets were designed via Primer3 and products were sequenced on an ABI 3700XL Genetic Analyzer. Primers used for amplification of PTEN are forward 5’-GGCTACGACCCAGTTACCATAG-3’ and reverse 5’-TGGGACAGGTTCTTCCATCATC-3’.

Results

Ocular findings

In initial examination in 2 years ago, the best corrected visual acuity was 20/50 in the right eye and 20/32 in the left. In recent visit, it was hand motion/10 cm and 20/200, respectively. The IOP had fluctuated around the normal range in the past, but 40 mmHg IOP was observed in recent visit, and was controlled well with brinzolamide-timolol eyedrops.

Bilateral and extensive retinal detachments were observed in OCT. The diffuse thickening of choroid was identified via B scan and MRI (Figure 2). Choroidal tumor was highly suspected, and following choroid biopsy demonstrated the histological diagnosis of choroidal ganglioneuroma. More details on the multimodal images can be found in our previous report[20].

Mutation screening

Genetic analysis documented the presence of a heterozygous PTEN frameshift mutation, c.498delA (p.Thr167LeufsTer16) in exon 6 (NM_000314). It was identified in the affected individual, but not in any of the unaffected family members; therefore, this was considered a de novo mutation. The identified mutation had not previously been reported, and was not observed in a large population cohort (Figure 3).

The c.498delA variant causes a frameshift starting with codon Threonine167, changing this amino acid to a Leucine residue, and creates a premature Stop codon at position 16 of the new reading frame, denoted p.Thr167LeufsTer16. This variant is predicted to cause loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay.

As most reported cases showed a medical history of NF-1, genetic changes in neurofibromatosis-related genes were evaluated. Genetic analysis showed that there was no mutant in NF1- or NF2-related genes.

Systemic workup

According to histological finding of ganglioneuromas and the mutation in PTEN, it is crucial to rule out the presence of ganglioneuromas, or PTEN related abnormalities in other tissues. Comprehensive systemic examinations were performed in the case, including general physical examination, ultrasound scan of the thyroid gland, liver and kidney, CT scan of the mediastinum and retroperitoneum, and no obvious abnormalities were detected.

Discussion

According to the reported studies (Table 1), most cases of choroidal ganglioneuroma share the same features. First, most cases of uveal ganglioneuroma co-occurred with NF-1, leading to the diagnosis as NF-1with orbit-facial involvement[19]. Second, ganglioneuroma leads blind and painful eye unilaterally, and end up with evisceration/enucleation in all cases. Third, ganglioneuroma were diagnosed unexpected only after subsequent histopathological examination. Genetic examination was performed in only one case diagnosed as Cowden syndrome and revealed a PTEN mutation.

In this case, retinal detachments were the major early manifestations, and choroidal tumor was suspected when choroidal thickness increases. There were no clinical features that supporting NF-1 throughout extensive examinations. Choroidal biopsy was performed and revealed the diagnosis of ganglioneuroma[20]. Interestingly, this is the first case of bilateral choroidal ganglioneuroma. Thus, whole exome sequencing was performed and identified a de novo PTEN mutation. PTEN is a tumor suppressor gene that classically dampens the PI3K/AKT/mTOR growth-promoting signaling cascade [21]. Loss of PTEN function results in increased cell proliferation, survival, and tumorigenesis [22, 23], manifesting with diverse human pathologies, and leading to the use of the umbrella term, PTEN hamartoma tumor syndrome (PHTS) [24, 25]. Thus, based on histologic features, this case is diagnosed as choroidal ganglioneuroma. On the other side, based on genetic finding, this case is diagnosed as PHTS manifesting with choroidal ganglioneuroma.

The most common features of PHTS patients are macrocephaly, gastrointestinal polyps with various histological patterns, and dermatological lesions including trichilemmoma and oral papillomatosis, which are each, found in around 90% of the PTEN mutation carriers [26, 27]. For instances, De Paris et al identified a PTEN mutation in a case of Cowden syndrome, who manifested with features including unilateral uveal

ganglioneuroma, macrocephaly, developmental delay, arteriovenous malformation, and a strong family history of early-onset uterine cancer. However, in current case, systemic examinations fail to identify any diagnostic criteria of PHTS. Thus, this is the first case of a patient with heterozygous PTEN mutation, who manifested as choroidal ganglioneuroma without any obvious abnormalities in other organs.

Although the pathogenic gene has been identified, the mechanisms are still unknown yet that why it causes bilateral choroidal ganglioneuroma, without any obvious changes throughout the whole body. This may be attributed to the young age of this patient. Studies have reported features with respect to age of onset in PTEN mutation carriers. For instances, the onset age of breast cancer of PHTS is believed to be around age 38–50 years, and average age of diagnosis are in the 40s for PHTS related cancers in thyroid, endometrial and colon [28-30]. Thus, genetic study is important the patients, as PTEN mutation has been considered to increase risks for multiple common cancers. Lifetime cancer risk estimates and close follow-up are highly required for the young boy in the present case.

One limitation of the present study is the limited case number. Choroidal ganglioneuroma is an extremely rare disease and most reported cases are not diagnosed until evisceration/enucleation. Therefore, it might be very difficult to recruit enough cases for a case-series study. To this point, biopsy and genetic testing are critical for the early and accurate diagnosis of choroidal ganglioneuroma.

Conclusion

A novel de novo PTEN mutation was identified in a bilateral choroidal ganglioneuroma case. Although PTEN mutation has been considered to induces multiple abnormalities, choroidal ganglioneuroma can be the first clinic manifestation without abnormalities in other organs. Further studies are needed to confirm this new association between choroidal ganglioneuroma and the PTEN mutation.

Availability Of Data And Materials

The datasets used for the current study are available from the corresponding author on reasonable request.

Abbreviations

IOP
Intraocular pressure
OCT
Optical coherence tomography
NF-1
Neurofibromatosis type 1
PTEN
Phosphatase and tensin homolog

PHTS
PTEN hamartoma tumor syndrome

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Declarations

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Contributions

DXY conceived and designed the study. JZX, ZT, CCL and LSS participated in data collection, laboratory analysis and interpretation. JZX and SLM analyzed the data and wrote the first draft of the manuscript. DXY and CCC critically reviewed the manuscript. All authors approved the submitted version.

Ethics approval and consent to participate

All procedures adhered to the tenets of the Declaration of Helsinki, and local approval was received from the Investigational Review Board of Zhongshan Ophthalmic Center, Sun Yat-sen University.

Written informed consent for participation in the study was obtained where participants are children (under 16 years old) from their parent or guardian

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Table

Table 1. Clinical profiles of choroidal ganglioneuroma cases in literature.

	Case	Sex	Age (Years)	Eye	Clinical presentation	NF-1	Treatment
1	Wolter	Femal	42	Left	Cataract, glaucoma, buphthalmos	Yes	Enucleation
2	Woog et al	Femal	21	Left	Cataract, retinal detachment, buphthalmos	Yes	Enucleation
3	Browstein et al	Femal	<1	Left	Congenital glaucoma, buphthalmos, plexiform neurofibroma	Yes	Enucleation
4	Shome et al	Male	11	Left	Glaucoma, buphthalmos, plexiform neurofibroma, sphenoid wing dysplasia	Yes	Evisceration
5	Ishijima et al	Male	7	Left	Cataract, retinal detachment, buphthalmos	Yes	Enucleation
6	Lad et al	Femal	<1	Right	Cataract, buphthalmos, sphenoid wing dysplasia	Yes	Enucleation
7	Yazici et al	Femal	17	Left	Cataract, glaucoma, buphthalmos plexiform neurofibroma	Yes	Evisceration
8	Goyal et al	Femal	<1	Right	Buphthamos, hypoglobus, plexiform neurofibroma	Yes	Exenteration via craniotomy access.
9	Chang et al	Male	42	Right	Cataract, optic nerve glioma, neurofibroma,	Yes	Enucleation
10	Mbagwu et al	Femal	5	Right	Proptosis, glaucoma, sphenoid wing dysplasia	Yes	Enucleation
11	Abdulkader et al	M	50	Right	Cataract, glaucoma, buphthalmos, plexiform neurofibroma, frontoethmoidal encephalocele	Yes	Orbital exenteration
12	DeParis et al	Femal	5	Right	Glaucoma, retinal detachment, macrocephaly, developmental delay	No	Enucleation
13	Gilani et al	Male	7	Not	Invading plexiform	Yes	Enucleation

				mentioned	neurofibroma, choroidal layer expanding		
14	Present case	Male	6	Bilateral	Retinal detachment and diffuse choroidal thickening	No	IOP lowering medicines

Figures

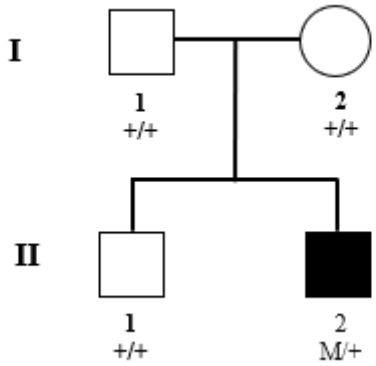

Figure 1

Pedigree of the family of the patient identified with choroidal ganglioneuroma. Squares represent males, circles represent females, arrows indicate proband, and black symbol identifies the clinically affected individual.

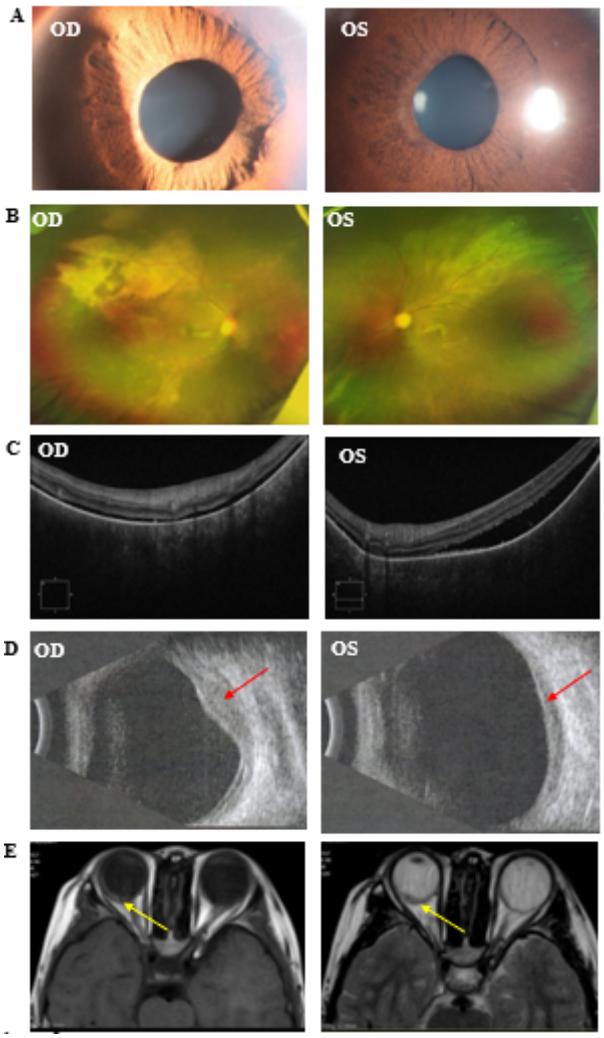

Figure 2

Images of comprehensive ophthalmic examinations. (A) The bilateral pupils were irregular and unable to dilate. (B) Funduscopy showed vasculitis and exudation bilaterally. (C) Bilateral retinal detachment was observed in optical coherence tomography. Choroidal vasculature was dramatically absent. (D) A dome-shaped mass bulging from choroid to retina was observed in B scan. (E) The diffuse thickening of bilateral choroids was verified by magnetic resonance imaging.

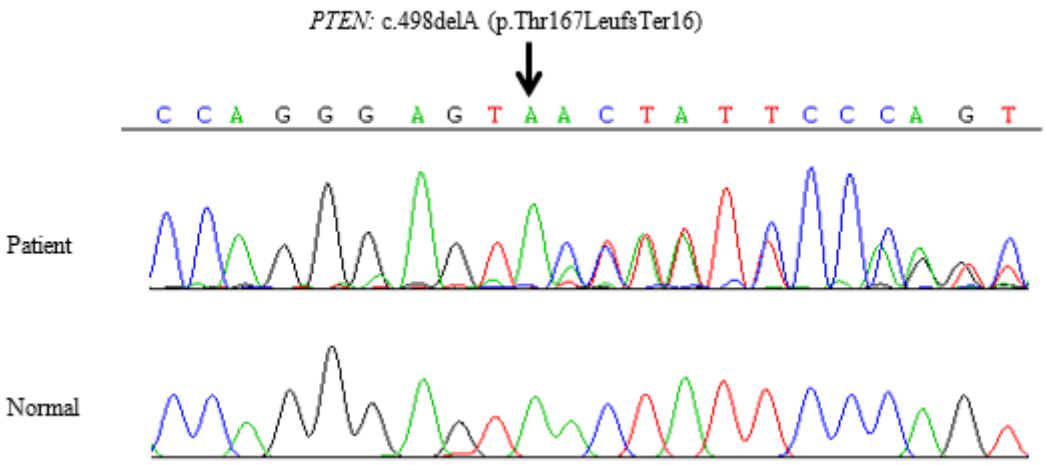

Figure 3

DNA sequence of a region of the PTEN gene in the patient and unaffected individuals. A heterozygous PTEN frameshift mutation c.498delA (p.Thr167LeufsTer16) in exon 6 (NM_000314) was identified in the affected individual, but not in the unaffected family members or unrelated control subjects.