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Evaluation of p.N46H mutation in ATOH7 gene in an Iranian patient affected by bilateral PHPV

KEYWORDS: Persistent Hyperplastic Primary Vitreous, ATOH7, p.N46H

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ABSTRACT *Background: Persistent hyperplastic primary vitreous (PHPV) is a rare congenital developmental malformation of the eye, caused by the failure of regression of the primary vitreous during third to nine months of gestation. In most cases PHPV is sporadic and unilateral while bilateral PHPV is rare. The disease can be of an isolated type but usually is complex and accompanied by other ocular abnormalities. PHPV can be inherited as an autosomal dominant or recessive trait. Some recent studies showed p.N46H mutation in ATOH7 gene on 10q21 in PHPV patients with autosomal recessive inheritance pattern.*

Materials and Methods: The analysis of c.136A>C mutation (p.N46H) of ATOH7 gene was performed by PCR amplification and sequencing using a standard Sequence Detection System.

Results: In this study, a 2.5 year old girl with bilateral1 PHPV was examined for genetic causes of her disease to determine the presence or absence of c.136A>C mutation in ATOH7 gene. The PCR product was sequenced through forward and reverse primers and the results shows no mutation in this point.

Conclusions: Despite the p.N46H mutation in ATOH7 gene that was reported in six-generation Pakistani family, the molecular analysis data in this patient shows no mutation in this locus. It seems other mutations maybe are involved in pathogenesis of PHPV in our Iranian patient.

INTRODUCTION

Persistent hyperplastic primary vitreous (PHPV), also known as persistent fetal vasculature (PFV) is a rare congenital developmental malformation of the eye, caused by the failure of regression of the primary vitreous during third to nine months of gestation (1).

When human eye is forming throughout embryogenesis, nutrients are provided by a hyaloid artery between the retina and crystalline lens, which is later replaced by the developing retinal vasculature (2).

The lens is comprised of the lens capsule, epithelium, and fibers. The lens is utterly surrounded by the lens capsule that is a collagen containing basement membrane structure. The lens epithelium is a simple cuboidal epithelium between the lens capsule and the lens fibers in the anterior portion of the lens. The lens fibers are long, thin, transparent cells filled with crystalline proteins to ensure lens transparency. Lens development begins in the ectoderm with formation of the lens placode. In the vertebrate eye, lens development come with the growth of the surrounding capillary network. This network is found within the anterior papillary membrane (APM) on the anterior surface of the lens and includes the tunica vasculosalentis (TVL) posterior to the lens and provides nutrients to intraocular components (2).

The retina is a highly metabolic tissue with a high oxygen consumption, which causes complex vascular formation to provide oxygen and nutrients during ocular development. Because the formation of retinal vessels is restricted in the inner retina and occurs only in the late gestation of development, the supplement of nutrients and oxygen to the developing retina of the early stage is dependent on the choroidal vessels and the hyaloid vessels. The hyaloid vessel, consisting of tunica vasculosa lentis (TVL) and hyaloids artery (HA), hyaloids vasa propria (HVP) is a transient intraocular circulatory system that provides all nutrients and oxygen to the developing eye and dramatically under goes a complete regression as retina is matured with retinal vascularization.If the complete involution of hyaloids vessels fails, there is a pathological persistence of these vessels, which is clinically significant as PHPV (3). In most cases PHPV is sporadic and unilateral while bilateral PHPV is rare (4).

The disease can be of an isolated type (4) but usually is complex and accompanied by other ocular abnormalities (2). The conditions that may mimic PHPV include microphthalmia, congenital cataract, ophtalmus, retinoblastoma, corneal opacity, uveal coloboma, retinal degeneration, leukocoria, and tractional detachment of the retina (3).

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Three types of PHPV have been observed: anterior, posterior, or a combination of the two. Getting useful vision in patients with posterior PHPV rarely happens (5).

PHPV can be inherited as an autosomal dominant or recessive trait (4). Although several genes, such as alternative reading frame (*Arf*) , norrie disease pseudoglioma (*NDP*), protein 53 (*p53*) , and genes in the int and wg (wingless; *Wnt*) signaling pathway have been implicated in the development of PHPV, the exact mechanisms have not been resolved (2).

The first locus for isolated nonsyndromic PHPV was identified by homozygosity mapping in a large inbred Pakistani pedigree, the linkage data presented in that study suggests that a gene for autosomal recessive PHPV is presented at the proximal part of the long arm of chromosome 10, most likely at 10q11-q21 by microsatellite mapping (6). In this fragment of chromosome 10 there are several genes including: CXCL12, RET, MBL2, MAPK8, CDK1, SIRT1.

In another article Su PH et al, reported on a newborn girl with partial trisomy (6)(p22 , this patient showed a unique condition for this syndrome that is PHPV with retinal detachment. They searched human genome database for candidate genes and reported nine genes that one of them (FOXC1) plays a role in the regulation of embryonic and ocular development. Mutations in this gene cause various glaucoma phenotypes. This gene belongs to the forkhead family of transcription factors which is characterized by a distinct DNA-binding forkhead domain (7).

A recent study in 2012 at university of Michigan showed ATOH7 mutations cause autosomal recessive PHPV. The vertebrate basic helix-loop-helix (bHLH) transcription factor ATOH7 (Math5) is specifically expressed in the embryonic neural retina and is required for the genesis of retinal ganglion cells (RGCs) and optic nerves. They identified a homozygous ATOH7 mutation (N46H) in a large family with an autosomal recessive PHPV disease trait linked to 10q21.

MATERIALS AND METHODS

Investigated in this study is a 2.5 year-old girl born in Shiraz (Iran). Her disease was diagnosed when she was 2 month. In the history, the girl was appeared normal at birth except the eyes that were closed until 10 days after birth. Her parents are related (second cousin). The results of right ocular B-mode sonography showed that the globe has normal shape. The axial length of the globe is about 11-12 mm. The anterior segment is disorganized. The anterior chamber depth is markedly decreased. The iris has abnormal shape. The lens capsule is thick and echogenic suggestive of cataract, and is slightly subluxated posteriorly. The globe wall thickness is slightly increased. A conical shape structure with lobulated margin and inhomogeneous echo pattern arising from the posterior lens capsule and extends posteriorly, which adheres to optic disc margin, and to posterior aspect of the globe wall below the optic disc suggestive of PHPV. The patient was found to have bilateral PHPV. At the close relatives, there is no history of PHPV. Other hematologic, hormonal, and biochemical tests were normal.

In this study, the patient with bilateral PHPV was examined for genetic causes of her disease. After genetic counseling and drawing the familial pedigree (Figure 1), informed consent for genetic studies of all participants was obtained. Consequently, we investigated the c.136A>C mutation (p.N46H) of ATOH7 gene as a main reason of the disease.

5ml of peripheral blood were collected from the patient and her parents in tubes containing EDTA. Leukocytes were separated from peripheral blood and DNA extraction was performed in accordance with standard phenol chloroform protocol (8). The fragment of ATOH7 gene which contains the c.136A>C mutation was amplified using PCR via following primers:

F: 5′-GCT CAG AAG AGG CAC GTT G-3′ and R: 5′-GAT TCT AAG GAT GCA ATC CTC G-3′. PCR process was performed in three phases. The first phase includes 94°C for 5 minutes. Afterwards, 30 cycles consists of 94°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute. The final phase comprises 72°C for 5 minutes. To determine the presence or absence of c.136A>C mutation in ATOH7 gene, the PCR product was sequenced through forward and reverse primers and the results shows no mutation in this point (Figure 2).

DISCUSSION

Malformation of the fetal eyes is scarce; they include anophthalmia, microphthalmia, cataract and PHPV. Persistent hyperplastic primary vitreous (PHPV) is a pathogenic entity resulting from abnormal hypertrophy of the fetal fibrovascular primitive stroma (hyaloid system) of the eye.

PHPV usually occurs as a unilateral eye disorder, also been reported in association with other anomalies, and cases with bilateral PHPV are rare (9). The imaging features in this patient point toward the diagnosis of bilateral PHPV.

Some articles suggest ATOH7 mutations as the main reason for autosomal recessive PHPV. The role of ATOH7 in RGC development is definitely specified and its second role is in retinal vascular development. ATOH7 mutations are predicted to cause human blindness, but the clinical spectrum and molecular mechanisms remain to be defined (10).

Studies recently carried out on ATOH7 mention p.N46H mutation as a causative factor in the arPHPV for several reasons, including the N46H mutation removes a highly conserved amino acid residue in the basic helix-loop-helix (bHLH) domain that directly contacts DNA, the mutant ATOH7 protein had no detectable activity in three independent assays and allelic ATOH7 variants have been identified in three families with similar retinovascular pathology (10).

Despite the p.N46H mutation in ATOH7 gene that was reported in six-generation Pakistani family (10), the molecular analysis data in this patient shows no mutation in this locus. It seems other mutations maybe are involved in pathogenesis of PHPV in Iranian patients.

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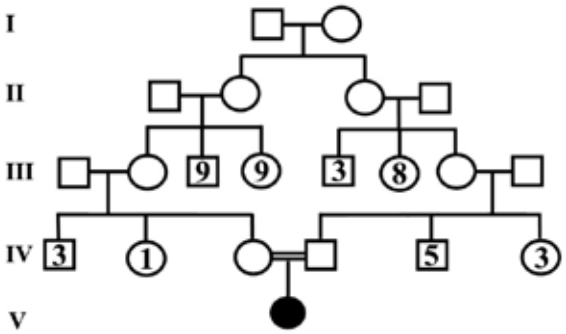

Figure 1) Pedigree of the patient's family

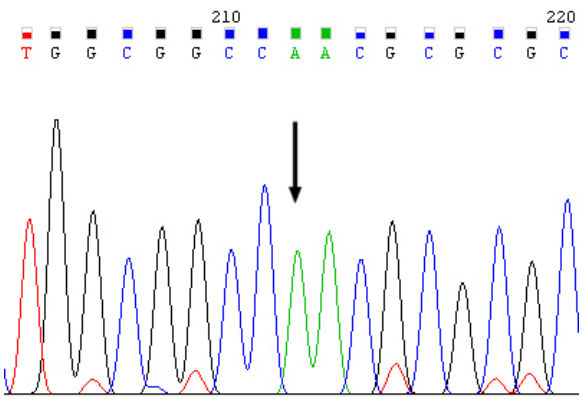

Figure 2) Sequence chromatogram from arPHPV family member

REFERENCE 1. Shastry BS. Persistent hyperplastic primary vitreous: congenital malformation of the eye. Clin Experiment Ophthalmol. 2009 Dec;37(9):884-T90. | 2. Huang TQ, Wang Y, Ebrahem Q, Chen Y, Cheng C, Doughman YQ, Watanabe M, Dunwoodie SL, Yang YC. Deletion of HIF-1α partially rescues the abnormal hyaloid vascular system in Cited2 conditional knockout mouse eyes. Mol Vis. 2012; 18: 1260–1270. | 3. Kim JH, Kim JH, Yu YS, Mun JY, Kim KW. Autophagy-induced regression of hyaloid vessels in early ocular development. Autophagy. 2010 Oct;6(7):922-8. | 4. Shastry BS. Persistent hyperplastic primary vitreous: congenital malformation of the eye. Clin Experiment Ophthalmol. 2009 Dec;37(9):884-90. | 5. Jain TP. Bilateral persistent hyperplastic primary vitreous. Indian J Ophthalmol. 2009 Jan-Feb;57(1):53-4. | 6. Khaliq S, Hameed A, Ismail M, Anwar K, Leroy B, Payne AM, Bhattacharya SS, Mehdi SQ. Locus for autosomal recessive nonsyndromic persistent hyperplastic primary vitreous. Invest Ophthalmol Vis Sci. 2001 Sep;42(10):2225-8. | 7. Su PH, Lee IC, Yang SF, Ng YY, Liu CS, Chen JY. Nine genes that may contribute to partial trisomy (6)(p22→pter) and unique presentation of persistent hyperplastic primary vitreous with retinal detachment. Am J Med Genet A.2012 Apr;158A(4):707-12. | 8. Sambrook J, Russell DW. Molecular Cloning, 2001. 3th ed. New York: Cold Spring Harbor Laboratory Press, 2001:6.1-6.10. | 9. Khaliq S, Hameed A, Ismail M, Anwar K, Leroy B, Payne AM, Bhattacharya SS, Mehdi SQ. Locus for autosomal recessive nonsyndromic persistent hyperplastic primary vitreous. Invest Ophthalmol Vis Sci. 2001 Sep;42(10):2225-8. | 10. Prasov L, Masud T, Khaliq S, Mehdi SQ, Abid A, Oliver ER, Silva ED, Lewanda A, Brodsky MC, Borchert M, Kelberman D, Sowden JC, Dattani MT, Glaser T. ATOH7 mutations cause autosomal recessive persistent hyperplasia of the primary vitreous. Hum Mol Genet. 2012 Aug 15;21(16):3681-94. |