Experimental Eye Research 110 (2013) 44–49

Contents lists available at SciVerse ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Transthyretin Ala36Pro mutation in a Chinese pedigree of familial transthyretin amyloidosis with elevated vitreous and serum vascular endothelial growth factor

Xuan Zou [a], Fangtian Dong [a], Shuying Zhang [b], Rong Tian [a], Ruifang Sui [a,*]

[a] Department of Ophthalmology, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, No. 1 Shuaifuyuan Road, Dongcheng District, Beijing 100710, China
[b] Department of Pathology, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, No. 1 Shuaifuyuan Road, Dongcheng District, Beijing 100710, China

ARTICLE INFO

Article history:
Received 11 July 2012
Accepted in revised form 4 February 2013
Available online 21 February 2013

Keywords:
familial transthyretin amyloidosis
transthyretin
vitreous opacities
retinal deposits
vascular endothelial growth factor

ABSTRACT

The familial transthyretin (TTR) amyloidosis (FTA) demonstrates variable penetrance of clinical features associated with mutations in the plasma thyroid hormone-binding protein TTR gene. The purpose of this study was to assess the ocular features, to analyze vitreous and serum vascular endothelial growth factor (VEGF) levels, and to identify the genetic defect in a Chinese family with TTR FTA. The pedigree of interest was a three-generation family with eleven members. The primary ocular signs were vitreous opacities, beginning from the third or fourth decade, accompanied by retinal vasculitis, hemorrhages, and widespread pinpoint deposits in the peripheral retina. Two patients underwent vitrectomy with marked improvement of visual acuity postoperatively. Vitreous and serum samples for VEGF were analyzed with an enzyme-linked immunosorbent assay (ELISA). Forty-eight healthy adult volunteers were enrolled as a control group for the analysis of serum VEGF. Eight subjects who underwent vitrectomy for a macular epiretinal membrane or macular hole were enrolled as control for the analysis of vitreous VEGF. Both serum and vitreous VEGF levels of patients were raised compared to that of controls. Venous blood was collected from family members and the genomic DNA was extracted. All exons and exon–intron boundaries of the TTR gene were sequenced. A previously-described pathogenic transversion in exon 2 (c.G106C, p.Ala36Pro) was identified. Within this family eight individuals were confirmed as affected. In conclusion, a Chinese family with TTR Ala36Pro associated FTA is characterized by early ocular involvement. Widespread pinpoint lesions indicate RPE lesions caused by TTR deposition. FTA is associated with increased VEGF levels, both in serum and vitreous.

1. Introduction

Familial transthyretin (TTR) amyloidosis (FTA) is characterized by slowly progressing peripheral sensorimotor neuropathy and autonomic neuropathy, as well as non-neuropathic changes of cardiomyopathy, nephropathy, vitreous opacities, and central nervous system (CNS) amyloidosis. FTA is inherited in an autosomal dominant mode with mutations affecting the TTR, which is a tetrameric serum protein comprising four identical subunits of 14 kDa. It acts as a transport protein for thyroxine and retinol, and is a cryptic protease for apolipoprotein AI (Liz et al., 2004).

More than 100 TTR mutations related to FTA have been reported (www.hgmd.org). Another feature of FTA is phenotype heterogeneity involving various tissues. Ocular involvement was documented in FTA patients with different TTR mutations, including the most common genotypes Val30Met, Ile84Ser, Glu54Gly, Trp41Leu, and Tyr114Cys. Ala36Pro associated FTA is relatively rare, and the clinical features, especially the ocular manifestations, have been seldom reported. Vitreous opacity is sometimes the first or the only manifestation of the disease. Vitreous hemorrhage, retinal neovascularization as well as amyloid deposition within the walls of retinal vessels were described previously in FTA (Kawaji et al., 2005). It has been hypothesized that retinal ischemia and the potential weakening of vessel walls caused by the deposition of the amyloid were etiological factors behind the observed retinal vascular abnormalities. To investigate this further, we reported a Chinese pedigree with bilateral vitreous opacities and retinal abnormalities caused by TTR Ala36Pro mutation, and analyzed serum and vitreous samples for the vascular endothelial growth factor (VEGF).

* Corresponding author. Tel.: +86 10 69156354; fax: +86 10 69156360.
E-mail address: hrfsui@163.com (R. Sui).

0014-4835/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.exer.2013.02.005

2. Materials and methods

The study was conducted in accordance with the tenets of the Declaration of Helsinki and had the approval of the Institutional Review Board, Peking Union Medical College Hospital. Informed consent was obtained from each of the family members after they were provided with sufficient information about the procedures to be used.

2.1. Clinical data analysis

A three-generation family with FTA was identified through outpatient visits to the Peking Union Medical College Hospital (Fig. 1). Six subjects (III:1, III:2, IV:1, IV:2, IV:3 and IV:4) from the family underwent detailed ophthalmic examination, including visual acuity testing, slit lamp biomicroscopy, tonometry and dilated fundus examination. Ultrasonography and fundus fluorescein angiography (FFA) were also performed for two index patients (III:1 and III:2). Optical coherence tomography (OCT) (Spectralis OCT; Heidelberg, Germany) was applied to show retinal structure changes (III:2 and IV:2). Electroretinogram (ERGs) and electrooculogram (EOG) (RetiPort ERG system; Roland Consult, Wiesbaden, Germany) were performed in patient IV:2 before the vitrectomy. The ERG and EOG protocol complied with the standards published by the International Society for Clinical Electrophysiology of Vision (ISCEV).

2.2. Pathological analysis

Two patients (III:2 and IV:2) had vitrectomy in the right eye. The pathology of vitreous sample was analyzed using the Congo red staining. Enzyme-linked immunosorbent assay (ELISA) analysis for VEGF was undertaken on undiluted vitrectomy specimens and serum of patient III:2 and IV:2 (R&D Systems, Quantikine, Minneapolis, Minnesota, USA). Initially, 100 μL of RDW1 assay solution was put into the microplate, and then 100 μL of a standard solution of serum and vitreous fluid was added to the microplate and incubated for 2 h at room temperature. After three rinses with a special solution, 200 μL of conjugate was added and re-incubated at room temperature, and washed three times; 200 μL of substrate was added and centrifuged at 25 rpm, and then incubated. Finally, 50 μL of stop solution was added and the plates were read at a wavelength of 450–570 nm. 48 healthy adult volunteers were enrolled as a control group for testing the serum VEGF. Eight patients who underwent vitrectomy for macular epiretinal membrane or macular hole were enrolled as control for the test of vitreous VEGF. Three assays were conducted for each subject to test the vitreous VEGF.

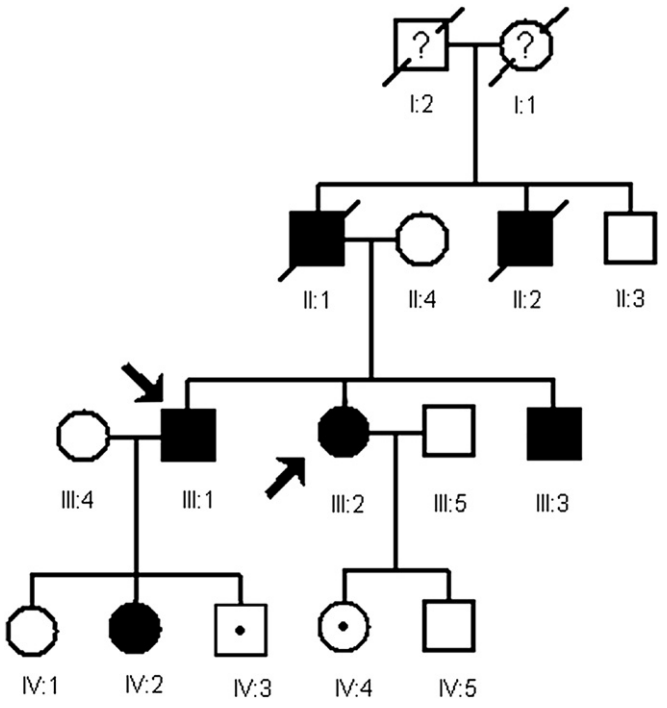

Fig. 1. Pedigree of the family with TTR Ala36Pro mutation. IV:3 and IV:4 were confirmed as asymptomatic carriers by gene analysis. □ Male. ○ Female. ■ Male patient. ● Female patient. ⊡ Male asymptomatic carrier. ⊙ Female asymptomatic carrier. ◪ Death of male patient. ◪ Death of female patient. ⊠ Death of unidentified male. ⊘ Death of unidentified female. ↗ Index patients.

2.3. Mutation analysis

Peripheral blood samples were collected from eleven family members. Genomic DNA was isolated from peripheral leukocytes using QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. All four coding exons of the TTR gene, including intron–exon boundaries, were amplified by polymerase chain reaction (PCR), and using the primers described by Long et al. (2011). After purification, amplicons were sequenced using both forward and reverse primers on ABI 3730 Genetic Analyzer (ABI, Foster City, CA, USA). Sequences were assembled and analyzed with Lasergene SeqMan software (DNASTAR, Madison, WI, USA). The results were compared with a TTR reference sequence (GenBank accession number: NM_000371.3).

3. Results

3.1. Clinical assessment

3.1.1. Case 1

A wheelchair-bound 55-year-old Chinese man (III:1, Fig. 1) was referred to the Peking Union Medical College Hospital's ocular genetics clinic after complaining of decreased vision and floaters for five years. Several months earlier he had been diagnosed as having uveitis. He also had paresthesia and weakness in his hands and legs to the level of the knees, with reflexes absent for seven years. A moderately severe autonomic neuropathy (alternating painless diarrhea and constipation) was present. At later stage the patient developed syncope, which responded to orthostatic hypotension. Cardiac ultrasound revealed mild degenerative membrane changes. One year later, the patient died suddenly without definite cause.

The visual acuity for patient III:1 was 20/1000 in both eyes. The ocular anterior segment and intraocular pressure were normal. Glass, wool-like vitreous opacities were observed in both eyes, which obscured the view to the posterior pole (Fig. 2A). Peripheral dot-blot hemorrhages were also observed (Fig. 2B). FFA showed small tufts of neovascularization, vascular fluorescein leakage and staining, and a possible non-perfusion area (Fig. 2C). Pars plana vitrectomy (PPV) was not considered because of the patient's poor general condition.

3.1.2. Case 2

A 49-year-old Chinese women (III:2), the younger sister of Case 1, had been complaining of decreased vision and floaters in her right eye for one year. Visual acuity was 20/35 in her right eye, and 20/15 in her left eye. The ocular anterior segment and intraocular pressure were normal. Glass, wool-like vitreous opacities were observed in both eyes, with the vitreous opacities more severe in the right eye. The fundus of both eyes illustrated small clumps of