Research Article

Current Trends in Ophthalmology

Ocular Phenotype of Mice with Impaired Fibrillin-1 Function on Hypercholesterolemic Apolipoprotein E-Deficient Background

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Abstract

Aim: Transgenic mice with an elastic fiber mutation (C1039G+/-) in the fibrillin-1 gene and apolipoprotein E deficiency express vulnerable atherosclerotic plaque formation in the aorta and coronary and carotid arteries. The fibrillin-1 gene mutation alone leads to impaired fibrillin-1 function common to Marfan syndrome. The aim was to study for the first time the potential effects of atherosclerosis and vulnerable plaques in mouse eye and spontaneous retinal vessel occlusions in these mice.

Methods: Apolipoprotein E-deficient and fibrillin-1 mutated mice (ApoE^{-/-}/Fbn1^{C1039G+/-}) were used for the study. ApoE^{-/-} littermates served as controls. Optical coherence tomography and fluorescein angiography were used to study the retina and retinal vessels before and after the 12-week high-fat diet. Series of staining were performed to study morphology, apoptosis, glial fibrillary acidic protein activation, collagen formation, inflammatory cell infiltration and drusen formation.

Results: No pathological changes were found in hypercholesterolemic ApoE^{-/-}/Fbn1^{C1039G+/-} mice in imaging studies nor in the histological stainings. There was neither abnormality in the retinal morphology nor any detectable biomarkers.

Conclusion: ApoE^{-/-}/Fbn1^{C1039G+/-} mice did not present the ocular signs of Marfan syndrome. 12-week high-fat diet is not sufficient to induce retinal vessel occlusion on 20 to 23 weeks old mice. This indicates that the mechanistic background of retinal vessel occlusion is more complex.

Keywords: Apolipoprotein E, Atherosclerosis, Eye, Fibrillin-1, Marfan syndrome, Mouse model, Retinal vessel occlusion

Abbreviations: AMD: Age-related Macular Degeneration; ApoE: Apolipoprotein E; Fbn1: Fibrillin-1; FA: Fluorescein Angiography; GFAP: Glial Fibrillary Acidic Protein; SD-OCT: Spectral Domain Optical Coherence Tomography; VEGF-A: Vascular Endothelial Growth Factor A

Introduction

Retinal vessel occlusions are vascular diseases in

which retinal arterial or venous circulation is blocked causing severe vision decline [1]. Retinal vein occlusion is the second most common retinal vascular disease after

diabetic retinopathy with the prevalence of 0.5-2.0% [2] with estimated 16 million people affected worldwide [3]. Occlusion in the retinal artery is much less common, with the estimated incidence of one person per 100 000 people [4]. Retinal vessel occlusions, as many other ocular vascular diseases, are strongly associated with increasing age [2],[5], predicting the number of patients rising in the future.

Currently there are few animal models for retinal vessel occlusions, despite the frequency of these vision-threatening diseases [6]. No animal models to date present spontaneous retinal artery or vein occlusions. In rodents, retinal vessel occlusions are the most commonly induced by using traumatizing laser photocoagulation with a photosensitizer [7-9]. The model is not optimal as the laser destructs the outer retinal layer and photoreceptors [10]. Limitations in current models hinder the elucidation of the pathogenic mechanisms of retinal vessel occlusions and the development of novel treatments.

To address the issue, we studied the ophthalmic status of mice with elastic fiber mutation (C1039G+/-) in the fibrillin-1 gene on hypercholesterolemic apolipoprotein E-deficient (ApoE^{-/-}) background. ApoE deficiency leads to impaired clearing of plasma lipoproteins, increased cholesterol levels and development of atherosclerosis in mice [11]. Together with fibrillin-1 mutation it leads to the formation of unstable atherosclerotic plaques on high-fat diet [12-16]. As both retinal artery and vein occlusions are associated with cardiovascular disease and atherosclerosis [1], we hypothesized that ApoE^{-/-}/Fbn1^{C1039G+/-} genotype affects the retinal vessels.

Besides retinal vessel occlusion, we also studied if the mice possess ocular features of Marfan syndrome. Heterozygous fibrillin-1 missense C1039G mutation in mice leads to impaired microfibrillar deposition common to Marfan syndrome [17]. Ocular features of Marfan syndrome include complications such as ectopia lentis, myopia and retinal detachment [18]. The effect of Fbn1^{C1039G+/-} on eyes has not been studied before, although the role of genetic defect in fibrillin-1 in Marfan syndrome is well established [19].

We report here the effect of fibrillin-1 C1039G mutation on Marfan syndrome related ocular features. This study also describes the first time the co-effect of fibrillin-1 mutation and ApoE deficiency to retinal vessels and ocular morphology.

Materials and Methods

Female and male ApoE^{-/-}/Fbn1^{C1039G+/-} mice [12],[15] (n=7) were fed a high-fat Western-type diet (TD88137, Harlan Teklad) starting at an age of 8 to 11 weeks. ApoE^{-/-} littermates without Fbn1^{C1039G+/-} -mutation served as controls (n=7). The genotype was determined by polymerase chain reaction. The mice were housed in a controlled environment with 12-h light/dark cycle and had access to food and water ad libitum. All animal procedures were approved by the Animal Experiment Board in Finland and carried out according to the guidelines of the Experimental Animal Committee of the University of Eastern Finland and in accordance with the European Communities Council Directive 2010/63/EU.

Spectral domain optical coherence tomography (SD-OCT) and fluorescein angiography (FA) were carried out using 30° lens and wide-angle 55° lens, respectively, at the beginning of the study and after 12-week high-fat diet. Animals were anesthetized with a mixture of ketamine (75 mg/kg; Ketaminol vet 50mg/ml) and medetomidine (1 mg/kg; Domitor vet 1 mg/ml) and reversed with atipamezole (1 mg/kg; Antisedan vet 5mg/ml). Pupillary dilation was achieved with 1% tropicamide eye drops (Oftan Tropicamide 5 mg/ml) and carbomer eye gel (Viscotears 2 mg/ml) was used as a lubricant. For fluorescein angiography 0.2 ml of 2% fluorescein (Fluorescite 100 mg/ml) was injected intraperitoneally. After 12 weeks on high-fat diet, cholesterol levels were 28.44 ± 17.48 mmol/l and 27.59 ± 3.06 mmol/l for ApoE^{-/-}/Fbn1^{C1039G+/-} and ApoE^{-/-}, respectively. Mice were sacrificed using CO₂ asphyxiation after 12 weeks.

After sacrifice, the mice's eyes were enucleated, fixed and embedded in paraffin and cut to 4-μm thick sections. Hematoxylin/eosin, Picro Sirius Red (ab150681; Abcam) and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TACS 2 TdT-Fluor *in situ* apoptosis detection kit; Trevigen) staining were performed. For immunostainings, the following primary antibodies were used: α-SMA-Cy3 (C6198; Sigma), CD34 (HM1015; Hycult Biotech), glial fibrillary acidic protein (GFAP) (Z0334; Dako), F4/80 (MCA497R; Bio-Rad) and vascular endothelial growth factor A (VEGF-A) (ab52917; Abcam). When needed, Alexa Fluorescent dyes 488 and 594 were used as secondary antibodies (ThermoFischer Scientific). Fluorescence imaging using wavelength 488 nm was used to study drusen-associated autofluorescence. Images were taken with Nikon Eclipse Ni electron microscope and Nikon DS-Ri2 and DS-Qi2 cameras.

Results and Discussion

No hyper- or hypofluorescent areas were seen in FA imaging in any of the mouse retinas (Figure 1). There were no changes between the groups at the beginning or end of the study in the mean retinal thickness compared with baseline values measured from SD-OCT images. Nor did the SD-OCT images show any retinal changes before and after the 12-week high-fat diet (Figure 1).

Figure 1: FA and SD-OCT images of ApoE^{-/-}/Fbn1^{C1039G+/-} and ApoE^{-/-} mice. ApoE^{-/-}/Fbn1^{C1039G+/-} mice had normal retinal structure in SD-OCT and vasculature in FA after 12-week high-fat diet.

Figure 2: Hematoxylin/eosin staining did not reveal abnormalities in the retina nor in the cornea of ApoE^{-/-}/Fbn1^{C1039G+/-} (A-C) and ApoE^{-/-} (D-F) mice. There were no differences in the corneal thickness (G) or in the ocular axial length (H) after 12-week high-fat diet. Scale bar is 100 μm.

Figure 3: Immunohistochemical and Sirius Red stainings of ApoE^{-/-}/Fbn1^{C1039G+/-} (A, C) and ApoE^{-/-} (B, D) mice. An ApoE^{-/-} mouse showed collagen formation in the anterior chamber (B) and vitreous (D). Both groups had normal retinal endothelium in superficial capillary plexus (arrow), deep capillary plexus (arrowhead) and choroid (*) in α-SMA (E, red) and CD34 stainings (F, green). ApoE^{-/-}/Fbn1^{C1039G+/-} and ApoE^{-/-} mice had a normal number of Brn3a-positive retinal ganglion cells (G, red). F4/80-positive macrophages (red) were mostly seen in the anterior chamber in the ciliary body (arrow) and in the iris (arrowhead) but not in the retina nor in the sclera (H). In total five mice from both groups showed increased GFAP-activation (red) in Müller cells (arrowhead) in addition to normal expression in astrocytes (arrow) (I). VEGF expression (green) was seen in the ganglion cell layer (arrow) and in inner segment of photoreceptors and outer nuclear layer (arrowhead) in both groups (J).

Hematoxylin/eosin staining and imaging of the eyes were used to study the retinal morphology and anatomy of the eyes. We did not detect any classical signs of fibrillin-1 impairment in the eye, such as lens dislocation, retinal detachment, thinning of the cornea or increased axial length. Hematoxylin/eosin staining of ApoE^{-/-}/Fbn^{1C1039G+/-} and ApoE^{-/-} mice did not show abnormalities in the morphology or in the measured features (Figure 2).

Immunohistochemical staining did not show anomalous morphology of the vasculature or ganglion cells nor abnormal VEGF expression (Figure 3). 1 out of 7 ApoE^{-/-} mice had positive collagen I/III staining to a lesser extent in the vitreous body and more intensively in the anterior chamber (Figure 3). Normal GFAP expression was seen in astrocytes in nerve fiber layer of all mice. Additional GFAP immunoreactivity in Müller cells in the outer retina was found in total of five mice from both groups. Apoptosis, F4/80-positive macrophages or autofluorescent drusens were not detected in the retina or in the choroid.

In conclusion, despite the success of the model to mimic vulnerable plaques in the aorta and coronary arteries, we show that the model does not have an ocular phenotype of Marfan syndrome nor retinal vessel occlusion in young mice even after high-fat diet.

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