NT5E mutation in sisters who underwent aortic valve replacements for aortic stenosis

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Abstract

Background and Aims: Mutations of the NT5E gene encoding the cluster of differentiation 73 (CD73) protein have been found in patients with characteristic calcification of joints and arteries (CALJA). CD73 plays a protective role against aortic valve calcification and its deletion results in aortic valve calcification. However, there have been no reports of a patient with CALJA with aortic stenosis. Methods: We describe two extremely rare cases of two sisters with identical NT5E gene mutation patterns, both of whom presented severe aortic stenosis and limb ischemia. Genetic examination for definitive diagnosis was also performed in both patients. Results: Both patients underwent aortic valve replacement and bilateral distal arterial bypass surgeries successfully. They were genetically diagnosed with CALJA based on the NT5E mutation. Conclusions: NT5E mutation should be considered in patients requiring aortic valve replacement for a calcified aortic valve and bypass surgery for specific calcified and occluded arteries.

Introduction

In 2011, mutation of the NT5E gene, that encodes cluster of differentiation (CD) 73, was found to result in specific calcifications in patients with aneurysmal dilatation of arteries as well as joint calcifications. These symptoms were termed calcification of joints and arteries (CALJA).[1] Since then, only 14 patients from 7 families have been reported as having CALJA.[2-5]Patients with CALJA often experience ischemic symptoms in the lower extremities secondary to severely calcified and occluded arteries. CD73 is also known to play a protective role against aortic valve calcification by hydrolyzation of adenosine monophosphate (AMP) to adenosine.[6] Although the deletion of the CD73 gene has been reported to cause aortic valve calcification leading to valvular stenosis in animal models,[7] there are no reports of patients with CALJA requiring an aortic valve replacement (AVR) for aortic stenosis (AS). We present two extremely rare cases of sisters with identical NT5E gene mutation patterns, both of whom underwent AVR for AS.

Materials and Methods

Both sisters underwent AVR for AS and distal bypass surgeries for chronic limb-threatening ischemia. As they presented with the typical features of CALJA, both patients underwent a genetic examination for definitive diagnosis of CALJA.

Genomic DNA was extracted from whole blood cells using a QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). We performed whole exome sequencing analysis as previously described.[8] In brief, we

used the SureSelect Human All Exon V6 kit (Agilent Technology, Santa Clara, CA) for capture and a HiSeq2500 (Illumina, San Diego, CA) for sequencing. Reads were aligned to GRC37 using Burrows-Wheeler Aligner (http://bio-bwa.sourceforge.net/). Variants were identified using the GATK Unified Genotyper and ANNOVA (http:// annover.openbioinfomatics.org/en/latest/).

This genetic analysis of the patients was approved by the Ethics Committee of Yamagata University. Both patients and their family members provided informed consent for clinical and genetic examinations and for the publication of this report.

Results

Patient 1 : An 81-year-old woman presented with a 50-year history of joint pain in her legs. She underwent bilateral distal bypass surgeries at 72 years of age for intermittent claudication. Plain radiography of the lower extremities revealed significant calcification with areas of arteriomegaly. Three-dimensional computed tomography revealed extensive occlusion of the bilateral femoropopliteal arteries with aneurysmal changes and severe calcifications, which are typical findings of CALJA (Fig. 1A). The patient's saphenous vein grafts were patent when she presented to us. During her recovery from the vascular reconstructive surgeries, the patient gradually developed exertional fatigue. Echocardiography revealed severe aortic valve stenosis with a peak pressure gradient of 50 mmHg. AVR was performed when the patient was 77 years old. The native aortic valve was tricuspid and the valvular leaflets and annulus showed significant calcification (Fig. 1B). However, no calcification was observed in the aortic wall. She underwent AVR with a Carpentier-Edwards PERIMOUNT bioprosthetic valve (Edwards Lifesciences, Irvine, CA) (Fig. 1C). Her postoperative course was uneventful.

Patient 2 : A 74-year-old woman, who is the younger sister of Patient 1, reported a 10-year history of bilateral intermittent claudication of the calves and chronic coldness of her lower extremities. At 71 years of age, she had developed dyspnea on exertion and was diagnosed with severe aortic valve stenosis with a peak pressure gradient of 90 mmHg. Her native aortic valve was tricuspid with severe calcification of the cusps and annulus (Fig. 2A). There were no calcified lesions in the aortic wall. She underwent an AVR with a Solo Smart stentless bioprosthesis (LivaNova PLC, London, UK) (Fig. 2B). She recovered from the operation well; however, the intermittent claudication in her calves gradually worsened. Three-dimensional computed tomography revealed occluded aneurysmal femoropopliteal arteries with severe calcification (Fig. 2C), which are typical of CALJA and were similar to the calcifications that had been found in her sister's lower extremities. She underwent bilateral distal bypass surgeries for claudication at the age of 73 years.

Currently, both patients are doing well after several cardiac and vascular operations.

Gene analysis

After filtering the variants between the patients and a non-patient control (the 76-year-old asymptomatic brother of the patients), we identified a homozygous variant of the NT5E (5'-ectonucleotidase) gene, NM_002526: c.3G>C, (p.Met1?), in the patients (Table 1). The homozygous variant was not found in the Japanese genome variant database (HGVD; http://www.hgvd.genome.med.kyoto-u.ac.jp) or the in-house whole exome sequencing data. The variant was confirmed by Sanger sequencing. Both patients were genetically diagnosed with CALJA based on the NT5E mutation.

Conclusions

CALJA is an extremely rare genetic disorder of isolated calcification that is characterized by late-onset calcification of the arteries and joints of the extremities. Mutation of the NT5E gene that encodes CD73 have been implicated in CALJA.[1] CD73 is a membrane-bound ecto-5'-nucleotidase catalyzing the conversion of AMP to adenosine.[1] The enzymic activity of CD73 plays an important role in the inhibition of inflammation, atherosclerosis, and ectopic tissue calcification. In patients with this genetic disorder, ischemic symptoms (including intermittent claudication and chronic limb-threatening ischemia) are common due to heavily calcified stenotic or occluded arteries.

Inflammation plays a key role in the pathogenesis of AS regardless of the presence of anatomical abnormalities. The deletion of the CD73 gene in mice resulted in almost complete loss of the pathway that degrades AMP to adenosine at a vascular surface, leading to the development of aortic valve dysfunction.[7] The NT5E gene mutation may also contribute to the pathogenesis of calcific aortic valve disease and is associated with an increased risk of AS.

We described the cases of two Japanese sisters aged 81 and 74 years, both of whom underwent AVR for AS and were diagnosed with CALJA and NT5E gene mutation. These are the first two reported cases of patients with the NT5E mutation who underwent surgical AVR for AS. The severity of AS in these patients was similar to that of genetically normal patients with AS despite the high-risk nature of aortic valve calcification.

Because the symptoms of the NT5E mutation have a late onset, other factors may also contribute to the severity and timing of CALJA. There may be undiagnosed patients with the NT5E mutation and CALJA, and the incidence of homozygous variants of NT5E may be underestimated. Genetic experts, vascular specialists, and cardiovascular surgeons should consider the possibility of NT5E mutations in patients who require AVR for AS and distal arterial bypass surgery for limb ischemia.

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Author contributions:

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Figure legends

Figure 1: CALJA characteristics in Patient 1 a A three-dimensional computed tomographic image shows occlusion of the bilateral femoropopliteal arteries with aneurysmal changes and severe calcification. The bilateral saphenous vein grafts are patent.**b** The patient's aortic valve has severe calcification of the leaflets and the annulus. **c** The patient underwent AVR with a bioprosthesis.

AVR: aortic valve replacement; CALJA: calcification of joints and arteries.

Figure 2: CALJA characteristics in Patient 2 a The patient's aortic valve has severe calcification. **b** The patient underwent AVR with a stentless bioprosthesis. **c** A three-dimensional computed tomographic image shows occlusion of the aneurysmal femoropopliteal arteries with dense calcifications.

AVR: aortic valve replacement; CALJA: calcification of joints and arteries.

Table 1: Clinical characteristics and results of the genetic analyses of both patients

Table 1

	Age/Sex	Aortic valve disease	Symptom of lower limb	Mutation of the NT5E gene		
				Gene site	Variant (zygosity)	Amino acid
Patient 1	81/F	Aortic valve stenosis	Intermittent claudication	exon 1	c.3G>C (homo)	p.M1I
Patient 2	74/F	Aortic valve stenosis	Intermittent claudication	exon 1	c.3G>C (homo)	p.M1I

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