Case Report

Only Characteristics Vascular Lesions of Williams-Beuren Syndrome in a Girl with a Novel Nonsense ELN Mutation

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Williams-Beuren syndrome (WBS) is caused by microdeletions of 7q11.23, including the ELN gene, and the characteristic vascular lesions include supravalvular aortic stenosis (SVAS) and peripheral pulmonary artery stenosis (PPS). We identified a patient who has the characteristic cardiovascular lesions of WBS but does not have the 7q11.23 deletion: analysis of ELN identified a nonsense mutation. This case is a baby girl, whose cardiovascular abnormalities were not identified during fetal life. She was admitted with systemic cyanosis after birth, and was diagnosed with SVAS and supravalvular pulmonary stenosis (SVPS). At 10 months of age she underwent cardiac catheterization and confirmed SVAS, which was accompanied by a thin ascending aorta, PPS narrowing from hilar, and SVPS. Due to high blood pressure and hyperreninemia, she was diagnosed with renal vascular hypertension caused by bilateral renal artery stenosis identified on MDCT and began an oral carvedilol regimen. Mental retardation and malformations associated with WBS were not observed and a FISH analysis excluded a microdeletion encompassing ELN. Since mutations in ELN have been identified in cases of isolated SVAS it was screened and a novel nonsense mutation in Exon 24 was identified. Thus, the cardiovascular lesions were considered to be caused by elastin deficiency due to this ELN mutation. We suggest that if a patient has the characteristic cardiovascular lesions of WBS, but is FISH negative, then ELN should be screened for mutations.

Keywords: Williams-Beuren syndrome, ELN gene, supravalvular aortic stenosis, peripheral pulmonary artery stenosis, supravalvular pulmonary stenosis

Introduction

Williams-Beuren syndrome (WBS) is contiguous gene syndrome caused by microdeletions of 7q11.23 and features cardiovascular lesions (supravalvular aortic stenosis [SVAS] and peripheral pulmonary artery stenosis [PPS]), distinctive facies (thick medial eyebrow, blepharophimosis, epicanthus, puffy eye, short and turned-up nose, long philtrum, opened thick lips) and specific cognitive profiles (specialty of the expressed languages, recognition of faces and music and poor visuospatial perception). The frequency of WBS has been estimated to be 1: 7500–20000, no gender differences, and the majority of them is sporadic. The prognosis of WBS without cardiovascular lesions is relatively favorable. The chromosome 7q11.23 includes several genes, ELN and GTF2I (Fig. 1), and ELN is responsible for expressions of the elastin which is the main component of elastic fibers.

Received: November 6, 2018; Accepted: January 4, 2019
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doi: 10.24509/jpccs.190110

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The elastin is comprised of systemic connective tissue, aorta, lungs, skins and ligaments, and the abnormal expressions of elastin according to \textit{ELN} mutation induce various symptoms, example for vascular wall thickening. The past reports of \textit{ELN} failure are accompanied with WBS and SV AS (familial and sporadic).

Here, we present the case of a girl with a nonsense mutation in \textit{ELN} that has never been reported before, while she has only characteristics cardiovascular lesions of WBS and fluorescence in situ hybridization (FISH) negative.

**Case**

A baby girl was not pointed out fetal abnormality and had nonconsanguineous healthy parents, two sisters and one brother. She was born at 40 weeks of gestation by vaginal delivery at previous hospital. After birth, she was systemic cyanosis and crying weakly, so needed manual ventilation (Apgar score: 6–8). She was pointed out systolic murmur and transferred our hospital.

Her clinical findings was as follows; body temperature was 37.1°C, pulse rate was 104 beats per minute, percutaneous saturation was 99–100% (oxygen flow 1 L per minute), blood pressure was 92/50 mmHg, height was 48 cm (−0.91 SD), body weight was 3.374 g (+0.61 SD) and head circumference was 33 cm (−0.39 SD). Her large fontanel was 2 cm and flat. She did not have cyanosis and distinctive facies. Her respiratory sounds were good, and systolic murmur (Levine III/VI) at three left sternal bone were noticed. Her abdomen was soft and flat, and good bowel sounds without hepatosplenomegaly, tumor and abdominal vascular sounds. An edema, joint contracture and skin lesion was not noticed. Laboratory routine investigations in blood were normal. A cardiothoracic rate was 0.66 without pulmonary lesions by X-ray investigation. ECG showed the right axis deviation and R-wave progression in V3 induction. 2D echo showed a right ventricular hyperplasia, a tricuspid regurgitation and a pressure gradient between right atrium and ventricle. A diameter of supravalvular pulmonary artery was 3.2 mm with a pressure gradient between the stenosis. A bifurcation of pulmonary trunk stenosis made prominent mosaic blood flow in peripheral pulmonary arteries. An Aortic valve was tricuspid without regurgitation. A stenosis of sinotubular junction, 3.9 mm (−2.27 SD), 2.7 meter per second and estimated 30 mmHg pressure gradient, made a mosaic blood flow after the stenosis. In conclusion, we diagnosed her SV AS, PPS and supravalvular pulmonary stenosis.

Her state was stable without particular interventions until echocardiography showed the signs of right heart failure at six days after birth, and we began diuretics (furosemide [1 mg/kg/day] and aldactone [1 mg/kg/day]). She was discharged at twenty days after birth since SVAS and PPS did not proceed. She was a normal psychomotor development, smiling at two months old, head controlling at four months old, sitting at seven months old and crawling at nine months old. The distinctive facies and the undeveloped recognition were not seen in follow-up periods. At ten months old, she was hospitalized and received cardiac catheterization for consideration of treatment policy. Cardiac cath-

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**Fig. 1** Schematic diagram at 7q11.23 region

\textit{ELN} is encoded at chromosome 7q11.23 region, and the \textit{LIMK1} and \textit{GTF2I} are contiguous.
eterization revealed that the pressure gradient was right atrium-right ventricle, bilateral peripheral pulmonary arteries-main pulmonary artery and left ventricle-asceding aorta. Estimated pressure gradient between left ventricle and ascending aorta was 40 mmHg and cardiac contractility was sufficient (Table 1), so we decided to manage her without surgical interventions. For hypertension, we performed Multi Detector-row Computed Tomography and discovered bilateral renal artery stenosis (Fig. 2). She had high renin/angiotensin ratio (plasma renin activity was 59.5 ng/mL/hr [reference range; 0.2-2.7 ng/mL/hr] and plasma aldosterone concentration was 128 ng/dL [reference range; 2-13 ng/dL]), and we diagnosed her renovascular hypertension and started carvedilol.

She is now one and a half years old, and height is 80.6 cm (+0.44 SD), body weight is 9.1 kg (-0.78 SD) and head circumference was 44.8 cm (-1.13 SD). She has good cardiac function without hypertension and is not psychomotor retardation (movement; 94, hand movement; 108, basic habit; 108, interpersonal relation; 108, speech; 94 and language understanding; 94 by using Enjoji Scale of Infant Analytical development).

**Molecular Genetic Study**

As SVAS and PPS were the characteristic cardiovascular lesions of WBS, we tried to detect a deletion at chromosome 7q11.23 by FISH (Elastin probe from Vysis), but no deletion was detected. So, we obtained informed consent from her parents and analyzed ELN which was responsible for cardiovascular lesions of WBS. We got approval for gene analysis from Toyama University Ethics Committee. We amplified the Exon coding region and the contiguous region including the introns for ELN by using the primer which was obtained from her peripheral blood lymphocyte genome DNA (we are ready to disclose the primer information when you need). We purified the amplified products by QIAquick polymerase chain reaction (PCR) purification method (QIAGEN, Hilden, Germany) and analyzed mutations by direct-sequencing using ABI 3130xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). As a result, we identified the nonsense mutation in heterozygote of ELN [c.1615C>T (p.Gln539X)] (Fig. 3). Using direct-sequencing, we also analyzed PTPN11, RAF1, SOS1, Shoc2, PKA and PKC, which were responsible for Noonan syndrome having PS with high probability, but no mutations were detected. We have not analyzed the mutations of her parents and brothers yet.

**Discussion**

WBS is a contiguous gene syndrome according to a partial deletion at chromosome 7q11.23. A cardiovascular lesions of WBS were SVAS, PPS, aortic hypoplasia, coarctation of aorta and mitral valve prolapse,21 SVAS and PPS are especially characteristic for WBS. As sporadic SVAS is rare, estimated to be 5–7:100000, so we need to take WBS into account in case of diagnosing SVAS.23 ELN is identified as a responsible gene for cardiovascular lesions of WBS.

ELN contribute to expression of the elastin. The elastin is a main component of elastic fiber and is widely distributed in artery, especially in a root of aorta, skin and lungs. It is advocated that decreasing of the elastin lead to increasing of the collagen in tunica media of artery and vessel wall becomes thicken and harden.

<table>
<thead>
<tr>
<th>Site</th>
<th>Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV</td>
<td>78/4</td>
</tr>
<tr>
<td>LV</td>
<td>148/10</td>
</tr>
<tr>
<td>aAo</td>
<td>112/48 (76)</td>
</tr>
<tr>
<td>main PA</td>
<td>74/10 (32)</td>
</tr>
<tr>
<td>rt. PA</td>
<td>17/6 (8)</td>
</tr>
<tr>
<td>lt. PA</td>
<td>16/10 (11)</td>
</tr>
<tr>
<td>Wedge mean</td>
<td>R = 8</td>
</tr>
<tr>
<td>L = 8</td>
<td></td>
</tr>
</tbody>
</table>

RV-MPA = 70 mmHg
lt.PA-main PA = 59 mmHg
rt.PA prox-main PA = 40 mmHg
rt.PA prox-RPA dist = 17 mmHg

aAo: ascending aorta, LV: left ventricle, PA: pulmonary artery, RV: right ventricle
基因敲除小鼠中，据报道，同源型小鼠在数天内因动脉梗阻与平滑肌的肥大而死亡，而杂合型小鼠表达弹性蛋白mRNA的一半量。基因ELN的点突变会导致心血管病变。5) 弗兰西基阿斯和塔萨贝希报告了多个家族性SVAS家族突变，因此认为ELN也是心血管病变的基因。6, 7) 心血管病变不仅限于大量突变，包括无义突变和移码突变，也限于错义突变，且表现模式是可变的。5, 7

我们报告了一例无义突变的杂合子，c.1615C>T (p.Gln539X)，从未被报道过。在ELN突变中，许多病例与无义突变或移码突变相关的病变非常严重，而无义突变或移码突变的病变非常严重。在该例中，除了SVAS、PPS和主动脉窝肺动脉间隔外，还观察到双侧肾脏动脉狭窄，肾脏动脉狭窄在过去的报告中很少见。无义介导的RNA降解也可能考虑无义突变，但在这种情况下，由于存在于ELN基因中具有重要角色的Exon 24无义突变，被认为导致了严重的量或质的变化，导致了WBS和其他广泛的血管病变。

图3  ELN基因的突变

我们报道了具有WBS特征的心血管病变的案例，其基因ELN中的无义突变从未被报道过。在过去的报告中，Exon 16和Exon 24包括了包含弹性蛋白相关的区域。

结论

我们报告了具有WBS特征的心血管病变的案例，其基因ELN中的无义突变从未被报道过。在过去的报告中，Exon 16和Exon 24包括了包含弹性蛋白相关的区域。
characteristic cardiovascular lesions of WBS, we need to consider the case having only ELN mutation with FISH negative and understand for pathological condition of cardiovascular lesion and extracardial lesion.

**Conflicts of Interest**

The authors declare no conflict of interest.

**Authorship Contribution**

S.T. designed and wrote the paper. S.T., H.N., K.I., S.O., K.H. and Y.A. practiced medicine. S.H. transferred the patient to our hospital and provided the information at birth. T.N. performed gene analysis. F.I. was responsible for the paper and gave a critical review on intellectual content.

**References**