A novel FOXC2 mutation in spinal extradural arachnoid cyst

Yoji Ogura1,2, Shunsuke Fujibayashi3, Aritoshi Iida1, Ikuyo Kou1, Masahiro Nakajima1, Eijiro Okada4, Yoshiaki Toyama2, Akio Iwanami2, Ken Ishii2, Masaya Nakamura2, Morio Matsumoto2 and Shiro Ikegawa1

Spinal extradural arachnoid cyst (SEDAC) is a cyst in the spinal canal, which causes spinal cord compression and subsequent neurological damage. We previously identified two FOXC2 mutations in two SEDAC families. The FOXC2 mutations have been shown to be responsible for lymphedema-distichiasis syndrome (LDS), which includes SEDAC as an occasionally associated phenotype. We encountered a non-familial patient with SEDAC associated with LDS, and identified a novel nonsense mutation in FOXC2, c.349C>T (p.Q117*).

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Spinal extradural arachnoid cyst (SEDAC) is a relatively rare spinal disease, representing 1% of all primary spinal tumors.1 Arachnoid mater protrudes from a small defect in the dura mater, forming a cyst in the epidural space (Figure 1); the cyst expands due to retention of cerebrospinal fluid in response to changes in spinal pressure, leading to compression of the spinal cord and subsequent neurological damage.2 SEDAC occurs predominantly in the posterior thoracic or lumbar area of the spine.3 The onset is generally after middle age and a SEDAC patient is sometimes asymptomatic since the cyst expands slowly.

Most cases of SEDAC are sporadic; however, a few cases of familial SEDAC have been described.4–7 Their studies showed that familial SEDAC was a part of phenotypes of lymphedema-distichiasis syndrome (LDS; OMIM 153400). LDS is an autosomal dominant disorder with variable expressivity. Its major phenotypes are lymphedema and distichiasis. Its minor phenotypes include ptosis, cleft palate, renal abnormalities, congenital heart disease, vertebral anomalies and SEDAC.6,8–11 LDS was caused by FOXC2 (forkhead box C2) mutations.6,12–16 We previously investigated two pedigrees with familial SEDAC and revealed that both families are related to LDS with variable expression of each phenotype. Subsequently, we identified novel FOXC2 mutations in each pedigree, giving solid evidence in the relationship between familial SEDAC and FOXC2.

In the present study, we encountered a 48-year-old female with SEDAC. No family history was recognized. However, we considered the possibility of SEDAC associated with LDS (syndromic SEDAC), since she had lymphedema in the lower extremities. We investigated the FOXC2 coding sequence of the subject and found a novel nonsense FOXC2 mutation.

Mutation analysis using Sanger sequencing was performed for the coding sequence of FOXC2. We identified a novel heterozygous nonsense mutation, c.349C>T (p.Q117*; Figure 2a). The mutation locates in the forkhead domain and the affected amino acid is highly conserved among species (Figure 2b). This alteration has not been reported and is not found in any databases, including UCSC genome browser, Human Gene Mutation Database and Human Genetic Variation Browser (WES database of 1,208 Japanese people).

Figure 1. Spinal extradural arachnoid cyst. T2-weighted image of magnetic resonance imaging (MRI) scan. There are multiple cysts (asterisk marks) dorsal to the spinal cord at the thoracic spine.

FOXC2 is one-exon gene encoding 501 amino acids (aa) with a forkhead domain locating from aa 71 to 162 (Figure 2c). We performed in silico analysis of c.349C>T using Mutation Taster
and the empty vector (NC). The activity of Q117* FOXC2 was significantly decreased compared with that of WT FOXC2 (Asterisk: P < 0.01 (t-test), thick bar: mean value, error bar: s.e.).

In conclusion, we identified a novel mutation, c.349C>T (p.Q117*), in a subject with SEDAC. The subject was shown to have a loss-of-function mutation. The current mutation would produce a significantly truncated protein, losing half of the forkhead domain (Figure 2c). We performed in vitro experiment for FOXC2 transcriptional activity as previously described. The c.394C>T construct showed a significantly reduced promoter activity compared with the control (Figure 2d), indicating that p.Q117* is a loss-of-function mutation.

In the clinical information, the patient had lymphedema in the lower extremities. She had multiple cysts locating out of thoracolumbar junction of the spine (Figure 1), which is the characteristic of syndromic SEDAC. The location and number of cyst are very informative to distinguish sporadic SEDAC from syndromic SEDAC when a SEDAC patient has no family history.

In conclusion, we identified a novel mutation, c.349C>T (p.Q117*), in a subject with SEDAC. The subject was shown to be syndromic SEDAC although she had no family history. It is important to examine not only family history of SEDAC but also lymphedema, distichiasis, cyst location and number of cyst to distinguish syndromic SEDAC from sporadic SEDAC as a part of lymphedema-distichiasis syndrome.

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REFERENCES
1 Yabuki S, Kikuchi S, Ikegawa S. Spinal extradural arachnoid cysts associated with distichiasis and lymphedema. Am J Med Genet A 2007; 143A: 884–887.
2 Chang IC, Chou MC, Bell WR, Lin ZI. Spinal cord compression caused by extradural arachnoid cysts. Clinical examples and review. Pediatr Neurosurg 2004; 40: 70–74.
3 Cilluffo JM, Gomez MR, Reese DF, Onofrio BM, Miller RH. Idiopathic ('congenital') spinal arachnoid diverticula. Clinical diagnosis and surgical results. Mayo Clin Proc 1981; 56: 93–101.
4 Bergland RM. Congenital intraspinal extradural cyst. Report of three cases in one family. J Neurosurg 1968; 28: 495–499.
5 Chynn KY. Congenital spinal extradural cyst in two siblings. Am J Roentgenol Radium Ther Nucl Med 1967; 101: 204–215.
6 Sanchez-Carpintero R, Dominguez P, Nunez MT, Patino-Garcia A. Spinal extradural arachnoid cysts in lymphoedema-distichiasis syndrome. Genet Med 2010; 12: 532–535.
7 Ogura Y, Yabuki S, Ida A, Kou I, Nakajima M, Kano H et al. FOXC2 mutations in familial and sporadic spinal extradural arachnoid cyst. PLoS ONE 2013; 8: e80548.
8 Brice G, Mansour S, Bell R, Collin JR, Child AH, Brady AF et al. Analysis of the phenotypic abnormalities in lymphoedema-distichiasis syndrome in 74 patients with FOXC2 mutations or linkage to 16q24. J Med Genet 2002; 39: 478–483.
9 Corbett CR, Dale RF, Coiltart DJ, Kinmonth JB. Congenital heart disease in patients with primary lymphedemas. Lymphology 1982; 15: 85–90.
10 Johnson SM, Kincannon JM, Horn TD. Lymphoedema-distichiasis syndrome: report of a case and review. Arch Dermatol 1999; 135: 347–348.
11 Schwartz JF, O’Brien MS, Hoffman JC Jr. Hereditary spinal arachnoid cysts, distichiasis, and lymphedema. Ann Neurol 1980; 7: 340–343.

Figure 2. FOXC2 mutations in spinal extradural arachnoid cyst. (a) Electrophoreogram of the wild type FOXC2 (upper panel) and heterozygous nonsense mutation c.349C > T (Q117*) in the present patient (lower panel). (b) Amino-acid sequences around p.Q117 of the FOXC2 in different species. p.Q117 is highly conserved. (c) Protein structure of FOXC2 and positions of the present (blue letters) and previously identified (black letters) mutations in spinal extradural arachnoid cyst. (d) Dual luciferase assay. Promoter activities of wild type (WT) and Q117* FOXC2 vectors and the empty vector (NC). The activity of Q117* FOXC2 was significantly decreased compared with that of WT FOXC2 (Asterisk: P < 0.01 (t-test), thick bar: mean value, error bar: s.e.).
12 Brooks BP, Dagenais SL, Nelson CC, Glynn MW, Caulder MS, Downs CA et al. Mutation of the FOXC2 gene in familial distichiasis. J AAPOS 2003; 7: 354–357.

13 Erickson RP. Lymphedema-distichiasis and FOXC2 gene mutations. Lymphology 2001; 34: 1.

14 Fang J, Dagenais SL, Erickson RP, Arlt MF, Glynn MW, Gerski JL et al. Mutations in FOXC2 (MFH-1), a forkhead family transcription factor, are responsible for the hereditary lymphedema-distichiasis syndrome. Am J Hum Genet 2000; 67: 1382–1388.

15 Mellor RH, Tate N, Stanton AW, Hubert C, Makinen T, Smith A et al. Mutations in FOXC2 in humans (lymphoedema distichiasis syndrome) cause lymphatic dysfunction on dependency. J Vasc Res 2011; 48: 397–407.

16 Sutkowska E, Gil J, Stembalska A, Hill-Bator A, Szuba A. Novel mutation in the FOXC2 gene in three generations of a family with lymphoedema-distichiasis syndrome. Gene 2012; 498: 96–99.