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DATA REPORT

A novel *FOXC2* mutation in spinal extradural arachnoid cyst

Yoji Ogura[1,2], Shunsuke Fujibayashi[3], Aritoshi Iida[1], Ikuyo Kou[1], Masahiro Nakajima[1], Eijiro Okada[4], Yoshiaki Toyama[2], Akio Iwanami[2], Ken Ishii[2], Masaya Nakamura[2], Morio Matsumoto[2] and Shiro Ikegawa[1]

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).

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

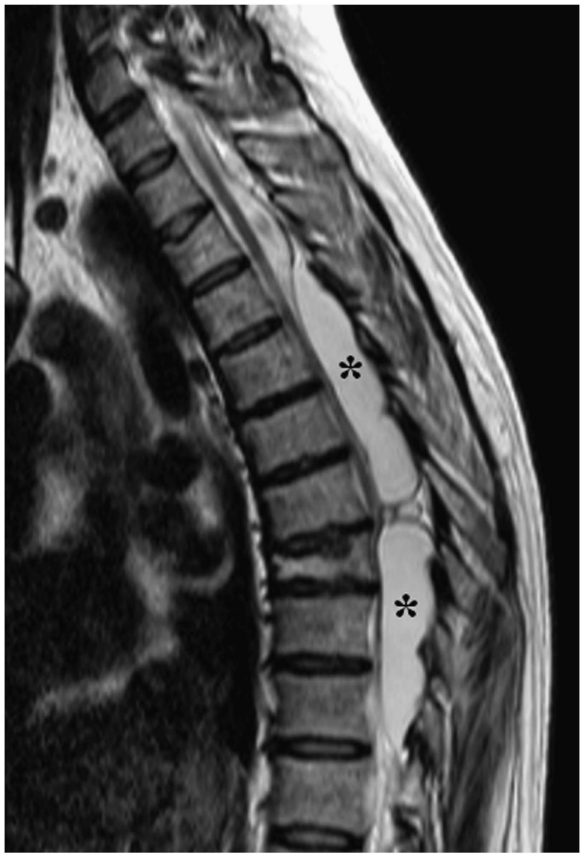

Figure 1. Spinal extradural arachnoid cyst. T2-weighted image of magnetic resonance imaging (MRI) scan. There are multiple cysts (asterisk marks) dorsal to the spinal cord at the thoracic spine.

[1]Laboratory of Bone and Joint Diseases, RIKEN Center for Integrative Medical Sciences, Tokyo, Japan; [2]Department of Orthopaedic Surgery, School of Medicine, Keio University, Tokyo, Japan; [3]Department of Orthopaedic Surgery, Kyoto University, Kyoto, Japan and [4]Department of Orthopaedic Surgery, Saiseikai Central Hospital, Tokyo, Japan.
Correspondence: S Ikegawa (sikegawa@ims.u-tokyo.ac.jp)
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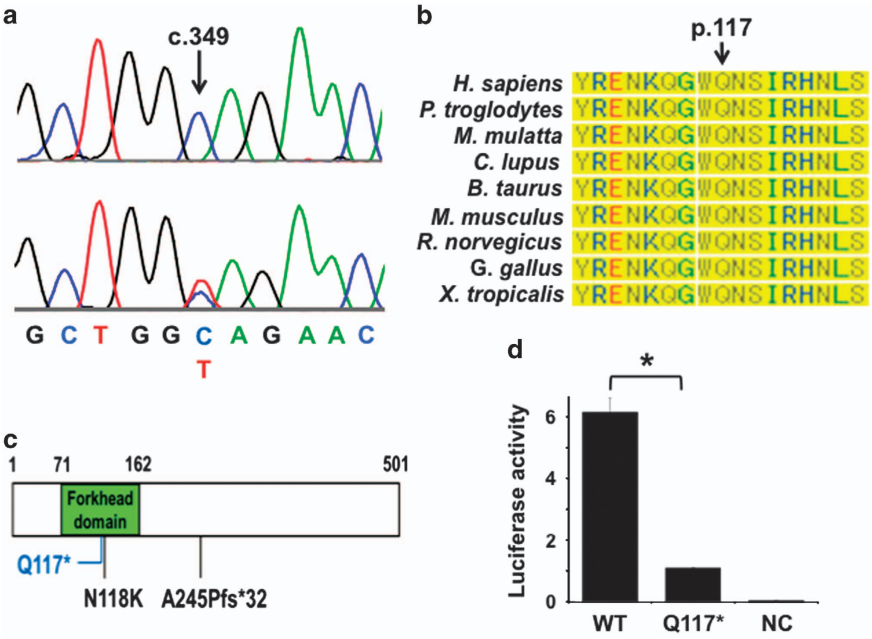

Figure 2. *FOXC2* mutations in spinal extradural arachnoid cyst. (**a**) Electrophoreogram of the wild type *FOXC2* (upper panel) and heterozygous nonsense mutation c.349C>T (Q117*) in the present patient (lower panel). (**b**) Amino-acid sequences around p.Q117 of the FOXC2 in different species. p.Q117 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.).

(http://www.mutationtaster.org/). It predicted that c.349C>T is disease causing. We previously conducted *in vitro* analysis of c.733delG (p.A245Pfs*32) and c.354C>G (p.N118K),[7] which were loss-of-function mutation. The current mutation would produce significantly truncated protein, losing half of the forkhead domain (Figure 2c). We performed *in vitro* experiment for *FOXC2* transcriptional activity as previously described.[7] 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.[7] 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 SEDAC patients are asymptomatic. An early detection enables surgery before irreversible neurological damage, providing significant benefits to other family members.

HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.figshare.hgv.690.

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COMPETING INTEREST

The authors declare no conflict of interest.

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