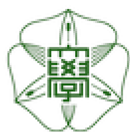

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NOTE

Examination of the *Lunatic fringe* and *Uncx4.1* expression by whole-mount *in situ* hybridization in the embryo of the CKH-*Jsr* (*jumbled spine and ribs*) mouse

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Abstract

The CKH-*Jsr* (*jumbled spine and ribs*) mouse was found as a spontaneous mutant with malformation of vertebrae, that is, a short trunk and kinky tail. We examined *Lunatic Fringe* (*Lfng*) and *Uncx4.1* expression in the presomitic mesoderm (PSM) and somites of *Jsr*-mutant (CKH-*Jsr*/+) embryos to elucidate pathogenesis of the *Jsr* mutation. Expression pattern of *Lfng* in the PSM of *Jsr*-mutant embryos was similar to that of the normal (C57BL/6) embryos. However, expression pattern of *Uncx4.1* in the somites of *Jsr*-mutant embryos was impaired to be irregular and mosaic, suggesting that the anterior-posterior (A-P) polarity is disordered in the *Jsr* mutant. These results indicate that the *Jsr* mutation disrupts the A-P polarity of somites during the somitogenesis without altering *Lfng* expression pattern in the PSM.

Key words: *Jsr*, *Lfng*, *Uncx4.1*

In vertebrates, the paraxial mesoderm corresponds to the bilateral stripes of mesodermal tissue flanking the notochord and neural tube. The paraxial mesoderm comprises the head or cephalic mesoderm anteriorly and the somitic region throughout the trunk and the tail of the vertebrates. Soon after gastrulation, the somitic region of vertebrates, termed presomitic mesoderm (PSM), starts to become segmented into paired blocks of mesoderm, termed somites. This process lasts until the number of somites reaches the intrinsic number that each species has. The newly formed somites have differentiated to

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anterior and posterior halves. The anterior and posterior halves of the somites later give rise to all skeletal muscles of the body, the axial skeleton, and part of the dermis[7]. Abnormalities in axial skeleton are caused by somite segmentation defects, anterior-posterior (A-P) polarity defects, or defects in a bone metabolism.

The developmental genes associated with the Notch signaling pathway have recently been studied by using knockout mice. The results from these mice have made it clear that the genes involved in the Notch signaling pathway play an important role during somitogenesis[2,3,4,7,9,11,12]. Further, one of the components of the Notch signaling pathway, *Lunatic Fringe* (*Lfng*) is periodically expressed in the PSM and *Lfng*-knockout mice show severe defects in axial skeleton[2,12]. Additionally, a loss of cyclic expression of *Lfng* in the PSM by *Lfng*-transgenesis causes the abnormality in somitogenesis and consequently vertebral defect[9]. Moreover, anterior and posterior patterning of the somites in both the *Lfng*-knockout and -transgenic mice shows a mosaic pattern[2,9,12].

The *jumbled spine and ribs* (*Jsr*) mutation in mice, which causes a short trunk and kinky tail, originated spontaneously in cataract-bearing mouse strain (CTA/Idr) at the Institute for Developmental Research in Aichi, Japan. We, then, established the CKH strain carrying the *Jsr* mutation as an inbred strain[6]. A mating experiment showed that these *Jsr* abnormalities were due to a single autosomal dominant gene[6]. At the result of the genetic analysis, the *Jsr* locus was mapped to distal region of Chr 5, where the *Lfng* gene locates[6,8]. In the recent study, it has been shown that *Lfng* is involved in somite segmentation and *Lfng*-knockout mice show very similar phenotype to that of *Jsr* mutants[2,12]. These data suggest that *Lfng* is a candidate gene responsible for the *Jsr* mutation. However, the sequence of the *Lfng* cDNA showed no substitution between the CKH-+/+ and CKH-*Jsr*/*Jsr* mice[8].

Lfng, the glycosyltransferase that modifies the Notch ligand-receptor interaction[10], is periodically expressed in the PSM. A loss of expression of *Lfng* during somite segmentation in *Lfng*-knockout mice causes closely similar phenotype to that of the *Jsr* mutant except for the mode of inheritance; the *Lfng*-knockout mice are recessive, while the *Jsr*-mutant mice are dominant[2,6,12]. On the other hand, a persistent expression of *Lfng* in the PSM of the *Lfng*-transgenic mice causes the same aberrant phenotype with the dominant inheritance as does the *Jsr* mouse[6,9]. These previous results raise a possibility that the *Lfng* expression in *Jsr*-mutant embryos is not periodic in the PSM. Therefore, we examined *Lfng* expression by whole-mount *in situ* hybridization with the *Lfng*-specific probe in the PSM of mouse embryos. In addition, we examined expression of *Uncx4.1*, since *Uncx4.1* is expressed in the caudal domain of the somites and the useful marker to assess if the somite segmentation occurs normally[5].

Total RNA from the adult mouse brain was isolated by use of TRIZOL (Invitrogen Corp., USA) and used for synthesis of first strand cDNA by using ReverTra Ace-a- (TOYOBO CO., LTD., Japan). The partial cDNAs of the *Lfng* and *Uncx4.1* genes were amplified by polymerase chain reaction (PCR) with TAKARA Ex Taq (TaKaRa BIO INC., Japan). The forward and reverse primers for *Lfng* were 5'-TCGATCTGCTGTTCGAGACC (nt 490-509) and 5'-ACGACTGCTAGAAGATGGAA (nt 1229-1248), respectively. The forward and reverse primers for *Uncx4.1* were 5'-TCGCAAGGAACTGGAGAAGA (nt 647-666) and 5'-GTTAGGTCCCGGAAAGAAGC (nt 1419-1438), respectively. The

PCR cycling profile was composed of an initial denaturing step at 95℃ for 2 min, followed by 35 cycles at 95℃ for 30 sec, 58℃ for 30 sec, 72℃ for 1 min, and final extention at 72℃ for 4 min. These *Lfng* and *Uncx4.1* cDNA fragments were purified with a GENECLEAN Ⅲ kit (Qbiogene, Inc., USA), and cloned into pGEM T-easy vector (Promega Corp., USA). Sequencing was performed with an ABI Prism 377 DNA Sequencer (Applied Biosystems INC., USA) and an ABI Prism Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems INC., USA). To produce antisense digoxigenin (DIG)-labeled RNA probe, the plas-

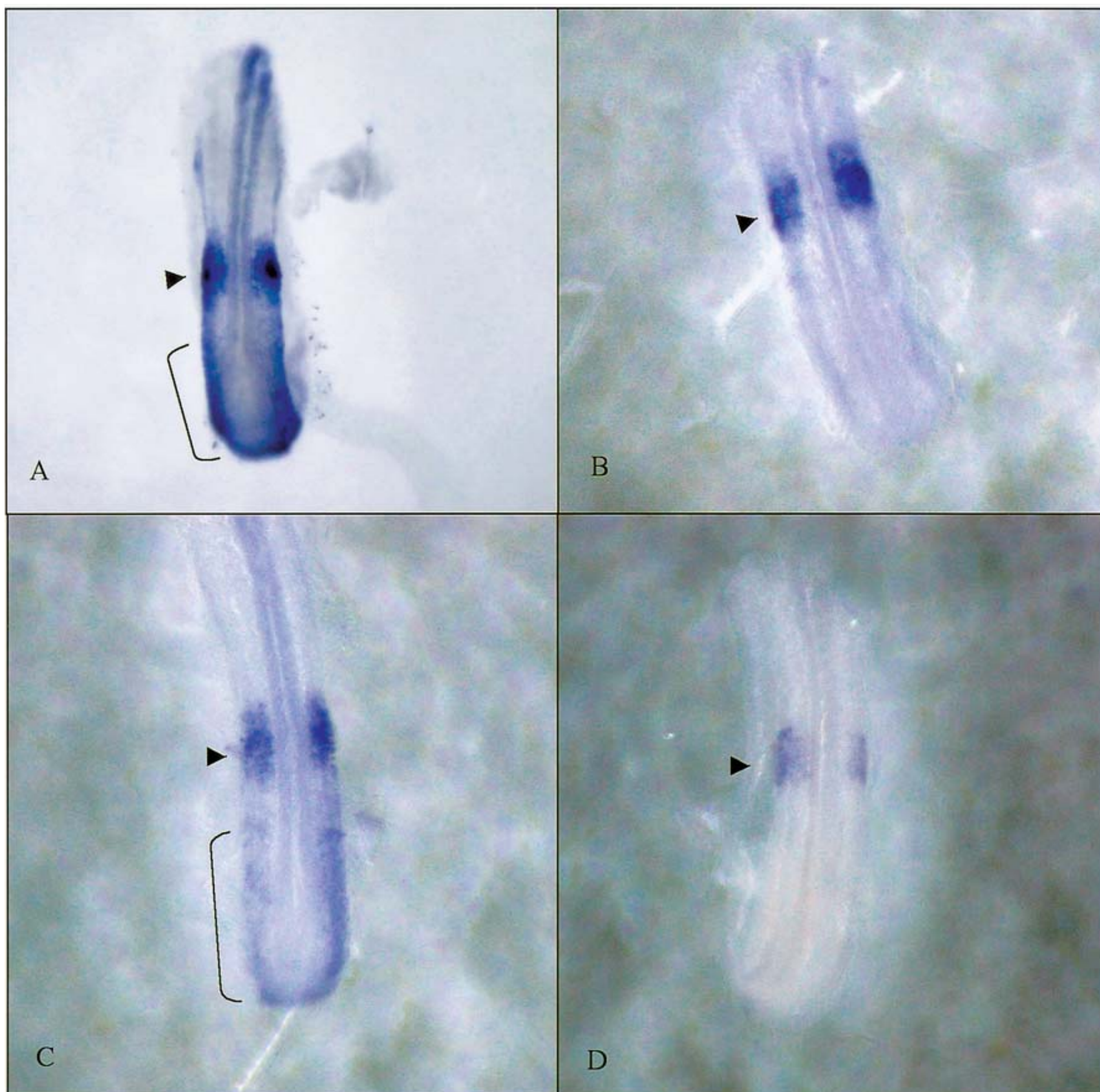

Figure 1. *Lfng* expression in 9.5 dpc normal (C57BL/6) and *Jsr*-mutant (*Jsr*/+) embryos. A and B, *Lfng* expression in normal embryos. A, *Lfng* is expressed in both the anterior PSM (arrowhead) and posterior region (bracket) (type Ⅰ). B, *Lfng* is expressed in the anterior PSM only (type Ⅱ). C and D, *Lfng* expression in *Jsr*-mutant embryos. C, *Lfng* is expressed in both the anterior PSM (arrowhead) and posterior region (bracket) (type Ⅰ). D, *Lfng* is expressed in the anterior PSM only (type Ⅱ). The similar expression pattern was observed between normal and *Jsr*-mutant embryos.

mid carrying the reverse insert was chosen. The DIG-labeled *Lfng* and *Uncx4.1* antisense RNA probes were synthesized with a DIG RNA Labeling Kit (Roche LTD., Switzerland).

The female C57BL/6 and male (C57BL/6 ×CKH-*Jsr*/+) F_1 mutant mice were mated to obtain the 9.5 day of post-coitus (dpc) mouse embryos. Whole-mount *in situ* hybridization analysis was carried out according to the protocol reported previously[1]. The genomic DNA was extracted from embryo yolk sac to genotype embryos. The genotyping was performed with the microsatellite marker *D5Mit327* by PCR. PCR cycling profile was the same as mentioned above.

Expression of *Lfng* in normal embryo PSM showed two patterns as previously reported[2], that is, expressed in both anterior region and posterior half of the PSM (type I) and in anterior region only (Type II) (Fig. 1A and B). *Lfng* expression in *Jsr* mutants showed similar pattern to that of the normal embryos (Fig. 1C and D). In addition, the level of *Lfng* expression was not different from that of normal PSM. These results indicate that *Lfng* expression in the *Jsr*-mutant PSM is periodic and normal.

Uncx4.1 is shown to be expressed at the posterior halves of the somites[5]. The result of the whole-mount *in situ* hybridization in normal mice showed regular pattern throughout all somites as reported previously[5] (Fig. 2A). The result of *Uncx4.1* expression in *Jsr*-mutant somites, however, showed irregular and mosaic pattern with a tendency of lower expression level when compared to that of normal embryonic somites (Fig. 2B). This result suggests that *Jsr*-mutant embryos have a defect in maintenance of the A-P boundary in somites as observed in both *Lfng*-knockout and-transgenic mice[2,9,13]. The failure in A-P polarity has been reported in three cases. The first is a loss of posterior specification such as *Delta like 1* (*Dll1*)-knockout mice[3], the second is a loss of anterior specification such as *Mesp2*-knockout mice[11] and the third is a fail-

Figure 2. *Uncx4.1* expression in 9.5 dpc normal (C57BL/6) and *Jsr*-mutant (*Jsr*/+) embryos. A, *Uncx 4.1* expression in a normal embryo. *Uncx4.1* expression is restricted to somite posterior halves. B, *Uncx4.1* expression in a *Jsr*-mutant embryo. *Uncx4.1* expression is observed in the whole somites, however, the expression pattern is disordered.

ure in maintaining the A-P boundary such as *Lfng*-knockout and -transgenic mice[2, 9, 12].

We have previously reported that *Lfng* and *Jsr* located in the same chromosomal region[8]. Either null-mutation or persistent expression by *Lfng*-trangenesis causes similar phenotype to that of the *Jsr* mutation[2, 9, 12]. However, the nucleotide sequence of the coding region of the *Lfng* gene in the *Jsr* mutant is identical to the normal[8]. These lines of evidence strongly suggest that expression of *Lfng* in the PSM is impaired in *Jsr*-mutant mice. In this study, however, expression of *Lfng* was normal in the PSM of *Jsr*-mutant mice. Expression of *Uncx4.1* in *Jsr*-mutants was, on the other hand, impaired as observed in *Lfng*-knockout or -transgenic mice. These results still support a hypothesis that *Lfng* is a gene responsible for the *Jsr* mutation. To dissolve this mysterious problem, it should be needed to perform more detailed expression analysis of *Lfng* in the PSM and further complete sequence analysis of the putative promoter, enhancer, silencer, and locus control region that regulate the periodic expression of *Lfng* in the PSM.

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