京都大学	博士（ 医 学 ）	氏 名	郭 龍
論文題目	Functional investigation of a non-coding variant associated with adolescent idiopathic scoliosis in zebrafish: elevated expression of the ladybird homeobox gene causes body axis deformation （ゼブラフィッシュを用いた思春期特発性脊柱側弯症に関連するノンコーディングバリアントの機能解析： ladybird homeobox 遺伝子の発現亢進は体軸変形を誘導する）		

（論文内容の要旨）

Adolescent idiopathic scoliosis (AIS) is the most common type of spinal deformity with a lateral spinal curvature of at least 10 degrees, affecting 2–4% of children aged between 10 and 16 years. AIS is known as a complex polygenic disease influenced by more than one allele at different loci. Previously, an AIS susceptibility locus near human *ladybird homeobox 1* (*LBX1*) and *FLJ41350* has been identified in Japanese population by a genome-wide association study, and the most significantly associated single nucleotide polymorphism (rs11190870) in the locus has been replicated consistently in independent studies using Chinese and Caucasian populations. *LBX1* and *FLJ41350* are located approximately 0.6 kb apart in a head-to-head arrangement on human chromosome 10, and rs11190870 is a non-coding variant, lying 7.5 kb downstream of *LBX1*. *FLJ41350* is a hypothetical gene that is found only in the human genome, and its function is uncharacterized. *LBX1* was first identified as a gene with homology to the *ladybird late* (*lbl*) gene in *Drosophila*. The ladybird protein is a member of the homeobox transcription factor family with an engrailed repressor domain. Previous *in vivo* studies using *Lbx1* knockout mice and *lbx* gene knockdown morphants in zebrafish or *Xenopus* did not reveal phenotypes associated with scoliosis. In this study, characterization of rs11190870 and the function of *LBX1* as well as its zebrafish homologues and *FLJ41350* were conducted.

In this study, a chromosome conformation capture assay revealed that the genome region with rs11190870 physically interacted with the promoter region of *LBX1-FLJ41350*. Electrophoretic mobility shift assay revealed that some nuclear proteins bound specifically to the genome sequences around rs11190870 with a higher affinity to the risk allele. In luciferase assays, significantly higher promoter activity was detected in the direction toward *LBX1*, but not toward *FLJ41350*. The promoter in the direction of *LBX1*, combined with a 590-bp sequence around rs11190870 that is highly conserved across species, had higher transcriptional activity with the risk allele than that with the non-risk allele in HEK 293T cells. The ubiquitous overexpression of human *LBX1* or either of the zebrafish *lbx* genes (*lbx1a*, *lbx1b*, and *lbx2*), but not *FLJ41350*, in zebrafish embryos caused body curvature followed by death prior to vertebral column formation. Such body axis deformation was not observed in zebrafish ubiquitously overexpressing a dominant-negative form of *lbx1a, lbx1b, or lbx2*, and transcription activator-like effector nucleases mediated knockout zebrafish of *lbx1b* or *lbx2*. Mosaic expression of *lbx1b* driven by the *GATA2* minimal promoter and the *lbx1b* enhancer in zebrafish significantly alleviated the embryonic lethal phenotype to allow observation of the later onset of the spinal curvature with or without vertebral malformation. Deformation of the embryonic body axis by *lbx1b* overexpression was associated with defects in convergent extension, which is a component of the main axis-elongation machinery in gastrulating embryos. In embryos overexpressing *lbx1b*, *wnt5b, a* ligand of the non-canonical Wnt/planar cell polarity (PCP) pathway, was significantly downregulated. Injection of mRNA for *wnt5b* or *RhoA*, a key downstream effector of Wnt/PCP signaling, rescued the defective convergent extension phenotype and attenuated the *lbx1b*-induced curvature of the body axis. Thus, as a step towards better understanding of the genetic pathophysiology of scoliosis, the study provide a new evidence for a pathological role of *LBX1* and its zebrafish homologs in body axis deformation at various stages of embryonic and subsequent growth in zebrafish.

（論文審査の結果の要旨）

側弯症の多くは、多因子遺伝病である思春期特発性側弯症（AIS）に分類され、その発症原因は特定されていない。これまでにゲノム相関解析によって AIS と非常に強い相関を示す一塩基多型（SNP）rs11190870 が見出され、疾患感受性遺伝子としてその近傍にある *LBX1* が同定されている。本研究では、側弯症研究モデルとしてゼブラフィッシュを用い、側弯の発症と *LBX1* 遺伝子との機能生物学的な関連を明らかにした。

rs11190870 を含むゲノム領域は、上流に存在する *LBX1* 遺伝子のプロモーター領域に働きかけて、その転写活性を上昇させることを明らかにした。ゼブラフィッシュ胚に *LBX1* またはそのゼブラフィッシュ相同遺伝子（*lbx1a*、*lbx1b*、*lbx2*）を全身性に過剰発現させると、いずれの場合も胚の体軸弯曲を誘導したが、胚性致死であった。そこで、*lbx1b* の内在性エンハンサーを用いて *lbx1b* を発現させたところ、一部の胚は成魚まで生存し、成長とともに椎骨奇形を伴う側弯と椎骨奇形を伴わない側弯を発症した。後者は、メスでの発症率が高く AIS の特徴と類似していた。*lbx1b* を過剰発現した胚では体軸形成に重要な役割を担っている収斂伸張運動が遅延しており、その要因は非古典的 *Wnt* シグナル経路のリガンドである *wnt5b* の発現低下であることを明らかにした。

以上の研究は、体軸弯曲における *LBX1* の役割の解明に貢献し、AIS 発症の分子メカニズムの理解に寄与するところが多い。

したがって、本論文は博士（医学）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、平成28年2月15日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日： 年 月 日 以降