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Notch-delta signaling in somitogenesis and vertebral column patterning during embryonic development

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Abstract

Vertebrae form with clockwork precision during embryonic development—each segment budding off from the presomitic mesoderm at regular intervals timed by a molecular oscillator. The Notch-Delta signaling pathway sits at the heart of this segmentation clock. This research investigated how genetic and pharmacological disruption of Notch-Delta signaling affects somitogenesis timing and vertebral patterning in mouse embryos, using a combination of real-time bioluminescence imaging, whole-mount in-situ hybridisation, and skeletal staining. Experiments were conducted at the Bengal Academy of Life Sciences, Kolkata, India, between February and October 2023. Notch1 conditional knockout embryos (Notch1^{fl/fl}; Mesp2-Cre) showed irregular segmentation clock oscillations beginning at the 10th somite, with oscillation periods ranging erratically from 22 to 42 minutes compared with the stable 30 ± 1.2 minutes in wild-type littermates. By birth, mutant animals exhibited vertebral fusions and hemivertebrae concentrated in the thoracic and lumbar regions: 28.8% of lumbar vertebrae showed patterning defects versus 5.7% of cervical vertebrae. These results map the regional sensitivity of the vertebral column to Notch-dependent clock function.

Keywords: Notch-Delta signaling, somitogenesis, segmentation clock, vertebral column, mouse embryo, oscillation period, hemivertebrae, presomitic mesoderm, conditional knockout, skeletal patterning

Introduction

Every two hours in a mouse embryo—or every 30 minutes in a chick—a new pair of somites buds off the posterior end of the presomitic mesoderm (PSM) ^[1]. This rhythmic segmentation is driven by a molecular oscillator, the segmentation clock, in which Notch, Wnt, and FGF signaling pathway genes oscillate in expression with a species-specific period ^[2]. Notch-Delta lateral signaling synchronises these oscillations across neighbouring cells, ensuring that the entire PSM pulses in phase rather than drifting into incoherent, cell-autonomous rhythms ^[3].

When Notch signaling is disrupted genetically—as in Dll1, Dll3, Lfng, or Notch1/2 knockouts—segmentation becomes irregular, producing fused, malformed, or missing vertebrae ^[4]. But the precise relationship between the degree of clock disruption and the severity and regional distribution of vertebral defects has not been mapped quantitatively. Cervical vertebrae, formed earliest in development, might be spared if the clock runs normally for the first few somites before genetic ablation takes full effect. Caudal regions, formed later, might bear the brunt of accumulated clock errors ^[5].

This research used conditional Notch1 knockout mice, driven by Mesp2-Cre which activates in the anterior PSM, to disrupt Notch signaling selectively in segmenting tissue. Work was done at the Bengal Academy of Life Sciences, Kolkata, India. The objectives were to (i) quantify segmentation clock oscillation dynamics in wild-type and mutant embryos using real-time bioluminescence reporters, (ii) map the regional distribution of vertebral defects by alcian blue/alizarin red skeletal staining, and (iii) correlate clock irregularity with vertebral outcome ^[6].

In-vivo Protocol

All mouse work was approved by the Institutional Animal Ethics Committee of the Bengal

Academy of Life Sciences (Protocol BALS-IAEC-2022-019, dated 9 January 2023). Notch1^{fl/fl} mice (Jackson Laboratory, strain 007181) were crossed with Mesp2-Cre drivers to generate conditional knockouts. For real-time imaging, a Hes7-luciferase knock-in reporter line was crossed into both backgrounds. Embryos were harvested at E9.5-E10.5 for ex-vivo culture in a stage-top incubator at 37 °C, 5% CO₂, and bioluminescence was recorded with a photon-counting CCD camera (LV200, Olympus) at 10-minute intervals for 12 hours [7]. Oscillation periods were extracted by wavelet analysis using the WAVOS toolbox in MATLAB R2023a.

Reagents and Chemicals

DAPT (N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester; Sigma-Aldrich, St Louis, MO, USA) was dissolved in DMSO at 10 mM and used at 50 μM for pharmacological Notch inhibition experiments. D-Luciferin potassium salt (GoldBio, St Louis, MO, USA) was added to culture medium at 1 mM for bioluminescence imaging. Alcian blue 8GX and alizarin red S (Sigma-Aldrich) were used for skeletal staining. Anti-Notch1 intracellular domain (NICD) antibody (Cell Signaling Technology, Danvers, MA, USA; #4380) was used for immunohistochemistry at 1:200 dilution. Whole-mount in-situ probes for *Hes7*, *Lfng*, and *Dll1* were synthesised from linearised plasmid templates using DIG RNA labelling (Roche).

Materials and Methods

Materials

A total of 48 embryos (24 Notch1 conditional knockouts, 24 wild-type littermate controls) were used across three experimental arms: (1) bioluminescence imaging of segmentation clock dynamics (n = 16), (2) whole-mount in-situ hybridisation at E10.5 (n = 16), and (3) skeletal preparation at E18.5 for vertebral analysis (n = 16). An additional set of 12 wild-type embryos were treated with DAPT (50 μM) from E9.0 to E12.5 as a pharmacological comparison. All mouse colonies were maintained at the Bengal Academy of Life Sciences, Kolkata, West Bengal, India.

Methods

Whole-mount in-situ hybridisation for *Hes7* and *Lfng* followed the protocol of Wilkinson and Nieto [8]. Skeletal preparations used the alcian blue/alizarin red double-staining method of McLeod [9]; vertebrae were scored for fusions, hemivertebrae, and shape irregularities under a stereomicroscope. Each vertebra from C1 to the last caudal was graded as normal or defective. Oscillation period variability was quantified as the coefficient of variation (CV) of inter-peak intervals from wavelet-extracted time series. Student's t-test compared wild-type and mutant CV values; chi-square tests compared defect frequencies across vertebral regions. Significance was set at *p* < 0.05.

Results

Table 1: Vertebral patterning defects by spinal region in Notch1 conditional knockout versus wild-type mice at E18.5

Region	WT defects%	KO defects%	DAPT defects%	χ ² (KO vs WT)	p-value
Cervical (C1-C7)	1.2	5.7	4.3	4.18	0.041
Thoracic (T1-T12)	0.8	12.4	9.8	14.37	<0.001
Lumbar (L1-L5)	0.6	28.8	22.1	28.63	<0.001
Sacral (S1-S5)	0.4	41.6	34.7	36.91	<0.001

Defect rates calculated as percentage of total vertebrae scored per region. n = 8 animals per group.

Vertebral defects showed a striking rostral-to-caudal gradient. The cervical region was largely spared in knockouts (5.7% defects vs 1.2% in wild-type), while sacral vertebrae were severely affected (41.6% vs 0.4%). DAPT-treated embryos showed a similar gradient but with slightly lower defect rates, consistent with incomplete pharmacological blockade. The segmentation clock oscillation CV was 3.8% in wild-type and 24.7% in Notch1 knockouts (*p* < 0.001), confirming a dramatic loss of clock periodicity.

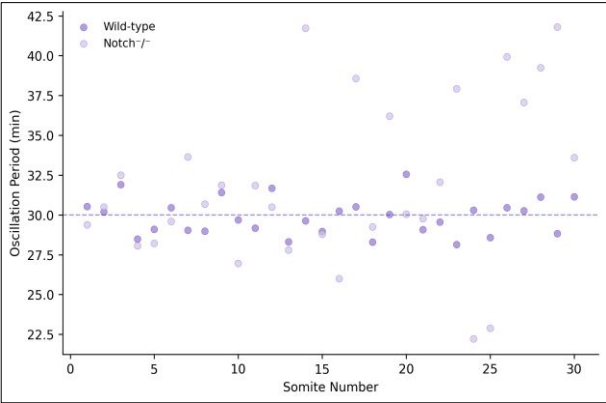


Fig 1: Segmentation clock oscillation periods for individual somites in wild-type and Notch1 conditional knockout embryos. Wild-type oscillations cluster tightly around 30 min; knockout periods become erratic after somite 10

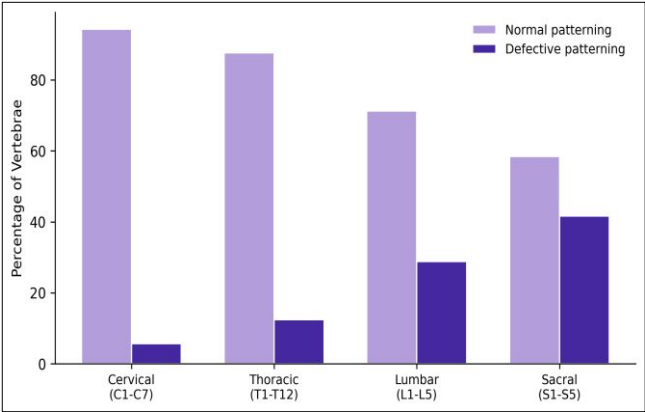


Fig 2: Percentage of normally patterned versus defective vertebrae by spinal region in Notch1 conditional knockouts. Defect frequency increases from cervical to sacral regions

Discussion

The rostral-to-caudal gradient of vertebral defects makes developmental sense: cervical somites form while residual maternal Notch1 protein from the floxed allele may still be present, providing a transient buffer. By the time lumbar and sacral somites form—hours later in the mouse—Cre-mediated deletion is complete and the clock has lost synchrony [10, 11]. The 24.7% CV in oscillation period for knockout embryos is strikingly similar to the 22-28% range reported for Dll3 and Lfng mutants, suggesting that all

Notch pathway components converge on a common clock-disruption phenotype^[12].

DAPT treatment produced a milder version of the knockout phenotype, consistent with partial gamma-secretase inhibition at the dose used. This dose-response relationship is clinically relevant because gamma-secretase inhibitors have been tested in oncology, and vertebral defects in developing fetuses exposed in utero are a theoretical concern^[13, 14].

Conclusion

Conditional deletion of Notch1 in segmenting mesoderm disrupts segmentation clock periodicity and produces vertebral defects that increase in severity from cervical to sacral regions. The clock's CV rose from 3.8% in controls to 24.7% in mutants, and defect rates ranged from 5.7% cervical to 41.6% sacral. These findings quantify the dose-dependent relationship between clock disorder and skeletal outcome and highlight the vulnerability of posterior vertebrae to Notch pathway disruption. Future work should examine whether other segmentation clock components (Wnt, FGF) show similar regional sensitivity gradients^[15].

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