

Impairment of endochondral ossification by *Hoxa2* overexpression: A plausible molecular explanation of idiopathic proportionate short stature

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Introduction. Using transgenic mice ectopically expressing *Hoxa2* all along chondrogenesis, we showed that *Hoxa2* exerts a negative influence in the earliest step of endochondral ossification (1,2) and demonstrated that this effect was due to a significant decrease in the number of mesenchymal cells entering chondrogenic differentiation. Furthermore, this endochondral ossification impairment was associated with an overall size reduction phenotype (Fig. 1) which could be referred to human idiopathic proportionate short stature (PSS).

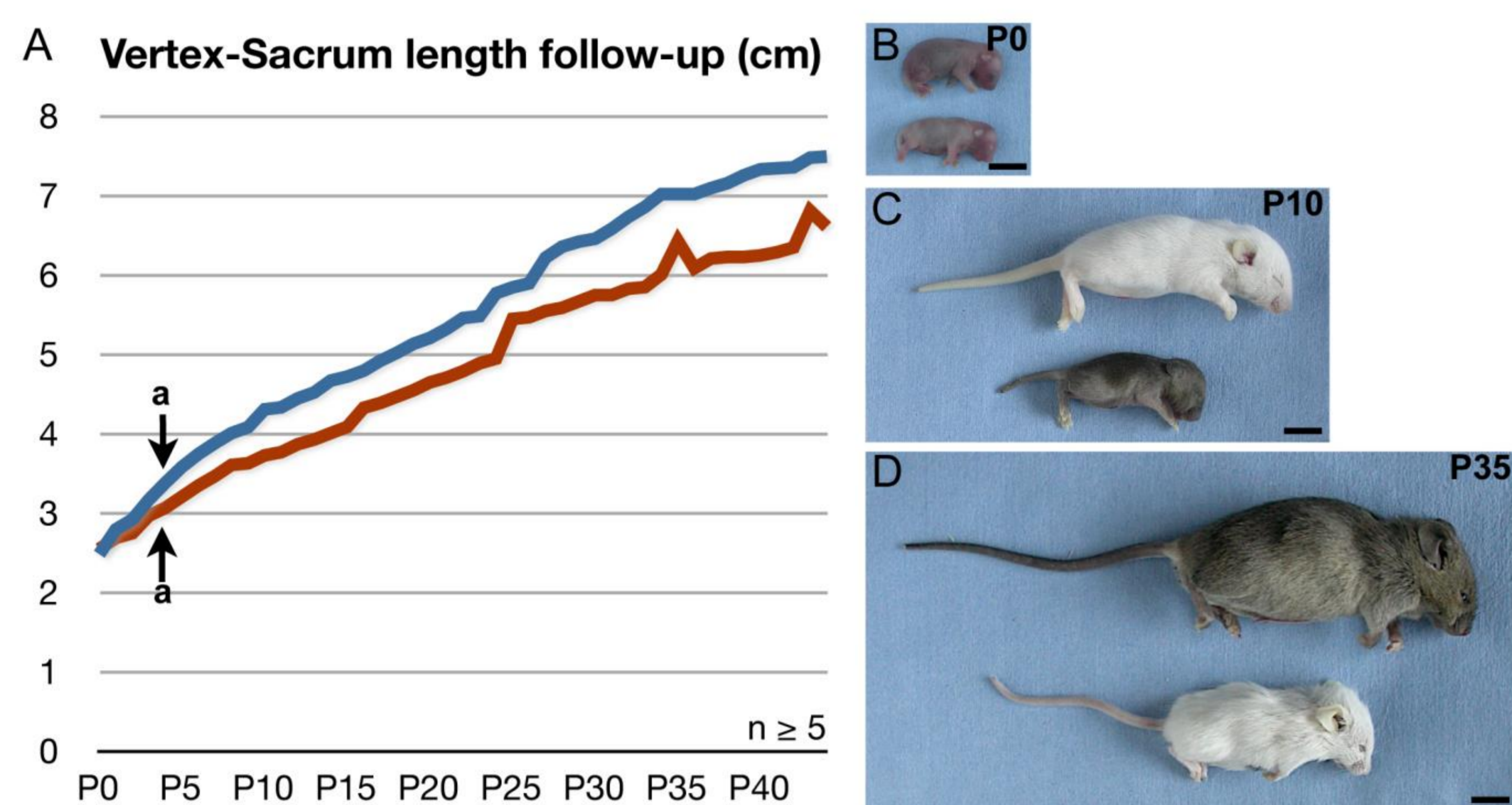


Fig. 1. *Hoxa2* expression induces vertex-sacrum length reduction during post-natal life (2). A. Growth curves of *β5-Hoxa2-lacZ* (black) and *Col2a1-Hoxa2-lacZ* (grey). B-D. Pictures of *β5-Hoxa2-lacZ* (top) and *Col2a1-Hoxa2-lacZ* (bottom) mice at P0 (B), P10 (C) and P35 (D) (Scale bar = 1 cm). a = P<0.05.

Objective. The molecular pathways underlying the human idiopathic proportionate short stature (PSS) pathogenesis are still unknown. With our murine transgenic model, we here tried to identify the impact of *Hoxa2* overexpression on the main factors involved in endochondral ossification in order to evaluate their possible role in idiopathic PSS.

Materials and methods. In our transgenic mice (*Col2a1/Hoxa2-lacZ*; Fig. 2), *Hoxa2* expression was induced in *Col2a1* expressing territories and maintained thereafter, i.e. all over the endochondral bone elements. Mice bearing the *hβ-actin-lox-STOP-lox-Hoxa2-lacZ* transgene only (*β5-Hoxa2-lacZ*) were considered controls.

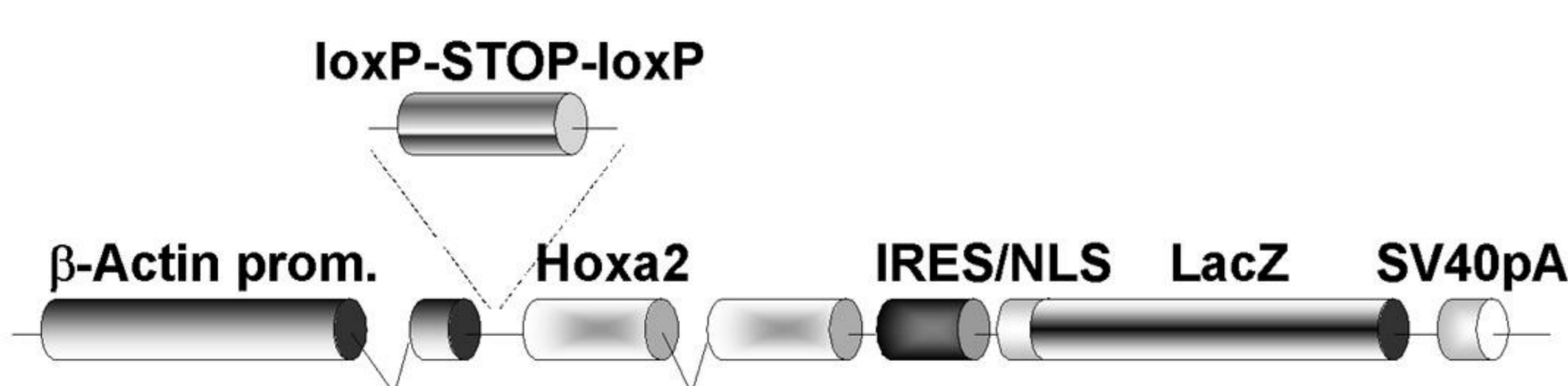


Fig. 2. Construct consists in the *Hoxa2* genomic sequence placed under the control of the human β -actin promoter and translationally coupled to a lacZ reporter by an Internal Ribosome Entry Site (IRES). It was further modified by inserting a « floxed » cassette blocking transcription between the β -actin promoter and the *Hoxa2* coding sequence, to give rise to a silent *hβ-actin-STOP-Hoxa2-IRES-lacZ* transgene (*β5-Hoxa2-lacZ*). Transgenics were mated with mice for a collagen IIIa1 promoter directed Cre expression (*Col2a1-Cre*) (3).

Using immunohistochemistry and Western blotting, we compared the protein levels of Bapx1, Runx2, Sox5, Sox6, Bmpr1a, Foxc2, β 1-integrin, Bmp7, Gdf10, Gdf5, Ihh, Wnt5a, Gdf3, Bmp4, Fgfr3, Gdf6, Meox1, Meox2, Pax1, PthrP, Msx1, Msx2, Osteopontin, Pax9, S-100 and Sox9 in E13.5, E15.5 and E16.5 transgenic and control mice. Immunohistochemistry was performed on sagittal paraffin sections through the spine.

References

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Results. Persistent expression of *Hoxa2* in chondrogenic territories provokes a general down-regulation of several factors known to control the endochondral differentiation cascade, i.e. Sox9, Bapx1, Bmp7, Bmpr1a, Msx1, Pax9, Sox6 and Wnt5a. Furthermore, later molecular agents, such as Ihh and Runx2, are also reduced. As a consequence, *Hoxa2* misregulation in mice, which induces a proportionate short stature phenotype mimicking human idiopathic conditions, is associated with a decrease of factors whose effects are distributed along the endochondral ossification cascade.

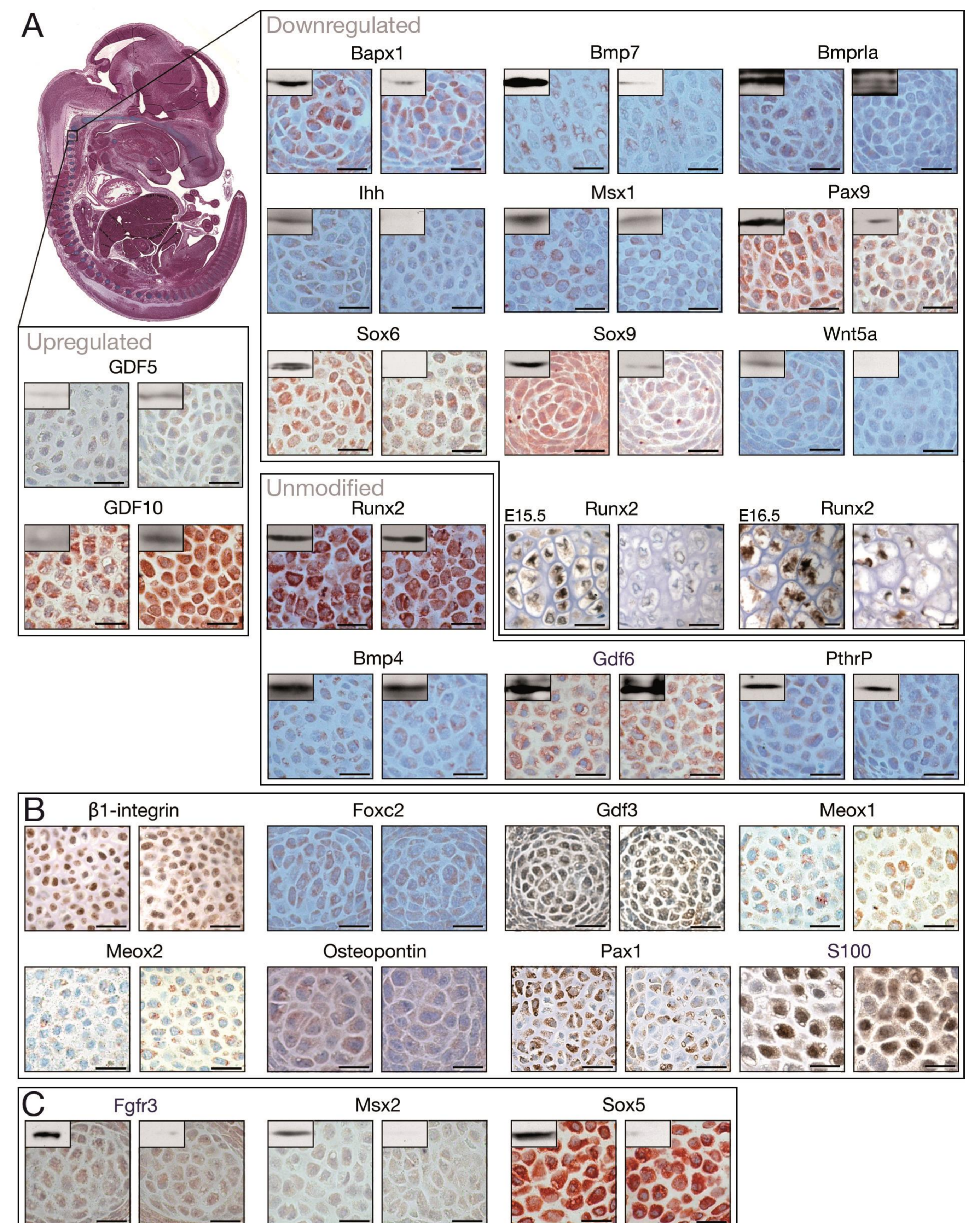


Fig. 3. Immunohistochemistry and western blotting on E13.5 *β5-Hoxa2-lacZ* (left) and *Col2a1/Hoxa2-lacZ* (right) vertebral bodies. Consistent results for both techniques are featured in panels A. Molecules that showed results in immunohistochemistry only are presented in panel B. Panel C depicts unfitting results. Runx2 was unmodified at E13.5 but reduced in pre- and hypertrophic chondrocytes as shown in E15.5 and E16.5 limbs, respectively.

Conclusions. Our results reveal molecular mechanisms linking the activity of *Hoxa2* to a negative impact on endochondral skeleton development (Fig. 4). These data give insights for understanding idiopathic proportionate short stature pathogenesis.

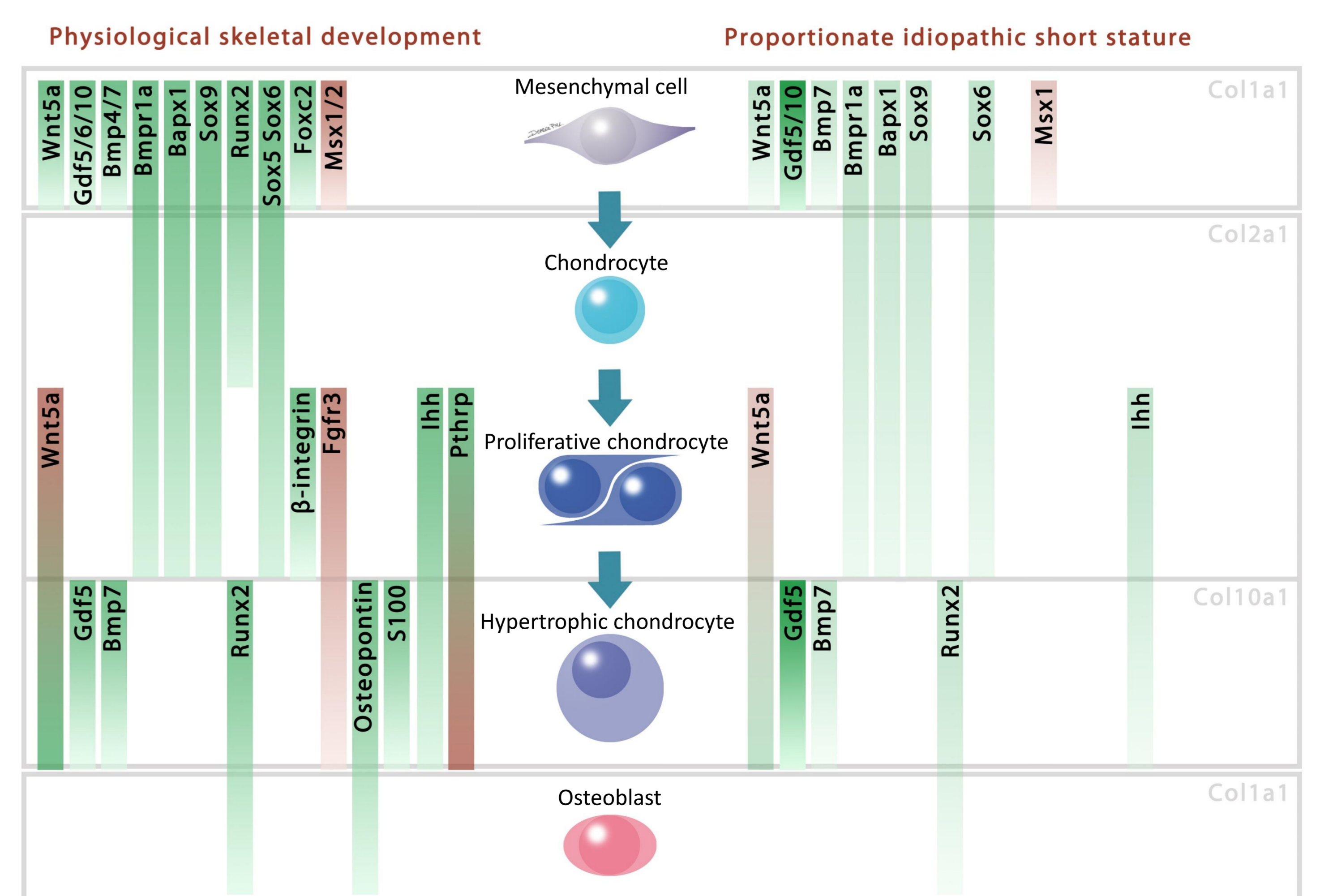


Fig. 4. Model featuring the proposed mechanism leading to idiopathic proportionate short stature. Physiological and pathological molecular expressions are featured on the left and right panels, respectively, according to the mesenchymal (*Col1a1*), differentiating (*Col2a1*) and hypertrophic (*Col10a1*) stages of endochondral ossification. Positive and negative influence of molecules over differentiation and hypertrophy are shown in green and red. Lighter and darker colors highlight inhibition or stimulation respectively, in the right panel.