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Protein and gene expression analysis of *Phf6*, the gene mutated in the Börjeson–Forssman–Lehmann Syndrome of intellectual disability and obesity

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Abstract

The *Plant homeodomain finger gene 6 (PHF6)* was identified as the gene mutated in patients suffering from the Börjeson–Forssman–Lehmann Syndrome (BFLS), an X-linked mental retardation disorder. BFLS mental disability is evident from an early age, suggesting a developmental brain defect. The PHF6 protein contains four nuclear localisation signals and two imperfect plant homeodomain (PHD) fingers similar to the third, imperfect PHD fingers in members of the trithorax family of transcriptional regulators. The *PHF6* gene is highly conserved in vertebrate species. Despite the devastating effects of mutation of the *PHF6* gene, nothing is known about the cellular function of PHF6. In order to lay the base for functional studies, we identify here the cell types that express the murine *Phf6* gene and protein during prenatal and postnatal development. The *Phf6* gene and protein are expressed widely. However, expression levels vary from strong to barely detectable. Strongest *Phf6* gene expression and nuclear localisation of Phf6 protein were observed in the developing central nervous system, the anterior pituitary gland, the primordia of facial structures and the limb buds. Expression levels of both mRNA and protein decline over the course of development. In the adult brain moderate Phf6 expression is maintained in projection neurons, such as mitral cells in the olfactory bulb, cerebrocortical pyramidal cells and cerebellar Purkinje cells. *Phf6* gene expression and nuclear localisation of Phf6 protein correlate with clinical symptoms in BFLS patients, namely mental disability, pan-anterior pituitary hormonal deficiency and facial as well digit abnormalities.

Keywords: *Phf6*, *Plant homeodomain finger gene 6*; BFLS, Börjeson–Forssman–Lehmann Syndrome; Mental retardation; X-linked syndrome; PHD; Nucleus; Nuclear protein; Brain development; Cerebral cortex; Pituitary gland; Hypophysis; Spermatogenesis; Hypotonia; Facial abnormalities; Digit abnormalities; Genital hypoplasia; Growth retardation; Gynecomastia; Obesity

The *Plant homeodomain finger gene 6* (*PHF6*, GenBank Accession No.: AY157622) was first described as the gene mutated in patients suffering from the Börjeson–Forssman–Lehmann Syndrome (BFLS, MIM 301900), an X-linked mental retardation disorder (Lower et al., 2002). The *PHF6* gene is located on the non-autosomal region of the X chromosome, such that male BFLS patients have only one, mutated copy of the *PHF6* gene. In BFLS patients, mental disability ranges from mild (IQ60) to severe (IQ20). The mental deficits are obvious before school age affecting both language and motor skills. Besides the mental deficits, consistently occurring symp-

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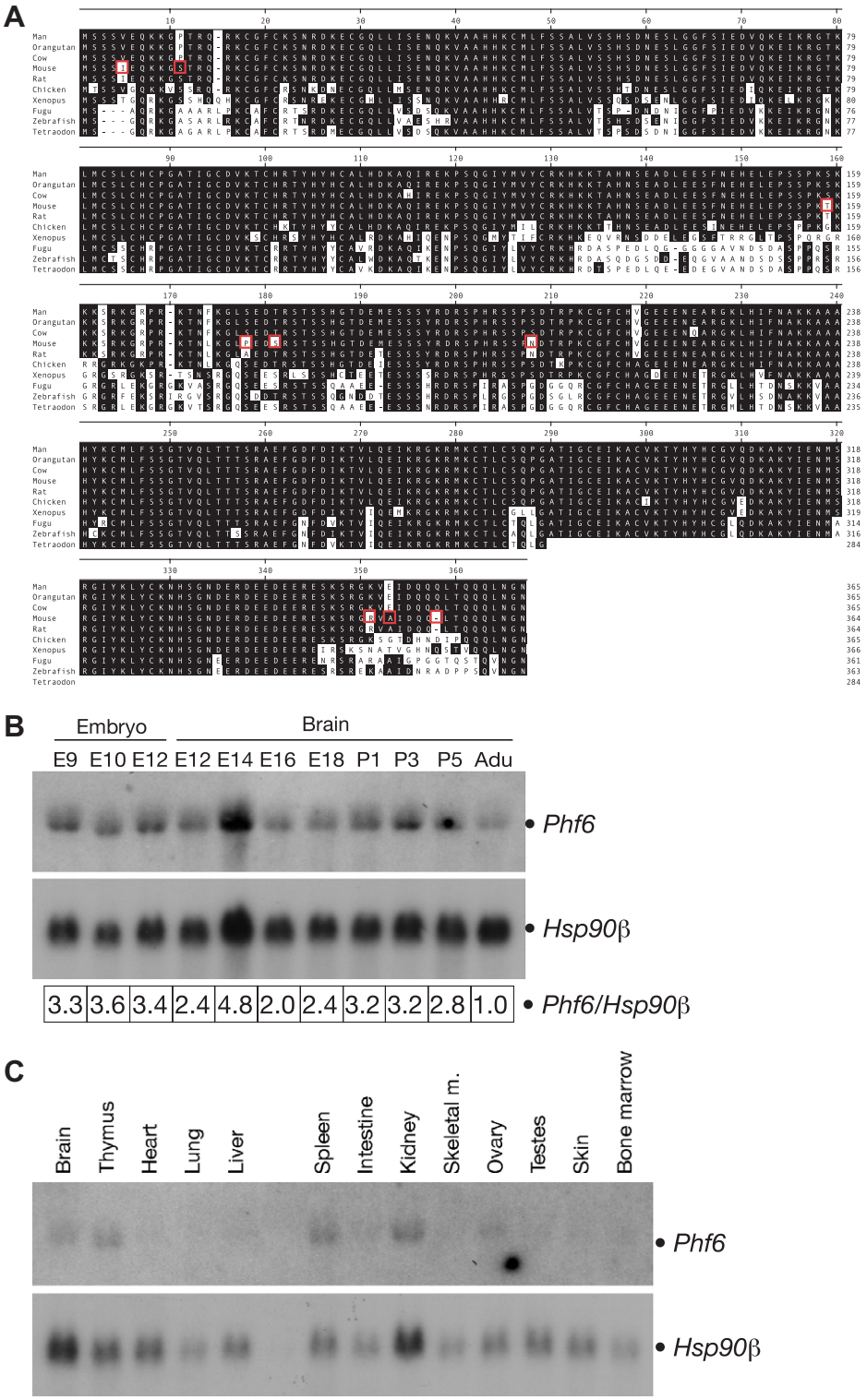

Fig. 1. Amino acid sequence conservation of the Phf6 protein in diverse vertebrate species and *Phf6* mRNA distribution. (A) Phf6 protein sequences were aligned with CLUSTALW (Combet et al., 2000) for comparison. Note the high degree of sequence identity particularly among mammals: 100% between human and orangutan, 99.7% between human and cattle, 97.5% between human and rodents (mice and rats). (B and C) Northern blot analysis of total RNA of E9.5 to E12.5 whole embryo and E12.5 to adult (adu) brain (B) and adult organs (C) as indicated above blot. Ten microgram of total RNA were loaded per lane. Blots were first probed with a *Phf6*-specific [^{32}P]dCTP-labelled cDNA probe, washed and exposed to film for 3 weeks. Subsequently blots were probed using a *Hsp90β*-specific probe as a loading and transfer control and exposed for 3 days. Relative ratios of densitometry readings for *Phf6* to *Hsp90β* mRNA are shown below the blot in (A) with the lowest recorded value displayed as "1.0". Higher levels of expression of the *Phf6* gene were observed during embryonic development and in the prenatal brain as compared to adult brain.