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# Developmentally regulated expression of the *LRRTM* gene family during mid-gestation mouse embryogenesis

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#### Abstract

We have analysed the expression during mid-gestation mouse development of the four member LRRTM gene family which encodes type 1 transmembrane proteins containing 10 extracellular leucine rich repeats and a short intracellular tail. Each family member has a developmentally regulated pattern of expression distinct from all other members. LRRTM1 is expressed in the neural tube, otic vesicle, apical ectodermal ridge, forebrain and midbrain up to a sharp central boundary. LRRTM2 is expressed in a subset of progenitors in the neural tube. LRRTM3 is expressed in a half somite wide stripe in the presomitic mesoderm adjacent to the boundary with the most recently formed somite. Additional expression is seen in the neural tube, forebrain and hindbrain. LRRTM4 is expressed in the limb mesenchyme, neural tube, caudal mesoderm and in three distinct regions of the head. Later expression occurs in a subset of the developing sclerotome. Each family member has a unique expression domain within the neural tube.

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Keywords: LRRTM; Leucine rich repeat; Transmembrane protein; Presomitic mesoderm; Somite; Sclerotome; Neural tube; Limb; Apical ectodermal ridge

#### 1. Results and discussion

There are four multi-member gene families (NLRR, FLRT, LERN and LRRTM) in mammals that encode type I transmembrane proteins containing 10–12 extracellular leucine rich repeats and short intracellular tails (Lacy et al., 1999; Carim-Todd et al., 2003; Lauren et al., 2003; Hamano et al., 2004; Haines et al., 2005). From their predicted secondary structure these proteins are thought to be involved in the regulation of cell adhesion and/or signalling. They are often expressed in the nervous system (Carim-Todd et al., 2003; Lauren et al., 2003) and have been associated with neural regeneration and neurite outgrowth (Ishii et al., 1996; Bormann et al., 1999; Robinson et al., 2004; Tsuji et al., 2004). Xenopus FLRT3 (XFLRT3) was identified as a gene with a similar expression pattern to FGF signalling molecules, particularly at the midbrain hindbrain boundary (Bottcher et al., 2004). It was shown to be regulated by

FGF signalling, be able to modulate FGF signalling and to interact with the FGF receptor. Rat NLRR-3 may be involved in the regulation of EGF receptor signalling through an intracellular clathrin adapter interaction motif (Fukamachi et al., 2002) and the structurally similar protein vasorin has been shown to interact with TGF- $\beta$  and inhibit signalling (Ikeda et al., 2004). These data suggest that leucine rich repeat transmembrane proteins may function in the regulation of cell signalling.

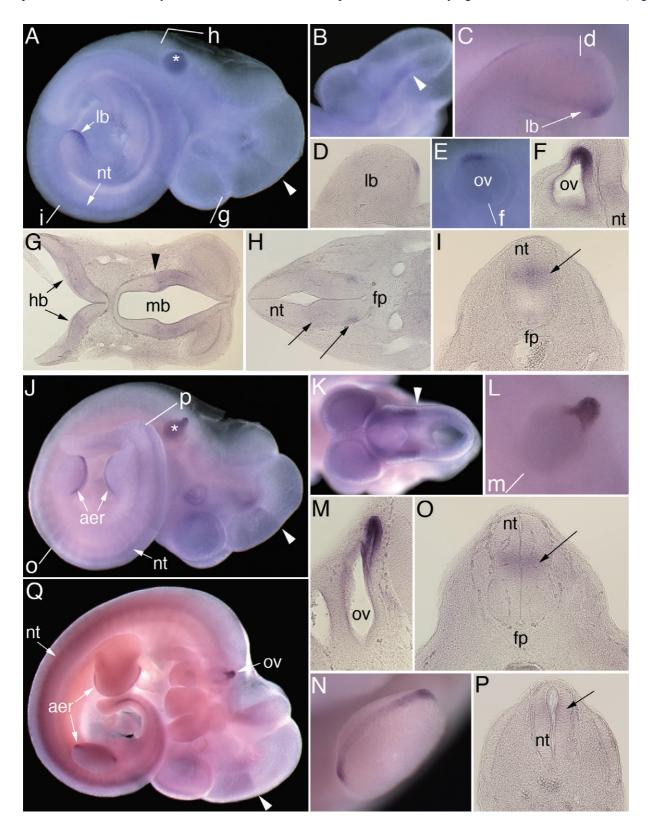
Expression analysis of the *NLRR* and *FLRT* gene families showed that they are highly regulated during mammalian development (Robinson et al., 2004; Haines et al., 2005, 2006). The leucine rich repeat transmembrane (*LRRTM*) gene family contains four genes that encode proteins containing 10 external leucine rich repeats, with no additional extracellular domains, and a short intracellular tail containing a conserved C-terminal domain with a consensus sequence for interaction with PDF motifs (Lauren et al., 2003). They are all expressed in adult brain with different expression profiles in other tissues. Analysis of expression in adult mouse brain by radioactive in situ hybridisation

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showed widespread but specific expression of different *LRRTM* transcripts (Lauren et al., 2003). We have examined the expression of this gene family during embryonic development using whole-mount in situ hybridisation on mouse embryos from 8 to 11 dpc. We found that LRRTM gene expression in mouse embryos occurs at low levels as

in situ hybridisations require long development times, which is consistent with lower levels of expression in embryonic brain compared to later developmental stages (Lauren et al., 2003).

*LRRTM1* is initially detected at 9 dpc with expression present in the overlying ectoderm of the limb bud (Fig. 1A,



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