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Gene expression pattern

Expression pattern of the lipocalin Apolipoprotein D during mouse embryogenesis

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Abstract

Apolipoprotein D (ApoD) is a secreted protein that belongs to the lipocalin family. We describe the expression pattern of ApoD during mouse embryogenesis by in situ hybridization using RNA probes. ApoD is expressed at E9 in mesenchymal cells in the rombencephalic–mesencephalic region. At E9.5 the cephalic ApoD-positive cells appear in the mesenchyme, and at later stages (starting at E10.5) ApoD expression is seen in meninges. Within the neuroepithelium, ApoD is expressed in pericytes surrounding brain and spinal cord capillaries from E10.5 to birth. Other places of expression of ApoD are the mesenchyme surrounding the olfactory epithelium and semicircular canals, as well as chondroblasts of skull and vertebrae.

Keywords: Apolipoprotein D; Lipocalin; Pericyte; Meninges; Cephalic mesenchyme; Chondroblasts; Vascular

1. Results and discussion

ApoD is a secreted lipocalin expressed in a variety of mammalian adult tissues. Quantitative Northern analyses (Yoshida et al., 1996) show the central nervous system (CNS) as the highest site of expression in rodents. In situ hybridizations to adult mammalian brains have located ApoD mRNA in pia matter cells and perivascular fibroblasts in the subarachnoidal space (Smith et al., 1990), but other cell types, such as astrocytes and oligodendrocytes, have also been shown to express ApoD (Smith et al., 1990; Navarro et al., 1998; Ong et al., 1999). Furthermore, immunocytochemistry studies show ApoD localization in cerebellar Purkinje neurons of 1–2 week postnatal rats (Ong et al., 1999). In spite of the broad knowledge on ApoD biology (reviewed by Rassart et al., 2000) and the confirmed presence in rat and human embryos (Drayna et al., 1986; Ong et al., 1999), a detailed account of the expression pattern of ApoD during mammalian embryogenesis has not been reported. Because of the suspected homology of ApoD to a particular group of lipocalins (Ganfornina et al., 2000) involved in axon guidance (Ganfornina et al., 1995; Gutierrez et al., 2000; Sanchez et al., 2000), we studied the expression pattern of ApoD during mouse embryogenesis by in situ hybridization using RNA probes.

We used an ApoD riboprobe to perform in situ hybridizations on cryostat sections of mouse embryos ranging 8.5–18.5 dpc. The hybridizations shown in Fig. 1 to sagittal sections of mouse embryos (E10.5, E11.5, and E13) reveal that ApoD is expressed mainly in tissues closely related to the CNS. However, other tissues also express this gene (see below).

The onset of ApoD expression is at E8.5–9 in cells located in the mesenchyme, ventral to the neural fold in the rombencephalic–mesencephalic region (Fig. 1D). At E9.5 ApoD mesenchymal cells appear to migrate to the mesencephalic flexure, where they continue to express ApoD throughout embryogenesis (Figs. 1A,C,F and 2A). At these stages, ApoD is absent in trunk cells (Fig. 1G). However, at E10.5 some ApoD-positive cells are seen in the caudal neural tube (arrows in Fig. 1H–J). Also, cells expressing ApoD are seen in the dermomyotome (arrowheads in Fig. 1I,J). The ApoD-positive mesenchymal cells are seen ubiquitously in the cephalic mesenchyme at later developmental stages (Fig. 2C). Fig. 2D shows ApoD in the mesenchymal cells of the choroidal plexus. ApoD-expressing cells appear in the mesenchyme surrounding the olfactory epithelium (Fig. 2E), and also as a condensed group of mesenchymal cells close to the semicircular canals (Fig. 2F), that contribute to the cartilaginous otic capsule and ossicles.

Another place of ApoD expression are the meninges. Cells that will eventually constitute the menix primitiva

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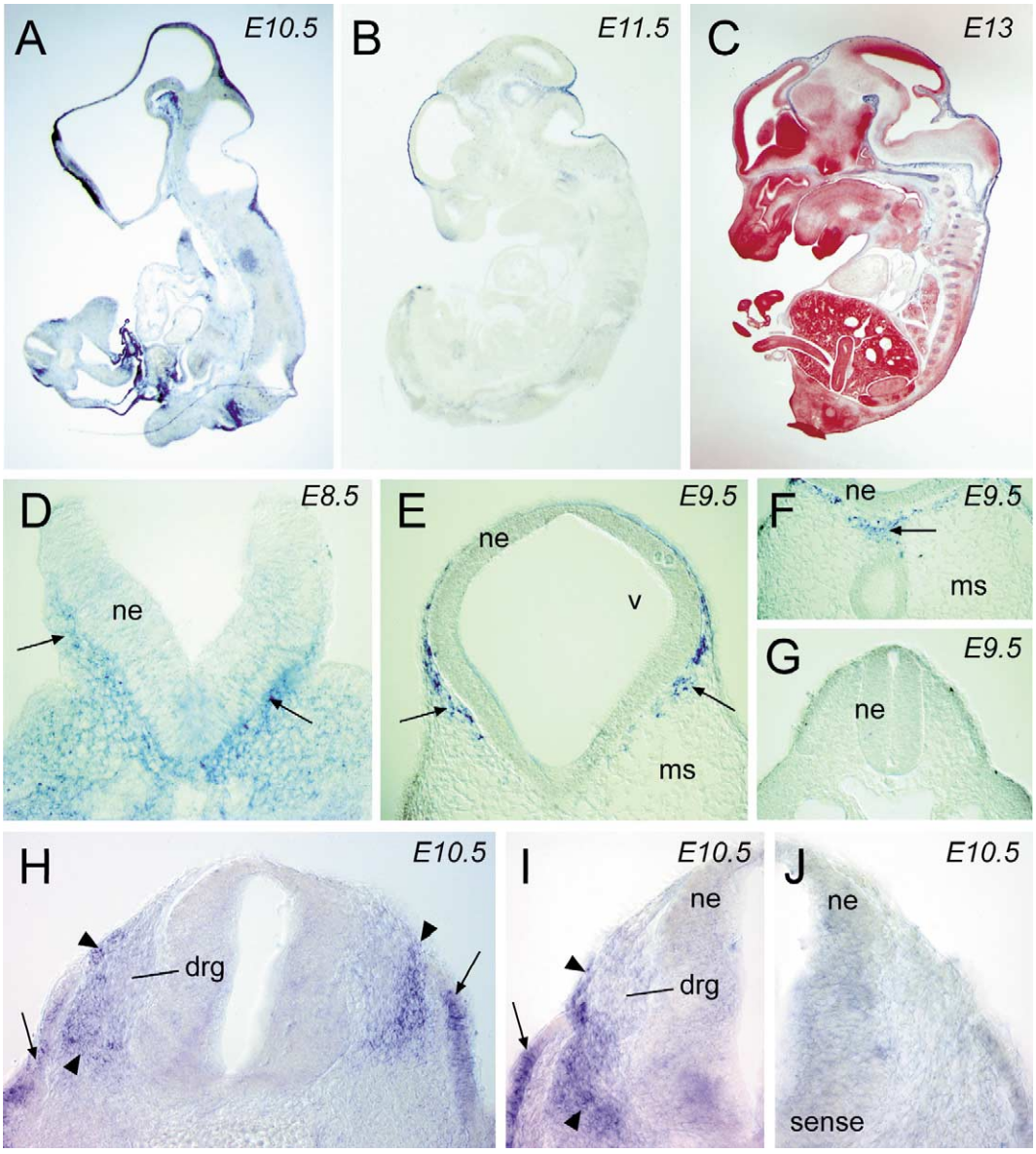
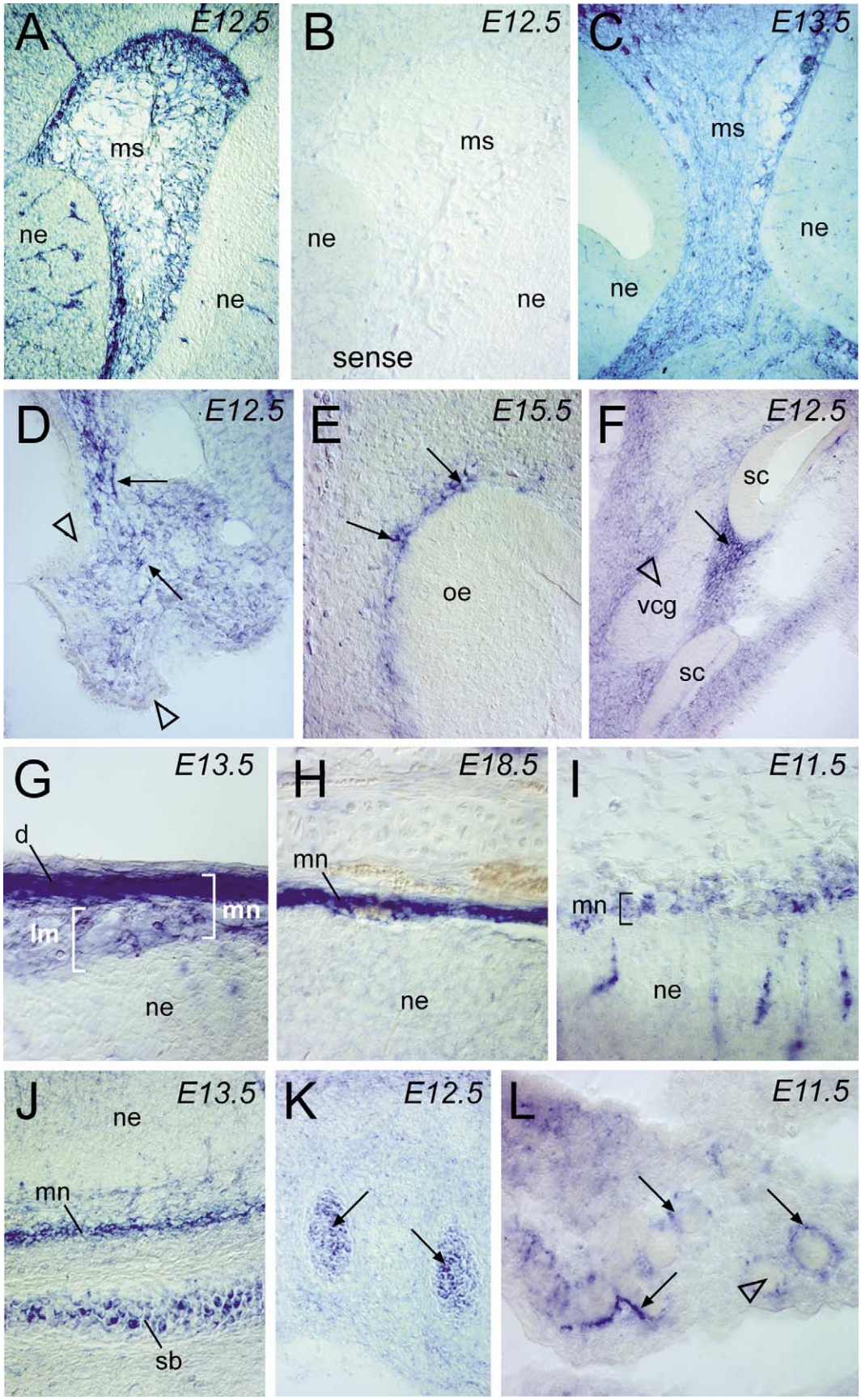


Fig. 1. (A–C) In situ hybridizations with a Dig-labelled ApoD antisense riboprobe to sagittal cryostat sections of mouse embryos. Developmental stages are indicated in each panel. Anterior is up, and dorsal is right. The E13 section was counterstained with neutral red. ApoD expression is mainly detected in the CNS. (D–J) Transverse sections. Dorsal is up. Since E8.5 cells expressing ApoD in the cephalic mesenchyme (arrows in D,E) are seen migrating towards the cephalic flexure (arrow in F). (G) Absence of labelling in the trunk at E9.5. (H,I) ApoD expression in cells (arrowheads) in the caudal neural tube at E10.5. Arrows point to ApoD-expressing dermomyotome. (J) Absence of labelling with ApoD sense riboprobe. drg, dorsal root ganglion; ms, mesenchyme; ne, neuroepithelium; v, cephalic vesicle.

Fig. 2. (A–F) ApoD expression in the cephalic mesenchyme. Developmental stages are indicated in each panel. Anterior is up, and dorsal is right. All micrographs are from sagittal or parasagittal sections. (A) Mesenchymal cells in the cephalic flexure expressing ApoD. (B) Negative labelling obtained with a sense ApoD riboprobe. (C) ApoD expression in lateral cephalic mesenchyme. (D) ApoD expression in mesenchymal cells in the IV ventricle choroidal plexus (arrows), but not in the epithelium (triangles). (E) ApoD expression in mesenchymal cells surrounding olfactory epithelium (arrows). (F) A localized group of mesenchymal cells express ApoD in the developing ear. Notice the absence of labelling in the vestibulocochlear ganglion (triangle). (G–I) Meningeal expression of ApoD in the brain (G,H) and the spinal cord (I). ApoD is expressed in chondroblasts of basioccipital bone (J) and vertebral primordia (K). (L) ApoD is expressed in cells (arrows) around the mesonephric ducts, whose epithelium is not labelled (triangle). b, basioccipital bone; d, duramater; lm, leptomeninx; mn, meninges; ms, mesenchyme; ne, neuroepithelium; oe, olfactory epithelium; sb, skull bone; sc, semicircular canals; vcg, vestibulocochlear ganglion.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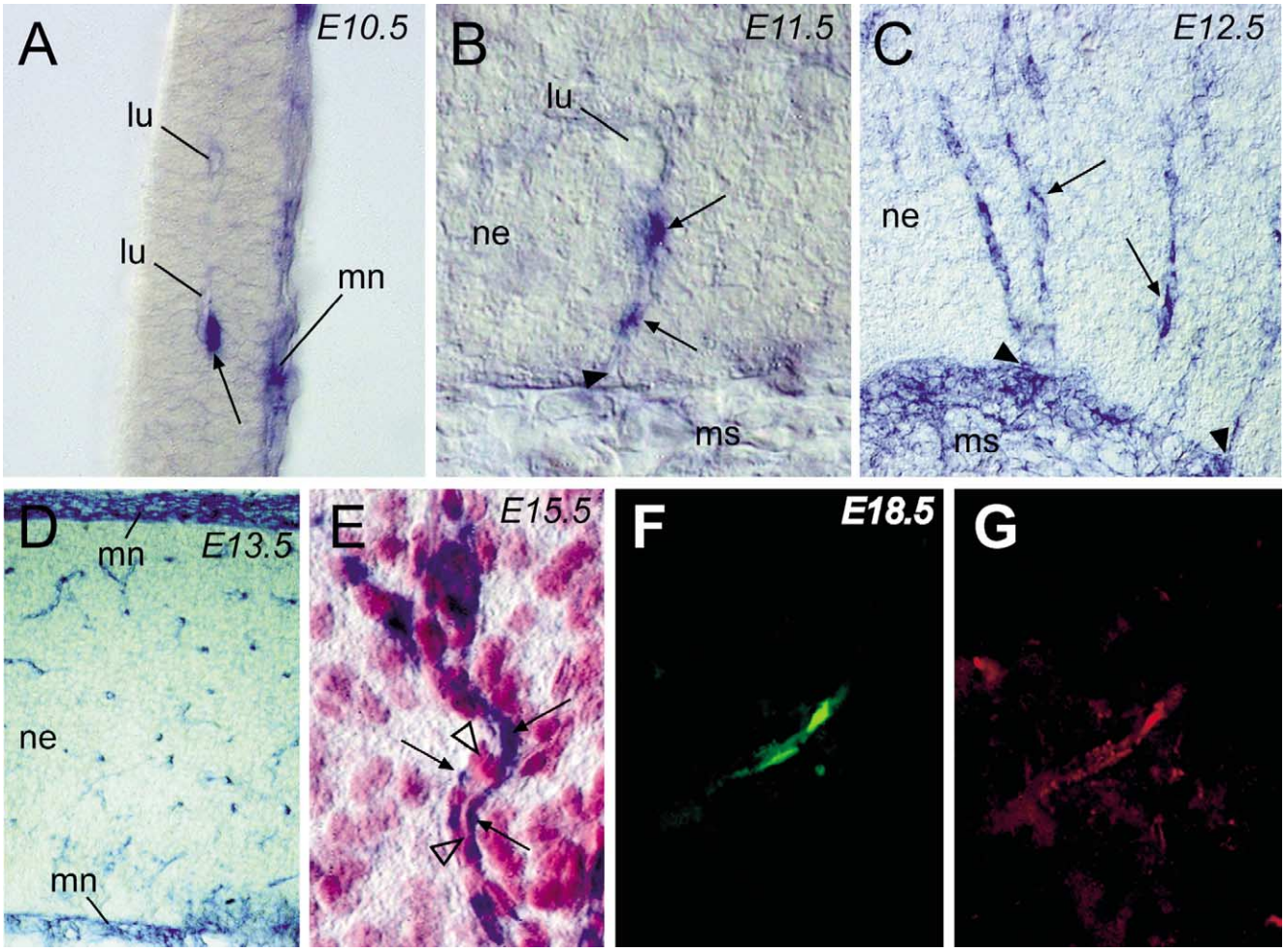

Fig. 3. ApoD expression in pericytes. Developmental stages are indicated in each panel. Anterior is up, and dorsal is right. All micrographs are from sagittal or parasagittal sections. (A) ApoD-positive cells located in the brain neuroepithelium, surround the lumen of developing blood capillaries. (B,C) These cells (arrows) are seen attached to the basal membrane of the neuroepithelium (arrowhead). (D) Low magnification of mesencephalic neuroepithelium showing capillary-associated ApoD-positive cells. (E) ApoD-expressing cells (arrows) are located outside the endothelial layer (triangles) of capillaries. (F,G) Co-localization of ApoD (F), and nestin (G) in pericytes. lu, blood vessel lumen; mn, meninges; ms, mesenchyme; ne, neuroepithelium.

(organized at E12–13) are seen to express this gene from E10.5 (see Figs. 1A and 3A). At latter stages, both the outer dura mater, and the inner leptomeninx are expressing ApoD (Fig. 2G), and this expression persists throughout embryogenesis (Fig. 2H). The spinal cord meninges, express ApoD as well (Fig. 2I).

ApoD is also expressed in the precartilaginous condensations, such as the primordium of the basioccipital bone (Fig. 2J), the Meckel's cartilage (not shown), and the sclerotomal condensations of vertebral bodies (Fig. 2K). Moreover, cells surrounding the mesonephric ducts express ApoD (Fig. 2L). A light expression is also observed in the mesenchyme surrounding the lung, stomach, intestine, and kidney (not shown), and in the umbilical cord (Fig. 1A).

We also found ApoD expression in the neuroepithelium, starting at E10.5. The ApoD-positive cells showed irregular shape at early stages (Fig. 3A). They extend long cytoplasmic processes and organize radially in the neuroepithelium at E11.5 (Fig. 3B), while maintaining attachment to the basal membrane (Fig. 3C). These cells appear to be migrating cells from the surrounding mesenchyme, and they locate close to developing blood vessels since early stages (arrows in Fig. 3A,B). At E11.5 these cells are seen along the whole span of the neuroepithelium (Fig. 3D). Their situation in blood vessels, immediately adjacent to the endothelial cells, can be seen in neutral red counterstained sections (arrowheads in Fig. 3E). This location points to pericytes, a layer of cells surrounding the endothelium of microvasculature, that are involved in maturation, remodelling and maintenance of blood vessels through secreted growth factors (reviewed by Allt and Lawrenson, 2001). The identity of the ApoD positive cells as pericytes was demonstrated by double labelling of the intermediate filament nestin (arrowheads in Fig. 3F,G), that is present in endothelial cells and pericytes (Alliot et al., 1999).

2. Methods

The ApoD digoxigenin-labelled riboprobes (sense and antisense) were synthesized from a fragment of the mouse cDNA clone comprising the coding sequence and part of the 3′-UTR. Mouse embryos were fixed in 4% paraformaldehyde and stored in methanol at −70°C until processed. In situ hybridizations on 30 μm cryostat sections were performed as previously described (Myat et al., 1996). Hybridization temperature was 63°C for all experiments, and the riboprobes were used at 1 μg/ml. Non-specific hybridization of the ApoD riboprobe was evaluated with an ApoD sense probe, as well as with other control genes. Double fluorescent in situ-inmunos were performed with anti-dig FITC-labelled Fab fragments (Roche), followed by incubation with anti-nestin antibody (Chemicon) developed with rhodamine-labelled secondary antibody (Sigma). The hybridized sections were observed with a Leica microscope, photographed with a Leica DC200 camera, and processed with Adobe Photoshop.

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