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Cellular Expression of the Monocarboxylate Transporter (MCT) Family in the Placenta
of Mice

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ABSTRACT

Lactate plays an important role as an alternative energy substrate, especially in conditions with a decreased utility of glucose. Proton-coupled monocarboxylate transporters (MCTs) are essential for the transport of lactate, ketone bodies, and other monocarboxylates through the plasma membrane and may contribute to the net transport of lactate through the placental barrier. The present study examined the expression profile and subcellular localization of MCTs in the mouse placenta. An *in situ* hybridization survey of all MCT subtypes detected intense mRNA expressions of MCT1, MCT4, and MCT9 as well as GLUT1 in the placenta from gestational day 11.5. The expression of MCT mRNAs decreased in the intensity at the end of gestation in contrast to a consistently intense expression of GLUT1 mRNA. Immunohistochemically, MCT1 and MCT4 showed a polarized localization on the maternal side and fetal side of the two cell-layered syncytiotrophoblast, respectively. The membrane-oriented localization of MCTs was supported by the coexistence of CD 147 which recruits MCT to the plasma membrane. However, the subcellular arrangement of MCT1 and MCT4 along the trophoblastic cell membrane was completely opposite of that in the human placenta. Although we cannot exactly explain the reversed localization of MCTs between human and murine placentas, it may be related to differences between humans and mice in the origin of lactate and its utilization by fetuses.

1. Introduction

Intermediate metabolites including L-lactate, acetate, and ketone bodies provide a “ready-to-use” fuel in most cells or are converted into glucose (gluconeogenesis) and fatty acids (lipogenesis). These metabolic monocarboxylates can compensate glucose as an energy source under several physical conditions such as fasting, diabetes, and the neonatal period. It is a fact that neuronal activities even in the normal and adult brain depend on lactate, which is produced by adjacent astrocytes, rather than glucose [1]. In humans and other mammals, the concentration of lactate in the fetal circulation is known to be higher than in the maternal circulation [2-6]. Some researchers consider the major source of lactate in the fetal circulation of humans to be the fetus itself due to the active anaerobic metabolism of fetus—that must be discharged into the maternal circulation [3-5]. On the other hand, fetal tissues of sheep effectively utilize lactate as a metabolic energy source, supplied by placental lactate production or transfer from maternal blood [2,6]. The most likely mechanism of lactate transfer through the placenta involves a specific carrier-mediated system, being coupled with proton transfer [4,7,8].

In the human and rodent placenta, arborized villi or complex labyrinths derived from the fetus directly contact the maternal blood (hemochorial placenta). Each part of the villous and labyrinthine tissues accommodating the fetal blood vessels is covered by continuous cell layers of syncytiotrophoblasts along the entire length, forming an epithelial barrier against the maternal immune system (the blood-placental barrier). Since glucose, a major nutrient source for fetal development, is supplied only from the maternal blood, the placental barrier develops a facilitated-diffusion transport system of glucose (GLUT). Among the glucose transporter isoforms, GLUT1 and GLUT3 are

localized in the cell membrane of syncytiotrophoblasts in mammals [9,10]. Another set of placental perfusion studies and isolated plasma membrane studies have demonstrated that lactate is transferred across the placental barrier by means of stereospecific (between L- and D-lactate) transporters [11-13], and the movement is mediated by a carrier-dependent transporter resembling the lactate/H⁺ co-transporters with a broad distribution throughout the body.

A representative lactate transporter is the monocarboxylate transporter (MCT) and classified SLC16 gene family. MCT is a H⁺-coupled, electroneutral, and bi-directional transporter composed of twelve transmembrane domains with intracellular N and C terminal domains [14,15]. To date, fourteen MCT isoforms—each having a unique distribution and different sequence homology—have been identified in mammals, though only four (MCT1–MCT4) have been demonstrated experimentally to facilitate the proton-linked transport of metabolically important monocarboxylates [16]. Northern blot and quantitative PCR analyses for the MCT family have demonstrated significant mRNA expressions of MCT1, MCT4, MCT5, MCT6, MCT8, and MCT10 (T-type amino acid transporter-1, TAT1) in the human placenta [16-19]. An immunohistochemical study reported that MCT1 and MCT4 were localized respectively on the basal plasma membrane and apical microvillous membrane of syncytiotrophoblasts in the human term placenta [20]. However, no histochemical data are available concerning the expression of MCTs in laboratory animals and the topographical relationship with glucose transporters.

In the murine hemochorial placenta, a complex trophoblastic labyrinth is formed between the maternal blood and fetal blood flows. The trophoblastic layer is composed of three cell-layers: an outer single cytotrophoblast layer and two inner

syncytiotrophoblastic layers (termed syncytiotrophoblasts I and II from the cytotrophoblastic side). The cytotrophoblastic layer facing the maternal blood is highly permeable, due to the numerous large pores crossing its cytoplasm. The syncytiotrophoblasts I and II form a double-layered firm epithelium and function essentially as a barrier unit against the maternal circulation, differing in the barrier composition from human placenta. The present study aimed to investigate the expression profile of MCT1–14 and the subcellular localization of MCT1 and MCT4 in the murine placenta with special reference to GLUT1. The present histochemical study revealed a predominant expression of MCT1, MCT4, and MCT9 among the MCT family and showed that the subcellular localization of MCT1 and MCT4 in the mouse differed from that in the human placenta.

2. Materials and methods

2.1. Tissue samplings

The placentas were obtained from three pregnant ddY mice at each stage, which were all sacrificed by bleeding after being deeply anesthetized by an intraperitoneal injection of pentobarbital, at days 9.5, 11.5, 14.5, 17.5 and 18.5 of gestation. More than three placentas were obtained from each mother and embedded in a freezing medium (OCT compound; Sakura FineTechnical Co. Ltd., Tokyo, Japan) and quickly frozen in liquid nitrogen for *in situ* hybridization analyses. The fresh tissues were also fixed in Bouin's fluid for 12 h and processed to paraffin sections for conventional immunohistochemistry. For immunohistochemistry at the electron microscopic level, the tissues were fixed overnight with 4% paraformaldehyde plus 0.2% picric acid in 0.1 M phosphate buffer, pH 7.4. All experiments using animals were performed under protocols following the Guidelines for Animal Experimentation, Hokkaido University Graduate School of Medicine.

2.2. *In situ* hybridization

Two non-overlapping antisense oligonucleotide DNA probes (45 mer in length) were designed for each mRNA of mouse MCT1–MCT12, GLUT1–GLUT5, and CD147, as described in our previous studies [21–23]. CD147 is an important chaperone protein of MCT1 and MCT4 [18]. Antisense probes for mouse MCT13, MCT14, GLUT6, and GLUT7 were newly designed to be complementary to the following sequences: 621–665 and 1201–1245 of MCT13 (NM_172371); 901–945 and 1521–1565 of MCT14 (NM_027921); 731–775 and 1391–1435 of GLUT6 (NM_172659); and 599–643 and 1091–1153 of GLUT7 (NM_001085529). The

probes were labeled with ^{33}P -dATP using terminal deoxynucleotidyl transferase (Invitrogen, Carlsbad, CA). Fresh frozen sections, 14- μm -thick, were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer for 15 min and then acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine-HCl (pH 8.0) for 10 min. Hybridization was performed at 42°C for 10 h with a hybridization buffer containing ^{33}P -labeled oligonucleotide probes (10,000 cpm/ μl). The sections were rinsed at room temperature for 30 min in 2 \times SSC (1 \times SSC: 150 mM sodium chloride, 15 mM sodium citrate) containing 0.1% *N*-lauroylsarcosine sodium, then rinsed twice at 55°C for 40 min in 0.1 \times SSC containing 0.1% *N*-lauroylsarcosine sodium, dehydrated through a graded series of ethanol, and air-dried. Sections were either exposed to BioMax MR film (Kodak, Rochester, NY) for 10 days or dipped in an autoradiographic emulsion (NTB-2; Kodak) at 4°C for 8–10 weeks. The hybridized sections used for autoradiography were counterstained with hematoxylin after development.

An *in situ* hybridization technique using the two non-overlapping antisense probes for each mRNA exhibited identical labeling in all the tissues examined. The specificity of the hybridization was also confirmed by the disappearance of signals upon the addition of an excess of an unlabeled antisense probe.

2.3. Immunohistochemistry

Bouin-fixed paraffin sections were dewaxed and immersed in water and 0.01 M phosphate buffered saline (PBS, pH 7.2). After preincubation with normal goat serum, the sections were incubated with a chicken anti-rat MCT1 antibody (AB1286; Chemicon International, Temecula, CA) at a concentration of 0.5 $\mu\text{g}/\text{ml}$, a rabbit anti-rat MCT4 (0.5 $\mu\text{g}/\text{ml}$; AB3314P, Chemicon), or a rabbit anti-rat GLUT1 antibody (1: 4,000

in dilution; AB1340, Chemicon) overnight. The sites of the antigen-antibody reaction were detected by incubation with biotin-conjugated goat anti-chicken IgY (Santa Cruz Biotechnology, Santa Cruz, CA) or goat anti-rabbit IgG (Nichirei, Tokyo, Japan), followed by incubation with the avidin-peroxidase complex (Vectastain ABC kit; Vector, Burlingame, CA). The reactions were visualized by incubation in 0.01M Tris-HCl buffer (pH 7.6) containing 0.01% 3,3'-diaminobenzidine and 0.001% H₂O₂. The specificity of immunoreactions on sections was confirmed according to a conventional procedure, including absorption tests. The immunoreactivities were completely abolished using the primary antibodies preabsorbed with corresponding antigens (human MCT1, MCT4, and GLUT1 from Santa Cruz Biotechnology and Yanaihara Institute, Shizuoka, Japan).

For double immunofluorescence, sections were stained with the anti-MCT1 (4 µg/ml) and anti-MCT4 antibodies (2 µg/ml), followed by incubation with FITC-labeled anti-chicken IgY (Santa Cruz Biotechnology) or Cy3-labeled anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA). The stained sections were mounted with glycerin-PBS and observed under a confocal laser scanning microscope (Fluoview; Olympus, Tokyo, Japan).

2.4. Silver-intensified immunogold method for electron microscopy

The paraformaldehyde-fixed tissues were dipped in 30% sucrose solution overnight at 4°C, embedded in OCT compound, and quickly frozen in liquid nitrogen. Frozen sections of 15 µm in thickness were mounted on poly-L-lysine-coated glass slides and incubated with a rabbit anti-human MCT1 antibody (3 µg/ml; Biogenesis Ltd, Poole, UK), the rabbit anti-MCT4 antibody (1 µg/ml), or rabbit anti-GLUT1 (1:800 in

dilution) overnight and subsequently reacted with goat anti-rabbit IgG covalently linked with 1-nm gold particles (1: 200; Nanoprobes, Yaphank, NY). Following silver enhancement using HQ silver (Nanoprobes), the sections were osmificated, dehydrated, and directly embedded in Epon (Nisshin EM, Tokyo, Japan). Ultrathin sections were prepared and stained with both uranyl acetate and lead citrate for observation under an electron microscope (H-7100; Hitachi, Tokyo, Japan).

2.5. Western blot analysis

The placenta (E17.5) and cecum were homogenized with an ice-cold 10 mM Tris-HCl buffer (pH 7.0) containing 1 mM ethylenediamine-tetraacetic acid, 20 mM KCl, and a protease inhibitor cocktail (Complete Mini; Roche, Mannheim, Germany). The cecum intensely expressing MCT1 was used as a positive control in the immunoblotting of MCT1. Soluble fractions were processed according to Garcia et al. [15] and were subjected to 12% sodium dodecyl sulfate polyacrilamide gel electrophoresis under reducing conditions. The proteins were then transferred to polyvinylidene difluoride membranes (Hybond-P; GE Healthcare Biosciences, Buckinghamshire, UK) and incubated with the chicken antibody against MCT1 (0.1 µg/ml) or the rabbit anti-MCT4 (0.08 µg/ml). The bound antibodies were visualized using peroxidase-labeled anti-chicken IgY (1: 2,000 in dilution; Santa Cruz Biotechnology) or anti-rabbit IgG (1: 10,000 in dilution; DAKO, Glostrup, Denmark) and an enhanced chemiluminescence system (ECL-plus; GE Healthcare Biosciences) according to the manufacturer's instructions.

3. Results

3.1. *In situ* hybridization of MCT and GLUT families

The mRNA expressions of all MCT members (from MCT1 to MCT14) and all GLUT members (GLUT1–GLUT7) were visualized on tissue sections of the mouse placenta obtained at the embryonic days (E) 11.5, 14.5, 17.5, and 18.5. No significant expression for MCTs and GLUTs was found at E9.5, due to a lack of development of the functional placenta. X-ray film images of hybridized serial sections showed significant expressions of six MCT members and two GLUT members with different intensities (Fig. 1). Comparatively intense signals of MCT mRNA were obtained for MCT1, MCT4, and MCT9 when judged at E11.5 and E14.5; there were less intense signals for MCT5, MCT10, and MCT12. The signals of MCT1 appeared to be most intense among the MCT family but were still weak as compared with those of GLUT1. Among the GLUT family, the expression of GLUT1 mRNA was extremely intense, while GLUT3 mRNA was weakly expressed, as confirmed by a light microscopic observation of hybridized sections (Fig. 2a, b). In earlier gestational stages, the junctional region developed between the placental labyrinth and maternal decidua and intensely expressed GLUT1 mRNA (an asterisk in Fig. 2a), but no expression of other transporters was found in most of the junctional regions, including MCT1 (Fig. 2b–d). Faint signals at best were detectable for MCT2, MCT3, MCTs6–8, MCT11, MCT13, and MCT14 and for GLUT2 and GLUTs4–7 throughout the placenta (data not shown). An intense expression of CD147 mRNA was found broadly in the placenta and surrounding tissues (Fig. 1).

The expressions of GLUTs and MCTs mentioned above were detectable throughout the gestational stages from E11.5. However, the signal intensities of

MCT1 and MCT4 tended to decrease at the end of gestation (E17.5 and E 18.5) as compared with the consistently intense expression of GLUT1.

3.2. Immunohistochemistry of MCT1, MCT4, and GLUT1 in the mouse placenta

Immunoreactivities for GLUT1 and MCT1 were found in the labyrinthine zone, which occupied the majority of the placental volume, as gestation progresses (Fig. 2e, f). At a higher magnification, the immunoreactivity for GLUT1 was visualized as two lines bordering the double-layered syncytiotrophoblasts (Fig. 3a), while the immunoreactivity for MCT1 was seen to be restricted to the apical plasma membrane of the syncytiotrophoblast I, being a thick single-line which faced the maternal blood sinuses (Fig. 3b). In contrast, MCT4 immunoreactivity was restricted to the basal plasma membrane of the syncytiotrophoblast II, appearing as a single thin line facing the fetal blood vessels (Fig. 3c). CD147 immunoreactivity occurred on the both maternal and fetal sides of trophoblasts and corresponded to the overall localization of MCT1 and MCT4 (Fig. 3d). Double immunofluorescence staining of single sections confirmed a different polarization between MCT1 and MCT4 in the syncytiotrophoblasts (Fig. 3e). The junctional region between the labyrinth and decidua contained many spongiotrophoblasts and deeply localized trophoblast giant cells. The spongiotrophoblasts, though not all cells, were immunolabeled for GLUT1 along the entire length of each cell surface (Fig. 2e), in agreement with the expression pattern of GLUT1 mRNA (Fig. 2a) and with previous immunohistochemical studies for GLUT1 in the rat [24]. The junctional region lacked immunoreactivities for MCT1 (Fig. 2f) and MCT4.

The specificity of the antibodies was confirmed by an immunoblot analysis using

extracts from the placenta and cecum—the tissue for a positive control (Fig. 3f). The antibody against MCT1 detected the major immunoreactive band around 43 kDa in the placenta and cecum, in agreement with the original data by Garcia et al. [15]. The immunoreactive band for MCT4 appeared around 40 kDa, slightly lower than MCT1, as reported in previous studies [25].

3.3. Immunohistochemistry at the electron microscopic level

Gold particles showing the immunoreactivity for MCT1 gathered along the apical plasma membrane of syncytiotrophoblast I, which formed a tuft of microvilli underneath the cytotrophoblast layer (Fig. 4a). In contrast to MCT1, gold particles for MCT4 were restricted to the basal plasma membrane of syncytiotrophoblast II cells facing the fetal blood vessels (Fig. 4b). More dense aggregations of gold particles for MCT4 were found in the microfolding regions. Immunogold particles for GLUT1 were densely distributed along both the apical microvillous membrane of syncytiotrophoblast I and the invaginated basal membrane of the syncytiotrophoblast II (Fig. 4c). The apical surface of syncytiotrophoblast I cells was partially covered by cytotrophoblasts and formed junctional complexes such as desmosomes or adherens junctions against the cytotrophoblasts. These spotty junctional regions in the apical plasma membrane of syncytiotrophoblasts were free of the MCT1 and GLUT1 immunoreactivities. Neither MCT1, MCT4, nor GLUT1 was found at the apposing plasma membranes of syncytiotrophoblasts I and II, which formed numerous gap junctions (Fig. 4). The cytotrophoblast and the endothelium of fetal blood vessels were both lacking in the immunoreactivities for MCT1, MCT4, and GLUT1.

3.4. MCT1, MCT4, and GLUT1 in human placenta

The MCT1 immunoreactivity was localized essentially in the basal but not apical plasma membrane of syncytiotrophoblasts, including the plasma membrane facing scattered cytotrophoblasts (Langhans cells) (Fig. 5a). The cytotrophoblasts often appeared to be encircled by an MCT1-positive membrane, possibly belonging to syncytiotrophoblast (indicated by an arrow in Fig. 5a). Immunostaining with the MCT4 antiserum specifically labeled the apical plasma membrane of the syncytiotrophoblast, although the immunoreactivity was not intense (Fig. 5b). GLUT1 immunoreactivity, on the other hand, was localized in both the apical microvillous and basal plasma membranes of the syncytiotrophoblast. Notably, the GLUT1 immunoreactivity in the apical plasma membrane tended to be less intense than that in the basal plasma membrane and was lacking in some areas (Fig. 5c). The intense labeling of GLUT1 in the basal plasma membrane of the syncytiotrophoblast almost encircled the cytotrophoblasts, like the MCT1 immunoreactivity. Desidua cells also showed immunoreactivities for both GLUT1 and MCT1 along the cell surface.

4. Discussion

Many studies using isolated plasma membrane vesicles of the placental trophoblast have demonstrated the transport of lactate through the placental barrier. Multiple expressions of MCT isoforms which can transport lactate and other monocarboxylates have been shown in the human placenta by Northern blot analyses. Only one study by Settle et al. [20] reported the cellular localization of MCTs in the trophoblast using human term placenta. The present study thoroughly investigated the expression profiles of all MCT members in the mouse placenta together with GLUTs and revealed the subcellular localizations of MCT1 and MCT4, which were reversed for the fetal or maternal sides of trophoblast as compared with those in the human placenta. Furthermore, we could demonstrate the co-localization of MCTs and CD147, the latter being an important chaperone protein, facilitating the trafficking of MCT molecules to the appropriate plasma membrane.

4.1. Expression profiles of MCT and GLUT families in the mouse placenta

To date, the first four members of MCT family have been shown to transport monocarboxylates, whereas MCT-5–7, 9, and 11–14 are orphan transporters whose substrates are unknown. MCT8 transports thyroid hormones, and MCT10 belongs to the System T aromatic amino acid transporter (TAT1) [18] and is also transporter of thyroid hormone [26]. Northern blot analyses of the human placenta demonstrated significant mRNA expressions of MCT-1, 4, 5, 6, 8 [17], and MCT10 [27]. The present *in situ* hybridization study is the first to reveal the expression profile of all MCT members in the rodent placenta. Namely, we detected comparatively intense expressions of MCT1, MCT4, and MCT9 with less intense expressions of MCT5,

MCT10, and MCT12. The results obtained in the mouse largely confirmed the expression pattern of MCT reported by Northern blot analyses in the human placenta, except for MCT6 and MCT8 whose signals were undetectable in the mouse. The placenta must be another organ with the multiple expressions of MCT members comparable to the intestine [28] and kidney [21], which are engaged in the active uptake and reabsorption of monocarboxylates as nutrients.

GLUT1 is a ubiquitous house-keeping transporter for regulating the entry of glucose into the cytoplasm in most animal cells. It is generally believed that GLUT1 is the major glucose transporter isoform in the human placenta with GLUT3 having a smaller role in transport [9,10]. This was confirmed by the present *in situ* hybridization analysis for GLUT1–GLUT7 in the murine placenta. Expressions of both GLUT1 and GLUT3 in the labyrinthine part and the expression of GLUT1 alone in the junctional region of rat placenta were reported by earlier immunohistochemical studies [24,29,30], and the results are consistent with findings by the present analysis at the mRNA and protein levels in the mouse. Subcellularly, previous immunohistochemical analyses in the rat placenta have demonstrated the presence of GLUT1 in both the apical microvillous membrane of the double-layered syncytiotrophoblast facing the maternal blood and the basal plasma membrane facing the fetal circulation [9,24]. This subcellular localization was also confirmed in the mouse placenta by the present study at the light and electron microscopic levels. In syncytiotrophoblast I of these rodents, GLUT1 was abundant at the microvillous plasma membrane, while GLUT1 in the syncytiotrophoblast II was condensed in the infolded basal plasma membrane. Thus, the trophoblastic glucose transporter is characterized by the expression of one GLUT subtype (GLUT1) along both surfaces of the trophoblastic barrier.

No significant expression of mRNA for GLUT and MCT families was found in the placenta at E9.5. It is known that, by E10.5, the placenta has developed a mature structure and exhibits the inner labyrinth where nutrient transfer occurs between the maternal and fetal blood spaces [31]. Thus, it is reasonable that the GLUT and MCT responsible for transport of nutrients has not yet been provided in the placental labyrinth at E9.5.

4.2. Subcellular localization of MCT1 and MCT4 and the transfer of monocarboxylates

Numerous studies have demonstrated that the lactate concentration in fetal blood is higher than that in the maternal blood. Lactate in fetal circulation has been considered to result from glycolysis in fetal tissues, due to low concentrations of fetal blood oxygen. However, there is strong evidence of net lactate uptake and consumption by ovine and bovine fetuses as a whole [2,6,32,33]. Lactate and other monocarboxylates not only serve the major carbon sources for fatty acid synthesis but also are utilized as a metabolic energy source in various fetal tissues, such as the porcine heart [34], rat brain [35], and ovine liver [36]. The H^+ gradient-dependent lactate transport occurs in the brush-border membrane of the trophoblast in rat [37] and human placentas [5,7,8] and in the basal plasma membrane of the trophoblast in humans [5,38]. Inuyama et al. [5] demonstrated that the apical brush-border and basal plasma membranes in the human trophoblast shared a H^+ -dependent transport system for lactate but differed in some aspects between apical and basal plasma membranes. A previous immunohistochemical study by Settle et al. [20], who used human term placenta, reported a more pronounced localization of MCT1 in the basal plasma membrane than apical plasma membrane of syncytiotrophoblasts, while the apical microvillous

membrane expressed MCT4. The present study confirmed the polarized expression of MCT1 and MCT4 in the syncytiotrophoblast of human placenta with antibodies against rat MCT1 and MCT4. By use of the same antibodies, we revealed the reversed topology of two transporters in mouse trophoblasts: MCT1 and MCT4 were localized respectively to the apical and basal plasma membranes of the syncytiotrophoblast layer (Fig. 6). The immunoreactivities for MCT1 and MCT4 in the mouse were restricted to the plasma membranes of trophoblasts, as confirmed at the electron microscopic level. Furthermore, the co-existence of CD147, involved in the trafficking of MCT molecules, indicated the functional significance of the polarized arrangement of two MCTs in the mouse placenta.

The reverse localization of MCT1 and MCT4 between humans and mice is a mystery but may be important for considering the direction of transport as well as functional significance of MCT. MCT transports lactate across the plasma membrane in both inward and outward directions, according to the concentration gradient of lactate and H^+ [14, 15]. Indeed, lactate can be transported in both directions by the same carrier system under certain experimental conditions using placental membrane vesicles of rats [37]. Inuyama et al. [5] noted the lower affinity of lactate carriers on the fetal side as compared with the maternal side of the trophoblast in human placenta. The characterization of MCT4 transfected in *Xenopus* oocytes revealed a transporter with much lower affinities for monocarboxylates than MCT1 [39,40]. These findings agree with the fact that the murine placenta provides low-affinity MCT4 and high-affinity MCT1 on fetal and maternal sides, respectively (Fig. 6). Taking the relationship of an affinity to substrates and the localization of transporters into consideration, MCTs in the murine placenta may drive lactate *from the mother or placenta to fetus*. The placental

origin of lactate in fetal circulation is suggested by an intense expression of lactate dehydrogenases in the trophoblast of human [41] and murine placentas (our unpublished data). However, a definite determination of the direction of lactate transport requires further confirmation, including the direct visualization of isotope-labeled lactate injected *in vivo*.

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Figure legends

Fig. 1 X-ray film images showing mRNA expressions of major subtypes for MCT and GLUT using serial sections from the mouse placenta. Placental samples were obtained at embryonic days 11.5, 14.5, 17.5, and 18.5. The sample for E11.5 contains three placentas and uteruses. Labyrinthine zones on the fetal side of placenta are shown with asterisks. Comparatively intense expressions are seen for GLUT1, MCT1, MCT4, MCT9, and CD147; the expressions of GLUT3, MCT5, MCT10, and MCT12 are less intense. A negative control for MCT1 is shown in as “MCT1/cold”. *Bar 5 mm*

Fig. 2 Light microscopic dark field images of GLUT1, GLUT3, MCT1, and MCT4 mRNAs in serial sections from the E11.5 mouse placenta. The labyrinth intensely expresses GLUT1, MCT1, and MCT4, while the signals for GLUT3 are less intense (**a–d**). Immunohistochemistry for GLUT1 and MCT1 (**e, f**) immunolabels the placental labyrinth in two serial sections at E14.5. Further expression of GLUT1 is seen in the junctional region indicated by asterisks at the mRNA and protein levels (**a, e**). *Bar 200 μ m* A higher magnification of spongiotrophoblasts immunoreactive for GLUT1 is shown in an inset of Fig. 2**e**. *Bar 10 μ m*

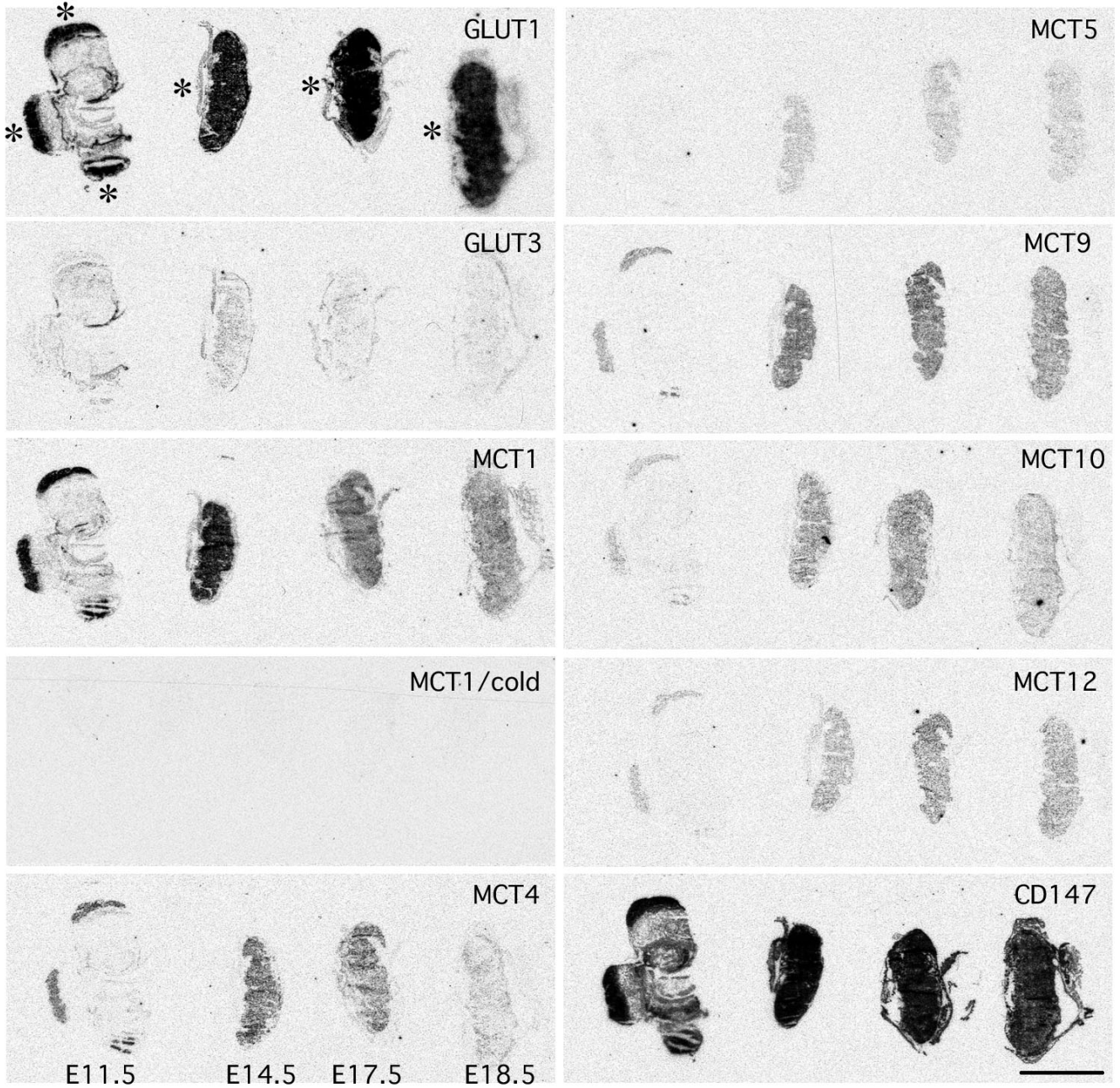
Fig. 3 Immunoreactivities for GLUT1, MCTs, and CD147. Higher magnification of four thin (2 μ m in thickness) serial sections stained with four antibodies (E11.5 mouse placenta). GLUT1 and CD147 immunoreactivities in each section appear as double lines at the syncytiotrophoblast (arrows in **a** and **d**). The MCT1 immunoreactivity (**b**) is seen as a thick line facing the maternal blood (M), while the MCT4 immunoreactivity (**c**) exhibits a thin line facing the fetal blood (F) containing nucleated red blood cells. Double staining of MCT1 (green) and MCT4 (red) in a section from the E11.5 mouse

placenta (**e**) shows that two immunoreactivities are localized separately in the apical plasma membrane and basal plasma membrane, respectively. *Bar 10 μ m* Figure 3**f** shows an immunoblot analysis of MCT1 and MCT4 in the extracts of the E17.5 mouse placenta (p1). The sample from the cecum (ce) was used for a control of MCT1 immunoreactivity. The antibody against MCT1 detects an intense single band around 43 kDa, in agreement with the original data by Garcia et al. [15]. The MCT4 antibody labels a protein band at a slightly lower molecular weight than MCT1, as detected in the skeletal muscle by Wilson et al. [25].

Fig. 4 Electron-microscopic observation of MCT1, MCT4, and GLUT1 in the E14.5 mouse placenta. Particles showing the immunoreactivity of MCT1 are located at microvilli on the apical side of syncytiotrophoblast I (arrows in **a**), while the MCT4 immunoreactivity is along the basal plasma membrane of syncytiotrophoblast II (arrows in **b**). Both plasma membranes of syncytiotrophoblast are labeled for GLUT1 (arrows in **c**). The cytotrophoblast (CT) and endothelium (E) of fetal blood vessels are free of any immunoreactivities. *R* red blood cells of fetus *Bar 1 μ m*

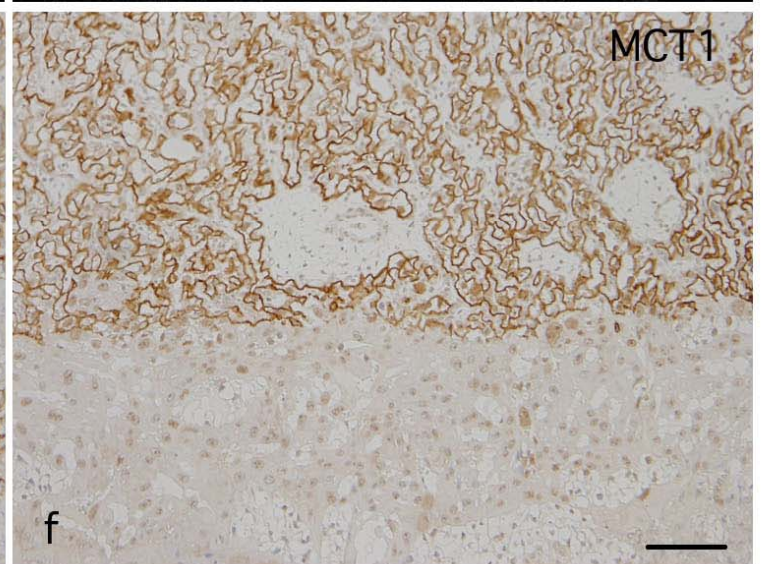
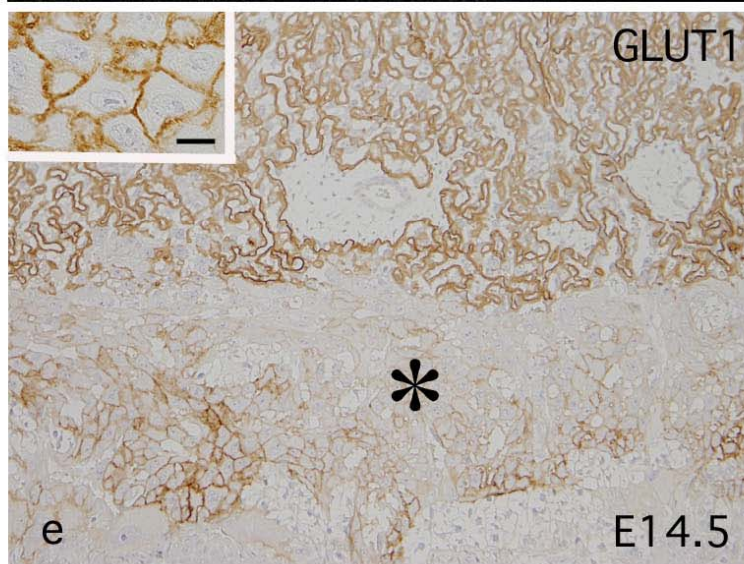
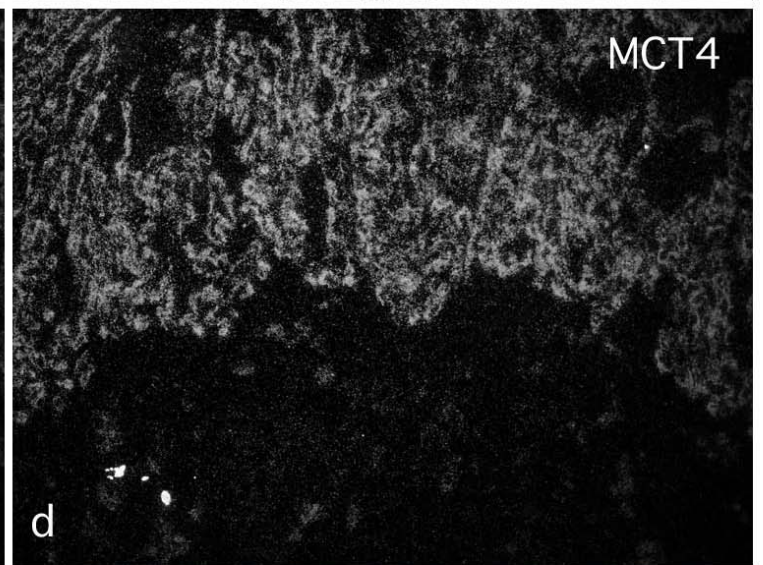
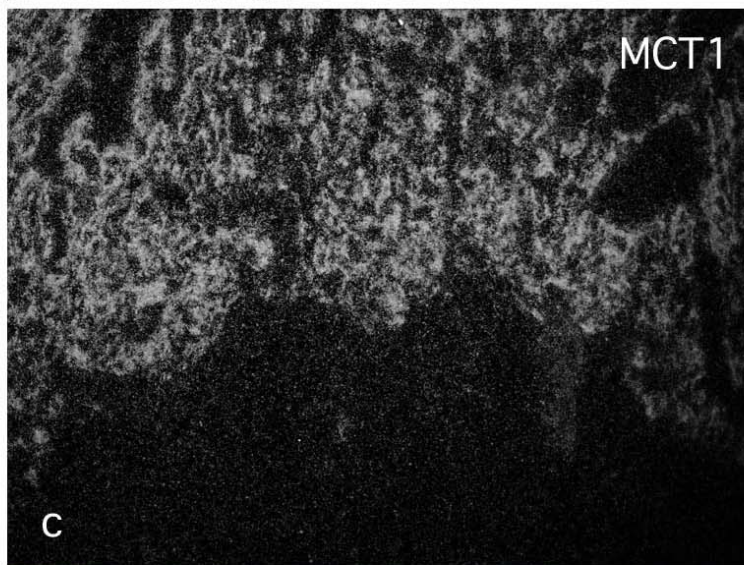
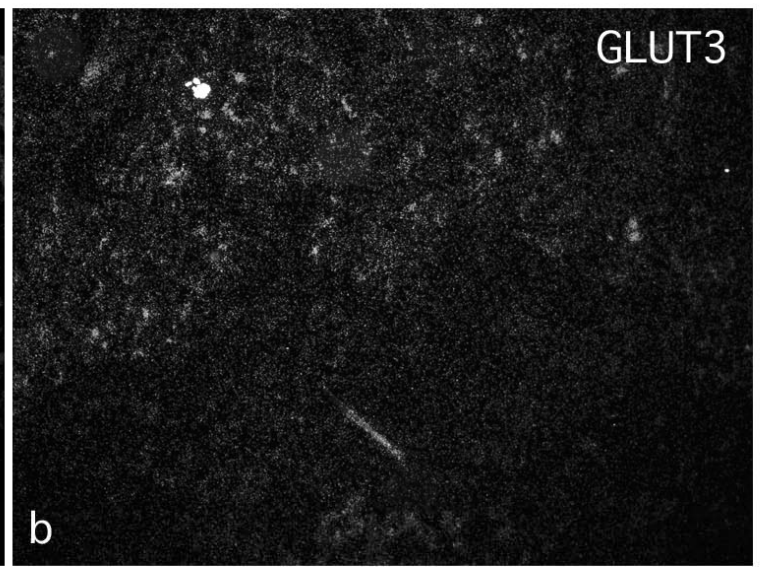
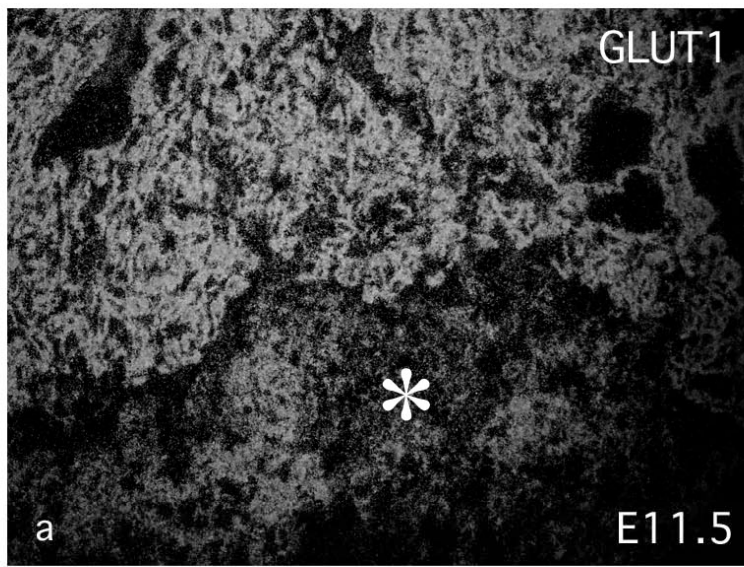
Fig. 5 MCT1, MCT4, and GLUT1 immunoreactivities in the human term placenta. The MCT1 immunoreactivity is distributed at the basal plasma membrane of the trophoblast (**a**), while the MCT4 immunoreactivity, though less intense, is localized at the apical plasma membrane facing the maternal blood (**b**). The GLUT1 immunoreactivities are seen in both the apical plasma membrane and basal plasma membrane (**c**). The GLUT1 and MCT1 immunoreactivities appear to encircle the scattered cytotrophoblasts (arrows). *Bar 10 μ m*

Fig. 6 A figure showing the arrangement of transporters and possible direction of lactate transfer in the mouse trophoblast.

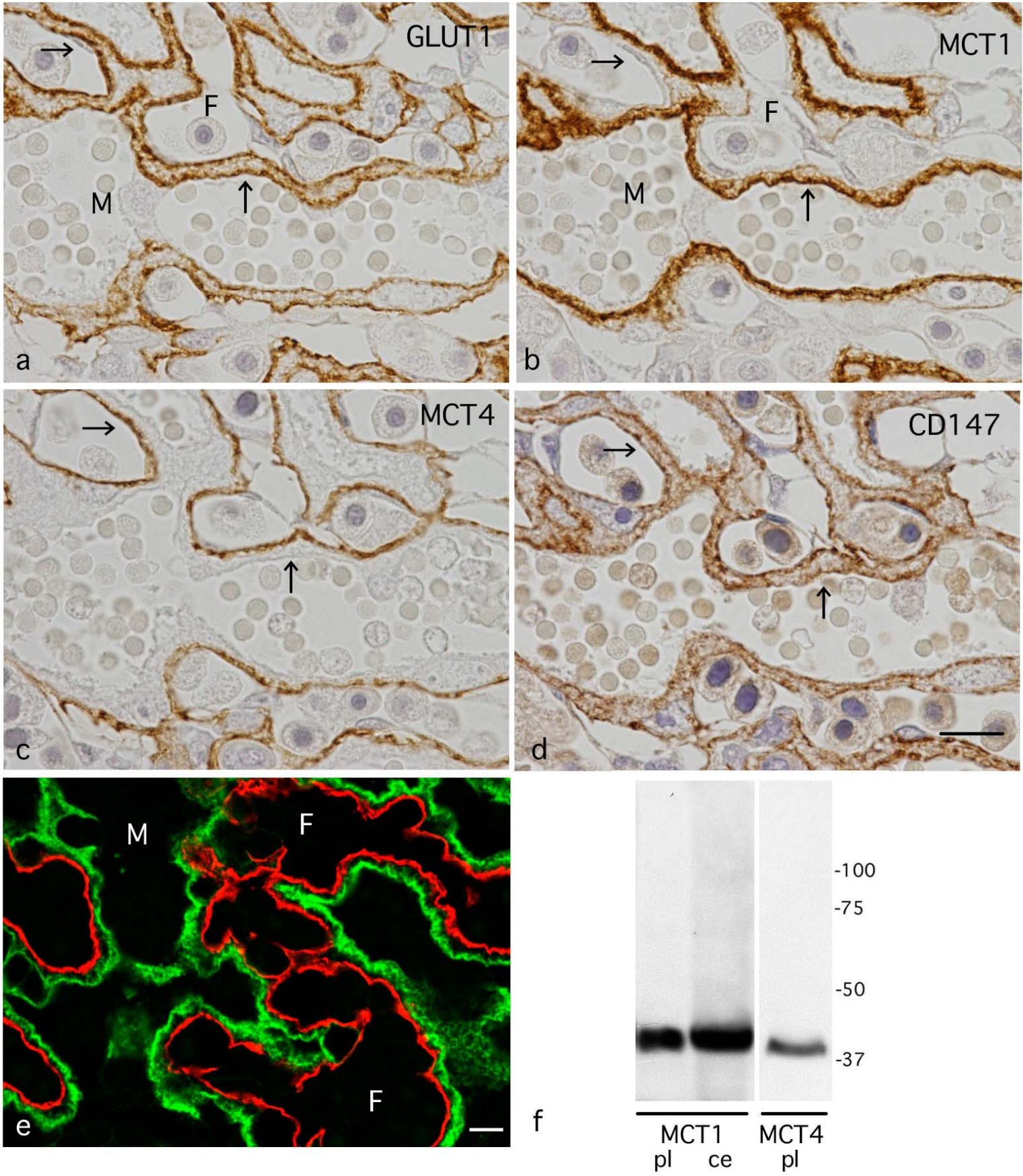


5 mm

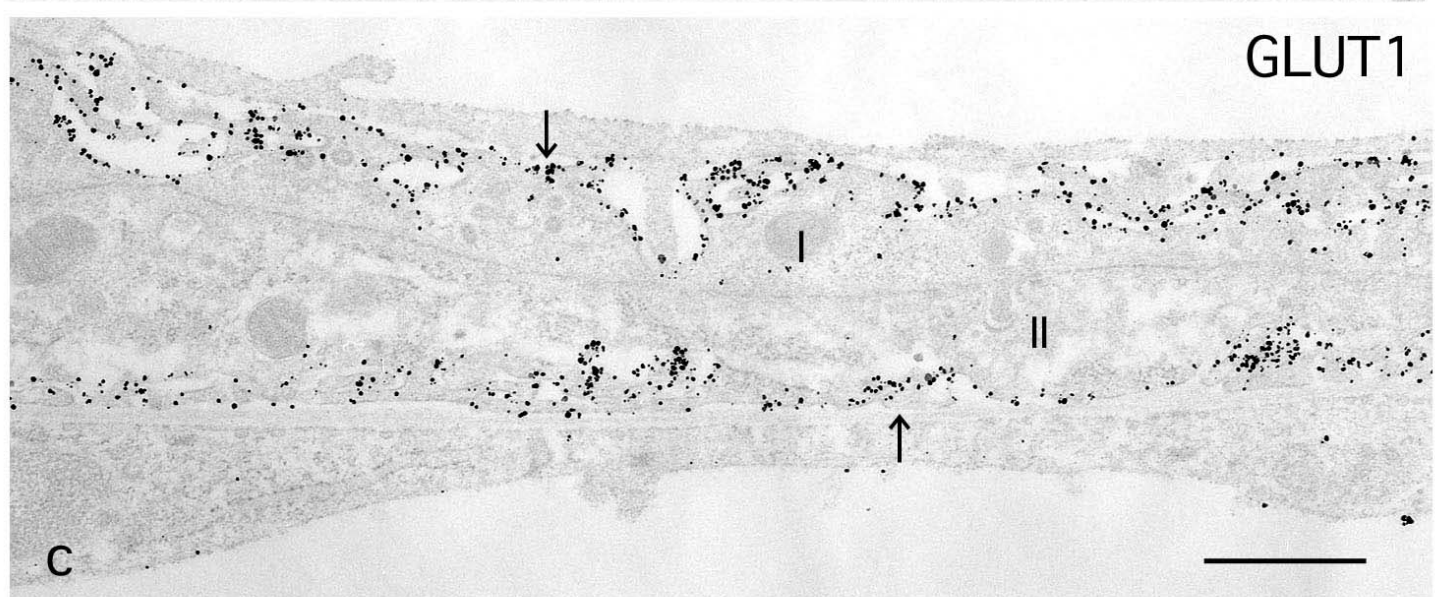
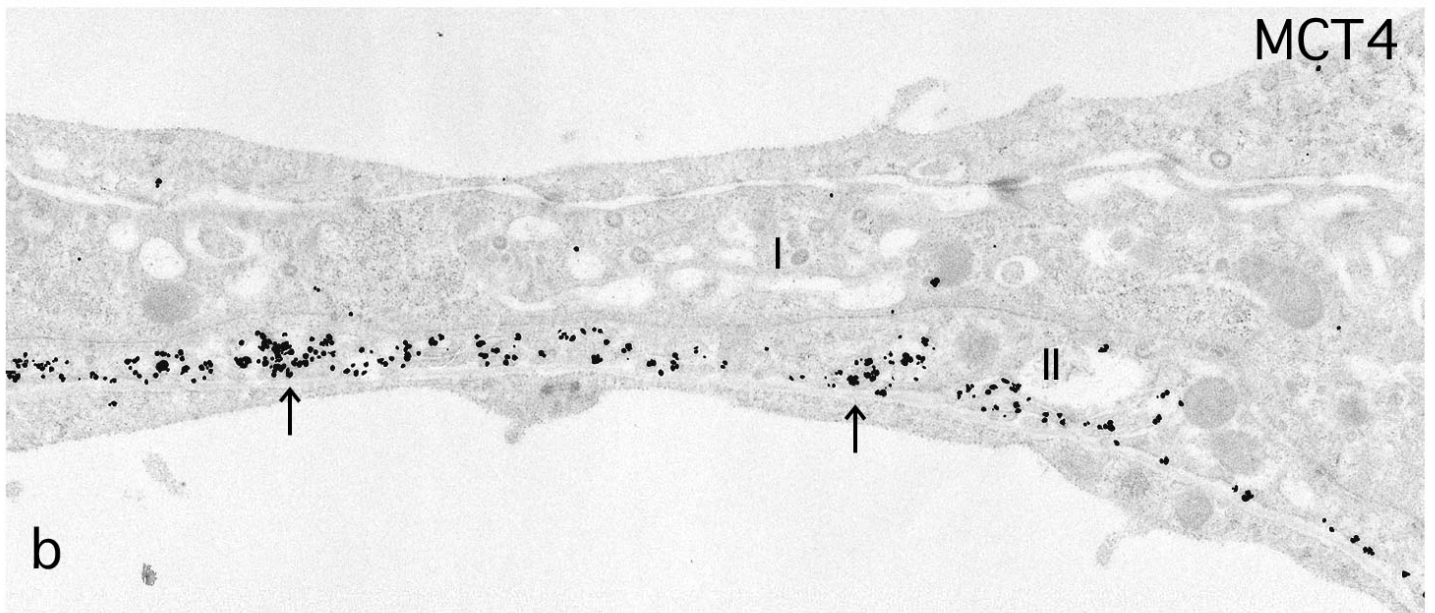
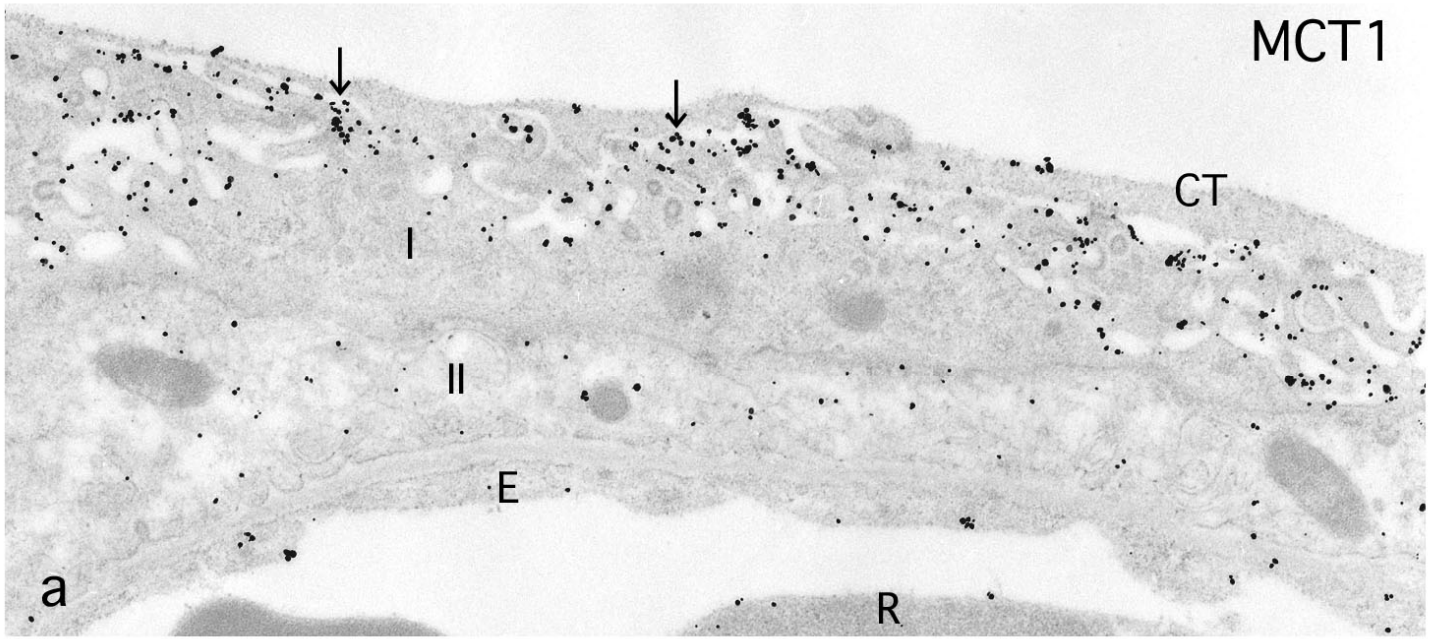
Nagai et al. Fig. 1



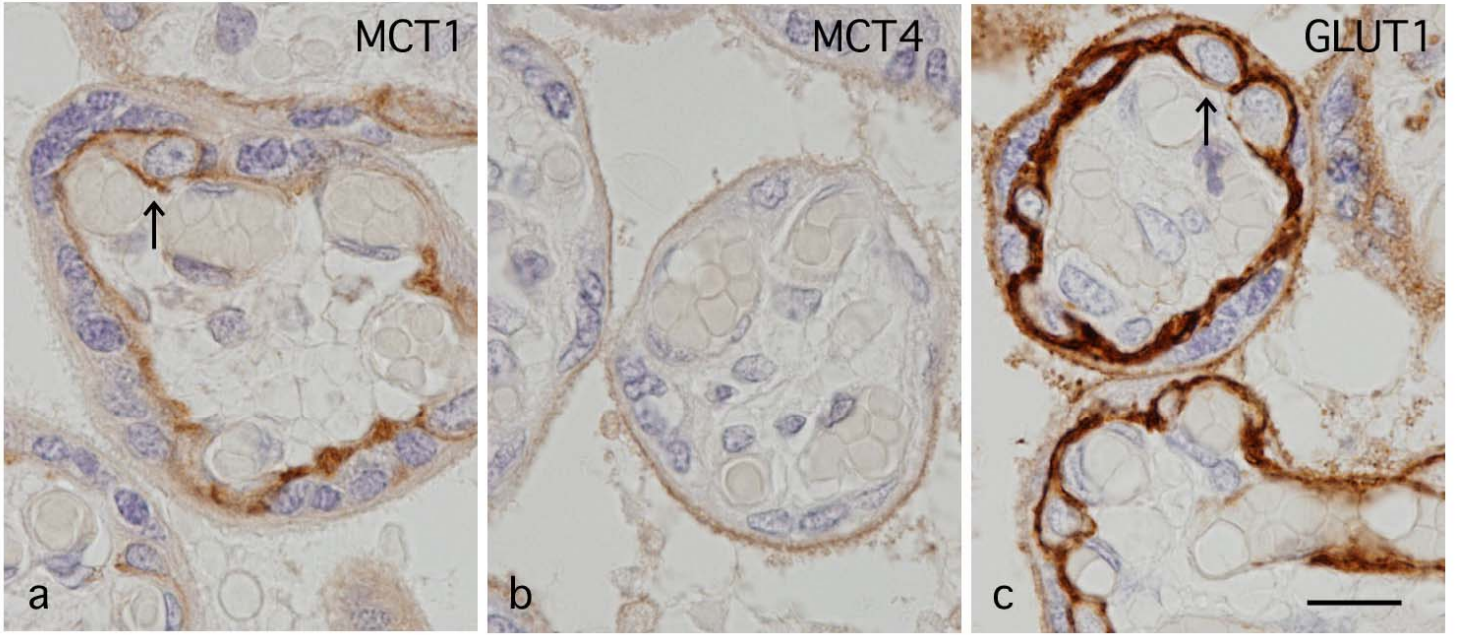
Nagai et al. Fig. 2



Nagai et al. Fig. 3



Nagai et al. Fig. 4



Nagai et al. Fig. 5

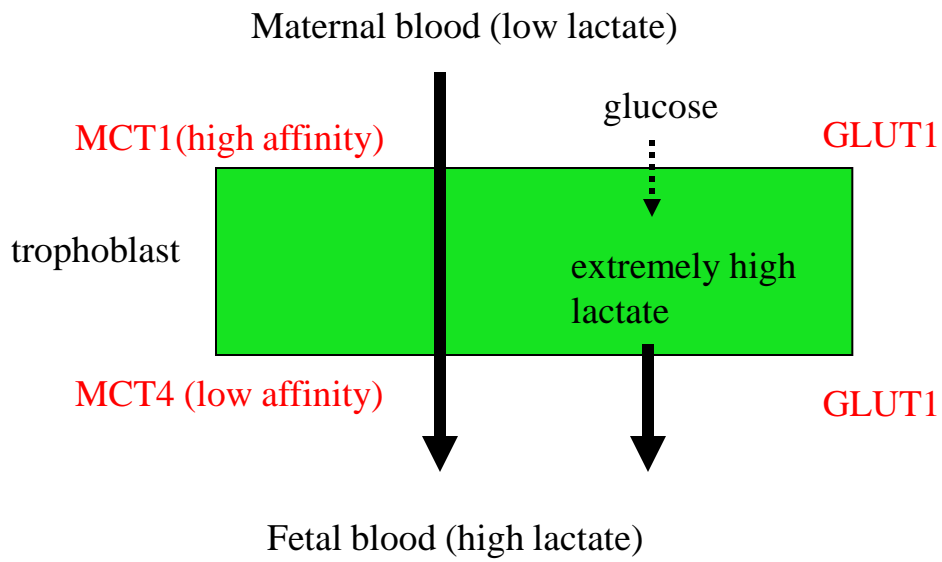


Fig. 6